UV-B and Biosphere

Advances in vegetation science 17

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UV-B and Biosphere

Edited by

J. ROZEMA
Free University, Amsterdam, The Netherlands

W.W.C. GIESKES
State University of Groningen, The Netherlands

S.C. VAN DE GEIJN
Research Institute of Agrobiology and Soil Fertility (AB-DLO), Wageningen, The Netherlands

C. NOLAN
European Commission – DGXII-E-1, Science & Technology for Environm. Protection, Brussels, Belgium

and

H. DE BOOIS
Netherlands Research Organisation, The Hague, The Netherlands

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Ecosystem experiment of effects of enhanced UV-B radiation and atmospheric CO_2 enrichment on a subarctic heathland, Abisko Research Station, Sweden.
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Contents

Preface

Current phase-out schedules of the production and emission of CFC's indicate that chlorine loading in the stratosphere is not yet at its maximum. The recovery of stratospheric ozone is estimated to take time and elevated levels of UV-B radiation are expected to occur throughout most of the next century. Despite numerous physiological studies of UV-B effects on plants, often grown in climate chambers, knowledge of UV-B effects on organisms and processes in natural aquatic or terrestrial ecosystems is poor. Currently it appears that UV-B radiation is not just an environmental stress factor to plants. In various ways, which are incompletely understood, UV-B affects a wide range of physiological and ecological processes. Remarkably, recent field studies indicate that enhanced UV-B does not markedly affect photosynthesis, growth and primary production, but rather interferes with plant morphogenesis and plant and ecosystem functions relating to the secondary metabolism. This special issue and book UV-B and Biosphere is an attempt to cover this range and to report the progress made in the research of ecological effects of enhanced solar UV-B radiation.

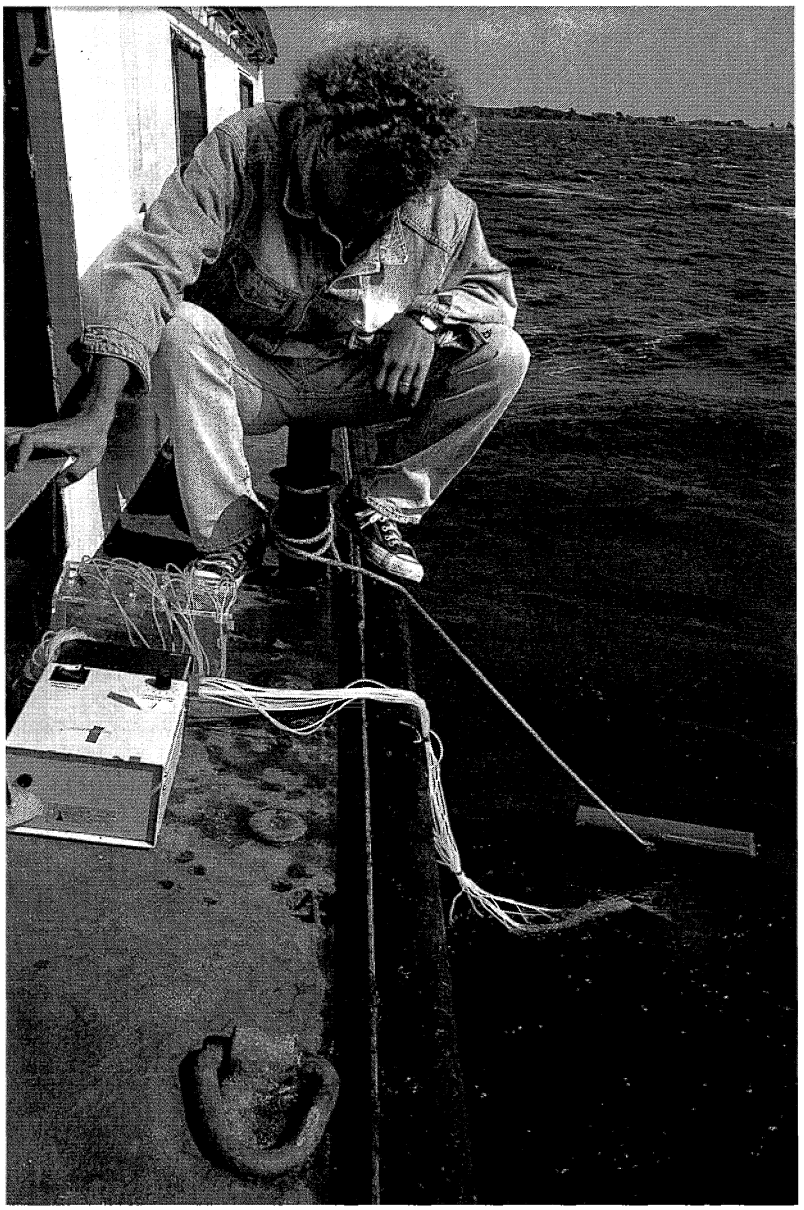
The papers in this book formed the basis of an international workshop entitled 'UV-B and Biosphere', December 15–18, 1995, in Wageningen, The Netherlands. A first reaction of Hans de Boois on the number of papers and sessions scheduled from Friday to Sunday morning was: far too many. Winfried Gieskes convinced us all in saying that the exciting progress in UV-B research to be presented, would no doubt catch and hold the attention of the audience. And so it was. The numerous papers were alternated by lively discussions. There was time for a Dutch 'borrel', a visit to Burgers Bush Zoo and Sunday evening a workshop dinner completed the conference.

The workshop was sponsored by the European Commission, the Dutch Ministry of Housing, Physical Planning and Environment (VROM), The Netherlands Organization for Scientific Research (NWO), the Royal Netherlands Academy of Arts and Sciences (KNAW), and the Department of Ecology and Ecotoxicology, Vrije Universiteit, Amsterdam. The organizers and editors are indebted for this financial support. We also acknowledge Mrs. Desirée Hoonhout, Mrs. Karin Uyldert, Mr. Arno Gregoor for preparation of the announcement, the workshop booklet, registration and financial administration.

We are indebted to Hendrik Prins, Noeline Gibson and Julian Sienkiewicz (Kluwer Academic Publishers) for their stimulating interest and professional support in the publication of the special issue and book UV-B and Biosphere.

Jelte Rozema
Winfred Gieskes
Siebe van de Geijn
Canice Nolan
Hans de Boois

I. UV-B and aquatic ecosystems



Water samples are taken simultaneously from different depths using submerged pumps during a Baltic sea research cruise. (Photograph: D. P. Häder)

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J. Rozema, W. W. C. Gieskes, S. C. van de Geijn, C. Nolan and H. de Boois (eds), UV-B and Biosphere.

Penetration and effects of solar UV-B on phytoplankton and macroalgae

D.-P. Häder
Institut für Botanik und Pharmazeutische Biologie, Friedrich-Alexander-Universität, Erlangen, Germany

Key words: Aquatic ecosystems, Light penetration, Macroalgae, Phytoplankton, Solar UV-B radiation

Abstract

The effects of short wavelength solar radiation on aquatic ecosystems were studied in several marine and freshwater systems. The spectral distribution and the penetration of solar radiation into different water types (coastal and oceanic waters of the Baltic Sea, North Sea, Atlantic and Mediterranean) were investigated. Penetration of short wavelength solar radiation strongly depends on the content of dissolved and particulate substances as well as the concentration of phytoplankton. The primary producers often show a typical vertical distribution within the euphotic zone and are reached as well as affected by the penetrating UV-B radiation. The effect of this radiation was both determined in phytoplankton and macroalgae. Measuring pulse amplitude modulated (PAM) fluorescence indicated that major biomass producers were severely inhibited by surface radiation and even impaired at their natural growth site. Likewise, photosynthetic oxygen production was affected by extended exposure to solar radiation.

Introduction

Biomass productivity of aquatic ecosystems equals that of terrestrial ecosystems on our planet. An annual total of about 100 Gt (10^9 tons) of atmospheric carbon has been estimated to be incorporated into organic material (Houghton & Woodwell 1989; Siegenthaler & Sarmiento 1993). Thus, aquatic ecosystems play a major role in controlling carbon fluxes and atmospheric concentrations. It is of eminent importance to study and understand the potential effects of increased solar UV-B irradiation on marine productivity (Prézelin et al. 1993; Smith 1989) since any substantial decrease in carbon uptake by this major sink would increase the effect of the greenhouse gas CO_2.

The largest share in biomass production can be attributed to phytoplankton as its habitat represents vast areas of the oceans. As primary producers they constitute the first level for the intricate food web in the oceans and are the basis for the crop of fish, crustaceans and mollusks. In contrast, most macroalgae are restricted to coastal areas, but their productivity should not be underestimated both for ecological and economic reasons.

Investigations in the past few years have indicated that many aquatic ecosystems are under considerable UV-B stress even at current levels (Acevedo & Nolan 1993; Biggs & Joyner 1994; Cullen & Lesser 1991; Cullen & Neale 1994; Häder 1993a, b; Holm-Hansen et al. 1993a, b; Karentz et al. 1994; SCOPE 1992a, b; Smith & Cullen 1995; Smith et al. 1992; Tevini 1993; Weiler & Penhale 1994; Williamson & Zagarese 1994). Being dependent on solar radiation for energetic reasons primary producers occupy the upper layers in the water column, where they are simultaneously exposed to high levels of ultraviolet radiation.

UV-B radiation affects cellular DNA, impairs photosynthesis, enzyme activity and nitrogen incorporation, bleaches cellular pigments and inhibits motility and orientation (Döhler et al. 1991; Häder et al. 1989, 1991, 1995; Worrest & Häder 1989; Häder & Worrest 1991).

In addition to exact predictions of future levels of UV-B radiation on a global basis, a number of questions needs to be answered in order to quantify the effects of solar UV-B radiation on natural aquatic ecosystems:

– What is the spectral penetration of UV-B radiation as a function of depth in different water types?

– What is the vertical distribution of the aquatic organisms in the water column for major water types?

– What is the biologically and spectrally weighted sensitivity of the organisms toward solar UV-B?

– What are the extent and limits of UV repair and adaptation in major biomass producers?

Materials and methods

Penetration of solar radiation into the water column

The transparency of the water strongly depends on the amount of seston (particulate substances) and gelbstoff (yellow dissolved organic substances). Jerlov (1970) suggested to describe the optical properties of bodies of water by classifying them into several oceanic and coastal types. In eutrophic ponds and lakes as well as in turbid coastal waters UV-B may penetrate less than 1 m to the 1% level; in contrast, in clear oceanic waters penetration to 30 or 40 m has been measured (Smith & Baker 1979; Smith et al. 1992). Measurements were performed in different coastal lagoons near the islands of Hiddensee and Rügen (Baltic Sea), in the North Sea off Helgoland as well as in the Mediterranean (off Sardinia, Greece and Spain).

The irradiation data were measured with a double monochromator spectroradiometer (type 752, Optronic Laboratories, Orlando, FL, USA). For the measurements of irradiance above the surface a commercially available Ulbricht sphere was used (Piazena & Häder 1994, 1995). For the measurements in the water column a novel 4π sensor was developed connected to the entrance slit of the radiometer by a 20 m long quartz fiber cable. The optical spherical entrance of the 4π sensor is realized by the polished ends of 72 quartz fiber cables of 250 μm diameter each, which were oriented radially and isotropically at the surface of a sea water resistant PVC hollow sphere. The deviation of the sensor response from an ideal 4 geometry is smaller than 10%. Before each measurement series the wavelength stability of the spectroradiometer was checked against a mercury calibration lamp (Optronic Laboratories, Orlando, FL, USA).

Vertical distribution of phytoplankton in the water column

The vertical distribution of phytoplankton in the water column was estimated by analysis of 20 water samples of 1 l each, which were sampled synchronously at equidistant intervals between surface and maximal depth (6 and 25 m, respectively) by submersible electric pumps during 1 min.

The organisms contained in each sample were concentrated by tangential flow filtration (Filtron) into a volume of 1.5 ml. Cell density was determined by automatic image analysis using a video digitizer (PIP 1024 B, Matrox, Québec, Canada) and an AT type computer (486 CPU) running the program COUNTC (Häder & Vogel 1991; Häder & Griebenow 1989). Five counts each were performed in two aliquots of each sample in a quartz cuvette (1 mm deep, Hellma, Mülheim); means and standard deviations were calculated.

Measurements of PAM fluorescence

A portable pulse amplitude modulated fluorometer (PAM 2000, Waltz, Effeltrich, Germany) was used to determine *in vivo* chlorophyll fluorescence on site (Schreiber et al. 1986). Macroalgae were freshly harvested and mounted in open UV-B translucent Plexiglas frames submersed in shallow water. Photoinhibition was induced by exposing the specimens to solar radiation after previous dark adaptation. Subsequently, the samples were shaded again, and the recovery of the quantum yield was determined at predefined time intervals for up to 6 h. In another type of experiment thalli were collected every hour from sunrise to sunset, and the fluorescence parameters were determined immediately after harvest.

Oxygen exchange measurements

Oxygen exchange was measured at the surface or in the water column with a submersible device using solar radiation as actinic light source (Häder & Schäfer 1994a, b). Oxygen concentration, PAR irradiance, temperature and depth were measured and after amplification the signals were routed to an analog/digital card located in a laptop computer. The computer program determines the data at frequent intervals, displays and stores them on the hard disk drive. The kinetics of photoinhibition was determined in thalli exposed to solar radiation immediately after harvest. After an initial measurement of dark respiration, net oxygen production was assayed until photoinhibition was manifest.

Results

Penetration of solar radiation into the water column

The Baltic Sea waters around the island of Rügen have to be classified as typical coastal waters with high concentrations of yellow substance and seston between C9 and C13 in the extended Jerlov system (C-coastal water type). High attenuation coefficients were found especially in the short and long wavelength ranges of the solar spectrum. Secchi depth varied between 0.5 and 1.9 m. Figure 1a shows spectra of total and diffuse solar UV irradiation at the sea surface for cloudless sky, total atmospheric ozone of 348 DU and different solar elevations. Using the action spectrum for inhibition of motility in *Euglena gracilis* (Häder & Liu 1990) the associated spectral efficiency was calculated from these data (Figure 1b). The maximal efficiency of solar irradiation centers around 310 nm. Figure 2 shows the penetration of solar irradiance integrated both over the range 290–320 nm and 290–400 nm into the water column for two Bodden waters off the island of Hiddensee and for the clear waters in the near shore zone off the island of Sardinia (Mediterranean Sea).

The waters of the Mediterranean Sea near Malaga were identified as typical oceanic with low concentrations of yellow substance and seston (about 0.0122 g l^{-1}), Secchi depth of 14 m and water type OIII in the Jerlov system. The attenuation coefficient depends on yellow substances and particles and, in addition, on the absorption and scattering caused by organisms contained in the column. The attenuation coefficients at 440, 510 and 680 nm increase with the concentration of phytoplankton organisms due to the absorption by chlorophylls and carotenoids. In contrast, the attenuation coefficient at 560 nm does not depend on the organism concentration because of the absence of accessory pigments like phycoerythrin in the species populating these waters.

Vertical distribution of phytoplankton

The stratification of phytoplankton is caused by active or passive movements of the organisms but affected by strong wind and waves. Vertical distributions have been investigated in the Baltic Sea, North Sea and the Mediterranean over long periods of time. Three different types of vertical distribution of the phytoplankton communities were found during noon time and at low winds in the investigated waters in the Baltic Sea and in the Mediterranean: maxima of phytoplankton concentration at different depths for Baltic Sea waters of type C9-C13 (Figure 3a), concentration of the organisms at the surface (Figure 3b) and increasing phytoplankton concentration with depth including two minima at 3 and 4 m depth, measured in the Mediterranean Sea near Malaga (Figure 3c). The figures show typical examples of many consecutive measurements.

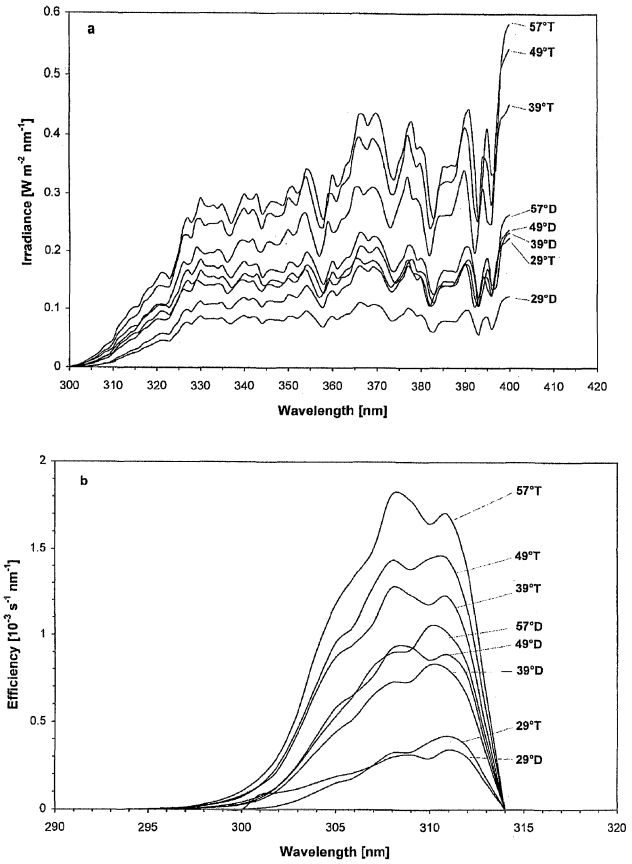

Figure 1. Spectra of total (T) and diffuse (D) solar UV irradiation measured above sea surface (**a**) and calculated spectra of inhibition of motility in *Euglena gracilis* (**b**) determined at Kloster/Hiddensee, 29 June 1993, cloudless sky, total ozone column: 348 DU.

A massive phytoplankton bloom occurred near the island of Helgoland in July 1993. Most phytoplankton organisms were dinoflagellates. The bulk of the biomass was represented by several *Ceratium* species, which could easily be distinguished by the image analysis system used to count the cells, as they were much larger than the other species; their distribution followed that of the other flagellates and that of the total count. Samples were taken northeast of the island during calm weather. At 11 a.m. local time an almost exclusive accumulation of phytoplankton at the surface

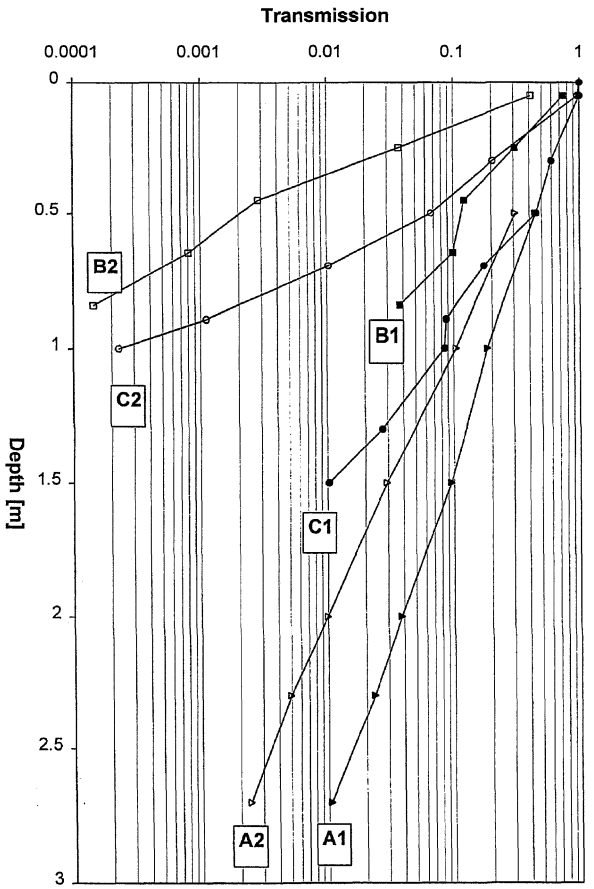

Figure 2. Transmission of solar UV irradiation between 290 and 400 nm (1) and between 290 and 320 nm (2) in the waters near Sardinia (Mediterranean Sea) (A: Baia di Tramariglio, 5 October, 1993) and in different coastal waters near the island of Hiddensee (Baltic Sea) (B: Kloster/harbor, 27 August, 1993; C: Klosterloch, 14 May, 1993.

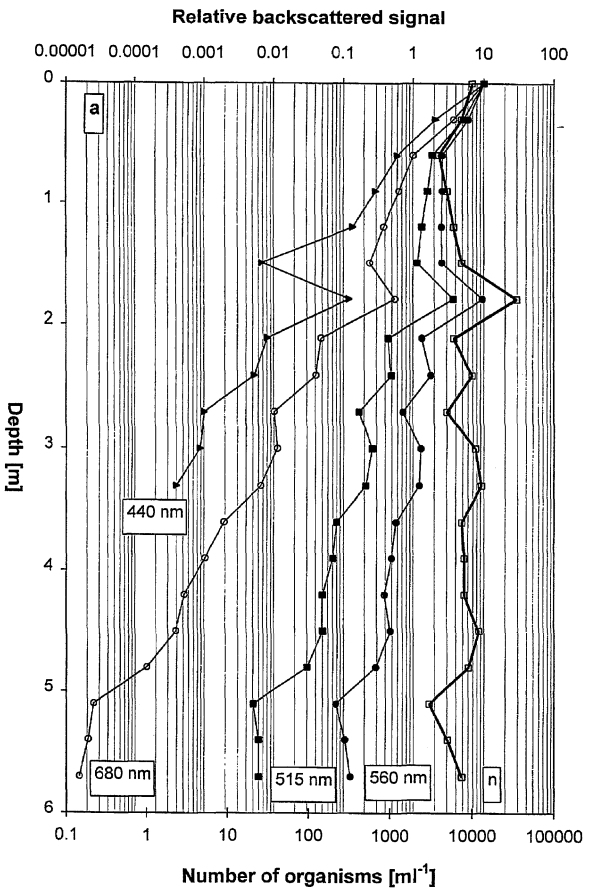

Figure 3a.

Figure 3. Concentration of phytoplankton (n) and the relative backscattered signals at the wavelengths 440, 515, 560 and 680 nm in dependence of depth (z_i) for the Baltic Sea lagoon stations FTL, 11 May, 1994 (**a**), SG, 11 May, 1994 (**b**) and for the near shore waters off Malaga (Spain) on 15 March, 1995 (**c**).

was found with a smaller maximum between 2 and 3 m and some more organisms below 5 m. At 1 p.m. the dense population at the surface had moved to greater depth forming a maximum at about 2 m and a larger one below 4 m. On the following day there were high wind and waves. Consequently, the *Ceratium* species and the other flagellates were almost randomly distributed throughout the water column down to 6 m which in summer is well above the pycnocline.

PAM fluorescence of phytoplankton and macroalgae

Thalli of *Padina pavonia* were harvested from close to the surface and kept in a shallow rock pool suitable for on-site measurements with the PAM instrument. The optimal quantum yield was determined after 30 min of dark adaptation (Figure 4). Then the thalli were exposed to solar radiation for 30 min during which time the yield had decreased substantially. After exposure the thallus was shaded again and recovery measured at predetermined intervals. The same experiment was repeated with thalli harvested from 5 m depth. In this experiment the thalli were exposed in a UV-transmitting Plexiglas container which kept the algae in place so that exposure and measurement area could be controlled; but sea water circulated through the container. When the UV-B component of solar radiation was removed by a cut-off filter WG 335 recovery was faster; this phenomenon was even more pronounced

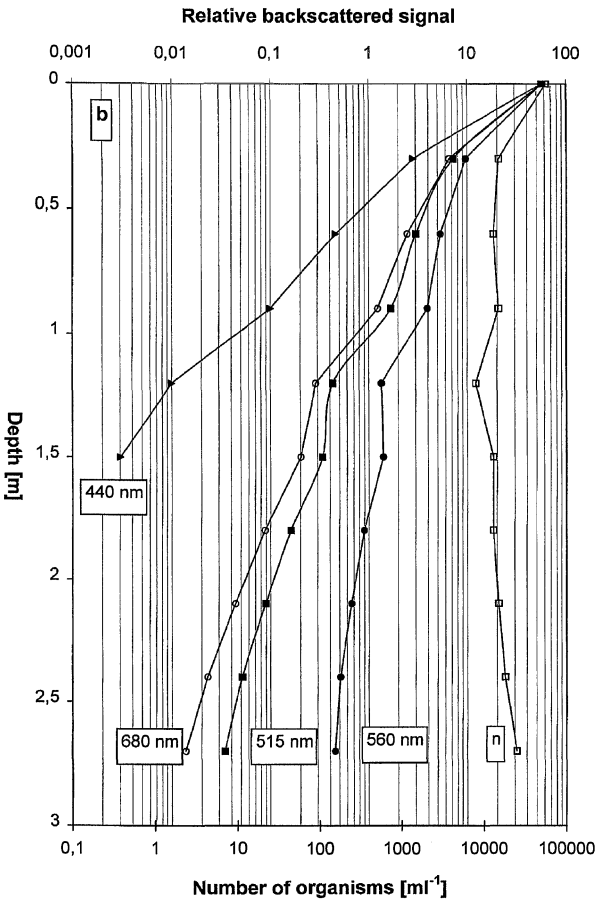

Figure 3b.

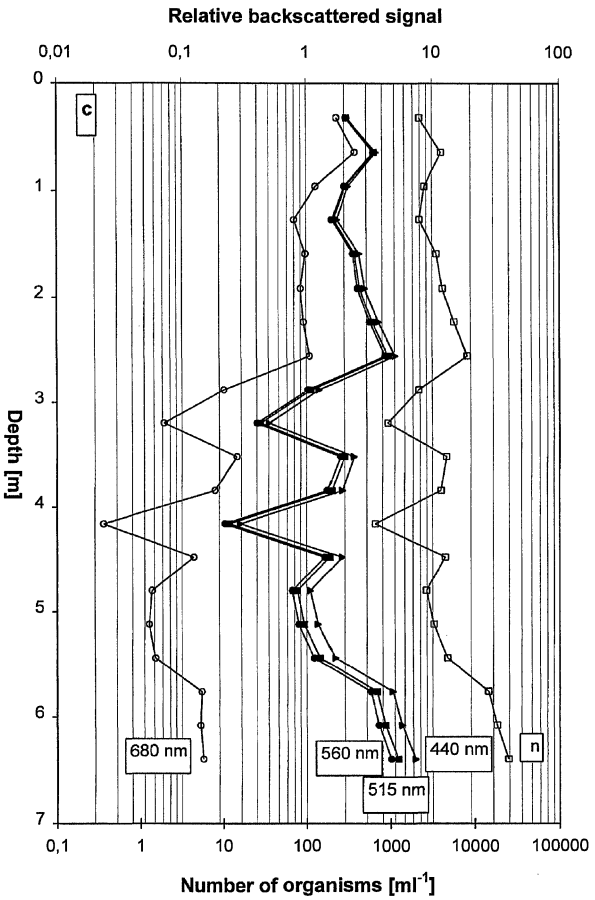

Figure 3c.

when also the UV-A component was removed (data not shown).

In the experiments described above the thalli were exposed to direct solar radiation in a shallow rock pool where they received unattenuated solar radiation. In order to determine whether photoinhibition occurs also at their natural growth site, algae were harvested from dawn to dusk at 1 h intervals and the yield was determined immediately after harvest (Figure 5). The samples of both algae showed maximal yield values of more than 0.7 for the first few hours. After 9.00 h the yield decreased significantly and recovered only very slowly over the day in specimens harvested from a site close to the surface. In contrast, specimens harvested from 6 m were only little affected. The same experiment was repeated with tufts of the cyanobacterium *Rivularia* spec. (Figure 6). This species grows in the spray water zone and is exposed to unattenuated solar radi-

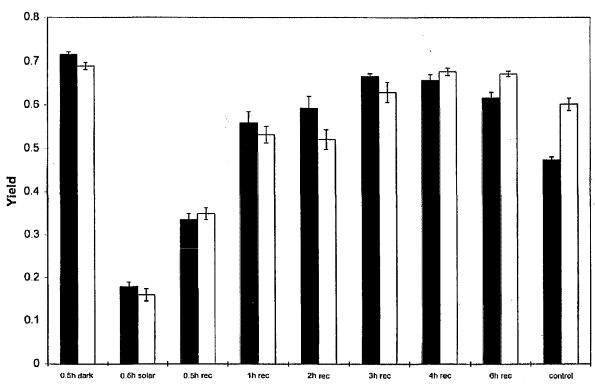

Figure 4. Photosynthetic yield as measured by PAM fluorescence in *Padina pavonia* harvested from 0 m (closed bars) and 6 m depth (open bars) before and after exposure to unattenuated solar radiation as well as during recovery in dim light. Bars indicate the mean values of eight measurements with S.E.

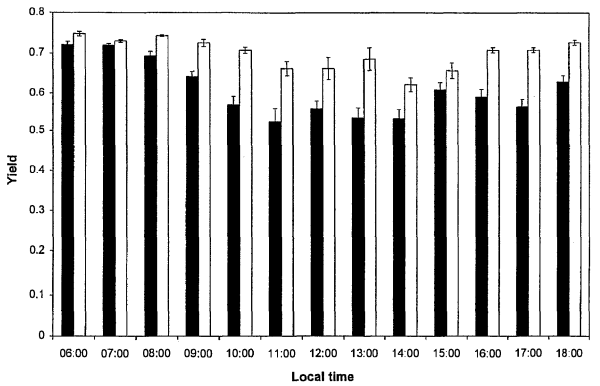

Figure 5. Photosynthetic quantum yield of *Padina pavonia* harvested from 0 m (closed bars) and 6 m depth (open bars) measured at 1 h intervals from dawn to dusk. Thalli were retrieved from their growth site and measured immediately after harvest. For each data point at least eight measurements were averaged and standard deviation calculated.

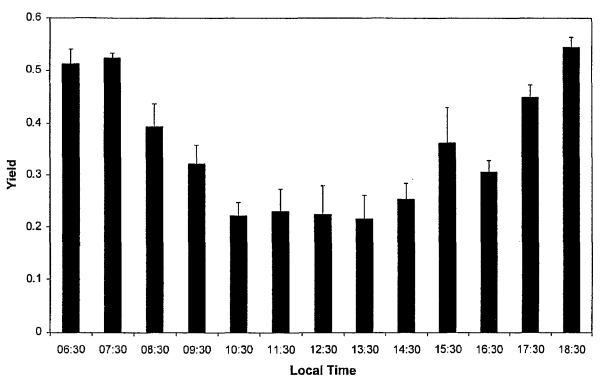

Figure 6. Photosynthetic quantum yield of the cyanobacterium *Rivularia* spec. measured at their growth site at 1 h intervals from dawn to dusk. For each data point at least eight measurements were averaged and standard deviation calculated.

ation. Even though, the organisms show a dramatic inhibition during most of the day.

Oxygen production of phytoplankton and macroalgae

Thalli of *Rivularia* were harvested and immediately transferred into the instrument to measure oxygen exchange. The thalli showed a significant dark respiration. When exposed to solar radiation close to the surface, oxygen production started to decline after a few minutes of exposure; however, complete photoinhibition was not reached even within 80 min (Figure 7).

In another type of experiment a sample of *Padina* was harvested from a rock pool and after measuring dark respiration it was exposed at different depths

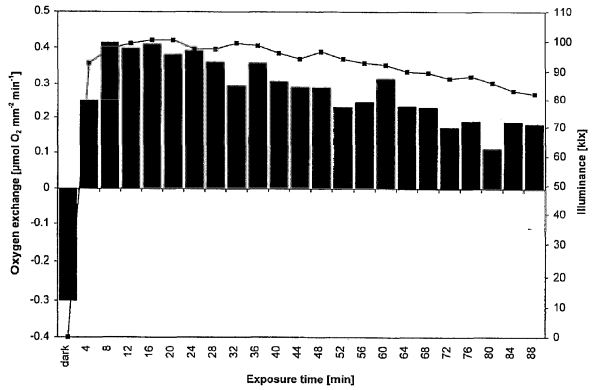

Figure 7. Oxygen exchange (bars) in *Rivularia* spec. as affected by solar radiation at the surface in comparison to the illuminance (solid squares and solid line). Temperature was 23 °C.

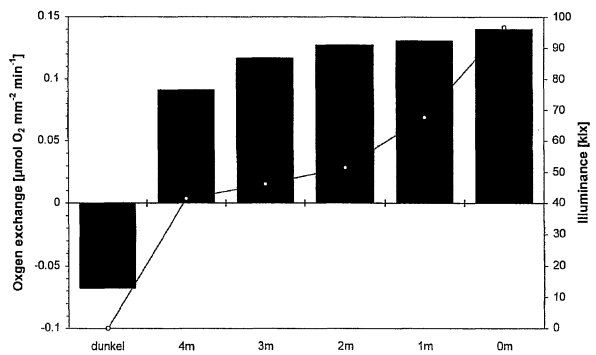

Figure 8. Photosynthetic oxygen exchange of *Padina* measured under solar radiation between 4 m and 0 m depth in comparison to the illuminance measured at that depth. Before exposure, dark respiration was determined and then oxygen exchange measured integrated over 2 min periods each. Temperature was 23 °C.

between 4 m and 0 m starting at the lowest level (Figure 8). It is interesting to note that the highest net photosynthetic oxygen production was found at 0 m at full sunlight. Differences between the various depths were not very striking even though the irradiances differed by a factor of more than two between surface and 4 m. Exposure time at the surface was too short (4 min) to induce photoinhibition.

Discussion

Although there is ample evidence that increased UV-B is harmful to aquatic ecosystems, quantitative estimates are scarce at the current stage (Acevedo & Nolan 1993; Biggs & Joyner 1994; Cullen & Lesser 1991; Cullen & Neale 1994; Häder 1993a, b; Karentz et al.

1994; SCOPE 1992a, b; Smith & Cullen 1995; Smith et al. 1992; Weiler & Penhale 1994; Williamson & Zagarese 1994).

Solar UV-B radiation penetrates well into the euphotic zone of major aquatic ecosystems. The convolution of the solar radiation in the water column with a published action spectrum indicates that the major inhibition is expected around 310 nm, even though the action spectrum increases toward shorter wavelength and extents into the UV-A (Häder & Liu 1990). This is due to the fact that the emission energy of solar radiation decreases dramatically below this wavelength; toward longer wavelengths the action spectrum indicates a steep decline in the sensitivity of the organism even though it extents well into the UV-A as do other action spectra in several phytoplankton.

Data on the penetration of solar radiation into the water column are only meaningful when the vertical distribution of the major biomass producers is known. The phytoplankton distribution shows a pronounced vertical profile unless disturbed by extreme winds and waves. Only under the action of high wind and waves the organisms are equally distributed throughout the mixing layer. A comparison of the vertical distribution with the UV-B penetration indicates that the organisms are well affected by solar short wavelength radiation. However, it is unclear how organisms are affected by the irregular, repetitive exposure to near surface radiation when they undergo pronounced mixing.

Solar irradiation of high fluence rates has been found to induce photoinhibition in higher plants (Björkman & Demmig 1987; Schreiber et al. 1994), macroalgae (Franklin et al. 1992; Hanelt et al. 1992, 1993; Larkum & Wood 1993) and phytoplankton (Helbling et al. 1992; Herrmann *et al.* 1995; Leverenz et al. 1990). Excessive solar radiation results in photooxidative stress caused by the generation of active oxygen species. These are produced by transfer of excessive excitation energy from excited chlorophyll molecules to ground state (triplet) oxygen (Foyer et al. 1994). The mechanism of photoinhibition is still under debate (Crofts & Yerkes 1994). However, it can be regarded as an active physiological regulatory process to protect the photosynthetic apparatus from excessive radiation. A key component is the D1 protein located in photosystem II (Sundby et al. 1993). Photosynthetic quantum yield and photochemical quenching decrease and often the non- photochemical quenching increases during photoinhibition. The PAM fluorescence measurements in this study indicate that both microalgae and macroalgae are affected by high solar radiation. The experiments using cut-off filters indicate that both visible and ultraviolet radiation induce massive photoinhibition. It is, however, important to note that even solar radiation at current levels affects both macroalgae and microalgae at their growth site when the sun is at high angles. In contrast to *Padina* and other algae adapted to direct solar radiation, recovery in *Rivularia* from exposure to direct sunlight was slower and not complete (Häder et al. 1995a,). One important result is that the photosynthetic quantum yield is not optimal even at the natural habitat of the algae. It is even more surprising that recovery takes several hours after direct solar radiation stopped.

It is interesting to note that inhibition of oxygen production and photosynthetic yield followed different kinetics. A similar result was found in the brown alga *Dictyota dichotoma* (Hanelt et al. 1994). In this alga an increase in zeaxanthin was observed. This carotenoid is supposed to play an important role in the photoprotection mechanisms under light stress (Uhrmacher et al. 1995). Algae seem to differ from higher plants in their regulatory mechanisms and capacity (Büchel & Wilhelm 1993). Further investigations, including inhibition and recovery kinetics are necessary to elucidate the mechanisms of photoinhibition and photodamage.

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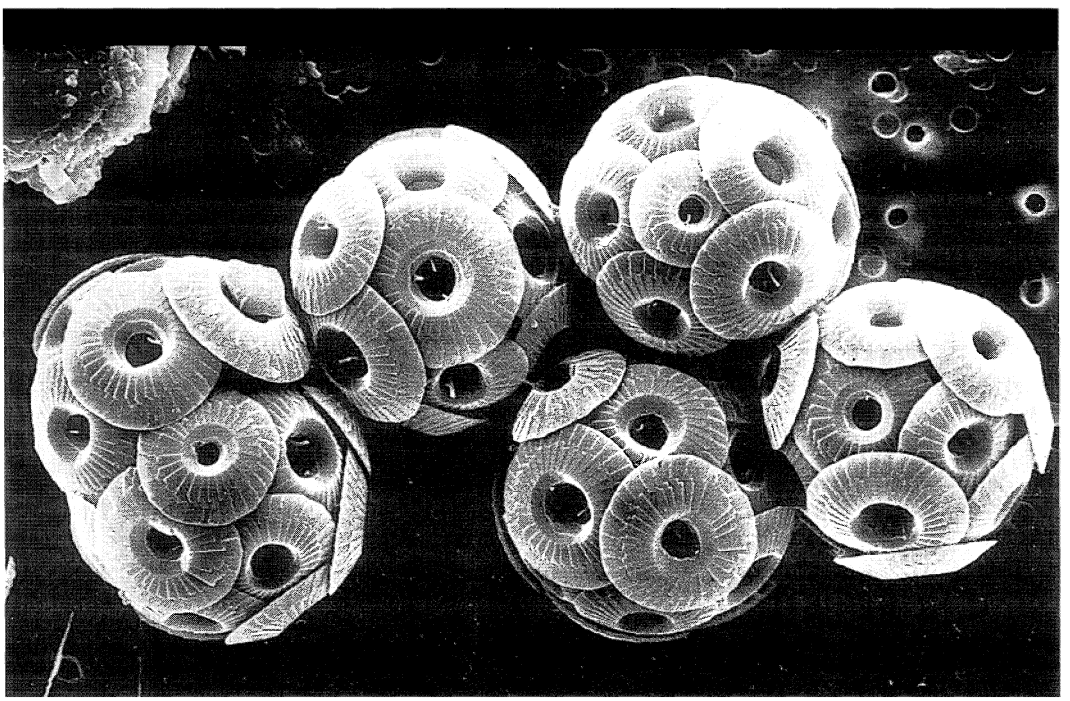

A coccolithophorid microalga (cell diameter 5 micron). These organisms are shown to be very sensitive to UV-B radiation in laboratory experiments. They can become highly abundant near the ocean's surface; the $CaCO_3$ platelets make blooms visible on satellite remote sensing images. (SEM by J. Zagers, own collection)

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UV damage to plant life in a photobiologically dynamic environment: the case of marine phytoplankton

W.W.C. Gieskes & A.G.J. Buma
University of Groningen, Department of Marine Biology, 9750 AA Haren, The Netherlands

Key words: DNA damage, Microalgae, Ocean, Photoproducts, Thymine dimers, UV-B, UV-A

Abstract

The effect of UV-B radiation on growth of marine phytoplankton was investigated in relation to DNA damage induced by a range of biologically effective doses (BEDs). *Emiliania huxleyi* (Prymnesiophyceae) was chosen as a model organism of the ocean's phytoplankton because of its importance in global biogeochemical cycling of carbon and sulphur, elements that influence the world's climate as components of the trace gases carbon dioxide (CO_2) and dimethylsulfide (DMS). A marine diatom, *Cyclotella*, was studied for its capacity to repair the DNA damage, quantified as thymine dimers by the application of a monoclonal antibody against these photoproducts. DNA repair was shown to be complete after just a few hours of exposure to visible light; the repair rate increased with PAR intensity. *E. huxleyi* appeared to be most sensitive to UV-B radiation: growth was already affected above a dose of 100 J m^{-2} d^{-1} (biologically effective radiation, weighted with Setlow's DNA action spectrum), probably through effects on the cell cycle related to damage to nuclear DNA: mean specific growth rates were inversely correlated with thymine dimer contents in cells. Near the ocean's surface UV-B radiation conditions that induce the changes observed by us in cultures can be expected during the growing season of phytoplankton, not only in the tropics but also at higher latitudes. Nevertheles, blooms of species such as *E. huxleyi* are often excessive in the field. It is suggested that exposure duration of cells near the surface of the ocean can be shorter than our artificial 3 h in the laboratory due to vertical mixing, a phenomenon that is typical for the ocean's upper 50–100 m. When mixing reaches depths greater than the layer where most UV-B is attenuated, negative effects on cells through UV-A-induced inhibition of photosynthesis may prevail over DNA damage, the action spectrum of which has been shown to be limited to the UV-B part of the spectrum. Moreover, the radiation wavelengths that induce DNA damage repair (UV-A and visible) are attenuated vertically much less than UV-B. The photobiological situation in the upper ocean is much more complicated than on land, and effects of UV radiation on plankton biota can only be modelled realistically here when both the spectrally differential attenuation in the UV and visual part of the spectrum and the rate of vertical mixing are taken into account. Action spectra of both damage and repair of DNA and of photosynthesis inhibition of representative microalgal species are the second *conditio sine qua non* if we want to predict the effect of stratospheric ozone depletion on marine phytoplankton performance.

Introduction

The most recent update of the Polar Ultraviolet Irradiance Monitoring Network feeds the fear expressed repeatedly (Gleason et al. 1993 McElroy & Salawitch 1989; Towe 1992) that the trend of increase of UV-B radiation related to stratospheric ozone depletion will continue. This American monitoring programme, sponsored by the US. National Science Foundation[1], is run at several locations on Antarctica. The observation series of 1995 shows record high values early in November (the 'austral spring'), at station South Pole up to 1 μW cm^{-2} (298.507–303.03 nm integrated

[1] For questions about the data and the weekly published updates please contact Biospherical Instruments, e-mail UVGROUP@biospherical.com; WWW access http: //www.biosperical.com

spectral irradiance) and 0.09 μW cm^{-2} Setlow's DNA dose weighted irradiance; during the same months of 1995 highest-ever values were also registered at other stations (e.g., at Palmer: 10 and 0.7 μW cm^{-2}, respectively; at McMurdo: 3.3 and 0.26 μW cm^{-2}). High UV-B levels have also been recorded in the aftermath of the Pinatubo eruption of 1991 (Gleason et al. 1993), when the sulfate particle injection into the stratosphere was taken to be responsible for ozone depletion even at mid-latitudes. However, the recent and continued depletion, which is now a globe-wide phenomenon (Blumthaler & Almbach 1990), remains largely unexplained and is unexpected in view of the well-known Montreal Protocol that is now in operation for a decade.

Modeling of ozone trends to predict losses on a global scale is necessary but not simple (Towe 1992). A central problem is the unknown magnitude of existing reservoirs of chlorinated fluorocarbons (CFCs) near the earth's surface: directly in foams, refrigerators, and aerosol applicators, and indirectly 'in store' in the upper ocean, the deep ocean, the troposphere and the stratosphere. All reservoirs, not in the least the oceanic ones, have different transport and removal times, and the interplay between these processes determines future concentrations. A model constructed by Rasmussen & Khalil (1993) on the basis of this notion reveals the history and future of Freon-11 (F-11) from 1930 all the way to 2100. Direct measurements over the last 2 decades, when F-11 went up from 100 to 275 pptv, agree quite well with the hind-casting of the model. Interestingly, future concentrations appear to be affected by the fact that the ocean (now a sink) will become a CFC source once atmospheric concentrations drop below saturation values in its upper mixed layer. By 2050 F-11 concentrations will still be at the level of the early 80s! Thus, the danger of severe ozone depletion events due to chemical CFC reactions in the stratosphere will be with us for a long time. Transportation of ozone-poor air masses and cloud formation, both potentially affected by global warming, are wild cards in the deck.

Studies of the consequences of the increased ultraviolet-B radiation that is expected when stratospheric ozone is depleted (Calkins 1982; Nachtwey et al. 1975) for life on earth are therewith highly relevant; but they are also interesting in their own right since life has had to cope with UVR from the very beginning: photosynthesis had not yet provided the oxygen for ozone formation (Cloud 1968; Kasting, 1993) so protective mechanisms were necessary. In aquatic plants mechanisms developed to avoid a UV overdose include motility and migration (Häder 1993); UV screens by structural adaptations in cell walls or by pigment-containing mucus around cells (Garcia-Pichell et al. 1992); UV-absorbing amino acids in cells (Carreto et al. 1990); enzymes that eliminate radicals elicited by UV-B (Palenik et al. 1993; Vincent & Roy 1993); carotenoids that defend against photo-oxidation (Vincent & Quesada 1994); finally, damage to DNA is repaired by photoenzymatic repair and nucleotide excision repair (Bron 1972; Sancar & Sancar 1988). Recovery of photosynthesis inhibition is stimulated by UV-A in various ways (Quesada et al. 1995).

In the present paper we will not review all effects of UVR on unicellular algae: on photosynthesis (Cullen et al. 1992; Cullen & Lesser 1991; Cullen & Neale 1994; Helbling et al. 1992; Smith et al. 1980), possibly through effects on photosystem II (Friso et al. 1994; Melis et al. 1992) or Rubisco (Jordon et al. 1992; Strid et al. 1990); on nitrogen metabolism (Döhler et al. 1991); on growth through plasmolysis and cell death (Jokiel & York 1984; Karentz et al. 1991); on motility (Ekelund 1990); on algal community changes (Bothwell et al. 1993). Rather, the two main and in a way integrating aspects of UV-B interference with phytoplankton activity, the impact on growth and photosynthesis, will be juxtaposed to highlight photobiological differences between aquatic plant life on the one hand and vegetation on land on the other. The differences are inherent to the light exposure regime in these opposite environments: aquatic versus terrestrial. Both growth (Buma et al. 1995; Calkins & Thordardottir 1980; Karentz et al. 1991) and photosynthesis (Holm-Hansen et al. 1993) of marine microalgae suffer from overexposure to UV radiation, just as is the case in terrestrial plants (Bornman & Teramura 1993), but the differential attenuation of spectral radiation with depth in seas and oceans potentially creates a gradient in relative damage in the euphotic zone. Near the surface growth and cell division would be affected most by UV because the action spectrum of UV-induced DNA damage is very steep, but limited to wavelengths shorter than 320 nm (Quaite et al. 1992; Setlow 1974); while deeper down in the euphotic zone photosynthesis would be affected most since the action spectrum (Coohill 1991) extends into the wavelength range that penetrates farthest under water (Smith & Baker 1979) so UV-AR damaging to photosynthesis (Cullen et al. 1992) can exert its influence at depths where UV-BR is down to extremely low levels.

The pronounced spectral variation with depth in the water is the most striking optical difference with the

situation on land. The consequences of this phenomenon for marine phytoplankton are discussed in this contribution. Another characteristic typical of aquatic environments, passive vertical motion of microalgal cells due to wind and tide induced turbulent mixing (Helbling et al. 1994; Sverdrup 1953), is also considered. Vertical mixing (more the rule than the exception in seas and oceans) causes rapid temporal shifts both in intensity and in the spectrum of light and UV the drifting cells are exposed to (Lorenzen 1979). The influence of solar elevation on the spectral distribution of light and UV is not considered although its effect is no doubt far more pronounced in water than on land (Karentz et al. 1994).

One model organism that is central in our considerations is *Emiliania huxleyi* (Prymnesiophyceae), an oceanic microalgal species that plays a central role in the global carbon and sulphur cycle (Brown 1995). As such it is an important object of study since the influence of UV-B radiation on its activity may induce feedback mechanisms that affect global warming through changes in the concentration of two trace gases (Coohill 1991): among the oceanic phytoplankters *E. huxleyi* populations tend to sequester a relatively high proportion of the greenhouse gas CO_2, and more DMS (dimethylsulphide) is released than from most other groups in the plankton, potentially providing cloud condensation nuclei over the extensive blooms in the open ocean (Falkowski et al. 1992). Global warming and the accompanying cooling of the stratosphere may in turn enhance ozone destruction (Austin et al. 1992). Another model organism used in this study is the marine diatom *Cyclotella*, a species that has been studied by us since the early nineties (Buma et al. 1996; Veen et al. 1995).

Material and methods

As mentioned in the Introduction, DNA is considered one of the prime targets of UV-BR (Karentz et al. 1991; Regan et al. 1992). Pyrimidine bases are especially susceptible to damage by UV-B; most of this damage is the consequence of cyclobutane thymine dimer formation (Tyrell 1986; Bron 1972). The dimerisation inhibits its DNA synthesis and transcription in *E. coli* (Smith 1969). The dimers also can upset the cell cycle because cells cannot perform mitosis until the DNA changes have been repaired (Weinert & Hartwell 1989). The link between DNA damage and other cell characteristics is not well established in marine microalgae but it is held responsible for cell killing, mutation, and other cell transformations in other organisms (Coohill et al. 1987; Doniger et al. 1981). This link is analysed here, using the following methods.

Cultures of *Emiliania huxleyi* and *Cyclotella* sp. were grown on artificial media (Guillard 1975) in quartz cuvettes of 0.5 l under growth-saturating conditions of up to 600 μmol m^{-2} s^{-1} PAR (photosynthetically active radiation) provided by 50W halogen lamps, in a dark-light regime of 14 h light and 10 h dark. UV-A was provided in the experiments with *E. huxleyi* using Philips 09 N fluorescent lamps. Emission spectra of PAR and UVR sources were measured with an Optronics OL752. UV-B treatments were given mid-daily for 3 h with Philips TL 12/20W lamps; dose rates were biologically weighted with Setlow's DNA action spectrum (Setlow 1974), normalised at 300 nm. UVC was eliminated with 3 mm Schott WG cut-off filters 305. The total weighted daily UV-B dose spanned a range of 0–2 kJ m^{-2}, which is within the limits of natural daily Biologically Effective Doses (DNA 300 nm) at the ocean surface (cf. Behrenfeldt et al. 1993).

In the experiments with *E. huxleyi*, cultures were adapted to a PAR intensity of 400 μmol m^{-2} s^{-1} and the light-dark regime for over a week prior to the experiments. Then UV-BR treatments were given for 5 consecutive days, during which growth was monitored. Different UV-BR doses were given by adjusting the distance between the cultures and the UVR source. At the end of the experiments, cultures were fixed and stored for flow cytometric analysis of thymine dimer content.

In the first experiment with *Cyclotella* sp., cultures were adapted to the experimental conditions (400 μmol m^{-2} s^{-1} of PAR, 14 h light, 10 h dark period) for more than a week, after which one UV-BR treatment was given for 3 h, in the middle of the light period. The total weighted UV-BR dose was 1.05 kJ m^{-2} d^{-1} ($BED_{DNA300nm}$). Samples were taken for thymine dimer analysis during UV-BR exposure and in the period following the UV-BR treatment, i.e. when the culture was exposed to PAR only and was followed by a dark period.

In the second experiment, a culture of *Cyclotella* sp. was exposed to UV-BR only for 3 h (UV-BR dose of 2 kJ m^{-2}, $BED_{DNA300nm}$). No PAR was supplied to avoid induction of repair during the UV-BR exposure period. After 3 h, the UV-BR lamp was switched off and the culture was subdivided into three separate samples. These three samples were exposed to various levels of PAR (100, 400 and 800 μmol m^{-2} s^{-1}).

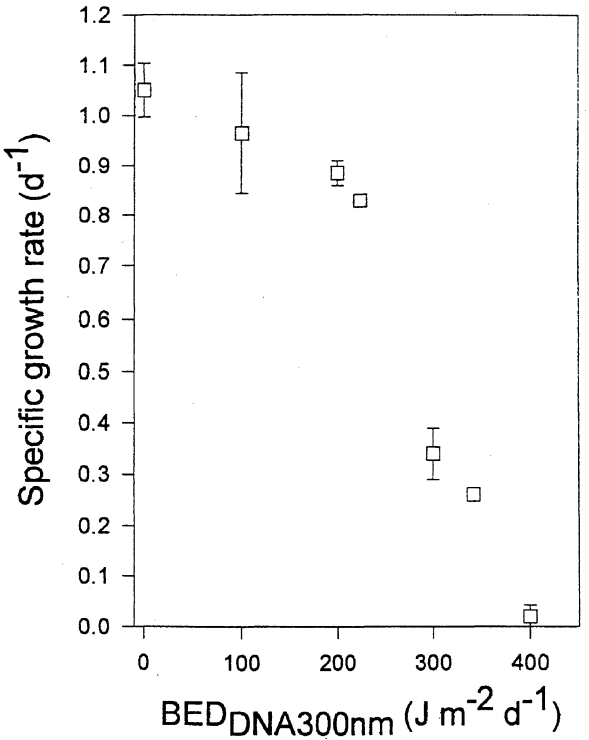

Figure 1a.

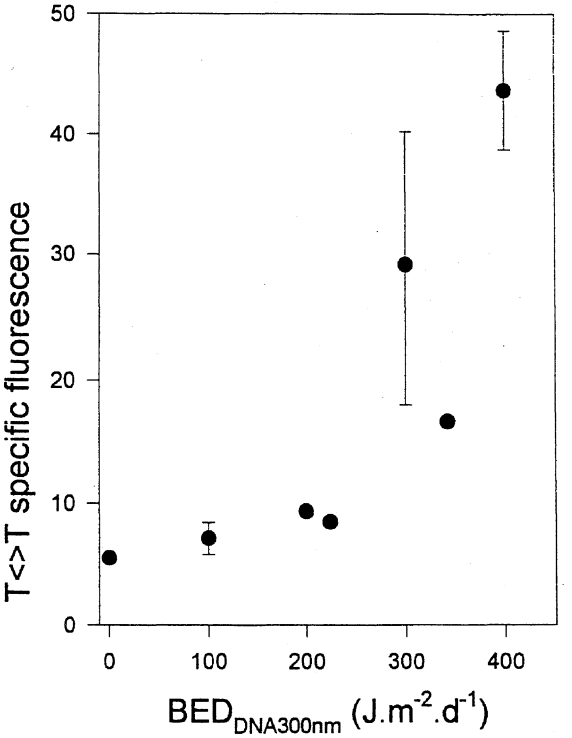

Figure 1b.

Figure 1. Effect of UV-BR exposure on growth and thymine dimer formation in *Emiliania huxleyi*. **A**: Specific growth rate; **B**: thymine dimer specific fluorescence (detection by flow cytometry). Vertical bars represent standard deviations of the mean, derived from duplicate or triplicate series. Biologically effective daily doses (BED) were calculated using the DNA action spectrum of Setlow (1974), normalised at 300 nm.

Removal of thymine dimers was monitored over a subsequent period of 5 h.

Cells were counted alive in Sedgwick counting chambers. Specific growth rates were calculated from the slopes of the regressions of semilog-transformed data. Each regression line was based on at least four data points.

Thymine dimers in DNA of cells were detected using a monoclonal antibody developed by Roza et al. (1988); such monoclonals have also been raised against these photoproducts by Mori et al. (1988) and Mizuno et al. (1991). Our method is based on the description by Berg et al. (1993) as adjusted for microalgae by Buma et al. (1995). After a number of steps (pigment extraction to get rid of autofluorescence; DNA denaturisation; cell wall permeabilisation) primary antibody binding took place, followed by binding of the secondary antibody, FITC conjugated rabbit-antimouse. To measure DNA content of the cells, propidium iodide was used as a dye. This is not DNA-specific but also binds to double-stranded RNA so RNAses were added before flow cytometric measurements. For these, an EPICS- C Coulter flow cytometer was used. Green fluorescence (detection of FITC binding) was used as a measure of thymine dimer formation, red fluorescence (propidium iodide detection) for DNA contents.

Results

The effect of the different UV-BR exposures on the specific growth rate of *Emiliania huxleyi* cultures is shown in Figure 1A. At 400 J m^{-2} d^{-1} growth was completely inhibited. In Figure 1B the mean thymine dimer specific fluorescence per cell is plotted against the weighted dose rate. At 400 J m^{-2} d^{-1} $BED_{DNA300nm}$ T<>T (thymine dimer) specific fluorescence was almost 10 times higher than in the control; even higher values were found at the highest dose applied (1800 J m^{-2} d^{-1}, not shown). Both dose-response relationships show a clear increase in the

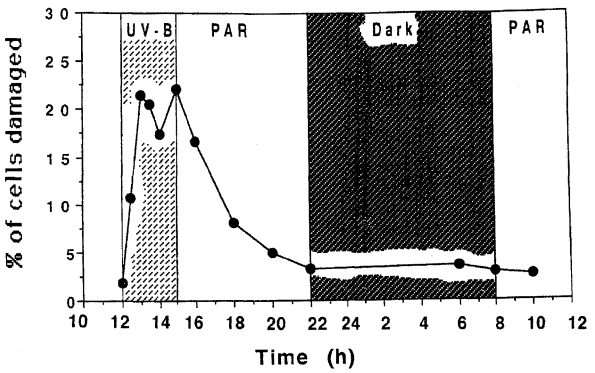

Figure 2. The formation and removal of thymine dimers in the marine diatom *Cyclotella* sp. during and after UV-BR exposure. UV-BR exposure: 1.05 kJ m^{-2} d^{-1} $BED_{DNA300nm}$); PAR: 450 μmol m^{-2} d^{-1}. Damage is expressed as the fraction of the population with detectable damage (increase in T<>T specific fluorescence).

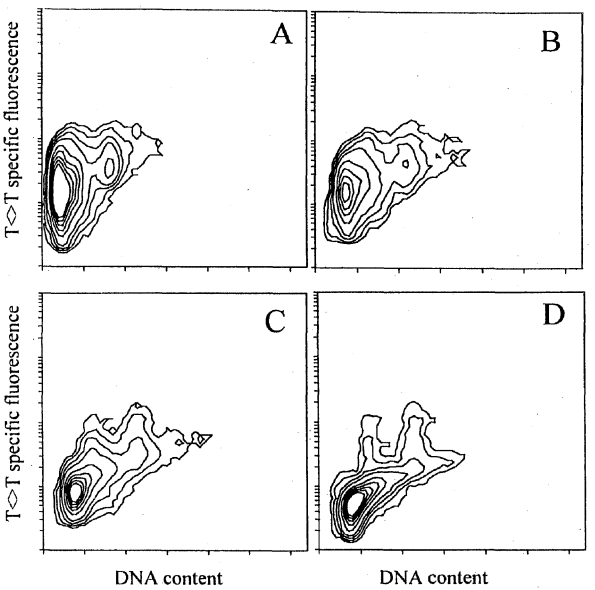

Figure 3. Removal of thymine dimers in a culture of *Cyclotella* sp. Flow cytometric contour plots of DNA content (propidium iodide staining; relative units) versus T<>T specific fluorescence (relative units) of a population of *Cyclotella* sp. cells exposed to UV-BR followed by exposure to 800 μmol m^{-2} s^{-1} of PAR only. Panel A: population of *Cyclotella* sp. cells after 3 h of exposure to UV-BR (cells with maximum damage: total UV-BR dose appr. 2 kJ m^{-2} $BED_{DNA300nm}$); panel B: after 1 h of PAR; panel C: after 3 h of PAR; panel D: after 5 h of PAR. The two clusters in panel A represent cells with one set of DNA (cells in G1, large cluster on left side), and cells with a double set of DNA (cells in G2, small cluster on right side).

measured effects with increasing UV-BR dose, but neither one was linear (Figure 1). The results imply an acceleration of the effect with higher doses, which might be caused by more efficient repair of damage (see below) at the lower UV-BR doses.

That thymine dimers can be removed readily is demonstrated by the first experiment with *Cyclotella* sp. (Figure 2) where a UV-BR treatment was accompanied by a PAR intensity of 450 μmol m^{-2} d^{-1}. The increase in thymine dimers appeared to level off already after one h of UV-BR exposure, presumably through the induction of repair systems (Figure 2). After the UV-BR lamps had been switched off, removal of dimers was complete after approximately 7 h of PAR exposure (Figure 2).

In the second experiment with *Cyclotella* sp., cultures were exposed to various levels of PAR to study the dependence of thymine dimer removal (photorepair) on PAR irradiance. Figures 3 and 4 show that removal of thymine dimers was particularly fast in the culture exposed to 800 μmol m^{-2} s^{-1} PAR following the UV-BR treatment (2 kJ m^{-2} $BED_{DNA300nm}$) that was clearly damaging to the DNA of cells. Repair is obviously PAR dependent (Figure 4): its rate was highest at the higher irradiance of PAR, and lowest at 100 μmol m^{-2} s^{-1}. However, relationships such as these can also be caused by PAR dependent division of the cells in the cultures that have not suffered thymine dimer formation. In fact, cell division is normally higher at higher PAR conditions. This could cause a 'dilution' of damaged cells if in a population cell division occurs. However, the G2/G1 ratio in the cultures remained stable during all experiments (cf. Figure 3), which implies that no DNA synthesis and cell division took place. The initial damage level was probably very high, enough to largely preclude the 'dilution' effect just mentioned.

Discussion

The observations suggest that the interference of even the lowest doses of UV-B radiation with growth of marine phytoplankters such as *Emiliania huxleyi* were caused by damage to DNA, namely induction of the photoproducts recorded in the cells. As described earlier, many cells that enter the cell cycle with damaged DNA do not seem to be able to complete the cycle before the next UV-B treatment (Buma et al. 1995, 1996). Delayed mitosis has also been observed in UV-irradiated *Saccharomyces cerevisiae*, where it allows repair of damaged chromatin in the G2 phase (Weinert & Hartwell 1989). When due to a deficient DNA repair

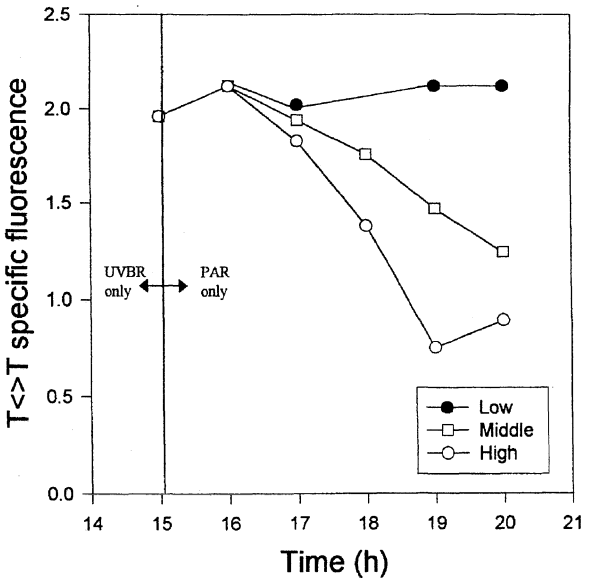

Figure 4. Time course (incubation in h) of thymine dimer removal, expressed as the decrease in mean thymine dimer specific fluorescence in a culture of *Cyclotella* sp. exposed to different levels of PAR. Closed circles: 100 μmol m^{-2} s^{-1} PAR; open squares: 400 μmol m^{-2} s^{-1} PAR; open circles: 800 μmol m^{-2} s^{-1} PAR. The cultures were exposed to a UV-BR dose of appr. 2 kJ m $BED_{DNA300nm}$ prior to PAR exposure.

mechanism cells are not able to remove damage before the next exposure period, photoproducts accumulate so cells remain unable to perform mitosis and cytokinesis, although chromatin duplication may go on.

The sensitivity of *Emiliania huxleyi* to UV-B radiation is strikingly high. It is also high in planktonic diatoms (Buma et al., 1996) where complete growth inhibition has been recorded at 1 kJ m^{-2} d^{-1} BED. This is in sharp contrast with the high tolerance level of microalgae isolated from high UV-B environments, e.g. in arctic and subarctic regions (Calkins & Thordardottir 1980; Helbling et al. 1992), the tropics (Jokiel & York 1984), and on tidal flats such as those in the Dutch Wadden Sea where the diatoms can divide normally up to a daily UV-B dose of 3.5 kJ m^{-2}, DNA-weighted (Peletier et al. 1996; cf. Sullivan et al. 1992 for a similar range in sensitivity in terrestrial plants). In the clear waters of the open ocean, where *E. huxleyi* is the main primary producer (Brown 1995), over 80% of the pyrimidine dimer inducing wavelengths are attenuated in the top few meters (Regan et al. 1992), so in the field the DNA damage and the related growth change reported for our cultures may remain restricted to the very surface. As we have argued above, DNA repair in planktonic microalgae is very efficient, the more so at higher extensities of PAR (Figure 3). However, near the ocean's surface photoreactivating wavelengths (in the UV-A part of the spectrum) may not elicit a sufficient photolyase response for complete recovery before the next day's exposure to UV-B when DNA damage is as severe as in *E. huxleyi*.

This may be quite different just *below* the surface, in excess of depths of 3 to 5 m. Even in the clearest ocean water (Gieskes et al. 1987) the attenuation coefficient (measured with a cosine collector) is 0.12 m^{-1} for 305 nm UV-B, 0.15 m^{-1} at 300 nm – so relatively much UV-A remains at depth in the open ocean, where UV-A attenuation is at most 0.05 m^{-1} (Smith & Baker 1979). In sea regions with much coloured dissolved organics, which attenuates preferentially in the UV-B part of the spectrum (yellow substance: Warnock et al. 1997), UV-A radiation may even dominate close to the very surface. This is the wavelength domain where interference with photosynthesis may become more important than UV-B-enhanced damage to growth, in view of the action spectrum of photosynthesis inhibition (Cullen et al. 1992) that runs all the way up to wavelengths in the visible. Thus, a negative response of algal cells to UV radiation just below the top layer of the ocean may be due to interference with photosynthesis (Cullen & Neale 1994; Greenberg et al. 1989), not with DNA, since the photon fluence rate of UV-A at those depths is so much higher than photon fluence in the UV-B part of the spectrum due to the differential attention of UV. The level of UV-A that reaches the surface in the mid-latitude regions where *E. huxleyi* dominates is far higher than of UV-B anyway: 40–80 kJ m^{-2} d^{-1} UV-B versus 800–1400 kJ UV-A (at a column ozone level of 300–350 DU: U.S. Ultraviolet Monitoring Network).

However, much uncertainty remains on the actual exposure of phytoplankton cells under *in situ* conditions in oceans and seas. The fact that huge blooms of *Emiliania huxleyi* develop near the ocean's surface (Brown 1995) is certainly not expected if one considers the very high sensitivity of this organism to UV-B: in the laboratory cell growth is seriously affected at doses far below the ones that are normally encountered near the surface at mid-latitudes in spring and summer. The radiation exposure of cells *in situ* must somehow be quite different from the conditions in the laboratory, which we thought were rather close to reality. The passive vertical movement of phytoplankton in the upper mixed layer of the ocean is probably responsible for such discrepancies. This has also been suggested by Cullen & Neale (1994). Unfortunately,

mixing rate and depth are largely unknown physical-hydrographical characteristics of nearly all sea areas. Biologists and photochemists know that processes such as photooxidation and chlorophyll bleaching, observed in enclosed samples kept near the sea surface, are mitigated *in situ* (Gieskes & Kraay 1982; Zafiriou 1988). This is related to the unrealistic duration of exposure to radiation in bottles or other enclosures that are incubated at fixed depths which creates a quite artificial situation: in the field, cells move vertically with the wind-induced water motion, and receive thereby a variable irradiance (Figure 5). Any model of the effect of UV radiation on phytoplankton must include information on the rate of change in the exposure regime of individual cells.

Variable light and UV exposure is simulated only rarely (Veen et al. 1995) but the natural situation remains elusive: mixing rates can be high and may reach to 100 m deep in the open ocean – but in the absence of winds or tides they can also be close to zero. We expect a high induction level (DNA damage) near the surface when the latter conditions prevail; when mixing, on the other hand, covers the upper 10 or even more meters, cells with detectable DNA damage can be transported to depths where thymine dimers cannot possibly have been induced by UV-B due to its rapid attenuation in the surface layer (of at most 5 m: Regan et al. 1992). Dr W Jeffrey (USA) has recently found differences in the depth distribution of DNA damage in bacteria under different mixing regimes (Abstract, ASLO meeting in Reno, USA, June 1995). We intend to do similar work with microalgae in various ocean regions, detecting the photoproducts induced by UV-BR by our immunochemical method that works on a single cell level (Buma et al. 1995).

To summarize: in the field the photobiology is highly complicated for two reasons: depth-differential induction of damage *and* repair because of differences in attenuation of PAR, UV-BR and UV-AR; and variable rates of mixing. In a vertically moving cell, DNA may be damaged by UV-BR near the surface where it can also be repaired, especially at high PAR irradiance (Figure 4); while during descendence to depths where no significant UV-B penetrates UV-A and PAR may maintain DNA repair – while UV-A intensity may still be high enough to inhibit photosynthesis. Realistic estimates of the effect of UV-BR increase on phytoplankton performance can only be obtained on the basis of good data on mixing rate, combined with action spectra of DNA damage, of DNA repair and of photosynthesis inhibition.

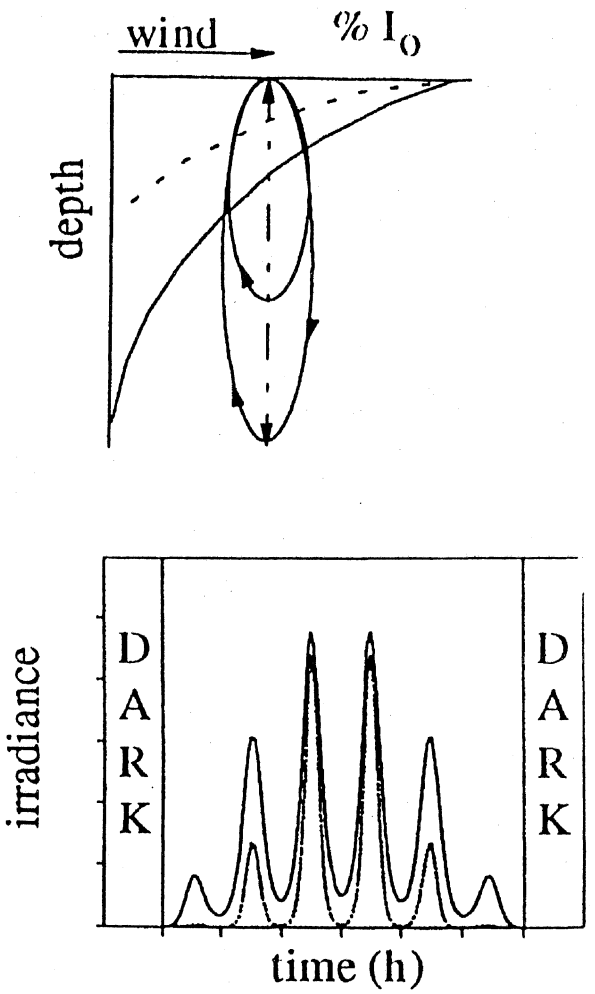

Figure 5. Attenuation of PAR and UV-BR and wind induced mixing (two mixing depths) in a water column (upper panel) and PAR and UV-BR received by an algal cell vertically moving through the water column by mixing (lower panel). Upper panel: I_0: incident irradiance at the sea surface. Attenuation of PAR (solid line) and UV-BR (broken line) with depth; two wind induced mixing regimes (arrows). Lower panel: daily course of PAR (solid line) and UV-BR (broken line), experienced by an algal cell that is subject to vertical mixing. Taken from Veen et al. (1995).

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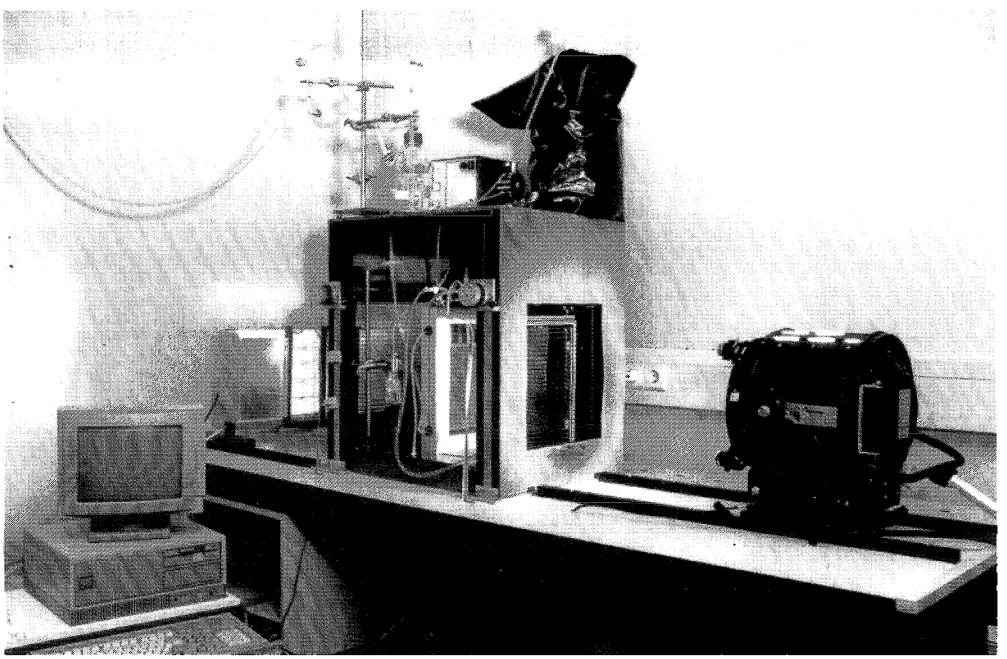

Continuous-culture system with computer-controlled dynamic light-regime for the study of micro-algal respons to UV-B exposure (for explanation see text). (Photograph: E. H. Rozendal)

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Effects of acute and chronic UV-B exposure on a green alga: a continuous culture study using a computer-controlled dynamic light regime

A. Veen[1], M. Reuvers & P. Ronçak[2]
National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands ([1]present address: Netherlands Institute of Ecology, Centre for Limnology, Rijksstraatweg 6, 3631 AC Nieuwersluis, The Netherlands; [2]joint affiliation and address: IHE, P.O. Box 3015, 2601 DA Delft, The Netherlands)

Key words: Algal growth, Algal photosynthesis, Green alga, Phosphorus limitation, UV-B inhibition

Abstract

The green alga *Selenastrum capricornutum* was grown in a specially developed continuous culture system to study long-term effects of chronic UV-B exposure. The new system improves upon previous laboratory culture approaches. It is demonstrated that short-term experiments underestimate UV-B effects. It is also shown that photoinhibition cannot explain the effects under chronic exposure. Under both nutrient-replete and phosphorus-limiting conditions a UV-B mediated delay in cell division rate and an increase in the cellular content of proteins, carbohydrates and chlorophyll *a* was measured. Transition experiments showed that complete acclimatisation to UV-B took several cell cycles. DNA damage appears to be the major cause of the observed long-term UV-B effects.

Introduction

Except for tropical latitudes, a global decrease in ozone concentration has been detected over the last decade (Madronich & De Gruijl 1993). This depletion, which is most distinct during the Antarctic spring ('ozone hole'), raises concern for negative effects of the resulting increase in UV-B (290–320 nm) radiation on aquatic ecosystems.

Research during the last decades revealed overwhelming evidence for negative effects of enhanced UV-B radiation on phytoplankton production (Häder 1993). Even ambient levels of UV-B radiation form a natural stress for phytoplankton. Inhibition of photosynthesis by UV-B (and UV-A: 320–400 nm) radiation has been measured frequently (Belay 1981; Gala & Giesy 1991; Jokiel & York 1984; Lorenzen 1979; Maske 1984; Smith et al. 1992; Smith & Baker 1980; Worrest et al. 1981). Worrest (1983) estimated that if no UV-B radiation was incident at the earth's surface, phytoplankton primary production would increase by about 12%. Despite these investigations, the exact quantitative impact of UV-B radiation is still unknown. The assessment of UV-B effects is complicated by spatial and temporal variation of the damaging radiation and concurrent effects of UV-A radiation and visible light. Experimental time scales and light conditions are often different from natural ones. In laboratory studies, simple on/off lighting conditions are usually applied and culture geometry (round culture flasks) makes irradiance difficult to quantify. Effects of the growth condition of an algal cell on its sensitivity to UV-B damage is rarely considered. Several investigations showed that growth conditions might determine the final effect level of UV-B damage. Cullen & Lesser (1991) found that nitrogen limitation increased the UV-B sensitivity of algae considerably. On the contrary, Behrenfeld et al. (1994) did not find a measurable effect of UV-B radiation on growth rate or cell volume of *Phaeodactylum tricornutum* under both nitrogen- and carbon limitation, whereas under nutrient-replete conditions the same UV-B dose decreased the growth rate of *P. tricornutum* by 16%. Also, Bothwell et al. (1993) found that UV-B inhibition of diatom growth rate was independent of the degree of phosphorus limitation.

As microalgae are unicellular, the complete organism is exposed to UV-B radiation and each cell com-

ponent can undergo direct or indirect damage. Indeed, during the past years, effects on almost all metabolic processes have been described. Besides inhibition of photosynthesis (photoinhibition), evidence has been found for DNA damage (Buma et al. 1995; Karentz et al. 1991), decreased motility (Donkor & Häder 1991), impairment of phototaxis (Blakefield & Calkins 1992), changes in pigments (Zündorf & Häder 1991), altered amino acid composition (Döhler 1984) and reduced nitrogen metabolism (Döhler 1985). As protection, algae have a specific repair mechanism (photoreactivation) for UV-B induced DNA damage (Van Baalen & O'Donnell 1972; Werbin & Rupert 1968; Williams et al. 1979). Some algae are able to decrease damage by producing UV-B absorbing pigments (Garcia-Pichel & Castenholz 1991; Garcia-Pichel et al. 1993; Shibata 1969). The enzyme superoxide dismutase (Eloff et al. 1976) and several carotenoids (Paerl et al. 1983; Siefermann-Harms 1987) can neutralise radicals formed by UV-B radiation. The combination of direct damage, indirect changes in cell metabolism and specific and non-specific repair and protection mechanisms further complicates UV-B effects research. To assess quantitatively the effects of enhanced UV-B radiation on primary production and hence on aquatic ecosystems, the identification of primary UV-B targets is necessary. The majority of studies on the impact of UV-B radiation on phytoplankton, both in the field and laboratory, has only addressed the effects of acute exposure. Natural phytoplankton assemblages or isolated species are usually exposed to modified levels of UV-B radiation for only a few hours when effects are measured. Mostly, attention is directed towards photosynthetic activity as measured by oxygen production or ^{14}C uptake. Within a time frame of several hours, neither DNA damage nor adaptation or induction of repair mechanisms are likely to become manifest. Only rarely have effects been studied after a prolonged (several days) exposure (Behrenfeld et al. 1992; Döhler 1984; 1989; Jokiel & York 1984).

Considering the aforementioned, we aimed our studies on long-term effects using improved methods of dosimetry. Our continuous-culture system derived from Kroon et al. (1991) is characterised by a computer-controlled dynamic light system and flat geometry of the culture vessel. Besides a brief description of the main characteristics of the newly developed system, a first series of experiments, directed to establish the differences in effects caused by acute and chronic exposure to UV-B radiation is presented here. Additionally, we attempted to identify the primary targets of UV-B radiation. To investigate the influence of physiological conditions of the algae on their sensitivity to UV-B radiation, experiments were performed under both nutrient-replete and phosphorus-limiting conditions.

Materials and methods

The culture system consisted of a flat rectangular continuous culture vessel with a 2.5 cm light path and a light facing surface of 25 × 31 cm. The windows (25 × 31 cm) of the culture vessel were made of quartz-glass (4 mm) to secure a constant and optimum transmission of UV-B radiation. At the bottom of the vessel, a 22 cm long sintered-metal air filter (0.2 μm SS 316 Fujiflo) with a diameter of 1 cm was placed horizontally. Air was circulated at a flow rate of 150 l h^{-1} to provide excess carbon dioxide and to assure complete mixing.

Light was provided by two separate light sources positioned at opposite sides of the culture vessel. A high-irradiance PAR light source (OSRAM HMI 1200W/GS) was used with a spectral composition resembling the solar spectrum. A set of 5 fluorescent light tubes (Philips TL12/20W) were used as UV-B source. Cut-off filters (Schott WG305/3mm) were placed in front of the source to remove low wavelength radiation (UV-C). To regulate irradiances, a Venetian blind (Luxaflex Raffinette 0.5 × 0.5 m, with 39 slats 16 mm wide) was positioned in front of each light source. Each blind was connected to a computer through a stepper motor (1.8° per step). In this way, UV-B and PAR light intensities were regulated independently by angular slat displacements of the specific blinds.

The computer program (Pascal) originally developed by Kroon (1991) was adjusted to simulate both PAR and UV-B radiation. To assure a realistic relation between total dose and maximum irradiance of both PAR and UV-B radiation, we used a modified sine function

$$\mathrm{Im}_t = \mathrm{Im}_{\max} \sin\left(\Pi \frac{t}{TL}\right)^{K_a}, \qquad (1)$$

where Im_t is the mean irradiance at time t, $\mathrm{Im}_{\max}$ is the maximum irradiance at noon, TL is the length of the light period and K_a is a shape constant which corrects the sine function for slightly dimmed subsurface irradiance at low solar angles. K_a values were different for both light sources to simulate natural daily changes in

the ratio between solar UV-B and PAR radiation. This ratio changes throughout the day mainly by changes in pathlength through the stratospheric ozone layer as related to solar angle. It was calculated that a simple sine function may result in an overestimation of the daily dose of UV-B radiation of about 10–15%. The blinds used 75 steps from minimum to maximum light transmittance.

The shape constant (K_a), irradiances and day lengths were calculated according to Veen (1996). To calculate mean (integrated) scalar irradiance inside the culture, the UV-B or PAR irradiance was measured at 96 points equally distributed over the front surface of the vessel. PAR intensities were recorded with a quantum sensor (LiCor) connected to a data logger (LiCor Li 1000). Relative UV measurements were performed with a UV-B sensor (International Light SEL 240/IL1400A). The spectral light distribution of the light sources was measured with a spectroradiometer (Optronics OL752) equipped with a quartz fibre optic probe and right angle Teflon cosine receptor. The spectroradiometer system was calibrated using a 200 W tungsten coiled-coil filament lamp. The spectral irradiance values of the standard lamp are based on the NIST 1973 scale of spectral irradiance. UV-B irradiances were weighted with the 'General Plant' action spectrum of Caldwell and the DNA action spectrum of Setlow using their analytical representation as given by Worrest et al. (1978). Action spectra were normalised to 1 at 300 nm. Integrated scalar irradiances inside the culture were derived from Monte-Carlo analysis of the trajectory of photons through the culture vessel (Veen 1996). Daily doses were calculated by numerical integration of Equation (1).

A culture of the green alga *Selenastrum capricornutum* was used to study chronic effects of UV-B exposure under dynamic light conditions. The alga was cultured in WC medium (Guillard 1975) at a pH of 8 and a temperature of 19±1 °C. A 12:12 LD light regime was applied. For phosphorus-limiting conditions the phosphorus concentration in the input medium was decreased to 3 μM. Five consecutive steady-state conditions (Table 1) were used to test the effects of a chronic exposure to UV-B radiation on cell metabolism. An interval of two weeks between different steady-states was sufficient for complete acclimatisation of the cells to the new condition. A K_{PAR} of 1.4 and a K_{UV} of 3.8 resulted in a balance between PAR and UV-B similar to that found during spring at temperate latitudes (Figure 1). Low background UV-B radiation in the reference culture was caused by the high irradiance PAR source. Between the nutrient-replete conditions and phosphorus-limiting conditions the algae were again adapted to optimum conditions (nutrient-replete, no UV-B) to check for effects of exposure history. During each steady state, the culture was sampled for several days. Each day protein concentration was sampled as a reference. As light regimes were dynamic, variation in the different parameters during the light period was considered. All parameters were analysed every two hours during the light period.

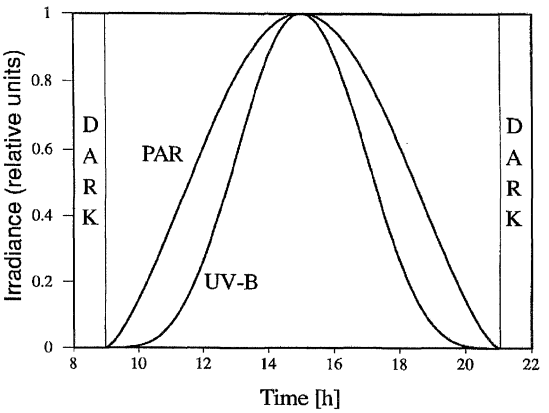

Figure 1. Relative irradiance conditions for cultures of *S. capricornutum* according to Equation (1) with K_{PAR} of 1.4 and K_{UV} of 3.8.

Chlorophyll *a* was measured spectrophotometrically after extraction in 90% acetone using a CO_2 cooled cell homogenizator (Braun MSK). Algae were filtered onto a 2 cm glass fibre filter (Whatman GF/F) and homogenised in 5 ml 90% acetone for 1 min using 12 g of 1 mm glass beads. Chlorophyll *a* concentrations were calculated according to Jeffrey & Humphrey (1975). Carbohydrate was determined with the anthrone reagent according to Hassid & Abraham (1957). Protein was determined according to Herbert et al. (1971). Bovine serum albumin was used as a standard. Prewashed 2 cm glass fibre filters (Whatman GF/F) dried at 80 °C for 6 h were used for dry weight measurements. Cell counting was done in a Türk counting-chamber. All chemical, dry-weight analyses and cell counts were performed in triplicate.

In vivo absorption coefficients (a_λ) were measured with a Perkin Elmer Lambda 2 spectrophotometer equipped with an optional integrating sphere (Labsphere RSA-PE-20). Absorption data were spectrally corrected for residual scattering by

$$a_\lambda = a^*_\lambda - \frac{a^*_{750}}{b^*_{750} - a^*_{750}}(b^*_\lambda - a^*_\lambda)\,, \qquad (2)$$

Table 1. Culture conditions for different UV-B treatments of *S. capricornutum*. K_{PAR} and K_{UV} are the shape constants for the sinusoid light regimes as given in Equation (1). I_{max} is the calculated mean scalar irradiance according to Veen (1996). $PLANT_{EFF300}$ and DNA_{EFF300} refer to the biologically effective radiation, normalized to 1 at 300 nm, according to the 'General Plant' action spectrum of Caldwell and the DNA action spectrum pf Setlow as given by Worrest et al. (1978)

	μ	K_{PAR}	K_{UV}	I_{max}			Daily dose		
	day^{-1}			PAR (μmol m^{-2} s^{-1})	$PLANT_{EFF}$ (W m^{-2})	DNA_{EFF} (W m^{-2})	PAR (E day^{-1})	$PLANT_{EFF}$ (KJ day^{-1})	DNA_{EFF} (KJ day^{-1})
Nutrient replete									
No UV-B	0.92	1.4		549	0.0076	0.0032	13.50	0.12	0.05
Low UV-B	0.84	1.4	3.8	552	0.10	0.0760	13.58	1.65	1.26
High UV-B	0.64	1.4	3.8	551	0.28	0.22	13.55	4.63	3.64
P-limitation									
No UV-B	0.41	1.4		557	0.0076	0.0032	13.70	0.12	0.05
Low UV-B	0.41	1.4	3.8	552	0.11	0.08	13.58	1.82	1.32

where (*) denotes the measured apparent scattering (b_λ) or absorption coefficient. Scattering coefficients were measured with a Perkin-Elmer Lambda 2 using a photodiode entrance angle of 0.3° (Bricaud et al. 1983). The spectrally weighted absorption in the PAR range was calculated by

$$\bar{a} = \frac{\int_{400}^{700} a_{ph}(\lambda) I(\lambda)}{\int_{400}^{700} I(\lambda)}, \qquad (3)$$

where $I(\lambda)$ is the irradiance and $a_{ph}(\lambda)$ is the specific absorption coefficient.

Oxygen production was measured polarographically (Dubinsky et al. 1987). The incubator was kept at culture temperature. P-I curves were determined after dark adaptation of the algae for at least 10 min. Prior to dark adaptation, the oxygen concentration was reduced to 80–90% of saturation level by gently bubbling the sample with N_2 gas. Dark adaptation was assumed when the respiration rate became constant. Algae were exposed to a series of 10 increasing irradiances. Irradiance was modulated by Schott neutral density filters. The initial slope was estimated by linear regression of the first 5 data points. Instantaneous O_2-production rates were measured by transferring the algae to the incubator within 1 min. In this case the algae were not dark adapted and were directly exposed to the calculated mean light irradiance inside the culture at the moment of sampling. The short incubation time of less than 10 minutes prevented supersaturation of O_2 and obviated the need for N_2 bubbling.

Results

Under nutrient replete conditions exposure to a low and high UV-B dose inhibited the growth rate of *S. capricornutum* by 9% and 28%, respectively (compare μ in Table 1). This decrease was reversible. After removing the high UV-B exposure, cells resumed their initial growth rate of 0.92 day^{-1}. Although the optical density of the culture was set to 0.1 in all steady states, cell counts were 20–30% lower under UV-B exposure because cellular scattering and absorption was increased. Under phosphorus limitation the dilution rate was kept constant. So in contrast to the nutrient-replete conditions there was no difference in growth rate between the two steady state conditions (Table 1).

Among the three photosynthetic parameters examined (maximum production (P_{max}), initial slope (α) and specific absorption coefficient ($\bar{a}_{Chl}$), only P_{max} showed a significant response to UV-B (Table 2) and decreased with an increasing UV-B exposure. Under nutrient-replete conditions in the absence of UV-B radiation P_{max} was about 20% higher around noon than at the start and end of the light period (Figure 2), while under UV-B exposure P_{max} was 10% higher around noon. Under phosphorus-limiting conditions P_{max} reached its maximum value before midday, then decreased steadily throughout the light period. Under UV-B exposure the decrease in P_{max} started two hours earlier (Figure 2). Neither α or $\bar{a}_{Chl}$ showed a trend over the light period. For all conditions the minimal quantum requirement ($\bar{a}_{Chl}/\alpha$), was close to the theoretical value of 8 (Table 2).

Cellular constituents of protein, carbohydrate and chlorophyll *a* increased with increasing UV-B expos-

Table 2. Averaged daily photsynthetic characteristics (±S.D.) of *S. capricornutum* under the different steady-state conditons ($\overline{a}$ Chl is the weighted mean specific absorption coefficient, α is the initial slope of the P-I curve, Q_{req} is the minimum quantum requirement for O_2 production and P_{max} the maximum production capacity).

	$\overline{a}$ Chl (m^2 mg chl^{-1})	α $\left(\frac{mg\ O_2\ mg\ Chl^{-1}\ h^{-1}}{\mu mol\ m^{-2}\ s^{-1}}\right)$	Q_{req} (phot O_2^{-1})	P_{max} (mg O_2 mg chl^{-1} h^{-1})
Nutrient replete				
No UV-B	0.008 ± 0.0005	0.04 ± 0.003	11.0 ± 1.59	11.5 ± 0.94
Low UV-B	0.006 ± 0.0003	0.04 ± 0.003	9.1 ± 0.86	9.0 ± 0.51
High UV-B	0.007 ± 0.0003	0.04 ± 0.003	9.4 ± 0.61	8.2 ± 0.57
P-limitation				
No UV-B	0.008 ± 0.0004	0.04 ± 0.004	11.7 ± 1.52	8.2 ± 0.54
Low UV-B	0.008 ± 0.0003	0.04 ± 0.005	10.6 ± 0.87	7.4 ± 0.76

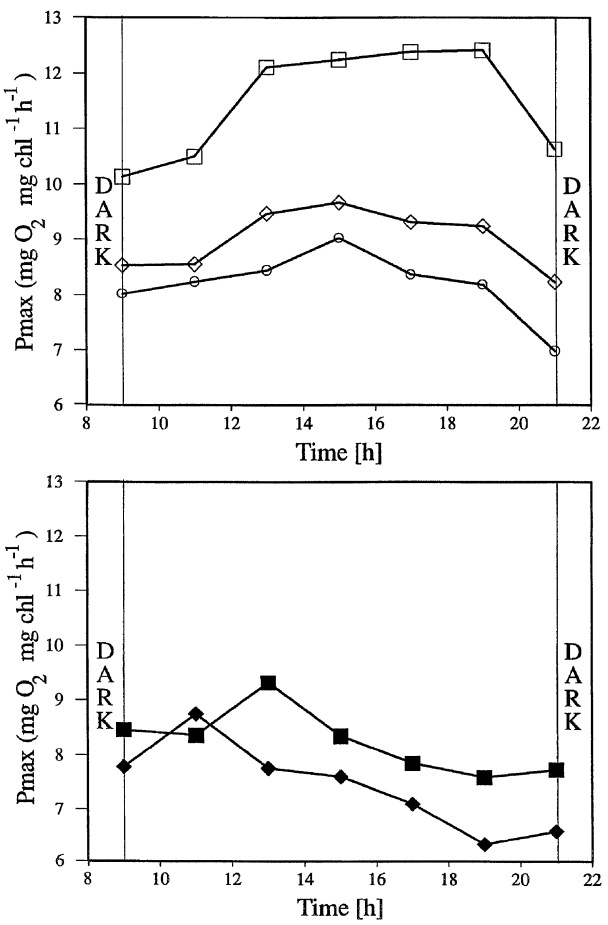

Figure 2. Steady-state maximum O_2 production of *S. caprocornutum* in continuous culture under nutrient-replete (open square – no UV-B, open diamond – low UV-B, open circle – high UV-B) and phosphorus-limiting (closed square – no UV-B, closed diamond – low UV-B) conditions. Culture conditions given in Table 1.

ure (Figures 3A, 3B, 3C). Cellular dry weight was also elevated by UV-B (Figure 3D). These increases were independent of the nutritional state of the cells. Trends in concentration of cell components during the light period were comparable at all conditions. In Figure 4 the daily variation in the concentration of cell components is shown for no and high UV-B exposure. Protein, carbohydrate and dry weight increased constantly during the light period. Chlorophyll *a* showed a minimum three to four hours before the end of the light period under both phosphorus-limiting and nutrient-replete conditions.

To mimic short-term exposure experiments often performed in the field, O_2 production was measured on the first day of the transition from one UV-B treatment to the other. Transition O_2 production rates were intermediate between the corresponding steady-states (Figure 5). When going from no UV-B exposure to high UV-B exposure, maximum production rates were reached before noon whereas during the reversed transition, a maximum production was measured after noon. During the transition to high UV-B, protein and carbohydrate both showed slight decreases in cellular accumulation, whereas chlorophyll *a* did not change at all (not shown). Also the increase in dry weight during the light period was only slightly less than in the absence of UV-B.

Under phosphorus limitation no significant change in production was measured during the transition between no and low UV-B (Figure 6). Only chronic UV-B exposure decreased production per unit chlorophyll *a*. After switching on UV-B under phosphorus-limiting conditions, the specific growth rate decreased instantaneously resulting in an exponential decrease in cell numbers at the fixed dilution rate of 0.41 day^{-1}

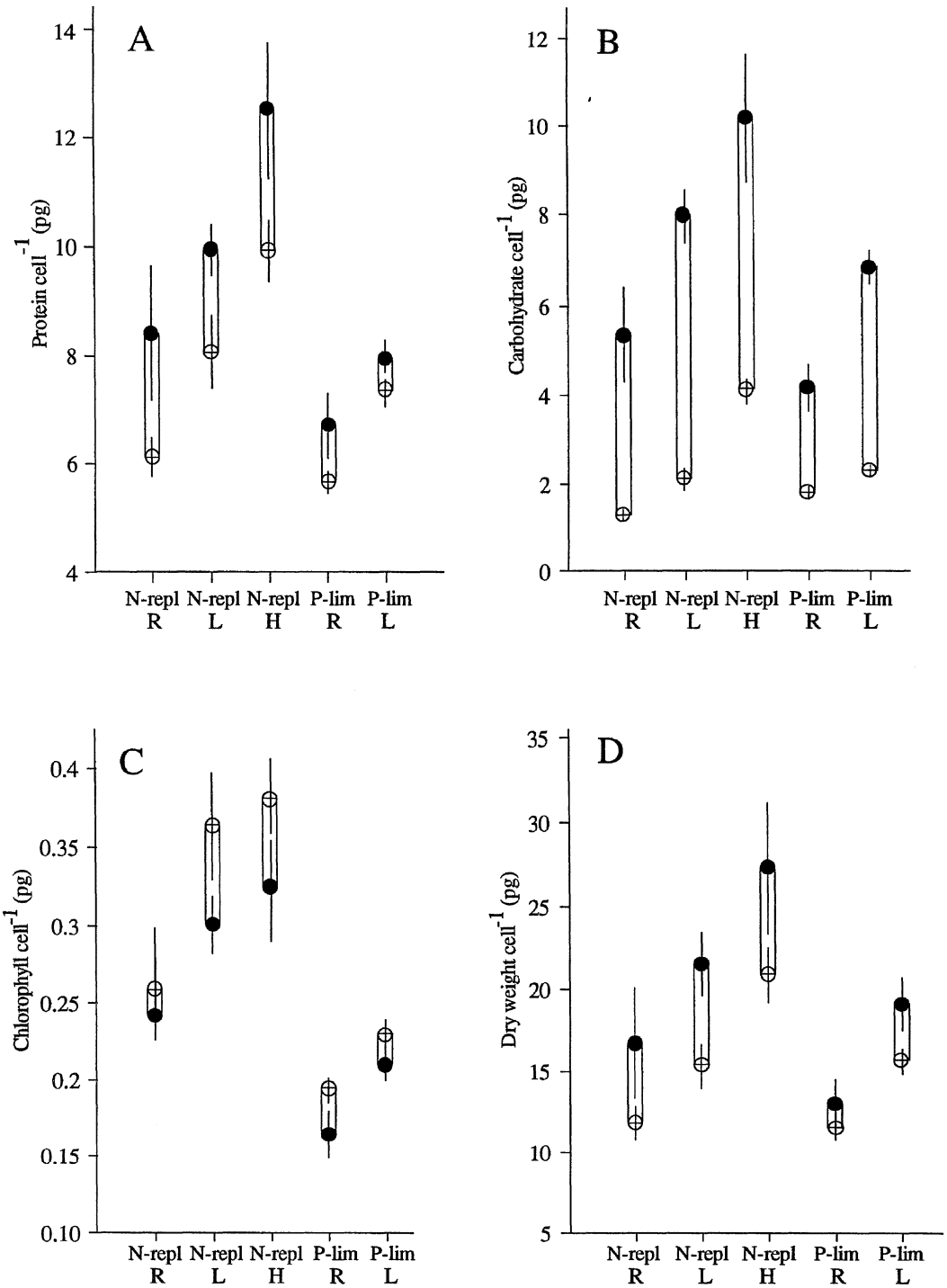

Figure 3. State-state cell content of protein (**A**), carbohydrate (**B**) and chlorophyll *a* (**C**) and total cellular dry weight (**D**) for *S. capricornutum* in continuous culture (N-repl = nutrient-replete conditions, P-lim = phosphorus-limiting conditions, R = no UV-B, L = low UV-B, H = high UV-B). Open circles refer to content at the start of the light period and closed circles refer to those at the end of the light period. Bars represent standard deviation ($n = 3$). Culture conditions given in Table 1.

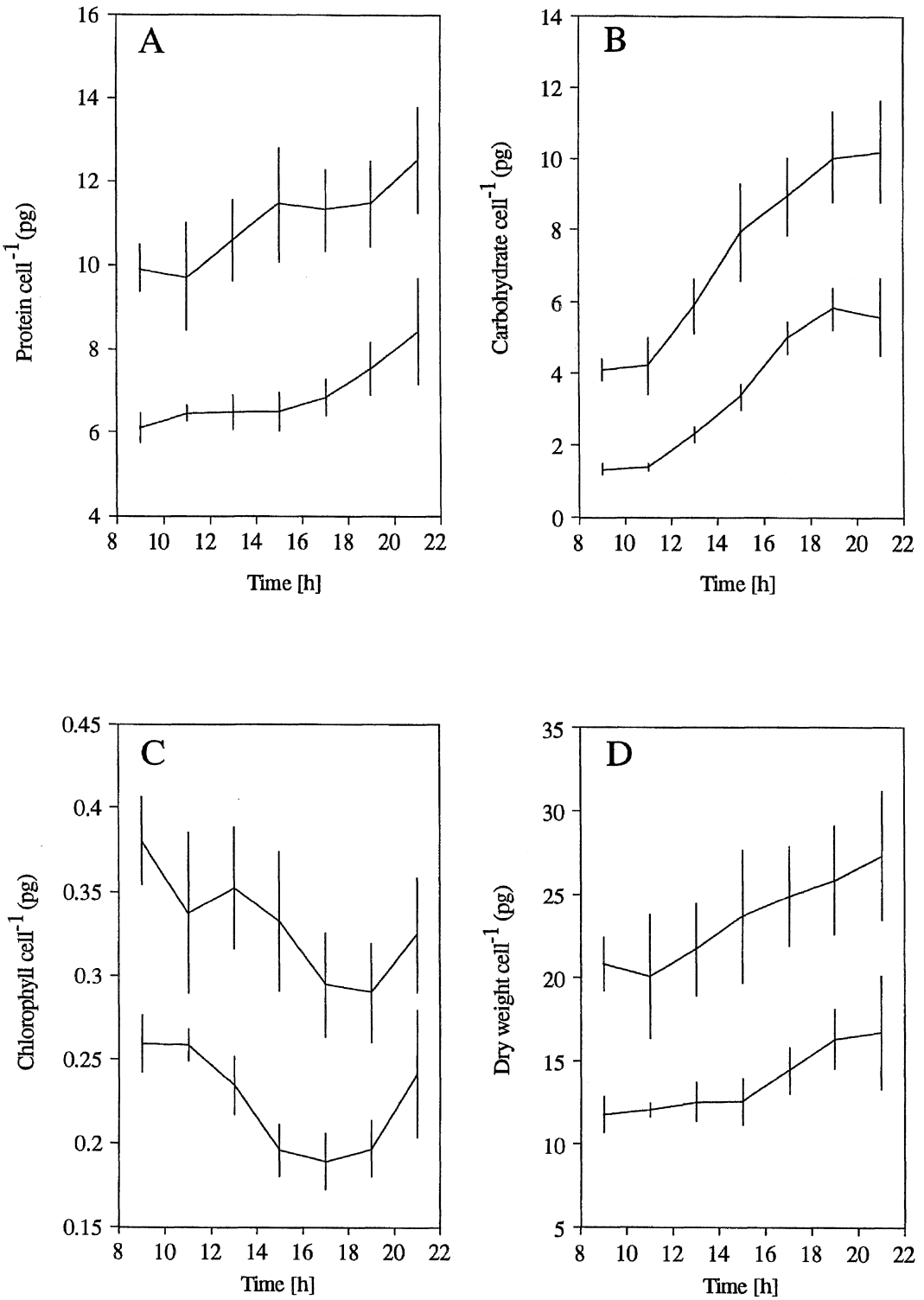

Figure 4. Steady-state cell content of protein (**A**), carbohydrate (**B**) and chlorophyll-*a* (**C**) and total cellular dry weight (**D**) for *S. caprocornutum* in continuous culture under nutrient replete conditions (upper curve – high UV-B, bottom curves – no UV-B exposure). Bars represent standard deviation ($n = 3$). Culture conditons given in Table 1.

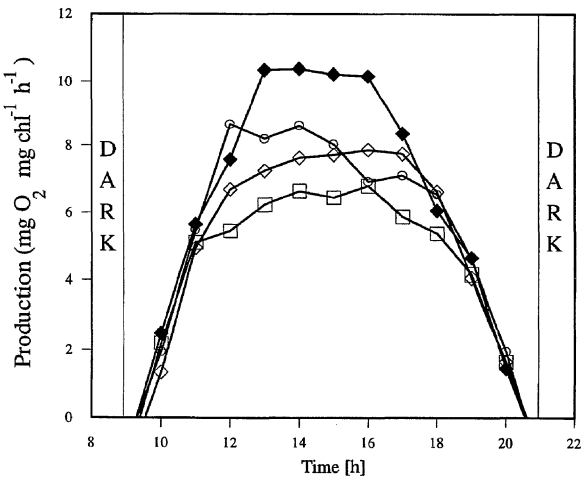

Figure 5. Instantaneous O_2 production by *S. capricornutum* in continuous culture under nutrient-replete conditions (close diamond – steady-state no UV-B, open square – steady-state high UV-B, open circle – first day of transition from no UV-B to high UV-B, open diamond – first day of transition from high UV-B to no UV-B). Culture conditons given in Table 1.

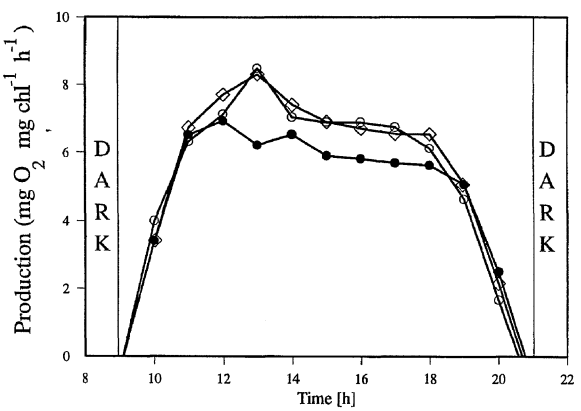

Figure 6. Instantaneous O_2 production by *S. capricornutum* in continuous culture under phosphorus-limiting conditions (open diamond – steady-state no UV-B, open circle – first day of transient from no UV-B to low UV-B, closed circle – steady-state low UV-B). Culture conditions given in Table 1.

(Figure 7). At the same time the cell dry weight showed a logarithmic increase (Figure 8). Within 10 days *S. capricornutum* regained its initial phosphorus-limited growth rate of 0.41 day^{-1} and a new steady-state was obtained. As the cellular scattering and absorption increased during the transient the decrease in cell number had no effect on the light regime inside the culture. Under nutrient- replete conditions a change in UV-B radiation required comparable adaptation periods before a new steady state was obtained (not shown).

Discussion

Our results show a clear difference between short-term and long-term UV-B effects. After prolonged exposure to UV-B radiation three main effects are induced in *S. capricornutum*: diminished cell division rate, increased cell size and reduced photosynthetic activity. On the contrary, in short-term experiments (1 day) UV-B damage became only manifest by a decrease in photosynthetic activity, whereas no significant increase in cellular protein, carbohydrate, chlorophyll *a* or cellular dry weight was detected. Under phosphorus-limiting conditions short-term exposure did not even decrease photosynthesis (Figure 6). The short-term changes in photosynthetic activity were also not representative of the long-term changes under prolonged exposure. Integration of O_2 production rates (Figure 5) yielded a 14% increase in daily production when UV-B was switched off and a 13% decrease when UV-B was switched on. The daily production was 29% less under chronic exposure to UV-B radiation. It must be noted here that the instantaneous light-saturated production rates (Figures 5 and 6) are lower than the P_{max} values determined from the P-I curves (Figure 2). This discrepancy is caused by disturbance of the short-term light history in the P-I measurements. In P-I measurements recovery of short-term depression of P_{max} can occur during the dark adaptation period and the initial low light irradiances (Horton et al. 1988; Neal & Marra 1985). Therefore, instantaneous production rates were measured to compare short-term with long-term experiments. Our measurements show restrictions on the interpretation of short-term effect studies. Not only are important UV-B effects not obvious but the decrease in photosynthetic activity is underestimated.

It seems somewhat remarkable that despite the early findings about DNA as the critical factor in the killing of micro-organisms exposed to ultraviolet radiation (Calkins & Barcelo 1982), research is still mainly concentrated on short-term production measurements. Recently, Karentz et al. (1991) concluded, after studying the sensitivity of twelve species of Antarctic diatoms to UV-B, that DNA is the primary lethal target. The results of our chronic exposure experiments with *S. capricornutum* also suggest that DNA might be a primary target for sublethal effects. A combination of a decrease in growth rate with an increase in cellular biomass is incompatible with photoinhibition but can be related to an arrest in the cell division process. Under the influence of UV radiation several photoproducts can be formed in the DNA. Under UV-B radiation

pyrimidine (thymine) dimers are the most important products. The intrastrand pyrimidine dimers can not be replicated and induces finally a cell cycle arrest towards the G2 phase until the dimer has been deleted by photoreactivation (Buma et al. 1995; Karentz et al. 1991). Under the assumption of the above mechanisms the increase in cell biomass can be explained by accumulation of cells at the end of the G1 phase, S phase, or G2 phase. Before actual cell division takes place, cells have attained about twice their minimum biomass. This shift only explains part of the doubling in cell biomass. The rest must be ascribed to some continued cell growth not directly blocked by DNA-damage. A change in growth rate accompanied by a change in cell size has also been observed by Veen (1991), Behrenfeld et al. (1992) and Buma et al. (1994), for various algal species. These findings strongly suggest that the response is common among (eukaryotic) algae.

Steady-state, defined by a constant growth rate was maintained under high UV-B exposure for at least two months. Growth can continue under UV-B radiation because the DNA-dimers do not lead to mutagenic and lethal effects. Except for very high damage rates, the photoreversal of the dimers is impeccable and does not result in changes in the DNA base sequence (Witkin 1976). Acclimatisation was also demonstrated by the daily O_2 production (Figure 6). The shape of the production curve under high UV-B exposure is comparable to that measured in the absence of UV-B radiation. Conversely to the transitions some balanced production, achieved by an enhanced repair rate, was accomplished during steady state. It is generally accepted that photoinhibition is brought about by a small alteration in the D1 protein which is than removed by a protease (Krause 1988; Ohad et al. 1984). So, the decrease in P_{max} (Table 2, Figure 2) under UV-B exposure reflects a decrease in the amount of active PSII reaction centres. As photorecovery is light dependent (Skogen et al. 1986), repair should not be necessarily completed during one light-dark period. Nevertheless, steady-state production rates are not exclusively explained by a decrease in active PSII reaction centres but will also be determined by the overall change in physiological conditions of the algal cells.

Our experiments on long-term effects showed no increased sensitivity to UV-B radiation under phosphorus limitation. Due to an initial decrease in cell division rate, the cell number in the culture decreased simultaneously. However, when the amount of cells at a constant dilution rate is diminished, phosphorus availability per cell increases, thereby relieving the phosphorus limitation. The decrease in cell numbers was partly compensated for by the increase in cellular biomass. In this way a new steady-state developed at a lower cell concentration. The change in cell numbers observed during the transition period (Figure 7) did not indicate an initial decrease in growth rate exceeding 10%. Changes in cell composition were also comparable to those under nutrient-replete conditions. These results seem on first sight contradictory to the results of Behrenfeld et al. (1994). They found for *Phaeodactylum tricornutum* no long-term effect of prolonged UV-B exposure under carbon or nitrogen limitation. They concluded that UV-B reacted by competitive interaction. Thus growth rate and biomass were determined by the most limiting factor which was nitrogen or carbon. Actually, under phosphorus limitation the biomass per unit of culture volume of *S. capricornutum* also did not change significantly. Furthermore, it must be noted that Behrenfeld et al. (1994) measured cellular volume as an estimate of cellular biomass. So *P. tricornutum* might still have demonstrated a change in cellular weight by an increase in density of the cell. The 8.6 times enhanced susceptibility of nitrate-limited cultures of *Thalassiosira pseudonana* to UV-B radiation (Cullen & Lesser 1991) seems best be explained by the short-term (0.5–4 h) character of their experiments. Bothwell et al. (1993) suggest as an explanation for enhanced UV-B susceptibility under nitrogen limitation, that under nitrogen limitation the reduction in protein free amino acids and chlorophyll *a*, all UV-absorbing compounds, increase the exposure risk of nuclear DNA. It is however questionable if this also holds for acute photoinhibition under short-term exposure. As nutrient limitation is a common phenomenon under natural conditions this topic certainly needs further investigation.

Our results confirm that under chronic exposure to UV-B DNA damage is the dominant factor determining the growth delay in phytoplankton. Additional evidence for DNA as the primary target should be obtained by action spectrum analyses. Such action spectra for algal growth and biomass/size are currently being developed (A Buma, pers. comm.).

In the experiments, the full potential of the culture system was not yet used. Though not shown here, the system is especially suitable to simulate vertical mixing (Kroon 1991) and is an ideal system to analyse the reciprocity between dose rate and exposure time. Nevertheless, the application of realistic PAR irradiances and natural light-dark dynamics yields optimal experimental conditions for the study of UV-B effects.

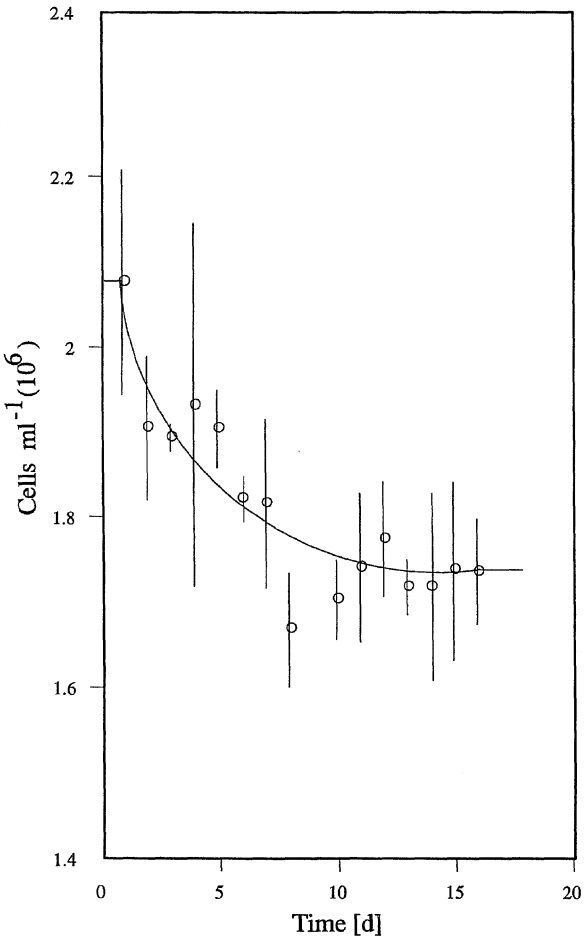

Figure 7. Change in cell concentration of *S. capricornutum* during transient from no UV-B to low UV-B under phosphorus-limiting conditions. On day 1 UV-B was switched on. Culture conditions given in Table 1. Bars represent standard deviations ($n = 3$).

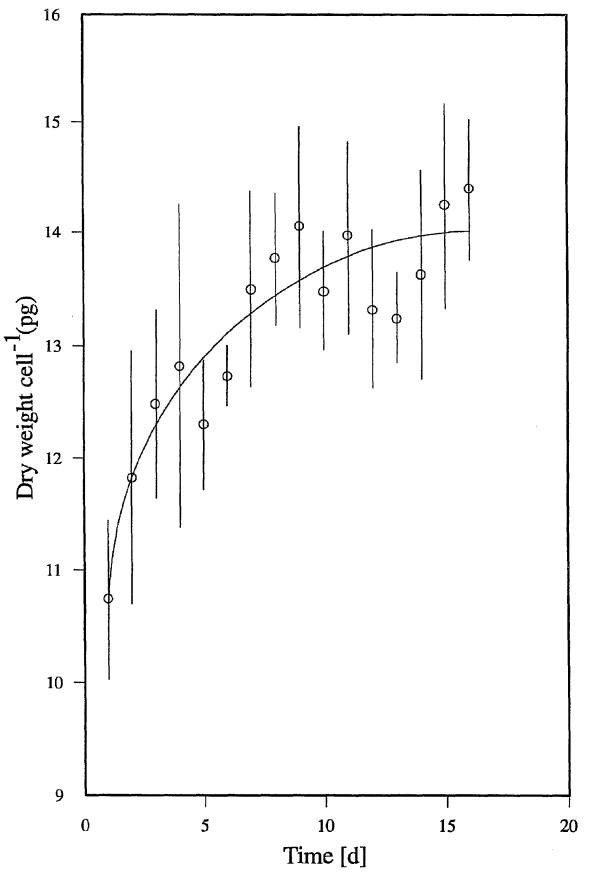

Figure 8. Change in cellular dry weight of *S. capricornutum* during transient from no UV-B to low UV-B under phosphorus-limiting conditions. On day 1 UV-B was switched on. Culture conditions given in Table 1. Bars represent standard deviations ($n = 3$).

It offers the possibility to simulate natural light conditions.

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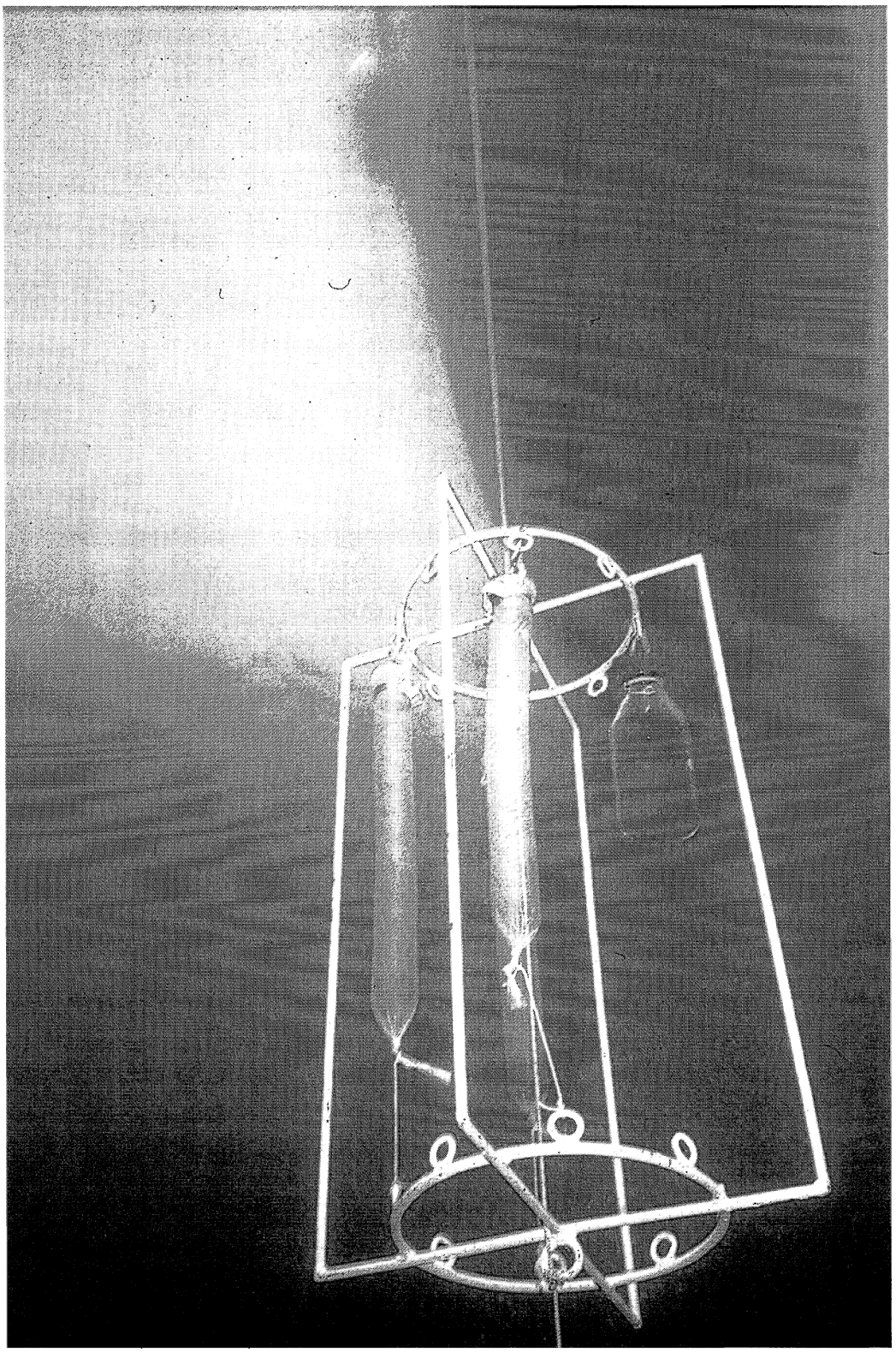

In situ incubations of bacterioplankton in the water column of the northern Adriatic Sea following exposure to surface solar radiation levels to mimic processes of the water column mixing. (Photograph: G. J. Herndl)

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Role of ultraviolet-B radiation on bacterioplankton and the availability of dissolved organic matter

Gerhard J. Herndl, Albert Brugger, Silvia Hager, Edith Kaiser, Ingrid Obernosterer, Bettina Reitner & Doris Slezak
Dept. of Marine Biology, Institute of Zoology, University of Vienna, Althanstr. 14, A-1090 Vienna, Austria

Key words: Bacterial utilization, Bacterioplankton, Dissolved organic matter (DOM), Radiation, Ultraviolet-B (UV-B)

Abstract

Attenuation of ultraviolet (UV)-radiation into the water column is highly correlated with the concentration of the dissolved organic matter (DOM). Thus UV penetrates deeper into marine waters than into freshwater systems. DOM is efficiently cleaved by solar surface radiation levels consuming more oxygen than bacterial metabolism. This photolytically cleaved DOM exhibits higher absorbance ratios (250/365 nm) than untreated DOM. Natural bacterioplankton reach higher abundance if inoculated in previously solar-exposed DOM than in untreated DOM; during bacterial growth the absorbance ratio declines steadily indicating the utilization of the photolytically cleaved DOM. On the other hand, bacterioplankton are greatly reduced in their activity if exposed to surface solar radiation levels. Photoenzymatic repair of DNA induced by UV-A radiation, however, leads to an efficient recovery of bacterial activity once the UV-B stress is released. Turbulent mixing of the upper layers of the water column leads to a continuous alteration of the UV exposure regime. Close to the surface, bacteria and DOM are exposed to high levels of UV-B leading to a reduction in bacterial activity and to photolysis of DOM. Once mixed into deeper layers where UV-B is attenuated, but sufficient UV-A is remaining to allow photoenzymatic repair, the photolytically cleaved DOM is efficiently taken up by bacterioplankton leading to even higher bacterial activity than prior to the exposure. Thus, the overall effect of UV on bacterioplankton is actually an enhancement of bacterial activity despite their lack of protective pigments.

Introduction

The depletion in stratospheric ozone leads to an increase in ultraviolet-B (UV-B) radiation (300 to 320 nm) reaching the Earth's surface not only over Antarctica but also in temperate regions (Blumthaler & Ambach 1990; Hofmann et al. 1989, 1992). Although it has been known for a long time that UV-B exhibits detrimental effects on organisms, aquatic ecology has neglected this radiation range until recently. This is mainly because it has been assumed that UV-B penetration in the water column is restricted to the top layers of the particular environment (Jerlov 1950). Only recently, it has been demonstrated that UV-B is penetrating deeper into the water column than hitherto assumed; in open marine waters UV-B can penetrate as deep as 20 m (Baker & Smith 1982; Fleischmann 1989; Smith & Baker 1981). With the development of new instruments to precisely measure the UV-B and UV-A (320 to 400 nm) wavelength range, the direct relationship between the concentration of dissolved organic matter (DOM) and the penetration-depth of UV light into the water column becomes obvious (DeHaan 1993; Kirk et al. 1994; Scully & Lean 1994).

Over the last 2 decades, information on the role of UV radiation on aquatic organisms is accumulating (Behrenfeld et al. 1992; Buckley & Houghton 1976; Cullen & Usser 1991; Cullen et al. 1992; Döhler 1984; Ekelund 1991; Häder & Liu 1990; Jagger 1975; Worrest 1983; Worrest & Häder 1989). The bulk of the literature concentrates on the impact of UV on phytoplankton primary production (Behrenfeld et al. 1993;

Cullen & Neale 1994; Häder et al. 1995; Helbing et al. 1992, 1994; Karentz et al. 1991 a; Lorenzen 1979; Neale et al. 1994; Prezelin et al. 1994; Smith 1989; Vernet et al. 1994). Surprisingly little, however, is known on the role of UV radiation on the microheterotrophic components of the aquatic food webs although their overall importance for the carbon- and energy flow has been well documented over the last decade leading to a revision in our concept of aquatic food webs (Azam et al. 1983, Ducklow & Fasham 1992). Most members of the autotrophic component of the aquatic microbial community produce protective pigments to shield off detrimental UV-radiation (Karentz et al. 1991 b; Karentz 1994) leading to an adaption to UV-B radiation as shown by Helbling et al. (1992). These authors demonstrated that Antarctic phytoplankton reduces photosynthetic activity more dramatically upon UV-B stress than tropical phytoplankton adapted to higher doses of UV-B (Helbing et al. 1992). In hetero- and mixotrophic flagellates, UV impairs their motility and orientation leading to a reduced grazing activity (Ekelund 1991; Häder & Liu 1990). From the 3 living components of the microbial loop - phytoplankton, bacteria and protists, least information is available on the role of UV on bacterioplankton.

It has been shown recently, that bacterioplankton activity is significantly reduced under UV radiation levels typical for the top meter layers of the ocean (Herndl et al. 1993; Müller-Niklas et al. 1995). Furthermore, no evidence was found that bacterioplankton are able to adapt to UV-B radiation (Herndl et al. 1993). More recently, Garcia-Pichel (1994) calculated that, due to the small size, bacteria are affected more by UV than any other planktonic component and that bacterioplankton are too small to protect the cells from UV radiation by pigmentation. Thus, we are left with the question to what extent bacterioplankton are affected by UV and whether there are differential bacterial responses detectable between freshwater and marine systems. This question is of particular importance if we consider that bacterioplankton dominates over phytoplankton in terms of biomass even in the euphotic zone of oligotrophic waters (Cho & Azam 1990; Fuhrman et al. 1989; Herndl 1991) and that bacteria, due to there small size represent by far the largest living surface in aquatic systems.

The limited information available on the interaction between UV and bacterioplankton has been recently compiled by Karentz et al. (1994) and the interaction between UV and DOM by Zepp et al. (1995). In this contribution we discuss the ecological role of UV on bacterioplankton and their interaction with DOM based on recent work done by the Aquatic Microbial Ecology Group at the University of Vienna. Specific attention will be paid to identify fundamental differences between freshwater and marine systems.

Effects of UV on bacterioplankton

Exposure of bacterioplankton to surface solar radiation results in a significant reduction in bacterial activity as measured by thymidine and leucine incorporation as well as ectoenzymatic activity (Herndl et al. 1993; Müller-Niklas et al. 1995). In a recent experiment (Kaiser & Herndl), we followed the decline in activity (measured by thymidine as well as leucine incorporation into bacterial biomass) of natural bacterial communities after exposure to artificial UV-B and to natural surface solar radiation levels. In both, the laboratory and the *in situ* experiments, bacterial activity was declined by 20 to 42% of the pre-exposure level (Figure 1). Upon UV-B stress, reduction in bacterial activity was significantly lower under nutrient-depleted conditions than in freshly-collected seawater (Kaiser & Herndl subm.) which is contrary to findings of Cullen & Lesser (1991) who detected a higher reduction of photosynthesis in marine diatoms following UV-stress under nutrient-depleted conditions. Generally, exposure of organisms to UV-B results in the formation of thymine dimers (Buma et al. 1995; Karentz et al. 1991b; Mitchell 1988; Mitchell et al. 1990). Quite recently, it has been actually demonstrated that bacterioplankton receive more UV-induced damage of DNA (measured as cyclobutane dimer formation) than phytoplankton (Jeffrey et al. 1996), confirming earlier, more theoretical considerations (Garcia-Pichel 1994). Thus we have reasons to assume that the size of the organism is inversely correlated to the extent of UV-induced damage it receives.

There are several mechanisms available to repair DNA damage; the most important are: the photoenzymatic repair (PER), the nucleotide excision repair (NER) and the postreplication repair (Friedberg 1985; Sancar & Sancar 1988). While the PER is activated by UV-A, which induces the expression of the enzyme photolyase all the other repair mechanisms require additional energy input by the organism in the form of ATP (Karentz 1994; Miller & Kojohn 1990). In order to evaluate the potential role of the different repair mechanisms in bacterioplankton, we transferred natural bacterial communities after UV-B exposure or sur-

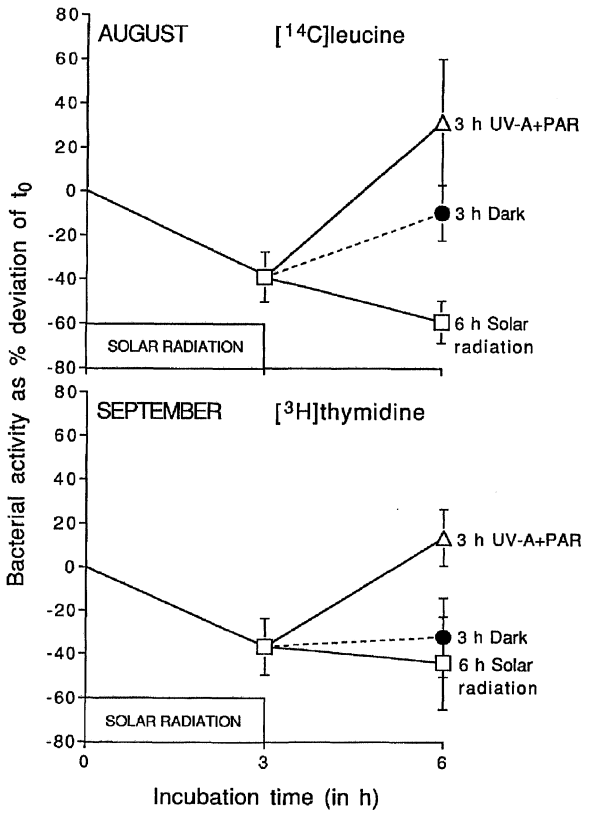

Figure 1. Development of the bacterial activity in 0.8μm filtered seawater under different solar wavelength ranges as compared to the activity prior to starting the experiment. Bacterial activity was measured by the dual labeling technique using [^{3}H]thymidine and [^{14}C]leucine incorporation into bacterial macromolecules. Examples are given for [^{14}C]leucine in August (upper panel) and for [^{3}H]-thymidine in September (lower panel). Water was collected from 3 m depth in the northern Adriatic Sea early in the morning. Thereafter, 1-1 samples (0.8 μm filtered) were incubated in quartz tubes and exposed to surface solar radiation for 3 h around noon (only cloudless days were used, radiation at 305 nm varied between 0.67 and 1.5 μW cm^{-2} nm^{-1} in August and between 0.6 and 0.91 μW cm^{-2} nm^{-1} in September). After assessing the bacterial activity (incubation time 30 min), the sample was split and exposed either to the full solar radiation spectrum, to surface solar radiation where UV-B was excluded by wrapping the quartz tubes in Mylar D-foil, or held in the dark. Measurements were performed 5 times in both August (between 12–24 August) and September (21–27 September). Bacterial production varied between 2.18 and 17.86 μg C l^{-1} h^{-1} at t_0 in August and from 2.65 to 6.78 μg C l^{-1} h^{-1} in September. All radiation measurements were performed with a Biospherical PUV 500 and 510 radiometer. Data points represent the mean of 5 experiments; vertical bars indicate the standard error. For August, the UV-A + PAR treatment was not significantly different from the dark treatment (Wilcoxon, p=0.07), while for September the UV-A + PAR treatment was significantly different (Wilcoxon, p=0.022).

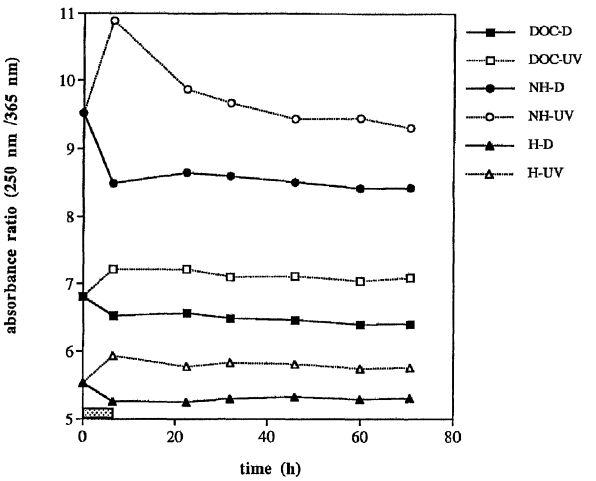

Figure 2. Development of the absorbance ratio (250 : 365 nm) of the humic and the non-humic fraction as separated by XAD-extraction and of the bulk DOC during exposure to surface solar radiation levels for 4 h (indicated by the stippled bar) and subsequent inoculation with natural bacterioplankton. Note the increase of the ratio during solar exposure and the subsequent decline indicating bacterial utilization of labile components of the DOM pool. Open symbols indicate absorbance ratios of solar exposed DOM, full symbols stand for DOM held in the dark; DOC-bulk DOC pool, NH-non humic fraction, H-humic fraction.

face solar radiation levels to different radiation regimes (outlined in Figure 1). Based on these experiments, the most efficient repair mechanism in bacterioplankton is PER; only a short exposure of UV-A is sufficient to yield a significantly higher bacterial activity than in the dark control (Figure 1).

Since bacterioplankton obviously lack pigmentation (Garcia-Pichel 1994; Herndl et al. 1993), efficient repair mechanisms are essential for sustaining surface water bacterial communities. One might assume, however, that different bacterial species exhibit different efficiencies in their repair of UV-induced damage. These differences would ultimately lead to shifts in bacterial species within the community. Seawater cultures of 0.8 μm filtered natural bacterial communities collected in 3 to 5 m depth of the northern Adriatic Sea were exposed to surface solar radiation levels on cloudless days for a total period of 2 days. Controls were held in the dark; an additional set of quartz incubation tubes was wrapped in Mylar D foil® to shield off UV-B. Thereafter, the bacterial community was filtered onto a filter, the DNA extracted and applied to a denaturing gradient gel electrophoresis (DGGE, (Wawer & Muyzer 1995). No differences in the band patterns are detectable between the dark control and the bacterial

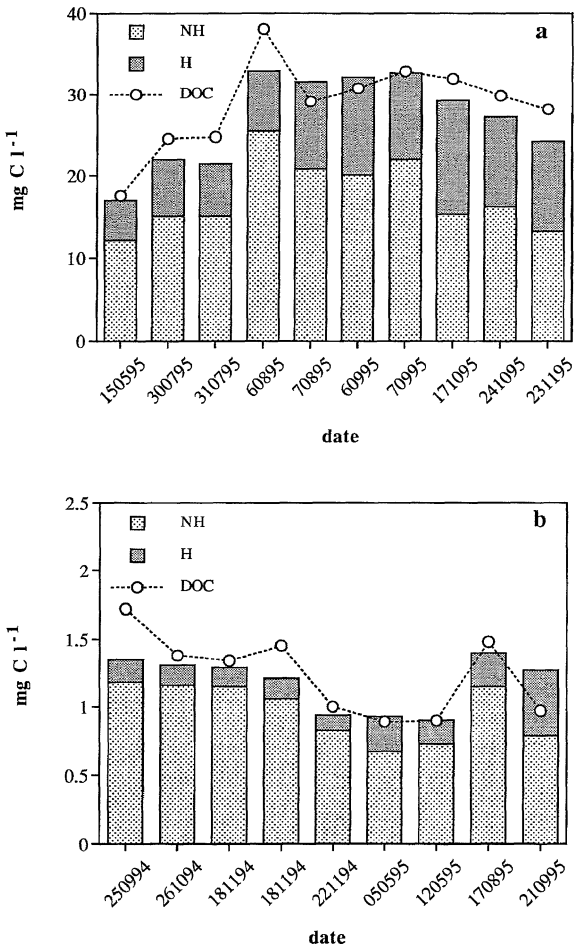

Figure 3. Contribution of the non-humic and the humic fraction to the DOC pool in (a) Lake Neusiedl and (b) in the northern Adriatic Sea on different sampling dates.

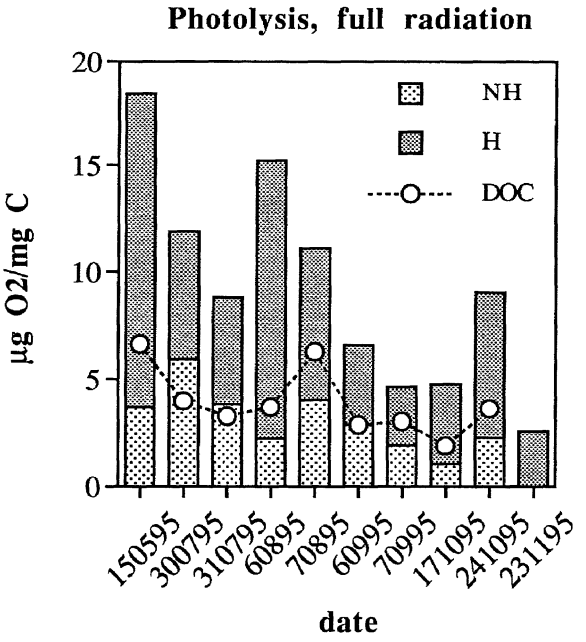

Figure 4. Photolytic activity (expressed as oxygen consumption per available mg C) of surface solar radiation on the humic and the non-humic fraction; for comparison the photolytic activity on the bulk DOC pool is also given.

community exposed to the full surface solar radiation although the number of bacteria as determined by direct counting, was significantly lower in the UV-exposed community. Thus, we have preliminary evidence that UV radiation does not induce shifts in the bacterial community. Further experiments using other molecular approaches will be necessary, however, to further substantiate this finding.

Effects of UV on DOM

Surface solar radiation has been shown to cleave bulk DOM and to produce low molecular weight (carbonyl) compounds (Kieber et al. 1990; Mopper & Stahovec 1986; Mopper et al. 1991). This photolytic activity, measured as oxygen consumption, can even exceed bacterial oxidation as discussed in more detail in the following chapter.

An important class of DOM, the humic substances, are ubiquitously distributed in aquatic systems; they vary considerably, however, in their major components and chemical constituents depending on their predominant origin (Charriere et al. 1991; Chin et al. 1994; Davies-Colley 1992; Fründl et al. 1994). Several methods exist to isolate them from the bulk DOM, among the most widely used technique is the extraction with the macroporous XAD resin. Using this method, it has been shown that humic substances are important sources of nitrogen (Hubberten et al. 1994; Lara et al. 1993; Moran & Hodson 1992, 1994) in neritic seas and that they comprise between 10 and 60% of the bulk DOC (Hubberten et al. 1994; Lara et al. 1993; Moran & Hodson 1992). In the oligotrophic northern Adriatic Sea, humic substances contribute between 10 to 15% to the bulk DOC while their contribution to the DOC pool range between 15 to 47% in the shallow Lake Neusiedl, a lake surrounded by a reed belt of *Phragmites australis*. These humic substances are efficiently altered upon exposure to solar radiation as shown by the absorbance

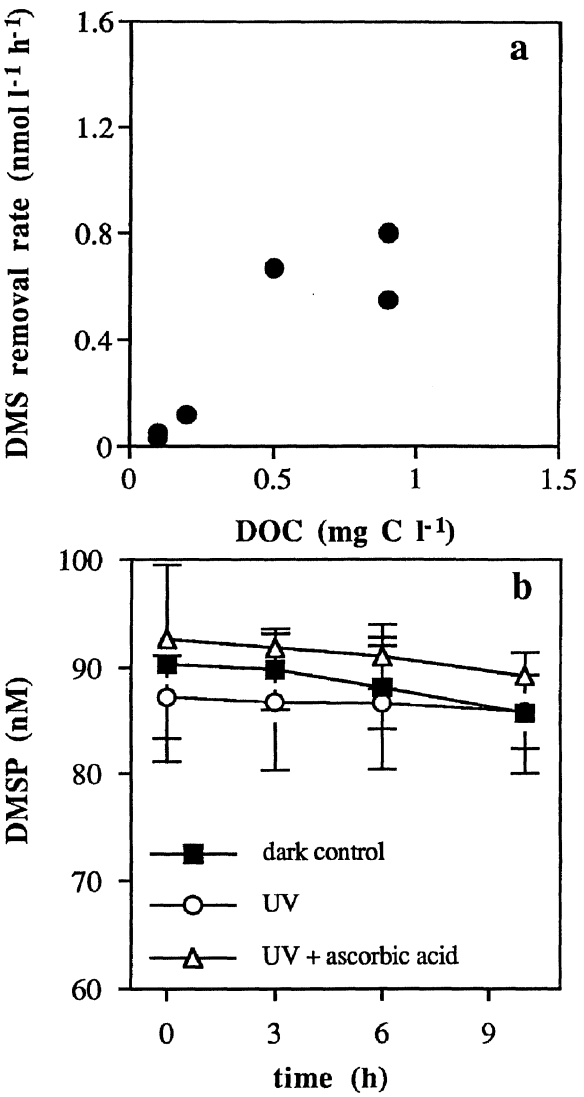

Figure 5. (**a**) Dependence of dimethylsulflde (DMS) removal rates on the dissolved organic carbon (DOC) concentration at a constant UV-B radiation level of 0.4 W m^{-2} provided by Philips TL 100 W/01 lamps (range 300 to 320 mn); (**b**) dimethylsulfoniopropionate (DMSP) is not affected by UV-B radiation.

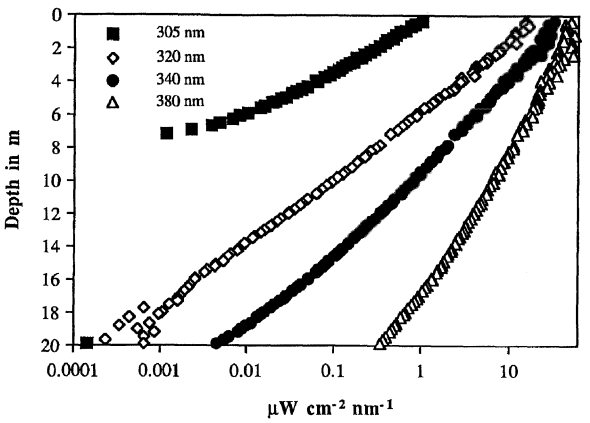

Figure 6. Penetration of several UV wavelengths into the coastal marine water column of the northern Adriatic Sea.

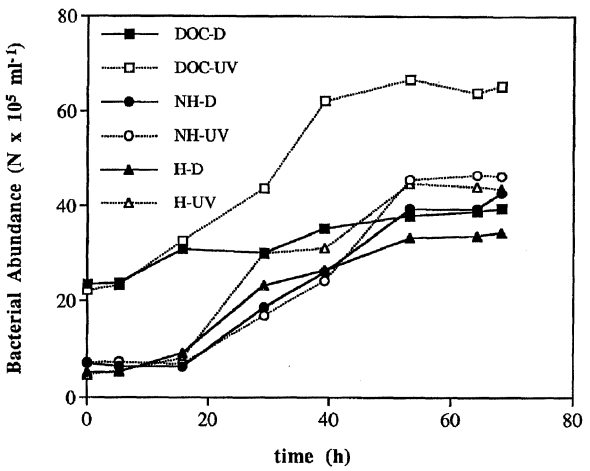

Figure 7. Development of bacterial abundance in batch cultures of fractionated DOM either exposed to surface solar radiation for 4 h or held in the dark prior to inoculating natural bacterioplankton from Lake Neusiedl; for abbreviations see Figure 2.

ratio (250/365 nm) for waters from Lake Neusiedl (Figure 2). A higher 250/365 nm absorbance ratio indicate higher availability of this particular DOM fraction. As shown in Figure 2, this ratio increases during exposure to solar radiation and thereafter, declines slowly again as bacterioplankton are utilizing the more labile components of this DOM-fraction.

It has been shown that humic substances play an important role in scavenging and immobilizing essential molecules and elements such as enzymes, phosphorus or iron (Wetzel 1992). As discussed in the following chapter, these compounds immobilized by humic substances are hardly available to the biota unless they are cleaved from the humics by UV radiation. Figure 3 shows 2 examples of the humic versus non-humic fraction of the DOM pool (separated by XAD extraction); in Lake Neusiedl the contribution of the humic fraction to the overall DOC pool is much higher (Figure 3a) than in coastal marine environments (Figure 3b). As shown in Figure 4, the humic fraction appears to be the predominant target for photolysis. Overall, about one third of the bulk DOC is cleaved by the UV-B wavelength range, while two third of the

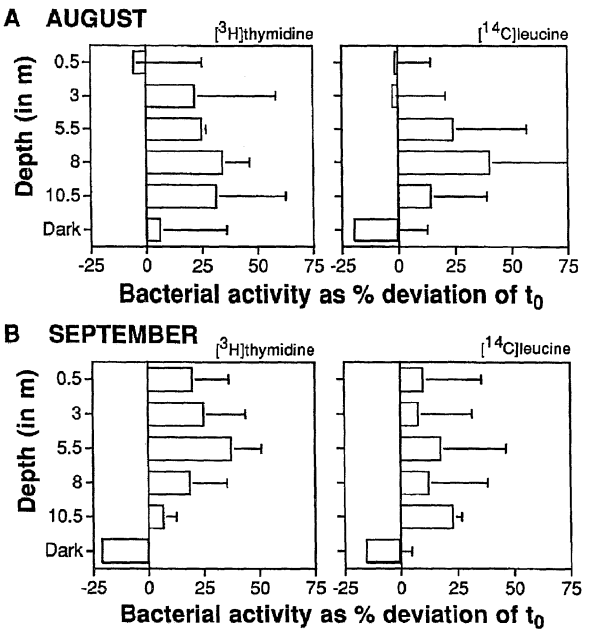

Figure 8. Pattern of the bacterial activity after a 3 h exposure to surface solar radiation levels and subsequent incubation at different depth layers of the northern Adriatic Sea. Bacterial activity is given as the percentage of the activity measured before exposing the samples to surface solar radiation levels. For comparison, bacterial activity of a sample held in the dark after exposure to surface solar radiation is also given. Bacterial activity was measured by the dual labeling technique. Bars represent the mean of 5 measurements in August and 6 measurements in September 1995; horizontal bars indicate standard error.

bulk DOC is cleaved by radiation >320 nm (Reitner et al. subm.).

Another important role of ultraviolet radiation on DOM is the formation of radicals (Zepp et aL 1995). For example, it has been shown that the concentration of hydrogen peroxide is highest in the upper layers of the ocean and declines with depth (Amador et al. 1989; Backlund 1992). An important although largely unsolved problem is the formation of photosensitizers from DOM which make substances available to oxidation, which do not absorb UV radiation. An example for such a substance which does not absorb in the UV range is dimethylsulfide (DMS) which is together with acrylic acid the cleavage product of dimethylsulfoniopropionate (DMSP; Andreae & Barnard 1984). Under UV radiation, the disappearance rates of DMS is correlated with the presence of DOC (Figure 5a), however, the exact chemical pathway of this photochemical reaction is unknown at present while its precursor, DMSP is not cleaved by UV radiation (Figure 5b). It is likely that similar photochemical reactions as shown for DMS take place in a variety of other compounds in the aquatic environment.

UV and the microbial loop in marine versus freshwater systems

The ecological role of UV radiation on aquatic microbes depends first on the penetration depth of UV radiation. Since the attenuation of UV is largely dependent on the concentration of DOM (Scully & Lean 1994), UV penetration in freshwater systems is usually much lower than in marine systems although exceptions have been reported for extremely oligotrophic, high mountain lakes (Psenner, personal communication). Figure 6 shows a typical example for coastal marine systems. In humic-rich Lake Neusiedl (concentration of humic substances 5 to 10 mg C 1^{-1}; DOC concentration between 25 to 32 mg C 1^{-1}) the wavelength of 305 nm is attenuated within 1–3 cm. For comparison, the concentration in humic substances in the northern Adriatic Sea ranges between 0.2 to 0.5 mg C 1^{-1} (Figure 3b). Although the concentration of humic substances is much lower in marine systems, the importance of photolytic cleavage of humics might be equally or even more important in marine systems since the lower humic concentration is more than compensated by the deeper penetration of the UV-B wavelength range. Upon photolysis, humic substances release low carbonyl compounds (Miller 1994; Mopper & Stahovec 1986). These compounds are likely to be responsible for the observed elevated bacterial growth in the humic fraction exposed to surface solar radiation (Figure 7). Ultraviolet radiation, however, does not only make humics more easily available for bacteria but also the non-humic fraction. The development of the absorbance ratios during bacterial growth (shown in Figure 2) points to the formation of low molecular weight DOM during exposure to solar radiation and subsequent uptake of these substances by bacterioplankton (compare Figures 2 and 7).

As shown above, bacterioplankton exposed to surface solar radiation are significantly retarded in their activity. Upon transfer of the solar-exposed bacterioplankton to deeper water layers (3 to 10 m in the northern Adriatic Sea), they recover rapidly exhibiting even higher metabolic activity after 4 h of incubation in these depth layers than prior to the exposure to solar stress (Figure 8). Thus, exposure to surface solar radiation levels for a certain period of time leads to an overall net increase in bacterial activity, most likely

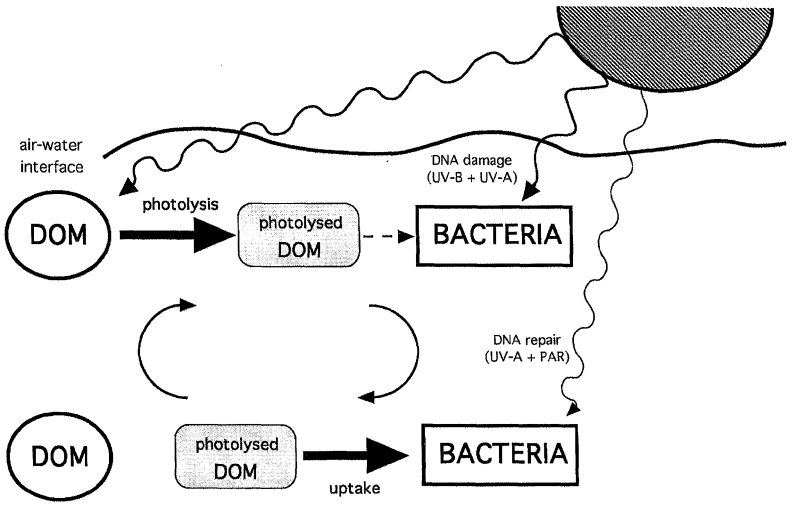

Figure 9. Major role of solar radiation on bacterioplankton and dissolved organic matter (DOM) in the upper mixed water column. In the top surface layers of the water column DOM is photolytically cleaved and bacterioplankton activity retarded due to high levels of UV-B radiation. If mixed into deeper layers, bacterioplankton repair damage via photoenzymatic repair mechanisms induced by UV-A and the photolysed DOM is taken up.

due to the concurrent photolysis of DOM and the production of easily metabolizable DOM in the uppermost layers of the water column.

Since the upper layers of the water column are basically always mixed due to wind-induced turbulence, DOM and bacterioplankton are both exposed to high UV radiation levels which cause in bacteria, a reduction in their activity and thus reduced uptake of DOM and, at the same time, an increased production of photolytically cleaved DOM as depicted in Figure 9. Turbulent mixing of the water column brings both bacterioplankton and the photolytically cleaved DOM into deeper layers where bacterioplankton efficiently repair UV-induced damage (DNA repair via the photoenzymatic repair, Figure 1) using the available UV-A radiation and concurrently by taking up the easily utilizable, photolytically cleaved DOM (Figure 9). Thus, taking the dynamic nature of the upper water column into account, bacterioplankton may actually benefit from UV-B radiation despite the fact that they do not have any protective pigmentation to shield off UV-B radiation.

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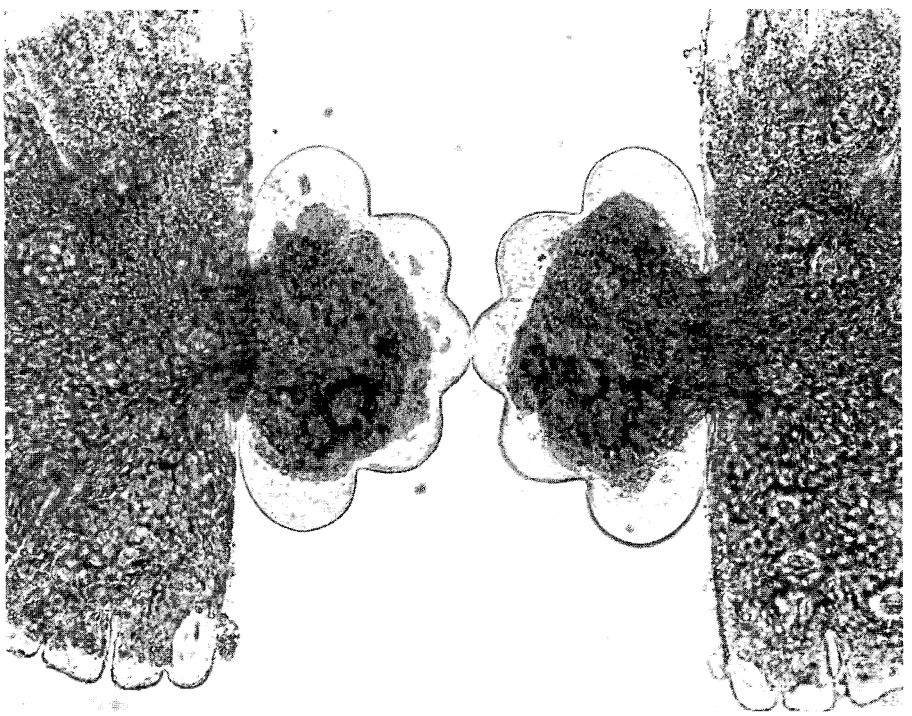

Growing cell pair of *Micrasterias denticulata* with chloroplast migrating from non-growing into growing semicell. (Photograph: U. Meindl)

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Physiological and structural changes in the chloroplast of the green alga *Micrasterias denticulata* induced by UV-B simulation

Cornelius Lütz[1], Harald K. Seidlitz[1] & Ursula Meindl[2]
[1]*GSF Forschungszentrum München, Expositionskammern, Oberschleißheim, Germany;* [2]*Institute of Plant Physiology, University of Salzburg, Salzburg, Austria*

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Abstract

Exposure of postmitotic growing and non-growing cells of the unicellular green alga *Micrasterias denticulata* to different UV-B cut-off wavelengths together with simulated sunlight in a sun simulator has revealed a marked resistence of the algae against strong irradiation. While down to a cut-off wavelength of 284 nm irradiated during the most sensitive stage of cell development chloroplast ultrastructure remains unaffected, severe changes in arrangement and structure of stroma and grana thylakoids occur only at the lowest cut-off wavelengths of 280 and 275 nm. The structural alterations end up in a more or less complete desintegration of grana and stroma thylakoids with the remaining membraneous structures appearing in negative staining thus indicating drastic changes in membrane composition. Photosynthetic activity determined by chlorophyll fluorescence (ratio of variable to maximal fluorescence) and oxygen evolution responded more sensitively to UV-B irradiation. With decreasing UV cut-off wavelengths and prolonged incubation a decrease of photochemistry of PS II occured reaching its lowest values after 60 min at 275 and 280 nm. Oxygen production was even maintained under strong UV irradiation with a cut-off wavelenght of 275 nm up to 15 min. With prolonged UV-B treatment any activity was lost. HPLC separations of pigments exhibited the appearance of break-down products (mainly derivatives of chl b and chl a) with decreasing cut-off wavelength and increasing exposure time. The xanthophyll cycle pigments seemed to be unaffected at least for an irradiation period of 60 to 90 min at low UV cut-offs. Possible mechanisms of UV stress avoidance or protection are discussed with regard to the varying altitudes of the natural habitats of the algae.

Introduction

Solar radiation reaching the earth's surface, contains UV-B which on the one hand may press evolution acting as stress factor, but on the other hand has detrimental effects on plants, animals, microbes and humans. This holds especially, if there is not enough time to develop resistance mechanisms or to escape. Anthropogenic influences have triggered a long lasting process of stratospheric ozone depletion. This depletion, which enhances UV-B radiation, has been observed not only in the Antarctic (as reviewed by Marchant 1994), but also in Central Europe (Blumthaler et al. 1993; Seckmeyer et al. 1994), with an increasing tendency (Madronich et al. 1995). UV-B absorption due to tropospheric ozone may somewhat counteract the UV-B increase but with increasing efforts to reduce tropospheric pollution its contribution will probably become insignificant. The effects of UV-B on terrestrial and aquatic ecosystems are described in numerous papers (e.g. Gold & Caldwell 1983; Häder and several contributions in Tevini et al. 1993; Ziska et al. 1993).

However, none of the known reports describes the response of organisms in alpine aquatic ecosystems to UV-B, neither in field studies nor in closed environment experiments. A reason for the lack of simulation studies is the amount of complicated and expensive equipment needed both for the simulation of global radiation and the measuring devices. Presently UV-B research is one of the principle research areas at the GSF Research Center. The GSF has therefore developed large scale facilities designed for UV-B studies (Seckmeyer &

Payer 1993; Seidlitz et al. 1995). Various scenarios corresponding to ozone related UV-B enhancement can be simulated by means of a flexible adaptive filter technique (Döhring et al., 1996).

Unicellular green algae of the family Desmidiaceae (Conjugatae) represent abundant and frequently predominant organisms in the small aquatic ecosystems of alpine moorlands. They are found in acid bog ponds at different altitudes and are thus exposed to different UV-B conditions. Whereas some species are only observed in lower regions, some species may occurr at different altitudes. Because of their confinement to extremely oligotrophic waters large desmids are regarded as suitable bioindicators for water quality (Brook 1981). On sunny summer days desmid cells can be found in large quantities directly underneath the water surface where they are exposed to nearly full sun light. They can regulate their vertical position in the water column by slime excretion through numerous pores in their secondary cell wall (Kiermayer 1981), a process which has been shown to depend on blue light (Nossag and Kasprik 1993).

The cell biology of the large-sized cells (100–300 μm) of this group of algae, like *Micrasterias* have been investigated since many decades (for summary see Meindl 1993). The highly ornamented, symmetrical cell pattern of *Micrasterias*, manifested in two half cells which are arranged like a mirror image, represents an ideal system for studies on morphogenesis and multipolar tip growth. Since the cell pattern has to be reformed in one half cell after each mitosis while it is maintained in the other one, influences of environmental and intracellular parameters can be easily detected by changes in cell symmetry, location and size of cell lobes and position of indentations. The short period of time required to complete the morphogenetic course (3 to 5 h) gives evidence of slight or severe injuries of the organisms shortly after exposure. The exactly defined shape of each developmental stage allows an accurate description of the time course of growth and a detection of even minor retardations at a light microscopical level. Detailed analysis of their ultrastructure at different growth stages, including studies on organelle development and positioning (Meindl 1993) represent a basis for electron microscopical observation of changes induced by environmental conditions. We used *Micrasterias denticulata* as a test object to follow ultrastructural and photosynthetic changes after different UV-B treatments in the presence of high intensity white light. Because of its distribution from lowland to high altitudes this alga is expected to have evolutionary developed resistance mechanisms against strong irradiation. On a cellular level the reactions might be comparable to the stress avoidance as is described for higher plants from high altitude locations (Lütz 1996; Wildi & Lütz 1996).

Materials and methods

Growth conditions

Cells of *Micrasterias denticulata* were cultivated under semisterile conditions in a 'desmid nutrient solution' (Schlösser 1982) at 20 °C and a light-dark rhythm of 14 to 10 h. Light was provided by fluorescent tubes (Philips 40W-1/29) at a light intensity of 30 μMol m^{-2} s^{-1}.

UV-B simulation

Growing and non-growing algae, freshly collected from the culture flasks, were exposed to the light conditions in the sun simulator for varying periods of time from 15 min up to 6 h. During exposure, cells were kept in small glass vials to avoid side shading at a constant temperature of 24 °C. The light absorption of the nutrient solution was negligible down to 270 nm. Four different UV-B treatments were simultaneously applied to the samples using approx. 600 μMol m^{-2} s^{-1} of PAR (400–700 nm), and a UV-B free control using a WG 360 filter (Schott) (Figure 1). We designate the four treatments as '275 nm', '280 nm', '287 nm', '306 nm' cut-offs according to the interception of the respective spectral curve with the 0.1 mW m^{-2} nm^{-1} abscissa. Table 1 gives the integrated spectral data for the different spectral regions. Spectral measurements and quality control of irradiation and the possible ageing of filters were made using a Bentham TM 300 double monochromator with a quarz fiber and a cosine corrected diffusor as input optics (Thiel et al. 1996).

Measurements of photosynthetic parameters

Fast chlorophyll fluorescence (Kautsky effect) was determined with an algae adapter to the Plant Efficiency Analyser (PEA) from Hansatech Ltd, Kings Lynn, England. A predarkening period of 20 min was applied to empty the electron pool in photosystem II. Fluorescence was recorded from 10 μs to 3 s, and data were calculated mainly for Fv/Fm values as a measure of the primary photochemistry of photosystem II

Table 1. Integrated data of the irradiation regimes used in the UV-B simulation. The weighting function is the generalized action spectrum for plant damage (UV-B_{BE}), normalized at 300 nm (Caldwell 1971). Compare spectral curves in Figure 1

Spectrum No.	(1)	(2)	(3)	(4)	
Filter	Tempax®	Sanalux®	Pyra®	Float glass	
Filter thickness	2 mm	4 mm	6.5 mm	4 mm	
UV-C (250–280 nm)	5.4	0.16	–	–	$\mu W\ m^{-2}$
UV-B (280–320 nm)	14.7	9.0	4.9	0.08	$W\ m^{-2}$
UV-B_{BE} (plant damage)	6.36	2.48	0.770	0.013	$W\ m^{-2}$
UV-A (320–400 nm)	27.8	26.8	24.0	15.4	$W\ m^{-2}$
PAR (400–700 nm)	approx. 600 μMol $m^{-2}\ s^{-1}$				
Cut-off wavelength	275 nm	280 nm	287 nm	306 nm	

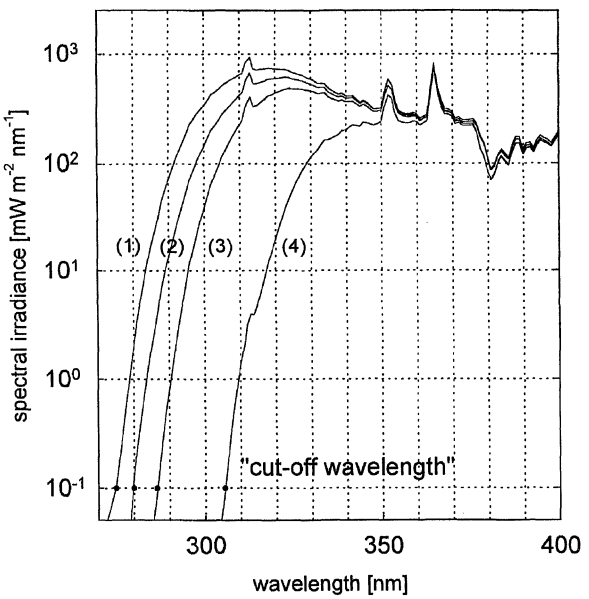

Figure 1. Spectral irradiance of the four UV-B treatments under Tempax (1), Sanalux (2), Pyran (3) and floatglass (4). The cut-off wavelenghts of 275,280,287 and 306 nm were defined by the interception with the 10^{-1} mW m^{-2} nm^{-1} irradiance value (closed circles).

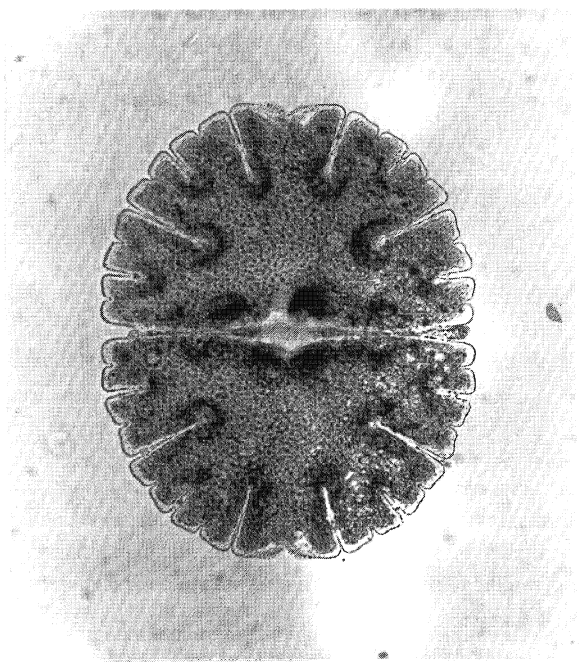

Figure 2. Light microscopical view of a *Micrasterias denticulata* cell. Main diameter amounts to ca. 200 μm. Each semi-cell contains one chloroplast.

(Strasser et al. 1995). Immediately after different times of irradiation under filters with the cut-off's of 306, 287, 280 and 275 nm the samples were measured. The latter two treatments were followed from 5 min up to 90 min, and the former two treatments from 15 min up to 180 min.

Photosynthetic activity was recorded as oxygen development using an oxygen suspension electrode (Hansatech, England) according to Delieu & Walker (1972). Before measurements, the algae were concentrated by gentle centrifugation and resuspended in nutrient solution plus 0.1 *M* bicarbonate buffer at pH 6.5 as CO_2 supply. Samples were irradiated with a saturating light intensity of 200 μMol photons $m^{-2}\ s^{-1}$, at a constant temperature of 24 °C. Oxygen development was recorded using a self-written computer program designed for use with the electrode (Lütz 1996).

Plastid pigments were prepared from the samples used in oxygen development assays and from samples

frozen in liquid nitrogen immediately after the incubation in the sun simulator. Pigments, extracted into Dimethylformamide (Bergweiler and Lütz 1986), could be separated on a Waters HPLC equipped with a photodiode array (Type 990+) as is described in Wildi and Lütz (1996).

Electron microscopy

For transmission electron microscopy growing *Micrasterias* cells exposed to 275 nm, 280 nm and 306 nm cut-off wavelengths in the presence of PAR for 1 h were fixed in 1% glutaraldehyde followed by a mixture of 2% osmium tetroxide and potassium ferricyanide for best preservation of membranes (for details see Meindl 1990). The cells were poststained with 2% aqueous uranyl acetat for 2 h, dehydrated in increasing concentrations of ethanol and embedded in Epon 812. After thin sectioning on an Ultracut (Reichert) the samples were examined in a Philips 400T electron microscope at 80 kV.

Results

Chloroplast structure

Untreated cells or cells from irradiations down to 306 nm cut-off did not show any changes in chloroplast structure (Figures 2 and 3), within 6 h incubation, which covers the complete growth period of a single cell. This was also shown in an additional simulation experiment for the 287 nm cut-off (Meindl & Lütz, unpublished). Figure 3 shows the network of grana and stroma lamellae of the single chloroplast of *Micrasterias* after a 306 nm irradiation (cf. Figure 2) in more detail. Numerous starch grains are embedded in the stroma; the content of plastoglobules is low. Visible changes in plastid ultrastructure developed under the '280 nm' irradiation (Figure 4). Stroma and grana lamellae appear less clear, grana tend to disintegrate and the net-like arrangement of thylakoids is lost. Some grana develop a negative stain appearance. Under '275 nm' irradiation the defined ultrastructure of the plastid was completely lost (Figure 5). Also, starch grains and plastoglobules are not clearly visible. Some remaining membraneous structures appear negatively stained – a possible consequence of drastic changes in the composition of the membrane.

Photosynthetic activities

Chlorophyll fluorescence

The primary photochemistry of photosystem II (PS II) was measured as the ratio of variable to maximal fluorescence (Fv/Fm) (Figure 6). Samples from the culture flasks had Fv/Fm values of 0.76 (±0.02 SD). The different treatments showed a gradual decrease of photochemistry in PS II with time, which reached very low values around 0.1 after 60 min under '275' and '280' nm filters. At a cut-off of 287 nm, even 180 min irradiation could not lower Fv/Fm more than 0.26, with a 60 min value of 0.44. Until 30 min incubation the UV-B (306 nm cut-off) lowered Fv/Fm slightly (c. 0.7), and prolonged incubation resulted in values between 0.5 and 0.6. The initial reduction of values by about 0.1 indicates an adaptive photoinhibition due to the change from culture light (30 μMol) to sun simulator irradiation (600 μMol).

Oxygen evolution

The measurement of oxygen evolution includes photosynthesis as well as respiration. In case of photosynthesis the values were not corrected for respiratory losses to show the possible positive energy balance of the whole cell. The measured values were calculated on a total chlorophyll basis, determined in the photometer before HPLC separation of pigments, because in some treatments new chlorophyll forms developed (see below), which could not be quantified in the HPLC system. Figure 7 shows the oxygen assays for the treatments '275', '280', and '287' nm. The '306' nm samples developed oxygen similar to the non-exposed controls with an average of 3.7 ± 0.3 μMol oxygen mg^{-1} chl min. Even with strong UV-B, including some UV-C, the '275' nm sample shows photosynthesis at least for 15 min irradiation. Then only oxygen consumption occurs; at '275' nm after 90 min any activity has gone. Some resistance is seen in the '287' nm sample up to 30 min irradiation, later a more or less constant oxygen consumption is observed.

Pigment changes

HPLC separations of pigments from culture cells or from '306' nm cells reveal the normal composition of chlorophylls and carotenoids known from higher plants and green algae (Figure 8a). The change from culture PAR conditions to simulation PAR intensity only resulted in the expected shift in the xanthophyll cycle pig-

Figure 3. Ultrastructure of *Micrasterias* cells from cultures treated with 306 nm cut-off irradiation (control): cytoplasmic area with part of the plastid, showing grana stacks (G), starch grains (S) and plastoglobuli (P). Dictyosome: (D). Vacuole: (V). No structural changes occur. Magnif.: 18 800×. *Figure 4.* Chloroplast structure after 60 min irradiation with '280 nm'. The net-like arrangement of thylakoids is lost. Most grana stacks (G) tend to disintegrate. Also stroma lamellae loose membrane structure. Some grana appear in negative contrast. Magnif. 33 300×. *Figure 5.* Chloroplast structure after 60 min irradiation with '275 nm'. Normal staining of thylakoids is completely lost. Remaining grana (G) are negatively stained as a result of severe chemical changes. Magnif. 51 300×.

ments (higher content of zeaxanthin). With decreasing cut-off wavelenghts and increasing time of exposure the separations show more and more additional peaks; their possible parent compounds can be assumed by comparing the photodiode spectra with known pigments. It is not possible to describe a continuous decrease of one compound ending in an increase of one or two new pigments, because a complete destruction of molecules (original and newly formed) may also occur to an unknown extent. Therefore in Fig-

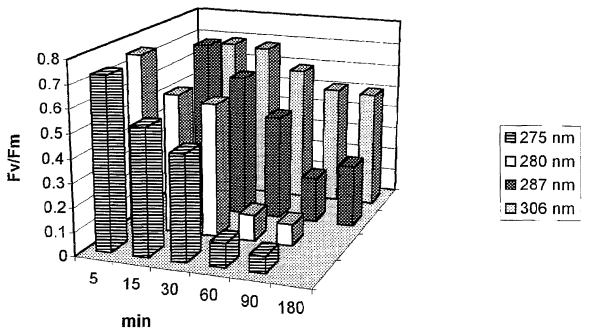

Figure 6. Influence of different irradiations for times from 5 min to 180 min on the photochemical efficiency (Fv/Fm). The nm values represent the filter-cut off's in the UV-B range. PAR-irradiation: 600 μMol m^{-2} s^{-1}. The treatments '287' and '306' are measured beginning with 15 min, the '275' and '280' samples end at 90 min. All values are means of five determinations; the SD is 0,02 or less. Samples from cultures have a Fv/Fm ratio of 0.76.

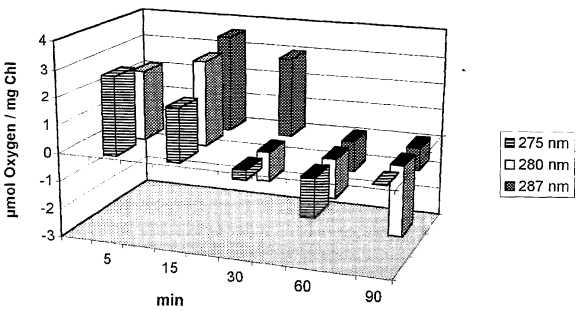

Figure 7. Rate of oxygen production at a light intensity of 200 μM m^{-2} s^{-1} or consumption in relation to the treatments (see Figure 6). Values are means of three determinations ($\pm$0.2 SD). The average oxygen formation in controls directly from the cultures and in the '306' nm treatment is in the same range of 3.8 ($\pm$0.3 SD) μMol O_2 mg^{-1} chl h. No activity (0,0) has been found at 90 min under '275' nm treatment.

ures 8b–d we only want to present examples of typical HPLC patterns. However, some general patterns can be observed: (a) at the expense of chl b new chl b-like compounds are formed easily (Figure 8d, c), mostly with shorter retention times. Chl a is affected only after longer exposure and by shorter wavelenghts, also with hydrophilic break-down products (Figure 8c). One compound is formed with a spectrum similar to protochlorophyll(ide) (Figure 8d, c, main absorptions at 451 nm and 634 nm). Nearly in parallel to chl a destruction ß-carotene is almost completely removed. The carotenoids remain remarkably stable: in relation to other pigments, lutein does not seems to change considerably; the influence on neoxanthin is low and not clearly to correlate with a treatment. The carotenoids of the xanthophyll cycle (violaxanthin, antheraxanthin, zeaxanthin) react in dependance of the duration of the light (PAR) treatment, but obvioulsy independent of UV-B irradiation. These pigment shifts indicate an undisturbed xanthophyll cycle at least for 60–90 min even at low UV-B cut-off's. In none of the treatments a complete bleaching of chlorophylls and carotenoids was observed. No attempts have been made to characterize unesterified porphyrins, if they may be formed by any treatment.

Discussion

Exposure of growing and non-growing cells of *M. denticulata* to UV light of different cut-off wavelengths has revealed a high resistence of the green algae against strong irradiation. Down to a cut-off wavelength of 287 nm which is not reached at the natural habitats of the algae, chloroplast structure does not show any changes even after a 6 hours treatment during the most sensitive cell division stages. Only lower wavelengths produce a loss in the arrangement and structure of stroma and grana thylakoids combined with a marked inhibition of photosynthesis. An increase in starch content of the chloroplast after UV-B exposure as Malanga & Puntarulo (1995) describe for *Chlorella* is not observed in our studies.

The severe structural damage of the chloroplast we found at low cut-off wavelengths (below 287 nm) corresponds to changes in ultrastructure of other organelles, especially dictyosomes and ER cisternae including an inhibition of secretion (Meindl & Lütz 1996) and demonstrate that several physiological activities of the cells are disturbed. These effects are different from the photodestruction which occurred as a combined action of high irradiation in the field at 2300 m altitude together with subzero air temperatures (Lütz 1996): thylakoid structures were found to be destroyed, but the remaining cell structures, even the plastid envelope, remained intact.

The negative staining of thylakoids, which developed with increasing radiation stress, indicates strong changes among membrane lipids, which alter the reactivity to the fixation and staining media possibly as a consequence of lipid peroxidation (Kochevar 1990).

Photosynthesis has been shown to be affected at different levels by high light, cold events and especially UV-irradiation in various organisms (Caldwell & Flint

1994; Häder & Worrest 1991; Malanga & Puntarulo 1995). In *M. denticulata* it responded more sensitive to the different treatments when compared to plastid structural changes.

Photochemical efficiency in PS II (measured as Fv/Fm) is slightly reduced after 5 min under '275 nm' or '280 nm' irradiation, a stronger decrease occurs until 30 min of incubation, followed by a steep fall down to values around 0.1 (control samples: 0.76). The decrease in the '306 nm' treatment down to Fv/Fm of 0.6–0.5 after 3 h is not paralleled by photosynthetic activity: oxygen development did not change considerably compared to shorter times or the untreated samples. A clear photoinhibition of photosynthesis after 30 min incubation at the two shortest wavelenghts regimes developed, resulting in accelerated respiration. However, longer incubation in the UV-C containing 275 nm cut-off variant also stopped this activity. These observations may indicate that the cells try to reduce photochemical reactions to avoid photodestruction, but with increasing radiation load detrimental effects on physiology are unavoidable.

A first description of pigments from desmids was given by Herrmann (1968) by means of extensive thin layer chromatography separations. Our HPLC separations of the control cells revealed the same pigment pattern. Additionally, we tried saponification with methanolic KOH to test for esterified carotenoids, but no further pigments were found. Considerable oxygen evolution activities occur in samples containing some altered chlorophyll and carotenoid molecules. The influences of the irradiation regimes on the pigments proper seem to follow several steps: (a) partial change of chl b into more hydrophilic chl b derivatives (hydroxylation in the porphyrin?), (b) reduction in chl a content, yielding chl a molecules with different retention times and a protochlorophyll (ide) comparable compound, (c) nearly complete decomposition of β-carotene.

The numerous breakdown products separated by HPLC indicate that their hydrophobic nature is only to a minor extent affected. In case of chlorophylls, predominantly the porphyrin part is changed. Because chlorophyll b derivatives develop first, and β-carotene and most of chlorophyll a seems to be more stable against UV-B stress we suggest that a destructive irradiation effect occurrs firstly in the light harvesting domains and not in the reaction centers. Published data on UV-B effects on PS II-D-1 protein were mostly raised in experiments with UV-treated isolated thylakoids under spectral irradiation and intensity conditions far from ecological relevance (e.g. Friso et al. 1994).

In comparison to higher plants, *Micrasterias* cells contain less xanthophyll cycle pigments in relation to chlorophylls or other carotenoids. The xanthophyll cycle pigments are not seen as a main target of irradiation, because their relative changes were observed only under conditions when whole cell effects are visible. This observation is different from studies with higher plants that UV-B may block the formation of zeaxanthin (Pfündel et al.1992). Photodestruction as a consequence of high irradiation plus low temperature in the field is described for a high alpine plant (Lütz 1996), and this study also shows a minor reaction of xanthophyll cycle compounds, but of chlorophylls, ß-carotene and of tocopherol. Under conditions of high PAR, the xanthophyll cycle is thought to protect photosynthesis from excess energy (Pfündel & Bilger 1994; Siefermann-Harms 1977). The destructive influences of increasing UV-B obviously did not destruct the xanthophyll-cycle pigments suggesting that these pigments were not involved and thus not able to protect against this kind of radiation.

An important requirement of UV-B simulation studies with ecological significance is to respect the ratio UV-B/UV-A/PAR (Caldwell & Flint 1994). This is a critical parameter for damage or survival of organisms in such studies. Our sun simulation studies were designed especially to address this problem and to avoid non-ecological irradiations (Thiel et al. 1996). Most UV studies with aquatic or terrestrial plants were not much concerned by the influence of the different spectral regions. Perhaps this is one reason that causes contradicting results on algae; for instance Häder & Worrest (1991) mention a strong negative effect of UV-B on photosynthesis, whereas Ryan (1992) found only a minor effect.

Our results thus strongly suggest that desmid cells have developed stress avoidance mechanisms against UV irradiation, like other organisms have towards extreme environments characterized by high irradiation and low temperatures (Caldwell et al. 1982; Karentz et al. 1991; Lütz 1996).

The mechanism of UV stress avoidance in *Micrasterias* is not yet clear. Motile algae living in large water bodies have the opportunity to escape from enhanced UV-B irradiation, which in the case of *Micrasterias* cells is limited due to the shallow bog ponds they inhabit. The continuous production of slime may protect this alga against short wavelengths. Some first measurements of isolated slime showed strong absorp-

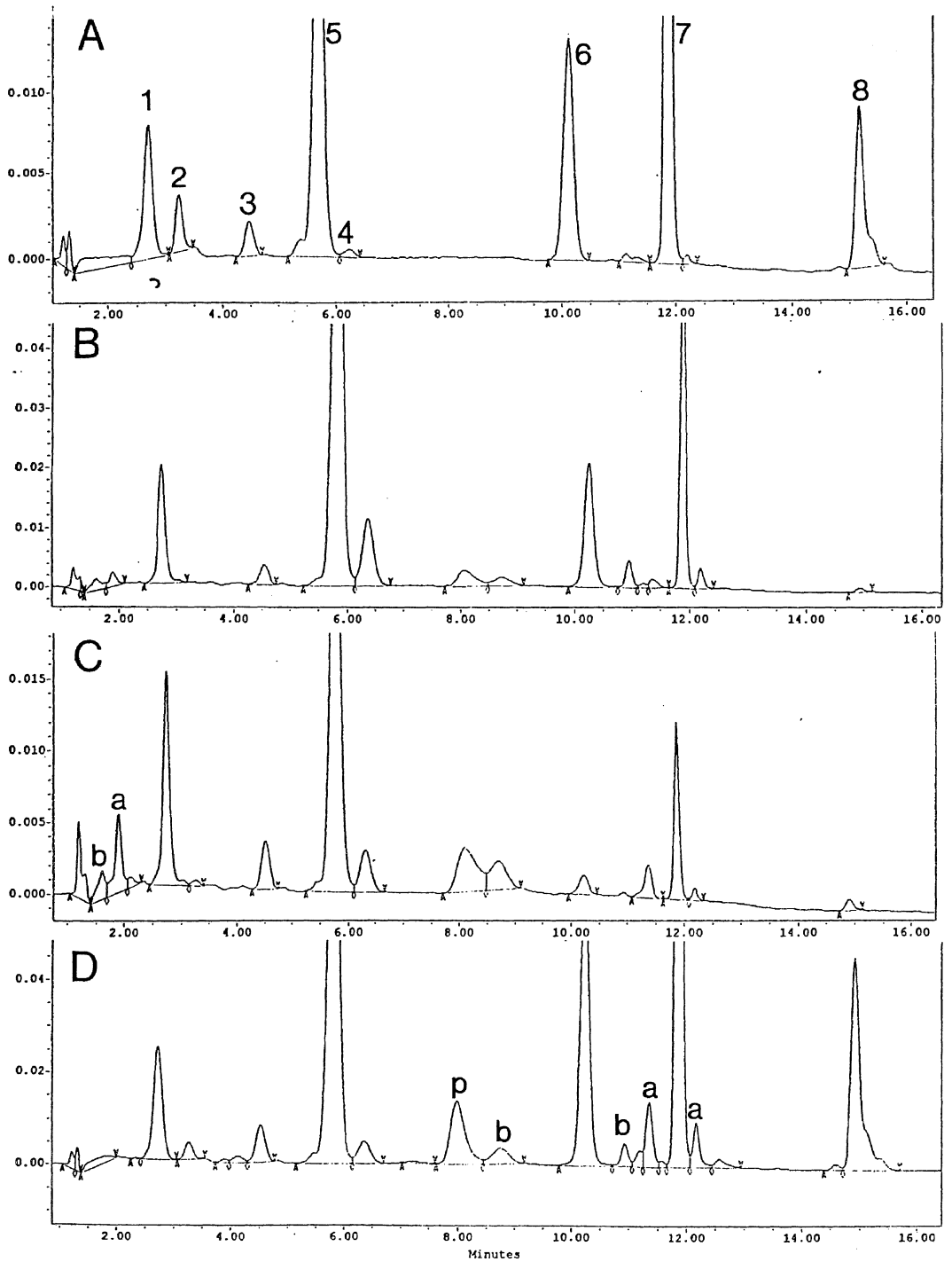

Figure 8. Chlorophylls and carotenoids separated by HPLC: samples from different irradiations and different times of incubation as **A**: control (no UV-B treatment), **B**: '287 nm' cut-off, 90 min; **C**: '280 nm' cut off, 60 min; **D**: '275 nm' cut-off, 15 min. 1 = neoxanthin; 2,3,4 = xanthophyll cycle pigments violaxanthin, antheraxanthin, zeaxanthin; 5 = lutein; 6 = chl b; 7 = chl a; 8 = β-carotene. Pigments formed by irradiation show spectra similar to: p = protochlorophyll(ide); a = chlorophyll a; b = chlorophyll b.

tion in the UV-B range. The thick mucilage layer which is secreted through cell wall pores and surrounds the *Micrasterias* cell may have a similar function and contain similar compounds as in cyanobacteria (e.g., Garcia-Pichel & Castenholz 1991; Scherer et al. 1988).

Except one early investigation using an UV wavelength of 253.7 nm (Kallio 1963) our study represents first evidence of UV effects on growth, ultrastructure and photosynthetic activities in *Micrasterias*. Further experiments are required to elucidate especially protection or avoidance mechanisms which are the basis for the high resistance of the algae against strong irradiation.

The extreme environments found in high alpine regions press all living matter with multiple stresses. High alpine flora obviously has therefore developed multiple stress avoidance mechanisms, especially towards high irradiation and low temperature (Larcher 1983; Lütz 1996). The well balanced life of the flora in alpine ecosystems is not used to environmental changes like the relatively fast increase in short wavelength UV-B. They may live close to the edges of their zones of tolerance. If there is any effect of increased UV-B on alpine aquatic systems, work on this question requires a well designed simulation and an exact measurement of irradiation (Seidlitz et al. 1995). Here, unicellular algae like *Micrasterias* offer best possibilities for mechanistic as well as for ecophysiological studies on UV-B effects.

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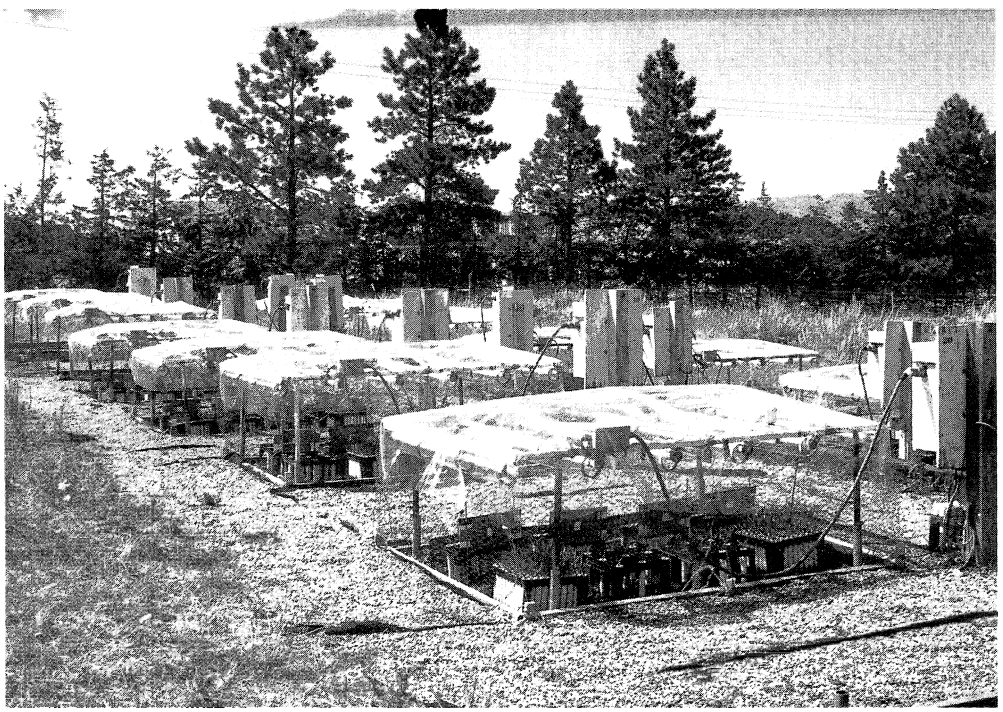
II. UV-B and terrestrial ecosystems: general and methodological aspects



Field experimental plots using different combinations of filters with electronically modulated UV lamp systems to provide different spectral combinations for scaling spectral responses. These plots are typically replicated by five for each spectral combination. (Photograph: R. Robberecht)

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J. Rozema, W. W. C. Gieskes, S. C. van de Geijn, C. Nolan and H. de Boois (eds), UV-B and Biosphere.

Uses of biological spectral weighting functions and the need of scaling for the ozone reduction problem

Martyn M. Caldwell & Stephan D. Flint
Department of Rangeland Resources and the Ecology Center, Utah State University, Logan, Utah, USA

Key words: Action spectra, Global change assessment, Higher plant response to UV, Solar ultraviolet, Stratospheric ozone layer, UV-B radiation

Abstract

In several phases of assessing implications of stratospheric ozone reduction for plants, biological spectral weighting functions (BSWF) play a key role: calculating the increase of biologically effective solar ultraviolet-B radiation (UV-B_{BE}) due to ozone reduction, assessing current latitudinal gradients of UV-B_{BE}, and comparing solar UV-B_{BE} with that from lamps and filters in plant experiments. Plant UV action spectra (usually determined with monochromatic radiation in the laboratory with exposure periods on the order of hours) are used as BSWF. Yet, many complicating factors cloud the realism of such spectra for plants growing day after day in polychromatic solar radiation in the field. The uses and sensitivity of BSWF in the stratospheric ozone reduction problem are described. The need for scaling BSWF from action spectra determined with monochromatic radiation in laboratory conditions over periods of hours to polychromatic solar radiation in the field is developed. Bottom-up mechanistic and top-down polychromatic action spectrum development are considered as not satisfactory to resolve realistic BSWF. A compromise intermediate approach is described in which laboratory results are tested under polychromatic radiation in growth chambers and, especially, under field conditions. The challenge of the scaling exercise is to resolve disagreements between expected spectral responses at different scales of time and radiation conditions. Iterative experiments with feedback among the different experimental venues is designed to reduce uncertainties about realistic BSWF in the field. Sensitivity analyses are employed to emphasize characteristics of BSWF that are particularly important in assessing the ozone problem. Implications for use of realistic BSWF both for improved research design and for retrospective analysis of past research is described.

Introduction

There is understandably much concern about the potential effects of a decreased ozone layer on terrestrial vegetation. Yet, biological effects of the increased solar ultraviolet-B (280–320 nm)[1] radiation (UV-B) resulting from ozone reduction are only significant if organisms are sensitive to the UV-B flux rates received, and if the spectral sensitivity (i.e., the relative response to different wavelengths) of these effects has rather specific characteristics. Most research addressing plant effects deals with the absolute sensitivity and very little with spectral response (Caldwell & Flint 1994a, b; Tevini & Teramura 1989). Plant spectral response information is needed in several aspects of assessing plant response to stratospheric ozone reduction, but in this context, the spectral responses must be relevant to plants exposed to solar radiation over long periods of time.

The challenge is to scale biological spectral responses determined in the laboratory (usually with monochromatic radiation over a period of hours) to spectral responses of plants in the field where plants are exposed day after day to the full solar spectrum, the flux density of which continually changes. Scaling, used here, denotes the process of linking laboratory results to more realistic field conditions and involves

[1] As originally defined (Coblentz 1932), the UV spectrum is UV-A 315–400 nm, UV-B 280–315 nm, and UV-C <280 nm. However, the division between UV-A and UV-B is often taken as 320 nm.

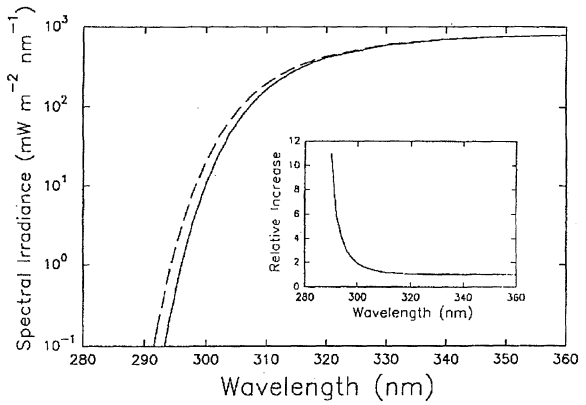

Figure 1. Solar global (direct beam plus diffuse) spectral irradiance computed for normal ozone concentrations and solar zenith angles appropriate for midday in the summer at temperate latitudes (40°) with normal (continuous line) and a 20% reduction of the ozone column (dashed line). In the inset is the factor for relative increase of spectral irradiance at each wavelength due to the ozone column reduction (from Caldwell & Flint 1994b).

both a series of iterative experiments and sensitivity analyses.

Why are spectral responses needed in assessing ozone reduction? The absorption coefficient of ozone increases by orders of magnitude with decreasing wavelength. A reduction of the ozone layer results in a very specific increase in solar UV-B and this is primarily in a waveband of about 30 nm – between 290 and 320 nm. At shorter wavelengths, absorption by ozone is so great that a small fraction of the present ozone layer is sufficient to block all radiation; at longer wavelengths, absorption is so weak that changes in ozone result in diminishing increments of radiant flux relative to background solar radiation. Within this 30-nm waveband, however, the solar irradiance decreases by over four orders of magnitude, due to ozone absorption (Figure 1). With ozone reduction, the relative enhancement of UV-B is also highly wavelength specific; the relative enhancement increases decidedly with decreasing wavelength (Figure 1 inset). The additional radiation resulting from even an appreciable ozone reduction (Figure 1) is trivial when considered against the background of total solar UV. The enhanced solar radiation becomes important only if biological responses are much more sensitive to shorter than to longer wavelength radiation.

To incorporate the biological importance in expressing UV-B, a biological spectral weighting function (BSWF) is convoluted with spectral irradiance and integrated with respect to wavelength. The weighted radiation is usually expressed in units such as biologically effective W m^{-2} or effective J m^{-2} day^{-1}. The weighting function itself is dimensionless and is normalized to a reference wavelength, usually 300 nm. The same weighting can be applied to ultraviolet radiation from sources other than sunlight, such as from lamps used in experiments. A common abbreviation for the weighted radiation is UV-B_{BE}.

The increment of solar UV-B_{BE} resulting from ozone reduction, i.e. ΔUV-$B_{BE}/-\Delta O_3$, is called the radiation amplification factor (RAF). Because of the very specific manner in which solar UV-B changes with wavelength (Figure 1), BSWF must have particular characteristics in order to result in an appreciable RAF. As developed later, only BSWF that decrease very steeply with increasing wavelength will result in an appreciable RAF with ozone reduction. Therefore, biological responses that do not result in BSWF of this character should receive much less attention in the assessment of ozone reduction consequences. While the foregoing is well known and has been discussed in reviews (e.g. Caldwell 1981; Caldwell et al. 1986, 1989; Coohill 1989, 1991, 1992), it is not always fully appreciated.

Are weighting functions involved in other aspects of the UV-B assessment?

In addition to calculating RAF, BSWF are used in three other aspects of assessing plant response to stratospheric ozone reduction (Figure 2).

Latitudinal gradients of UV-B

A potentially steep latitudinal gradient of effective solar UV-B irradiance exists currently on the earth's surface – much steeper than that of total solar radiation, but only if BSWF used in evaluating solar UV-B_{BE} have certain characteristics. The natural latitudinal gradient of UV-B radiation serves as a basis for study of organism response to solar UV-B. For example, it has been used in the analysis of human skin cancer incidence and predictions of cancer incidence resulting from ozone reduction. Study of plant adaptation to UV-B radiation at different latitudes can also be instructive (e.g. Barnes et al. 1987; Caldwell et al. 1982; Robberecht et al. 1980). The relationship between BSWF and latitudinal changes in UV-B_{BE} will be portrayed later.

Comparing UV-B from lamps and the sun

Since spectral irradiance received from lamp systems commonly used for UV-B studies does not match that of solar irradiance (Caldwell & Flint 1995), it is only possible to draw comparisons by calculating UV-B_{BE} using BSWF. Characteristics of BSWF will play a large role in the amount of radiant flux that should be delivered by lamp systems in experiments designed to evaluate potential consequences of ozone reduction (Caldwell et al. 1986). Even if one demonstrates plant effects to be specifically elicited by a certain UV-B flux from lamps, this still does not necessarily denote its significance for the ozone reduction problem. Based on spectral criteria, the dose used in such experiments might be greatly over- or underestimated.

Evaluating solar UV attenuation experiments

Filtering materials used to attenuate solar UV in most experiments of this nature do not abruptly filter solar radiation as does ozone in the atmosphere. Therefore, analogous to experiments with lamps, BSWF are needed to evaluate the degree of effective solar UV attenuation. One might, for example, wish to equate partially attenuated solar UV at a low latitude with normal solar UV at a higher latitude.

A short history of plant action spectra used in assessing UV-B changes

The purpose of this section is to depict briefly how action spectra have been used for BSWF in assessing the ozone reduction problem. Two so-called generalized action spectra have been widely used in this field, a generalized plant action spectrum (Caldwell 1971) and a DNA-damage spectrum (Setlow 1974). Both are composite spectra, the former is based on several plant responses to UV while the latter is derived from several data sets of DNA lesions, both *in vivo* and *in vitro*, and is not restricted to plants. While both spectra are widely used and may continue to have utility in allowing workers to compare UV-B_{BE} in various experimental systems, they are limited in several respects. Furthermore, it is not reasonable to expect that a single action spectrum should represent the likely diverse responses of plants to UV-B, since many chromophores are suspected to be involved (Beggs et al. 1986; Bornman 1989; Caldwell 1981; Caldwell et al. 1986). The generalized plant action spectrum goes to zero at 313 nm, which happens to be a mercury vapor emission line commonly used in monochromators employed in experiments available at the time this spectrum was compiled. Even though the spectra used in this composite generally ceased to exhibit effects above 313 nm, in reality there were likely tails of lower order response at longer wavelengths. Such tails may or may not be significant depending on how abruptly they decline with increasing wavelength. (For example, the DNA-damage spectrum does not have this abrupt limit and the tail is taken to about 365 nm. However, this tail is sufficiently depressed at longer wavelengths that it is not particularly important when used as a BSWF for calculating RAF.

Why be concerned with the tails at longer wavelengths? Traditionally, analytic action spectra (as termed by Coohill 1991) used in photobiology to identify chromophores focus much more on fine structure and tend to be little concerned with tails. And with good reason, since this emphasis is most pertinent to identifying chromophores. However, for assessment purposes in the ozone reduction problem, the tails at longer wavelengths can become very important if they do not decline markedly with increasing wavelength since sunlight increases by orders of magnitude at longer wavelengths. Ozone reduction effectively influences only a narrow waveband (ca. 290–320 nm), as discussed above. However, rather than being constrained to this waveband, in which ozone reduction influences solar UV, the limits to the wavelength integral in calculating UV-B_{BE} should be set by where solar spectral irradiance or the BSWF decrease to such low levels that further contributions to the integral for UV-B_{BE} are negligible. This can be particularly important in calculating RAF. The sensitivity analyses discussed below indicate how BSWF whose slopes differ result in very disparate results for calculating RAF or the amount of UV that would be needed from lamps in plant experiments.

Sensitivity of biological spectral weighting functions in assessing UV-B

RAF and latitudinal gradients

Virtually all UV action spectra (and therefore, BSWF) possess the general characteristic of decreasing with increasing wavelength since the more energetic photons at shorter wavelengths are more photochemically effective. However, the slopes can differ consider-

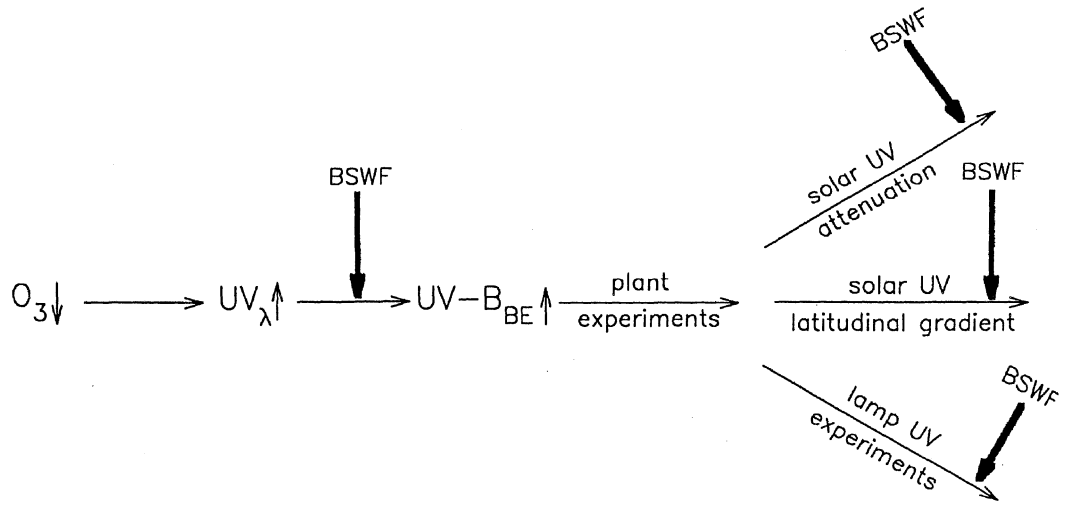

Figure 2. Scheme for analysis of the consequences of stratospheric ozone layer reduction for plants and how biological spectral weighting functions (BSWF) are needed. Ozone column reduction, $O_3 \downarrow$, causes an increase in solar ultraviolet spectral irradiance, $UV_\lambda \uparrow$, which results in an increase of biologically effective solar UV-B, $UV\text{-}B_{BE}$, according to a BSWF. Plant experiments can involve attenuating the existing solar UV, experiments with UV-emitting lamps (either with or without background solar radiation), or studies of plant response or adaptation along an existing latitudinal gradient of solar $UV\text{-}B_{BE}$. In all three lines of research, BSWF are usually needed to evaluate their meaningfulness with respect to ozone reduction.

ably. A comparison of three BSWF differing in slope is shown in Figure 3 and the implications of these differences in calculated RAF and latitudinal gradients in Figure 4. The relative increase of solar $UV\text{-}B_{BE}$ due to ozone depletion (RAF) and also the difference in solar $UV\text{-}B_{BE}$ with latitude (in the absence of ozone reduction) are computed using the three BSWF. With the first spectrum (Figure 4, top), there is little increase of $UV\text{-}B_{BE}$ due to ozone reduction and the $UV\text{-}B_{BE}$ at different latitudes is essentially the same (only in the Arctic at 67° is it somewhat lower). As BSWF are steeper, both the relative increase of $UV\text{-}B_{BE}$ resulting from ozone reduction and the latitudinal gradient become more appreciable.

Comparing lamp and solar UV-B_{BE}

In virtually all plant experiments involving lamp systems, the generalized plant action spectrum has been used to evaluate how much lamp UV is needed to simulate a given ozone reduction scenario (whether in growth chamber, greenhouse or field conditions). It is not generally appreciated, even by workers in this area, that very sizeable differences in lamp UV for plant experiments will result by using different BSWF. The discrepancies are least for experiments in the field in which lamp UV is used to supplement solar radiation and much greater under greenhouse or growth

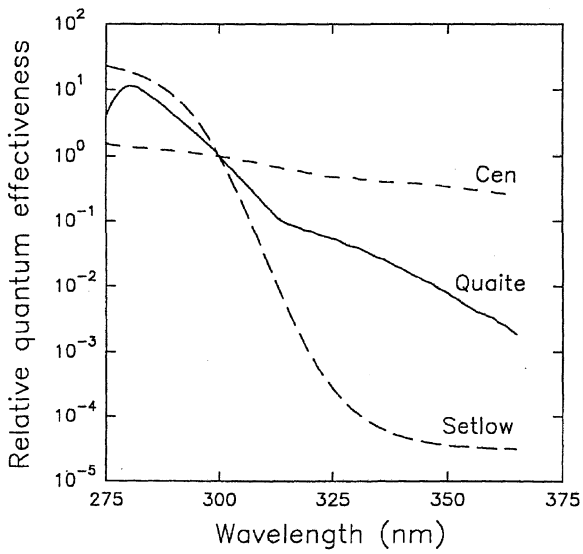

Figure 3. Three UV action spectra used as BSWF in the proposal for sensitivity analysis: The Cen & Björn (1994) spectrum for enhancement of ultraweak luminescence in leaves of *Brassica napus*, the Quaite et al. (1992) spectrum for DNA-dimer formation in *Medicago sativa* seedlings, the Setlow (1974) composite spectrum for DNA damage. All of these have been normalized at 300 nm.

chamber conditions (Caldwell et al. 1986). To demonstrate this with the most conservative case in a field experiment, the three BSWF of Figure 3 are used to

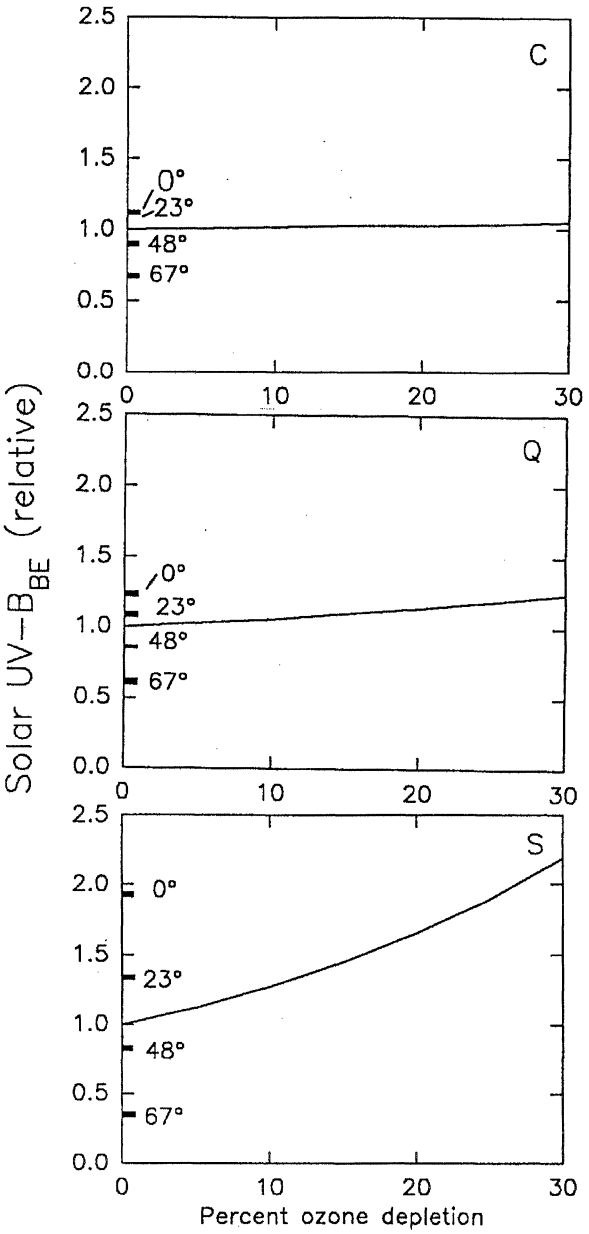

Figure 4. The relationship between ozone reduction and ΔUV-B calculated with the three BSWF of Figure 3 [top (C), Cen & Björn (1994); middle (Q), Quaite et al. (1992); bottom (S), Setlow (1974)]. These represent relative total integrated daily UV-B_{BE} on the summer solstice at 40° latitude. Also shown are the relative daily integrated UV-B_{BE} values for current ozone conditions at different latitudes at the time of year of maximum solar irradiation.

estimate how much UV from commonly used lamp systems will be needed to supplement solar radiation in an experiment designed to simulate 20% ozone reduction. Although it would appear that use of filtered UV

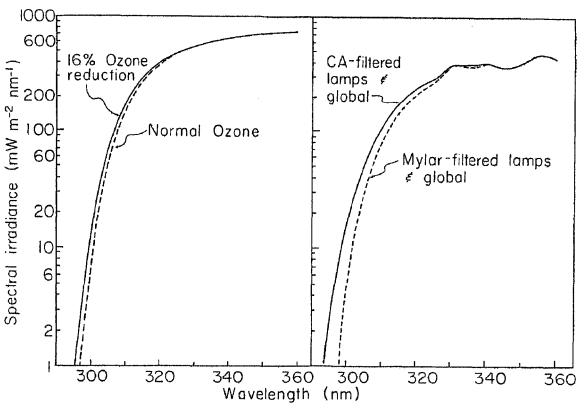

Figure 5. (left) Solar spectral global irradiance with normal ozone column thickness (dashed line) and with a 16% ozone column reduction (continuous line). (right) The UV spectral irradiance measured under lamp systems (with background solar global irradiation) commonly used in plant experiments to augment solar UV to simulate solar radiation with a reduced ozone column (continuous line) (fluorescent UV-B emitting lamps with a cellulose acetate filter to remove shorter UV-B and UV-C wavelengths). Also shown (dashed line) is radiation under a control lamp system (also with background solar irradiation) (the same lamps, but filtered with a Mylar film to remove all UV-B) (Adapted from Caldwell et al. 1986).

lamps to supplement solar radiation simulates ozone reduction rather well on a commonly used logarithmic plot (Figure 5), one notes that the lamps supply somewhat more shortwave, and less longwave, UV-B than would come from the sun with ozone reduction. Therefore, depending on the BSWF used to calculate the needed lamp UV supplement, very large differences emerge (Figure 6). With the steepest BSWF (Setlow DNA damage), the least lamp UV would be applied, but with the ultraweak luminescence BSWF, more than three-fold greater lamp UV would be needed. Thus, the resulting scenarios of ozone reduction can be greatly changed simply by using different BSWF. In growth chamber and greenhouse experiments these discrepancies are greater still, because the normal background solar UV is not present (Caldwell et al. 1986).

Solar UV attenuation experiments

Though much less frequently performed than lamp UV supplementation experiments, some plant research has involved exposing plants to current levels of solar UV attenuated to different degrees (Searles et al. 1995). Analogous to lamp UV experiments, the degree to which filters attenuate solar UV-B_{BE} is dependent on the BSWF used.

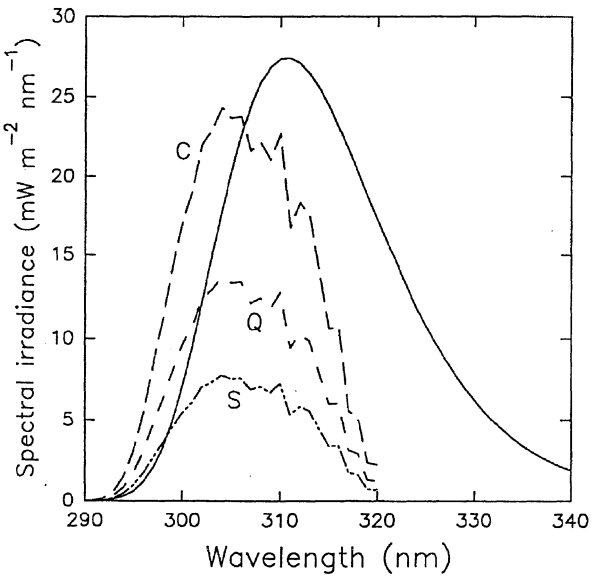

Figure 6. The difference in solar spectral irradiance, ΔI_λ, for a 20% ozone reduction as in Figure 1 (continuous line). The other lines represent the supplemental UV-B lamp spectral irradiation (unweighted) used to simulate the 20% ozone reduction using the three BSWF from Figure 3 to calculate the amount of supplement. (The lamps are filtered with cellulose acetate filter, as is normally done in such experiments – see Figure 5). (If a BSWF were perfectly flat, the total irradiance from the lamps would equal ΔI_λ, although the wavelength distributions would not be the same.)

Biological spectral weighting functions, traditional action spectra and problems of scaling

Clearly, BSWF play a central role in the assessment of biological consequences of stratospheric ozone reduction. There are numerous plant action spectra available, including a few reported in the last few years (e.g., two of the spectra in Figure 3). Unlike most of the earlier spectra, at least a few of the newer spectra involve intact leaves or seedlings. Can one simply use some of these as BSWF? Most of these spectra, as well as those used in the composite action spectra mentioned above, have been developed with monochromatic radiation under laboratory conditions. If it could be demonstrated that such action spectra are indeed relevant for plants growing for long periods under the full solar spectrum reaching the ground, these action spectra could be used as BSWF. The great challenge is to link biological spectral responses conducted in the laboratory (usually as action spectra with monochromatic radiation) to spectral responses of plants in the field where plants are exposed day after day to the full solar spectrum, the flux density of which is constantly changing. Time scales of exposure are vastly different – a matter of hours (or less) in the laboratory to weeks and even months in the field. Of course, many other environmental factors differ between laboratory and field conditions. In a more general context of global change (e.g., elevated CO_2, global warming, etc.) scaling is one of the greatest challenges in linking experiments and theory at different spatial and temporal scales (Ehleringer & Field 1993).

A good case has already been made that plant response to polychromatic radiation will be different than to monochromatic radiation, at least in terms of sensitivity. Interactions between UV-B and PFD (photosynthetic photon flux density, i.e., total photon flux in the 400–700 nm waveband) have been investigated over several years (e.g., Cen & Bornman 1990; Mirecki & Teramura 1984; Warner & Caldwell 1983), and many recent papers also address this topic of interactions between radiation at different wavebands (Kramer et al. 1992; Ensminger & Schäfer 1992; Kumagai & Sato 1992; Fernbach & Mohr 1992; Krizek et al. 1993, 1994; Wilson & Greenberg 1993; Adamse et al. 1994; Britz & Adamse 1994; Deckmyn et al. 1994; Caldwell et al. 1994; Takayanagi et al. 1994). Most of this work shows that at greater ratios of PFD/UV-B there is much less damage or effect of the UV-B than at lower PFD/UV-B ratios. Very little higher plant research addresses interactions between UV-A and UV-B or all three spectral ranges (UV-B, UV-A and PFD) (e.g., Middleton & Teramura 1993, 1994). In a field study Caldwell et al. (1994) showed that both PFD and UV-A could have pronounced mitigating influence on UV-B effects, but not in a simple additive manner.

However, an important question is not whether the UV-B sensitivity will be changed by the presence of UV-A and PFD, but whether longer wavelength radiation leads to a different spectral response than occurs with monochromatic radiation (Figure 7). For example, one result that could emerge is that although sensitivity to UV-B is reduced by simultaneous exposure to UV-A and PFD, the BSWF could become steeper (which would lead to greater RAF and have implications for plant experiments as well).

Bottom-up mechanistic scaling

Theoretically, if all the photobiological mechanisms, their wavelength dependencies, their sensitivities and their interactions were known, one could calculate how different spectral combinations of solar radiation

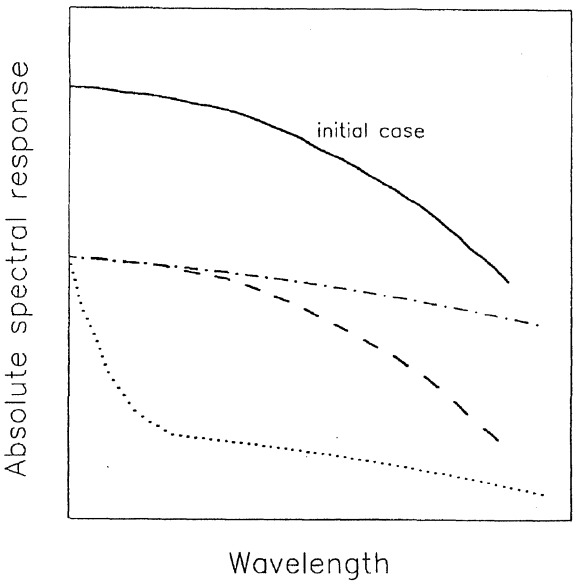

Figure 7. Hypothetical changes in absolute plant sensitivity to UV spectral irradiance due to treatments such as simultaneous application of UV-A or PFD. Reduced sensitivity is generally expected, however, the question of interest is whether the relative spectral sensitivity changes. The heavy dashed line represents reduced sensitivity from the initial case (continuous line), but no change in relative spectral response. The othr two lines represent reduced sensitivity and either flattening (dash-dot line) or steepening (dotted line) of the spectral response.

would affect plants in nature. Although much is known about UV photobiology, predicting plant response in polychromatic radiation is not possible. For phenomena such as plant growth and morphological response to UV, several basic chromophores interact: these responses may involve DNA damage (Beggs et al. 1986; Pang & Hays 1991; Quaite et al. 1992), free radical formation (Cen & Björn 1994; Panagopoulos et al. 1990), photoreceptors such as flavin (Ballaré et al. 1991, 1995; Wilson & Greenberg 1993), or other compounds. The manner in which UV-A and PFD might interact with UV-B can also involve several possibilities. For example, UV-A and blue light can drive photolyase enzymes to repair at least two common lesions of DNA caused by UV-B (Beggs et al. 1986; Jagger et al. 1969) in the process of photoreactivation (PR); UV-B can induce PR and other DNA repair systems (Menezes & Tyrrell 1982; Pang & Hays 1991), but phytochrome control has also been shown (Langer & Wellmann 1990); UV-A can cause growth delay (at least in bacteria) which allows more time for dark DNA repair systems to operate (Jagger 1981), but UV-B and UV-A at high flux rates can damage DNA repair systems (Buchholz et al. 1995; Webb 1977). If free radical damage is involved, UV-A can stimulate carotenoid quenching of free radicals (Webb 1977). Kramer et al. (1992) suggested that polyamine accumulation may be important in ameliorating UV-B damage, and greater PFD can lead to increased polyamines. The blue light receptor also appears to be important in mitigating UV-B damage (Adamse et al. 1994). Interactions between the UV-B, UV-A/blue, and phytochrome receptors have also been demonstrated (Beggs et al. 1986; Gaba & Black 1987; Mohr 1986). Thus, a quantitative prediction of plant spectral response over periods of weeks in sunlight is not likely to be forthcoming based purely on photobiological mechanisms.

Top-down development of BSWF using polychromatic radiation

The spectral response of plants can be assessed by exposing plants to several combinations of polychromatic radiation (often using the same radiation at longer wavelengths and then sequentially adding increments of shorter wavelength radiation). Based on the biological fluence-response slopes for the different polychromatic radiation combinations, an action spectrum, or BSWF, can be deconvoluted (Rundel 1983). A few plant UV action spectra have been developed in this manner (e.g., Caldwell et al. 1986, Steinmüller 1986). This approach has the advantage of simultaneously exposing biological systems to radiation at several wavelengths while assessing spectral dependency. However, it is necessary to have a sufficient number of radiation combinations with similar relative wavelength distributions that span the complete range in which the deconvoluted spectrum will have a signal. Defining the longwave tails of spectra can be problematic, and several possible spectra can emerge from the same data set (Rundel 1983). In growth chamber or field conditions, the appropriate number and type of polychromatic radiation combinations for successful deconvolution is usually not practical. Furthermore, even if sufficient combinations are feasible, these tests are still usually developed over short periods of time with high flux densities of radiation in the laboratory. Despite this, attempts to develop action spectra in the field environment using various lamp and filter combinations are apparently meeting with some success (Holmes, personal communication).

Scaling using an intermediate approach

In general, scaling is at best a compromise among several approaches and clear rules do not exist (Ehleringer & Field 1993). For scaling BSWF, we suggest an iterative, compromise approach using three venues of experimentation, rather than to use only monochromatic radiation in the laboratory in classical action spectra development or to depend entirely on use of polychromatic radiation in the field. Plant responses to monochromatic and polychromatic radiation combinations in laboratory, growth chamber and field environments are used to develop ecologically relevant BSWF (Flint & Caldwell 1996). Rather than use a physiological or molecular-level manifestation of UV irradiation as the parameter for action spectrum and BSWF development, we have chosen whole-plant morphological changes. Interesting UV action spectra have been recently developed using DNA cyclobutane dimer production (Quaite et al. 1992) and ultraweak luminescence (an indicator of free radical formation) (Cen & Björn 1994). However, the link between these molecular and physiological indicators and whole-plant performance has not been established.

Field tests exposing plants to different polychromatic combinations of UV-B and UV-A, showed that in addition to an inhibiting effect of UV-B on oat stem elongation, UV-A had both some inhibiting effect itself and also a mitigating influence on the UV-B inhibition (Flint & Caldwell 1996). This obviously requires more resolution. We feel that particular emphasis needs to be placed on the transition regions between the UV-B and UV-A and on interactions of UV-B, UV-A and PFD in determining the slope of BSWF into the UV-A region. These are important because it is this part of the solar spectrum that is increasing by orders of magnitude with increasing wavelength. This is also the general waveband where differences in BSWF have a great influence on how they are applied in at least four aspects of the ozone reduction problem – see Figures 2–6.

The challenge in this scaling exercise arises if predictions from the laboratory measurements do not predict responses to polychromatic radiation. If this happens, since BSWF are intended for use in assessing the ozone reduction problem with polychromatic solar radiation in the field, greater credibility must be given to the results with polychromatic radiation, especially under field conditions. To further resolve the nature of the BSWF in polychromatic radiation, adjustments in BSWF need to be made and then tested with a new set of polychromatic conditions. Obviously, in all phases of such research, assessment of data and refinement of BSWF takes on an iterative character.

Implications for assessing stratospheric ozone reduction

Realistic BSWF provide the biological research community with more relevant estimates of RAF and functions to simulate different scenarios of ozone reduction. In addition, new retrospective assessments of previous research using lamps and filters can be made. For example, if BSWF turn out to be less steep than originally thought, RAF and latitudinal gradients would be also accordingly smaller. However, the less steep BSWF also means that in earlier experiments, the investigators were applying less effective UV-B than they anticipated at the time. For example, if the DNA-damage weighted spectrum of Setlow (Figure 6, Curve S) were used in a such an experiment and it was later determined that the Quaite et al. DNA-damage spectrum (Figure 6, Curve Q) was more appropriate, the amount of supplemental UV-B supplied would have been 50% of what was intended. The degree of underestimated UV-B supplementation is generally a more sensitive function of BSWF steepness than is the RAF (Caldwell et al. 1986). Thus, the retrospective analysis in this scenario might lead to the conclusion that much less ozone reduction had been simulated in particular experiments than claimed at the time.

Acknowledgements

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Pedunculate oak *Quercus robur* exposed to elevated UV-B radiation, Monks Wood, Cambridgeshire, UK. (Photograph: A. R. McLeod)

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Outdoor supplementation systems for studies of the effects of increased UV-B radiation

A.R. McLeod
Institute of Terrestrial Ecology, Monks Wood, Abbots Ripton, Huntingdon, PE17 2LS, UK

Key words: Action spectra, Radiation amplification factor, Ultraviolet-B radiation, UV-B exposure systems

Abstract

Studies of the effects of increases in ultraviolet-B (UV-B) radiation on plants and terrestrial ecosystems have been undertaken using a variety of methods including: controlled-environment cabinets, glasshouses, outdoor filtration and outdoor supplementation using fluorescent UV-B lamps. Outdoor supplementation systems provide a method of study which creates only small alterations to the microclimate and the number of such studies has increased during the past 3 years. These supplementation systems differ in their methods of operation, equipment, UV-B exposure regime and experimental design. This essay surveys the systems currently developed, considers problems associated with their use and discusses these in relation to the interpretation of biological effects.

Introduction

A wide range of experimental methods have been used to provide information on the responses of plants to increases in UV-B radiation that would result from stratospheric ozone depletion. These have included exposing plants to UV-B from artificial lamps in controlled environment studies (e.g. Nouchi 1993), in glasshouses (e.g. Sullivan & Teramura 1988), and outdoor filtration using plastic (Rozema et al. 1995) or an ozonized air layer (Tevini et al. 1990). Studies inside enclosures have greatly increased knowledge of mechanisms for UV-B effects but do not simulate the microclimate and ambient radiation environment of the field. Neither do they enable naturally occurring interactions to be observed between different organisms and trophic levels. Studies have therefore been undertaken in the field using fluorescent UV-B lamps to simulate increases in solar UV-B radiation (e.g. Caldwell et al. 1983; Teramura et al. 1990). Arrays of fluorescent lamps have been suspended above plants to maintain measured levels of UV-B, either switched on for a fixed period, or with a modulated output to provide a controlled level of exposure. Such outdoor supplementation systems have now been established for over 20 different studies worldwide (Tables 1 and 2) and many differ notably in their method of operation, UV-B exposure regime and experimental design. The spectral composition of ultraviolet radiation from lamps does not precisely match the spectral changes caused by ozone depletion (Björn & Teramura 1993; Sullivan et al. 1994) and this necessitates the use of biological weighting functions or action spectra in order to relate experimental treatments to a particular level of ozone depletion. Outdoor supplementation systems, like other UV-B exposure methods, require the use of lamp filters and calculation of the UV-B dose. This review aims to examine the design and operation of outdoor supplementation systems, to consider their problems and to discuss these in relation to the interpretation of biological effects.

Technical performance

Lamps and filters

Fluorescent UV-B lamps used in field supplementation studies operate on the principle of an electric discharge in argon containing mercury vapour. The alternating electric current, limited by a choke, stimulates the production of shortwave UV which is converted to the

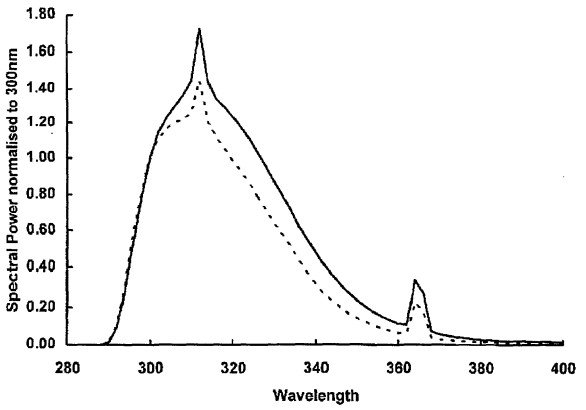

Figure 1. The relative spectral power of Q-Panel UVB-313 (—) and Philips TL40/12 (– – –) fluorescent lamps filtered through cellulose diacetate and normalised at 300 nm.

observed lamp emission by a fluorescent "phosphor" layer on the inner surface of the glass. The wavelengths produced by fluorescent lamps are dependent upon the nature of the phosphor coating and the transmission of the glass envelope. Although many lamp types have been used for UV-B studies (see e.g. Gehrke et al. 1996; Nouchi 1993; Nouchi & Kobayashi 1995; Sisson & Caldwell 1975; Thimijan et al. 1978) the two most frequently used lamps in recent outdoor supplementation studies are the Q-Panel UVB-313 (The Q-Panel Company, Cleveland, OH, USA) and the Philips TL40W/12 (Philips Lighting, Croydon, UK). Until 1994, the cathode filaments at each end of a fluorescent lamp, necessary to release electrons to start the current, operated at a different voltage in North America and Europe. However, both the UVB-313 and TL40/12 are now available with the same cathode voltage and recent supplies are interchangeable electrically. The spectral emission of the two types is different. When used with a cellulose diacetate filter (see below) the UVB-313 has a lower ratio of UV-A (315-400 nm) to UV-B (280-315 nm)(Figure 1) being 0.78 for the UVB-313 and 0.99 for the TL40/12 in unweighted units of energy (W m^{-2}). When lamp output is normalised to provide the same UV-B dose, weighted with the plant action spectrum using the equation of Green et al. (1974), the UVB-313 lamps provide 36% more unweighted UV-A. This difference should be borne in mind when establishing a system and interpreting published data.

The radiant flux from fluorescent lamps is dependent upon temperature. Björn & Teramura (1993) provided an example of radiant flux changes with temperature and this effect should be considered when planning field experiments particularly during the winter months. Low temperature may also affect the actual starting of the lamps and the choice of the type of electrical system described below. A new design recently developed in the USA (Charles Ashurst, pers. comm.) can provide reliable lamp starting at temperatures down to –25 °C.

The spectral output of fluorescent UV-B lamps includes wavelengths in the UV-C range (<280 nm) which do not occur at the earth's surface due to atmospheric absorption of solar UV-C by oxygen and ozone. Consequently, these unwanted wavelengths must be eliminated from experiments on changes in UV-B arising from stratospheric ozone depletion. This has typically been undertaken in field studies using filters of cellulose diacetate (CA) which does not transmit wavelengths below 280 nm (e.g. Caldwell et al. 1983). Fluorescent UV-B lamps also emit some UV-A wavelengths which are not modified in the solar spectrum by ozone depletion. These have also been taken into account in many experiments by using additional 'control' lamp arrays with different plastic filters, usually polyester (PE) (e.g. Mylar (various types), DuPont Co., DE, USA) (e.g. Sisson & Caldwell 1975) but sometimes also window glass (e.g. Johanson et al. 1995a, b). These filters of polyester or window glass absorb all wavelengths below about 318 and 320 nm respectively (Gehrke et al. 1996; Nouchi & Kobayashi 1995). The UV-B effect is then determined as the difference in response beneath UV-B 'treatment' lamp arrays and 'control' lamp arrays. It has often been suggested (e.g. Petropoulou et al. 1995) that the UV-A contribution from lamps is a small fraction of ambient UV-A exposure and may therefore be considered to be negligible. Perhaps for this reason there has been a tendency for some recent studies to omit PE-filtered 'UV-A control' treatments and to use only unenergised lamps as a 'control' against which to compare the UV-B treatment effects (Tables 1 and 2). Only the Monks Wood exposure system (described below) and the system of Zipoli & Grifoni (1994) have utilized CA-filtered UV-B treatments, and both PE-filtered UV-A controls as well as unenergised lamp controls (see Figure 3).

The ageing and changing spectral characteristics of lamps and filters of cellulose diacetate and polyester have been well described by Adamse & Britz (1992), Krizek et al. (1990), Middleton & Teramura (1993) and Sisson & Caldwell (1975). The importance of these effects for simple switched or 'square-wave' treatments is noted below. Certain investigations have used UV-B transmitting Plexiglas (Röhm 2458,

Röhm GmbH, Germany) to supplement CA- filters (e.g. Johanson et al. 1995a, b) while in Japan, where CA was difficult to obtain, UV-B treatment lamps have been filtered using polyvinyl chloride film (Nouchi 1993). Cellulose triacetate has also been used as a filter (Caldwell et al. 1994) and transmits more UV-B than cellulose diacetate (C. Ashurst, pers. com.). One particular outdoor system at Cambridge, UK, designed to determine polychromatic action spectra in the field, has used a number of different lamp types and glass filter combinations to produce a range of treatments which differ in spectral bandwidth (M.G. Holmes, pers. comm.). The importance of UV-A wavelengths in photorepair, photoprotection and damage processes (Caldwell 1984), and the demonstrated effect of UV-A in ameliorating UV-B damage (Caldwell et al. 1994) should be borne in mind when interpreting published work using the variety of different filter types in outdoor supplementation systems.

Lamp operating frequency

Fluorescent lamps can typically be operated at the low frequency (LF) of the mains electricity supply (e.g. 50/60 Hz) and also at a much higher frequency (HF) (e.g. 30 kHz). The light output from lamps decreases towards zero twice every cycle so that a LF-operated lamp flickers at 100/120 Hz. The LF flicker is barely detectable by the human eye but may influence insect behaviour (Shields 1984) and consequently be of considerable importance in field studies. High frequency lamp operation may consequently be preferred as flicker is greatly reduced. However, until recently, HF lamp operation required electrical cables of no more than a few metres between the lamp and control equipment. This influences choice for field experiments where minimal shading of solar radiation is also desirable. The choice of high or low frequency equipment is particularly important in modulated systems as described below.

Shading and spacing

Arrays of fluorescent lamps have been suspended above plants in a variety of patterns. Equally spaced parallel lamps have often been used (e.g. Caldwell et al. 1983, Nouchi & Kobayashi 1995) but do not provide the largest area of uniform irradiance. Alternative spacing with the tubes closer at the ends of arrays have been used (e.g. Yu et al. 1991) to improve spatial uniformity. Gehrke et al. (1996) used four small tubes which formed the sides of a square. Björn & Teramura (1993) examined the theoretical aspects of lamp patterns and provided a computer programme for calculating the distribution of light from arrays of fluorescent lamps. They also pointed out that different lamp patterns are necessary in order to optimise either the irradiance field or the fluence rate fields. Field supplementation studies have so far operated and reported in terms of irradiance and not fluence rate.

The irradiance field can also be optimized by differential shading along the lamp length. An unshaded lamp array produces the highest irradiance in the centre (e.g. Zipoli et al. 1994) with gradients towards the lamp ends. This gradient can be minimized by shading out the central portion of the lamps (e.g. Johanson et al. 1995a,b). Partial shading of entire lamps may also be necessary in order to modify the maximum irradiance from the array in order to achieve a suitable operating range throughout the year as described below.

Switched or 'square-wave' systems

Although one of the first outdoor supplementation systems utilized lamp modulation (Caldwell et al. 1983) many of the field experiments that have taken place have had a simple on/off or so-called 'square-wave' treatment in which the lamps were turned on at full output for a specific period centred around solar noon (Table 1). The resulting UV-B treatment has been calculated from measurements with a spectroradiometer and often related to the theoretical ambient UV-B exposure for the site calculated for clear sky conditions using a mathematical model (e.g. Björn & Murphy 1985; Green et al. 1980) and expressed in terms of an equivalent ozone depletion based upon annual means and a chosen action spectrum (e.g. Johanson et al. 1995a, b). In some instances the diurnal pattern of exposure has been improved by switching on alternate lamps in two stages. The seasonal pattern has also been adjusted by varying the duration of exposure every few weeks (e.g. Johanson et al. 1995). Most studies have made no adjustment (manual or automatic) to the lamp output to compensate for overcast days versus clear sky conditions, so that the values of the ratio UV-B:UV-A:PAR varies not only as a result of the "square-wave" exposure, but between days with different cloud conditions. In one study an estimate of the effect of cloud cover on the equivalent ozone depletion was made (Johanson et al. 1995b). The significance of the UV-B:UV-A:PAR ratio has often been noted

Table 1. Switched or square-wave outdoor supplementation systems for studies of increased UV-B radiation

Reference	Lamp Type	High/low frequency	Filters		Species studied	Description
			Treatment	Control		
Johanson et al. (1995a,b) Gehrke et al. (1995)	Q-Panel UVB-313	LF	CA plus Plexiglas (Röhm 2458)	Window glass	Subarctic heathland. Dwarf shrubs including decomposition	Timer controlled in two steps to simulate 15% O_3 depeltion at Abisko, Sweden calculated using the model of Björn & Murphy (1985). Duration adjusted biweekly. Also with CO_2 in small open-top chambers
Gehrke et al. (1996)	Philips TL/4W	LF	CA	PE 'Mylar-S'	Peatland ecosystem dominated by *Sphagnum fuscum*	Timer controlled to simulate 15% O_3 depletion at Abisko, Sweden calculated using the model of Björn & Murphy (1985). Duration adjusted biweekly.
Teramura et al. (1990)	Westing-house FS-40	LF	CA	PE 'Mylar'	*Glycine max*	Timer controlled in 2 steps for six hours daily to simulate 16 and 25% O_3 depletion at Beltsville, USA calculated using the model of Green et al. (1980). Different dose by varying distance from lamps.
Sullivan & Teramura (1992)	Q-Panel UVB-313	LF	CA	PE	*Pinus taeda*	Timer controlled for 6 h per day to simulate 16 and 25% O_3 depletion at Beltsville, USA calculated using the
Sullivan et al. (1994)					*Liquidambar styraciflua*	model of Green et al. (1980). Different dose by varying distance from lamps.
Booker et al. (1992)	Q-Panel UVB-313	LF	CA	PE	System description	Inside PVC open-top chambers. Timer controlled. Flux and duration adjusted biweekly. Manual intervention if overcast.
Fiscus et al. (1994)					*Glycine max*	Three year study with gaseous O_3 interaction simulating a 15, 20 and 35% O_3 depletion at Beltsville, USA calculated using the model of Green et al. (1980).
Petropoulou et al. (1995)	Philips TL40/12	LF	CA	LNE	Mediterranean pines	Timer controlled exposure in 2 steps to simulate 15% O_3 depletion at Patras, Greece calculated using model of Björn & Murphy (1985) Daily exposure duration adjusted monthly.

Table 1. Continued

Reference	Lamp Type	High/low frequency	Filters		Species studied	Description
			Treatment	Control		
H. Ro-Poulsen (pers. comm.) Zeuthen (1995)	Philips TL40/12	LF	CA	Window glass	*Fagus sylvatica* *Quercus robur*	Field study near Copenhagen, Denmark on 6-year old, 1m high trees. Simulated 15 & 30% O_3 depletion using model of Björn & Murphy (1985).
H. Ro-Poulsen (pers. comm.)	Philips TL20/12	LF	CA	Window glass	*Cladonia mitis*	Field study at Qaanaaq, Greenland. Simulated 15 & 30% O_3 depletion using model of Björn & Murphy (1985)
Dai et al. (1995) Olszyk et al. (1996)	Q-Panel UVB-313	LF	CA	PE 'Mylar'	Rice (*Oryza sativa*) cultivars	Field study at IRRI, Phillipines using 6 hour switched treatment to simulate 20% O_3 depletion.
Rozema et al. (1995)	Philips TL40/12	HF	CA	PE 'Mylar'	Crop species Species of natural vegetation	Switched exposures. Modulated system also under development.
Zipoli & Grifoni (1995) Antonelli et al.(1996)	Q-panel UVB-313	HF	CA	PE and LNE	System description and *Phaseolus vulgaris*	Manually modulated treatment for 7 h per day simulating 20% O_3 depletion at Pistoia, Italy calculated using the model of Björn & Murphy (1985.)
Paul, N. D. (pers. comm.)	Philips TL40/12	LF	CA	PE 'Mylar'	Fungal pathogens of crops	Timer controlled exposure to study wheat pathogen *Septoria tritici*.

LF, low frequency (50/60 Hz); HF, high frequency; PE, polyester; CA, cellulose diacetate; LNE, lamps not energized.

(Adamse & Britz 1992b; Caldwell et al. 1994) particularly with respect to the ameliorating effect of PAR and UV-A on UV-B damage resulting from photorepair. The square-wave nature of treatments may have significance for these ratios and UV-B effects and warrants further investigation.

One of the major disadvantages of unmodulated field exposure systems is the need for frequent changing of filters (typically CA and PE) that is necessary in order to avoid serious effects of aging on UV-B transmission and the calculation of exposure. The effects of ageing and shifts in the spectral transmission of CA and PE have been described by Adamse & Britz (1992) and by Middleton & Teramura (1993). Some studies have used window glass as an alternative 'control' filter instead of PE and UV-B transmitting perspex has been used together with CA (e.g. Johanson et al. 1995a). However, in general the prior solarization of CA for 8 hours to achieve a more stable UV-B transmission and the regular filter changes required after every 50 hours of exposure necessitate a considerable work load and remains a serious disadvantage of the 'square-wave' treatment protocol.

Modulated systems

Modulated outdoor systems are capable of varying the radiant flux from the UV-B lamps to supply a treatment dose that is proportional to ambient levels. The first such modulated system was employed by Caldwell et al. (1983) followed by the design of Yu et al. (1991) and subsequently by many others (Table 2). These systems all employ a closed-loop feedback control either effected using electronics or by control software in a computer. The UV-B level is monitored both below a treatment array and also at a suitable location for an ambient measurement. The control system continuously adjusts lamp output to maintain the chosen elevation of the treatment exposure above the measured ambient value. In this way local changes in irradiance caused by the weather, diurnal and seasonal variations are accommodated by the control system. The ratios of UV-B: UV-A: PAR are maintained relatively constant compared to those achieved with a switched system.

There are a number of variations of the methods of lamp dimming for modulated control but these are typically phase-angle control for LF systems and pulse width modulation for HF systems. The 100/120 Hz flicker of LF systems is particularly apparent at low lamp output whereas no visible flicker is seen with HF systems even at their lowest output level. The operating frequency also influences the range of lamp output. The first modulated system (Caldwell et al. 1983) operated over a 25–100% maximum output range. Until recently, commercially available dimming systems typically only reduce output to 5% of maximum for HF systems or 1% for LF systems. The range of LF systems can often be extended by the use of a base-load resistor in parallel to the lamps to achieve 0.3–100% of maximum output. Only the YMT-UIMS modulated system (Yu et al. 1991; Björn & Teramura 1993) has reported the capability of a wide dynamic range of 1:300. Recently, commercially available HF dimming systems have become available operating over a 1–100% range and are being utilized in a number of systems whilst a new HF system (C. Ashurst, pers. comm.) has a specially designed control circuit to optimise performance for field studies.

The dynamic range is particularly important for outdoor supplementation studies in order to permit maintenance of a given elevation above ambient levels from sunrise to sunset (e.g. Yu et al. 1991). A more limited output range requires that a certain threshold ambient UV-B level is crossed before lamps are turned on in order to permit a correct supplementation at midday under clear skies and to avoid an excessively elevated dose at the ends of the photoperiod. Such limitations are rarely reported but can be observed in the data for the Monks Wood supplementation system (see below). In order to optimise the range of control for experiments that extend throughout the year it is also necessary to apply shading to the lamps (e.g. perforated mesh) during the winter period and to adjust the threshold at which tubes turn on and off.

Sensors

The use of a modulated lamp system that provides a UV-B irradiance that is related to short-term fluctuations in ambient UV-B necessitates the use of a broad-band sensor to monitor both the ambient and treatment levels. A range of sensor types have been used in such experiments (see Table 2). A key feature of sensor choice is spectral weighting. Many commercially available broadband UV-B sensors have a spectral response which is unrelated to biological weighting functions and include a significant response to UV-A wavelengths, which may strongly limit the detection of changes in UV-B levels. The biologically weighted broad-band sensors that have been chosen fall into 2 groups: those weighted with the CIE erythemal

Table 2. Modulated outdoor supplementation systems for studies of increased UV-B radiation

Reference	Lamp type	High/low frequency	Filters		Sensor type	Species studied/experimental details
			Treatment	Control		
Caldwell et al. (1983)	Westing -house FS-40	HF	CA	PE 'Mylar-D'	Filtered Hamamatsu UV photodiodes	Dynamic range of modulation 50:1. R-B meter measured ambient UV-B.
Flint et al. (1985)				No lamp control in *V. faba*	attached to master lamps.	Study of *Vicia faba* stimulating 6 and 32% O_3 depletion calculated using the model of Green et al. (1980)
Caldwell et al. (1994)	Q-Panel UVB-313 & UVB-351	HF	Cellulose triacetate	PE	As above	*Glycine max*. Four experiments using different lamps and filter around the lamps and the lamp frames to vary the ratios of UV-B:UV-A:PAR. UV-B exposure simulating 36% O_3 depletion at Logan, USA calculated using the model of Green et al. (1980).
Barnes et al. (1994) Barnes et al. (1995)	Q-Panel UVB-313	HF	CA	PE	As above	Mixtures and monocultures of *Triticum aestivum* and *Avena fatua*. Dose equivalent to different O_3 depletions calculated for Logan, USA using the model of Green et al. (1980)
Yu et al. (1991)	Q-Panel UVB-313	LF	CA	PE 'Mylar-S'	YMT, Interscience Tech, MD, USA	Dynamic range of modulation 300:1. Sensor response similar to plant plant action spectrum (Caldwell 1971).
Sullivan et al. (1994)	Q-Panel UVB-313	LF	CA	PE 'Mylar-S'	YMT As above	Comparison of square-wave and modulated exposure of *Glycine max* at Beltsville, USA.
Mepsted et al. (1996)	Philips TL40/12	LF	CA	LNE	BW-100, Vital Tech, Ontario, Canada. UV-B	Study of *Pisum sativum* at Wellesbourne, UK. Simulated 15% O_3 depletion calculated using model of Björn & Murphy (1985) and following seasonal variations in the O_3 column. Sensor cross-calibrated to PAS.

Table 2. Continued

Reference	Lamp type	High/low frequency	Filters		Sensor type	Species studied/experimental details
			Treatment	Control		
Anonymous (1995)	Philips TL40/12	LF	CA	LNE	BW-100. UV-B version as above	Field study of limestone grassland at Buxton, UK using model of Björn As above & Murphy (1985) to simulate 15, 20, 25 & 30% O_3 depletion and following seasonal variations variations in the O_3 column. Sensor cross-calibrated to PAS.
Campbell et al. (1995)	Philips TL40/12	HF	CA	LNE	BW-100 UV-B version As above	Simulation of six O_3 depletion levels (0–25%). Dynamic range of modulation 100:1. 100:1.
N.D. Paul (pers. comm.)	Philips TL40/12	LF	CA	LNE	BW-100, UV-B version As above	Decomposition study of *Calluna vulgaris* and *Rubus chamaemorus* at Lancaster, UK. Simulated 15% O_3 depletion calculated using model of Björn and Murphy (1985) and following seasonal variations in the O_3 column. Sensor cross-calibrated to PAS.
Coop et al. (1995)	Philips TL40/12	LF	CA	LNE	BW-100, UV-B version As above	Study at Lancaster, UK of *Calluna vulgaris* and *Vaccinium myrtillus* in teflon tunnlels to permit CO_2 interaction studies. Simulated 0, 15 and 25% O_3 depletion calculated using model of Björn & Murphy (1985).
Nouchi & Kobayashi	Philips F40UVB	LF	CA	PE 'Mylar-D'	MS-210D Eiko Seiki Japan	Rice cultivars. Modulated increase in ambient UV-B to 2.67 times measured ambient. Sensor response similar to DNA action spectrum.
Newsham et al. (1996)	Q-Panel UVB-313	LF	CA	PE 'Mylar' and LNE	BW-100 CIE erythemal version	Modulated 30% increase above ambient UV-B. Study of *Hyacinthoides non-scriptus, Anemone nemorosa* and *Quercus robur* at Monks Wood, UK.

LF, low frequency (50/60 Hz); HF, high frequency; PE, polyester; CA, cellulose diacetate; LNE, lamps not energized.

response (McKinley & Diffey 1987) e.g. McLeod et al. (1996), Zipoli et al. (1994) and those with a weighting which approximates to the plant action spectrum (PAS) (Green et al. 1974) e.g. Anonymous (1995), Campbell et al. (1995), Mepsted et al. (1996) (Table 2). One study (Nouchi & Kobayashi 1995) has used a sensor stated to approximate to a DNA action spectrum. As sensor responses often do not perfectly match the action spectrum (e.g. PAS), periodic calibration against a spectroradiometer may be necessary (e.g. Mepsted et al. 1996) and this calibration assumes no significant spectral change in ambient UV-B between clear sky and overcast conditions during each calibration period.

Action spectra and radiation amplification factors

Examples of action spectra used as biological weighting functions for plant response are shown in Figure 2A. The generalised plant action spectrum (PAS) of Caldwell (1971) has been characterized by two different equations: that of Green et al. (1974) and also that of Thimijan et al. (1978). Both have been used to quantify UV-B exposures from outdoor supplementation systems, typically using an empirical model (e.g. Björn & Murphy 1985; Green et al. 1974;) to determine an appropriate dose for a particular level of ozone depletion.

Also shown is a function determined for the response of seedling growth by Steinmuller (1986) which notably shows biological effects at UV-A wavelengths. These action spectra can be utilized together with empirical models to calculate the radiation amplification factor (Madronich et al. 1995), which relates a level of ozone depletion to an equivalent percentage increase in UV-B, weighted by the chosen action spectrum. Annual changes in RAF's in the UK using the PAS according to Green et al. (1974) have been examined by (Paul 1996). Figure 2B shows the pattern of annual changes in RAF, calculated using the power rule (Madronich et al. 1995), for 55° N and a constant 15% ozone depletion using the model of Björn & Murphy (1985). The difference in the absolute values and the annual pattern between the two different mathematical fits to the PAS is notable and should be considered when evaluating biological results in relation to UV-B dose and ozone depletion. It is also important to note that the action spectra with a response component extending into the UV-A tend to have lower RAF's and a less pronounced annual variation. The choice of a broad-band sensor, its response spectrum and the timing and duration of an experiment can all profoundly modify the UV-B treatment elevation in outdoor supplementation studies.

Experimental design

The experimental layout of outdoor supplementation systems places intrinsic constraints on statistical analysis and the ability to detect UV-B effects with confidence. Field experimental design and analysis has often been confounded by inappropriate replication or 'pseudoreplication' of samples (Hurlbert 1993). The true level of replication for correct statistical analysis is based upon the treatment or lamp array replication and not on the number of pots or plants beneath each rack. Thus, four treatment arrays and four control arrays each irradiating 30 plants or pots beneath has a replicate number of four and the analysis must be based upon the eight means (or other statistic) for each array and not on the 240 values for each pot or plant. Unfortunately, pseudoreplication is not infrequent in the analysis of biological field experiments, including UV-B studies (e.g. Deckmyn & Impens 1995; Petropoulou et al. 1995).

Field experiments, more so than controlled environment facilities, may be subject to variation in response parameters with a spatial component or a feature of the exposure equipment which may confound the detection of treatment effects if incorrect statistics are applied. For example, few studies replicate the broad band-sensor used to modulate lamp output and the replicated lamp arrays are usually 'slaves' of one 'master' lamp array (e.g. Mepsted et al. 1996; Newsham et al. 1996). It is rarely established (and unlikely) that separate dimming systems will produce the same lamp output over the entire control range. Independent sensors under each replicate treatment array requires regular cross-calibration of sensors. An alternative method is being able to adjust the output of individual lamps in each array and hence to balance arrays, as achieved by the YMT system of Yu et al. (1991).

In order to reduce confounding variation in irradiance from lamp arrays there are also other system configurations that may be adopted. For example, Nouchi & Kobayashi (1995) utilized separate dimming systems for the control and treatment arrays whereas McLeod et al. (1996) (see Figure 3) utilized one common dimmer for each pair of treated and control arrays.

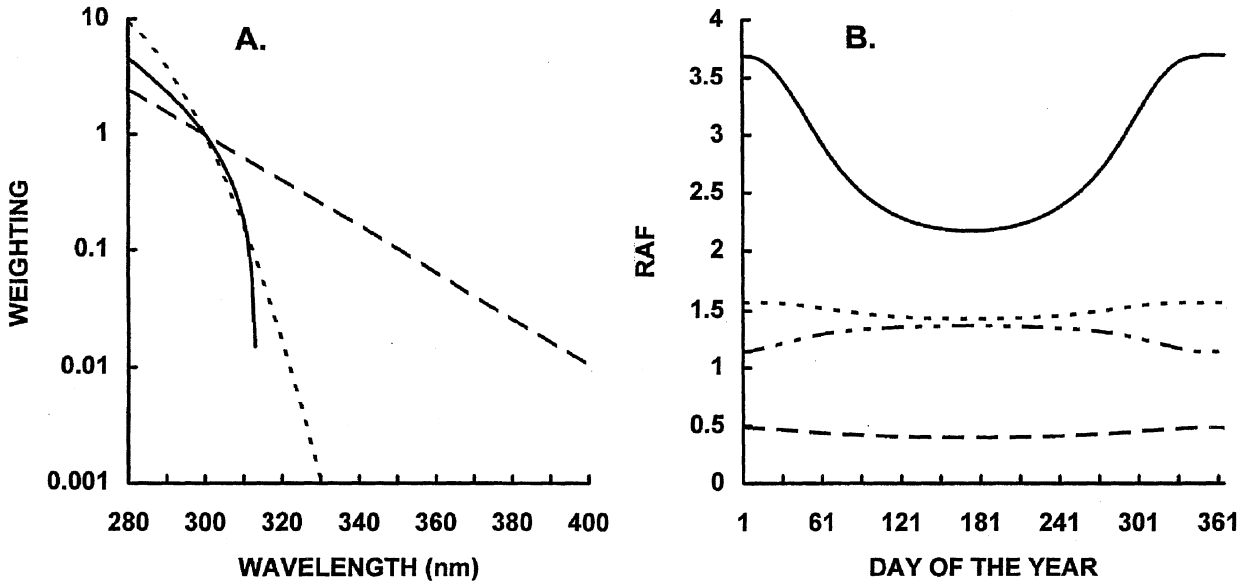

Figure 2. (a) Action spectra for the biological effects of UV-B: — plant action spectrum (PAS),(equation of Green et al. (1974); - - - - - PAS (equation of Thimijan et al. (1978); — — — plant growth inhibition (equation of Steinmuller (1986)). (b) Annual cycle of the radiation amplification factors for the action spectra shown in (a) and (- - —) that for the CIE erythemal action spectrum of McKinley & Diffey (1974) at 55°N. RAF's were calculated using the model of Bjorn & Murphy (1985) as modifed by Fiscus & Booker (Fiscus et al. 1994) for rural land type, green farmland, a total ozone column of 400 DU and with other parameters held constant.

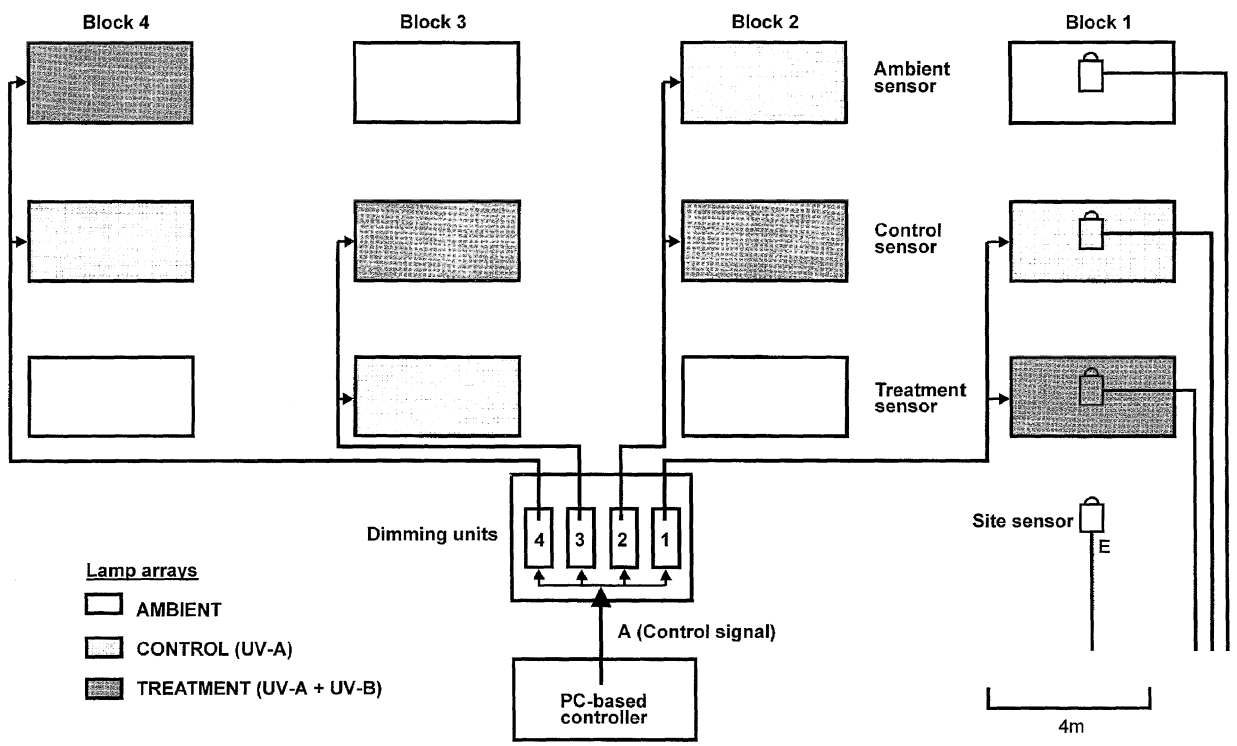

Figure 3. The experimental layout of one set of 12 lamp arrays, sensors and modulation control of the ITE Monks Wood UV-B supplementation system. The algorithm used to control lamp output is $\Delta A = -G\,(B-D)$ where G is a gain factor.

ITE Monks Wood supplementation system

An outdoor supplementation facility, with some features typical of other systems and many of the features described above, was constructed at the Institute of Terrestrial Ecology, Monks Wood, UK to provide fully modulated control of lamp output for studies of UV-B effects on natural vegetation (Newsham et al. 1996). The facility consists of two separate groups of 12 lamp arrays (Figure 3) each adjustable in height over a range of 0–2.5 m using a suspension system of ropes and pulleys connected to a hand-winch. Each lamp array consists of 12 lamps (Q-Panel UVB-313, The Q-Panel Co., Cleveland, OH, USA) mounted on a frame (1.4 × 4 m) of aluminium channel using fully waterproof end connectors. Lamps were spaced along the rack with a 'cosine-distribution' in which the distances vary as if the tubes had been equally spaced on half a cylinder and their position projected onto a plane (see Björn & Teramura 1993). The central 30 cm of the lamps is occluded using aluminium tape in order to produce a central area (2.6 × 0.7 m) within which the supplemental irradiance at canopy height varies by <10% of maximum. The lamps are electrically powered in pairs using cathode transformers (Type T40RS, Oy Helvar, Helsinki, Finland) and modulated using thyristor controlled dimmers (Type RDF110, Oy Helvar). When used with base-load resistors this provides an operating range of 0.3–100% maximum output. A closed-loop feedback control is operated using a successive approximation algorithm (see Figure 3) programmed using control software (Viewdac, Keithley Instruments Ltd., Reading, USA) in a personal computer. Ultraviolet-B irradiation is measured beneath lamp racks using sensors (Type BW-100, Vital Technologies Inc. Ontario, Canada) with a response which closely matches that of the CIE erythemal action spectrum (McKinley & Diffey 1984). As this is a LF electrical system, sampling errors caused by lamp flicker are avoided by sampling in bursts of 50 readings at 1000Hz and the feedback control loop is executed 135 times every minute. The one-minute mean exposure values are logged for subsequent analysis.

The Monks Wood experimental design incorporates both UV-B treatment lamp arrays (filtered with 125 μm thick cellulose diacetate), UV-A 'control' lamp arrays (filtered with 125 μm thick polyester, 'Mylar') and unenergised lamps as 'ambient' controls. The filters are applied in contact with the lamps in order to avoid any shading beneath the arrays caused by supporting structures. Each type: 'treatment', 'control' and 'ambient' lamp array is replicated four times (Figure 3) and there are 2 separate groups of these 12 lamp arrays. Further details of the system design and operation are given by Newsham et al. (1996). Figure 4 shows an example of the diurnal performance for a 30% elevation in UV-B in which a small step change can be seen near the beginning and end of the day as described earlier. This diurnal performance is typical of many modulated supplementation systems.

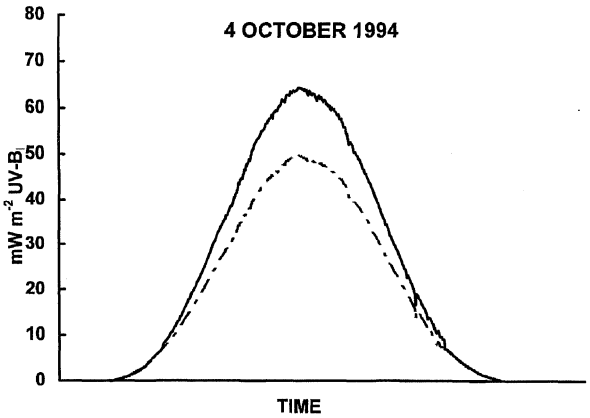

Figure 4. The diurnal cycle of UV-B irradiation measured with BW-100 broad-band sensors beneath a UV-B treatment lamp array (—) and an unenergised lamp array (- - - - - -) of the ITE Monks Wood outdoor supplementation system on 4 October 1994.

Discussion

The choice of a type of broad-band sensor and its spectral response has profound implications for the operation and interpretation of an experimental study. Sensors which respond to UV-A (including the erythemally-weighted examples) introduce some differences to the diurnal and seasonal patterns of UV-B exposure because UV-B is attenuated more strongly than UV-B at low solar angles. However, more profound changes are produced in the RAF's which express the percentage elevation in UV-B for a given ozone depletion scenario. Action spectra such as the PAS, which only weight wavelengths below 313 nm, show a marked sensitivity to seasonal patterns of ozone column thickness (irrespective of ozone depletion) producing, for the UK, RAF's that are higher during the winter months (Figure 2b). However, an action spectrum that includes a significant response to UV-A wavelengths, such as the CIE erythemal spectrum or the plant growth spectrum of Steinmuller (1986) (see

Figure 2a), result in much lower RAF's which exhibit much less seasonal variation. If seasonal trends in ozone depletion are considered, the seasonal changes in the RAF for the PAS are even more marked (Paul pers. comm.) compared to the other spectra noted above. The correct action spectrum for plant response has been the subject of much discussion (see e.g. Coohill 1989, 1992), but whatever function is used, a full description of the choice and details of the methods of exposure are particularly important for outdoor supplementation studies.

Outdoor supplementation systems can reveal interactions between plants and other organisms affected by UV-B, but they are also subject to some of the disadvantages inherent in creating 'islands' of isolated vegetation which may have been altered from the surrounding area (see McLeod et al. 1993). The vegetation beneath treated lamp arrays may be foci for herbivorous insects and pests not only because of the potential attraction to the lamps (e.g. flying insects), but also because of a potential attraction to the patch of altered vegetation. Regional increases in UV-B would affect all the surrounding vegetation and would not result in an 'island effect'. As a consequence of this, impacts of UV-B on mobile organisms might be overestimated. However, in contrast to this, any impact which reduces numbers (e.g. of an insect or microbe) may be underestimated in an experiment because of continual migration into the treated plot. Consequently, population changes of organisms of higher trophic levels within vegetation or changes in insect leaf damage observed using outdoor supplementation systems must be carefully evaluated. Outdoor systems are useful for determining which of the many potential interactions between organisms may occur but should then be used together with controlled environment studies to quantify the mechanisms involved.

There has only been one reported comparison of exposures with both a switched or square- wave protocol and a fully modulated exposure by Sullivan et al. (1994), who examined soybean. Both protocols were intended to simulate a 25% depletion in stratospheric ozone. The daily total UV-B irradiance was up to 30% higher with the square-wave protocol compared to the modulated exposure and this difference was reflected in some plant responses e.g. increases in leaf thickness and accumulation of UV-absorbing compounds. However, other parameters e.g. increases in photosynthetic pigments and changes in carbohydrate metabolism were observed only with the modulated treatment. It was unclear whether the difference in response arose solely from differences in total UV-B exposure or were the result of subtle differences in the timing of exposures or spectral differences between the two protocols. The results of this comparison further emphasise the need to carefully consider and report upon all aspects of the operation of outdoor supplementation systems to aid the correct interpretation and subsequent analysis of published results.

There are many differences between the large and increasing number of outdoor supplementation systems used for UV-B effects research. There are choices to be made in terms of lamps, filters, electrical systems, exposure regimes, sensors and action spectra. All these may have a profound effect upon the UV-B dose applied and the biological effects observed. It is important that all these aspects of experimental exposure are carefully evaluated and reported with the biological results. Outdoor UV-B supplementation systems have the potential to reveal many interactions between plants and other organisms under realistic microclimatic conditions, but observed effects must be considered with due attention to all aspects of the exposure technique.

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In assessing biological UV-B effects, natural fluctuations of solar radiation should be taken into account

Regas Santas[1], A. Koussoulaki[1] & D.-P. Häder[2]
[1]*OikoTechnics Institute, Kefallenias 50, 16342 A. Helioupolis, Greece;* [2]*Friedrich-Alexander Universität, 91058 Erlangen, Germany*

Key words: Outdoor UV-B, UV-A, PAR measurements, Spectral balance

Abstract

Daily and weekly fluctuations of PAR, UV-A, and UV-B have been continuously monitored for 5 months in Ancient Korinthos, Greece (37°58′ N, 23°0′ E) using a calibrated instrument based on 3 sharp band sensors. Daily dose ranged between 521–12 006 kJ m^{-2} for PAR; 52–1, 239 kJ m^{-2} for UV-A; and 0.66–22.5 kJ m^2 for UV-B. Weekly dose ranged between 16 778-81 788 kJ m^{-2} for PAR; 1 406–8 517 kJ m^{-2} for UV-A; and 18–151 kJ m^{-2} for UV-B. UV-B/PAR and UV-A/PAR ratio distribution, however, does not follow closely PAR fluctuations. Generally, the UV-B/PAR and UV-A/PAR ratios were high in bright light conditions (2.1×10^{-3}, 118×10^{-3}) and low in darker weeks (0.9×10^{-3}, 63×10^{-3}). The UV-B/UV-A ratio exhibits smaller fluctuations with season ($20x1\times10^{-3}$, 12×10^{-3}). Attention is drawn to the effects of sudden changes in ambient radiation and to the ratios of UV-B, UV-A, and PAR.

Introduction

In addition to CFC's, two new sources of ozone destruction were recently identified whose combined effects with anthropogenic reactive chlorine have led to record low levels of stratospheric ozone: volcanic eruption particles (McCormick 1995), and methyl bromide, used for the treatment of agricultural soils (Cox 1991; Yagi et al. 1993; 1995. The rapid decline in stratospheric ozone concentrations has been confirmed by satellite measurements (Molina & Molina 1992). As a result, scientific concern has culminated about the ongoing global-scale change, and much research focuses on predicting the biological effects of the expected increase in UV-B radiation. A standard experimental approach in studying UV-B effects is the exposure of organisms to a predicted level of increase in UV-B dose. In order to simulate the increase in solar UV, elaborate systems have been constructed using UV-lamp banks, O_3 filters, Plexiglass and/or plastic foil cutoff filters, etc. (Santas, 1989; Tezuka et al. 1994; Tendel & Häder 1995). Such systems, including growth chambers, greenhouses, and field enclosures, can achieve accurate replication of the desirable *mean* light intensity and spectral composition. However, the *fluctuations* of the above light parameters are very poorly, if at all simulated in most experimental setups. The data presented herein provide an assessment of the variation of daily and weekly dose of UV-A, UV-B and PAR, and the relative proportions of these three bands in the solar spectrum.

Methods and materials

Light measurements were performed in Ancient Korinthos, Greece (37°58′ N, 23°0′ E) in the period 17 June, 1995–17 November, 1995. The instrument used was a light dosimeter equipped with three sharp band sensors (Grubel, Ettlingen) for PAR (photosynthetically active radiation), UV-A and UV-B (Figure 1). The signals from the sensors are amplified, digitized by an analog/digital interface and stored in a dedicated computer. For this purpose, the 'Windose' program was developed specifically (Michael Lebert, Univ. of Erlangen). Each light sensor transmits 220 readings per

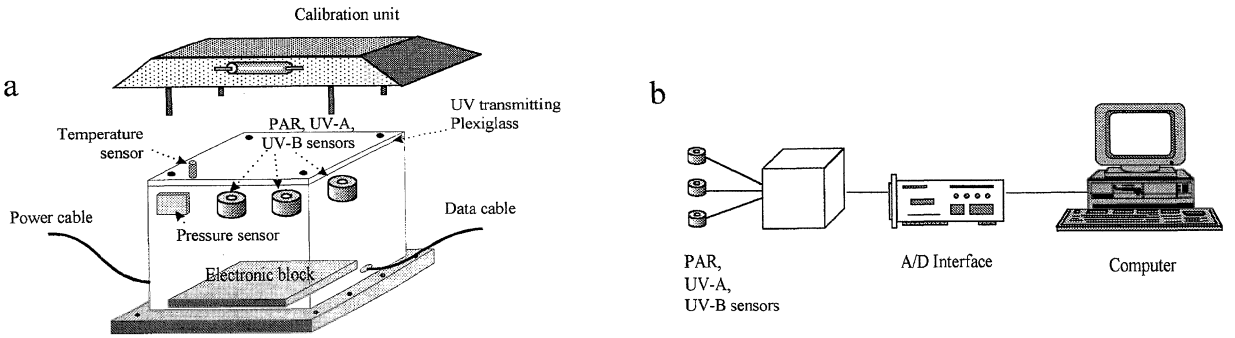


Figure 1. ELDONET light monitoring apparatus and data storage system: (a) Measurements are made with 3 sharp band sensors (PAR, UV-A, UV-B). The instrument can be operated on land or underwater (sensors here are shown inside the water tight chamber under a UV-transmitting Plexiglass top). A pressure sensor is used for indicating depth in the water column, while a temperature sensor is used for correcting erroneous readings due to overheating. (b) The analog signal from the sensors is digitized by an analog/digital (A/D) interface, and the data are fed in a dedicated computer. The data are displayed as a light intensity curve, and stored by 'Windose' software.

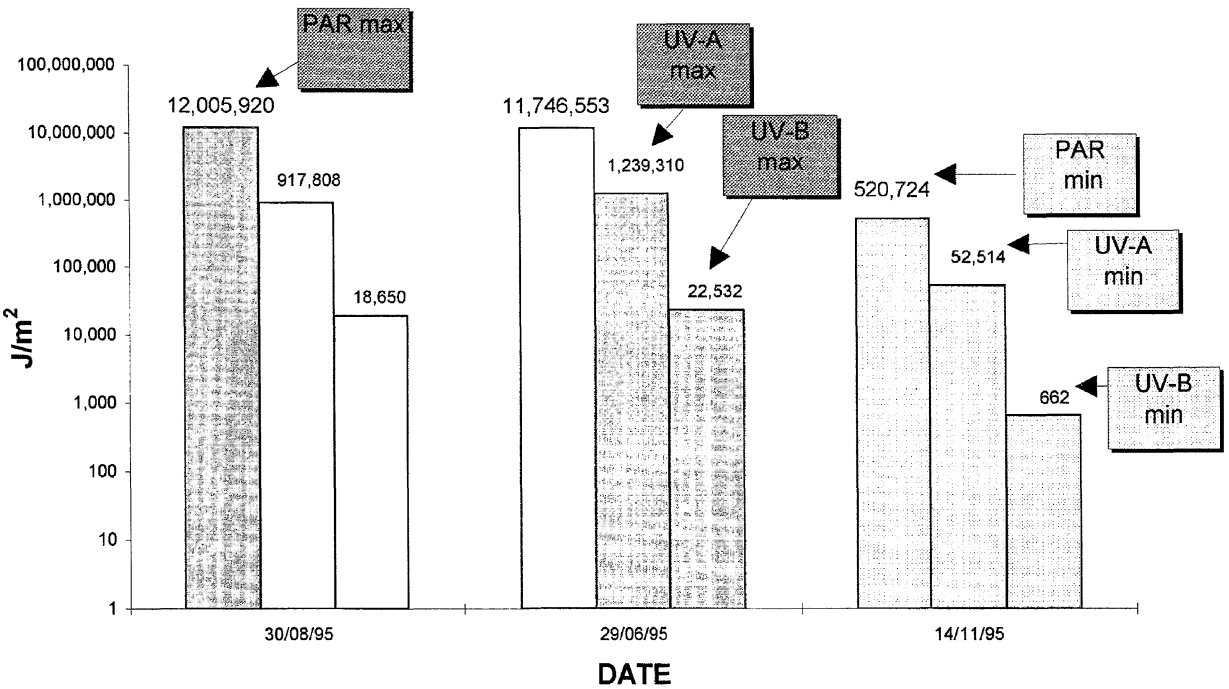
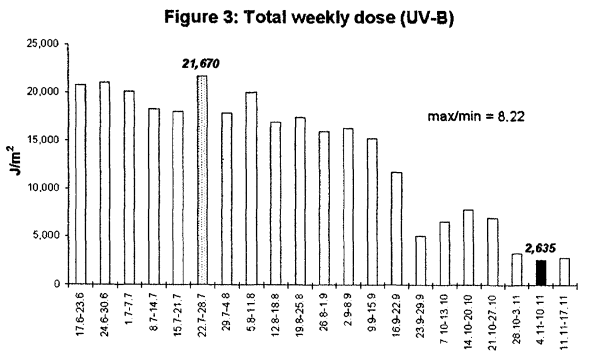
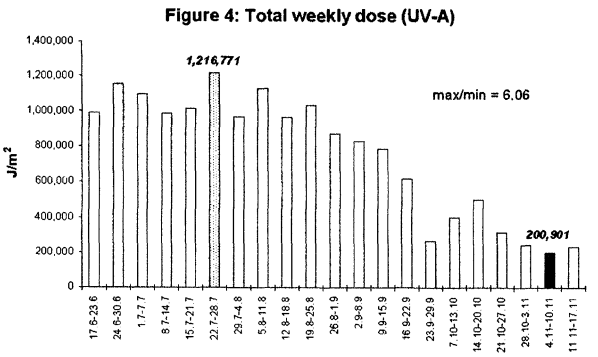


Figure 2. Daily dose maxima (dark columns) and minima (light gray columns) of solar PAR, UV-A and UV-B. The maximum daily PAR dose occurred on a different date (30 August, 1995) than the UV-A and UV-B daily maxima (29 June, 1995). All minima occurred on 14 November, 1995. The max/min ratios – a measure of daily dose variance during the course of this study – were 23 for PAR; 23.6 for UV-A; and 34 for UV-B.

minute which are then integrated and stored as minute doses. From the stored values larger duration doses (hourly, daily, weekly, etc.) can be easily calculated. The instrument was calibrated against a double monochromator spectroradiometer (Optronic OL 752). Specific care was taken to identify and correct for mechanical errors and deviation from the true values. For practical purposes, during data collection the instrument is placed in a horizontal position. Upon running the monitoring program, the user enters information about geographic latitude, date, and time of the day. A program subroutine uses the user-defined data for calculating sunrise and sunset time and solar angle of incidence. Data monitoring begins automatically





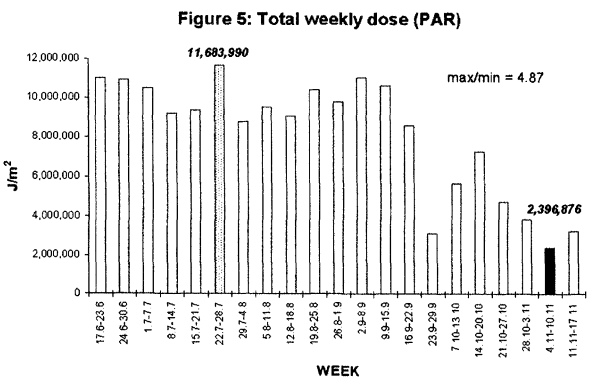


Figure 3–5. Total weekly dose fluctuations for UV-B (Figure 3), UV-A (Figure 4), and PAR (Figure 5). All maximum values (light gray columns) occurred during the period 22–28 July, 1995, while all minima (dark columns) occurred during 4–10 November, 1995. The max/min ratio increases with decreasing wavelength: 8.22 for UV-B; 6.06 for UV-A; and 4.87 for PAR.

one hour before sunrise, and ends one hour after sunset. The instrument's internal temperature during the course of the experiment ranged between 18–45 °C. To correct for errors in light readings due to overheating, temperature is monitored simultaneously with light. The program then performs the necessary adjustments according to an initial temperature calibration curve.

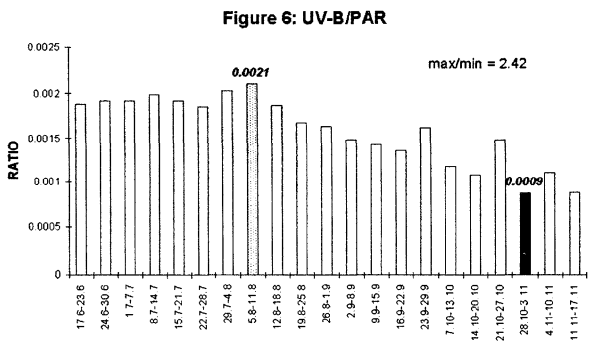
Results and Discussion

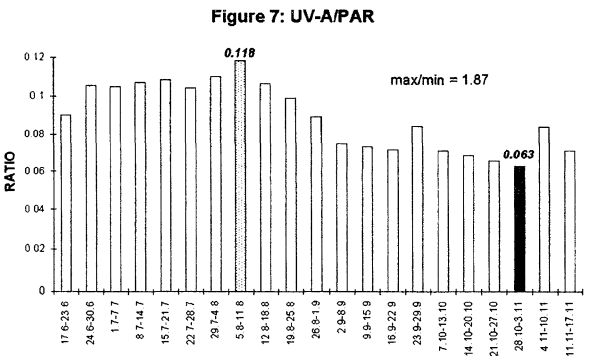
The daily maximum PAR value occurred on a different date (30 August, 1995) than the maximum values for UV-A and UV-B (29 June, 1995). Minima for all three bands occurred on 14 November, 1995 (Figure 2). The daily UV-B minimum dose (662 J m^{-2} on 14 November 1995) was 34 times lower than the maximum (22,532 J m^{-2} on 29 June, 1995). For UV-A and PAR, the minima are 23 times lower than the corresponding maxima.

The total weekly dose varies less than the total daily dose, while the maximum/minimum ratio decreases with increasing wavelength: 8.22 for UV-B, 6.06 for UV-A and 4.87 for PAR (Figures 3–5). Maximum and minimum doses for all three bands occurred on the fourth week of July and first week of November respectively (Figures 3–5). Spectral composition as measured by the relative proportion of the three bands at any time also varies considerably. In the 5th week of October the UV-B/PAR ratio was 2.42 times lower than in the 2nd week of August (Figure 6). In other words, during the summer months, there are more than twice as many UV-B photons per PAR unit dose than in the cold season. The UV-A/PAR and UV-B/UV-A ratios vary by a factor of 1.87 and 1.81 respectively (Figures 7, 8).

It is interesting to note that while the maximum of all other ratios occur in August, the UV-B/UV-A maximum occurred in late October (Figure 8). Unlike the UV-B/PAR and UV-A/PAR ratios, the UV-B/UV-A ratio did not decline during the two-month period of August-September.

These results point out the need for better simulation of light conditions in enhanced UV-B experiments





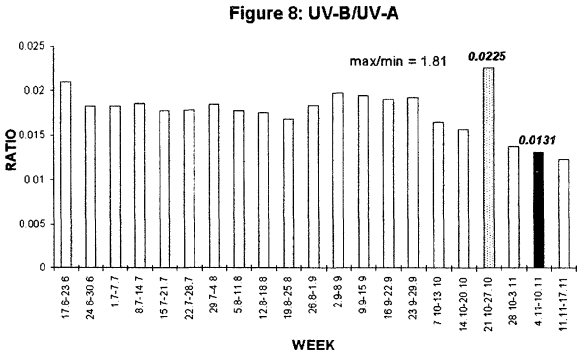


Figure 6–8. Spectral balance of solar UV-B, UV-A and PAR bands. Although total weekly dose was highest in the period 22–28 July 1995, (Figures 3–5), the relative proportion of UV/PAR photons was highest between 5–11 August 1995 (Figures 6 and 7; light gray columns). The minimum values were observed in the period 28 October–3 November, 1995 (Figures 6 and 7; dark columns). In other words, in the fall months, the weekly UV dose declines more rapidly than the weekly PAR dose. The UV-B/UV-A ratio (Figure 8) does not decline as much as the UV-B/PAR and UV-A/PAR ratios.

both in controlled environments and in the field. In most experimental setups, supplemental UV-B radiation is provided by steady output UV-B lamps. In the best of cases, the daily light cycle is simulated in a stepwise fashion, with irradiance periods centered midway through the photoperiod. Reports of ultraviolet irradiation experiments simulating light fluctuations due to sudden changes of atmospheric conditions are lacking from the literature, probably due to the practical limitations present in such attempts. Nevertheless, several researchers have pointed out the need to take account of spectral composition in photobiological studies (Caldwell et al. 1986; Smith & Baker 1989). In studying photobiological effects, it is important to keep in mind that the wavelength dependence of the biological photoprocess under consideration may vary with spectral balance. Bean plants, for instance, appear to be more resistant to enhanced levels of UV-B radiation when simultaneously exposed to high PAR conditions (Cen & Bornman 1990). Middleton and Teramura have pointed out the importance of UV-A control in UV-B experiments conducted using artificial lamps and filters (Middleton & Teramura 1993a). The same authors conclude that potential complications in the interpretation of plant responses exist when UV-B experiments are conducted under low ambient light conditions (e.g. growth chambers; glasshouse in winter) or high daily UV-B irradiances (e.g. greater than or equal to 14 kJ m^{-2}) for those plant responses that are sensitive to UV-A radiation (Middleton & Teramura 1993b). In some studies, shading of ambient radiation by lamp banks can be as high as 29% (Booker et al. 1992).

Light-dependent mechanisms such as photoreactivation, photorepair and photoprotection are driven by different wavelengths of the light spectrum including UV-A and PAR. In field studies, such mechanisms may alleviate or even reverse the impact of UV-B radiation on plant growth and development (Miller et al. 1994; Sinclair et al. 1990; Sullivan et al. 1992). It is also important to remember that the dose does not always cause an equal effect for a short exposure to a high intensity source as a long exposure to a low intensity

source even though the products are equal (Smith & Baker 1989). Therefore, in conducting long-term outdoor experiments, referring to the total dose may be of little value without examining the fluctuations of the biologically important light bands on a shorter time scale. Integrating or averaging, as practical as it may be, results in loss of valuable information.

Conclusions

For meaningful data interpretation in enhanced UV-B experiments, besides the monitoring of standard light parameters (intensity, spectral composition, total dose, biologically weighted dose), the natural radiation fluctuations – periodical and random – should also be accounted for. It is also necessary to examine the interaction of the different light bands (UV-A, UV-B and PAR), since photodamage and photorepair mechanisms occur simultaneously. Laboratory experiments should strive for closer simulation of the diurnal and seasonal changes in light intensity and spectral balance. In nature, darkness may not vary, but light does.

Acknowledgements

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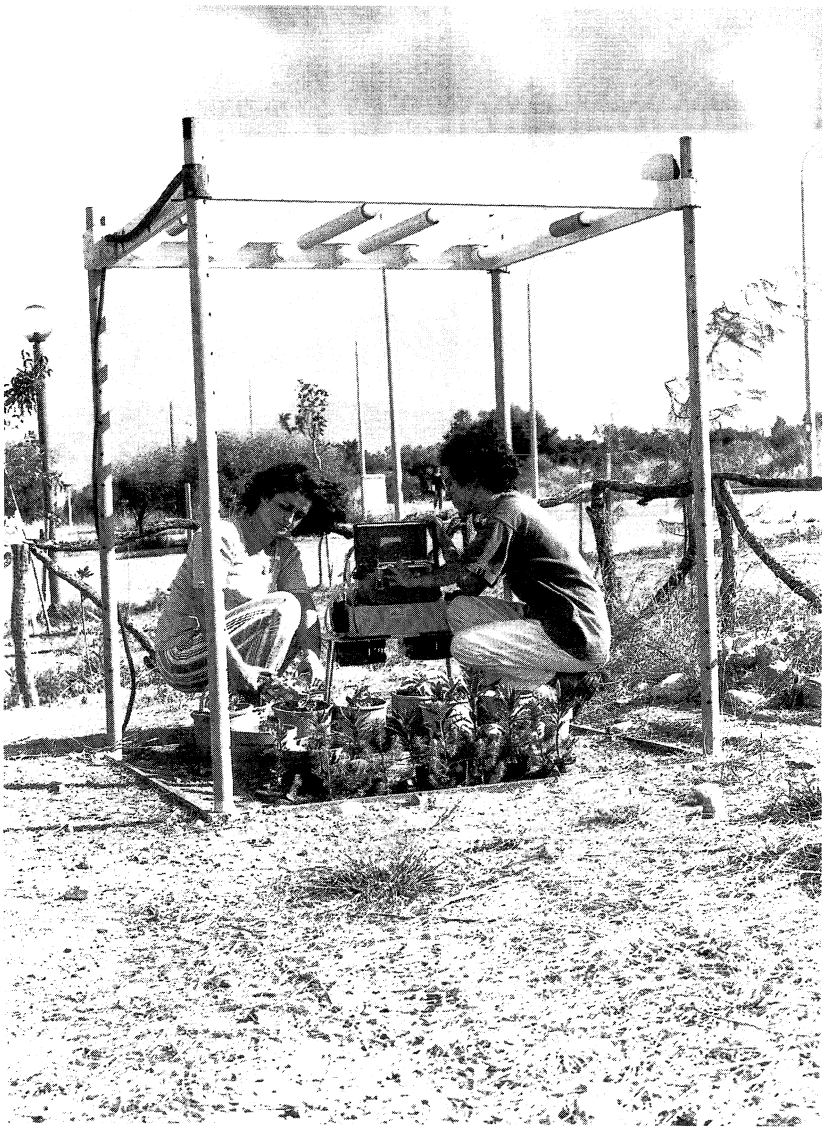
III. UV-B and physiology of terrestrial plants



Plants and students in action under a UV-B frame. (Photograph: P. Lambropoulos)

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J. Rozema, W. W. C. Gieskes, S. C. van de Geijn, C. Nolan and H. de Boois (eds), UV-B and Biosphere.

Beneficial effects of enhanced UV-B radiation under field conditions: improvement of needle water relations and survival capacity of *Pinus pinea* L. seedlings during the dry Mediterranean summer

Yiannis Manetas, Yiola Petropoulou, Kostas Stamatakis*, Dimostenis Nikolopoulos, Efi Levizou, George Psaras[1] & George Karabourniotis[2]
Laboratory of Plant Physiology, Department of Biology, University of Patras, Greece;
[1]Institute of General Botany, Department of Biology, University of Athens, Greece; [2]Laboratory of Plant Physiology, Department of Agricultural Biology and Biotechnology, Agricultural University of Athens, Greece
*(*present address: Institute of Biology, National Research Center Demokritos, Athens, Greece)*

Key words: Cuticle, *Pinus pinea*, UV-B radiation, Water relations

Abstract

The possible mechanism(s) by which supplemental UV-B radiation alleviates the adverse effects of summer drought in Mediterranean pines (Petropoulou et al. 1995) were investigated with seedlings of *Pinus pinea*. Plants received ambient or ambient plus supplemental UV-B radiation (biologically equivalent to a 15% ozone depletion over Patras, 38.3° N, 29.1° E) and natural precipitation or additional irrigation. Treatments started on 1 February, 1994 and lasted up to the end of the dry period (29 September). In well-watered plants, UV-B radiation had no influence on photosystem II photochemical efficiency and biomass accumulation. Water stressed plants suffered from needle loss and reduced photosystem II photochemical efficiency during the summer. These symptoms, however, were less pronounced in plants receiving supplemental UV-B radiation, resulting in higher total biomass at plant harvest. Laboratory tests showed that enhanced UV-B radiation did not improve the tolerance of photosystem II against drought, high light, high temperature and oxidative stress. Enhanced UV-B radiation, however, improved the water economy of water stressed plants, as judged by measurements of needle relative water content. In addition, it caused an almost two-fold increase of cuticle thickness. No such UV-B radiation effects were observed in well-watered pines. The results indicate that the combination of water stress and UV-B radiation may trigger specific responses, enabling the plants to avoid excessive water loss and, thereby, maintain a more efficient photosynthetic apparatus during the summer. The extent of this apparently positive UV-B radiation effect would depend on the amount of summer precipitation.

Abbreviations: DW – dry weight, F_v/F_m – ratio of variable to maximum fluorescence, A_{300} – absorbance at 300 nm, PAR – photosynthetically active radiation, PS II – photosystem II, RWC – relative water content, TCA – trichloroacetic acid, $UV\text{-}B_{BE}$ – biologically effective ultraviolet-B radiation

Introduction

Recent measured trends in stratospheric ozone concentration (Stolarski et al. 1992) allow the prediction that considerable increases in ultraviolet-B (UV-B) radiation reaching the surface of the earth may be expected within the next few decades. Accordingly, intensive research has been undertaken on the effects of enhanced UV-B radiation on plants. Earlier studies, mainly performed in growth chambers or glasshouses, showed that UV-B radiation was generally detrimental to sensitive species, causing reductions in growth, which in some cases could be ascribed to corresponding reductions in net photosynthetic rates (Teramura et al. 1983). Although field studies are rare and mainly restricted to cultivated plants, there is a general trend

for the UV-B radiation effects to be less pronounced in field grown plants (Fiscus & Booker 1995). A critical factor may be the visible light irradiance which, in growth chambers and similar enclosures, is only a fraction of natural sunlight. Indeed, it has been shown that for a given UV-B irradiance, the effects are magnified as the visible light is reduced (Teramura et al. 1980). In addition, various stress factors imposed concurrently with the supplemental UV-B irradiation have been shown to modify the UV-B radiation effects. Water stress, in particular, has been shown either to increase or mask the UV-B radiation effects (Sullivan & Teramura 1990; Tevini 1993).

It comes out that in long term experiments under field conditions, in areas with marked seasonal changes of the factors influencing plant growth, responses of plants may be season-specific. This was shown recently with Mediterranean pines. In *Pinus pinea* and *Pinus halepensis*, supplemental UV-B radiation had no effects during the wet winter period. During the summer drought, however, UV-B irradiated plants suffered significantly less needle drop and photosystem II inactivation, while the photon yield of O_2 evolution and the photosynthetic capacity were higher, when compared with plants receiving ambient UV-B radiation (Petropoulou et al. 1995).

The present paper investigates the possible mechanisms underlying this apparent alleviation by enhanced UV-B radiation of the adverse effects of summer drought in *P. pinea*. We have considered two hypotheses: pre-exposure of the seedlings to enhanced UV-B radiation during the wet period renders the photosynthetic machinery more resistant to the water-stress syndrome. Alternatively, enhanced UV-B radiation improves needle water relations and, accordingly, the effects on the photosynthetic properties are indirect. To this aim, pine seedlings receiving either natural precipitation or additional irrigation were used.

Materials and methods

Plant material and growth conditions were as described previously (Petropoulou et al. 1995). In particular, apparently uniform, one year old seedlings of *Pinus pinea* L. were donated by the local state Forest Service on November 1993. They were transferred to a small open nursery in the vicinity of the experimental site and their growth rate was recorded up to late January of the following year. Eighty similar seedlings, based on their growth rate, height and total branch length were selected for further experimentation. The bottom of the plastic pots (15 cm diameter) was removed to permit root growth and the pots buried at ground level in local, well-mixed soil under the appropriate control and UV-B frames, which were located in a horizontal, shade-free area with no reflecting objects around. A distance of 200 cm between the control and UV-B frames and a 20 cm, horizontal, metal shield at the height of the distant UV-B tubes were enough to exclude all supplemental UV-B radiation from the UV-B frames to reach the plants in the control frames, as shown with preliminary measurements of UV-B irradiance. Four frames (2 controls, 2 UV-B) with 20 seedlings per frame were used. The soil was slightly alkaline (pH 7.78) and consisted of sand, silt and clay (55.4/28.2/16.4) with negligible organic material. Repeated measurements of soil water potential showed that there was no difference in water availability between the control and UV-B plots. The experiment started on 1 February, 1994 and terminated on 30 September of the same year. Half of the plants in each frame were left undisturbed for biomass measurements at final harvest, while from the others, a minimum amount of needles was periodically removed and used for the appropriate measurements. Sampling did not exhaust the plants since it was estimated that a total of less than 10% of the needles was removed during the whole experimental period.

Growth conditions

UV-B radiation was given by 6 Philips TL 40/12 fluorescent tubes, 20 cm apart from each other, suspended approximately 170 cm above the plant apex. The tubes were wrapped with 0.1 mm cellulose acetate film (Courtaulds Chemicals, Derby, UK) to eliminate the UV-C radiation. Since UV-B transmission properties of cellulose acetate change with exposure time, the films were 'pre-burnt' with the same tubes for 12 h before use and were replaced after approximately 20 h of field tube function. In control frames, the TL 40/12 tubes were replaced by white, plastic tube effigies of the same diameter. In this way, the visible light environment under control and UV-B frames was the same. With our irradiation system, UV-A radiation under the UV-B frames was increased by 1.9% compared to ambient (Petropoulou et al. 1995). This slight increase, however, was valid as long as the tubes were on (around mid-day) and completely abolished when all the tubes were off (early morning and late afternoon). Accordingly, the total daily percent increase was very small. Much higher differences are needed for UV-A radi-

ation to modify UV-B radiation effects (Caldwell et al. 1994). Shading from the tubes and tube supports was estimated through daily measurements of photosynthetically active radiation (PAR) performed along 10 predetermined, equidistant lines, arranged perpendicularly to the tubes, with a linear (80 cm) quantum sensor (Decagon Sunfleck Ceptometer, Pullman, WA, USA). During a clear day near the summer solstice (June 8), with low zenith angle and, accordingly, maximum expected shading, the plants received at their apex 90% of the daily PAR found above the frames. Obviously, less shading is expected with increasing zenith angle.

The homogeneity of the UV-B radiation field was exhaustively checked at night (i.e. in the absence of ambient UV-B radiation) and was considerably improved by covering the central part of the two central and the distal UV-B tubes by 70 and 20 cm respectively with black, polyurethane material. In this way, the UV-B irradiance differences within the field were less than ±10%, provided that the very center and the corners of the square frame were not used.

Spectral irradiance was measured with an OL 752 Optronic (Orlando, FL, USA) spectroradiometer, calibrated against an OL 752-150 module for wavelength accuracy and an OL 752-10 spectral irradiance standard. In order to determine the supplemental, biologically effective ($UV\text{-}B_{BE}$) daily dose under the required 15% ozone depletion, the absolute spectral irradiance (measured at night) was weighted with the generalised plant action spectrum normalised at 300 nm (Caldwell 1971) and used in conjunction with the computer program of Björn & Murphy (1983). Accordingly, the duration the lamps should be on each day was computed. Lamps were on in groups of three, in order to minimise possible step effects and supplemental UV-B radiation was centred at solar noon. Lamp duration was modified monthly, in order to follow the natural march of ambient UV-B radiation change. Actual $UV\text{-}B_{BE}$ doses during the summer maximum were 8.55 and 11.21 kJ m^{-2} day^{-1} for the control and UV-B plants respectively.

Plants in two of the frames (one UV-B, one control) received only natural precipitation, the height of which was provided by the University of Patras weather station, located 0.5 km from the experimental site. The other two received natural plus additional watering of 17.6 mm per month given in two equal doses, starting from April. During the four summer months (September included) natural precipitation was 42.5 mm. Accordingly, watered plants received 112.9 mm. Mean annual precipitation at Patras is 766.6 mm, 5% of which (38.3 mm) falls during the four summer months. Only during exceptionally wet summers, precipitation can exceed 100 mm.

Measurements

Chlorophyll fluorescence induction was measured non-destructively with a Hansatech Plant Efficiency Analyzer. Excitation red light at 3000 μmol m^{-2} s^{-1} was used and the needles were pre-darkened for 30 min.

Relative water content (RWC) was measured with the floating method of Turner (1981).

For assessing needle resistance against various stresses, the needles were cut from the plants, transferred to the laboratory and subjected to the corresponding stresses as follows:

(a) Drought stress-needles were floated over water for 4 h in the dark to obtain full turgor and then left to lose water on a laboratory bench at room temperature and 40 μmol m^{-2} s^{-1} PAR.

(b) High light-needles were floated over water at room temperature and 2900 μmol m^{-2} s^{-1} PAR.

(c) High temperature-needles were floated over water at 40 μmol m^{-2} s^{-1} PAR and 45 °C.

(d) Oxidative-needles were floated over water containing 50 μM methylviologen at room temperature and 450 μmol m^{-2} s^{1} PAR.

When needed, the needles were put in a temperature regulated leaf chamber to avoid overheating from the irradiation source. Light was given with a quarz-halogen lamp. In all cases, time courses of F_v/F_m changes were followed. For drought stress, the RWC was also monitored.

Epicuticular UV-B absorbing compounds and waxes were measured at plant harvest. The intact needles from each individual plant were immersed in chloroform for 2 min. UV-B absorbing compounds were measured spectrophotometrically after transferring to methanol and waxes were measured gravimetrically.

For malonaldehyde measurements, the needles (0.13 g) were ground in a mortar with 3 ml of 0.5% TCA. After centrifugation, the absorbance of the clear supernatant at 532 nm was measured after reaction with thiobarbituric acid (Dhindsa et al. 1981).

Apart from malonaldehyde and epicuticular material, in all other cases 2 needles per plant were measured independently. Exposed needles, located 2–5 cm below the plant apex were always used.

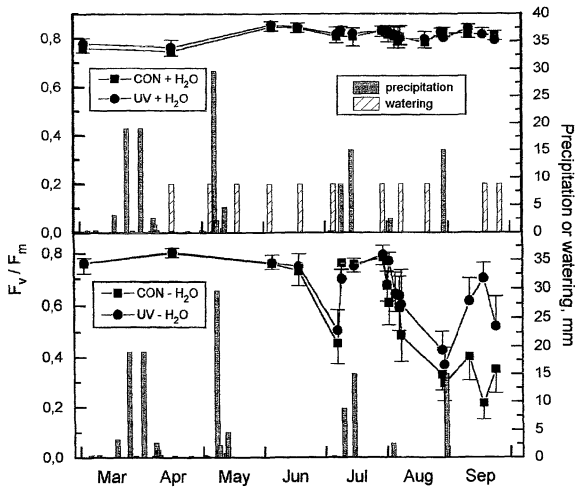

Figure 1. Effects of supplemental UV-B radiation and additional watering on mid-day F_v/F_m values of *P. pinea* needles. The height of natural precipitation and additional watering is also given. Supplemental UV-B radiation simulated that would have been received by the plants on the event of a 15% ozone depletion over Patras. CON, plants under ambient UV-B; UV, plants under ambient plus supplemental UV-B radiation. $-H_2O$, plants receiving only natural precipitation; $+H_2O$, plants receiving additional watering. Data are means ± SD from 20 needles (two needles per individual plant).

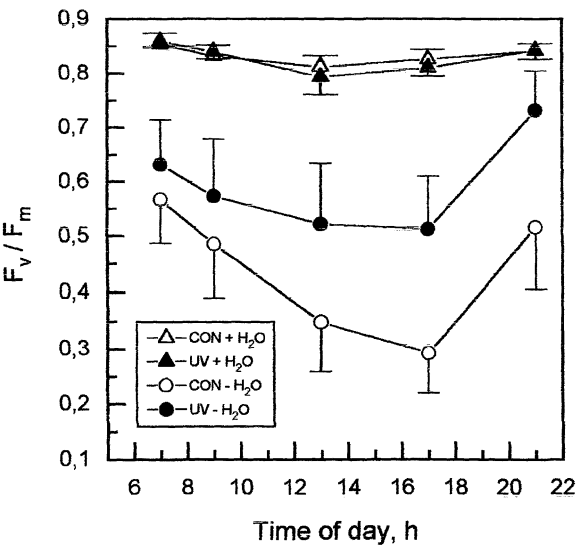

Figure 2. Daily F_v/F_m of pine needles under various treatments. One out of three daily measurements taken during mid-September, with similar trends. Data are means ± SD from 20 needles (two needles per individual plant).

For microscopic examination, transverse sections were handcut from fresh needles and viewed immediately under polarised light in an Axioplan (Zeiss) microscope without fixing.

Soil water potential was measured with a Decagon (Pullman, WA, USA) SC-10 thermocouple psychrometer.

Results and discussion

Figure 1 shows the effects of additional watering on PS II photochemical efficiency in plants receiving ambient or ambient plus supplemental UV-B radiation. There were three major rain storms in the summer of 1994 giving totals of 23.5 and 15 mm of rain during mid-July and late August respectively (Figure 1). Plants receiving only natural precipitation showed a decrease in mid-day F_v/F_m at early July and a complete recovery after the mid-July rain, with no differences between control and UV-B irradiated individuals. Statistically significant differences in F_v/F_m were observed after mid-August and, although a trend for recovery was evident after the late August rain, this was more complete in UV-B treated plants (Figure 1). In plants receiving additional water, F_v/F_m was high in both control and UV-B treated individuals for the whole experimental period (Figure 1).

Figure 2 shows the diurnal variations in F_v/F_m under various treatments during mid-September. In water-stressed plants, F_v/F_m was lower at mid-day but the afternoon and night recovery was not complete, indicating that the reduced mid-day values were not only due to an adaptive down regulation of PS II activity, but to irreversible PS II damage as well (Long et al. 1994). In any case, F_v/F_m was higher under UV-B supplementation during the whole day. Apart from the lower F_v/F_m, water-stressed control plants, as expected (see Petropoulou et al. 1995), suffered a heavier needle loss compared to UV-B treated, while needle loss in watered plants was negligible. As shown on Table 1, at plant harvest (29/09/94), needle dry mass in water-stressed, UV-B treated plants was almost 2-fold higher compared to controls receiving ambient UV-B radiation. In watered plants, supplemental UV-B radiation had no effect on dry mass accumulation. The results indicate that, under field conditions, *P. pinea* is not only resistant against UV-B irradiances in excess of that normally encountered in its natural environment, but acclimation to enhanced UV-B radiation improves the capacity of the seedlings to withstand the adverse conditions of summer drought. Apparently, the extent of this beneficial effect would depend on the amount

Table 1. Some effects of enhanced UV-B radiation on *P. pinea* seedlings receiving either natural (−) or natural plus supplemental (+) watering. CON, plants under ambient UV-B; UV, plants under ambient plus supplemental UV-B radiation. Irradiation started on 1 February, 1994 and measurements were carried out on 29 September, 1994. Values are means ± SD, $n = 10$

	CON+	UV+	CON−	UV−
Needle DW, g	2.69 ± 0.56	2.69 ± 0.70	0.59 ± 0.09	1.10 ± 0.26
Total shoot DW, g	6.41 ± 1.09	6.46 ± 1.68	2.81 ± 0.39	3.63 ± 0.55
A_{300}g DW^{-1}(external)	8.78 ± 1.43	10.65 ± 1.67	8.80 ± 0.65	9.48 ± 1.93
Epicuticular wax, % of DW	1.79 ± 0.48	1.84 ± 0.37	2.22 ± 0.15	2.48 ± 0.31
Cuticle thickness, μm	2.5 ± 0.2	2.5 ± 0.2	2.6 ± 0.2	4.3 ± 0.8
Needle dimensions, μm				
height	805 ± 55	794 ± 42	677 ± 90	728 ± 55
base	1045 ± 72	950 ± 100	981 ± 75	1012 ± 95

and frequency of summer rains, being completely abolished during exceptionally rainy summers.

During the Mediterranean summer, the absence of precipitation coincides with high temperatures and a cloudless sky. According to local records, the mean daily temperature and the mean daily sunshine hours at Patras for the 4 summer months (including September) are 24.8 °C and 10.6 h respectively (Kotini-Zambaka 1983). Evergreens respond to the increased evaporative demands by maintaining a strict stomatal regulation of gas exchange (Kyparissis & Manetas 1993; Lange 1988). Under such conditions, the photosynthetic machinery is prone to severe photoinhibitory damage (Long et al. 1994) which may be accompanied by the generation of oxy-radicals, lipid peroxidation and membrane deterioration (Smirnoff 1993). On the other hand, UV-B radiation is considered a potential oxidative stress (Larson 1988; Panagopoulos et al. 1990) and may induce increased activities of antioxidative enzymes (Balakumar et al. 1993). We speculated, therefore, that enhanced UV-B radiation given in advance of the summer drought, may harden the plants against the summer drought syndrome. We may add that heat, water and high light stress may be related to oxidative stress (Eickmeier et al. 1992; Freeman et al. 1990). Accordingly, acquired resistance to any of them may render cross-resistance for the others. Caldwell (1994) showed, for example, that UV-B radiation increased heat resistance in cucumber cotyledons. In our case, however, enhanced UV-B radiation had no statistically significant effects on the resistance against high light, drought, high temperature and oxidative stress, as judged by laboratory tests involving measurements of chl fluorescence parameters (data not shown). In addition, malonaldehyde contents of needles showed no significant differences (data not shown). Accordingly, we reject the hypothesis that enhanced UV-B radiation increases the resistance of the photosynthetic machinery against the water stress syndrome. Judjing *a posteriori*, we may note that UV-B radiation is almost completely attenuated by the epidermis of conifer needles (Day et al. 1992) and, accordingly, is not expected to exert its effects in the mesophyll.

We proceeded, therefore, in testing the second alternative, i.e. enhanced UV-B radiation improves needle water relations. In our previous study (Petropoulou et al. 1995), measurements of RWC were performed once during the summer (late August). Although a small increase (ca. 5%) of RWC under UV-B supplementation was observed, its importance was ignored, as detailed and frequent measurements were lacking.

In Figure 3, mid-day RWC is shown to be consistently and significantly higher in water-stressed, UV-B irradiated plants, compared with those receiving only ambient UV-B radiation, from mid-August and up to the end of the experiment (late September). In watered plants, RWC was high under both UV-B radiation regimes. We may accept, therefore, the second hypothesis and conclude that the higher PS II photochemical activities as well as the less pronounced needle loss are an indirect effect of improved needle water relations induced by enhanced UV-B radiation.

In growth chamber and greenhouse experiments, UV-B radiation has been shown to restrict stomatal opening (Grammatikopoulos et al. 1994; Negash & Björn 1986; Teramura et al. 1984) and increase leaf thickness (Cen & Bornman 1993) and epicuticular wax (Steinmüller & Tevini 1985). Each one or a combina-

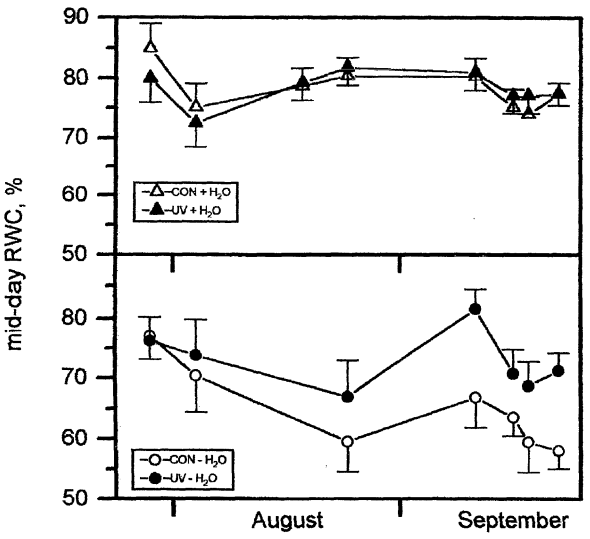

Figure 3. Mid-day relative water content of *P. pinea* needles receiving either natural ($-H_2O$) or natural plus supplemental ($+H_2O$) watering. CON, plants under ambient UV-B; UV, plants under ambient plus supplemental UV-B radiation. Data are means ± SD from 10 independent measurements (two needles per measurement).

tion of these effects, if they happen under field conditions, should restrict excessive water loss from the leaf, increasing stomatal, mesophyll or cuticular resistance to water vapour diffusion. In our case, however, needle dimensions were not affected under any circumstances (Table 1). Epicuticular wax increased in water-stressed plants and a trend towards further increase (ca. 11%) was observed under UV-B supplementation (Table 1). Although the difference was small and not statistically significant, its importance in reducing transpiration can not be assessed by simply measuring gross wax amounts, since wax is not homogeneously distributed on leaf surfaces. Scanning electron microscope observations are needed, as wax rods have been frequently observed to block stomata of pine needles (Fahn & Cutler 1992). Table 1 also shows that cuticle thickness considerably increased in water- stressed plants under UV-B supplementation (see also Figure 4). To our knowledge, this is the first report of a UV-B radiation effect on cuticle thickness.

Increased cuticle thickness may decrease cuticular transpiration rates. Although water loss through the cuticle is only a small fraction of the total transpired from the leaf, its contribution may become considerable during prolonged dry periods, when stomata are kept tightly closed. This is indeed the case with Mediterranean evergreens during the summer (Lange 1988; Kyparissis & Manetas 1993). Under such circumstances, cuticular transpiration becomes the major route for water loss and an increase in cuticle thickness may be of particular significance for the maintenance of a critical leaf water content. Our results do not exclude the possibility that stomatal closure by UV-B radiation may also contribute to the improvement of pine water relations during the summer. We may note, however, that if supplemental UV-B radiation had resulted in stomatal closure, reduced growth would have been observed in UV-B treated pines under additional irrigation.

An alternative explanation would be that water relations may have been improved due to an increase in the root : shoot ratio (Nikolopoulos et al. 1995). No evidence for such an increase, however, was found with pine seedlings (Petropoulou et al. 1995).

Reports for beneficial effects of UV-B radiation are not absent from the literature, but they are based on comparisons of plants in the presence and absence of UV-B radiation in green-house experiments (Barnes et al. 1990; Teramura et al. 1991; Tosserams & Rozema 1995). In such an experiment with temperate Pinaceae of different elevational origin, Sullivan & Teramura (1988) found that the high altitude *Picea engelmannii* was the only species to respond with increased biomass under UV-B radiation, compared to a minus UV-B control. Field studies with temperate Pinaceae have not shown any beneficial UV-B radiation effects (Naidu et al. 1993). This discrepancy with our results may be attributed to species differences or to the absence of co-occuring drought stress.

Conclusions

The results clearly show that Mediterranean pines under field conditions are particularly resistant against enhanced UV-B radiation, as no effects on growth and photosynthetic parameters could be observed during the wet period or during the whole year, if additional irrigation was provided during the summer. Under natural precipitation, however, the combination of enhanced UV-B radiation and water stress induced specific changes on needle surface (i.e thicker cuticles) leading to an improved water economy during the summer and, indirectly, to the maintainance of a satisfactory photosynthetic performance. The results also indicate the strong seasonality of enhanced UV-B radiation effects, stressing the need for long term field studies and emphasizing the view that apparently

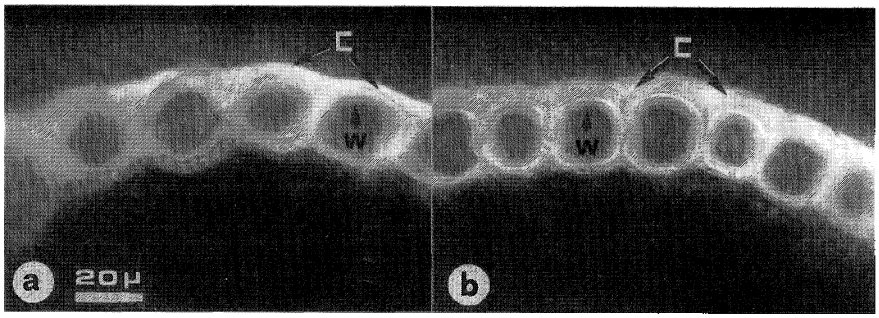

Figure 4. Transverse sections of *P. pinea* needles showing epidermal cells under polarised light. a, Control; b, UV-B. c, cuticle; w, cell walls. The needles were harvested at the end of the dry period. Sections from plants receiving only natural precipitation are shown. In well-watered plants, the cuticle thickness was equal to that of plants receiving ambient UV-B radiation and natural precipitation.

neutral effects during the favourable growth period, may become critical for plant survival under adversity. Obviously, the extent of UV-B radiation effects would depend on evaporative demand during the summer, i.e. on the amount of summer precipitation.

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Ultraviolet-B radiation effects on the Mediterranean ruderal *Dittrichia viscosa*

M. Stephanou & Y. Manetas*
Laboratory of Plant Physiology, Department of Biology, University of Patras, Patras 26500, Greece
*(*author for correspondence)*

Key words: Dittrichia viscosa, Epicuticular flavonoids, Growth, Photosynthetic pigments, UV-B radiation

Abstract

Young seedlings of *Dittrichia viscosa* L. (syn. *Inula viscosa* (L.) Aiton) (Asteraceae) were extensively treated with artificial rain in order to remove the water soluble component of their epicuticular UV-B absorbing compounds. As a result, 75% of the epicuticular absorbing capacity at 300 nm was lost. The seedlings were subsequently grown in a naturaly lit glasshouse for 80 days under 0.06, 6.41 and 10.14 kJ m^{-2} day^{-1} biologicaly effective UV-B radiation doses. The initial, pre-rain values of the water soluble, epicuticular UV-B absorbing potential was restored in about three weeks. During this transient period the plants were exposed to the enhanced UV-B radiation doses with part of their UV-B radiation screen removed. Although a trend for increased accumulation of epicuticular UV-B absorbing capacity was observed with increasing UV-B radiation doses, the allelopathic potential of the epicuticular material remained unchanged. Internal (cellular) UV-B absorbing compounds and chlorophylls were unaffected, but total carotenoids were increased, indicating a possible protective role against UV-B radiation damage. Leaf, stem and root dry mass were the same under all treatments but UV-B radiation caused a reduction in the dry mass invested per unit leaf area with a concomitant increase in leaf area. The importance of this UV-B radiation induced selective allocation of photosynthate to the production of assimilative surfaces is discussed.

Introduction

Dittrichia viscosa is a Mediterranean ruderal exuding on its leaves an epicuticular material rich in flavonoids (Wollenweber et al. 1991). Stephanou and Manetas (1995) showed that a considerable part (ca. 75%) of the ultraviolet-B (UV-B) absorbing capacity of this exudate can be removed by simple washing off the leaves and, apparently, it can be drained to the ground by rain. Short-term laboratory experiments (Stephanou and Manetas 1995) showed that removal of the epicuticular material did not render the leaves more sensitive to UV-B radiation, even at UV-B irradiances far exceeding those encountered by the plants in their natural environment. The epicuticular material was, however, strongly allelopathic, inhibiting seed germination in letuce and radicle growth in *Phlomis fruticosa* L. (Stephanou and Manetas 1995). The present study was therefore scheduled on a dual aim. First, to investigate the effects of UV-B radiation on the epicuticular UV-B absorbing compounds and their allelopathic potential. Second, to examine whether *D. viscosa*, temporarily devoided of the water soluble component of its UV-B radiation screen, could stand long term exposures to enhanced UV-B radiation.

Materials and methods

On early February, 1994, sixty, about 2 months old seedlings of *D. viscosa* were excavated from their natural environment within the Patras University campus, put in 6 liters plastic pots with local soil and transferred in a naturaly lit glass-house. Their growth rate was monitored and on March 15, 30 similar seedlings (based on growth rate, height and total leaf area) were selected for further experimentation. The seedlings were extensively treated with artificial rain in

order to remove the water soluble leaf exudate and subsequently grown for 80 days under 0.06, 6.41 and 10.14 kJ m^{-2} day^{-1} biologicaly effective UV-B radiation (UV-B_{BE}) doses. The last two doses correspond to those reaching the Patras area (38.3° N, 29.1° E) at 5 May under clear sky and with normal column ozone thickness and 10 July under clear sky and 10% ozone depletion. 10 seedlings per treatment were used and irrigated with 150 ml of tap water every other day. The rain treatment was applied once, at the start of the experiment.

Growth conditions, irradiation system and calculations of the UV- B_{BE} have been previously described (Petropoulou et al. 1995). In short, UV-B irradiation was given by Philips TL 40/12 fluorescent tubes wrapped with 0.1 mm cellulose acetate film, which was changed regularly. In control plants, the UV-B tubes were replaced by white, plastic tube effigies of the same diameter. In this way, the visible light environment under the control and UV-B frames was the same. Differences in UV-A radiation between the frames were negligible (Petropoulou et al. 1995). Spectral irradiance was measured with an OL 752 Optronic Spectroradiometer calibrated against an OL 752-150 module for wavelength accuracy and an OL 752-10 spectral irradiance standard. In order to calculate the time needed for the lamps to be on each day to achieve the desired UV-B_{BE} dose, the absolute spectral irradiance was weighed with the generalized plant action spectrum normalized at 300 nm (Caldwell 1971) and used in conjuction with the computer program of Björn & Murphy (1995).

Photosynthetic pigments were estimated spectrophotometrically in 100% methanolic extracts using the equations of Lichtenthaler & Wellburn (1983). UV-B absorbing compounds were assessed from UV-spectra of the same extract after appropriate dilution. Leaf area was measured with a LI-COR LI 3000 leaf area meter and leaf specific mass (LSM) was computed from dry mass and area measurements. Total epicuticular UV-B absorbing compounds were obtained by immersing the leaves in chloroform for 3 min (Wollenweber et al. 1991). The chloroform was evaporated and the dry residue was dissolved in methanol for spectrophotometry. The allelopathic potential of the water soluble epicuticular material was assessed against germination of letuce seeds as previously described (Stephanou & Manetas 1995). Fresh healthy leaves (14.5 g fresh weight) were immersed in 40 ml deionized water for 20 min under gentle shaking and the resulting solution was sterilized by passing through Corning, 0.45 μm sterile filters. The solution, after appropriate dilution, was used to test for allelopathic effects against letuce seed germination. Fifty seeds (surface sterilized with 4% v/v NaOCl) were evenly layered on filter paper and watered with 10 ml of either deionized water or test solutions in 9 cm Petri dishes. Germination tests were performed in a growth chamber under 26 μmol m^{-2} s^{-1} photosyntheticaly active radiation, 15 °C and a photoperiod of 12 h.

Results and discussion

Before rain treatment, the A_{300} cm^{-2} (see Table 1 for definition) of epicuticular material was 2.528 ± 0.382. As indicated in the legend of Table 1, artificial rain removed from the leaves almost 75% of their epicuticular UV-B absorbing capacity. However, the initial values were restored during the first 20 days of the experiment. The rate of accumulation of epicuticular UV-B absorbing compounds was the same under all treatments during this initial period (not shown). At plant harvest, however, a trend for increased A_{300} cm^{-2} (epicuticular) was evident with increasing UV-B_{BE} dose (Table 1). No such effect was observed on the internal UV-B absorbing compounds, although their level was doubled during the course of the experiment (Table 1). This selective increase of superficially located phenolics and flavonoids may be physiologically meaningful, if considered in conjunction with their proposed function as a UV-B radiation screen (Caldwell et al. 1983). Such a protective role, however, cannot be assessed for *D. viscosa*, since no UV-B radiation effects on leaf, stem or root dry weight were observed in plants which were initially devoid of their epicuticular UV-B absorbing capacity (Table 1). Phenolics and flavonoids yet have a variety of other ecologically important roles (Dacora 1995), and epicuticular compounds of *D. viscosa* in particular, are highly toxic against seed germination and seedling development of other species (Stephanou & Manetas 1995). This allelopathic potential, however, was not influenced by UV-B radiation (Table 1) and, accordingly, the significance of an increase of epicuticular phenolics and flavonoids for *D. viscosa* fitness can not be appraised at the moment.

UV-B radiation had no effect on chlorophyll (chl) content but increased considerably the levels of total carotenoids, thereby increasing the ratio of photoprotective to photoselective pigments (Table 1). UV-B radiation is considered a potential oxidative stress (Panagopoulos et al. 1990), and carotenoids have been

Table 1. UV-B radiation effects on various parameters measured after 80 days under the indicated UV-B_{BE} daily doses. Initial values were as follows: total leaf area (cm^2 $plant^{-1}$), 43 ± 10, 46 ± 10 and 40 ± 10; A_{300} cm^{-2} for epicuticular material, 0.645 ± 0.105, 0.603 ± 0.035, and 0.649 ± 0.120; A_{300} cm^{-2} for internal UV-B absorbing compounds, 1.543 ± 0.151, 1.453 ± 0.245 and 1.732 ± 0.297. Different letters in each row indicate statistically significant differences at $p < 0.05$ (**) or $p < 0.10$ (*). $n = 10$ in all cases

	UV-B_{BE}, kJ m^{-2} day^{-1}		
	0.06	6.41	10.14
A_{300} cm^{-2}, epicuticular[z]	4.008[a]	4.263[a]	4.808[b]*
A_{300} cm^{-2}, internal [z]	3.443[a]	3.334[a]	3.680[a]
% germination (lettuce)[y]	33[a]	30[a]	35[a]
chl, μg cm^{-2}	18.97[a]	18.48[a]	14.93[a]
chl, mg g^{-1} DW	2.189[a]	2.138[a]	2.329[a]
carotenoids, μg cm^{-2}	0.96[a]	1.39[b]**	1.03[c]*
carotenoids, mg g^{-1} DW	0.109[a]	0.151[b]**	0.155[b]
carotenoids / chl	0.051[a]	0.075[b]**	0.069[b]
Leaf DW, g $plant^{-1}$	2.17[a]	2.26[a]	2.13[a]
Stem DW, g $plant^{-1}$	1.49[a]	1.16[a]	1.22[a]
Root DW, g $plant^{-1}$	2.90[a]	2.70[a]	2.33[a]
Leaf area, cm^2	249[a]	284[a]	329[a]
LSM, g DW dm^{-2}	0.87[a]	0.80[a]	0.65[b]**

[z] A_{300} cm^{-2} is the 1 cm light path absorbance at 300 nm of a 1 cm^3 methanolic extract taken quantitavely from 1 cm^2 leaf area.
[y] After two weeks at 15 °C on filter paper moistened with 10 cm^3 of water rinse taken from 10 cm^2 of leaf area. Control values (% seed germination in deionized water), 84 ± 4.

shown *in vitro* to quench effectivelly active oxygen species (Larson 1988) and they have been implicated in the protection of the photosynthetic apparatus against UV-B radiation damage (Middleton & Teramura 1993). An increase in the carotenoid to chl ratio by enhanced UV-B radiation has been recently observed in a field experiment with the Mediterranean shrub *Phlomis fruticosa* (Nikolopoulos et al. 1995). Further experiments are needed to identify the specific carotenoids induced by UV-B radiation.

An unexpected finding was the considerable reduction in leaf specific mass (LSM) under the highest UV-B radiation dose (Table 1). LSM, expressing the mass invested per unit leaf area, is a function of leaf thickness and mesophyll compactness. Some authors have reported an increase in leaf thickness by UV-B radiation, suggesting that in this case the possibility for UV-B radiation penetration into deeper mesophyll layers is reduced (Bornman & Vogelmann 1991; Cen & Bornman 1990). In our case, the reduction in LSM was accompanied by a trend towards bigger leaf areas (Table 1), resulting in no net change of leaf dry weight and indicating that UV-B radiation favoured the selective allocation of photosynthate to the production of assimilative surfaces at the expense of leaf thickness. Similar results have been recently obtained by Saxten & Bornman (1994).

A thinner leaf and/or a less compact mesophyll may have a considerable influence on gas exchange, increasing the mesophyll diffusive conductance to both CO_2 and water vapour. The final effect on growth would obviously depend on the relative importance of CO_2 assimilation versus water loss, which varies with resource availability and, accordingly, with season. Further experiments are needed in order to assess the impact of these findings under field conditions.

Acknowledgements

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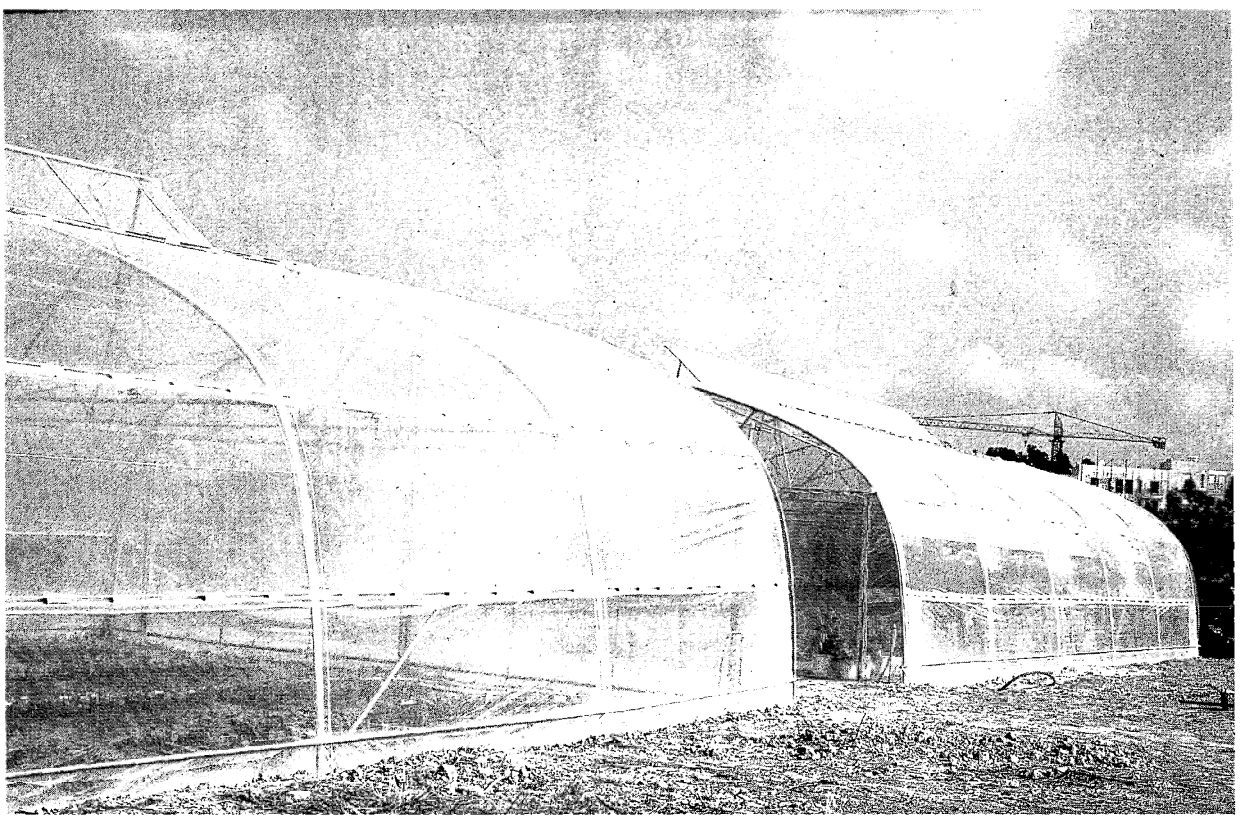

Greenhouses covered with differently absorbing plastic foils designed for UV-B attenuation experiments in Portugal.

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Effects of solar UV-B radiation on growth, flowering and yield of central and southern European bush bean cultivars (*Phaseolus vulgaris* L.)

M. Saile-Mark & M. Tevini*
*Botanical Institute II, University of Karlsruhe, D-76128 Karlsruhe, Germany (*author for correspondence)*

Key words: Bush bean cultivars, Flowering, Growth, Solar UV-B, UV-sensitivity index, Yield

Abstract

Different cultivars of bush beans (*Phaseolus vulgaris* L.) originating from Central and Southern Europe were grown from July to August/September 1993 up to 7 and 8 weeks, respectively, in two greenhouses covered by different UV-B-absorbing (280-320nm) plastic foils. By using the ambient UV-B radiation of the southern location (Portugal, 38.7°N, 9.1°W) in one of the greenhouses as intense UV-B radiation compared to the reduced radiation in the second greenhouse at the same place, a difference in UV-B of about 8–10% was simulated. All cultivars examined showed significant reductions in height of up to 31,8% in most growth phases under intense UV-B. Also fresh and dry weight as well as leaf area were reduced under intense UV-B in the cultivars Purple Teepee, Cropper Teepee and Goldstrahl, and in early growth phases also in Coco bianco, but with ongoing development this cultivar caught up. Cultivars Hilds Maja, Primel, Manata and Cannellino exhibited no UV-B effects on weight and leaf area. A flowering delay of up to 1 day was observed under intense UV-B in several cultivars. Probably due to this delay the yield (fresh weight of fruits) decreased in all cultivars up to 55% under intense UV-B at harvest time, while the potential yield (sum of buds, opened flowers and fruits) was reduced only in the cultivars Cropper Teepee, Purple Teepee, Cannellino and Goldstrahl. The UV-sensitivity index (UVSI) calculated according to the UV induced changes in growth, dry weight and yield at the second harvest date has shown that all cultivars are UV-sensitive, however the index was numerically higher for Southern European cultivars (average = 2.5) than for Central European ones (average = 2.3) which means that the first group was slightly less UV-sensitive than the second.

Introduction

The question of how enhanced UV-B radiation affects crop yield is still open. The answer, however, is of great importance for evaluating the potential effects of higher UV-B radiation on global agriculture and food supply in case ozone depletion will continue to progress. Enhanced UV-B radiation affects crop species and varieties in a differential way depending on their sensitivity, which may be determined by their genetic and enzymatic ability to accumulate UV-protecting pigments and to repair UV-B damage (Robberecht et al. 1980; Pang & Hays 1991; Takayanagai et al. 1994; Tevini et al. 1991). In a greenhouse study simulating a 20% ozone depletion scenario, one quarter of the 16 rice cultivars examined showed reduced biomass, leaf area and tiller number (Teramura et al. 1991). In another study in the Philippines (at the International Rice Research Institute) with 22 rice cultivars simulating a 5% ozone depletion biomass and leaf area were reduced under enhanced UV-B, but not the tiller number (Barnes et al. 1993). In a very extended greenhouse study in the Philippines with 188 rice cultivars, more than two thirds were sensitive to enhanced UV-B radiation (Dai et al. 1994). In the field the detrimental UV-B effects are often reduced, possibly due to the ameliorating action of UV-A and white light, especially by the repair of DNA-damage (Caldwell et al. 1994; Takayanagi et al. 1994). Nevertheless, in field experiments, in which solar UV-B is supplemented by artificial UV-B, the damage often observed can be modified by climatic conditions, such as drought and temperature. In a

six-year field study with Essex and Williams soybean cultivars yield reductions between 19 and 25% have been measured for Essex during 4 years, while, Williams cultivar increased its yield when a 25% ozone reduction was simulated. On the opposite, in two other years characterised by drought conditions Williams cultivar reacted with a yield reduction and Essex with a yield increase (Teramura et al. 1990).

Another critical point besides the variability of the experimental conditions in the evaluation of UV-B effects is the use of artificial radiation itself, which has to be weighted according to a suitable action spectrum. It has been discussed very often that the use of the wrong action spectrum not related to the plant response may lead to over or underestimation of the UV-B effects. For yield studies no action spectrum exists. There is an international consensus for the use of the generalized plant action spectrum according to Caldwell (1971). However, new results show that the use of this very steep action spectrum may overestimate the UV-B effect (Quaite et al. 1992). Another problem is that artificial UV radiation sources emit small but biologically very effective UV-C radiation, which is not present in the solar radiation at the earth surface. Cellulose acetate used to filter out the UV-C radiation is very unstable and has to be replaced very often during the experimental period. Even the best suntracking system supplementing solar UV-B by a defined amount of artificial UV-B will suffer from these inconveniences and uncertainties. For these reasons only solar radiation has been used to evaluate UV-B effects in the present study. Logically, this can be done only by attenuation experiments using a suitable and stable filter to reduce the ambient UV-B radiation to a certain amount. Considering this reduced radiation as the control radiation, the relatively enhanced UV-B radiation will supply information on the effects of a defined increment of only solar UV-B compared to the control. The paper describes the effects of a 8–10% difference in solar UV-B on growth, flowering and yield of 8 bush bean cultivars. The reasons for yield reduction may be alterations in the reproductive phase, e.g. flower suppression and/or delay of flowering as well as morphological changes. All these changes have been reported for plant species and cultivars under artificially enhanced UV-B radiation, but to our knowledge not under solely solar UV-B radiation (Barnes et al. 1990; Bornman & Teramura 1993; Caldwell & Flint 1994; Musil 1995; Tevini 1994; Tevini & Teramura 1989).

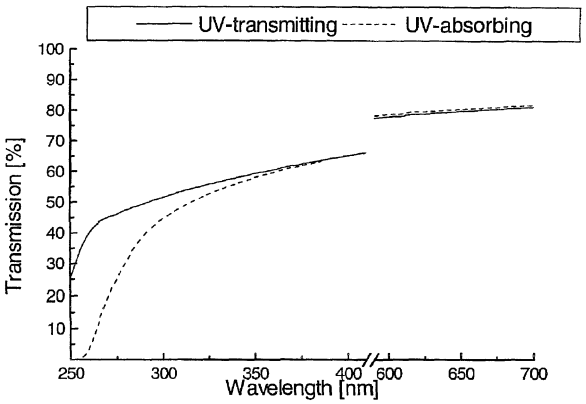

Figure 1. Transmission spectra of the two different greenhouse foils.

Materials and methods

Growing conditions

Four different Central European bush bean cultivars (*Phaseolus vulgaris* L. cv. Hilds Maja, cv. Primel, cv. Cropper Teepee, cv. Purple Teepee) and four Southern European cultivars (*Phaseolus vulgaris* L. cv. Coco bianco, cv. Cannellino, cv. Manata, cv. Goldstrahl) were grown in Portugal (38.7°N, 9.1°W) at the research station Quinta de São Pedro from July to August/September 1993 in two greenhouses covered with different UV-transmissible plastic foils (BP Chemicals PlasTec GmbH, D-64720 Michelstadt, FRG) generating a UV-B difference of about 8–10%. The transmission spectra are given in Figure 1. The irradiation conditions are indicated in the paper as follows:
–UV: plants grown under a partly UV-B absorbing foil,
+ UV: plants grown under UV-B transmitting foil.

For each cultivar two plots of 2 m^2 with 100 plants were prepared in each greenhouse. At the beginning of the growing season the soil was removed up to a depth of 50 cm and replaced by standard field soil. From both greenhouses soil samples were taken and analysed for nutrient and fertilizer content, which was the same in both greenhouses and adequate for beans. Plants were regularly watered by a computer controlled irrigation system.

Radiation conditions

To measure ambient radiation two Optronic radiometers were used. The measurements in Portugal (38.7°N)

Table 1. (a) Fluence rates (W m^{-2}) measured with an Optronic 742 at noon outside and inside the two greenhouses on the 3rd of August 1995. (b) Fluence rates (W m^{-2}) measured with an Optronic 752 at noon in Karlsruhe (49° N) on the 4th August 1994. (c) Comparison of the optical properties of new and used (for one year) plastic foils in percent of outside radiation.

	Outside			+UV			–UV		
	UV-B	UV-A	PAR	UV-B	UV-A	PAR	UV-B	UV-A	PAR
(a)									
03.08.95	2.61	44.3	399.1	2.10	39.1	342.4	1.94	37.6	347.1
(b)									
04.08.94	2.27	38.7	323.3	–	–	–	–	–	–
(c)									
new foil				–22.2	–16.8	–13.9	–27.4	–19.0	–11.9
used foil				–21.6	–16.8	–13.6	–26.1	–18.8	–12.0

were taken with an Optronic 742 and exemplary values for the radiation conditions at local noon are given in Table 1. As a comparison, the ambient radiation of Karlsruhe (49°N) at noon at the corresponding time of year is also provided. The radiation was measured in Karlsruhe with an Optronic 752. The radiation conditions in the greenhouse defined as –UV are comparable to the ambient conditions in Karlsruhe in absolute fluence rates as well as in the relation of UV-B to UV-A. Simulated was an increase of UV-B of about 8% which should be due to an ozone reduction of about 4–5%. The optical properties of the foils in respect to age were also checked with the Optronic 742. As shown in Table 1 there were only slight differences in the absorbance properties of new and used (one year old) foils.

Temperature conditions

The average of the daily mean temperature measured from the 1st to 14th of August inside the greenhouses was 24.6°C. Measurements from the Institute of Meteorology and Climate Research of the University of Karlsruhe revealed an average of 23.0 °C in Karlsruhe at that time period. Therefore, the plants were growing in Portugal under similar temperature regimes as in Central Europe.

Measurements

Every 7 days growth measurements (fresh and dry weight, height, leaf area) were performed until plants were fully developed and no further growth increases were obtained. For the determination of flowering the appearance of the first flower was recorded and the B_{50} value (B_{50}= days after sowing when 50% of the plants flowered) was calculated. Plants were harvested for the first time after 6 (*Phaseolus vulgaris* cv. Hilds Maja, cv. Primel, cv. Coco Bianco), respectively 7 weeks (*Phaseolus vulgaris* cv. Cropper Teepee, cv. Purple Teepee, cv. Cannellino, cv. Manata, cv. Goldstrahl) (first harvest) and the potential yield (sum of buds, opened flowers and fruits) was determined. At a second harvest, after 7 respectively 8 weeks, only the yield (fresh weight of fruits) was determined. Differences in the UV-sensitivity of the 8 cultivars were ascertained by a UV sensitivity index (UVSI) which was calculated according to the following equation (Saile-Mark 1993):

$$\text{UVSI} = \frac{\text{height}_{+\text{UV}}}{\text{meanheight}_{-\text{UV}}} + \frac{\text{dryweight}_{+\text{UV}}}{\text{meandryweight}_{-\text{UV}}} + \frac{\text{yield}_{+\text{UV}}}{\text{meanyield}_{-\text{UV}}}.$$

An UV-tolerant plant has an UVSI of 3 whereas UVSI values below 3 indicate an UV-sensitive plant.

Statistical analysis

All data are presented as means of 20 to 25 plants and the corresponding standard deviation. For estimating significant differences of the means a two-sample *t*-test with (α=0.05 was performed. A one sample t-test (α=0.05) was used to evaluate whether the UVSI of the plants are 3 or lower. In the Tables significant differences are indicated by an asterisk.

Results

Growth

All cultivars of bush beans grown under an 8–10% higher UV-B radiation showed reductions in plant

Table 2. Plant height (cm) (a) and leaf area (cm^2 (b) of four different Central European bush bean cultivars depending on age and radiation conditions

Cultivar		2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks
(a)								
Hilds	–UV	5.96	14.17	29.3	37.86	38.80	39.26	–
Maja		±1.25	±4.60	±7.28	±9.17	±9.33	±8.37	
	+ UV	5.39*	12.55*	26.67*	34.72*	35.17*	36.16*	–
		±1.16	±3.65	±7.07	±8.94	±8.40	±8.28	
Primel	–UV	4.62	10.06	23.31	43.74	48.01	49.15	–
		±0.89	±2.11	±4.71	±8.46	±7.78	±6.06	
	+ UV	4.19*	9.23*	21.28*	39.71*	44.56*	44.95*	–
		±0.92	±2.36	±4.74	±8.47	±7.28	±6.57	
Cropper	–UV	3.77	6.35	13.69	28.00	39.02	40.94	41.89
Teepee		±0.84	±1.66	±4.86	±7.60	±6.10	±8.57	±7.40
	+UV	3.35*	5.15*	10.13*	20.54*	30.98*	34.54*	36.00*
		0.89	±1.53	±4.24	±7.90	±7.02	±6.52	±7.05
Purple	-UV	2.80	4.35	8.10	17.28	25.32	26.72	28.05
Teepee		±0.79	±1.18	±2.47	±4.55	±5.41	±5.55	±5.52
	+UV	2.56*	3.70*	5.89*	11.78*	17.63*	18.73*	19.85*
		±0.66	±1.11	±2.22	±4.09	±4.68	±4.06	±4.26
(b)								
Hilds	–UV	145.32	296.04	539.50	1116.66	1498.84	1647.13	–
Maja		±42.98	±100.49	±158.73	±343.81	±278.42	±505.68	
	+UV	144.37	257.48	526.04	967.45	1288.21	1654.89	–
		±32.83	±46.93	±156.77	±240.15	±571.60	±354.41	
Primel	–UV	131.63	287.40	491.09	971.35	1286.57	1587.81	–
		±28.60	±69.99	±147.20	±186.95	±271.93	±380.42	
	+UV	138.52	256.97	441.97	850.18	1209.14	1432.83	–
		±24.95	±83.52	±159.63	±198.09	±249.62	±523.04	
Cropper	–UV	91.95	159.83	411.00	786.36	1329.69	1594.24	1685.47
Teepee		±19.94	±55.68	±150.95	±220.21	±448.35	±257.97	±368.77
	+UV	85.72	119.11*	224.18*	438.54*	703.15*	927.23*	995.08*
		±22.74	±20.56	±103.97	±132.11	±246.93	±381.68	±283.49
Purple	–UV	93.03	170.07	306.23	692.56	1147.13	1329.08	1339.3
Teepee		±31.62	±38.32	±113.53	±133.15	±293.13	±311.80	±308.68
	+UV	85.56	160.31	205.41*	450.44*	893.31*	962.86*	969.39*
		±16.25	±20.06	±64.64	±79.07	±239.95	±333.88	±336.80

height (Tables 2 and 3), which were not correlated with a reduction of fresh or dry weight (Tables 4 and 5) in the cultivars Hilds Maja, Primel and Cannellino. Only the cultivars Purple Teepee, Cropper Teepee and Goldstrahl showed major significant reductions in fresh weight caused by higher UV-B at nearly all ages, whereas Coco bianco and Manata showed only reductions after 3 and/or 4 weeks. Cultivars (e.g. Cropper Teepee, Purple Teepee, Goldstrahl) which had significant reductions in weight had also smaller leaf areas (Tables 2 and 3).

Onset of flowering

In all Central European cultivars the onset of flowering was delayed by about one day (Figure 2). The Southern European cultivars showed only an initial delay in flowering growing under higher UV-B, but the 100%

Table 3. Plant height (cm) (a) and leaf area (cm^2 (b) of four different Southern European maize cultivars depending on age and radiation conditions.

Cultivar		2 weeks	3 weeks	4 weeks	5weeks	6 weeks	7 weeks	8 weeks
(a)								
Coco	-UV	4.68	9.82	28.14	38.97	39.96	41.28	–
bianco		±0.94	±2.12	±6.87	±6.15	±7.88	±8.71	
	+UV	4.74	9.31	25.81*	33.63*	34.15*	36.91*	–
		±0.82	±1.88	±4.61	±5.22	±6.00	±5.88	
Cannellino	-UV	4.69	9.09	30.41	48.62	51.81	54.48	55.46
		±0.87	±2.41	±6.50	±9.56	±7.38	±8.05	±6.78
	+UV	4.53	8.40*	25.69*	45.25*	48.71*	49.71*	50.46*
		±0.84	±2.16	±7.16	±10.02	±8.89	±8.39	8.51
Manata	-UV	5.11	11.06	35.78	51.94	54.41	55.15	56.29
		±1.03	±3.25	±13.29	±10.58	±11.42	±9.94	±10.29
	+UV	4.93	10.47	34.12	46.38*	47.94*	49.47*	50.63*
		±1.16	±2.98	±10.89	±9.76	±9.76	±9.67	±9.49
Goldstrahl	-UV	5.54	8.84	49.09	113.92	143.19	158.32	169.68
		±1.50	±3.25	±21.13	±23.47	±26.45	±25.82	±25.63
	+UV	4.68*	6.60*	33.45*	98.89*	131.51*	148.09*	154.88*
		±1.59	±2.18	±17.31	±22.01	±24.50	±22.07	±20.70
(b)								
Coco	-UV	165.41	337.42	822.78	1675.29	2075.89	2197.09	–
bianco		±26.93	±57.54	±130.02	±453.55	±452.42	±657.89	
	+UV	162.24	277.3*	651.46*	1393.38*	1974.92	2048.76	–
		±33.61	±48.37	±101.78	±228.45	±430.74	±326.19	
Cannellino	-UV	126.27	242.54	515.89	1071.57	1616.49	2040.16	2127.65
		±27.77	±52.26	±53.97	±238.46	±181.00	±612.98	±590.70
	+UV	113.05	230.43	472.63	890.64	1482.06	1804.19	2080.22
		±24.76	±65.51	±144.21	±249.57	±344.33	±669.85	±593.86
Manata	-UV	169.21	287.22	649.34	828.72	1587.75	1777.42	1786.13
		±34.98	±61.12	±127.95	±105.23	±513.03	±399.76	±299.35
	+UV	153.64	272.76	572.76	883.25	1393.65	1692.90	1798.98
		±44.21	±41.78	±119.56	±229.18	±516.16	±538.72	±444.75
Goldstrahl	-UV	107.07	207.45	399.28	1020.34	1847.70	2269.08	3767.22
		±15.13	±45.54	±145.79	±223.37	±371.61	±580.97	±700.99
	+UV	102.84	164.12*	333.23	762.67	1510.46	1795.58	2491.80*
		±16.32	±42.18	±127.70	±254.39	±452.54	±223.62	±630.90

level was reached nearly at the same time under both growing conditions (Figure 3).

Yield

The potential yield of the Central European cultivars Cropper Teepee and Purple Teepee (Table 6) as well as the Southern European cultivars Cannellino and Goldstrahl (Table 7) was reduced under higher UV-B radiation at the first harvest after 7 weeks. At this time all Central European cultivars and the Southern cultivars Cannellino and Manata showed UV induced reductions in yield caused by smaller and/or less fruits. At the second harvest one week later all cultivars (Table 8) showed reductions in yield varied between 15% (cv. Coco bianco) and 55% (cv. Cropper Teepee and cv. Goldstrahl) depending on cultivar.

Table 4. Fresh (g) (a) and dry weight (g) (b) of four different Central European bush bean cultivars depending on age and radiation conditions

Cultivar		2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks
(a)								
Hilds	–UV	6.16	12.87	23.87	48.36	69.81	73.62	–
Maja		±1.91	±4.23	±7.41	±12.24	±15.47	±20.15	
	+UV	6.26	11.01	22.39	45.03	66.57	74.92	–
		±1.67	±2.29	±7.07	±12.63	±17.94	±17.39	
Primel	–UV	6.45	13.16	22.37	47.44	59.72	77.13	–
		±1.56	±3.36	±6.81	±9.15	±12.55	±19.86	
	+UV	6.38	12.11	21.46	41.51	56.31	71.79	–
		±1.15	±3.81	±6.06	±11.06	±10.73	±22.95	
Cropper	–UV	4.35	8.45	19.40	38.95	64.82	83.75	84.00
Teepee		±0.89	±3.48	±7.61	±17.78	±21.71	±14.72	±18.41
	+UV	3.82	5.45*	10.13*	20.97*	31.48*	43.06*	45.34*
		1.05	±1.09	±4.63	±8.32	±15.17	±12.22	±12.41
Purple	–UV	3.95	7.24	13.50	31.05	51.65	57.52	58.83
Teepee		±0.93	±1.46	±4.98	±6.15	±17.19	±15.02	±17.38
	+UV	3.60	6.61	8.16*	19.67*	37.44*	40.06*	40.28*
		±0.78	±0.82	±2.42	±3.55	±15.24	±14.11	±18.21
(b)								
Hilds	–UV	0.75	1.80	3.36	6.12	9.19	11.29	–
Maja		±0.24	±0.58	±1.05	±2.33	±2.19	±3.98	
	+UV	0.73	1.46	2.91	5.16	8.25	10.71	–
		±0.24	±0.39	±1.02	±2.08	±3.41	±3.21	
Primel	–UV	0.78	1.83	3.13	6.38	9.09	12.89	–
		±0.20	±0.44	±0.96	±1.77	±2.04	±3.84	
	+UV	0.79	1.72	2.62	5.09	8.26	10.37	–
		±0.16	±0.52	±0.82	±1.67	±1.54	±4.12	
Cropper	–UV	0.52	1.12	2.44	4.40	7.73	11.36	12.13
Teepee		±0.12	±0.50	±1.05	±1.86	±2.83	±1.33	±2.37
	+UV	0.46	0.69*	1.28*	2.36*	3.75*	5.80*	6.48*
		±0.13	±0.16	±0.60	±0.89	±2.29	±3.21	±2.09
Purple	–UV	0.47	0.97	1.74	3.34	5.93	6.81	7.09
Teepee		±0.12	±0.17	±0.64	±0.56	±2.31	±1.54	±1.65
	+UV	0.46	0.91	1.09*	2.24*	4.09*	4.52*	5.43*
		±0.10	±0.19	±0.36	±0.41	±1.95	±1.47	±1.90

UV sensitivity index

The sensitivity index of all cultivars was significantly less than 3, which means that all cultivars are UV sensitive to some extent (Table 8).

Discussion

Central European cultivars of bush beans as well as the Southern European cultivars showed reductions in plant size when grown under a relative enhancement of UV-B by about 8–10%. This could be due to a photooxidative destruction of the phytohormone indole acetic acid as demonstrated in sunflower seedlings under high UV and low white light conditions (Ros & Tevini 1995). UV-induced reductions of fresh

Table 5. Fresh (g) (a) and dry weight (g) (b) of four different Southern European bush bean cultivars depending on age and radiation conditions

Cultivar		2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks
(a)								
Coco	–UV	6.17	12.42	31.04	60.48	78.86	80.44	–
bianco		±0.90	±2.05	±4.97	±10.13	±17.64	±19.19	
	+UV	6.12	10.52*	23.83*	56.12	75.11	79.86	–
		±1.32	±1.85	±3.83	±7.93	±17.71	±14.79	
Cannellino	–UV	5.52	10.54	23.30	45.08	71.93	81.23	83.98
		±1.31	±2.20	±2.27	±11.54	±7.43	±19.68	±15.64
	+UV	4.96	9.96	21.04	37.93	64.71	70.99	74.03
		±1.08	±3.16	±5.95	±9.74	±16.89	±18.67	±13.78
Manata	–UV	7.19	12.66	28.47	40.99	65.68	78.72	79.43
		±1.51	±2.56	±3.88	±5.37	±16.37	±19.01	±14.11
	+UV	6.77	11.57	21.56*	37.25	59.75	79.68	81.48
		±2.00	±2.25	±3.21	±8.06	±21.46	±25.86	±19.13
Goldstrahl	–UV	4.41	8.29	17.91	45.77	78.62	103.52	172.06
		±0.69	±1.84	±7.19	±11.68	±15.61	±27.71	±35.80
	+UV	4.31	6.44*	14.05	34.73	58.82*	83.87	116.22*
		±0.76	±1.71	±5.81	±12.46	±13.44	±10.02	±40.62
(b)								
Coco	–UV	0.90	1.86	4.04	8.23	11.64	13.37	-
bianco		±0.12	±0.33	±0.58	±1.03	±2.22	±3.08	
	+UV	0.86	1.56*	3.32*	7.13*	11.00	12.74	-
		±0.15	±0.29	±0.60	±1.25	±2.89	±2.41	
Cannellino	–UV	0.69	1.68	3.13	6.24	11.00	14.79	16.40
		±0.19	±0.34	±0.43	±2.00	±1.12	±3.16	±2.96
	+UV	0.57	1.62	2.79	4.94	10.08	12.54	14.86
		±0.13	±0.48	±0.72	±1.47	±2.77	±3.04	±2.34
Manata	–UV	0.97	1.70	3.64	5.30	8.54	12.53	12.83
		±0.20	±0.41	±0.47	±0.58	±3.04	±1.86	±3.14
	+UV	0.88	1.51	3.24	5.12	7.66	12.06	12.82
		±0.26	±0.28	±0.68	±1.50	±2.73	±4.30	±3.73
Goldstrahl	–UV	0.50	1.19	2.19	5.59	9.70	15.47	28.68
		±0.07	±0.33	±0.98	±1.19	±1.68	±3.52	±6.65
	+UV	0.49	0.86*	1.75	4.33	7.86*	14.06	20.04*
		±0.10	±0.25	±0.71	±1.76	±1.58	±1.64	±5.83

and dry weight as well as leaf area were detected only in the cultivars Purple Teepee, Cropper Teepee and Goldstrahl. Decreases in dry weight under higher UV-B radiation may be caused by reduced photosynthetic rates due to reductions in enzyme activities (Jordan et al. 1992; Mark 1992), in the efficiency of the photosystem II (Strid et al. 1990; Bornman & Vogelmann 1991; Tevini et al. 1991) and in stomatal conductance (Teramura et al. 1983; Negash & Björn 1986).

In general, the results of the growth analysis confirm earlier findings under artificial or solar radiation supplemented by artificial UV-B radiation where general growth reductions were obtained (Biggs et al. 1981; Lydon et al. 1986; Teramura & Murali 1986). In this paper it was shown that even slightly higher solar UV-B radiation can cause similar growth reductions, mostly pronounced in younger plants and depending on the sensitivity of the cultivars. For example, the cultivars Coco Bianco and Manata showed only weight

Table 6. Distribution of buds, opened flowers and fruits (number/plant), as well as potential yield (PY=sum of buds, flowers and fruits) and yield (Y=fresh weight of fruits in g) of four different Central European bush bean cultivars

	Hilds Maja		Primel		Cropper Teepee		Purple Teepee	
	–UV	+UV	–UV	+UV	–UV	+UV	–UV	+UV
buds	10.50	16.10*	8.80	9.00	14.20	10.70	2.45	2.30
(number)	±4.76	±4.35	±4.44	±3.13	±8.81	±6.38	±2.93 ±2.05	
flowers	5.10	5.50	3.10	2.95	6.60	5.00*	1.30	1.65
(number)	±2.10	±1.79	±1.97	±2.04	±3.07	±2.51	±1.38	±1.87
fruits	22.35	19.50	13.05	12.25	26.20	18.5*	25.35	15.70*
(number)	±6.57	±7.11	±3.09	±2.94	±7.59	±6.68	±8.02	±5.48
PY	37.95	41.10	24.95	24.20	47.00	34.20*	29.10	19.65*
(number)	±9.31	±11.27	±6.51	±4.35	±14.30	±12.39	±10.31	±5.98
Y	6.43	3.51*	2.31	1.96	5.60	2.40*	28.56	8.30*
(g)	±2.87	±2.61	±1.37	±1.16	±3.29	±2.44	±13.26	±4.99

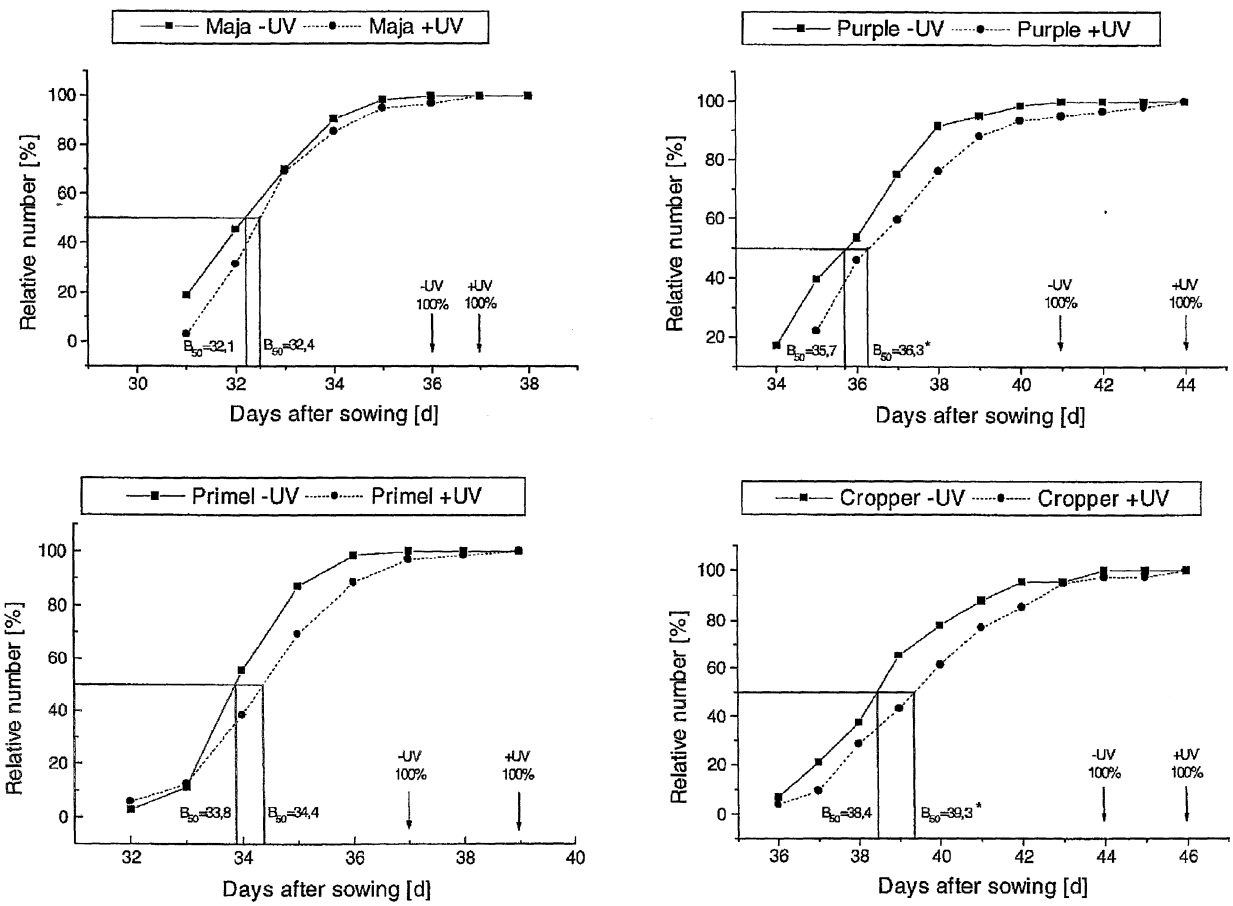

Figure 2. Onset of flowering of four different Central European bush bean cultivars under two UV irradiation conditions (B_{50} values = days after sowing when 50% of the plants flowered).

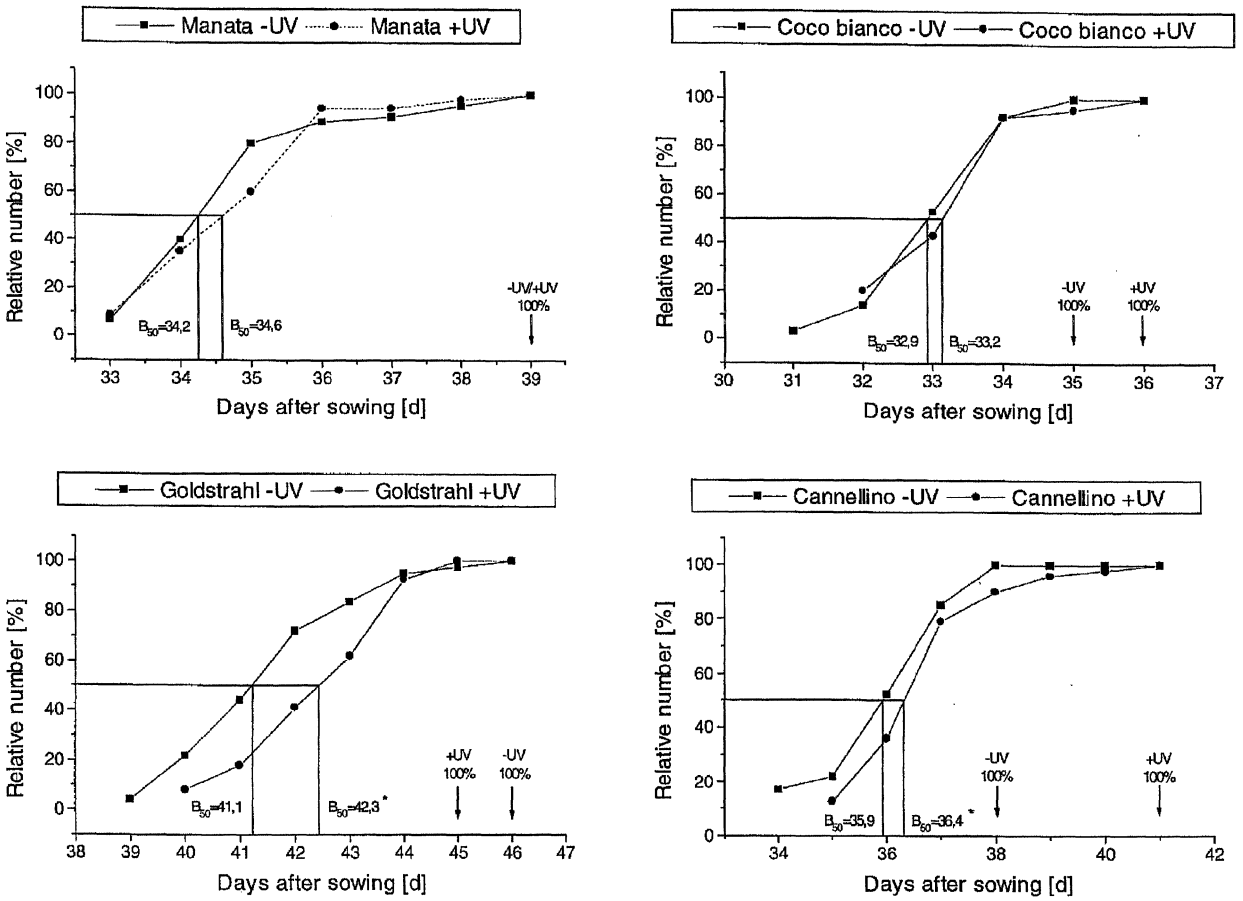

Figure 3. Onset of flowering of four different Southern European bush bean cultivars under two UV irradiation conditions (B_{50} values = days after sowing when 50% of the plants flowered).

Table 7. Distribution of buds, opened flowers and fruits (number/plant), as well as potential yield (PY=sum of buds, flowers and fruits) and yield (Y=fresh weight of fruits in g) after 6/7 weeks of four different Southern European bush bean cultivars

	Coco bianco		Cannellino		Manata		Goldstrahl	
	–UV	+UV	–UV	+UV	–UV	+UV	–UV	+UV
buds	12.20	13.55	6.90	2.60*	2.60	4.45	35.33	32.00
(number)	±4.35	±4.06	±4.35	±2.89	±2.54	±4.57	±9.43	±7.48
flowers	3.25	3.05	3.05	1.20*	2.45	2.40	9.50	9.94
(number)	±2.65	±3.27	±1.96	±1.06	±1.76	±2.21	±3.37	±3.39
fruits	18.60	16.15*	23.10	21.40	30.30	29.45	18.83	10.33*
(number)	±4.15	±4.66	±6.14	±5.00	±8.28	±6.72	±7.08	±4.84
PY	34.05	32.75	33.05	25.20*	35.35	36.30	63.67	52.28*
(number)	±7.69	±7.40	±10.13	±5.47	±7.86	±9.04	±11.09	±10.71
Y	6.24	5.41	34.39	23.28*	48.31	32.61*	1.70	1.40
(g)	±2.51	±2.70	±11.57	±9.63	±13.44	±11.49	±1.67	±1.44

Table 8. Yield (g fresh weight of fruits/plant), UV-sensitivity index (UVSI) of Central (a) and Southern (b) European bush bean cultivars at harvest time after 7/8 weeks

(a)

	Hilds Maja	Primel	Cropper Teepee	Purple Teepee
–UV	49.97±20.18	46.67±25.33	52.93±31.47	61.89±22.69
+ UV	40.87*±19.84	28.07*±13.87	23.85*±15.42	28.32*±14.57
% of control	81.79	60.15	45.06	45.76
UVSI	2.87*±0.38	2.52*±0.65	1.95*±0.52	1.94*±0.30

(b)

	Coco bianco	Cannellino	Manata	Goldstrahl
–UV	50.93±20.52	70.63±20.53	115.75±43.55	45.50±22.31
+ UV	43.28*±12.10	49.84*±14.98	91.71*±34.54	20.27*±15.30
% of control	84.98	70.56	79.23	44.55
UVSI	2.73*±0.41	2.51*±0.23	2.70*±0.40	2.09*±0.27

reductions in young plants which disappeared in further developmental phases. This indicates a delay of development at early phases and acceleration at later phases additionally caused by higher UV-B levels. The same effect was also observed in bush beans when artificial UV-B irradiation was applied in a glass house (Saile-Mark 1993). In this study it was shown that growth regulators inhibiting the gibberellin biosynthesis were delaying the flower induction as under enhanced UV-B.

The delay of the onset of flowering observed in nearly all cultivars was also demonstrated in earlier studies using artificial lamp systems (Rau et al. 1988; Ziska et al. 1992; Musil 1995). The reason for the delay is probably the impact of enhanced UV-B radiation on the biosynthesis of gibberellins as described for *Hyoscyamus niger* (Rau et al. 1988) and bush beans (Saile-Mark 1993).Due to the flowering delay and/or to potential yield reductions, reductions in harvestable yield were observed in all cultivars at the second harvest date. Teramura et al. (1990) found analogous yield reductions in the field under artificially enhanced UV-B in soybeans. In case that higher UV-B radiation delays flowering and thus harvestable yield in general, this may have severe economical and ecological consequences for many crops and natural ecosystems as well. In many geographical areas late harvests of crops might not be possible due to bad weather conditions. On the other hand, in natural ecosystems pollinators may not be available when the flowers are in their final reproductive phase. This may lead to changes in biodiversity.

All cultivars have a sensitivity index <3. Therefore, all of them are UV sensitive although the two Southern European cultivars Coco bianco and Manata as well as the Central European cultivar Hilds Maja have UVSI's >2.7 which means that they are affected much less by UV-B than e.g. the cultivar Purple Teepee with an UVSI of 1.9. This indicates that it might be possible with special selection and breeding of plant cultivars to compensate the negative UV-B effects in case increase of UV-B radiation is continuing due to ozone depletion.

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Morphological and physiological responses of bean plants to supplemental UV radiation in a Mediterranean climate

F. Antonelli, D. Grifoni, F. Sabatini & G. Zipoli
I.A.T.A.-C.N.R. P.le delle Cascine 18, 50144, Firenze, Italy

Key words: Bean, Field experiment, Plant growth, Stratospheric ozone depletion, UV-A radiation, UV-B radiation

Abstract

During the last few decades many experiments have been performed to evaluate the responses of plants to enhanced solar UV-B radiation (280–320 nm) that may occur because of stratospheric ozone depletion; most of them were performed in controlled environment conditions where plants were exposed to low photosynthetically active radiation (PAR) levels and high UV-B irradiance. Since environmental radiative regimes can play a role in the response of plants to UV-B enhancement, it appears doubtful whether it is valid to extrapolate the results from these experiments to plants grown in natural conditions. The objective of this work was to evaluate the effects on physiology and morphology of a bean (*Phaseolus vulgaris* L.) cultivar Nano Bobis, exposed to supplemental UV radiation in the open-air. UV-B radiation was supplied by fluorescent lamps to simulate a 20% stratospheric ozone reduction. Three groups of plants were grown: control (no supplemental UV), UV-A treatment (supplementation in the UV-A band) and UV-B treatment (supplemental UV-B and UV-A radiation). Each group was replicated three times. After 33 days of treatment plants grown under UV-B treatment had lower biomass, leaf area and reduced leaf elongation compared to UV-A treatment. No significant differences were detected in photosynthetic parameters, photosynthetic pigments and UV-B absorbing compounds among the three groups of plants. However, plants exposed to UV-A treatment showed a sort of 'stimulation' of their growth when compared to the control. The results of this experiment showed that plants may be sensitive to UV-A radiation, thus it is difficult to evaluate the specific effects of UV-B (280–320 nm) radiation from fluorescent lamps and it is important to choose the appropriate control. Environmental conditions strongly affect plant response to UV radiation so further field studies are necessary to assess the interaction between UV-B exposure and meteorological variability.

Introduction

During the last twenty years a reduction of the Earth stratospheric ozone layer caused by contamination with man-made chlorofluorocarbons (Anderson et al. 1991; Bojkov 1994; Manney et al. 1994) has occurred. The amount of solar UV-B radiation (280–320 nm) reaching the Earth surface is consequently increasing (Madronich 1993) and several studies were started to assess the possible impact of new levels of UV-B radiation on agricultural and natural ecosystems.

Almost 80% of these experiments (Caldwell & Flint 1994) were conducted in artificial environments (growth chambers or glasshouses) and they were often characterized by high doses of supplemental UV-B radiation, together with low levels of UV-A (320–400 nm) and photosynthetically active radiation (PAR). The radiative environment, especially the UV-B/PAR and the short-wavelengths UV-A (320–340 nm)/long-wavelengths UV-A (340–400 nm) ratios were very different from natural sunlight. Under such conditions UV-B supplementation negatively affected photosynthesis (He et al. 1993; Sisson 1981; Tevini & Iwanzik 1983; Ziska et al. 1992) and produced morphological alterations and growth inhibition (Barnes et al. 1990; Teramura & Sullivan 1987; Tevini et al. 1981). Very few studies have been performed under field conditions and only one was performed under Mediterranean field conditions (Nikolopoulos et al. 1995). These field experiments generally indicated that plant

effects induced by UV-B radiation were less prounounced than those observed in controlled environments (Caldwell & Flint 1994; Teramura 1983). The results obtained under the altered radiative conditions of controlled environment studies could lead to erroneous extrapolation of an amplified plant sensitivity to natural conditions. Field experimentation is needed to evaluate realistic consequences of increased solar UV-B, but to obtain consistent forecasts on future ozone depletion scenarios it is necessary to have a better understanding of the interaction between UV-B radiation and other meteorological parameters. In a six years field study on soybean Teramura et al. (1990) observed a great variability of UV-B plant responses depending on varied meteorological conditions.

An open-air experiment has been designed to test the sensitivity of the bean cultivar Nano Bobis to enhanced UV radiation in a Mediterranean climate. Plant responses were analyzed in terms of morphological and physiological parameters.

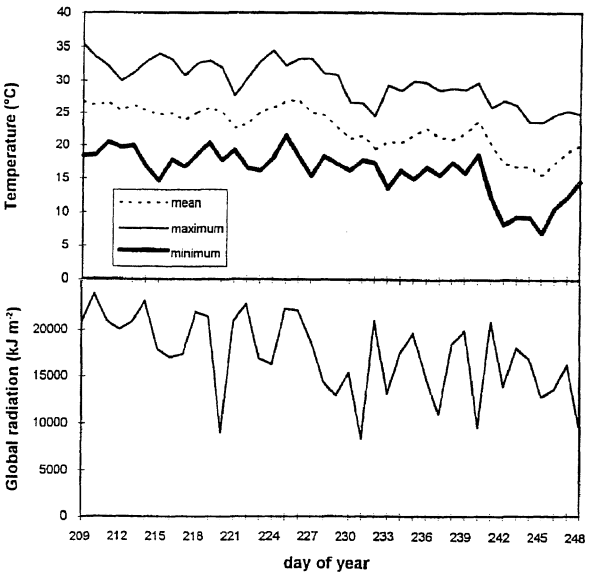

Figure 1. Meteorological conditions recorded in Pistoia, Italy, during the experimental period (28 July – 4 September 1995).

Methods

Experimental site, plant material and meteorological conditions

A 'field' experiment was conducted from 28 July (209th day of the year) to 5 September 1995 (248th day of the year) at the Nursery Experimental Centre (Ce.Spe.Vi), Pistoia, Italy, lat N. 43° 56', long E. 10° 54'.

Two seeds of bean (*Phaseolus vulgaris* L. cv. Nano Bobis) were sown in plastic pots (3.5 l) filled with a standard greenhouse mixture composed of 2:1 (v/v) mould:pumice and premixed with 1.4 g l^{-1} of a granular 15-9-15-2 (N-P-K-Mg) commercial fertilizer. When plants were 0.05 m tall they were manually thinned to 1 plant/pot and they were placed under UV treatments. During the experiment plants were automatically watered twice daily.

Global solar radiation, air temperature and other meteorological parameters were monitored at a station located approximatively 100 m far from the experimental site.

Daily global radiation (Figure 1) showed large variability and decreased throughout the experiment. Effectively, several days were overcast and solar radiation and air temperature decreased consequently. In fact the mean air temperature of August 1995 (the crucial period for the plant growth) was 22.6 °C while the average of August during the period 1951 to 1995 was 23.4 °C (Marzialetti 1995).

UV supplementation and experimental design

The apparatus for ultraviolet supplementation consisted of nine aluminium racks each one equipped with six commercially available fluorescent lamps (type UVB-313 Q-Panel, Cleveland, OH). Sixty plants were grown under each rack and an extra edge pot row was seeded to minimize border effects. Lamps were spaced in each rack at variable distances from each other in order to obtain an area (0.6 × 1.5 m) characterized by an uniform distribution of UV-B radiation (this area received at least 90% of the maximum UV radiation). Data presented in this paper were obtained from measurements performed on 30 plants grown within this area. Racks were adjustable in height to maintain a constant distance of 0.5 m above the top of vegetation.

Lamps were driven by high frequency ballasts and their emission was manually modulated by means of a fluorescent lamp dimmer which regulated the input voltage to the ballasts. Lamps were preburnt for 70 h to minimize irradiation changes in time (Adamse & Britz 1992) and they were wrapped with filters either of 75 m thick cellulose diacetate (c.a.) film (which absorbed radiation below 292 nm) for the UV-B supplementation treatment, or 130 m thick polyester plastic

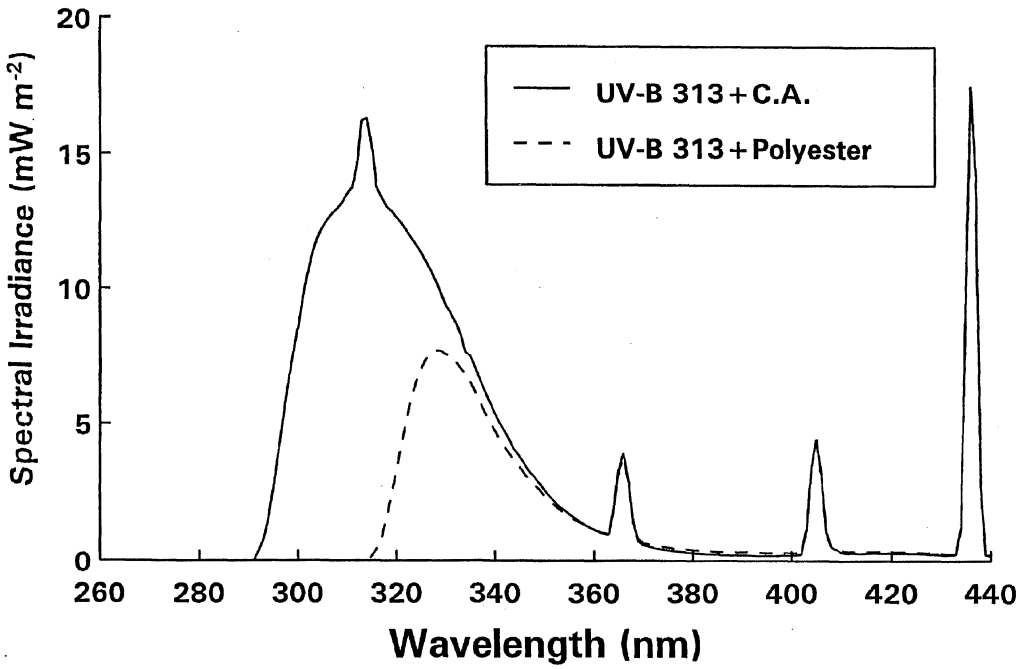

Figure 2. Unweighted spectral irradiances from filtered Q-Panel UV-B 313 fluorescent lamps measured at 0.5 m in a dark room. Lamps were wrapped with filters either of 75 μm thick cellulose diacetate film for the UV-B treatment or of 130 μm polyester plastic film for the UV-A treatment.

film (optically similar to mylar and absorbing all radiation below 315 nm) for the UV-A treatment. Plants under c.a. filtered lamps received both UV-B and UV-A supplemental doses (later on named UV-B treatment or plants) while plants under polyester filtered lamps received only UV-A supplemental radiation (later on named UV-A treatment or plants) at a dose approximately equal of the UV-B treatment (Figure 2). Many past experiments have considered these latter plants 'control'. The c.a. film was presolarized for 10 h to reduce transmittance variations and both plastic filters were changed three times/week.

The experiment simulated a 20% stratospheric ozone reduction in Pistoia (Italy). Biologically effective UV-B (UV-B_{BE}) doses were based on calculations by Björn & Murphy (1985) using the generalized plant action spectrum, normalized at 300 nm (Caldwell 1971) in accordance with the mathematical function elaborated by Thimijan et al. (1978). On the summer solstice with clear sky conditions the supplemental UV-B_{BE} dose was 2.8 kJ m^{-2} day^{-1} in addition to the effective 8.1 kJ m^{-2} day^{-1} UV-B_{BE} from the sky. UV radiation was supplemented 7 h daily centered at solar noon. UV-B measurements were performed with a double monochromator spectroradiometer (model SR9910-PC, Macam Photometrics Ltd, Livingstone, Scotland).

The experimental design consisted of three types of lamp rack: control (lamps turned off), UV-A treatment (polyester filtered lamps) and UV-B treatment (cellulose diacetate filtered lamps). The natural radiative regime under the three types of rack was identical. Each type of lamp rack was replicated three times according to a 3 × 3 Latin square design and a complete ANOVA analysis between blocks was performed.

Measurements of growth parameters

After 33 days of treatments (during early reproductive stage) 10 plants from each replicate were harvested and analysed for morphological parameters (plant height, internode length, petiole length, leaf area, number of leaves, number of lateral branches). Biomass was then partitioned into leaves, stems and roots and dry weights were obtained after oven drying at 80 °C for 48 h. Leaf area was determined with a portable leaf area meter (LI-COR, LI-3000, Lincoln, Nebraska, USA). Leaf extension was measured in the field for the first four trifoliate leaves by measuring the length of the central leaflet of the trifoliate leaves 2–3 times/week.

Table 1. Effect of UV treatments on net CO_2 assimilation (A_N), stomatal conductance (Gs) and transpiration (E) on bean cv Nano Bobis

	During leaf expansion(1)			At the end of leaf expansion(2)		
	UV-B	UV-A	Control	UV-B	UV-A	Control
A_N (μmol m^{-2} sec^{-1})	20.06 ± 0.5	21.13 ± 0.5	19.9 ± 0.47	22.44 ± 0.4	22.13 ± 0.6	23.22 ± 0.4
Gs (mmol m^{-2} sec^{-1})	891.87 ± 98	848.46 ± 77	739.42 ± 42	801.96 ± 38	715.04 ± 5	749.04 ± 38
E (mmol m^{-2} sec^{-1})	6.39 ± 0.22	6.42 ± 0.19	6.35 ± 0.16	5.11 ± 0.09	4.84 ± 0.13	4.94 ± 0.09

No significant differences ($p<0.05$) were found among values (n=30) in the same line according to ANOVA analysis. (1) mean leaf temperature = 29.5 °C; WPD (water pressure deficit) = 27.92 mbar; RH (relative humidity) = 13.45 mbar (2) mean leaf temperature = 25.7 °C; WPD = 20.25 mbar; RH = 13.05 mbar.

Table 2. Effect of UV treatments on the efficiency of the photosynthetic apparatus

	UV-B	UV-A	Control
V_{cmax}(1) (μmol m^{-2} sec^{-1})	194.17 ± 5.5	200.8 ± 13.9	201.7 ± 8.3
J_{max}(2) (μmol m^{-2} sec^{-1})	86.62 ± 7.3	82.6 ± 4.3	94.15 ± 6.7
TPU(3) (μmol m^{-2} sec^{-1})	12.44 ± 0.35	13.14 ± 0.83	12.45 ± 0.66

No significant differences ($p<0.05$) were found among values (n=6 for UV-B treatment and control, n=5 for UV-A treatment) in each line according to ANOVA analysis. (1) V_{cmax}=maximum rate of RuBP carboxylation; (2) Jmax=maximum rate of electron transport; (3) TPU=maximum rate of triose-phosphate utilization.

Gas exchange measurements

CO_2 net assimilation (A), transpiration (E) and stomatal conductance (Gs) were simultaneously measured on the central attached leaflet of the third fully expanded trifoliate leaf 12 and 20 days after their appearance. Measurements were taken using a portable open-system (CIRAS-1, PPS, Hitchin) under solar saturating irradiance (1500 μmol m^{-2} s^{-1}) and ambient CO_2 concentration (350 μmol mol^{-1}) in a temperature-controlled and ventilated cuvette. The cuvette cover was not transparent to UV radiation so the determination represents a cumulative response to UV irradiation rather than an instantaneous effect. Net CO_2 assimilation was obtained from gas exchange measurements using the equations of von Caemmerer & Farquhar (1981). Measurements were taken on 10 plants from each replicate lamp rack under the specific conditions shown in Table 1. The response of CO_2 assimilation (A) to intercellular CO_2 concentration (Ci) was obtained from gas exchange measurements at CO_2 concentrations (Ca) of 100, 250, 350, 700, 1500 and 2000 μmol mol^{-1} on the central attached leaflet of the third fully expanded trifoliate leaf. It was measured after 33 days of treatment on six plants for each rack. For measurements, the leaf was enclosed in an artificially illuminated, temperature controlled and ventilated cuvette; vapor pressure deficit was kept almost constant (1.7 kPa) by controlling both temperature (20–25 °C) and air humidity entering the cuvette; assimilation was recorded when all variables were steady. Each A:Ci curve was analysed according to the biochemical model developed by Farquhar et al. (1980) and Farquhar & von Caemmerer (1982). Values of maximum rate

of ribulose-1,5-biphosphate (RuBP) of carboxylation (V_{cmax}), light saturated rate of electron transport (J_{max}) and triose phosphate limitation (TPU) were iteratively screened to minimize the mean-square error between observations and model estimates. Parameters used in the model (V_{cmax}, J_{max} and dark respiration) were obtained from the original model as modified by Long (1991). Calculated values of V_{cmax}, J_{max} and TPU were statistically analysed to compare the A/Ci response.

Photosynthetic pigments

Photosynthetic pigments were determined after 33 days of treatment on 10 plants from each replicate rack, by direct extraction of intact leaf tissues in *N,N*-dimethylformamide (DMF) according to Moran & Porath (1980). The analysis used 1 cm^2 of the third trifoliate leaf with 10 ml of DMF and another 1 cm^2 of leaf was oven dried. The absorbance of extracted solutions was read at 647 and 664 nm with a DU-spectrophotometer (Perkin–Elmer) after 48 h of extraction and photosynthetic pigments were quantified by the equation of Moran (1982). The concentration of pigments was expressed per unit leaf area and per unit dry weight.

UV-B absorbing compounds

Leaf discs (1 cm^2) from the third trifoliate leaf were used for extraction of UV-absorbing pigments in acidified methanol (79:1:20; methanol:HCl:water). Absorption spectra of 10 leaves from each replicate were determined with a DU 640-spectrophotometer (Beckman) from 270 to 350 nm and absorbances at 270, 300, 305, 330 and 350 nm were used for comparison.

Results

Biomass production, plant morphology and leaf growth

After 33 days of treatment, UV-B plants showed a significant reduction of the whole plant biomass when compared to UV-A plants. The above-ground biomass was significantly reduced while no differences were detected in the root mass. Plants grown under turned off lamps had a lower biomass compared to UV-A treatment even if not statistically significant (Figure 3). Root biomass was almost the same among the three

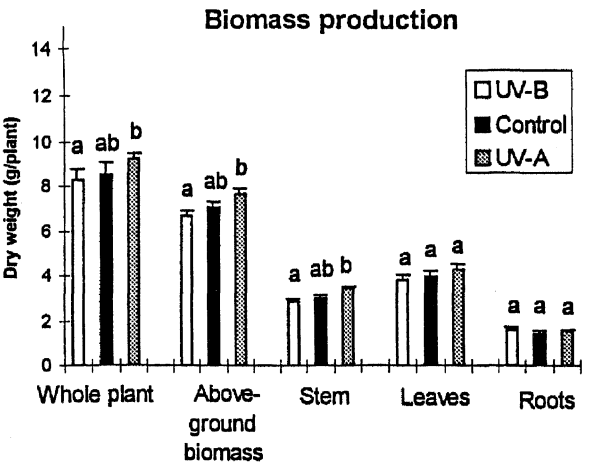

Figure 3. Effects of UV treatments on the growth parameters of the bean cv Nano Bobis after 33 days of supplemental irradiation. Bars represent the mean of 30 independent values except for roots and whole plant biomass where n=12. Different letters indicate significant differences ($p < 0.05$) according to ANOVA analysis.

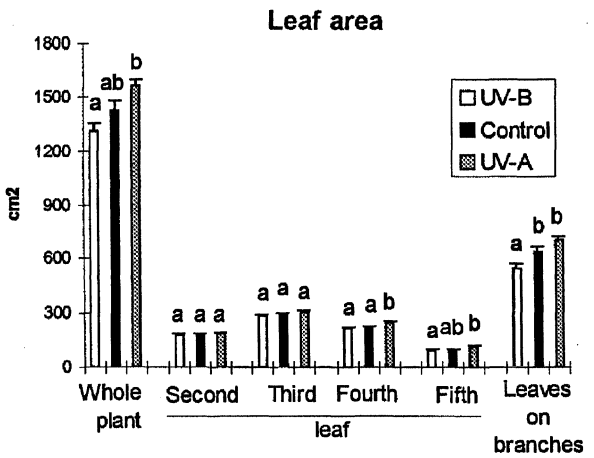

Figure 4. Effects of UV treatments on leaf development. Bars with different letters are significantly different ($p < 0.05$) according to ANOVA analysis.

groups of plants while the root/shoot ratio (data not shown) was lower in UV-B treated plant.

Morphological measurements (Figure 4) revealed a significant reduction in the whole plant leaf area of UV-B treated plants compared to UV-A plants. This was caused by the reduction of the size of leaves on branches and secondarily of the size of youngest leaves (fourth and fifth) of the main shoot. Control plants had a lower leaf area compared to UV-A plants, even if not statistically significant except for the fourth leaf of the main stem. UV-B plants, when compared to UV-A

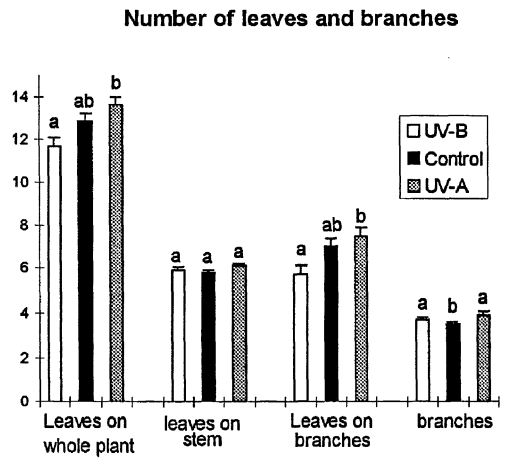

Figure 5. Effects of UV treatments on plant leaf number and plant branching after 33 days of supplemental irradiation. Bars with different letters are significantly different ($p < 0.05$) according to ANOVA analysis.

Table 3. Effects of UV treatments on the concentration of photosynthetic pigments in the bean cv Nano Bobis.

	Pigments/dry weight(mg g^{-1})		
	UV-B	UV-A	Control
Chl *a*	8.31 ± 0.32	9.06 ± 0.25	8.86 ± 0.29
Chl *b*	1.96 ± 0.08	2.09 ± 0.06	2.14 ± 0.08
total Chl	10.27 ± 0.4	11.15 ± 0.31	11.01 ± 0.37
Carotenoids	1.92 ± 0.06	2.05 ± 0.05	1.97 ± 0.05
	Pigments/area (μg cm^{-2})		
	UV-B	UV-A	Control
Chl *a*	34.18 ± 0.5	35.99 ± 0.36	35.48 ± 0.68
Chl *b*	8.05 ± 0.16	8.3 ± 0.12	8.59 ± 0.2
total Chl	42.23 ± 0.7	44.29 ± 0.46	44.07 ± 0.87
Carotenoids	7.93 ± 0.1	8.14 ± 0.1	7.92 ± 0.13

No significant ($p<0.05$) differences were found among values (n=30) in the same line according to ANOVA analysis.

treatment, had a significant lower number of leaves on the whole plant which resulted from a lower number of leaves on branches (Figure 5) since no differences were revealed in the number of leaves of the main stem. Control plants showed the same tendency to a reduced number of leaves compared to UV-A plants. Similarily, plant height of UV-B plants was significantly lower than UV-A plants (Figure 6); control plants were also shorter that UV-A plants.

UV-B treatment influenced leaf ontogeny, as leaf extension of the UV-B treated plants was generally lower than UV-A plants (Figure 7). In particular the third trifoliate leaf (on which photosynthesis measurements were performed) of the UV-B treatment was growing slower than UV-A during the rapid growth phase while they reached a similar final length. UV-A treatment and control plants were generally quite similar.

UV effects on photosynthetic apparatus

Net CO_2 assimilation (determined with gas-exchange measurements on the third trifoliate leaf) of UV-B treated plants was sightly lower than UV-A plants during leaf expansion (12 days after leaf appearance), even if not statistically significant. No differences at all were detected later at the end of leaf expansion (Table 1). Stomatal conductance (Gs) and transpiration (E) were also not statistically different among the treatments and these data were further confirmed by porometer measurements of leaf diffusion conductance performed simultaneously with gas-exchange measurements on the same leaves (data not shown). The values of the maximum rates of RuBP carboxylation (V_{cmax}) and regeneration (J_{max}) and of the phosphate limitations (TPU) calculated from the response of net CO_2 assimilation to different internal CO_2 concentration showed no significant differences among the three groups of plants for these photosynthetic parameters (Table 2).

Photosynthetic pigments and UV-B absorbing compounds

The concentration of photosynthetic pigments of UV-B plants were always lower than UV-A plants, both in terms of dry weight and of leaf area (Table 3), even if not significant differences were found. Values of absorbance of the methanolic extracts were also almost identical among the three groups of plants (Table 4).

Discussion

Exposure of bean plants cv Nano Bobis to UV-B supplementation obtained by means of UV lamps covered with cellulose diacetate for 33 days under a natural Mediterranean radiative regime induced a moderate reduction in their growth when compared with UV-A treated plants; this result is consistent with the observation that plants growing under natural conditions were less sensitive to UV-B than plants cultivated in artificial

Table 4. Effect of UV treatments on the absorbance of methanolic extract.

λ	UV-B	UV-A	Control
270	0.64 ± 0.02	0.62 ± 0.01	0.64 ± 0.02
300	0.62 ± 0.01	0.61 ± 0.01	0.64 ± 0.01
305	0.63 ± 0.01	0.61 ± 0.01	0.64 ± 0.01
330	0.78 ± 0.02	0.75 ± 0.01	0.78 ± 0.01
350	0.7 ± 0.02	0.68 ± 0.01	0.72 ± 0.02

No significant ($p<0.05$) differences were found among values ($n=30$) in the same line according to ANOVA analysis.

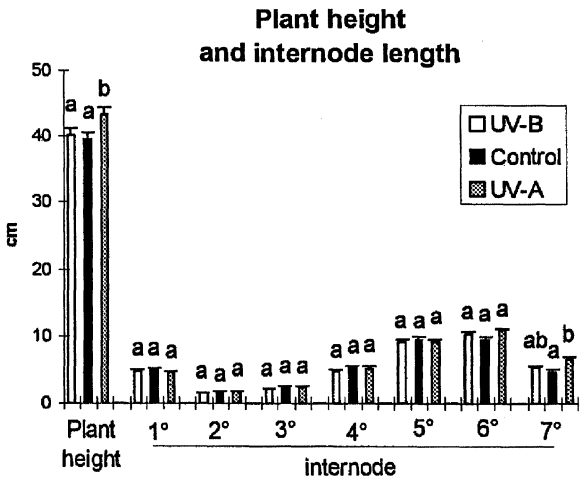

Figure 6. Effects of UV treatments on plant height and internode length after 33 days of supplemental irradiation. Bars with different letters are significantly different ($p < 0.05$) according to ANOVA analysis.

environments (Caldwell et al. 1994; Caldwell & Flint 1994; Teramura 1983).

We observed in fact a significant reduction in the biomass, leaf area and plant height induced by the UV-B treatment compared with the UV-A plants. Plant leaf area of UV-B treatment was significantly reduced when compared with UV-A plants, mainly because of a lower leaf area present on branches. This last observation is ascribed to the lower number of leaves on an equal number of branches (Figures 4 and 5). These data support the current hypothesis that the increase of UV-B radiation can induce changes in plant architecture since morphological parameters are often more sensitive than biomass production. These modifications in plant architecture and spatial geometry are very important since it has been shown (Barnes et al. 1990, 1993; Gold & Caldwell 1983) that they can produce alterations in the competitive interactions of species and consequently in the natural composition of species within an ecosystem.

Plants belonging to the UV-B treatment were shorter than UV-A plants. This difference was caused by the reduction of the length of the last internodes still in elongation (Figure 6) since no difference in the number of internodes was detected (data not shown). The final length of older internodes was similar in the three plant groups. Leaf ontogeny was also affected and UV-B treated plants extended their leaves slower (and generally less) than UV-A plants (Figure 7).

On the contrary, the root system was not affected by either UV treatments while UV-B treated plants had a significant lower above-ground biomass (Figure 3), suggesting that in these plants there was an higher allocation of biomass to the roots.

On the other hand we observed a sort of 'stimulating' effect on the growth of UV-A treated plants when compared with plants grown under turned off lamps. UV-A plants had, in fact, higher (even not statistically significant) dry matter production, leaf area, number of leaves and number of branches compared to the control plants (Figures 3–5). Moreover plants belonging to UV-A treatment were the tallest plants (Figure 6) and their leaves elongated faster than control (even if final lengths was generally similar) because of a possible acceleration of growth induced by this treatment. The 'stimulating' effect of UV-A radiation was unexpected since it has been generally supposed that the additional dose of UV-A radiation under field conditions is a low percentage increase of the incident solar UV-A radiation and for this reason it has been assumed to be negligible (Middleton & Teramura 1994; Nikolopoulos et al. 1995).

A possible explanation for our observations could be found in the particular meteorological trend occurred throughout the experiment (Figure 2). Effectively, the month of August 1995 did not show the typical Mediterranean summer conditions since it was characterized by 12 days with a clear sky (mean global radiation was 21 500 kJ m^{-2} day^{-1}), 20 partially overcast days (18 000 kJ m^{-2} day^{-1} of mean global radiation) and seven completely cloudy days (10 500 kJ m^{-2} day^{-1} of mean global radiation).

Daily unweighted supplemental radiation in the UV-A spectral band (320–400 nm) and in the short-wavelength UV-A (320–340 nm) supplied by lamps filtered with polyester were approximately ~ 5.0 and 2.9 kJ m^{-2} day^{-1}, respectively. During overcast days

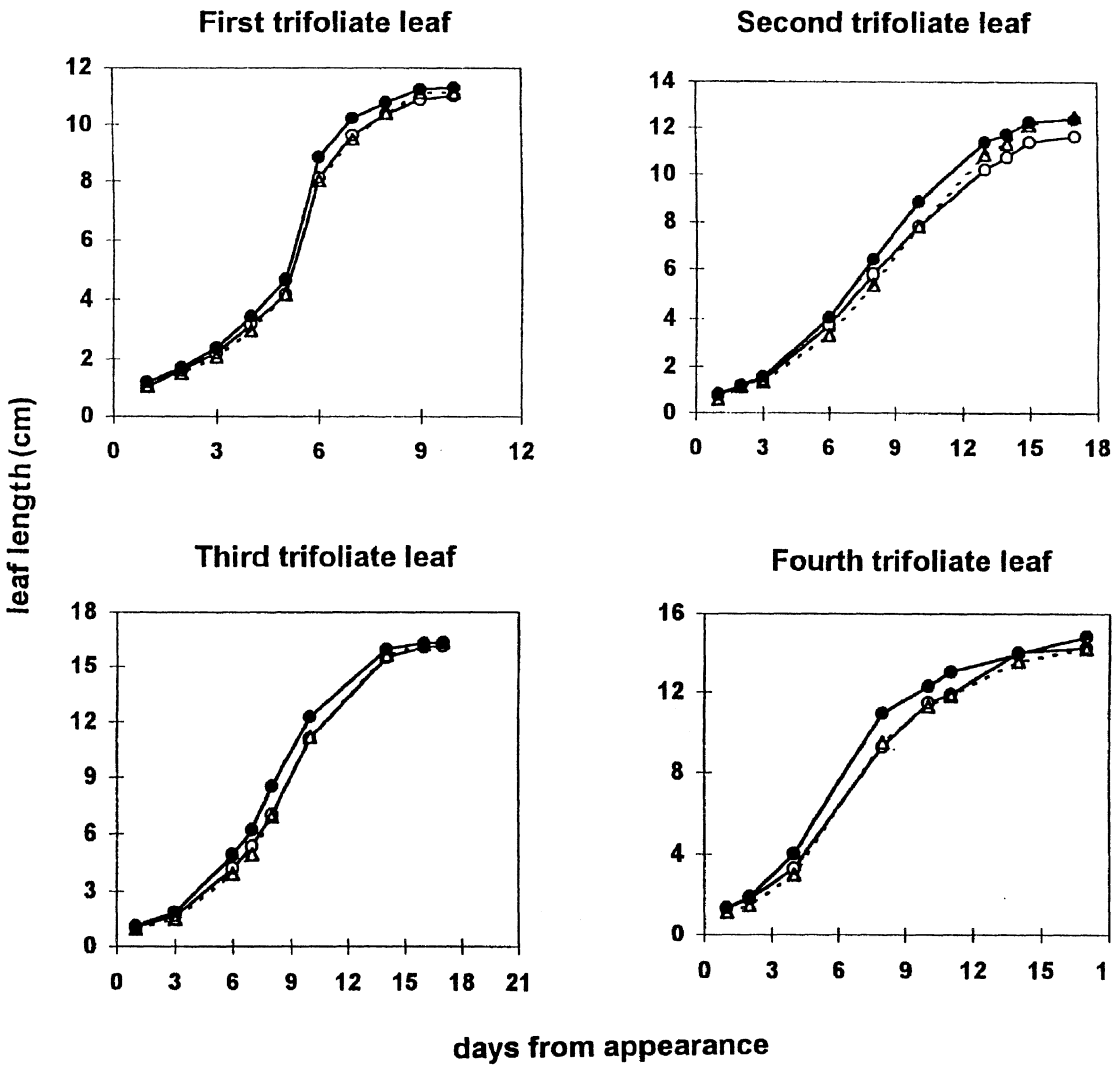

Figure 7. Effects of UV treatments on leaf ontogeny. For all points (n = 30) the standard error is smaller than the symbol and cannot be seen. Open (○) and closed (●) circles represent the UV-B and UV-A treatment, respectively; triangles (△) represent control leaves.

the supplemental UV-A radiation and the supplemental short-wavelength UV-A radiation were estimated to be ~ 2% and ~ 6% of the analogous incident UV-A components, respectively. Thus, in the cloudy days, supplemental radiation supplied by polyester-covered lamps could have been used for growth.

The effects of UV treatments on the photosynthetic apparatus were evaluated measuring the individual leaf gas exchange, the response of assimilation to different CO_2 internal concentration (A:Ci curves) and the concentration of photosynthetic pigments (Tables 1–3). The limited number of measurements we performed indicate that UV-B treatment marginally and not significantly influenced the functioning of photosynthetic apparatus. These data are not surprising since is well reported that the high levels of PAR, blue light, UV-A and UV-B:PAR ratio typical of experiments performed in natural radiative regimes (Adamse et al. 1994; Caldwell et al. 1994; Cen & Bornman 1990; Middleton & Teramura 1993; Mirecki & Teramura 1984), mitigate the negative effects of UV-B radiation on specific components of the photosynthetic apparatus, activating specific photoreceptors or photorepairing mechanisms.

In this experiment ultraviolet-B-absorbing compounds (measured as the total absorbance of acidified methanol at 270, 300, 305, 330 and 350 nm)

were almost identical among the three groups of plants (Table 4). Many higher plants are able to accumulate UV-absorbing phenylpropanoid-type pigments when irradiated with UV-B (Murali & Teramura 1985; Robberecht & Caldwell 1978; Ziska et al. 1992), but this behaviour can not be generalized, as it is known that there are intraspecific differences in response (Murali & Teramura 1986). Many plants are able to tolerate marked increases of UV-B radiation without producing an increase of UV-B absorbing compounds (Musil & Wand 1994; Sullivan & Teramura 1989; Ziska et al. 1992) probably because they are adapted to a higher UV-B environment during their relatively short evolutionary history. Our results could be explained considering (1) that bean is 'genetically' acclimated to high UV-B radiation since this species is native to high altitudes of South America and therefore the supplemental UV-B dose was too low to induce an additional synthesis; (2) that the cultivar of bean Nano Bobis used in this experiment is cultivated from May to October and therefore is well adapted to tolerate great natural variation in the UV-B regime; (3) that other mechanisms of protection from UV-B damage exist which were not investigated in the present work.

Data from this experiment indicate that the supplementation of UV-A radiation may produce effects on plant growth even under natural Mediterranean radiative regimes. This fact has to be carefully considered when evaluating the real impact of the radiation emitted by UV fluorescent lamps covered with cellulose diacetate because its two spectral components (UV-B and UV-A) may induce different and even opposite effects on plant growth.

Middleton & Teramura (1993) indicated that potential errors in the interpretations of data obtained in controlled environments could arise from the use of cellulose diacetate and polyester filters, because "a range of experimental conditions exists where polyester-covered lamps do not provide an adequate control for UV-A irradiance relative to the cellulose acetate treatment for glasshause/growth chamber experiments". In conditions of low ambient UV-A radiation like growth chambers, glasshouses and overcast skies at any season, Middleton & Teramura (1993) assumed that 'the polyester covered lamps do not always serve as appropriate controls in UV-B experiments' because the differences in UV-A fluences from cellulose diacetate and polyester filtered lamps could induce different plant responses.

Under field conditions with high ambient radiation, similar responses to UV-A supplementation and to solar UV-A are generally expected, 'on the assumption that the UV-A/blue response is saturated at a fluence below that found in natural sunlight. In this case the additional UV-A lamp irradiances would be considered neutral in effect and their careful control unnecessary' (Middleton & Teramura 1994).

On the basis of these considerations we conclude that even in a Mediterranean climate it is more appropriate to compare the effects induced on vegetation by the supplementation with UV lamps covered with c.a. filters (the so-called UV-B treatment) with those (if present) induced by the same type of lamps covered with mylar-type filters.

From this prospective, our results indicate that even under Mediterranean field conditions a supplemental dose of UV-B_{BE} (280–320 nm) radiation simulating a 20% decrease of the stratospheric ozone produced a reduction of growth on bean plants.

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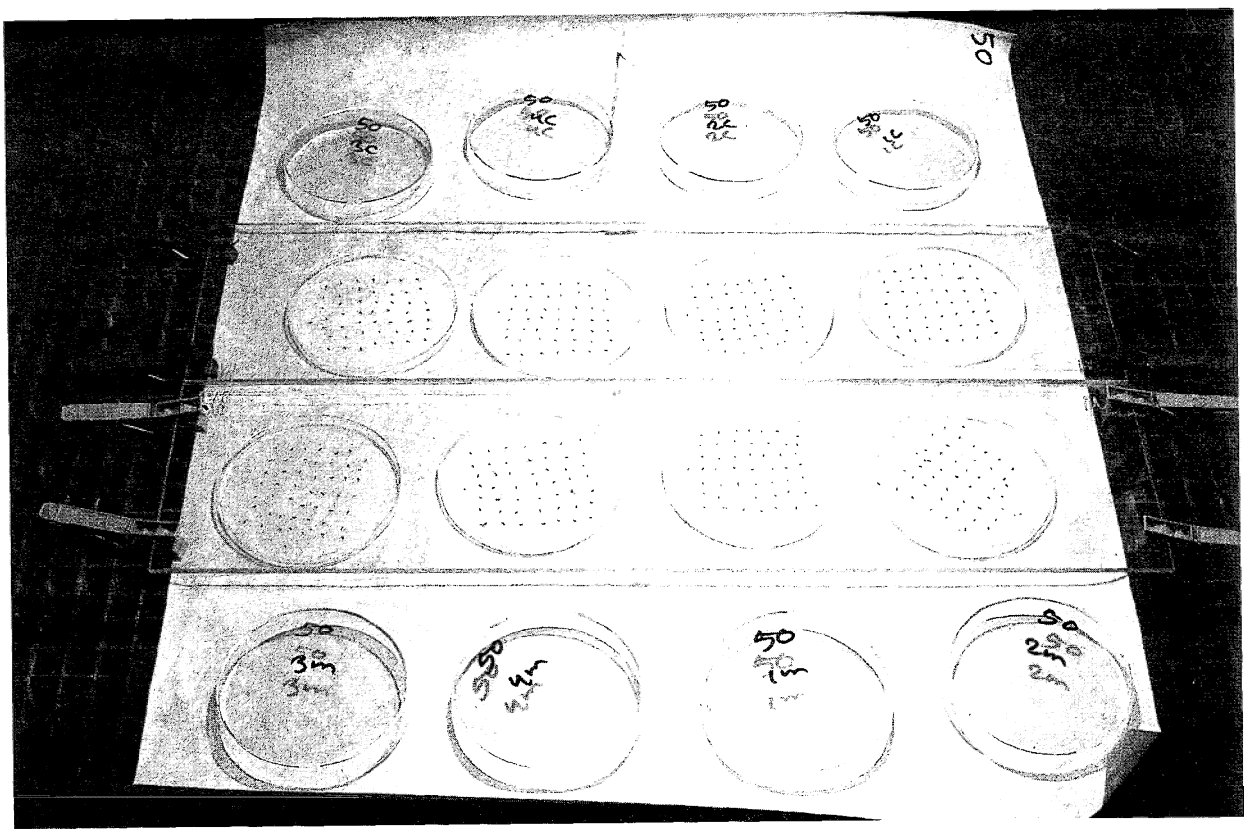

Germination experiment with seeds of dune grassland species in petridishes, exposed to enhanced ultraviolet-B radiation. (Photograph: M. Tosserams)

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The effect of enhanced ultraviolet-B radiation on germination and seedling development of plant species occurring in a dune grassland ecosystem

Marcel Tosserams, Esther Bolink & Jelte Rozema
Department of Ecology and Ecotoxicology, Faculty of Biology, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

Key words: Development, Dune grassland ecosystem, Germination, Ozone depletion, Plant growth, Seedlings, UV-absorbing pigments, UV-B

Abstract

The germination of seeds of seven plant species occurring in a dune grassland vegetation of the Netherlands, was studied at four levels of UV-B radiation simulating unto 45% stratospheric ozone reduction during April. With the exception of seeds of *Senecio jacobaea*, germination of the dune grassland species was not affected by enhanced UV-B irradiance. Although a clear UV-B fluence-response relationship was not observed, the germination rate of *S. jacobaea* seeds and maximal germination percentage were reduced at enhanced UV-B. Germination rate in the dark was higher than germination in the light for *Oenothera biennis*, *Plantago lanceolata*, *Rumex obtusifolius* and *S. jacobaea*. Total dry biomass accumulation of seedlings was not affected by increased UV-B radiation in any of the species tested. Clear-cut differences in UV-absorbance of methanolic extracts were observed between species. Enhanced UV-B irradiance stimulated UV-absorbance of seedling extracts of *Holcus lanatus* and *Verbascum thapsus*. A clear UV-B fluence-response relationship was observed for both species. The results indicate that germination of the studied plant species probably will not be adversely affected by the expected stratospheric ozone reduction in The Netherlands.

Introduction

Since a decrease of the earth's stratospheric ozone column thickness was observed and reports on the causes of this phenomenon were published, a considerable amount of research has focused on the implications of increased solar UV-B radiation for life on earth (Caldwell & Flint 1994; Teramura & Sullivan 1994).

Growth chamber studies as well as greenhouse and field experimentation have led to a better understanding of how enhanced solar UV-B radiation may affect plant life. There are considerable differences in the UV-B response of different plant species (Krupa & Kickert 1989, Tosserams et al. 1997) and between cultivars of the same species (Biggs et al. 1981; Teramura & Murali 1986; Barnes et al. 1993; Dai et al. 1994).

UV-B radiation may reduce plant growth and yield (Tevini & Teramura 1989; Teramura & Sullivan 1994). To avoid damage by UV-B radiation, plants evolved protective mechanisms. Photorepair is reported to be an efficient mechanism for plants to ameliorate UV inflicted damage (Pang & Hays 1991; Chen et al. 1994). In addition, the synthesis of UV absorbing compounds in the epidermal layer of leaves and flowers, reduces the UV-B fluence rate before it can cause damage to underlying tissues (Flint & Caldwell 1983; Tevini et al. 1991; Stapleton & Walbot 1994; Lois & Buchanan 1994). These absorbing compounds, mainly flavonoids, also act as scavengers counteracting radical formation by UV-B radiation (Larson 1988; Lonchampt et al. 1989).

It has been suggested that enhanced solar UV-B radiation might directly or indirectly affect natural vegetation resulting in alterations of species composition (Tevini & Teramura 1989). However, experimental data concerning effects of enhanced UV-B radiation on plants in their natural environment is scanty (Rozema et al. 1991; Caldwell & Flint 1994). Most of the reports describe the effects of UV-B on seedlings

and individual plants grown in pots. Other developmental stages like germination, flowering and seed-set are less well documented. These stages in plant development however, might prove to be susceptible to UV-B. In a recent study by Musil (1995), dicotyledonous *Asteraceae* exhibited delayed flowering, decreased flower production and reduced numbers of seeds set in response to elevated UV-B. In addition, the latter study as well as several earlier studies (Flint & Caldwell 1984; Feder & Shrier 1990), demonstrated the UV-B sensitivity of pollen germination and pollen tube growth. It is reasonable to assume that developmental stages may have differential UV-B sensitivity, regarding that the maximal UV-B exposure of different developmental stages may vary considerably (Teramura & Sullivan 1994).

In order to obtain a better understanding of the consequences of increased solar UV-B radiation on natural ecosystems it is important to evaluate effects of UV-B radiation on all developmental stages during the life history of a specific plant species. The aim of the present study was to determine to what extent enhanced UV-B radiation influences seed germination and initial seedling development of different plant species of a dune grassland ecosystem in The Netherlands.

Material and methods

Experimental design. Caryopses of the grass species *Bromus hordeaceus* L. and *Holcus lanatus* L., seeds and fruits of the herbaceous species *Oenothera biennis* L., *Plantago lanceolata* L., *Senecio jacobaea* L., *Rumex obtusifolius* L. and *Verbascum thapsus* L., were collected in a dune grassland (Heemskerk, Noord-Holland, The Netherlands, 52° N, 4° E). Experiments were conducted in a greenhouse from January till April 1993. For each species 32 petri-dishes each containing two paper filters (Schleicher and Schuell, 90 mm) and fifty seeds (6 rows of 7 seeds and one row of 8 seeds), were used. During the course of the experiment, the filtration papers were humidified regularly with demineralized water. The distance between adjacent seeds was sufficient (> 5 times seed diameter) to avoid mutual influences (Justice 1972). The top of all petri-dishes was removed after which the dishes were divided into 4 groups of 8 dishes. Per group 4 petri-dishes were covered with UV-B transparent acrylic glass (Type 006; 50 × 12 × 0.3 cm) and cellulose acetate foil (50 × 12 × 0.01 cm). This combination transmits radiation > 290 nm (Figure 1). The remaining 4 petri-dishes

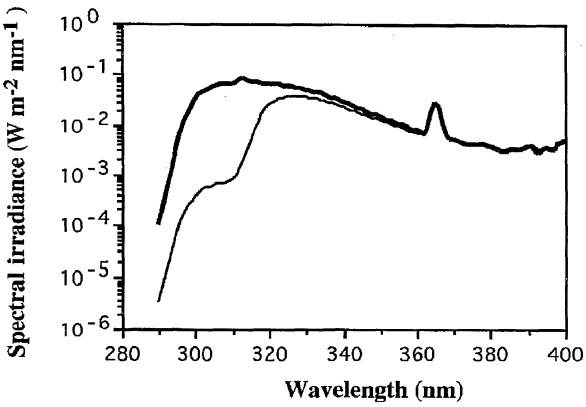

Figure 1. Spectral irradiance as received by the seeds of the UV-B treatment and the respective control. (——) Acrylic glass covered with cellulose acetate (absorbance < 290 nm); (——) Acrylic glass covered with polyester foil (absorbance < 313 nm). Measurements were conducted using an Optronics OL 752 Spectroradiometer.

were also covered with acrylic glass but the cellulose acetate was replaced by polyester foil (Mylar, Type S; 12 × 50 × 0.013 cm) this combination transmits radiation > 313 nm (Figure 1) and served as the UV-A control treatment. Each group was placed underneath a lamp system consisting of two Philips 40W/12 UV-B fluorescent tubes covered with cellulose acetate (0.1 mm, transmittance > 290 nm; Tamboer & Co. Chemie B.V., Haarlem, The Netherlands), suspended on both sides of a Philips 400W HPI/T lamp. Because of photodegradation, the cellulose acetate wrapped around the UV-B tubes was replaced twice a week. All treatments were screened from adjacent treatment by a sheet of polyester foil. The UV-B lamps operated from 1000 till 1600 h daily. The spectral irradiance underneath the filters was measured with a double-monochromator spectroradiometer (Optronic Model OL 752, Orlando, FL, USA). The spectroradiometer was calibrated for absolute responsivity against a 200 W tungsten-halogen standard lamp (Optronic Model OL 752-10, Orlando, FL, USA). Before the measurements, wavelength accuracy was checked with a dual calibration and gain check source module (Optronic Model OL 752–150, Orlando, FL, USA), by scanning a low-pressure mercury vapour lamp with a known peak emission at 312.9 nm.

Using the generalised plant action spectrum (Caldwell 1971) normalised at 300 nm, the daily biologically effective UV-B radiation ($UV\text{-}B_{BE}$) levels received by the seeds of the different groups were 2.46, 4.35, 5.75 and 9.18 kJ m^{-2} day^{-1}. According to an empirical

model designed by Green et al. (1980), the lowest UV-B_{BE} level is comparable to the ambient UV-B_{BE} (2.98 kJ m^{-2} day^{-1}) on a clear day during April in The Netherlands. The other UV-B_{BE} levels simulate a situation of 17, 28 and 45% stratospheric ozone reduction respectively in the same month. The UV-B_{BE} received by the seeds in the UV-A control groups was 0.033, 0.052, 0.070 and 0.105 kJ m^{-2} day^{-1} respectively. In addition, four petri-dishes each with fifty seeds of the same species, were wrapped in aluminium foil. One of these dishes was placed underneath each lamp system to test whether microclimate at the different sites in the greenhouse influenced germination. The 400W HPI/T lamps provided 250 μmol m^2 s^{-1} additional photosynthetically active radiation (PAR) during 16 hours a day. The maximal value of PAR measured on a clear day was 800 μmol m^2 s^{-1}. Temperature during the experiment varied between 13.9 °C (night) and 34.5 °C (day).

Measurements. To determine the weight and the water content of the seeds, fresh and dry weight (48 hr, 70 °C) of 50 seeds per plant species were determined. The number of germinated seeds was counted twice a day. A seed was defined germinated at the moment the radicula appeared. When all seeds had germinated or no further germination was observed, counting was stopped. After the germination experiment, the UV absorbance of the seedlings was determined. UV absorbing compounds were extracted with 5 ml of an acidified methanolic solution (CH_2OH:HCl:H_2O, 79:1:20 v/v) from approximately 15 mg of fresh plant material per petri-dish. Because seedlings of *V. thapsus* were very small, all plant material was used for the determination of UV absorbance. The extracts were stored for one night (8 °C) after which they were boiled for 10 minutes at 90 °C in a waterbath. The absorbance of the solution was measured with a Perkin-Elmer Lambda 15 UV/VIS Spectrophotometer at either 300 or 310 nm. Total dry weight (48 hr, 70 °C) per petri-dish was determined for all species except *V. thapsus*.

Statistics. All data were subjected to analysis of variance (ANOVA; Sokal & Rohlf, 1981). Dry weight data were transformed to their natural logarithms. Analysis of variance for repeated measurements was used for the analysis of germination data.

Table 1. Biomass and water content of 50 seeds of the plant species used. Values presented are means of 2 replicate measurements ±SEM

Plant species	Fresh weight (mg)	Dry weight (mg)	Water content (%)
Monocotyledons			
Bromus hordeaceus	204 ± 4.0	182 ± 3.4	10.8 ± 0.1
Holcus lanatus	11.2 ± 0.3	10.1 ± 0.6	10.1 ± 3.0
Dicotyledons			
Oenothera biennis	26.2 ± 0.7	24.4 ± 0.7	6.9 ± 0.1
Plantago lanceolata	85.1 ± 3.0	76.2 ± 2.7	10.5 ± 0.1
Rumex obtusifolius	62.5 ± 0.9	55.5 ± 0.8	11.2 ± 0.1
Senecio jacobaea	12.1 ± 0.2	11.3 ± 0.2	6.7 ± 0.2
Verbascum thapsus	8.3 ± 0.0	7.5 ± 0.2	9.5 ± 2.3

Results and discussion

Large differences of seed biomass were observed between the different species (Table 1). Fresh weight of reproductive units varied between 166 (*V. thapsus*) and 4,080 μg (*B. hordeaceus*). According to Harrington (1972), the water content of seeds should ideally be 5-14%. The water content of all seeds used in this experiment lies well within this range.

Receiving 7.1 kJ m^{-2} day^{-1} UV-B_{BE}, seed germination of radish was reduced by 26%, while germination of cucumber and bean was reduced by 23% when compared with control plants receiving 0.2 kJ m^{-2} day^{-1} UV-B_{BE} (Tevini et al. 1983). In contrast, Krizek (1975) observed no adverse effects on the percentage of germination for a range of crop species after 72 h of continuous exposure to UV-B irradiance. Germination of *Silene vulgaris* seeds originating from a highland and a lowland population was also unaffected by enhanced UV-B irradiance (van de Staaij 1994). Musil (1995) reported enhanced germination for several dicotyledonous and monocotyledonous arid-environment ephemerals. In the present experiment maximal seed germination (Figures 2 and 3) of control treatments varied between 74% (*P. lanceolata*) and 97% (*B. hordeaceus*, *R. obtusifolius* and *V. thapsus*). Enhanced UV-B only affects the maximal germination percentage of *S. jacobaea* (Figure 3). For this species, both germination rate and the maximal germination percentage were negatively affected by enhanced UV-B radiation. A clear UV-B fluence-response relationship however, was not observed. A significant UV-B effect ($p=0.008$) was only present at 4.35 kJ m^{-2} day^{-1} UV-B_{BE}. Possibly,

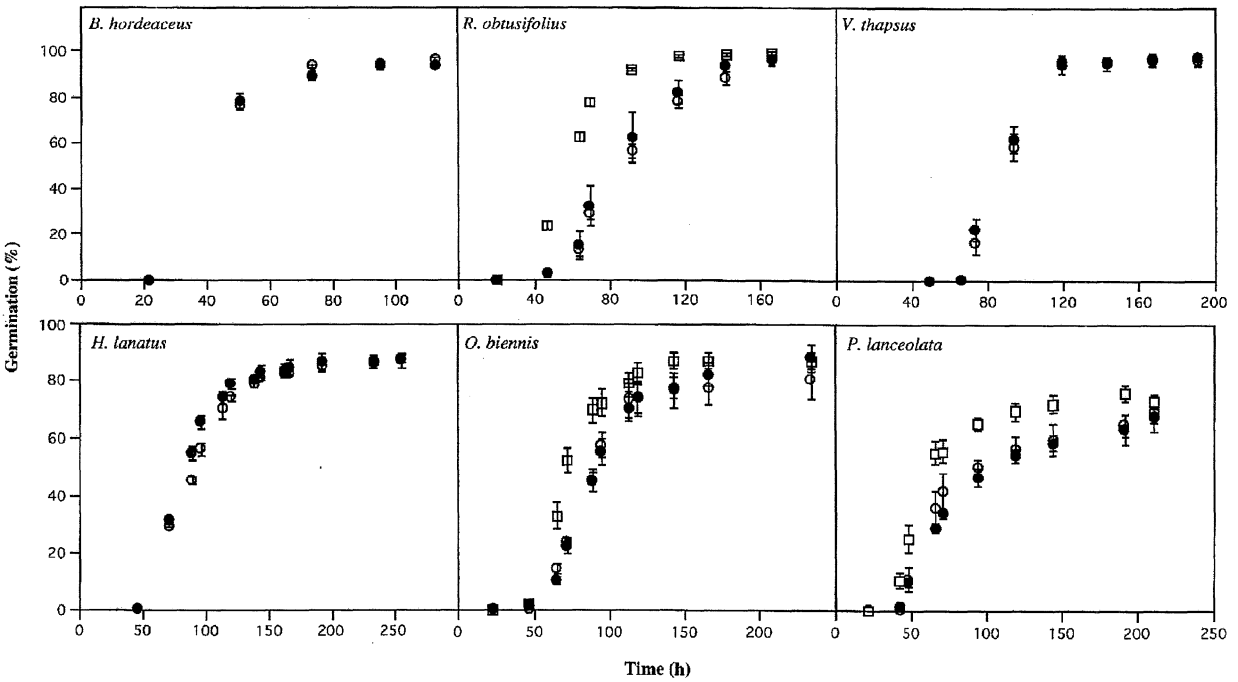

Figure 2. The effect of UV-B irradiance on germination of dune grassland plant species . In each treatment seeds either received 9.18 kJ m^{-2} day^{-1} $UV\text{-}B_{BE}$ (●) or did not receive UV-B (○). The other $UV\text{-}B_{BE}$ treatments are not presented because they did not significantly differ from the presented data. Germination data of dark controls (□) was only included when it was significantly different from the other treatments. All presented data are means of 4 replicates ± SEM.

the seed coat of most species provides sufficient protection against deleterious UV-B effects (Krizek 1975).

Dark controls of *O. biennis*, *P. lanceolata*, *R. obtusifolius* and *S. jacobaea* had a higher germination rate as compared to germination in the light (Figures 2 and 3). Dark and light germination of the other plant species tested was comparable (data not shown).

Total seedling dry weight of all species tested was not affected by enhanced UV-B irradiance (Figure 4). In accordance, Krizek (1975) reported no adverse effects on dry weight accumulation of a variety of crop species after 72 h of continuous UV-B irradiance. A marked decrease of dry weight was reported by Tevini et al. (1983) for bean and cucumber seedlings.

The accumulation of UV absorbing compounds (e.g. flavonoids) in epidermal tissue appears to be an important protective mechanism, which effectively reduces the detrimental action of UV-B irradiance on plants (Middleton & Teramura 1993; Kootstra 1994; Lois & Buchanan 1994; Stapleton & Walbot 1994). The absorbance of UV-B radiation by flavonoids and related phenolic compounds varies between cultivars and species (Teramura et al. 1991; Day et al. 1994; Wand 1995). In agreement, clear-cut differences in UV-B absorbance between seedling extracts of the control treatments were observed in the present experiment (Figure 5). Controls of *O. biennis* had the highest absorbance (± 0.65) while the lowest absorbance (between 0.1 and 0.15) was observed in *R. obtusifolius* and *V. thapsus* seedlings.

In many plant species UV-B irradiation enhances the accumulation of absorbing compounds (Tevini & Teramura 1989). This UV-B-enhanced accumulation is due to a higher activity and/or higher rate of biosynthesis of L-phenylalanine ammonia-lyase (PAL). This enzyme regulates the diversion of L-phenylalanine into precursors for secondary phenolics. In barley primary leaves UV-B irradiance prolonged the activity of PAL resulting in an enhanced accumulation of UV absorbing compounds (Liu & McClure 1995). The influence of UV-B on the stimulation of UV-B absorbance varied between species. Absorbance of *B. hordeaceus*, *O. biennis*, *P. lanceolata*, *R. obtusifolius* and *S. jacobaea* remained unaffected by increasing UV-B levels, whereas the UV-B absorbance of *H. lanatus* and *V. thapsus* was increased by UV-B enhancement. The

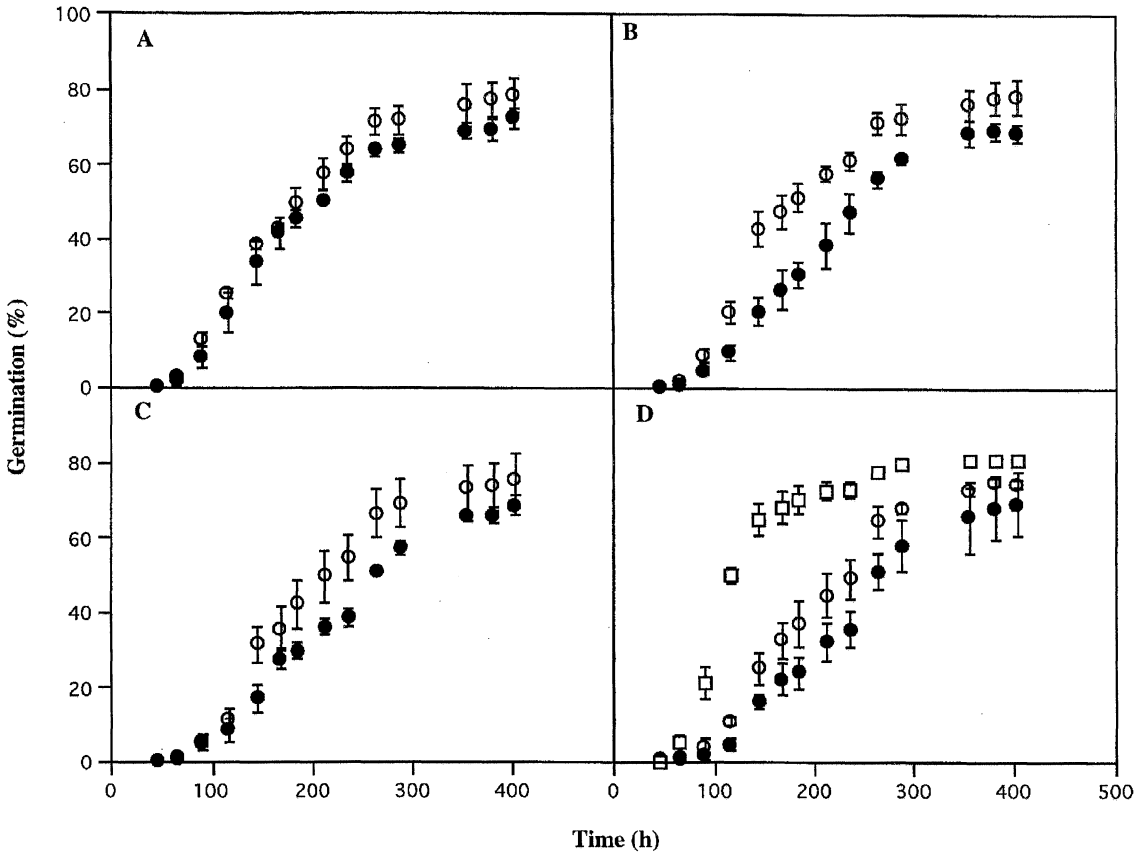

Figure 3. The effect of UV-B irradiance on germination of *S. jacobaea* seeds. Four UV-B_{BE} levels were applied: (A) 2.46 kJ m^{-2} day^{-1}; (B) 4.35 kJ m^{-2} day^{-1}; (C) 5.75 kJ m^{-2} day^{-1}; (D) 9.18 kJ m^{-2} day^{-1}. In each treatment seeds were either irradiated with UV-B (●) or did not receive UV-B (○). Germination of dark controls (□) is only included in D. All presented data are means of 4 replicates ± SEM.

UV-B absorbance of *H. lanatus* seedlings was stimulated by 37, 61, 74, and 99% when the different UV-B treatments were compared to their respective controls. This is in agreement with results of Wellmann (1985) who demonstrated the linear dependency of flavonol accumulation with UV-B fluence. Seedlings of *V. thapsus* also exhibited a clear UV-B fluence-response relationship. For this species the maximal stimulation of absorbance was 28% at the highest UV-B treatment.

Although large differences between species were observed, the presence and/or accumulation of absorbing compounds may already protect the seedlings against detrimental effects of UV-B radiation directly after emergence from the soil. Enhanced UV-B fluence rates might however stimulate the accumulation of phenolic compounds in some species, which may affect herbivory (Coley et al. 1985), litter quality and decomposition processes (Gehrke et al. 1995, Rozema et al. 1997) in natural environments. However, other environmental factors like drought and mineral deficiency have also been shown to stimulate the accumulation of phenolic compounds in plants (Balakumar et al. 1993; Murali & Teramura 1985). Therefore, further studies on the interaction of these factors with enhanced UV-B radiation are necessary to elucidate the contribution of UV-B in the accumulation of these compounds in natural ecosystems.

Our results indicate that germination and early seedling development are not adversely affected by enhanced UV-B irradiance. Even at the relatively high UV-B_{BE} levels used in this experiment, no reduction of germination rate and capacity was observed for most species tested. In natural environments several factors may influence the maximal UV-B exposure of germinating seeds. First of all timing of germination is important. In the field, germination may occur largely in the dark, avoiding solar irradiance. Secondly, the

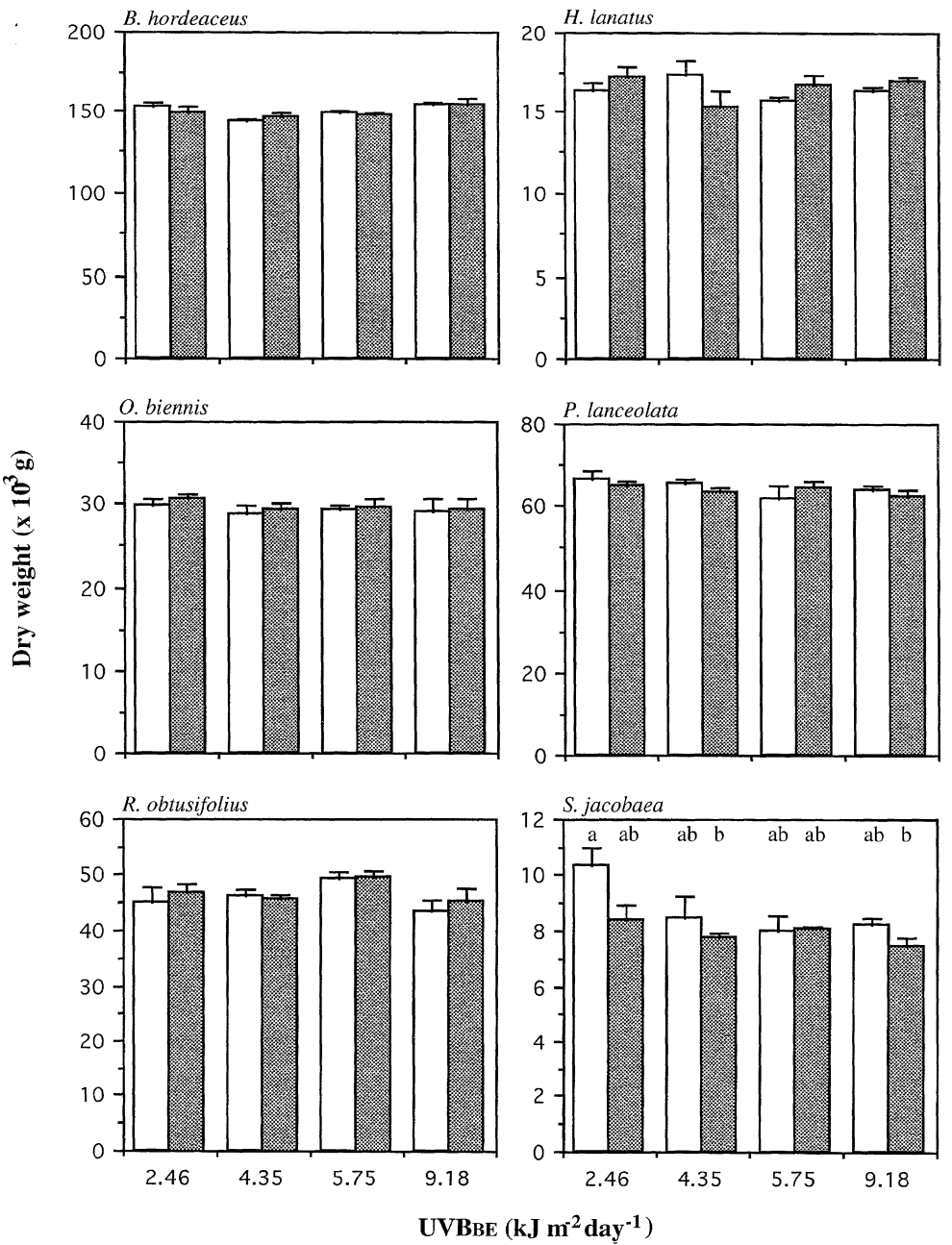

Figure 4. The influence of enhanced UV-B irradiance on dry matter accumulation of seedlings. Seedlings received no UV-B (□) or additional UV-B (▨). Values presented are means of 4 replicates ± SEM. Bars marked with the same letter are not significantly different ($p>0.05$). Data for *V. thapsus* was not available because all seedlings were used for the determination of UV-absorbance.

position of the seed in the soil profile is important. Thirdly, vegetation density may markedly influence the UV-B climate at soil level. Even in the relatively sun exposed dune grassland ecosystem, germination is expected to occur at a relatively low $UV\text{-}B_{BE}$ level. Therefore, we conclude that the expected ozone reductions for The Netherlands (WMO 1994) will probably not negatively influence germination of plant species of the dune grassland ecosystem.

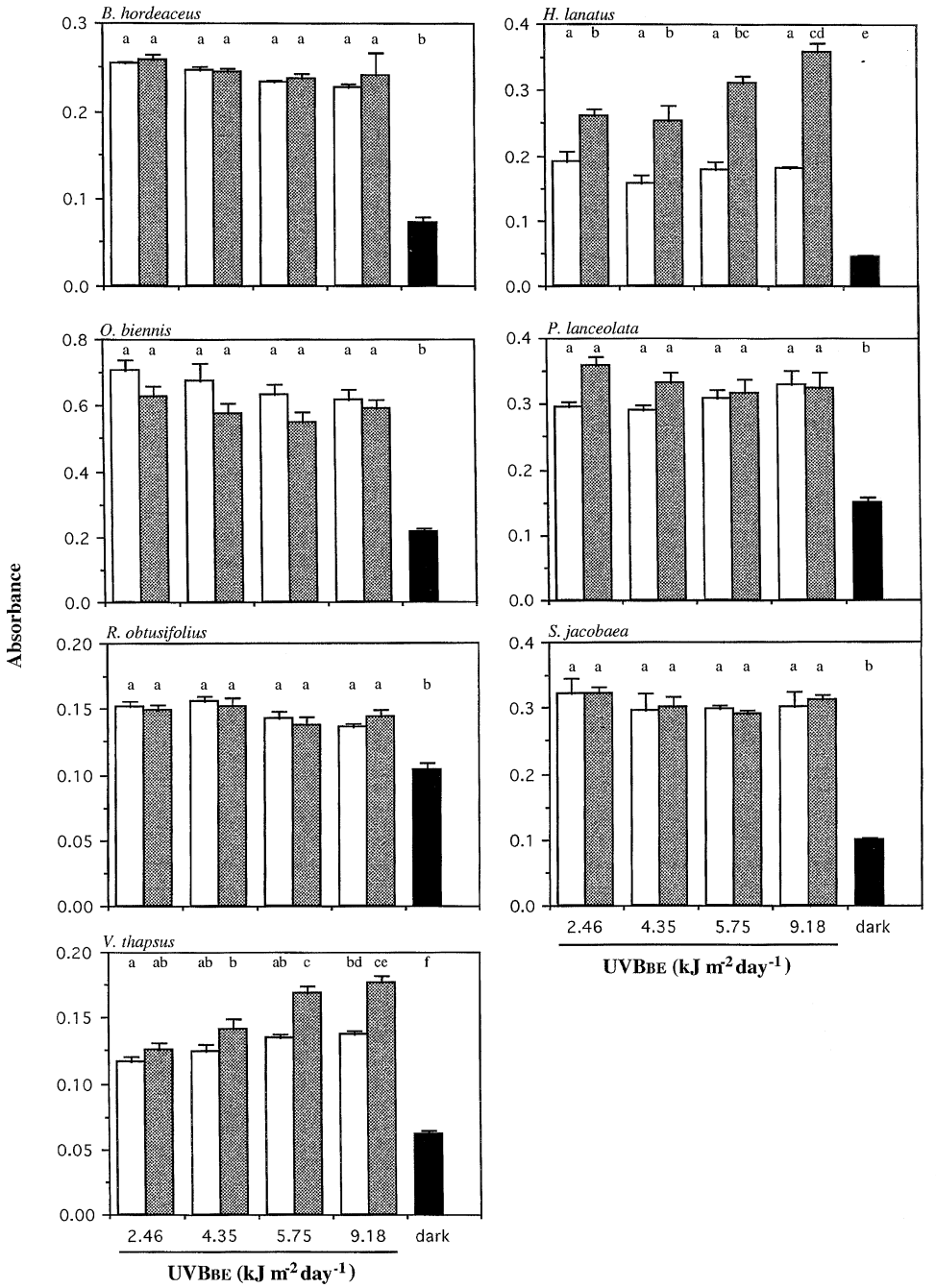

Figure 5. The influence of enhanced UV-B irradiance on UV-B absorbance of methanolic seedling extracts. Seedlings received no UV-B (□), additional UV-B (▨) or were kept in the dark (■). Absorbance was measured at 300 (*B. hordeaceus*, *O. biennis*, *S. jacobaea* and *V. thapsus*) or 310 nm (*H. lanatus*, *P. lanceolata* and *R. obtusifolius*) and was recalculated to a concentration of 1 mg fresh material per ml extraction solution. Values presented are means of 4 replicates ± SEM. Bars marked with the same letter are not significantly different ($p>0.05$).

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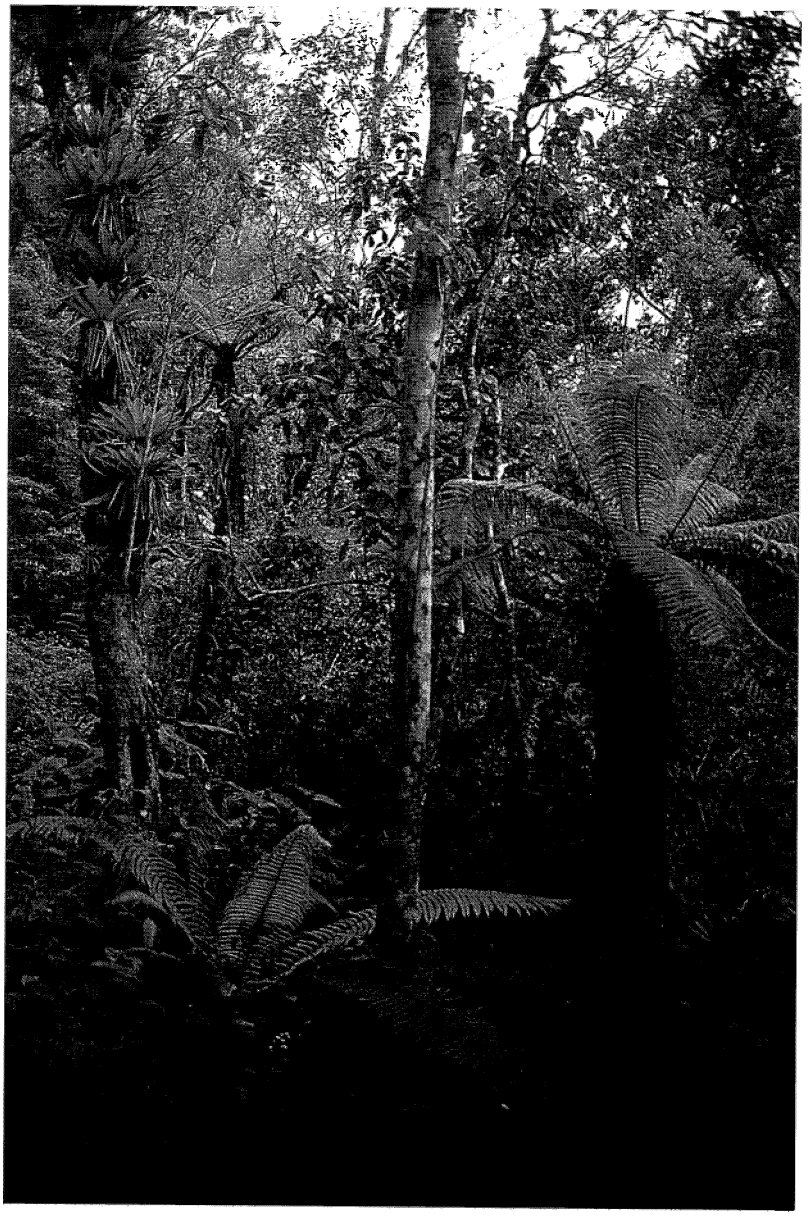

Treeferns and epiphytes in the Tropical montane cloud forest of the Blue Mountains, Jamaica. (Photograph: J. Rozema)

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J. Rozema, W. W. C. Gieskes, S. C. van de Geijn, C. Nolan and H. de Boois (eds), UV-B and Biosphere.

Leaf thickness and UV-B absorbing pigments of plants in relation to an elevational gradient along the Blue Mountains, Jamaica

Jelte Rozema[1], Agnes Chardonnens[1], Marcel Tosserams[1], Raemond Hafkenscheid[2] & Sampurno Bruijnzeel[2]
[1] *Department of Ecology and Ecotoxicology, Faculty of Biology, Vrije Universiteit;* [2] *Department of Hydrology, Faculty of Earth Sciences, Vrije Universiteit, Amsterdam, The Netherlands*

Key words: Elevation, Jamaica, Leaf thickness, Tropical montane cloud forest, UV-B absorbing compounds, UV-B radiation

Abstract

Terrestrial plant species vary widely in their adaptation to (increasing) solar UV-B radiation. Among the various responses of higher plants to enhanced UV-B are increasing leaf thickness and increasing concentrations of UV-B absorbing compounds. In some (UV-B resistant) plant species increased leaf thickness and UV-B absorbance may form part of mechanisms protecting plants from UV-B damage. However, in UV-B sensitive plant species leaf thickness and UV-B absorbance may increase as well with enhanced UV-B radiation. In the latter case however, this response cannot prevent plant damage and disturbance. In the present field study the relationship between these plant parameters and a natural elevational UV-B gradient on the tropical island of Jamaica was described. Four plant species of the Blue Mountain Tropical Montane Forest, occurring on open forest sites along the roadside and paths were studied along an elevational gradient. Plant species studied are Redbush (*Polygonum chinense*), Wild ginger (*Hedychium gardneranum*), John Crow Bush (*Bocconia frutescens*) and White clover (*Trifolium repens*). The elevational sites were at 800, 1000, 1200, 1400 and 1600 m above sea level. Leaf thickness was measured of leaves of intact plants around midday in the field. Leaf disks (5 mm) were sampled and extracted with a methanol/HCl mixture. UV-B absorption of these leaf extracts was measured spectrophotometrically. For all species leaves from higher elevations were thicker than those from lower elevations. In addition, the absorption of UV-B of leaf extracts increased with increasing elevations. It is assumed that the calculated gradient of the $UV\text{-}B_{BE}$ from 800 m above sea level: 9.45 kJ m^{-2} day^{-1} to 9.75 kJ m^{-2} day^{-1} at 1600 m is related to the measured increase of leaf thickness and UV-B absorbing compounds.

The responsiveness of these plant parameters to the elevational gradient does not necessarily imply that the plant species are UV-B resistant. One possibility is that the species studied, which are growing on open, disturbed sites on the forest floor and along mountain-roads, are relatively sensitive to UV-B. In addition to clear sky conditions, mist and clouds occur frequently in this tropical mountane forest at Jamaica. Also, the low nutrient status of the soil (low pH, nutrient deficiency) and the high content of polyphenols in leaves of many plant species of the tropical montane rain forest may relate to the marked response of the species studied with increasing elevation.

Abbreviations: asl – above sealevel, UV-B – ultraviolet-B radiation (280–320 nm), TMCF – Tropical Montane Cloud Forest.

Introduction

Reduction in stratospheric ozone due to anthropogenic emission of chlorofluorocarbons and nitrogen oxides leads to an increase of UV-B radiation reaching the earth's surface and to a shift towards shorter wavelengths (Blumthaler et al. 1985; Blumthaler & Ambach 1990, 1991; Madronich et al. 1995). Stratospheric ozone depletion occurs not only over the southern hemisphere, but also over the northern hemisphere (Peter 1993). In addition, not just northern arctic latitudes, but also more southern latitudes are affected (Stolarski et al. 1991). It is now generally accepted that depletion of stratospheric ozone will continue for some more decades, although measures to ban the emission of deleterious trace gases have been taken (Acevedo & Nolan 1993). There is great concern about the biological and ecological consequences of enhanced UV-B radiation (SCOPE 1993).

Enhanced UV-B radiation may cause damage to plants and animals resulting in reduced biomass (Caldwell 1977) and depressed primary production of ecosystems (SCOPE 1993). In addition, UV-B radiation affects, in a more subtle manner, plant morphogenic parameters such as biomass allocation, branching, reproduction and phenology (Caldwell et al. 1995; Kendrick & Kronenberg 1994; Rozema et al. 1996). Herbivory and decomposition are also affected by UV-B (Gehrke et al. 1995; Gwynn-Jones et al. 1997; Rozema et al. 1997). As a result, UV-B radiation will both directly and indirectly cause changes in vegetation structure, ecosystem composition and functioning.

To a large extent, knowledge of UV-B effects on plants and adaptations of plants to UV-B, is based on experiments in the laboratory, climate rooms, greenhouses and on outdoor experimental fields (Caldwell & Flint 1994; Rozema et al. 1993, 1995a, b). Most of these UV-B studies relate to crop plants (Rozema et al. 1992; Visser et al. 1996) while only few relate to plants of natural ecosystems (Rozema et al. 1995).

Experimental studies of terrestrial plants to enhanced UV-B radiation often indicate enhanced leaf thickness (Cen & Bornman 1990; Johanson et al. 1995; Murali & Teramura 1985) and an increase of UV-B absorbing compounds in leaf extracts (Barnes et al. 1987).

Both responses can, in principle, be viewed as functional adaptations to enhanced UV-B. A longer optical path through thicker leaves and absorption of penetrating UV-B radiation by UV-B absorbing compounds will help to prevent damage to the metabolism. Accumulation of UV-B absorbing secondary metabolites in epidermal cells of rice has been demonstrated to reduce UV-B damage (Lois & Buchanan 1994; Tevini et al. 1991).

However, a high or an increasing content, of UV-B absorbing compounds does not necessarily imply resistance against UV-B radiation. In (stunted) Tropical Montane Cloud Forests (TMCFs), such as at the study site at Jamaica, leaves may have a high content of (UV-B absorbing) phenolic compounds (Bruijnzeel et al. 1993). This may be a response to nutrient deficiency of the soil. Such an increased content of phenolic compounds may help Jamaican mountain trees and shrubs to adapt to UV-B also.

A rise in UV-B absorbing compounds in response to increasing UV-B radiation has been reported for a number of UV-B sensitive species e.g.: *Phaseolus vulgaris* (Cen & Bornman 1990); *Vicia faba* (Visser et al. 1997) and for soybean (Murali & Teramura 1985). Alternatively, UV-B resistant plant species may not show increased (overall) leaf UV-B absorbance in response to raised UV-B (Tosserams & Rozema 1995; van de Staay et al. 1995). Possibly, the same will hold for changes in leaf thickness and UV-B resistance. This means that there will be no general relationship between leaf thickness, leaf UV-B absorbance and plant resistance to UV-B radiation. Yet, leaf thickness and leaf UV-B absorbance can be used as plant parameters, which may be sensitive (or responsive) to changes of solar UV-B radiation. In such an approach, primarily disregarding UV-B resistance or sensitivity of species involved, leaf thickness and leaf UV-B absorbance may be considered as parameters to monitor variation of UV-B radiation.

In addition to an experimental analysis of the dose response relationship between leaf thickness and UV-B absorbing compounds and UV-B radiation levels (in a climate room or a greenhouse), elevational gradients offer the opportunity to test plant responses to natural gradients of solar UV-B radiation. More in particular, mountains in tropical regions, such as the Blue Mountain area on Jamaica, receive a high natural level of solar UV-B radiation. Elevational gradients of UV-B radiation have been used to assess UV-B sensitivity of plant species (Sullivan et al. 1992) and to assess responses of physiological parameters to UV-B (Barnes et al. 1987; Ziska et al. 1992). In these studies plants originating from various elevations were exposed to different levels of UV-B radiation, artificially supplied by UV-B lamps, in climate rooms or greenhouses. In the present study, variation of leaf thickness and UV-B absorbance

of leaf extracts of plant species actually growing along an elevational gradient of UV-B radiation in the Blue Mountain National Park at Jamaica, were studied.

Material and methods

Area description and elevational gradient

In the Blue Mountains and John Crow Mountains National Park on Jamaica, Tropical Montane Cloud Forest (TMCF) occurs (Bruynzeel & Proctor 1994). Clouds, mist or seafog are frequently present. Jamaica is situated in the Caribbean sea between 18° 32° N, 78° 22[1] E and 17° 48[1] N, 76° 19[1] E (Figure 1). The highest peak in the Blue Mountains is Middle Peak (2256 m) (Hodges 1993). The elevational gradient of the present study was from 800 m above sea level (asl), along the road to the Grand Ridge of the Blue Mountains, to New Haven Gap, 1550 m asl.

Soils of the TMCF have a high organic matter content and a low soil pH (H_2O) at the surface of 3.5–5.5 (Grubb & Tanner 1976). The soil nutrient content is low. Leaves of the Tropical Montane Cloud Forest are hard and leatherly (Bruynzeel & Proctor 1994; Tanner & Kapos 1982). However, these xeromorphic characteristics (Kapos & Tanner 1985) do not relate to limited water supply. Annual precipitation on the forest hills ranges from 2600 mm–4270 mm (Tanner 1977). In fact, the soils of the TMCF are subject to considerable leaching of nutrients and erosion. The canopy height of the TMCF ranges from about 5–13 m. Epiphytes are abundant particularly in forests with a less closed canopy. Montane cloud forests on the continents of the tropics may have a canopy height which significantly exceeds that of the TMCF of the island of Jamaica. Despite considerable debate (Bruijnzeel & Proctor 1994) nutrient deficiency seems to be the most likely cause of stunting of the montane rainforests on Jamaica.

Plant measurement and sampling

For the purpose of the present study, i.e. to test the relationship between the plant parameters leaf thickness, UV-B absorbance of leaf extracts and the UV-B fluence rate with increasing elevation, four plant species of open forest sites and the road verge, were chosen. These plant species were

(a) generally occurring along the elevational gradient (800–1600 m asl),

(b) more or less fully exposed to solar radiation (including UV-B radiation 280–320 nm),

(c) easily accessible for sampling and measurement

The species chosen were:

– Wild ginger (*Hedychium gardneranum* Sheppard ex Ker-Gawl, Zingiberaceae; On sheltered, steep roadside banks). Introduced to Jamaica from the Himalayan region.

– John Crow Bush (*Bocconia frutescens* L., Papaveraceae) Shrub 2–3 m, weed of clearings and roadsides from 350–2200 m asl.

– White clover (*Trifolium repens* L. Papilionaceae, Fabaceae). Weed of roadsides from about 800–2220 m asl. Native of Europe and Asia, introduced to Jamaica.

– Red bush (*Polygonum chinense* L., Polygonaceae). Introduced to Jamaica from East Indies and Japan. On open or shaded sheltered banks.

(Nomenclature and notes according to Adams, 1972.)

In addition, leaf thickness was measured of 5 shrub and tree species of the Tropical Montane Cloud Forest at New Haven Gap (1550 m asl) at man height (1.50 m) and at canopy height (5.7 m).

The estimated UV-B fluence rate along an elevational transect from 0 m–2400 m above sea level for the Blue Mountains on Jamaica ranges from 9.26 to 10.1 kJ m^{-2} day^{-1} (Figure 2). The estimated values were calculated for May 1, 1995 using the generalized plant weighting function (Caldwell 1977) and an empirical model (Green et al. 1980). Ozone thickness was taken 300 Dobson units and the aerosol scaling coefficient 1 for the Green calculation program.

Leaf thickness measurements

Measurements of the leaf thickness were done during the period March–May 1995 at open forest sites and the roadside between 11.00 and 14.00 h. These sites were generally fully exposed to the sun. Leaf thickness measurements were made with a dialpipe gage Mitutoyo with a range from 0.01 to 10 mm. The resolution of this thickness-meter is 0.01 mm. Thickness measurements were made in the middle of the leaf, next to the main vein, of at least twenty replicate leaves similarly exposed to the sun of the same developmental stage: just fully expanded leaves. Care was taken that each leaf was measured only once.

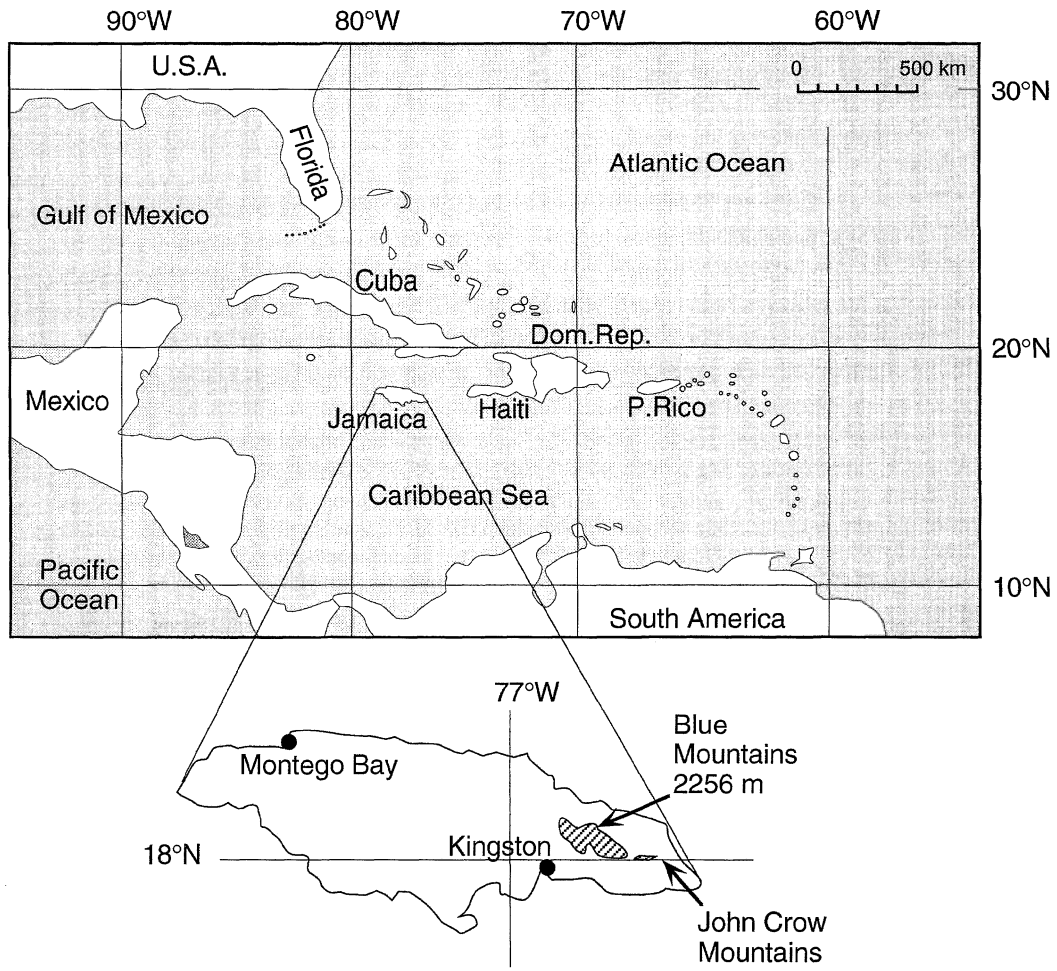

Figure 1. Location of Jamaica in the Caribbean Sea and the location of Blue Mountain and John Crow National Park on Jamaica.

Leaf disks and UV-B absorbing compounds

After leaf thickness measurement, leaf disks (0.5 cm diameter) were taken with a stainless punch, and put in screw-on tubes containing 5 ml extraction medium for UV-B absorbing compounds. The extraction medium consisted of acidified ethanol (79:20:1, v:v:v, ethanol: water: hydrochlorid acid) (Tosserams & Rozema 1995; van de Staaij et al. 1995). The tubes with leaf disks were heated in a waterbath for at least two hours until the leaf disks had completely bleached. The absorption of the leaf extract was measured spectrophotometically at 280, 300 and 320 nm using quartz cuvettes. Ten or more replicate leafdisks were sampled per species, per elevation. Depending on the sampling site, the time period between leafdisk collection, fixation in the extraction medium and absorption measurement varied between 1 and 7 hours. Absorption was expressed both per m^2 leaf area, and per m^3 leaf volume, correcting for differences in leaf thickness per litre extraction medium. The absorption of the leaf extract was calculated as follows:

Absorption
$(E/m^2/L)$ = Extinction/leaf area (mm^2) × 5 ml.
Absorption
$(E/m^2/L)$ = Extinction/leaf area (mm^3) × 5 ml.

Leaf volume was calculated as leaf area (3.14 × 2.5 mm^2 × leaf thickness (mm)).

Results of leaf thickness and UV-B absorbance measurements were statistically analysed with a one-way analysis of variance according to Sokal & Rohlf (1981).

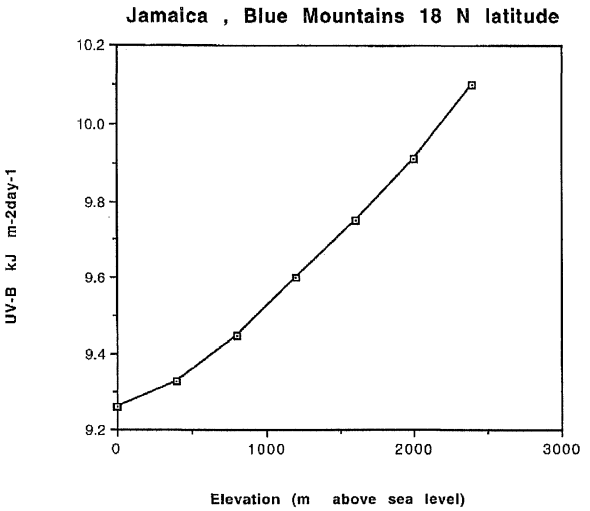

Figure 2. The relationship between the UV-B fluence rate (kJ m^{-2} day^{-1}) along an elevational transect 0–2400 m above sea-level in Jamaica (18° N) latitude. Fluence rate values have been calculated based on an empirical model (Green et al. 1980) and using the Generalized Plant Weighting function (Caldwell 1977) for May 1, 1995.

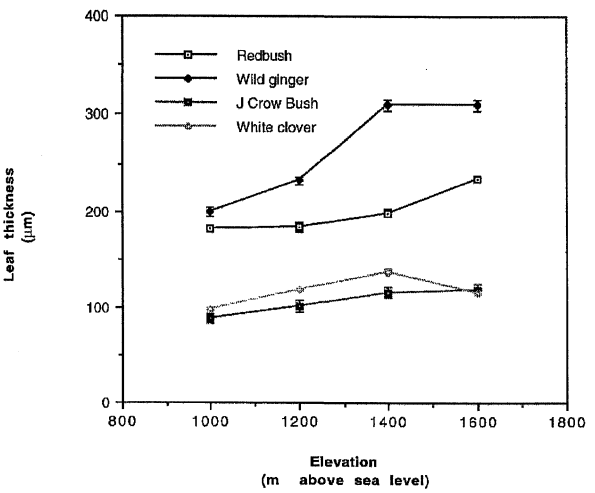

Figure 3. Leaf thickness (μm) of Redbush, Wild ginger, John Crow Bush, and White clover along an elevational gradient of approximately 800–1600 m above sea level. Average values with standard error of the mean.

Results

Leaf thickness of the four species studied increases with increasing elevation (Figure 3). For all the species, differences in leaf thickness between the different elevations are statistically significant ($p < 0.001$). One deviation is that leaf thickness of White clover, which increases over an elevational gradient 1000 m–1400 m asl, shows a decline from 1400 m–1600 m asl. Leaf thickness of Wildginger and Redbush is between 200 μm and 325 μm, which is in the range of leaf thickness of trees and shrubs of the TMCF measured at New Haven Gap (Table 1).

Leaf thickness of trees and shrubs of the TMCF measured at canopy height (5–7 m) always exceeds the values measured at man-height (1.50 m). This indicates that increased solar irradiance (PAR, UV-B radiation), in addition to other environmental parameters (e.g. temperature, vapour pressure deficit) at the canopy, directly relates to leaf thickness for all the TMCF species studied.

Also, this marked difference in leaf thickness between leaves at man height and canopy height, measured at one elevation (New Haven Gap, 1550 m asl), implies that differences in leaf thickness along an elevational gradient can only be detected when leaves with the same or similar exposure to solar radiation are measured and a standardized procedure is followed.

In addition to leaf thickness, the content of UV-B absorbing compounds of leaves of all four species increase statistically significantly with increased elevation (Figure 4). As for leaf thickness,the only somewhat deviating value refers to UV-B absorbance of leaves of White clover at 1600 m asl compared to 1400 m asl.

There is no significant difference in the elevational relationship with UV-B absorbance of leaf extracts expressed on a per leaf area or per leaf volume basis.

Discussion

The calculated elevational gradient of biologically effective UV-B radiation ranged from 9.25 at sealevel to 10.1 kJ m^{-2} day^{-1} at 2500 m asl. For the elevational range of 800–1600 m asl the range is 9.45–9.75 kJ m^{-2} day^{-1} (Figure 2). Measured values of UV-B radiation at Jamaica are unknown to us. In the Tropical Montane Cloud Forest environment, measurement of solar UV-B radiation can be disturbed by the frequent occurrence of seafog, mist and clouds. Yet, the UV-B fluence rate (9–10 kJ m^{-2} day^{-1}) in this tropical area is high, in comparison for example with a summer value for the Netherlands measured at clear sky (4–5 kJ m^{-2} day^{-1}) (Tosserams & Rozema 1995). Naturally occurring plant species in such a high UV-B radiation environment may well be adapted to increased or changing UV-B fluence rate (Caldwell et al. 1980; Häder & Tevini 1987). More generally,

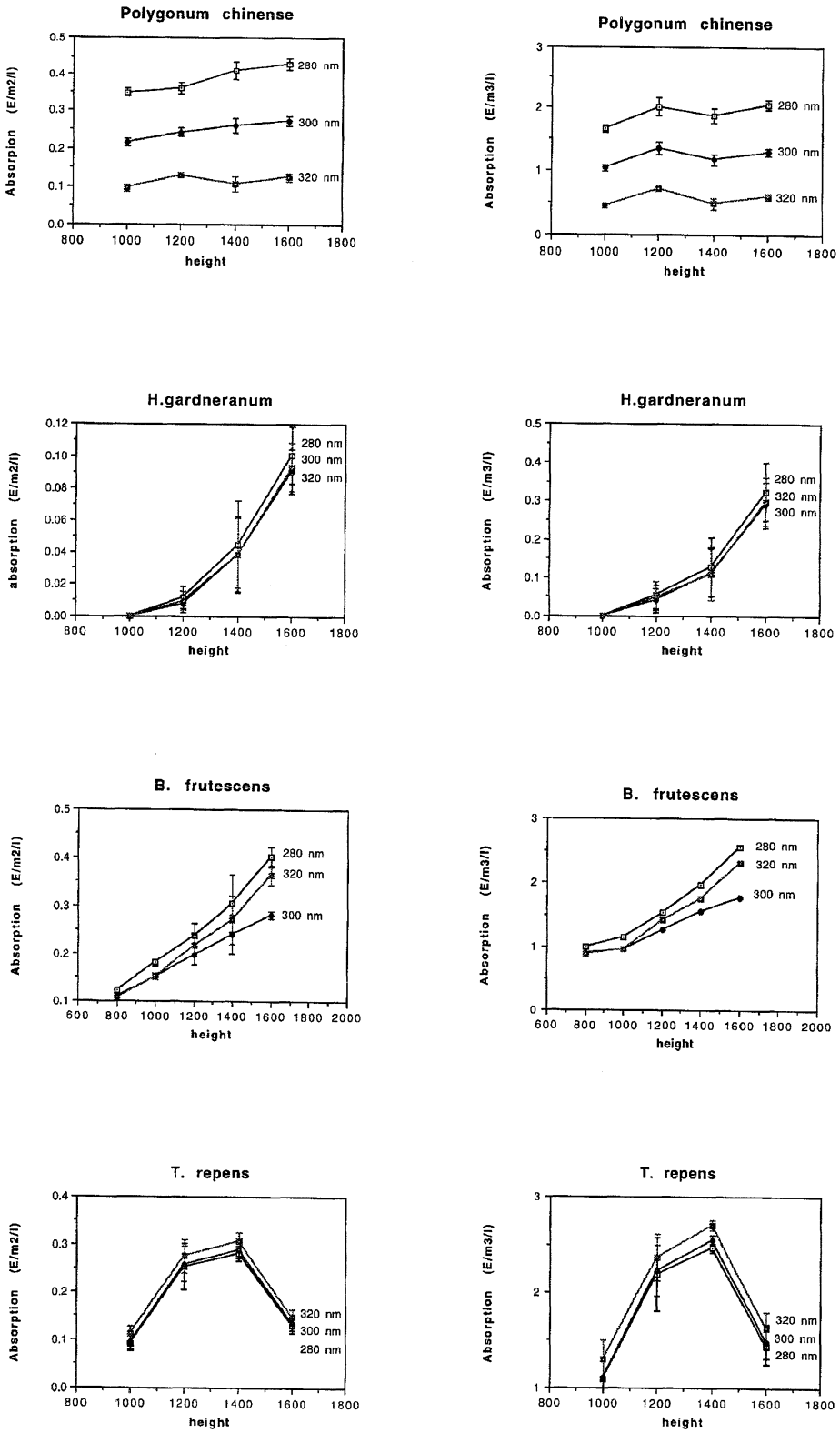

Figure 4. UV-B absorbance at 280, 300 and 320 nm of leaf extracts of Redbush, Wild ginger, John Crow Bush and White clover expressed as $E/m^2/l$ and as $E/m^3/cl$.

Table 1. Leaf thickness (μm) of plant species in a Tropical Montane Cloud Forest at New Haven Gap, approximately 1550 m above sea level, measured at man-height (1.50 m) and at canopy-height (5–7 m). Average values and standard deviation. Number of replicated leaves in brackets.

Species	Family	Leaf thickness man height	Leaf thickness canopy height
Podocarpus urbanii Pilger	Taxaceae, Gymnospermae	483 ± 30 ($n = 218$)	495 ± 28 ($n = 192$)
Chaetocarpus globosus (SW.) F&R	Euphorbiaceae	270 ± 65 ($n = 198$)	390 ± 50 ($n = 46$)
Cyrilla racemiflora L.	Cyrillaceae	225 ± 30 ($n = 258$)	320 ± 65 ($n = 97$)
Alchornea latifolia SW.	Euphorbiaceae	210 ± 40 ($n = 35$)	234 ± 28 ($n = 25$)
Lyonia octandra (SW.) Griseb	Ericaceae	208 ± 54 ($n = 247$)	275 ± 38 ($n = 148$)

form and function of plants in montane environments have attracted the attention of ecologists (Rundel et al. 1994).

Leaves of many shrubs and trees of the (stunted) Tropical Montane Cloud Forest of Jamaica are characterized as sclerophyllous (Kapos & Tanner 1985; Tanner & Kapos 1982). The mesophyllous leaves of John Crow Bush and White clover are significantly thinner (Table 1 and Figure 3). The marked decrease in leaf thickness measured from canopyheight (5–7 m) to man height (1.5 m) indicates the responsiveness of this parameter to an environmental gradient of solar radiation (Table 1). However at canopy height there will not only be enhanced solar UV-B radiation (280–320 nm), but also raised levels of photosynthetically active radiation (PAR; 400–700 nm) compared to more shaded conditions at 1.5 m. The observed increase of leaf thickness when comparing leaves at man-height and canopy-height indicates that the method we used for leaf thickness measurement appears to be sufficiently sensitive to assess thickness changes over such radiation gradients. At the same time it implies that in our research, aiming at a relationship between leaf thickness and elevational UV-B gradient, leaf thickness measurements should be standardized and other sources causing leaf thickness changes should be avoided. By studying plants of open forest sites and roadsides and equally exposed to solar radiation and measurement of leaf thickness between 11.00 and 14.00 hours, we have attempted to prevent disturbance of leaf thickness changes by other factors.

In a study of sclerophyllous mountain fynbos vegetation near Hermanus (34° S, 19° E), South Africa, Wand (1995) reached conclusions related to ours. Wand (1995) analysed Specific Leaf Mass (SLM, expressed as g leaf biomass per m^2 leaf area), which is a measure of leaf thickness, and UV-B absorbance of acidified methanol leaf extracts. Leaves of 38 plant species occurring over an elevational gradient from 100 to 824 m above sea level were analyzed. Leaves collected at high elevation exhibited higher UV-B absorbances than those from lower elevations. Although no significant increase was found for SLM (leaf thickness) with increasing elevation, there was a positive overall correlation between SLM and UV-B absorbance per unit leaf area. Lovelock et al. (1992) found a positive relationship between soluble phenolic compounds in the leaf and leaf thickness. For the four plant species selected in the present study, with all species occurring over the whole elevational gradient 800–1600 m, both leaf thickness and UV-B absorbance of leaves increased with increasing elevation. Plant species from a 3000 m elevational gradient in Hawaii, with an estimated range of UV-B fluence rate of 10.8–12.4 kJ m^{-2} day^{-1} (Sullivan et al. 1992) appeared to differ in UV-B sensitivity. In general, sensitivity to UV-B radiation was reduced as the elevation of the plant's origin increased (Ziska et al. 1992). Similarly, Barnes et al. (1987) demonstrated that plants grown from seed collected from equatorial, alpine sites (with high solar UV-B irradiance) showed no UV-B induced damage.

Obviously, the *in situ* study of plants along an elevational UV-B gradient, as in our present study, has the advantage of avoiding many methodological problems linked with UV-B supplementation with lamps (Caldwell & Flint 1994). The disadvantage of an elevational gradient is that other environmental factors than UV-

B (a.o. soil nutritional status, water availability) may change as well with elevation. Yet, it is tempting to hypothesize that increase of leaf thickness and content of UV-B absorbing compounds with higher elevations as observed for mediterranean species in South Africa (Wand 1995), and plant species from a tropical montane environment (this paper) relates to protection against (enhanced) UV-B radiation.

Leaves of the Tropical Montane Cloud Forest of Jamaica are generally sclerophyllous, leatherly, hard, relatively thick and high in phenolic compounds (Bruijnzeel et al. 1993; Kapos & Tanner 1985). This may be linked with the nutrient deficient soil conditions with a low pH (3.5–5.5) (Grubb & Tanner 1976; Murali & Teramura 1985) report soybeans to have smaller and thicker leaves with increased concentration of flavonoids, when grown in shortage of phosphorus and at enhanced UV-B. This led to reduced sensitivity of soybean to elevated UV-B irradiance. So, changes of leaf thickness and leaf UV-B absorbance along an elevational transect may not only reflect a response to increasing UV-B radiation, it may also relate to the soil nutrient status. From an ecological-evolutionary point of view, a combination of various functions of thick leaves, high in UV-B absorbing compounds, will be advantageous. Cyanogenic glycosides in *Trifolium repens* for example help to prevent herbivory by snails and slugs. Absorbance of UV-B radiation by such compounds may also prevent UV-B damage (Harborne 1993). The road-side and forest gap species we studied apparently prefer open habitats (cf. Ballaré 1994). Therefore, they should not be regarded as typical TMCF species. All species studied have been introduced to Jamaica and behave more or less as weeds (Adams 1972).

The marked increase of leaf thickness and leaf UV-B absorbance of four species along an elevational UV-B gradient at Jamaica indicates that a natural UV-B gradient provides a useful tool to assess plant responses to natural variation of UV-B radiation. Leaf thickness and leaf UV-B absorbance of the four road-side species represent responsive morphological and physiological plant parameters monitoring natural variation in UV-B radiation. Further field and experimental research is needed to understand in more detail the ecological and physiological function of increased leaf thickness and UV-B absorbance in these species of a Tropical Montane Cloud Forest environment.

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Acacia tortilis in an overgrazed savanna in Botswana. (Photograph: W. H. O. Ernst)

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Reaction of savanna plants from Botswana on UV-B radiation

Wilfried H.O. Ernst, Jos W.M. van de Staaij & Hans J.M. Nelissen
Department of Ecology and Ecotoxicology, Faculty of Biology, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

Key words: Acacia tortilis, *Chloris virgata*, Nutrient content, Photosynthesis, Seedling growth, *Tragus berteronianus*

Abstract

The annual savanna grasses *Chloris virgata* (C_4) and *Tragus berteronianus* (C_3) and the tree *Acacia tortilis* were exposed in a greenhouse to elevated UV-B radiation (16.8 kJ m^{-2} d^{-1} $UV\text{-}B_{Be}$) and to no UV-B and grown on a poor and a rich soil for one life-cycle (grasses) and one growing season (*Acacia*). UV-B radiation had no effect on biomass production and caryopses mass of both annual grasses. The longevity of the cotyledons of *A. tortilis* was shortened by 4 to 10 days under enhanced UV-B radiation, which also hampered the translocation of Fe, Mg and Mn from the cotyledons to the seedling and the retranslocation of Mn on both soil types and that of P on fertile soil out of senescent leaves. At the end of the growth period (190 days after germination), photosynthesis of UV-B radiated leaves of *A. tortilis* was significantly decreased and supported the tendency of decreased biomass of UV-B radiated plants. It is concluded that from the investigated savanna species the grasses are relatively well adapted to increased UV-B due to their actual exposure to high UV-B radiation under Botswana conditions, whereas saplings of *A. tortilis* are more sensitive to UV-B radiation.

Introduction

The depletion of the atmospheric ozone layer, mostly due to emitted CFC's (Fisher et al. 1990) and the expected increase in UV-radiation at the earth's surface (Caldwell et al. 1989) may affect plant growth. Negative or neutral effects of enhanced UV-B radiation, i.e. radiation between 280 and 330 nm have been reported for agricultural crops and wild plant species, often in a species-specific and genotype specific way (Larson et al. 1990; Lovelock et al. 1992; Murali et al. 1988; Teramura et al. 1990, Teramura & Sullivan 1994; Tevini 1994; Van de Staaij et al. 1990). Generally, exposure to increased UV-B radiation is known to inhibit plant growth and photosynthesis of plants from regions with a relatively low UV-B radiation (Sisson & Caldwell 1977, Teramura 1983, Van de Staaij et al. 1990). Other environmental factors, e.g. water deficits (Murali & Teramura 1986), phosphorus deficiency (Murali & Teramura 1987) and high irradiation levels of visible light (Cen & Bornman 1990) reduce the negative impact of UV-B radiation to pulses.

The stronger destruction of the ozone layer over the antarctic and the southern hemisphere (Crutzen 1992) than over the northern hemisphere may expose plants in subtropical ecosystems more to UV-B radiation than in temperate ecosystems (Frederick et al. 1989). In the present study plant species from a savanna of southern hemisphere with a well-known ecology (Ernst & Tolsma 1989), i.e. that of Botswana, were exposed to increased UV-B radiation to test their growth performance in the vegetative and reproductive stage. The leaves of the grasses and trees are not protected from UV-B penetrance by sclerophylly like those of the South African fynbos (Wand 1995).

Based on model calculation of the increase of UV-B radiation after a 20% reduction of stratospheric ozone (Green 1983), the UV-B radiation in the semi-arid savanna in Botswana (1000 m above sea-leavel, 18°S-25°S) will increase from presently 13.5 kJ m^{-2} d^{-1} (Musil 1995) to 16.8 kJ m^{-2} d^{-1} $UV\text{-}B_{Be}$. Due to the shortage of major nutrients in most savanna soils in Botswana (Ernst & Tolsma 1989) the possibly modifying effect of poor soil on UV-B exposure was tested by

growing grasses with C_3 and C_4 type of photosynthesis and a dominant C_3 savanna tree under nutrient-poor and nutrient-enriched conditions.

Material and methods

Plant material

Seeds of the wide-spread savanna tree *Acacia tortilis* (Forsk.) Hayne and caryopses of the annual savanna grasses *Chloris virgata* Swartz, a C_4 grass (Veenendaal et al. 1993), and *Tragus berteronianus* Schulten, a C_3 grass (Ernst & Tolsma 1992) were collected in a tree savanna near Gaborone/Botswana (for site description see Tolsma et al. 1987) and stored for one year to break the dormancy of the caryopses (cf. Ernst & Tolsma 1988, Veenendaal & Ernst 1991). The high variation of seed mass of *A. tortilis* and the high degree of infestation by bruchid beetles and seminivorous hymenopterans (Ernst et al. 1989) made it necessary to test first the relationship between cotyledon mass and seed mass and the influence of cotyledon mass on sapling performance.

Plant growth

For the seed selection experiment 100 seeds of *A. tortilis* were slightly filed (Tietema et al. 1992), imbibed for one day and separated into seed coat and embryo. The seed coat was dried on silical gel for 1 week and weighed. The embryos were differentiated according to their colour, i.e. yellow, green and white, dried at 90 °C, weighed, and the relation between embryo mass and seed mass calculated. The impact of seed (embryo) mass on seedling vigor was analysed by dividing the seed population of one tree after discarding infected seeds in 10 mass classes from 19.6 to 53.6 mg and a separate class below 19.6 mg. Ten seeds were selected from each mass class. To avoid mass-dependent dormancy effects and delayed germination, seeds were filed, placed in petridishes on a grid, moistened with demineralized water and kept at a temperature regime of 25/20 °C (day/night, 12/12 hours). Within two days all seeds germinated, so that nearly even-aged seedlings were transplanted to 750 cm^3 pots, filled with coarse sand. Pots were randomly placed in a greenhouse at 300 $\mu E\ m^{-2}\ s^{-1}$ (12 hours per day) irradiation at a temperature regime of 25/20 °C (day/night) and a relative air humidity of 50 ± 10%. After 4 weeks of growth, plants were harvested, dried at 90 °C for 48 hours and weighed.

For the UV-B experiments seeds of *A. tortilis* with a mass between 42 and 47 mg were selected and pretreated as described above. Just after germination seedlings of *A. tortilis* and the two annual grasses were transfered to plastic pots (500 ml), two seedlings per pot, and filled with dune sand for the nutrient-poor condition or with a commercial garden soil for the nutrient-enriched conditions (cf. Ernst 1983). All pots were placed in a greenhouse, with 14 hours natural sun light, filtered by window glass. The UV-B treatment for half of the experimental plants (5 at each nutrient condition and each UV-B level) was given with UV-lamps at a daily dose of 16.8 kJ $m^{-2}\ d^{-1}$ as biologically effective radiations with 6-hr irradiance periods centered midway through the photoperiods, normalized at 300 nm. The dose is comparable with expected UV-B conditions in Botswana. The control treatment was with UV-B-lamps, but filtered by mylar foil. The UV-lamps (Philips TL 12/40 tubes) were covered with 0.1 cellulose acetate foil to filter radiation below 290 nm. UV-measurements were carried out with a UV-X radiospectrometer (Optronics OL752; van de Staaij et al. 1993). In addition to the natural light, plants received supplementary irradiation (200 $\mu E\ m^{-2}\ s^{-1}$ at plant level) from 400 W Philips HPI/T lamps 14 h daily. Temperature in the greenhouse was maintained between 18–25 °C, relative humidity between 60% (day) and 75% (night).

In the case of *A. tortilis*, the soil was covered by aluminium foil, so that cotyledons and shed leaflets could be collected without soil contamination. Plants of all species and all treatments were watered every third day (demineralized water). They were grown up to caryopsis ripeness for 90 days (*Tragus berteronianus*) and 125 days (*Chloris virgata*), respectively. *A. tortilis* saplings were grown for 190 days, simulating a natural growth period in the savanna.

Photosynthesis and transpiration

Both parameters were measured on individual leaves of *A. tortilis* after 185 days of growth with a Parkinson Leaf Chamber of a portable ADC-LCA-3 system (Analytical Development Company, Haddesdon, UK) at a photon flux density of about 250 $\mu E\ m^{-2}\ s^{-1}$ (PAR). Leaf area was recorded with a LICOR 3100 area meter.

Leaflet movement

Leaflets of *A. tortilis* have a daily rhythm in folding up and expanding, as many leguminous species in Southern Africa (Ernst 1988). This daily rhythm can be disturbed by water shortage and high temperature. Due to a reported impact of UV-B radiation on photonastic movement of bean leaves (Tendel & Häder, 1995), we investigated the leaflet movement of *A. tortilis* between day 170 and 175 conform the method described by Ernst (1988).

Harvest

At harvest roots were carefully washed in demineralized water. Plants were dissected into leaves, stems, roots, root nodules (for *A. tortilis* only) and caryopses (for the grasses only) and except for caryopses, dried at 80 °C for 48 hours. The caryopses were stored over silica gel for 10 days prior to weighing, and then stored at 21 ± 2 °C for afterripening (Veenendaal & Ernst 1991) and further experiments.

Chemical analysis

Dried plant parts were wet-ashed in Teflon bombs at 140 °C (HNO_3: $HClO_4$, 7:1, v/v) over night. After dilution of the digest, the mineral nutrients calcium, copper, iron, magnesium, manganese, potassium and zinc (calcium and magnesium after addition of lanthanun nitrate (1% v/v)) were analysed by atomic absorption spectrophotometry (Perkin Elmer 4000). Phosphorus was determined by spectrophotometry after formation of a blue ascorbic acid-phosphorus complex (Chen et al. 1956). Nitrogen was analysed after burning the dried plant material with oxygen, separation of the gases by column chromatography (Kirsten 1979) and detection by IR in a Carlo Erba HNC-analyzer. As reference material of analytical procedures the olive leaf standard (BCR) and an own laboratorium-standard of *A. tortilis* were used.

Statistical analysis

Data were – if possible – statistically analyzed using a one way or two way analysis of variance (ANOVA) to test the significance of treatments, and correlation coefficients (Sokal & Rohlf 1981). Due to interdependence of cumulative losses of leaves (Figure 3) statistical testing is only allowed for the final value.

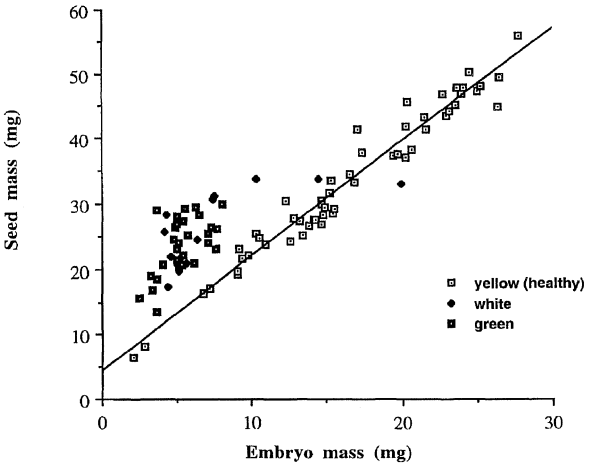

Figure 1. The relationship between seed mass and embryo mass in seeds of *Acacia tortilis*, collected at Gaborone. Prior to germination embryos were classified according to the colour of their cotyledons as yellow (healthy), white and green (both non-vital).

Results

The seedling selection experiment

Non-infested seeds of *A. tortilis* contained three different types of cotyledons: white and spongy, green and compact, yellow and compact. Seeds with white and green cotyledons, making up between 1 and 3% of non-infested seeds, were not able to germinate and to develop to a seedling. For seeds with yellow cotyledons there was a highly significant correlation between seed mass and embryo mass ($P < 0.001$; $r = 0.97$) which can be described by the linear function y (seed mass) = 4.21 + 1.77 x (embryo mass), as shown in Figure 1.

Seeds with a mass below 18 mg produced seedlings which died within two weeks after germination. Seeds above 18 mg gave viable seedlings, although the mortality of seedlings derived from seeds with less than 33 mg, was high. All seedlings from seeds above 45 mg survived. These seedlings produced the highest biomass after 4 weeks of growth (Figure 2). Seedlings derived from a seed mass of less than 23 mg had only a quarter of the biomass produced by seedlings from large seeds. Over the total seed mass range, there was a linear relationship between seed mass and seedling mass with y (seed mass) = 20.75 + 0.92 x (seedling mass after 4 weeks) and a correlation coefficient r = 0.74. The correlation was greater in the range from 18 to 33 mg (r = 0.83). Above a seed mass of 40 mg, seedling mass was only weakly (r = 0.45) linearly related

Table 1. Biomass (mg) of various plant parts and total saplings of *Acacia tortilis* grown for 190 days with or without UV-B radiation on nutrient-poor and nutrient-enriched soil. n=5 per UV-B-treatment and per nutrient condition. Values are means ± S.E. None of the biomass of plants (parts) grown in the nutrient-enriched soil was significantly ($P < 0.05$) higher without UV-B radiation than that with UV-B radiation.

Treatment	Root nodule	Roots	Stems	Dead leaves	Green leaves	Total sapling
nutrient-poor soil						
–UV	5.7	41.9	39.6	43.1	23.6	153.9
	±1.3	±4.0	±7.1	±8.2	±9.5	±18.3
+ UV	5.1	49.4	43.8	48.8	28.4	175.5
	±0.9	±6.6	±2.4	±10.2	±13.3	±19.4
nutrient-enriched soil						
–UV	38.6	411.1	292.2	52.8	303.8	1098.4
	±19.9	±202.8	±142.5	±27.7	±109.8	±493.4
+ UV	29.2	143.4	193.9	45.4	302.7	714.5
	±11.8	±78.6	±91.4	±28.5	±142.2	±292.3

Table 2. Photosynthesis, transpiration and water use efficiency of *Acacia tortilis* plants 185 days after germination, when grown with (+) and without (–) UV-B radiation on nutrient-rich soil. Values are the means of 6 measurements per treatment (± S.E.) (one way ANOVA).

Parameters	–UV-B	+ UV-B	Significance
Photosynthesis (μmol CO_2 m^{-2} s^{-1})	6.78±0.90	4.94±0.90	$P = 0.014$
Transpiration (mmol H_2O m^{-2} s^{-1})	7.51±1.39	8.56±3.34	N.S.
Water use efficiency (μmol CO_2/mmol H_2O)	0.93±0.27	0.65±0.28	N.S.

to its seed mass. Therefore it was decided to take only seeds above 40 mg for the UV-B experiments.

The UV-B experiment

Annual savanna grasses

Growth and seed production of the two investigated savanna grasses *Chloris virgata* and *Tragus berteronianus* were not affected by the UV-B treatment. The caryopsis mass was not different between control and UV-B treated plants, being for *C. virgata* 155 ± 21 μg and 157 ± 29 μg, respectively, and for *T. berteronianus* 186 ± 39 μg and 179 ± 27 μg, respectively. The caryopsis mass of both grasses is in good agreement with field-collected caryopses (Veenendaal & Ernst 1991).

Acacia tortilis

Independent of soil nutrient conditions, seedlings of *A. tortilis* under UV-B treatment started to shed their cotyledons 50 days after germination, 4 days earlier

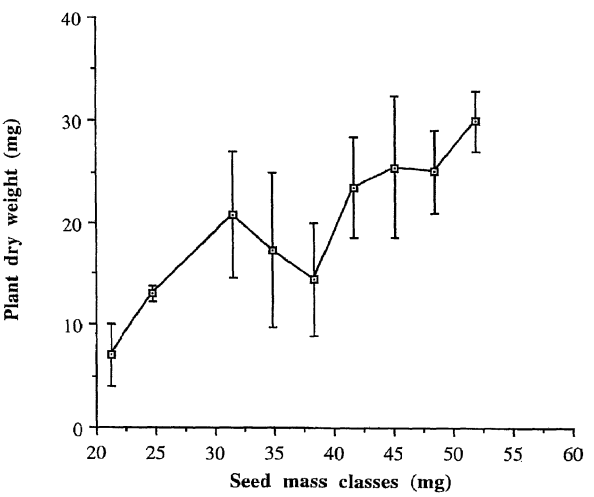

Figure 2. The relationship between initial seed mass and biomass of 4-weeks-old seedlings of *Acacia tortilis*. The mean of each class is based on 8 seedlings. The error bars present ± 1 S.E.

than seedlings without UV-B radiation ($P < 0.001$). After 56 (UV-B) and 60 days (control) of growth all seedlings had lost their cotyledons. The necromass of shed cotyledons of UV-B treated seedlings did not significantly ($P > 0.05$) differ from control seedlings. On nutrient-enriched soil, the necromass of shed cotyledons was twice as much as those on nutrient-poor soil ($P < 0.001$), independent of UV-B treatment. During the development of the saplings there was a continuous loss of old leaves (Figure 3) which was slightly higher in UV-B radiated plants. At the end of the growth period necro-leaf mass of UV-B radiated plants tended to be higher than in control plants.

After 190 days of growth, total biomass and the biomass of the various plant parts, except for dead leaves, strongly differed between saplings grown on nutrient-poor and nutrient-enriched soil (Table 1, $P < 0.001$). Within each nutrient level, the exposure to UV-B radiation did not result in a significant effect on the overall biomass. However, there is a tendency that saplings on nutrient-poor soil grew slightly better under UV-B radiation whereas samplings on nutrient-enriched soils were slightly affected by UV-B. There was also no significant interaction between UV-B treatment × soil fertility.

At day 185 after germination, photosynthesis of plants grown without UV-B radiation was 37% higher than that of UV-B radiated plants (Table 2). Transpiration of plants grown with or without UV-B did not significantly differ, so that the water use efficiency was slightly better in non-radiated plants than in radiated ones. Between day 170 and 175, the daily closure movement of leaflet was delayed by 1.3 ± 0.1 h without any effect on the opening movement the next morning.

With regard to mineral nutrition *A. tortilis* as a leguminous plant is characterised by two phases: (1) the mobilization of the nutrients in the cotyledons and their transfer to the developing seedling; (2) the nutrient uptake from the soil, distribution over the various plant parts and the retranslocation out of senescent leaves as well as the nitrogen supply by symbiosis with *Rhizobium* species. During the first phase the nutrient amount translocated from the cotyledons to the seedling (Table 3) was nearly the same for copper, nitrogen, phosphorus, and zinc in both treatments. UV-B radiation decreased the translocation of iron, magnesium, manganese and potassium and increased that of calcium ($P < 0.05$). During the second phase differences in soil nutrient status had stronger effects on mineral concentration of *Acacia* saplings and their plant parts (not shown) than UV-B radiation (Table 4). Saplings on rich

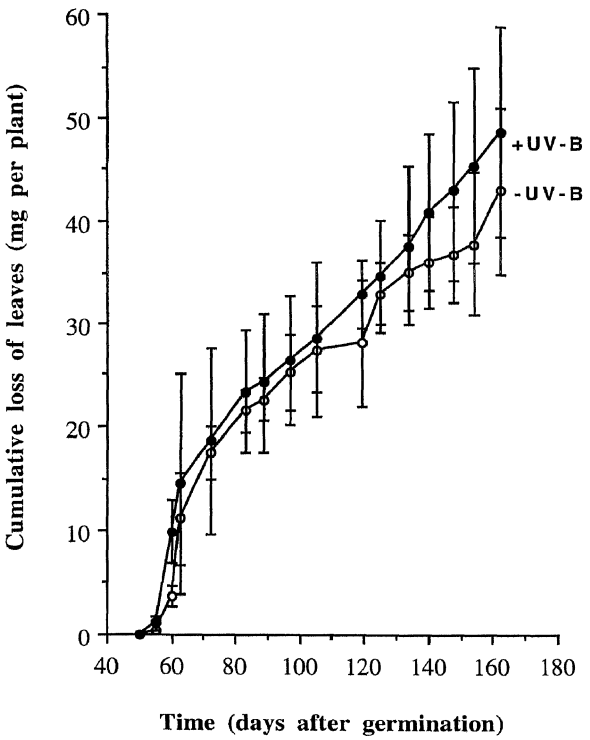

Figure 3. Cumulative loss of senescent leaves of *Acacia tortilis* saplings grown with (open circle) and without (closed circle) UV-B radiation. Differences between treatments are not significant.

soil contained nearly twice the nitrogen concentration than those on poor soil. For all other elements, except potassium, saplings on rich soil contained less nutrients than on poor soil: phosphorus and zinc decreased by a factor of 3 to 5, Fe, Mg, Mn, and Zn by a factor of 2 to 3. UV-B radiation let increase the manganese concentration of saplings at both fertility levels, and the phosphorus concentration of saplings on fertile soil. This effect of UV-B radiation was due to a strong increase of manganese and phosphorus in the shoot (Mn: 348 ± 29 vs 658 μmol g^{-1} dry wt; P: 24.5 ± 7.8 vs 44.5 ± 11.8 μmol g^{-1} dry wt in saplings grown without and with UV-B radiation, respecitvely). UV-radiation had also an impact on the retranslocation of phosphorus and manganese from dying to living leaves of saplings grown on a fertile soil by diminishing the retranslocation efficiency (Figure 4) so that the leaf litter of irradiated saplings contained significantly ($P < 0.05$) higher concentrations of manganese and phosphorus than that of non-irradiated plants.

Table 3. Mobilization of nutrients (μmol per cotyledon) in the cotyledons and their translocation to the growing seedling of *Acacia tortilis* grown with and without UV-B radiation in a greenhouse. The nutrient amount in the plumula and radicula of non-germinated seeds are too small, so that they are taken together with the cotyledons. *

	Biomass (mg)	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
non-germinated seeds	20.5	124.4	2.13	5.98	3.77	3.26	0.0160	0.0141	0.0162	0.00185
cotyledon after shedding										
without UV-B	7.1±1.0	9.6	0.22	0.52	2.83	2.71	0.0131	0.0134	0.0064	0.00071
with UV-B	6.6±0.9	8.9	0.25	0.75*	2.59	3.09	0.0155	0.0140	0.0065	0.00071
Transfer to seedlings										
(% of the non-germinated seeds)										
without UV-B	–	92.3	89.7	91.3	24.9	16.9	18.1	5.0	60.5	61.6
with UV-B	–	92.8	88.3	87.5	31.3*	5.2*	3.1*	0.7*	59.9	61.6

*Significant difference at $P<0.05$

Table 4. Mean biomass and mean nutrient concentration of saplings of *Acacia tortilis* grown on soil poor or rich in nutrients and with (+) and without (–) UV-B radiation in a greenhouse for 190 days. Elements with a significant ($P < 0.05$) difference between UV-B treatments and soil nutrition are indicated by different letters.

Soil condition	UV-B	Biomass	μmol g^{-1} dry wt					nmol g^{-1} dry wt			
		(mg)	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
Poor	–	154^a	1219^a	148^a	441^a	573^a	180^a	7130^a	910^a	1450^a	134^a
	+	176^a	1099^a	120^a	435^a	509^a	197^a	6315^a	1294^b	1336^a	157^a
rich	–	1098^b	1976^b	28^b	313^b	357^b	91^b	2565^b	386^c	492^b	45^b
	+	715^b	2248^b	43^b	391^{ab}	386^b	114^b	2340^b	613^d	631^b	35^b

Discussion

UV-B radiation had no effect on the annual grasses *Chloris virgata* and *Tragus berteronianus*, as far as biomass production and caryopses mass is taken as parameter. There was no difference between the C_3-grass *T. berteronianus* (Ernst & Tolsma 1992) and the C_4-grass *C. virgata* (Veenendaal et al. 1993). These responses fit quite well to those of monocotyledonous Iridaceae species in South Africa (Musil 1995). In contrast to semi-arid monocots UV-B radiation had an impact on life history and physiological processes in the dicotyledonous savanna tree *Acacia tortilis*. The increase of seeds with white cotyledons (Figure 1), as observed between the years 1980 and 1990 (Ernst, unpublished), may be not the result of enhanced radiation but of increased self-fertilization or geitonogamy due to the decrease of mature trees by firewood cutting. Although there was a very good correlation between cotyledon mass and seed mass (Figure 2), there was still a high genotypic variability of the selected seed mass classes of *A. tortilis* (J. Schroten, pers. commun.) and phenotypic plasticity. Both parameters gave a lot of treatment-independent variation, which had reduced the significance level of the experiments with *A. tortilis* saplings.

The acceleration of cotyledon shedding of *A. tortilis* under UV-B radiation does not influence the mobilization of carbohydrates and proteins in the cotyledons (Table 3) and their export to the seedlings, as can be judged by the remnant dry matter and the concentration of nitrogen and phosphorus of the shed cotyledons. The significant decrease of the translocation of iron, magnesium and manganese from UV-B radiated cotyledons to seedlings will finally have no ecological consequence for the sapling, because these elements are sufficiently plant-available in savanna soils (Ernst & Tolsma 1989).

As observed in soybeans under phosphorus stress (Murali & Teramura 1987), saplings of *A. tortilis* grown on nutrient-poor soil were not affected by UV-B radiation, whereas those on nutrient-rich soil showed a not-yet significant decrease of biomass production (Table 1). This small reaction of *A. tortilis* to UV-B radiation may be explained by the already high-UV radiation in Botswana, which may be in the vicinity of

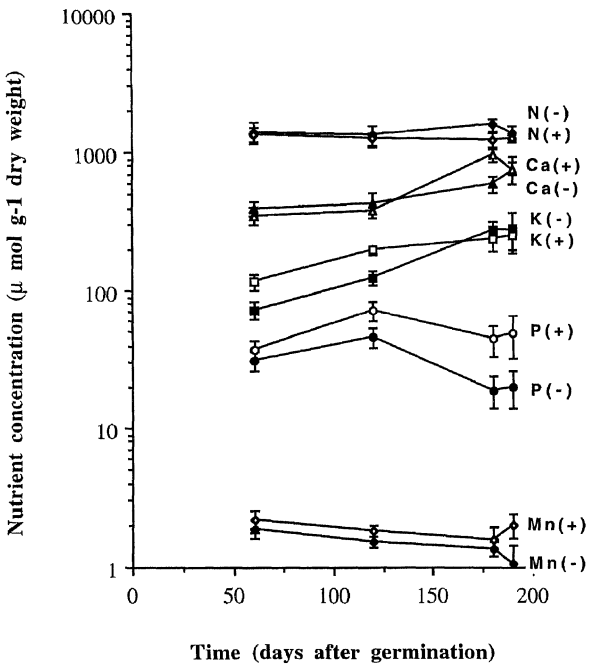

Figure 4. Nutrient concentration in shedded leaves during the growth of *Acacia tortilis* with (open circle) and without (closed circle) UV-B radiation. The first data set concerns shed cotyledons. The bar presents ± S.E.

13.5 kJ m^{-2} d^{-1} $UV\text{-}B_{Be}$, measured in Nambia (Musil 1995), i.e. nearly double as high as the 7.1 kJ m^{-2} d^{-1} $UV\text{-}B_{Be}$ in temperate areas at maximum solar position according to the Green model (Green 1983). Genetically based adaptation to increased UV-B radiation is known from plant populations under a naturally high exposure to UV-B, i.e. high elevation above sea level (Larson et al. 1990).

In saplings of *A. tortilis* the adaptations to 20% increased UV-B exposure as applied in our experiment is not yet complete although UV-B radiation did not inhibit hypocotyl elongation as observed in de-etiolating tomato seedlings (Ballaré et al. 1995). UV-B radiation enhances not only the timing of cotyledon shedding, but it do affect the translocation of nutrients: (1) the mobilization of nutrient reserves from the seed to the seedling; (2) the retranslocation from phosphorus and manganese from senescent leaves to growing ones, as demonstrated by leaf litter analysis and (3) an increase of the manganese concentration in the UV-B radiated saplings. In savanna ecosystems, as that of Botswana, an obstruction of the age-dependent retranslocation of phosphorus, which is strongly expressed in savanna trees (Tolsma et al. 1987) may affect a phosphorus imbalance on the long term. To our knowledge, this is the first study which shows an impact of UV-B on mineral cycling within the plant. Therefore it is too early to speculate on its further impact on plant survival and reproduction. No effect of UV-B radiation was found on *Acacia* root nodulation by *Rhizobium*, which is only related to photosynthetic leaf mass and thus soil fertility. In contrast to a positive effect of UV-B radiation on peas (Hatcher & Paul 1994), negative effects of UV-B radiation on photosynthesis have been reported from various plant species (Caldwell et al.1989; Cen & Bornman 1990; Flint et al. 1985; Murali & Teramura 1986, 1987; Rozema et al. 1991). The reduction of photosynthesis of *A. tortilis* leaves under UV-B radiation is in good agreement with the data of other plant species and with the tendency to decrease biomass production under UV-B radiation. In addition the delayed closure of leaflets by UV-B is in accordance with leaf movement of other leguminous species (Tendel & Häder 1995). Such a retardation may diminish water loss at times of water stress, if the UV-B effect is not overruled by the water stress trigger. Physiologically, the impact of UV-B on leaflet movement may be transmitted not directly by UV-B, but it may be the effect of enhanced UV-A fluxes.

The investigated two annual grasses may be better adapted than the saplings of *A. tortilis* to the high UV-B radiation in Botswana. If this adaptation of grasses will also be present in plant species which are preferentially growing under the crown of savanna trees (Veenendaal et al. 1993), and in mature savanna trees remains to be investigated. In unicellular soil algae of the genus *Chlorococcum* from South Africa UV-B radiation had species-specific positive or negative effects on photosynthesis (Xiong et al. 1996), emphasizing once more species-specific reaction patterns.

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Silene vulgaris exposed to UV-B in a greenhouse compartment. (Photograph: J. van de Staaij)

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The impact of elevated UV-B (280–320 nm) radiation levels on the reproduction biology of a highland and a lowland population of *Silene vulgaris*

J.W.M. van de Staaij, E. Bolink, J. Rozema & W.H.O. Ernst
Department of Ecology and Ecotoxicology, Faculty of Biology, Vrije Universiteit, Amsterdam, The Netherlands

Key words: Fertilisation, Flowers, Germination, Mutation, Seed

Abstract

A highland (altitude 1600 m) and a lowland (altitude –2 m) population of the perennial herb *Silene vulgaris* were tested on the effects of elevated levels of UV-B radiation on their reproductivity. Highland populations receive higher natural UV-B doses than lowland populations. Therefore adaptation to high UV-B levels of the highland population is to be expected. The lowland population showed a decrease in the number of seed producing flowers and the number of seeds produced per plant under elevated UV-B levels. The highland population increased the number of seeds per plant under elevated UV-B levels. In both populations individual seed mass as well as seed germination percentages were unaffected by the UV-B flux received by the parental plant. Possible effects of UV-B induced alterations in reproductivity on the geographical distribution of the different populations are discussed.

Introduction

Increasing concentrations of trace gases such as chlorofluorocarbons (CFCs) and NOx in the stratosphere lead to an enhanced breakdown of ozone (Folkins & Brasseur 1992; Krupa & Kickert 1989, 1993). This results in increasing levels of UV-B radiation on the surface of the earth (Blumthaler & Ambach 1990; Madronich et al. 1995; Peter 1993).

Elevated UV-B radiation levels affect plant performance in various ways, ranging from reduced biomass (Rozema et al. 1990, 1991; Van de Staaij et al. 1990, 1993), to changes in the concentration of UV-B absorbing compounds in various plant parts (Barnes et al. 1987; Liu et al. 1995; Tevini et al. 1991; Van de Staaij et al. 1995a). Great differences in UV-B sensitivity among cultivars, populations and species exist however (Barnes et al. 1993; Caldwell et al. 1995; Dai et al. 1994; Krupa & Kickert 1989; Rozema et al. 1995; Sullivan et al. 1992).

The impact on reproductive processes in plants is one of the less known aspects of elevated UV-B radiation levels (SCOPE 1992, 1993). However the existing studies on this topic indicate that UV-B effects on the reproductivity in higher plants are likely. Flint & Caldwell (1984) reported partial inhibition of *in vitro* pollen germination in *Scrophularia peregrina*, *Geranium viscosissimum*, *Papaver rhoeas* and *Cleome lutea* under elevated UV-B radiation, Musil (1995) reported reduced pollen germination in 2 out of 8 species tested. Reduced *in vitro* pollen tube growth under UV-B radiation has been reported for *Nicotiana tabacum* and *Petunia hybrida* (Feder & Shrier 1990), for four out of four tested dicotyledonous Asteraceae and for one out of four tested monocotyledonous Iridaceae (Musil 1995). UV-B induced reductions in seed yield in agricultural as well as in natural plant species have been reported (Teramura & Murali 1986).

The first aim of this study is to investigate effects of elevated levels of UV-B radiation on plant reproductivity. The perennial herb *Silene vulgaris*, a widely distributed species of temperate climate zones of Europe (Tutin et al. 1964), is used as a model species.

The second aim of this study is to investigate possible changes in the geographical distribution of different *Silene vulgaris* populations over Europe under elevated UV-B conditions. Experimental work indicates that species and populations originating from nat-

urally high UV-B sites (high altitude, low latitude) are less sensitive to enhanced levels of UV-B radiation than species and populations from low UV-B sites (low altitude, high latitude) (Caldwell et al. 1982; Larson et al. 1990; Robberecht et al. 1980; Sullivan et al. 1992). Differences in UV-B adaptability can determine in part the distribution of species under elevated UV-B conditions (Ziska et al. 1992). Therefore the use of *S. vulgaris* populations originating from locations with different natural UV-B fluxes makes it possible to address the question: can UV-B effects on reproductivity influence the distribution of *S. vulgaris* populations over Europe? In the present study the reproductive response of two *S. vulgaris* populations, a sub-alpine population originating from Austria and a lowland one originating from The Netherlands to enhanced levels of UV-B radiation was compared.

Materials and methods

Plant material

Seeds of the lowland population of *Silene vulgaris* (Moench) Garcke, were collected at Hoogezand, The Netherlands (altitude −2 m, 52° N, 4° E), August 1989. Plants of the highland population were collected as taproots at Gaschurn, Vorarlberg, Austria (altitude 1600 m, 47° N, 15° E), July 1990. Calculations (according to Green et al. 1980) reveal a 7.5–10% higher natural UV-B flux for the Austrian population. Care was taken to select populations growing at fully sun exposed sites. Plants were taken to Amsterdam and cultured to the stage of seed set. The seeds of at least 15 individual plants per population were harvested and pooled.

General growth conditions

Plants were grown from seed in a greenhouse and received supplementary PAR light (250–350 μEinstein m^{-2} s^{-1} at plant level) from 400 W Philips HPI/T lamps, 16 h daily. Temperature was maintained between 18–25 °C, during a few extremely hot days temperature increased to 30 °C in the early afternoon. Relative humidity varied from 60% (light period) to 80% (dark period).

UV-B radiation levels

UV-B radiation was generated by Philips TL 12/40 tubes 70–90 cm above the plants. Radiation transmitted by the UV-B tubes was filtered using 0.1 mm cellulose acetate foil (transmission down to 290 nm, own measurements). The tubes burned 6 hours daily from 10.00 a.m. until 4.00 p.m. Plants within groups were randomised weekly to minimise site effects within the greenhouse compartments.

The daily UV-B radiation levels applied were 0, 6 and 16.2 kJ m^{-2} biologically effective radiation ($UV\text{-}B_{BE}$), weighed according to the generalised plant action spectrum (Caldwell 1971), normalised at 300 nm. The low dose, 6 kJ m^{-2}, simulates the present UV-B flux for the Netherlands in June. The high dose, 16.2 kJ m^{-2}, simulates a reduction of 45% (calculated according to Green et al. (1980) for a stratospheric ozone thickness of 330 Dobson units). UV-B measurements were taken at half plant height. Radiation was measured at 2 nm intervals using an Optronics OL752 spectroradiometer (Optronics Industries, Orlando, USA), calibrated against a National Institute of Standards and Technology (N.I.S.T.) traceable 1000 W quartz halogen lamp, powered by a model 65 DS precision current source (Optronics Industries, Orlando, USA). Wavelength alignment and system response were checked against a dual calibration source (Optronics Industries, Orlando, USA), using mercury emission lines.

Experiment 1: Flower and seed production under different UV-B radiation levels

Plants of both the Dutch and the Austrian population were planted as seedlings (at the stage of two leaf-pairs) in 1.8 litre pots containing garden soil (Jongkind BV, Aalsmeer Holland) and placed directly under UV-B treatment; 0, 6 or 16.2 kJ m^{-2} $UV\text{-}B_{BE}$. Twelve individuals for each population/UV-B combination. After 21 days of growth for each population/UV-B combination 6 plants of the same dimensions were selected and grown to reproduction. *S. vulgaris* is an outcrossing, protandrous species, which flowers are fertilised by bumblebees and bees at its natural sites (Marsden-Jones & Turrill 1957). Therefore during the reproductive phase pollination has been carried out by hand, every other day; care was taken to pollinate only flowers among plants from the same population receiving the same UV-B radiation dose. Flowers were harvested every 2 to 4 days, in total 19 times over a period

ranging from April 10 to June 1, 1992. At the end of this period the last plants had completed flowering. Flowers were harvested when the seed-capsules turned brown, previous experiments had indicated that by then the seeds were ripe. At each harvest the number of flowers without seeds (no seed-capsule formation) was scored and the flowers were removed from the plant. The number of flowers with seeds was scored and the flowers were kept to analyse the number and weight of the seeds. After this analysis the seeds were stored at room temperature (20 ± 2 °C) to be used in the germination experiment.

Experiment 2: Germination rates of seeds produced by plants growing under different UV-B levels

To establish possible UV-B induced damage to seeds during seed formation in the parental plant, seeds harvested from individuals grown under the different population/UV-B treatments from Experiment 1 were used in this germination experiment.

Five hundred thirty-six seeds, selected on the same size and colour, were taken from the pooled seed production of each population/UV-B combination of Experiment 1. Each seed was sown individually in 3 cm diameter pots, made of garden soil (Jiffy products Ltd., Norway). Fifty-six of these were placed together in plastic containers, resulting in 6 replications of 56 seeds each per population/UV-B combination. The total of 36 (seeds produced by 2 populations under 3 UV-B levels, 6 replications) plastic containers, with 56 pots each, was placed randomly in the glasshouse. During this germination experiment no UV-B radiation was given to make sure all possible differences found were caused by UV-B influences on the seeds during their formation.

During a 21-day period the germination process was monitored daily. The first appearance above ground of the green seedling was considered as the moment of germination, this is about two days after penetration of the radicula through the seed coat. The number of abnormal seedlings (1 or 3 cotyledons, no chlorophyll) was scored to detect possible UV-B caused mutations.

Experiment 3: Flower development and seed production in plants cultivated outdoors

Seedlings of the Austrian and the Dutch population were planted in the spring of 1991 in the experimental garden of the Vrije Universiteit. During June 1992 plants reaching the end of their flowering season (few flower buds left) were selected and the flowers harvested; capsules which had already lost their seeds were not taken into account. A separation was made between capsules containing seeds and empty capsules. In both these groups the amount of capsules suffering from seed predation was established. For the group of flowers of which the capsules contained seeds at harvest, predation by micro-lepidoptera did not destroy all seeds within the calyx, some unpredated seeds were left. For the group of flowers containing no seeds at harvest a discrimination was made between flowers of which all seeds were destroyed by predation, and flowers in which no seed-capsule formation had taken place.

Statistical analysis

All data were statistically analysed using one or two way analysis of variance (ANOVA) to test significance ($p < 0.05$) of treatments and the interaction with populations (Sokal & Rohlf 1981). Flower and seed formation data were logarithmically transformed to obtain homogeneity of variances.

Results

Experiment 1: Flower and seed production under different level of UV-B

At the end of the experiment (after 51 days) all plants had reached the end of their flowering period. The total number of flowers produced, separated in empty capsules and those containing seeds, was calculated. Plants from the Dutch population produced significantly more capsules containing seeds than the Austrian population (Table 1). The Dutch population produced significantly less seed containing flowers under UV-B radiation (6 and 16.2 kJ m^{-2} UV-B_{BE}) compared to the non UV-B radiated group (Table 1). The Austrian population showed a positive effect of UV-B on fertile flower formation. Plants radiated with the highest UV-B dose (16.2 kJ m^{-2} UV-B_{BE}) produced significantly more seed containing flowers than the 0 and 6 kJ m^{-2} group.

This difference in reaction between the two populations towards UV-B radiation effects on fertile flower formation was statistically significant as the interaction between pop × UV-B shows (Table 1).

The number of infertile flowers was in both populations depressed by UV-B radiation, UV-B radi-

Table 1. The effect of different levels of UV-B radiation (kJm^{-2} $UV\text{-}B_{BE}$) on flower and capsule, with or without seeds, formation and seed production in a Dutch and an Austrian population of *S. vulgaris*. Values are averages of six plants per population and treatment. Significance of population, UV-B and the interaction population*UV-B from a two way analysis of variance is given. n.s. = not significant; * = $p < 0.05$

Population/ treatment	Number of flowers	Capsules with seeds	Capsules without seeds	Seeds per plant	Seed mass per plant (g)	Mass per seed (g)
NL 0	197	77	120	1161	0.82	0.69×10^{-3}
NL 6	65	37	28	535	0.30	0.70×10^{-3}
NL 16.2	127	41	86	556	0.40	0.92×10^{-3}
AU 0	95	24	71	134	0.15	1.10×10^{-3}
AU 6	35	12	23	145	0.12	0.85×10^{-3}
AU 16.2	105	45	60	347	0.27	0.81×10^{-3}
Pop	*	*	n.s.	*	*	n.s.
UV-B	*	*	*	n.s.	*	n.s.
Pop × UV-B	n.s.	*	n.s.	*	*	n.s.

ated groups of both populations produced less infertile flowers than the non radiated groups (Table 1).

For both populations the total number of flowers produced is lower under 6 kJ m^{-2} $UV\text{-}B_{BE}$ than under the 16.2 kJ m^{-2} $UV\text{-}B_{BE}$ treatment. In the Dutch population the total number of flowers was highest under 0 kJ m^{-2} $UV\text{-}B_{BE}$, for the Austrian population production was highest under the 16.2 kJ m^{-2} $UV\text{-}B_{BE}$ treatment.

At the end of the flowering period the total number of seeds produced per plant was counted, the total seed mass per plant and the mass per seed was determined. The total number of seeds produced per plant revealed a significant higher production for the Dutch population than for the Austrian population (Table 1). The effect of UV-B on seed numbers showed only a trend ($p = 0.06$) indicating a UV-B induced depression in the Dutch population, and a UV-B induced stimulation in the Austrian population on seed numbers produced. This contrasting reaction between both populations is significant as the interaction pop × UV-B showed (Table 1).

The total seed mass produced per plant showed a significant negative UV-B effect in the Dutch population while the Austrian population benefited from the UV-B radiation and increased the total seed mass per plant under 16.2 kJ m^{-2} $UV\text{-}B_{BE}$. The difference in reaction between populations was significant (Table 1).

The mass per seed did not differ between both populations, nor did it show a UV-B effect in either of the populations (Table 1).

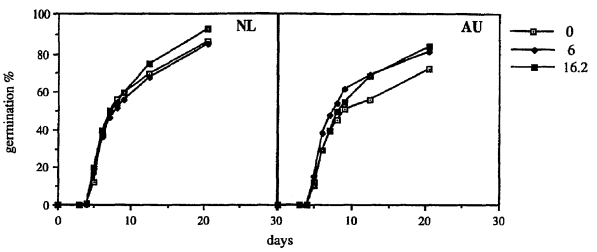

Figure 1. Germination percentages as function of the time for seeds produced by *S. vulgaris* plants of a Dutch and an Austrian population cultivated under 0, 6 or 16.2 kJ m^{-2} $UV\text{-}B_{BE}$.

Table 2. Statistics of germination percentages at day 12 and 20 and of the mutation percentages in *S. vulgaris* seedlings. P values of population, UV-B and the interaction population × UV-B from a two way analysis of variance

	Day 12	Day 20	Mutations
Pop.	0.294	0.189	0.088
UV-B	0.421	0.143	0.716
Pop*UV-B	0.536	0.850	0.796

Experiment 2: Germination rates of seeds produced by plants grown under different UV-B levels

The percentage seeds germinated was monitored for 21 days, after this period no germination occurred. Seeds produced by individuals originating from the two different populations did not differ in germination rates (Figure 1, Table 2).

The UV-B doses which the parental plants received during seed development did not affect the germination rates of the seeds (Figure 1, Table 2).

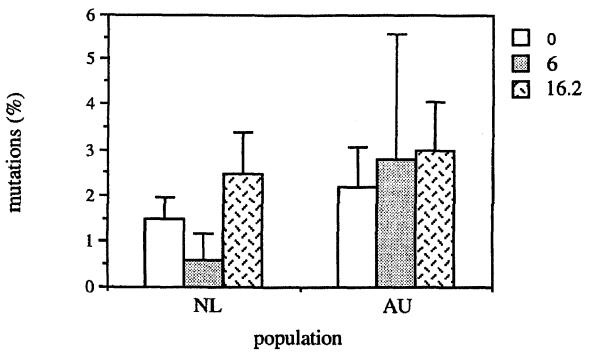

Figure 2. The percentages (±s.d.) of seedlings showing abnormalities (1 or 3 cotyledons, no chlorophyll) germinated from seeds produced by *S. vulgaris* plants from a Dutch and an Austrian population cultivated under 0, 6 or 16.2 kJ m^{-2} UV-B_{BE}.

The number of seedlings showing abnormalities (1 or 3 cotyledons, no chlorophyll) did not reveal a population nor a UV-B effect, none of the population/treatment groups differed significantly from the other (Figure 2, Table 2).

Experiment 3: Flower development in plants cultivated outdoors

In plants from both the lowland and the sub-alpine population grown outdoors under natural solar irradiance conditions about 30% of the flowers was infertile and did not form seeds (Table 3). Flowers which did form seeds suffered heavily from seed predation by caterpillars of micro-lepidoptera; about 50% of all the flowers containing seeds were predated. In about 40% this predation was so severe that no seeds survived (Table 3).

Discussion

Effects of elevated UV-B radiation levels on the reproduction of higher plants are hardly investigated (SCOPE 1992; Teramura 1993; Van de Geijn et al. 1993). Since DNA is one of the main targets of UV-B (Björn 1996; Giese 1976) it is likely that the haploid genomes of pollen are more susceptible to UV-B radiation than the diploid genomes of other plant cells. Reduced pollen growth under elevated UV-B radiation has been reported for some plant species (Feder & Shrier 1990; Musil 1995). The haploid egg cell and the developing embryo are surrounded by other tissues which can act as a UV-B shield (Van de Geijn et al. 1993). The results presented in this paper support this hypothesis; UV-B has an effect on the number of seeds produced per plant but has no influence on the individual seed mass, germination percentages nor on the mutation rates of emerged seedlings. If the developing embryo would suffer UV-B damage, germination percentages would drop and mutation rates would increase in seeds produced under the elevated UV-B regimes. However it has to be remarked that only mutations directly visible in seedlings have been investigated, therefore it is possible that some less devastating UV-B induced mutations to the genotype remained unnoticed.

The stimulative effect of the elevated UV-B treatment on seed production in the Austrian population must have resulted from higher fertilisation rates; only the number of flowers containing seeds increases, the number of infertile flowers remains the same under the elevated UV-B flux. Higher fertilisation rates can result from increased pollen germination and pollen tube growth under elevated UV-B radiation as reported for *Ixia viridiflora* (Musil 1995) and *Pinus sylvestris* (Zelles et al. 1977).

The depressive effect of the low (6 kJ m^{-2} UV-B_{BE}) UV-B treatment compared to the 0 UV-B treatment on the total number of flowers produced by individuals from both populations probably results from a direct negative UV-B effect on flower formation. The following rise in flower formation when comparing the 6 kJ m^{-2} UV-B_{BE} treated plants with the 16.2 kJ m^{-2} UV-B_{BE} treatment, may be related to increasing concentrations of UV-B absorbing pigments (flavonoids and related phenolics) in the plants with rising UV-B fluxes. These pigments act as an epidermal UV-B filter protecting the plant from damage (Schnitzler et al. 1996; Tevini et al. 1991). Flavonoids are known to stimulate flower formation (Nakanishi et al. 1995). Therefore protection against UV-B by increasing internal concentrations of flavonoids in plants may, as a side-effect, stimulate the number of flowers in plants under rising UV-B fluxes.

Experiments using various non-reproductive wild plant species revealed a limited influence of elevated UV-B levels on biomass production, morphological features and physiological processes of some plant species (Ernst et al. 1997; Sullivan et al. 1992; Tosserams & Rozema 1995). Previous experiments with seedlings and non-reproductive individuals of *S. vulgaris* showed that this species is relatively insensitive to elevated UV-B levels with regard to biomass production, morphology and photosynthesis. Populations from The Netherlands and Austria responded in the same way. From these results it was concluded that an UV-B induced

Table 3. Number of flowers produced by *S. vulgaris* and seed predation rates of plants growing outdoors under natural UV-B fluxes. Average values and standard deviation (in brackets) of five replications for the Dutch population and of two replications for the Austrian population are given.

Population	Capsules with seeds			Capsules without seeds		
	No predation	Predated	Total	No seed formation	Predated	Total
NL	27 (22)	8 (6)	35 (24)	29 (19)	20 (15)	49 (34)
AU	13 (11)	6 (6)	19 (17)	21 (23)	15 (15)	35 (38)

alteration in the geographical distribution of the different *S. vulgaris* populations is not to be expected (Van de Staaij et al. 1995b). The distinctive difference between the Dutch and the Austrian population in the reaction concerning their reproductive effort, less seeds per plant for the Dutch and more seeds per plant for the Austrian population under elevated UV-B levels, makes a revaluation of the possible effects of elevated UV-B levels on the geographical distribution of the different populations of this species necessary. In a high UV-B environment individuals of the Austrian population will increase the number of seeds produced, the number of seeds produced by the Dutch population will not alter from the production under ambient solar UV-B radiation levels. Even under elevated UV-B fluxes seed production of the Dutch population remains greater than the seed production of the Austrian population. Although elevated levels of UV-B influence seed production it seems unlikely that these changes would influence the distribution patterns of the different populations. In the indoor experiments reported here, seed predation did not occur. As the seed production of the individuals cultivated outdoors and data from literature (Ernst et al. 1990) made clear, seed predation is a major factor influencing the number of seeds available for germination in a natural situation. The influence of elevated UV-B radiation levels on predation rates is hard to predict, to the present day no studies on UV-B effect on micro-lepidoptra exist.

The effect of increased levels of UV-B on the seed production in a species not showing responses to UV-B in non-reproductive stages of growth, makes an assessment of UV-B damage to agricultural yields only possible when it is based on experiments in which the actual reproductive response of the species is tested. Assessments of UV-B induced effects on yields based on non-reproductive parameters can lead to an underestimation of elevated UV-B levels, especially when in agricultural production reproductive plant parts (grain etc.) are the final product.

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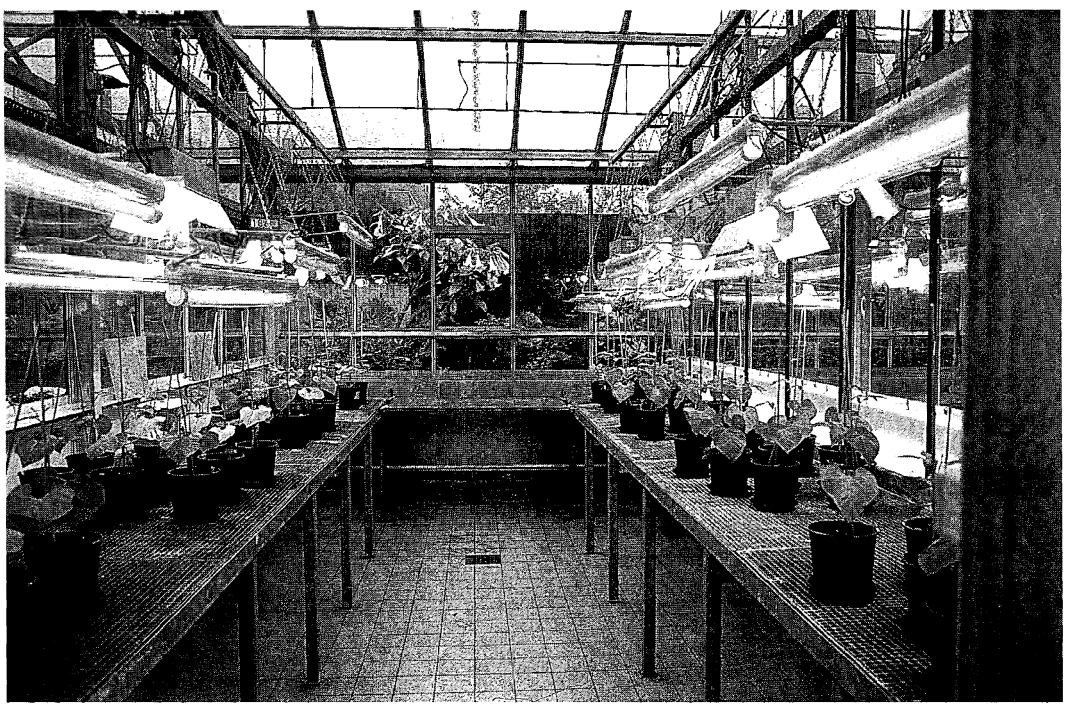
IV. Interactions of UV-B with environmental factors



UV-B lamp experiments in the greenhouse with bean (*Phaseolus vulgaris*). (Photograph: J. Rozema)

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Effects of UV-B radiation on terrestrial plants and ecosystems: interaction with CO_2 enrichment

J. Rozema, G. M. Lenssen, J. W. M. van de Staaij, M. Tosserams, A. J. Visser & R. A. Broekman
Department of Ecology & Ecotoxicology, Vrije Universiteit, Amsterdam, The Netherlands

Key words: CO_2 enrichment, *Elymus athericus*, Interaction, UV-B radiation

Abstract

UV-B radiation is just one of the environmental factors, that affect plant growth. It is now widely accepted that realistic assessment of plant responses to enhanced UV-B should be performed at sufficiently high Photosynthetically Active Radiation (PAR), preferably under field conditions. This will often imply, that responses of plants to enhanced UV-B in the field will be assessed under simultaneous water shortage, nutrient deficiency and variation of temperature. Since atmospheric CO_2 enrichment, global warming and increasing UV-B radiation represent components of global climatic change, interactions of UV-B with CO_2 enrichment and temperature are particularly relevant. Only few relevant UV-B× CO_2 interaction studies have been published. Most of these studies refer to greenhouse experiments. We report a significant CO_2 × UV-B interaction for the total plant dry weight and root dry weight of the C_3-grass *Elymus athericus*. At elevated CO_2 (720 μmol mol $^{-1}$), plant growth was much less reduced by enhanced UV-B than at ambient atmospheric CO_2 although there were significant (positive) CO_2 effects and (negative) UV-B effects on plant growth. Most other CO_2× UV-B studies do not report significant interactions on total plant biomass. This lack of CO_2 × UV-B interactions may result from the fact that primary metabolic targets for CO_2 and UVB are different.

UV-B and CO_2 may differentially affect plant morphogenetic parameters: biomass allocation, branching, flowering, leaf thickness, emergence and senescence. Such more subtle interactions between CO_2 and UV-B need careful and long term experimentation to be detected. In the case of no significant CO_2× UV-B interactions, combined CO_2 and UV-B effects will be additive. Plants differ in their response to CO_2 and UV-B, they respond in general positively to elevated CO_2 and negatively to enhanced UV-B. Moreover, plant species differ in their responsiveness to CO_2 and UV-B. Therefore, even in case of additive CO_2 and UV-B effects, plant competitive relationships may change markedly under current climatic change with simultaneous enhanced atmospheric CO_2 and solar UV-B radiation.

Climatic change: the combined increase of atmospheric CO_2 and UV-B radiation and global warming

The climate of the earth is currently changing as a result of the emission of CO_2, chlorofluorocarbons (CFCs), CH_4 and N_2O into the atmosphere (Stolarski et al. 1991; WMO 1994). Increasing levels of CFCs, methane and nitrous oxides play an important role in the depletion of the stratospheric ozone layer. As a result of the thinning ozone layer, solar ultraviolet-B radiation (280–320 nm) reaching the surface of the earth will increase (Caldwell et al. 1995). Growth of terrestrial plant species will generally be reduced under enhanced UV-B radiation (Caldwell & Flint 1994; Caldwell et al. 1989), although some positive responses have been observed (Caldwell & Flint 1994; Johanson et al. 1995; Tosserams & Rozema 1995). Increases in atmospheric CO_2 generally result in increased photosynthesis, growth and primary production (Rozema et al. 1993; Rozema 1995; Strain & Cure 1985).

Growth responses to elevated CO_2 and UV-B have been studied extensively in single stress mode, but not in combined action (Rozema 1993).

In theory, several mechanisms can be involved in a modification of the plant response to CO_2 enrichment by enhanced UV-B radiation. For example, a damaged photosynthetic apparatus under enhanced UV-B, may alter the photosynthetic response to elevated CO_2 (Ziska & Teramura 1992). The content of secondary metabolites may increase under enhanced CO_2 (Lambers 1993; Rozema 1993), which may increase the amount of UV-B absorbing compounds. This may reduce sensitivity of plant species to enhanced UV-B radiation. Since enhanced ultraviolet-B radiation, at least in the past, has been considered to cause environmental stress to plants, it was assumed that in many CO_2 × UV-B studies enhanced UV-B would reduce the responsiveness to CO_2 enrichment (Teramura et al. 1990; Ziska & Teramura 1991).

In this paper it is indicated that CO_2 and UV-B effects on plant morphogenetic parameters may also lead to interactions with large consequences for ecosystem processes.

Structure of this paper

The purpose of the present study is to briefly review previous studies on combined CO_2 and UV-B effects on plants and to report the response of the C_3-grass *Elymus athericus* to combined elevated atmospheric CO_2 and enhanced UV-B radiation. The review of UV-B× CO_2 studies considers some methodological aspects, effects on plant biomass, plant morphogenetic parameters, and consequences for ecosystem structure and functioning. Physiological parameters affected by combined CO_2 enrichment and enhanced UV-B radiation are discussed by Sullivan (1997) in this volume. Combined effects of CO_2, temperature and UV-B are considered by Mark & Tevini (1997) in this volume.

Methodology of combined elevated CO_2 and enhanced UV-B studies on plants

Both CO_2 enrichment and enhanced UV-B radiation studies require high (expensive) standards of instruments and experimental facilities (Caldwell & Flint 1994; Leadley & Drake 1993; McLeod 1997). Often research groups have specialised in high quality CO_2 enrichment *or* in UV-B radiation studies but only occasionally in both. The above helps to explain that only in relatively few cases have combined CO_2 and UV-B radiation been studied.

What are the basic requirements for studies of CO_2 enrichment and enhanced UV-B radiation?

Methods of CO_2 enrichment

Basically, there are three different ways of experimental CO_2 enrichment (Rozema 1995). In closed controlled environments such as greenhouses and climate rooms, the CO_2 concentration of the air inside can be relatively easily controlled. Temperature, light and humidity in this closed controlled environment may differ from outdoor conditions. CO_2 enrichment in so-called Open Top Chambers (OTC) is more expensive, but temperature, light and humidity of the CO_2 enriched air in the OTC are more natural. In Free Air CO_2 Enrichment Systems (FACE) (Hendrey et al. 1993) light, temperature and humidity are natural. Elevated CO_2 concentrations in the air within FACE rings are not always stable, moreover costs of CO_2 enrichment of FACE-systems are high (Hendrey et al. 1993).

Methods of enhanced UV-B radiation

Studies of enhanced UV-B radiation generally require a UV-lamp supplementation system either installed indoors or outdoors. A survey of outdoor UV-B supplementation systems is given by McLeod (1997). Caldwell & Flint (1994), surveying many recent experimental UV-B studies, conclude that a majority of experimental UV-B studies 'has been conducted as short-term experiments in climate rooms and greenhouses, where the unnatural balance of radiation can lead to unrealistic conclusions'.

Moreover, under indoor UV-B experiments, plants may suffer from insufficient Photosynthetically Active Radiation (PAR: 400–700 nm) and may therefore have a reduced activity of photolyase, repairing DNA damage caused by enhanced UV-B (Caldwell et al. 1995). Ideally, realistic assessment of combined CO_2 and UV-B effects should be studied under controlled conditions in the field (Rozema et al. 1995; Tosserams et al. 1997a). At the moment, this would imply an outdoor UV-B supplementation system, combined with Free Air CO_2 enrichment. To our knowledge no such CO_2 × UV-B experiments are performed at present.

Despite the unnatural spectral radiation balance in many indoor systems, most CO_2 × UV-B experi-

ment have yet been performed in growth chambers and greenhouses (Tosserams et al. 1997a) and only few in outdoor Open Top Chamber studies (Visser et al. 1997; Tosserams et al. 1997b; Gwynn-Jones et al. 1997).

Interactive effects of elevated CO_2 and UV-B on the C_3 grass *Elymus athericus*

Here we describe a greenhouse study of the combined effects of atmospheric CO_2 enrichment and enhanced ultraviolet B radiation on the C_3 salt marsh grass *Elymus athericus*. Primarily we focus on combined CO_2 and UV-B effects on total plant biomass, analysis of plant growth parameters LAR, LWR , SLA and shoot to root ratio. Finally, some physiological parameters have been determined (net photosynthesis, stomatal conductance and UV-B absorbance of leaf extracts) to interpret CO_2 and UV-B effects on biomass or growth parameters.

CO_2 enrichment, experimental set up

Tillers of *Elymus athericus* were collected in January 1991, at the salt marsh of Den Oever (Noord-Holland). Plants were grown for 6 days in commercial potting soil (one plant per pot) in a greenhouse. Thereafter, the plants were exposed to 380 ± 30 μmol^{-1} and 720 ± 30 μmol mol^{-1} CO_2 in two greenhouse components. The CO_2 level in the CO_2 enriched greenhouse compartment was recorded by an infrared gas analyser (Siemens type ZFP-DZ), linked with a West 2071 controller, regulating a Brookstype 5876 mass flow controller, which injected pure CO_2 from a cylinder (Hoekloos, type K 50 H) into the greenhouse. The CO_2 was mixed with air by a fan (S & P type HCFT). The CO_2 concentration in the ambient and elevated greenhouse was continuously recorded by a Binos Leybold-Heraeus infrared gas analyser (Hanau, Germany) or by an ADC/LCA2, (Analytical Development Co, Hoddesdon, Herts, United Kingdom).

Plants received 300 μmol m^{-2} s^{-1} PAR supplementary light from 400 W Philips HPI/T lamps, during 14 hours per day. Temperature was 25/18 °C (day/night). Relative humidity varied between 50 and 80%. Every two weeks, plants were rotated between the two compartments, reset to the desired CO_2 concentration to minimise compartment effects.

UV-B radiation, experimental set up

At both CO_2 levels, plants were exposed to 10.0 or 16.8 kJ m^{-2} day^{-1} biologically effective UV-B radiation (UV-B_{BE}) by varying the numbers of UV-lamps and adjusting the height of the lamps. the UV-B radiation was provided by Philips TL 40 W/12 tubes, wrapped in 0.1 mm thick cellulose acetate foil (cut off at 290 nm). Foil was renewed twice a week. UV-tubes burned six hours daily from 10.00–16.00 h. Every CO_2 × UV-B treatment consisted of 16 replicate plants. At both CO_2 levels 10 extra plants were grown without UV-B for the measurement of the content of UV-B absorbing compounds. At day 32 and 39, each plant received 100 ml of a 1% NPK (4:5:6) solution. Plants were harvested after 42 days. Leaf area was measured with a Licor −3100 area meter (Li, Corp. Inc. Lincoln, Nebraska, USA). Dry weight of root, stem and leaves was measured after drying for 24 hours at 80°C.

Gas exchange

Net leaf photosynthesis and stomatal conductance was measured at day 35 using ADC/LCA 3 equipment (Analytical Development Co., Hoddesdon, Herts, UK). Measurements were made at a light intensity of 300 μmol m^{-2} s^{-1}.

UV-B absorbing compounds

The content of UV-B absorbing compounds was measured nine times during the experiment using young leaves. Five mg fresh leaf material was placed in 5 ml of an ethanol/acetic acid (99:1, v/v) extraction medium and heated at 90 °C for one hour. Absorbance was measured at 300 nm with a Perking Elmer Lambda 15 spectrophotometer.

Statistical analysis

Data were subject to two-way analysis of variance, to test the significance of the CO_2 and UV-B treatment and their interaction, according to Sokal & Rohlf (1981). Leaf area and dry weight data were logarithmatically transformed to obtain homogeneity of variance, tested with Bartlett's test.

Effects of combined atmospheric CO_2 enrichment and enhanced UV-B radiation on *Elymus athericus*

The dry weight of all plant parts was significantly increased at elevated CO_2 and reduced with enhanced UV-B radiation (Table 1). The relative increase in total plant dry weight by CO_2 enrichment was 31% and 81% at 10.0 and 16.8 kJ m^{-2} day^{-1} UV-B respectively (Table 1), reflecting the significant interaction of the CO_2 and UV-B treatment on total plant dry weight. Total plant dry weight of the C_3-grass *Elymus athericus* is relatively more increased with elevated CO_2 at enhanced UV-B. Similarly, root dry weight is more increased with CO_2 enrichment with enhanced UV-B radiation (Table 1). Another way of expressing this interaction, is that biomass reduction with enhanced UV-B is less at elevated CO_2. LAR and SLA are reduced with CO_2 enrichment and increased with enhanced UV-B. The shoot to root ratio was not significantly affected by elevated CO_2 and elevated UV-B radiation. There were no significant interaction effects of CO_2 and UV-B on these plant parameters (Table 2). Increased total plant dry weight with elevated CO_2 may be associated with an increased rate of net photosynthesis. Averaged over both UV-B levels, CO_2 enrichment stimulated net leaf photosynthesis by 62% (Table 3). In addition to increased net photosynthesis, stomatal conductance is significantly (37%) reduced with elevated CO_2. Reduced total plant dry weight with enhanced UV-B cannot be ascribed to reduced CO_2 fixation, since net photosynthesis is not significantly affected by enhanced UV-B radiation (Table 3). Neither is stomatal conductance significantly changed with enhanced UV-B. There are no significant interaction effects of CO_2 and UV-B in net photosynthesis and stomatal conductance.

Absorbance (at 300 nm) of leaf extracts of *Elymus athericus* increased with enhanced UV-B radiation (Figure 1). In all treatments absorbance increased with time until maximum values were reached 10–15 days after the start of the experiment.

CO_2 enrichment had no significant effect on absorbance of UV- B radiation by the leaf extracts.

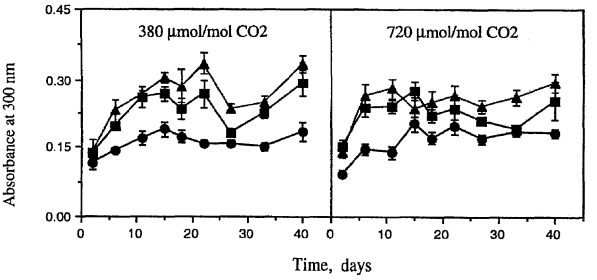

Figure 1. The effect of CO_2 and UV-B on absorbance (at 300 nm) of acid ethanolic extracts of fresh leaf material of *Elymus athericus* as a function of time . Plants were grown at 380 (**a**) or 720 (**b**) µmol mol^{-1} CO_2. $UV\text{-}B_{BE}$ levels were 0.0 (circles), 10.0 (squares) or 16.8 (triangles) kJ m^{-2} day^{-1}. Absorbance was recalculated to a concentration of 1 mg fresh leaf per ml extract Data are means of 4 replicates ±se. Effect of UV-B, time and the interaction UV-B x time, analysed with a three way analysis of variance, was significant ($p < 0.05$). The effect of CO_2 and other interactions were not significant.

Discussion of combined CO_2 and UV-B effects in the C_3-grass *Elymus athericus*

Van de Staaij et al. (1993) report significant CO_2 and UV-B effects on total plant dry weight, root, stem and leaf dry weight of *Elymus athericus.* There was, however, not a statistically significant interaction of CO_2 and UV-B on any of the biomass parameters assessed. In the present study with an almost identical experimental set up, there was a statistically significant interaction effect of CO_2 and UV-B on total plant dry weight and the root dry weight. An experimental difference of the present study with that of van de Staaij et al. (1993) is the increased supplementary PAR level (300 instead of 200 µmol m^{-2} s^{-1}) and increased supply of nutrients. After 42 days of growth total plant dry weight in the present study was more than double the total plant dry weight obtained by van de Staaij et al. (1993) after 65 days.

In the present study, with the C_3-grass *Elymus athericus* growing faster, the relative increase in total plant dry weight by CO_2 enrichment was small (31%) compared to 64% at low UV-B reported by van de Staaij et al. (1993). At ambient CO_2, the percentage dry weight decrease by elevated UV-B was 32% (van de Staaij et al. 1993) compared to 34% in the present study. The statistically significant interaction between CO_2 and UV-B in the present study, implies reduced sensitivity to enhanced UV-B at elevated CO_2.

We could not find a physiological explanation for this effect in terms of net photosynthesis, and stomatal conductance (Table 3). Neither the Leaf Area Ratio, Leaf Weight Ratio, Specific Leaf Area nor the shoot to root ratio could provide an explanation. Absorbance of leaf extracts at 300 nm increased with increasing UV-B radiation, but not with elevated CO_2 (Figure 1).

Table 1. The effect of CO_2 and UV-B on leaf area and dry weight of *Elymus athericus*. Plants were harvested after 42 days. Average values of 9–16 replicates. Significance of CO_2, UV-B and the interaction CO_2 × UV-B from a two way analysis of variance is given. n.s. = not significant; * = $p < 0.05$

CO_2 concent. (μmol mol^{-1})	UV-B_{BE} (kJ m^{-2} day^{-1})	Leaf area ×10^{-4} (m^2)	Dry weight (g)			
			Root	Stem	Leaf	Total plant
380	10.0	481	1.98	2.04	2.03	6.04
720	10.0	547	2.34	2.91	2.69	7.94
380	16.8	387	1.16	1.28	1.50	3.94
720	16.8	507	2.42	2.34	2.38	7.14
CO_2		*	*	*	*	*
UV-B		*	*	*	*	*
CO_2 × UV-B		ns	*	n.s.	n.s.	*

Table 2. The effect of CO_2 and UV-B on leaf area ratio (LAR), leaf weight ratio (LWR), specific leaf area (SLA) and shoot to root (S/R) ratio of *Elymus athericus*. Plants were harvested after 42 days. Average values of 9–16 replicates. Statistics similar as in Table 1. n.s. = not significant; * = $p < 0.05$

CO_2 conc. (μmol mol^{-1})	UV-B_{BE} (kJ m^{-2} day^{-1})	LAR (m^2 kg^{-1})	LWR (kg kg^{-1})	SLA (m^2 kg^{-1})	S/R ratio
380	10.0	8.2	0.34	23.8	2.41
720	10.0	7.2	0.34	21.0	2.58
380	16.8	10.2	0.39	26.0	2.50
720	16.8	7.5	0.34	22.0	2.09
CO_2		*	*	*	n.s.
UV-B		*	n.s.	*	n.s.
CO_2 × UV-B		n.s.	n.s.	n.s.	n.s.

In the following parts of this paper we discuss more generally combined CO_2 and UV-B effects and possible morphological changes caused by CO_2 which also affect UV-B susceptibility.

Combined CO_2 × UV-B effects on biomass parameters

A survey of studies of combined CO_2 and UV-B effects on plants is presented in Table 4. Interactions of effects of CO_2 of enrichment and enhanced UV-B radiation on total plant biomass are found in only 2 cases out of 15 plant species studied (Table 4). The case of *Elymus athericus* was discussed in detail earlier in this paper. The other case concerns soybean (*Glycine max*) cultivars, grown in a greenhouse with 80–85% of outdoor solar PAR (Teramura et al. 1990). In that study, growth of soybean decreased with enhanced UV-B at ambient CO_2, but increased with enhanced UV-B at elevated CO_2 . There was no increased UV-B absorbance of extracts with increased UV-B radiation in soybean. No explanation was given for the reduced sensitivity to enhanced UV-B at elevated CO_2.

Although there is variability in the response to enhanced UV-B among soybean cultivars, growth of many soybean cultivars is reduced at enhanced UV-B (Teramura & Murali 1986).

Many of the plant species surveyed in Table 4 are crop species, only a few are natural species. The species represent a variety of responsiveness to elevated CO_2 and UV-B, both UV-B sensitive and insensitive species are included. However, for almost all species tested, effects of CO_2 enrichment and of enhanced UV-B radiation were significant in single stress mode. Tosserams et al. (1997b) report clear-cut CO_2 and UV-B effects on faba bean in a greenhouse study, and significant interaction on total plant biomass. In an Open

Table 3. Net photosynthesis and stomatal conductance of leaves of *Elymus athericus* as affected by CO_2 and UV-B treatment. Measurements were made 35 days after the start of experiment. Average values of 6 replicates. Statistics similar as in Table 1. n.s. = not significant; * = $p < 0.05$

CO_2 conc. (μmol mol^{-1})	UV-B_{BE} (kJ m^{-2} day^{-1})	Net photosynthesis (μmol CO_2 m^{-2} s^{-1})	Stomatal conductance (mmol H_2O m^{-2}s^{-1})
380	10.0	9.5	387
720	10.0	14.5	230
380	16.8	9.3	434
720	16.8	15.8	293
CO_2		*	*
UV-B		n.s.	n.s.
CO_2 × UV-B		n.s.	n.s.

Table 4. Overview of combined effects of atmospheric CO_2 enrichment and enhanced ultraviolet-B radiation on total plant biomass on various crops and natural plant species

Authors	Plant species	PAR μmol (m^{-2} s^{-1})	CO_2 (μmol m^{-2} s^{-1})	UV-B levels (kJ m^{-2} day^{-1})	CO_2 effect	UV-B effect	CO_2 × UV-B	Indoor/ Outdoor/ remarks
Teramura et al. 1990	*Triticum aestivum*	80–85%	350/650	8.8/15.7	+	−	−	Greenhouse
	Oryza sativa	outdoors	350/650	8.8/15.7	+	−	−	Greenhouse
	Glycine max		350/650	8.8/15.7	+	+	+	Greenhouse
Rozema et al. 1990	*Pisum sativum*	200	350/760	0/22	+	−	−	Greenhouse
	Lycopersicum esculentum		350/760	0/22	+	−	−	Greenhouse
	Aster tripolium		350/760	0/22	o	−	−	Greenhouse
Ziska & Teramura 1991	*Oryza sativa*	1800–2000	130–1430	8.8/13.8	+	−	−	Greenhouse
Adamse & Britz 1991	*Cucumis sativa*	1000	450/750	0.05/18	+	−	−	Greenhouse
Van de Staaij et al. 1993	*Elymus athericus*	200	380/720	10.0/16.8	+	−	−	Greenhouse
Stewart & Hoddinott 1993	*Pinus banksiana*	−?	350/750	0.005–0.03	+	−	−	Greenhouse
				0.25–0.90 W m^{-2}	+	−	−	Very small pots (40 cm^3)
Sullivan & Teramura	*Pinus taeda*	80% outdoors	350/650	8.8/13.8	+	−	−	Greenhouse
Tosserams et al. 1997b	*Vicia faba*	250–700	380/750	0, 4.6, 7.6, 10.6	+	−	+	Greenhouse
Visser et al. 1997	*Vicia faba*	outdoors	350/700	0.7/3.0	+	−	−	Open Top Chamber
Rozema et al. 1997	*Elymus athericus*	300	380/720	10.0/16.8	+	−	+	Greenhouse
Gwynn-Jones et al. 1997	*Vaccinium myrtillus*	outdoors	350/600	4.6/5.8	+	−	−	Open Top chamber; UV-B lamps

CO_2 effects and UV-B effects + =positive effect ; − =negative effect , o =no effect; CO_2 × UV-B − = no interaction, or + =significant interaction.

Top Chamber study (Visser et al. 1997), there was a small (negative) biomass effect of enhanced UV-B radiation on biomass in early growth stages, but this disappeared when plants grew older.

Realising the limited number of CO_2 × UV-B studies, and the limitations of indoor studies, we provisionously conclude, that CO_2 enrichment and enhanced UV-B radiation do not interact in general on total plant biomass. This implies that effects of CO_2 enrichment and enhanced UV-B radiation can be regarded to be additive for total plant biomass. This -provisional- conclusion regarding plant biomass does not rule out that elevated CO_2 and UV-B may interact – in a more subtle way – on physiological and plant morphogenetic parameters (Tosserams et al. 1997b). Since many species tested differ in their responsiveness to elevated CO_2 and UV-B radiation (Table 4; Caldwell et al. 1989, 1995; Rozema et al. 1993; Tosserams et al. 1997a), additivity of (positive) CO_2 effects and (neutral or negative) UV-B effects, will lead to marked changes in competitive relationships in ecosystems. In ecosystem studies it will therefore be relevant to consider both effects of elevated CO_2 and enhanced UV-B in an assessment of consequences of global climatic change (Caldwell & Flint 1994; SCOPE 1993; Sullivan 1997).

Combined CO_2 × UV-B effects on plant morphogenetic parameters

Leaf thickness may increase with increased solar UV-B radiation (Bornman & Vogelmann 1991; Cen & Bornman 1990). Increased leaf thickness decreased Specific Leaf Area in m^2 per kg FW) under enhanced UV-B was observed in the field for *Calamagrostis epigeios*, particularly in early stages of leaf development. At the end of the growing season the effect had disappeared (Table 5). Increased leaf thickness may lead to reduced levels of internal UV-B radiation (Bornman & Vogelmann 1991). Cen & Bornman (1990, 1993) indicated that increased leaf thickness under elevated UV-B results from increased cell length of palisade parenchyma. Tosserams & Rozema (1995) report reduced transmission of visible light through intact leaves of *Calamagrostis epigeios* at an increased level of UV-B radiance. As a result of increased leaf thickness, lower internal UV-B radiation levels may be reached, at the cost of reduced photosynthetically active radiation and reduced photosynthesis. It will depend on the plant species, the radiation climate, and possibly other growth limiting factors involved, whether the reduced internal levels of UV-B (280–320 nm) and PAR (400–700 nm) will lead to reduced growth and fitness of the plant at elevated UV-B radiation. Johanson et al. (1995) report increased leaf thickness in two evergreen dwarf shrub species (*Vaccinium vitis-idaea* and *Empetrum hermaphroditum*) after two years of exposure to enhanced UV-B radiation in a field experiment in northern Sweden.However, leaf thickness of two deciduous species, *Vaccinium myrtillus* and *V. uliginosum* decreased. Johanson et al. (1995) suggested that UV-B effects accumulate in the evergreen dwarf shrubs over the years. Therefore, they indicate, no growth reduction occurred with enhanced UV-B radiation in the two deciduous dwarf shrub species, in contrast with the two evergreen *Vaccinium* species. It has frequently been reported that leaf thickness increases with atmospheric CO_2 enrichment (Rozema 1993, 1995). The physiological background of thicker leaves under elevated CO_2, such as increased leaf content of starch, may differ from that under higher UV-B such as increased cell length of palisade parenchyma (Cen & Bornman 1993). Leaf thickness might, however, be a relevant parameter for interaction of elevated CO_2 and UV-B effects.

It is clear that studies of UV-B effects on leaf thickness and relating ecological consequences need further laboratory and field research. Sullivan & Teramura (1994) report significant effects of elevated CO_2 (positive) and UV-B (12% biomass reduction) at higher UV-B radiation at ambient and elevated CO_2 in Loblolly pine (*Pinus taeda*). There was no significant interaction effect of CO_2 and UV-B on total plant biomass. However, dry matter was preferentially allocated to the shoot under enhanced UV-B radiation at ambient CO_2 and towards root biomass components at elevated CO_2. As far as we know no significant shift in root-shoot biomass allocation under elevated CO_2 has been reported (Rozema et al. 1993).

Table 5. The effect of enhanced solar UV-B radiation on Specific Leaf Area (m^2 leaf area per kg leaf biomass – fresh weight) of *Calamagrostis epigeios*, at day 15, 42 and 70 in 1994. Day 15 is June 30, 1994. Average values of 20, just fully expanded leaves exposed to 5 kJ m^{-2} day^{-1} (ambient) or 7.5 kJ m^{-2} day^{-1} $UV\text{-}B_{BE}$ (enhanced) growing in a dune grassland (Rozema et al. 1995).The effect of UV-B and time , analysed with a two-way anova , was significant ($p < 0.05$).The interaction effect of UV-B and time was not significant ($p = 0.06$)

Time	Ambient UV-B	Enhanced UV-B
Day 15	5.19 ± 0.20	4.96 ± 0.20
Day 42	5.99 ± 0.24	5.81 ± 0.26
Day 70	7.11 ± 0.20	6.43 ± 0.16

These subtle effects on biomass allocation may be important to competitive interactions in natural and agro-ecosystems. Effects of UV-B radiation on plant-morphogenetic parameters may relate to changes in levels of phytohormones under enhanced UV-B (Ros & Tevini 1995). At the moment there is no unequivocal information on UV-B effects on phytohormones and further research is essential.

Another way in which enhanced UV-B radiation and atmospheric CO_2 enrichment may interact, is via their mutual impact on carbon fluxes in ecosystems. Enhanced UV-B radiation may indirectly, and directly change decomposition of plant organic matter. This is considered by Rozema et al. (1997) elsewhere in this volume.

Outlook

Elevated CO_2 and UV-B affect plant growth in an opposite direction: growth is often stimulated at elevated CO_2 and may be reduced at elevated UV-B.

Of the few studies on combined effects of elevated CO_2 and UV-B radiation most do not report significant

CO_2 × UV-B interactions on plant biomass. This may relate to the fact that de direct metabolic targets of CO_2 and UV-B are different. However indirect and interactive effects of CO_2 and UV-B may occur (e.g. plant morphology, architecture, biomass allocation) with far reaching ecological consequences.

Elevated UV-B and CO_2 may also differentially affect the chemical composition of plants (e.g. flavonoids, tannins, lignin and other phenolic compounds). Such secondary plant compounds may change plant-animal relationships as well as plant litter decomposition. Regarding the latter process, enhanced solar UV-B may, indirectly, affect carbon fluxes and storage in ecosystems.

In this way enhanced UV-B and CO_2 may have important, but as yet unpredictable ecological consequences.

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Tree seedlings at experimental field site growing under supplemental UV-B irradiation. (Photograph: J. H. Sullivan)

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Effects of increasing UV-B radiation and atmospheric CO_2 on photosynthesis and growth: implications for terrestrial ecosystems

Joe H. Sullivan
Department of Horticulture and Landscape Architecture, University of Maryland, College Park, Maryland 20742, USA

Key words: Biomass allocation, Elevated CO_2, Global climate change, Ozone depletion, Photosynthetic acclimation

Abstract

Increases in UV-B radiation reaching the earth as a result of stratospheric ozone depletion will most likely accompany increases in atmospheric CO_2 concentrations. Many studies have examined the effects of each factor independently, but few have evaluated the combined effects of both UV-B radiation and elevated CO_2. In general the results of such studies have shown independent effects on growth or seed yield. Although interspecific variation is large, high levels of UV-B radiation tends to reduce plant growth in sensitive species, while CO_2 enrichment tends to promote growth in most C_3 species. However, most previous studies have not looked at temporal effects or at the relationship between photosynthetic acclimation to CO_2 and possible photosynthetic limitations imposed by UV-B radiation. Elevated CO_2 may provide some protection against UV-B for some species. In contrast, UV-B radiation may limit the ability to exploit elevated CO_2 in other species. Interactions between the effects of CO_2 enrichment and UV-B radiation exposure have also been shown for biomass allocation. Effects on both biomass allocation and photosynthetic acclimation may be important to ecosystem structure in terms of seedling establishment, competition and reproductive output. Few studies have evaluated ecosystem processes such as decomposition or nutrient cycling. Interactive effects may be subtle and species specific but should not be ignored in the assessment of the potential impacts of increases in CO_2 and UV-B radiation on plants.

Introduction

A considerable body of evidence has now demonstrated that atmospheric trace gases such as CO_2, nitrous oxides, chlorofluorocarbons (CFCs), etc. are increasing (WMO, 1986). For example, increases in atmospheric CO_2 concentrations are on the order of 1.5 μmol year^{-1} (Watson et al. 1990) and are due primarily to deforestation and the burning of fossil fuels. It has been projected that atmospheric levels of CO_2 will reach 600 μmol^{-1} sometime within the next century (Hansen et al. 1986). The consequences of the increases of CO_2 and other trace gases may include warming of regional or global mean temperatures, alterations in precipitation patterns and sea level rise over the next 100 years (WMO 1986).

In addition to their contribution to global warming, CFCs may also deplete the earth's protective stratospheric ozone layer (Molina & Rowland 1974). Reductions in this ozone column have led to increases in ultraviolet-B radiation (UV-B between 290 and 320 nm) reaching the earth's surface (e.g. Blumthaler & Ambach 1990, Kerr & McElroy 1993). Therefore, changes in global climate, such as increasing temperatures and alterations in precipitation patterns may occur within an environment of increasing UV-B radiation and atmospheric CO_2.

Numerous studies have examined the effects of CO_2 enrichment on plant growth (see Bazzaz 1990; Oechel & Billings 1992; Rogers & Dahlman 1993; Strain 1992, for recent reviews). In general, CO_2 enhancement tends to stimulate biomass production in most C_3 species. The effects of increases in UV-B radiation on plant growth or productivity tend to be more subtle than those of CO_2 enrichment. Although inter- and intra-specific variation is quite large, about one-

half of the species evaluated have exhibited alterations in physiology, biomass accumulation or morphology (see Caldwell & Flint 1994; Tevini 1993; Teramura & Sullivan 1994; Jordan 1996, for recent reviews).

In contrast to single factor studies, relatively few studies have evaluated the combined effects of increasing CO_2 and UV-B radiation. Elsewhere in this volume, Rozema et al. (1996a) also consider the combined effects of UV-B and CO_2 on biomass and terrestrial ecosystems and Mark and Tevini (1996) assess the combined effects of UV-B, CO_2 and increased temperatures on plants. In this review I will consider data currently available at the physiological and whole plant level, the experimental conditions under which it was obtained and the mechanisms of possible interactive effects between UV-B and CO_2, in order to present some hypotheses about potential consequences at the ecosystem level.

Previous studies

In most previous studies, the effects of CO_2 enrichment and UV-B radiation on growth have been found to be independent (see Rozema et al. 1996a). Few studies have demonstrated interactive responses and a generalization of the results suggests that exposure to high levels of UV-B radiation may reduce or eliminate the stimulatory effect of CO_2 enhancement on plant productivity. For example, Teramura et al. (1990) found that UV-B radiation reduced (in wheat) or eliminated (in rice) the CO_2 enhancement effect on total biomass accumulation. In contrast, enhanced levels of UV-B radiation did not alter the CO_2 response in soybean. Sullivan & Teramura (1994) found that the growth of loblolly pine seedlings was reduced by about 12% upon exposure to high levels of UV-B radiation. This effect was independent of CO_2 concentration. Other studies on Dutch marsh or crop species (Rozema et al. 1990, Van de Staaij et al. 1993), cucumber (Adamse & Britz, 1992), rice (Ziska & Teramura, 1992) and three northern coniferous species (Stewart & Hoddinott 1993; Yakimchuk & Hoddinott 1994) have demonstrated a similar lack of interactive effects on plant growth. The implication from these results is that overestimates of increased productivity, based upon future CO_2 enrichment, could result for some species if the solar radiation environment is not also considered.

While the present literature represents an important contribution to our knowledge base, some caveats must be considered before the results from these studies may be extrapolated to the ecosystem level.

First, the data are quite limited in that many plant groups are not represented. For example, data are lacking for representative species from many higher plant groups; and, with but few exceptions that I am aware of (e.g. Sonesson et al. 1995, 1996), there are essentially no data available for non-vascular species. In fact, few single factor studies exist for non-vascular species, such as mosses or lichens (Johanson et al. 1995). Consequently, a significant portion of many terrestrial ecosystems has not been evaluated.

Second, predictions of ecosystem responses based on studies on isolated plants may not be accurate. Caldwell & Flint (1994) suggest that species may respond quite differently to both UV-B and CO_2 enrichment in isolation than in an intact ecosystem. Very few studies have attempted to evaluate the responses of ecosystem processes in intact systems to either UV-B radiation or CO_2 enrichment (e.g. Johanson et al. 1995). Therefore, predictions of future effects on ecosystems are quite difficult even where UV-B radiation or CO_2 alone is considered. The difficulty in making accurate predictions most certainly increases where combined effects are considered.

Finally, the experimental conditions under which most previous studies have been conducted adds to our inability to extrapolate those results to the ecosystem level. The bulk of data available have been obtained under controlled environment conditions, e.g. growth chambers and glasshouses. Both UV-B and CO_2 effects may be modified by variations in other environmental conditions such as light quality or quantity (e.g. Warner & Caldwell 1983; Tolley & Strain 1984a; Mirecki & Teramura 1984; Cen & Bornman 1990; Rufty et al. 1994; Caldwell et al. 1994; Middleton & Teramura 1994), water availability (Sullivan & Teramura 1990; Tolley & Strain 1984b; Petropoulou et al. 1995), nutrients (Murali & Teramura 1985; Tissue et al. 1993) and temperature (Baker et al. 1989; Pang & Hayes 1991; Ziska & Bunce, 1993). Therefore, studies conducted under controlled environments may not realistically simulate natural conditions and the plant responses may not be indicative of effects under natural conditions.

Recent advances in CO_2 fumigation technology such as the free air CO_2 enrichment (FACE) technique will help to resolve microclimate issues where CO_2 enrichment is concerned. Almost all UV-B studies have been conducted in controlled environment settings with artificial irradiation systems. Caldwell & Flint (1994)

found reports of only 28 studies that evaluated the response of field-grown plants to increases in UV-B radiation. Of these, only 2 studies spanned more than a single season for perennial plants, which make up the vast majority of terrestrial vegetation. Some recent studies have modified natural levels of UV-B radiation by shading (e.g. Searles et al. 1995), with ozone filters (e.g. Tevini 1993), and using specially constructed UV-transmitting open-top chambers to either transmit or exclude ambient levels of UV-B radiation (Visser et al. 1993). These studies will add to our ability to assess the consequences of natural alterations in the UV-B component of sunlight.

An obvious conclusion from the above discussion is that further studies, conducted under realistic outdoor conditions are needed. However, it would be unrealistic to suggest that representatives from all plant groups should be studied. One possible approach would be to select and survey certain 'key' species or plant groups. For example, annual crop species have been rather extensively studied, compared to natural or perennial species, for their sensitivity to UV-B radiation. Unfortunately, the large degree of variation in responses to UV-B radiation, limits the utility of such data for extrapolation across species or major plant groups. Another approach would be to increase our understanding of the mechanisms of biochemical or physiological responses to CO_2 and UV-B radiation in order to augment our ability to make predictions about potential consequences of alterations in these factors. Such an increased understanding would be particularly important to modelling studies that would attempt to predict future responses to changes in these environmental factors.

Photosynthetic Responses to UV-B Radiation and CO_2

While instantaneous photosynthetic rate may not be well correlated with growth, total plant carbon gain is the fundamental process of plant or ecosystem productivity. An understanding of how environmental factors regulate or limit carbon assimilation is essential to making predictions about future alterations due to environmental change. For example, an understanding of the mechanisms of UV-B limitations on photosynthesis would allow the response to CO_2 enrichment to be predicted and tested. Although the data are limited, some data are available that can be utilized to speculate about the implications of increases in CO_2 and UV-B radiation on carbon assimilation.

The primary stimulatory effect of CO_2 enrichment on plant productivity is mediated through increases in photosynthetic carbon assimilation. This increase is due to an increase in the CO_2 diffusion gradient (i.e. higher ambient CO_2 or C_a) and to a reduction in the oxygenase component of ribulose-bisphosphate-carboxylase-oxygenase (Rubisco) (Eamus & Jarvis 1989; Long et al. 1993). Indirect effects such as increasing instantaneous water use efficiency, may also increase carbon assimilation under increased levels of CO_2 (e.g. Jacob et al. 1995).

Over the long-term, however, so-called photosynthetic acclimation may occur. This response can result in a reduction in the stimulatory effect of CO_2 on photosynthesis and may be due to reductions in Rubisco concentration or activity (e.g. Sage et al. 1989). This down-regulation of photosynthetic capacity may be due to lowered tissue N content and feedback inhibition by increased carbohydrates or starch on expression of the Rubisco genes (Jacob et al. 1995). The degree of photosynthetic acclimation present may be observed by analysis of the response of assimilation to internal CO_2 concentrations (the so-called $A*Ci$ response curve). Acclimation is generally associated with a reduction in carboxylation efficiency or assimilation rate at saturated C_i (Figure 1A).

Photosynthetic acclimation has not been found in all cases, however (Arp & Drake 1991; Ziska et al. 1991) and also may be reversible (Ziska et al. 1995). Ziska et al. (1995) found that renewed sink strength upon flowering reversed apparent photosynthetic acclimation in Swiss chard and sugarbeet. Other studies (Arp 1991; Thomas & Strain 1991) have found that acclimation appears to be linked to the availability of adequate sinks for increased photosynthate. Thus, plant growth conditions and other environmental factors (e.g. temperature, light quantity and quality) may be linked to the acclimation process.

The primary site of direct photosynthetic damage by UV-B radiation is thought to be associated with Photosystem II, where effects of reduced oxidative capacity (Renger et al. 1989), D1 polypeptide turnover (Greenburg et al. 1989) and photoreduction of PQ (Melis et al. 1992) have all been reported. Direct effects of high levels of UV-B radiation on Rubisco may include a reduced number of mRNA transcripts (Jordan et al. 1992) and reduced RUBISCO activity (Strid et al. 1990; Jordan et al. 1992). The molecular

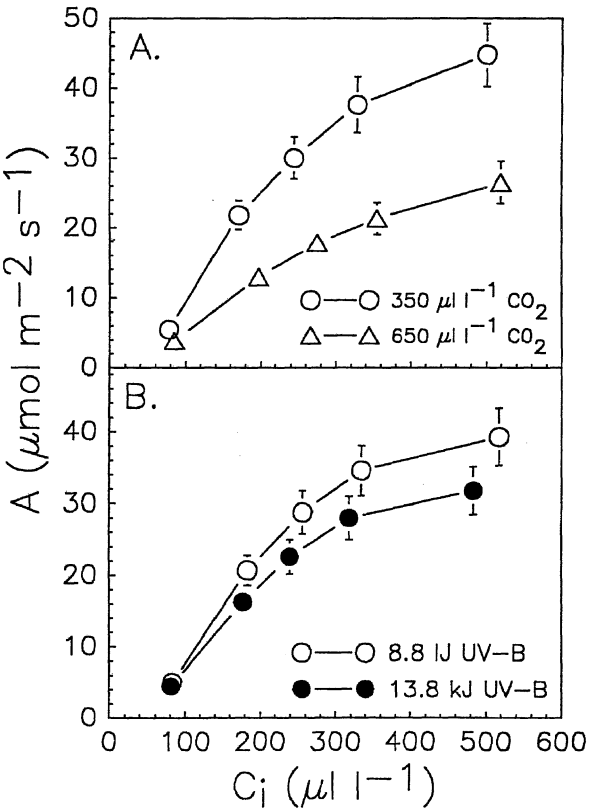

Figure 1. Photosynthetic response (at PPF of 1500 μmol m^{-2} s^{-1}) to internal CO_2 concentration (C_i) for rice plants grown for 15 weeks under either (A) 2 CO_2 levels (350 and 650 μl l^{-1}) and 8.8 kJ m^{-2} d^{-1} of UV-B radiation weighted with Caldwell's (1971) weighting function, or (B) under 2 levels of UV-B radiation (8.8 or 13.8 kJ m^{-2} d^{-1}) at 350 μl l^{-1} CO_2. Each point on the curve is the mean of 9–12 measurements ± SE. Additional details of experimental design may be found in Teramura et al. (1990).

effects of UV-B on photosynthesis have recently been extensively reviewed by Jordan (1996).

Photosynthetic damage by UV-B radiation may be detected by analysis of the functional responses of CO_2 assimilation to light or internal CO_2 concentration. For example, damage to PS-II by UV-B radiation may result in a reduction in quantum efficiency or a lower photosynthetic capacity (Figure 1B). Reductions in CO_2 saturated assimilation rates may be due to a reduced capacity to regenerate substrate (Ribulose bisphosphate or RuBP) for Rubisco. This substrate regeneration limitation to assimilation is thought to be dependent in part on so-called light reaction products, ATP and $NADPH^+$ (e.g. Harley et al. 1985; Sharkey 1985). Direct UV-B damage to Rubisco itself might also be detected by a lowering of the initial slope of the *A*Ci* response. The importance of these effects from a diagnostic standpoint is that the 'symptoms' may appear quite similar to the CO_2 acclimation response (Figure 1A and 1B). As a result these effects may not be readily partitioned by whole leaf gas exchange analysis.

Considering the discussion above regarding limitations to photosynthesis, one may hypothesize about the possible combined effects of UV-B radiation and CO_2 enhancement. If for example, UV-B radiation reduced electron transport and ultimately substrate regeneration, then CO_2 enrichment may not compensate for such damage. UV-B radiation may limit the capacity of the photosynthetic system to utilize the greater CO_2 supply through limitations on RuBP regeneration rates. Thus an apparent photosynthetic acclimation to high CO_2 would be observed on the *A*Ci* response curve. The ability of a plant to respond photosynthetically or in growth to CO_2 enrichment is clearly modulated by other factors such as light or nutrient availability. The magnitude of UV-B radiation present may also be a contributor to this regulatory system.

Are there experimental data to corroborate the above hypothesis? While few data are available, Teramura et al. (1990) found that apparent quantum efficiency (AQE) in rice, soybean and wheat was increased by elevated CO_2 only at the lower of two UV-B radiation levels (Figure 2). Ziska & Teramura (1992) found a similar effect on one of two rice cultivars studied when potential photochemical efficiency was assessed by chlorophyll fluorescence techniques. This suggests that for these species, even though there was no apparent photosynthetic damage by UV-B radiation at ambient CO_2 levels, there were limitations imposed by UV-B radiation that restricted photosynthetic capacity at elevated levels of CO_2.

The above studies suggest that there is a subtle difference between photosynthetic damage in response to UV-B radiation and a limitation imposed by UV-B radiation on the maximum rate of photosynthesis. In fact, damage to the photosynthetic system by sunlight and its repair is an ongoing process as in the continuing processes of DNA damage and repair (Quaite et al. 1994). Therefore, the suite of repair or protective mechanisms that have evolved in plants may in fact be sufficient to prevent damage under current environmental conditions. However, new limitations on carbon assimilation may become operative under more favorable environments, such as CO_2 enrichment. It should be pointed out that of the entire body of CO_2 literature, few if any studies have been conducted within a back-

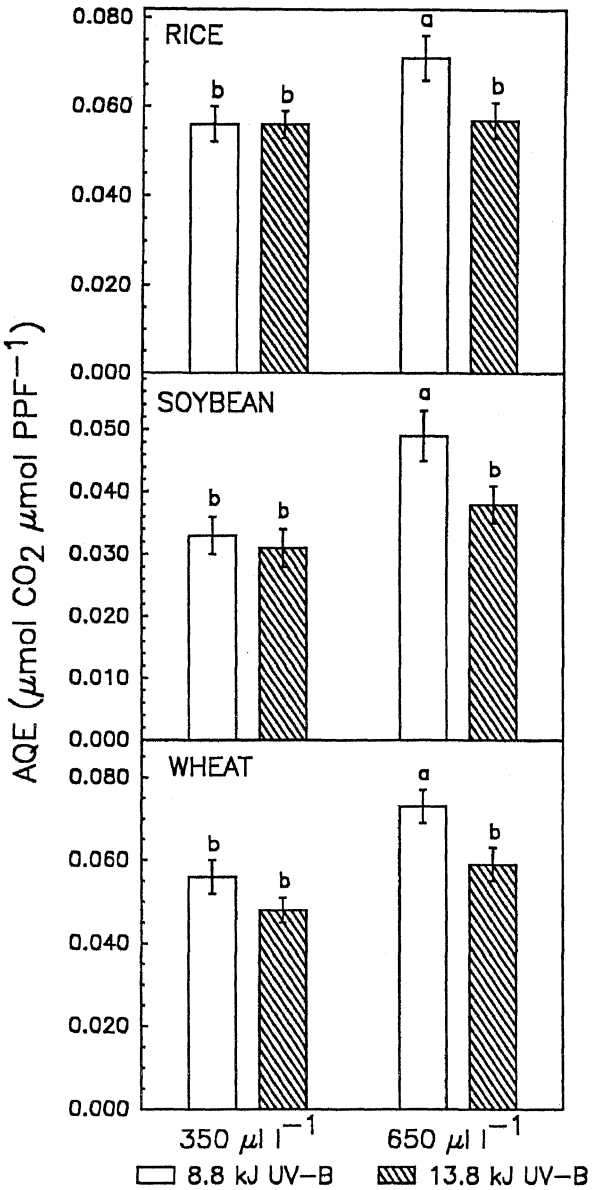

Figure 2. Apparent quantum efficiency (μmol CO_2 μmol PPF^{-1}) between 0 and 100 μmol m^{-2} s^{-1} for rice, soybean and wheat plants grown for 5 weeks in a factorial experiment with 2 CO_2 levels (350 and 650 μl l^{-1}) and 2 levels of UV-B radiation (8.8 or 13.8 kJ m^{-2} d^{-1} of UV-B radiation weighted with Caldwell's (1971) weighting function. Each bar is the mean of 9–12 measurements ± S.E. Additional details of experimental design may be found in Teramura et al. (1990).

ground of solar irradiation that contains realistic levels of UV-B radiation. Only FACE studies and a few other studies that have incorporated UV-transparent materials into open-top chambers, will allow the role of UV-B radiation, if any, in modifying the response to CO_2 to be investigated. Therefore, it would be premature to speculate on how widespread this limitation by UV-B radiation on the CO_2 response may be.

While UV-B radiation or other environmental conditions may reduce the magnitude of the photosynthetic response to CO_2, some data also suggest that CO_2 enrichment may provide protection to the photosynthetic apparatus or compensate for UV-B radiation damage. For example, if UV-B radiation damage is directed at Rubisco (e.g. Strid et al. 1990; Jordan et al. 1992), then this damage could potentially be compensated for by increased CO_2. Compensation might occur due to the reduction in the oxygenase activity of Rubisco under elevated CO_2 conditions. This could increase net carboxylation even in the presence of reduced Rubisco activity. Also Adamse & Britz (1992) hypothesized that increased photosynthetic rate in response to CO_2 enrichment could protect the plant from UV-B damage. They suggested that such protection might be provided by increased assimilate and potentially more energy available for repair processes, in plants grown under elevated CO_2. They found some evidence to support this in cucumber where UV-B radiation damage was mitigated under CO_2 enrichment.

Growth under elevated CO_2 may also provide protection from UV-B by increasing levels of flavonoids in the epidermis. Flavonoids and other polyphenolics that absorb in the UV-B region have been shown to reduce epidermal penetration of UV-B radiation (e.g. Robberecht & Caldwell 1978, 1983; Caldwell et al. 1983, Day 1993) and the accumulation of such compounds has been widely considered to be a protective response to UV-B radiation. CO_2 enrichment has been shown to increase foliar concentrations of UV-absorbing compounds (e.g. Teramura et al. 1990; Ziska & Teramura 1992; Lambers 1993; Sullivan & Teramura 1994), perhaps due to greater carbohydrate availability. Therefore, direct damage to components of the photosynthetic system might be avoided by increases in epidermal flavonoids that reduce UV-B penetration into the mesophyll (e.g. Caldwell et al. 1983; Tevini et al. 1991, etc.). Carbohydrate accumulation and associated leaf thickening in response to CO_2 enrichment (e.g. Adamse & Britz 1992) may also provide protection from UV-B radiation. Both Stewart & Hoddinott (1993) and Sullivan & Teramura (1994) found that exposure to high CO_2 concentrations appeared to protect photosynthesis from damage by UV-B radiation (Figure 3).

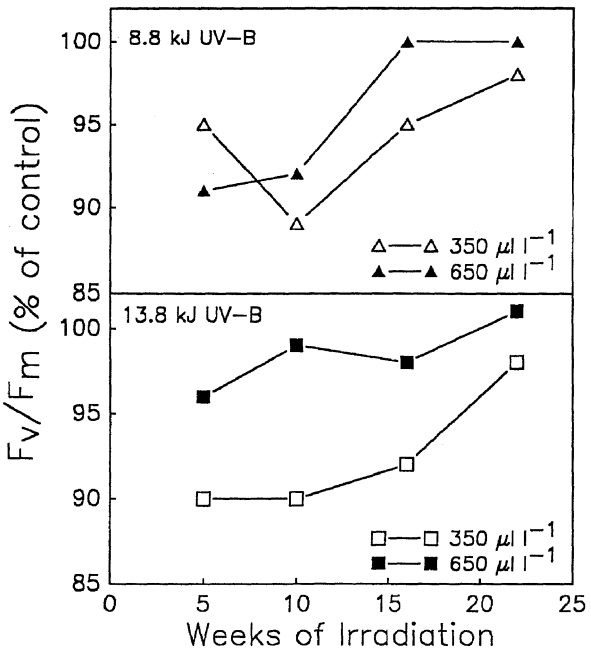

Figure 3. Changes in F_v/F_m ratio, expressed as % of control (0 UV-B radiation) values, for loblolly pine seedlings grown for 22 weeks in a factorial study with 2 CO_2 (350 and 650 $\mu l\ l^{-1}$) and 3 UV-B (0, 8.8 or 13.8 kJ $m^{-2}\ d^{-1}$) radiation levels. Measurements were made at approximately 5 week intervals and the values are shown at 8.8 kJ $m^{-2}\ d^{-1}$ (top panel) and 13.8 kJ $m^{-2}\ d^{-1}$ (lower panel). Each point is the mean of 15 measurements per treatment and the error bars are ±1 S.E. Additional details of experimental design may be found in Sullivan & Teramura (1994).

In summary, a survey of the data available finds examples of instances where UV-B radiation limits the photosynthetic response to CO_2 enrichment, has no effect on the response to CO_2 enrichment, and cases where elevated CO_2 results in protection from UV-B radiation damage. These response differences may be the result of variation in the CO_2 response and in the plant's relative sensitivity to UV-B radiation damage. Interspecific variation and alteration in the relative limitations to photosynthesis (e.g. diffusional versus photochemical) may have implications for plant growth under a future environment of increased CO_2 and UV-B radiation. An improved understanding of such subtle interactions will be important in determining the likely consequences of increases in CO_2 and UV-B radiation.

Effects on biomass and allocation

Even though CO_2 and UV-B radiation may both alter photosynthetic parameters, these changes do not always translate into effects on total biomass. As previously stated, effects of increased CO_2 and UV-B radiation on total biomass have generally been additive (e.g. Rozema et al. 1996a). However, such might not be the case under field conditions. For example, indirect effects of CO_2 enrichment on WUE could be more important under natural conditions where drought is a common stress.

Most previous studies have assessed biomass at a single point, usually at the conclusion of the study. Acclimation to CO_2 and variation in effects of UV-B radiation due to leaf age or developmental stage (e.g. Dillenburg et al. 1995) may lead to apparently inconsistent findings due to timing of harvests. In a study at the Duke University Phytotron (Teramura et al. 1990), we made three sequential harvests at approximately 5, 10 and 15 weeks after seed germination. In that study, significant CO_2 by UV-B interactions were observed for plant vegetative biomass of rice at the harvests made at 5 and 10 weeks but no effects of either treatment were detected at the final harvest (Figure 4). This loss of effectiveness of CO_2 may have been due to photosynthetic acclimation to CO_2 since assimilation rates at growth concentrations of CO_2 were reduced in the plants grown at high CO_2 concentrations (Figure 1A). In contrast, an interaction between CO_2 and UV-B was seen only at the final harvest in soybean (Figure 5) when UV-B radiation decreased biomass at ambient CO_2 and increased biomass at elevated CO_2. The mechanism of this increase in biomass remains unknown. Also soybean did not display any apparent acclimation to CO_2 in that study. Both of these examples indicate that the results of studies that use a single point measurement may be influenced by the timing of the sampling.

Previous studies have demonstrated considerable variation in the effects of CO_2 on biomass partitioning ranging from no effects (Tolley & Strain 1984a), increases in root to shoot ratio (RSR) (Ziska & Teramura 1992) and reductions in RSR (Wulff & Strain 1982). Background visible irradiance influences biomass partitioning (e.g. Kramer & Kozlowski 1979), and an interaction between visible irradiance and CO_2 on biomass partitioning has been demonstrated in loblolly pine and sweetgum (Tolley & Strain 1984a).

In UV-B sensitive plants the detrimental effects of UV-B radiation often lead to reduction in growth with no shifts in biomass allocation (e.g. Teramura 1983; Tevini & Teramura 1989; Caldwell & Flint 1994); however, biomass allocation has been altered in some species such as cassava (Ziska et al. 1993), Engelmann

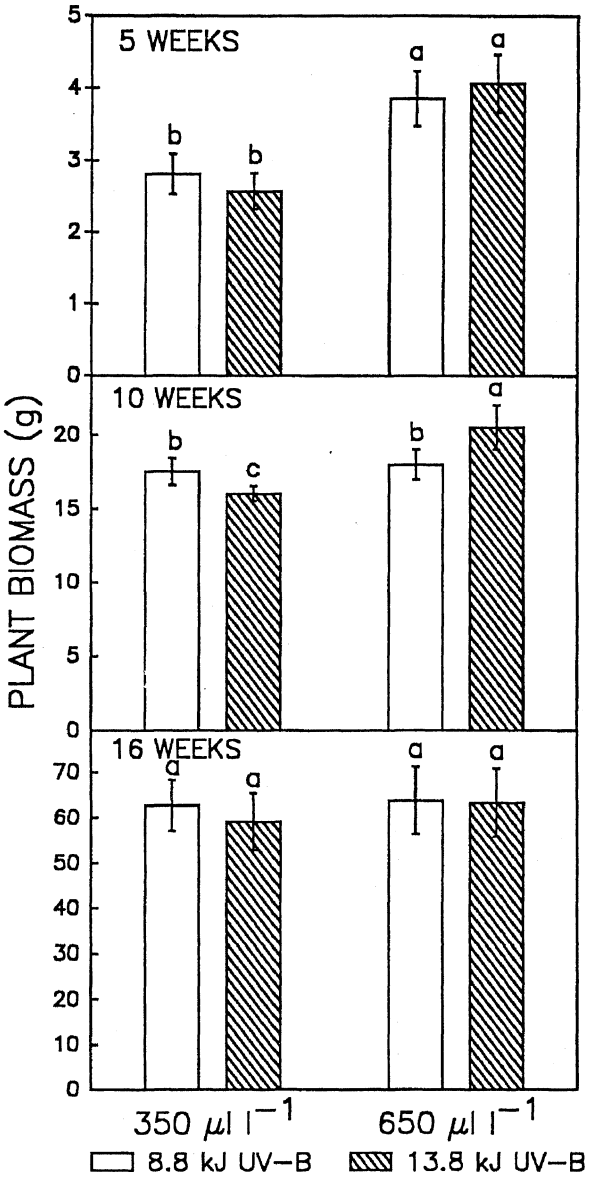

Figure 4. Changes in total vegetative biomass for rice seedlings grown to maturity in a factorial study with 2 CO_2 (350 and 650 μl l^{-1}) and 2 UV-B (8.8 or 13.8 kJ m^{-2} d^{-1}) radiation levels. Three harvests were made during the growing period and biomass was measured after oven drying. Each bar represents the mean of 15 plants ±S.E. Bars denoted by the same letter within a panel are not significantly different ($P < 0.05$) according to Student Newman-Keuls multiple range test. Additional details of experimental design may be found in Teramura et al. (1990).

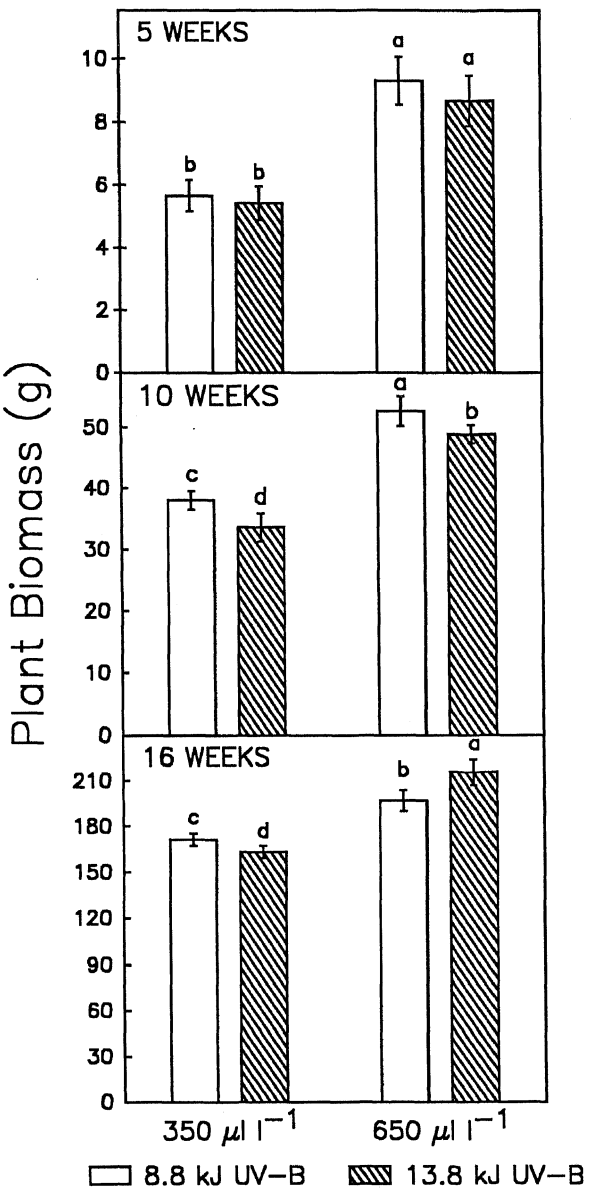

Figure 5. Changes in total vegetative biomass for soybean seedlings grown to maturity in a factorial study with 2 CO_2 (350 and 650 μl l^{-1}) and 2 UV-B (8.8 or 13.8 kJ m^{-2} d^{-1}) radiation levels. Three harvests were made during the growing period and biomass was measured after oven drying. Each bar represents the mean of 15 plants ± SE. Bars denoted by the same letter within a panel are not significantly different ($P < 0.05$) according to Student Newman-Keuls multiple range test. Additional details of experimental design may be found in Teramura et al. (1990).

spruce and Scots pine (Sullivan & Teramura 1988). Also leaf size and the rate of leaf development has been shown to be affected by UV-B radiation in both herbaceous and woody species (DeLucia et al. 1994; Dickson & Caldwell 1978; Dillenburg et al. 1995; Lindoo & Caldwell 1978; Murali et al. 1988; Searles et al. 1995; Sisson & Caldwell 1976).

It was hypothesized that a primary cause of growth reduction by UV-B radiation in loblolly pine is a reduction in needle size (Sullivan 1994; Sullivan et al. 1996). If UV-B radiation exerts a direct effect on leaf size as hypothesized above, then shifts in biomass allocation might occur under elevated CO_2. Sullivan & Teramura (1994) reported a significant UV-B by CO_2 interaction on biomass allocation in loblolly pine. In that study the root to shoot ratio was reduced by UV-B at ambient CO_2 levels but increased by UV-B radiation at the higher CO_2 concentrations. If UV-B radiation restricts needle size, perhaps through alterations in cell wall chemistry (e.g. Dale 1988; Liu & McClure 1995; Liu et al. 1995), then increasing the CO_2 supply or photosynthetic rate may not compensate for this effect. However, if sink strength is sufficient, then the increased photosynthetic carbon assimilation could favor root growth (Sullivan & Teramura 1994). They suggested that this possible uncoupling of the response to CO_2 enrichment between below and above ground components by alterations in needle growth by UV-B radiation would explain the interaction of UV-B radiation and CO_2 on biomass allocation. Specific studies designed to carefully monitor needle physiological and anatomical development under the combination of these factors and an understanding of the mechanisms responsible for reduced needle elongation would be needed in order to adequately test this hypothesis.

Ziska & Teramura (1992) also found an interaction between UV-B radiation and CO_2 on biomass allocation in rice. However, in that study RSR increased due to UV-B radiation at 350 $\mu l\ l^{-1}$ of CO_2 but decreased at the higher CO_2 concentration. This suggests that in rice, UV-B effects on leaf development may have been modified by increased CO_2 concentrations. Studies by Yakimchuk & Hoddinott (1994) on three boreal conifers failed to demonstrate any interactions on biomass accumulation. Therefore, the available literature suggests that biomass allocation may be altered in some species in response to UV-B radiation and CO_2 but that effects are species specific.

Implications for ecosystems

The obvious conclusion to be drawn from this paper is that our knowledge base is quite small where studies on the combined effects of UV-B radiation and CO_2 are concerned. Consequently, there is a great deal of uncertainty in any attempt to extrapolate findings to the ecosystem level. Clearly, the inter- and perhaps intraspecific variation observed in previous studies suggests that there is no universal response to UV-B and CO_2. It would be more surprising if there were such a response given our understanding of genetic diversity and the myriad of adaptations and acclimations present in the plant kingdom in response to environmental variation. Given the considerations above, however, some general hypotheses may be made with respect to ecosystem productivity and structure in a future environment of increased UV-B radiation and elevated CO_2.

First, the available data suggest that in most cases, the presence of high levels of UV-B radiation reduces the magnitude of the response to CO_2 enrichment. The modification of the CO_2 response could stem from photosynthetic limitations that are manifested in a similar fashion to CO_2 acclimation, from direct effects of UV-B radiation on growth or from other as yet unresolved mechanisms. Thus, even though increases in UV-B radiation may not reduce ecosystem productivity per se, such increases may place limitations on projected increases in productivity due to CO_2 enrichment.

A critical caveat to the above hypothesis is the expectation of a large degree of variation in response among species. This variation in response may itself lead to second order or indirect effects on ecosystem structure and function. For example, subtle changes in biomass allocation, such as those described in Sullivan & Teramura (1994) or Ziska & Teramura (1992), could have implications for water and nutrient relations and thus alter seedling establishment and competitive interactions in natural communities. Both CO_2 enhancement and UV-B radiation have individually been shown to alter competitive interactions in some species (e.g. Barnes et al. 1988; Carter & Peterson 1983; Gold & Caldwell 1983; Marks & Strain 1989; Zangerl & Bazzaz 1984). These changes in competition may be due to alteration in below ground resource allocation (e.g. altered root to shoot ratios) or through alterations in canopy structure and light interception (Ryel et al. 1990). These effects on competition have been observed in the absence of reductions in total productivity (Barnes et al. 1988, 1990).

Alteration in competition could influence successional dynamics, community makeup and potentially biodiversity. For example, results from previous studies (Carter & Peterson 1983; Zangerl & Bazzaz 1984, and others) have suggested that the lack of a response to CO_2 in C_4 plants could reduce their competitive ability against C_3 plants. However, in general, it would appear that C_3 plants are more susceptible to deleterious effects from UV-B radiation than are C_4 plants

(Van & Garrard 1976; Van de Staaij et al. 1990). Since no study that I am aware of has examined the effects of CO_2 enrichment on competition in the presence of natural or elevated levels of UV-B radiation, it is unclear whether or to what extent UV-B radiation might modify competition under elevated CO_2 concentrations. One hypothesis would be that if the response to CO_2 enrichment is limited by UV-B radiation in C_3 species, then the anticipated shift in competitive dominance of C_3 plants compared to C_4 plants under elevated CO_2 concentrations may not be as extensive as predicted by studies conducted in the absence of UV-B radiation.

In addition to alterations in community composition through effects on seedling establishment or competition, alterations in phenology or reproductive output could significantly affect ecosystem structure. Ziska & Teramura (1992) found that the number of panicles produced in one variety of rice was reduced by UV-B radiation and this reduction was independent of CO_2 concentration. Also Teramura et al. (1990) found that the stimulation of seed yield by elevated CO_2 was reduced in wheat and rice by UV-B radiation.

Elevated CO_2 may alter both vegetative and reproductive phenology in a variety of plant species (e.g. Reekie & Bazzaz 1991; Jarvis et al. 1994; Johnson & Seiler 1996). Also, UV-B radiation may alter both the magnitude of floral production (e.g. Tevini & Teramura 1989; Ziska et al. 1992) and the timing of leafing out and flower production (Rau et al. 1988; Staxen & Bornman 1994). Some recent studies (A. Visser et al., pers. comm.) have shown that UV-B radiation may alter the plastochron index and flower production under elevated CO_2 conditions. Changes in phenology could have indirect effects on growth, pollination and reproductive success. For example, early bud break in response to elevated CO_2 could predispose plants to frost damage (Jarvis et al. 1994).

No information is available with which to assess whether combined effects of UV-B radiation and CO_2 may alter interactions of terrestrial organisms of different trophic levels. However, Bothwell et al. (1994) demonstrated that differential sensitivity to UV-B radiation between algae and their herbivores led to a counter intuitive increases in algal productivity under UV-B enhancement. They concluded that ecosystem level responses could not be predicted by single trophic level assessments. Some data suggest that UV-B radiation and CO_2 may alter insect feeding. For UV-B radiation, this change could result from UV-induced changes in leaf chemistry (McCloud & Berenbaum 1994). Since CO_2 may also alter leaf chemistry and C:N ratios, it may also affect insect feeding (Lincoln et al. 1993).

Ecosystem processes such as decomposition or nutrient cycling may also be impacted by increases in UV-B radiation and CO_2. Some theoretical (Moorehead & Callaghan 1994) and experimental (Gehrke et al. 1995; Rozema et al. 1996b) studies have demonstrated that exposure to UV-B radiation may alter these processes, but no information is available on the combined effects of UV-B radiation and CO_2.

The direction and extent of effects of combined increases in UV-B radiation and CO_2 on trophic level interactions and ecosystem processes can only be speculated on at this time. Clearly such interactions could be important to issues of productivity, energy flow and species composition. Studies, conducted under realistic field conditions, that span trophic levels and that evaluate ecosystem processes are sorely needed in order to make more realistic estimates of the potential consequences of increases in UV-B radiation and CO_2 at higher levels of organization, such as communities and ecosystems. Further knowledge of how increased UV-B radiation might modify the effectiveness of CO_2 enrichment will be important to any estimation of the response of terrestrial ecosystems to future environmental change.

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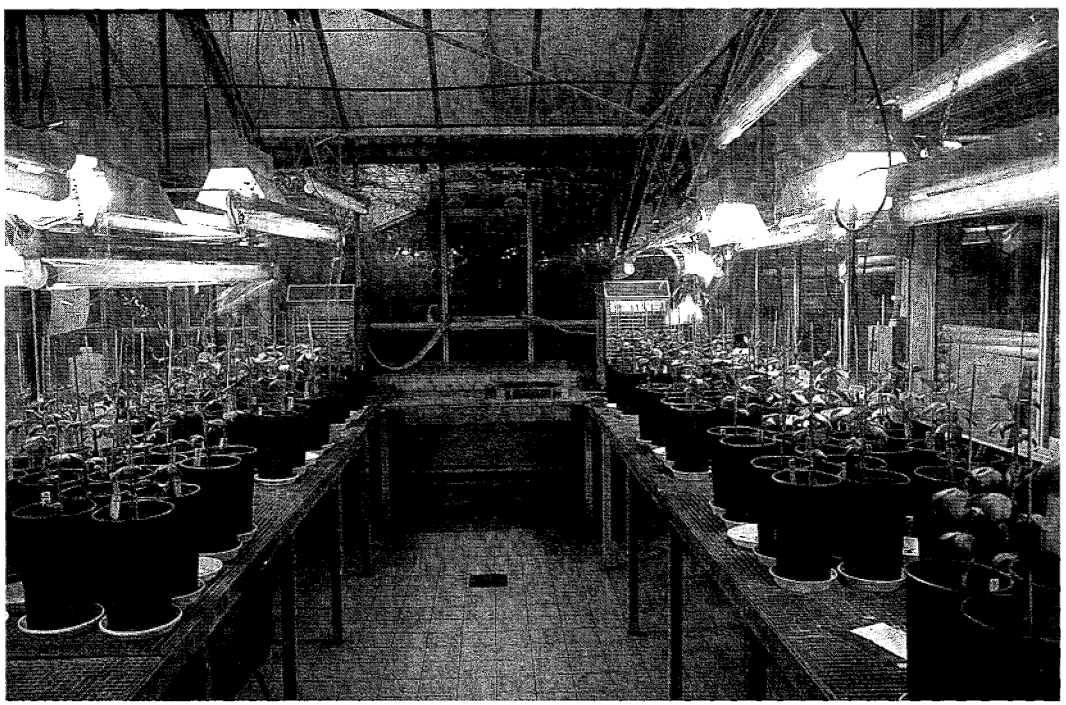

Faba bean (*Vicia faba*) grown at enhanced UV-B radiation and elevated CO_2. (Photograph: A. J. Visser)

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The combined effects of CO_2 concentration and enhanced UV-B radiation on faba bean. 3. Leaf optical properties, pigments, stomatal index and epidermal cell density

A. J. Visser[1], M. Tosserams[1], M. W. Groen[1], G. Kalis[1], R. Kwant[2], G. W. H. Magendans[1] & J. Rozema[1]

[1] *Department of Ecology and Ecotoxicology, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands;* [2] *Department of Plant Ecology and Evolutionary Biology, University of Utrecht, Utrecht, The Netherlands*

Key words: Chlorophyll, Elevated CO_2, Flavonoids, Leaf optical properties, Stomatal density, Stomatal index, UV-B radiation, *Vicia faba*

Abstract

Seedlings of *Vicia faba* L. (cv. Minica) were grown in a factorial experiment in a greenhouse. The purpose of the study was to determine whether CO_2 enrichment and supplemental UV-B radiation affect leaf optical properties and whether the combined effects differ from single factor effects. Seedlings were grown at either 380 μmol mol^{-1} or 750 μmol mol^{-1} CO_2 and at four levels of UV-B radiation. After 20 and 40 days of treatment, absorptance, transmittance and reflectance of photosynthetically active radiation (PAR) were measured on the youngest fully developed leaf. On the same leaf, the specific leaf area on a fresh weight basis (SLA_{fw}), chlorophyll content, UV-B absorbance, transmittance of UV light and stomatal index were measured. UV-B radiation significantly increased PAR absorptance and decreased PAR transmittance. The increased PAR absorptance can be explained by an increased chlorophyll content in response to UV-B radiation. Leaf transmittance of UV radiation decreased with increasing UV-B levels mainly caused by increased absorbance of UV absorbing compounds. UV-B radiation decreased both the stomatal density and epidermal cell density of the abaxial leaf surface, leaving the stomatal index unchanged. Effects of CO_2 enrichment were less pronounced than those of UV-B radiation. The most important CO_2 effect was an increase in stomatal density and epidermal cell density of the adaxial leaf surface. The stomatal index was not affected. No interaction between CO_2 and UV-B radiation was found. The results are discussed in relation to the internal light environment of the leaf.

Introduction

The amount of stratospheric ozone is being reduced due to anthropogenic emission of chlorofluorocarbons (CFCs) and nitrogen oxides (Molina & Rowland 1974; Rowland 1989). This reduction of ozone results in an increased flux of UV-B radiation reaching the earth's surface (Blumthaler & Ambach 1990). Under the current CFC phase-out schedules, global UV levels are predicted to peak around the year 2000 (Madronich et al. 1995). Simultaneously the atmospheric CO_2 concentration increases at a rate of 1.5 μmol mol^{-1} year^{-1} and will have doubled compared to pre-industrial levels around the year 2050 (Watson et al. 1990).

UV-B radiation and CO_2 enrichment have opposite effects on biomass production and yield in C3 plants; UV-B radiation in general decreases biomass production (Caldwell & Flint 1994; Tevini & Teramura 1989), whereas CO_2 enrichment results in an increase of biomass and yield (Kimball 1983; Poorter 1993). A decrease in specific leaf area (SLA), an indication for increased leaf thickness, is a common response to both supplemental UV-B radiation and CO_2 enrichment (Allen 1990; Teramura 1983). An increased leaf thickness may attenuate the deleterious

effects of UV-B radiation with the upper leaf areas acting as anatomical screens for sensitive underlying leaf areas (Teramura 1983). In this manner, CO_2 enrichment might provide protection against damaging UV-B radiation. Increases in leaf thickness can alter the distribution of photosynthetically active radiation (PAR) inside the leaf (Vogelmann 1994) and in this way affect photosynthesis (Bornman & Vogelmann 1991; DeLucia et al. 1996). The leaf internal light environment can be affected by other factors as well. According to Beer's law, absorbance of light is directly proportional to pigment concentration. Most pigments in plants are situated within organelles and not uniformly distributed within the tissue, which leads to a different absorption compared to a homogeneous distribution. This phenomenon is known as the sieve effect (Vogelmann 1994). Generally, UV-B absorbing compounds (e.g. flavonoids) increase in response to UV-B radiation (Caldwell et al. 1995; Tevini et al. 1991; Van de Staaij et al. 1995), but chlorophyll content and chlorophyll distribution in the leaf can change as well (Day & Vogelmann 1995; Teramura 1983). UV-B absorbing pigments may increase at elevated CO_2 (Sullivan & Teramura 1994; Visser et al. 1996) whereas chlorophyll content is often negatively affected (Cave et al. 1981; DeLucia et al. 1985). Steinmüller and Tevini (1985), showed that UV-B radiation increased the total amount of cuticular waxes of bean, barley and cucumber leaves by 25%. In contrast, Barnes et al. (1996) found reduced amounts of wax deposited on the adaxial side of the leaves of tobacco in response to UV-B radiation. Changes in amounts and in quality of cuticular waxes may affect the amount of light reflected from the leaf surface (Ehleringer 1981) and/or the quality of the reflected light (Mulroy 1979).

There is a large difference between the refractive indices of air (1.0) and plant cell walls (1.45). Consequently, the plant-air surface and the cell-intercellular interfaces form reflective boundaries (Vogelmann 1994). This means that if a treatment influences cell size or cell shape, the internal light environment might be altered as well. UV-B radiation stimulated cell-division in *Petunia hybrida* (Staxen & Bornman 1994) whereas the size and number of vascular xylem vessels decreased in *Vigna unguiculata* (Lingakumar & Kulandaivelu 1993). Furthermore, epidermal cells were severely damaged in cucumber when irradiated with UV-B. The number of stomata decreased, cell walls collapsed and cells became more flaccid (Tevini et al. 1983).

CO_2 enrichment can improve the water status of the plant through partial closure of the stomata (Morison 1993). As a result, a higher turgor pressure might stimulate leaf expansion (Lenssen & Rozema 1990). Indeed, Cure et al. (1987) reported increased expansion rates of soybean leaves. Woodward (1987) has shown that both stomatal density (SD) and stomatal index (SI) of leaves have decreased over the past 100 years, negatively correlated with increasing CO_2 levels. Ferris and Taylor (1994) observed increases in stomatal density in *Lotus corniculatus* but no effects on SI, indicating effects on cell division.

The effects of UV-B and CO_2 as described above, are sometimes in the same direction and sometimes in opposite direction, all influencing the internal light environment of the leaf. Combined effects may differ significantly from predicted changes based on a single factor assessment only (Teramura et al. 1990) and therefore interactions might occur. In general, the combined effects of UV-B radiation and CO_2 enrichment on biomass production have been found to be additive (Adamse & Britz 1992; Sullivan 1997; Van de Staaij et al. 1993). However, in some studies interactions on biomass allocation were found (Sullivan & Teramura 1994; Ziska & Teramura 1992). Although these subtle changes did not affect biomass under these experimental conditions, they may affect plant performance on an ecosystem level where for example light or nutrients are limiting (Sullivan 1997).

This study evaluates the combined effects of supplemental UV-B radiation and CO_2 enrichment on characteristics of faba bean leaves in relation to the internal light environment.

Material and methods

Growth conditions

Seeds of faba bean (*Vicia faba* L. cv. Minica) were sown in November 1993 in 4 dm^3 pots filled with commercial potting soil (Jongkind BV, Aalsmeer, The Netherlands) and cultivated in a greenhouse. The experimental conditions were as follows: light period, 14 h; temperature 22.5 ± 0.7 °C; relative humidity (RH) 62 ± 2%; night period, temperature 13 ± 0.5 °C; RH 82 ± 2%. Pots contained 3.5 g dm^{-3} Osmocote controlled release fertiliser (N:P:K:Mg:Fe, 13:13:13:3:2, Grace Sierra Int., Heerlen, the Netherlands). Following germination, seedlings were thinned to one per pot. Thirteen days after sowing, plants were divided

at random over eight groups of forty plants. Half of these groups were placed in a greenhouse compartment with an ambient CO_2 concentration (380 μmol mol^{-1}), while the remaining four groups were placed in a second greenhouse compartment with an elevated CO_2 concentration (750 μmol mol^{-1}). In both compartments plants received UV-B radiation which was artificially supplied by Philips 40 W/12 tubes. These tubes were attached at both sides of a 400W Philips HPI/T lamp providing 250 μmol m^{-2} s^{-1} additional PAR at canopy level. Maximal light intensity at plant level under clear sky conditions, measured with a Li-185b quantum sensor (LI-COR Inc., Lincoln, NE, USA), was 600 μmol m^{-2} s^{-1}. The UV light was filtered either with 0.10 mm thick cellulose acetate foil (Tamboer & Co. Chemie B.V., Haarlem, the Netherlands), which absorbs all radiation below 290 nm, or with 0.13 mm thick Mylar foil (Dupont Industries, USA), which absorbs all radiation below 313 nm for the control treatment. Mylar foil and cellulose acetate were replaced once and twice a week respectively. Plants received UV-B radiation during a six-hour period (10.00–16.00 hours). All UV-B treatments were carried out *in duplo* within each green house compartment and plants were rotated between duplicate treatments twice a week to minimise site effects within the greenhouse compartment. After two weeks of treatment plants were exchanged between green house compartments to avoid compartment effects. The spectral irradiance of the tubes was measured with an OL 752 Spectroradiometer (Optronics Laboratories, Orlando, FL, USA). The spectroradiometer was calibrated using the OL 752–150 dual calibration and gain check source module (Optronics Laboratories, Orlando, FL, USA). Biologically effective UV-B radiation (UV-B_{BE}) was calculated using the generalised plant action spectrum (Caldwell 1971), normalised at 300 nm. Four different UV-B treatments were applied with a weighted daily UV-B_{BE} dose of 0 for control plants and 4.6, 7.6 and 10.6 kJ m^{-2} day^{-1} for UV-B treated plants respectively. According to the model of Green et al. (1980), the UV-B_{BE} doses in the different UV-B treatments (excluding the control) simulated 0, 15 and 30% ozone reduction, respectively, during clear sky conditions on June 21 in Amsterdam (52°N). The long-term (1971–1993) mean stratospheric ozone thickness measured in June at the KMI in Ukkel (Belgium, 51°N) and the RIVM in Bilthoven (The Netherlands, 52°N), was used for the model calculations. The 4.6, 7.6 and 10.6 kJ m^{-2} day^{-1} UV-B treatments were obtained by adjusting the height of the UV tubes above the top of the plants. The UV tubes above the 0 kJ treatment were placed at the same height above the plants as those above the 7.6 kJ treatment. The absolute UV-A radiation emitted by the 400 W Philips HPI/T and the 40 W/12 UV-B lamps (excluding solar UV-A radiation) was 1.56 for control plants and 1.21, 1.56 and 1.91 W m^{-2} for the UV-B treated plants respectively.

Reflectance and absorptance measurements

The first leaflet of the youngest fully developed leaf was used for measurements of optical properties after 20 and 40 days of treatment respectively. The leaflet was detached from the plant and the fresh weight was determined. Immediately after this, the leaflet was placed in a LI-1800-12 integrated sphere connected to a LI-1800 spectroradiometer (LI-COR Inc., Lincoln, NE, USA). The reflectance (R) and transmittance (T) were measured with steps of 2 nm in the 400–700 nm range both at the adaxial and the abaxial surface of the leaflet. The adaxial transmittance and reflectance were always measured first. Absorptance (A) was calculated as $[A = 1 - R - T]$. Immediately after the measurements the area of the leaflet was measured using a Li-3100 leaf area meter (LI-COR Inc., Lincoln, NE, USA). In this way the specific leaf area on a fresh weight basis (SLA_{fw}, m^2 kg^{-1}) of the leaflet could be calculated.

Transmittance spectra (280–400 nm) were obtained according to the method of Tosserams and Rozema (1995) after 47 days of treatment. Intact, attached leaflets of the last fully developed leaf were clamped between two circular metal holders, which were horizontally attached on top of the optics head of an OL 752 spectroradiometer. In the middle of each metal holder an opening of 1 cm^2 allowed light emitted by four halogen lamps to pass through the exposed leaf area. Leaflets of plants grown in the 10.6 kJ treatment were not used for measurements because these leaves were severely damaged after 47 days of treatment.

Differences in leaf transmittance as induced by the various treatments were calculated by subtracting the transmittance of control leaves (380 μmol mol^{-1} CO_2, 0 kJ UV-B_{BE}) from those of leaves of the different treatments. Sensitivities were computed by dividing the transmittance of the different treatments by the transmittance of the control treatment (Carter et al. 1995).

Stomatal and epidermal cell counts

Prints of the adaxial and abaxial epidermis of the youngest fully developed leaf were obtained using transparent nailvarnish in combination with cellophane tape. Prints were made of the first leaflet of the youngest fully developed leaf. Of these prints, stomata and epidermal cells were counted under a phase contrast microscope under ×400 magnification. Four leaflets of each treatment were used for the counts and 5 replicate fields were counted on one leaflet. Stomatal density (SD) and epidermal cell density (CD) were calculated on a leaf area basis. Stomatal index (SI) was calculated as [SI = (SD/(SD + CD)) × 100].

Pigment analysis and statistics

Chlorophyll content and methanolic UV-B absorbance were measured on the leaflet used in the reflectance, transmittance and absorptance (RTA) measurements. The leaflets for pigment analysis were harvested at the same time as the RTA measurements were carried out. Samples for chlorophyll determination were weighed, frozen in liquid nitrogen and stored at −20 °C. Chlorophyll content was measured using the method of Arnon (1949). UV-B absorbing pigments were extracted from fresh leaf material with a MeOH:H_2O:HCl (79:20:1 v/v) solution. Samples were stored overnight (8°C) after which they were heated in a waterbath (90°C) for one hour. An absorbance scan (280–320 nm) of the solution was made with a Perkin-Elmer Lambda 15 UV/VIS scanning spectrophotometer. Absorbance differences and absorbance sensitivities were calculated in the same manner as described in the reflectance and absorptance measurements section.

The data were analysed using a two-way ANOVA (SYSTAT 5.0, Systat, Inc., USA). If necessary, percentages were $\sqrt{x}$ transformed before analysis to obtain homogeneity of variance (Gomez & Gomez 1984).

Results

UV-B radiation significantly affected the leaf optical properties 20 days after the start of the treatment (Table 1a). The largest effects were found when absorptance, transmittance and reflectance of PAR light were measured with the abaxial surface oriented towards the light source. Reflectance from the abaxial leaf surface decreased by 7% comparing the 10.6 kJ treatment with the control averaged over both CO_2 concentrations. Calculated in the same way, absorptance increased by 3% under the highest UV-B treatment. As a consequence, the transmittance decreased by 8% compared to the control treatments. Adaxial reflectance was not affected by the UV-B treatment. Adaxial absorptance increased slightly at the highest UV-B treatment (1%) compared to the control. Since transmittance of PAR light is a small amount of the total incoming PAR (±5%), this small increase in absorptance substantially decreased the adaxial transmittance by 12% when compared with the control treatment. The SLA_{fw} significantly decreased with increasing $UV\text{-}B_{BE}$ radiation (Table 1a), indicating that leaf thickness increased.

CO_2 enrichment only affected abaxial reflectance which decreased by 4% averaged over all UV-B treatments (Table 1a).

After 47 days of treatment differences in optical properties between treatments had disappeared (Table 1b). The absorptance of the highest UV-B treatment was still at the same level as on day 20, whereas the absorptance of the lower UV-B treatments had increased to the same level as in the highest UV-B treatment. The SLA_{fw} no longer differed between treatments on day 47. Comparing day 20 and day 47, the SLA_{fw} increased by 23% at the highest UV-B level. Since the optical properties at this UV-B level hardly changed, it seems that the thickness of the leaf did not alter the optical properties.

Chlorophyll content per unit leaf area significantly increased by 12% comparing the 10.6 kJ treatment with the control averaged over both CO_2 concentrations 20 days after the start of the treatment (Table 2a). Both chlorophyll *a* as chlorophyll *b* were affected in the same way, consequently no changes in the chlorophyll *a/b* ratio were observed. At this time CO_2 enrichment did not affect chlorophyll content. The situation was somewhat different 47 days after the start of the treatment (Table 2b). UV-B radiation still significantly affected the chlorophyll content, but in a different way, since the chlorophyll content did not increase gradually with increasing UV-B fluence as on day 20. The 0, 4.6 and 10.6 kJ treatments were the same, only the 7.6 kJ treatment showed a decreased chlorophyll content. CO_2 enrichment increased the chlorophyll content slightly at day 47.

Table 3 shows the transmittance of UV-B and UV-A light through intact attached leaves. At day 20, the transmittance of UV-B light was reduced by 72% at the 7.6 kJ treatment compared to the control (Table 3). The

Table 1. The effect of CO_2 and UV-B_{BE} on Transmittance (T), Reflectance (R) and Absorptance (A) of Photosynthetically Active Radiation (PAR) and the Specific Leaf Area (SLA, fresh weight basis). Measurements were conducted on the youngest fully developed leaf, 20 days after the start of the experiment (a), and 47 days after the start of the experiment (b). Values represent means of 5 replicate measurements. n.s. = not significant ($p > 0.05$)

CO_2 (μmol mol^{-1})	UV-B_{BE} (kJ m^{-2} day^{-1})	Abaxial surface			Adaxial surface			SLA (m^2 kg^{-1})
		T (%)	R (%)	A (%)	T (%)	R (%)	A (%)	
(a)								
380	0.0	5.50	17.96	76.54	5.07	10.29	84.63	4.33
380	4.6	4.35	17.61	77.99	4.43	10.20	85.37	3.97
380	7.6	3.92	18.27	77.80	3.86	10.21	85.93	3.76
380	10.6	4.78	16.84	79.05	4.46	9.79	85.75	3.74
750	0.0	5.21	17.39	77.40	5.03	10.42	84.54	4.36
750	4.6	4.51	17.38	78.11	4.58	10.01	85.42	4.07
750	7.6	4.23	17.21	78.55	4.12	9.85	86.03	3.89
750	10.6	5.08	16.08	78.84	4.52	10.14	85.34	3.66
CO_2		n.s.	0.006	n.s.	n.s.	n.s.	n.s.	n.s.
UV-B		0.009	0.001	0.008	0.024	n.s.	0.049	0.000
$CO_2 \times$ UV-B		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
(b)								
380	0.0	4.44	16.24	79.32	4.51	10.27	85.22	4.66
380	4.6	4.13	16.43	79.44	3.97	10.16	85.57	4.62
380	7.6	4.56	16.02	79.42	4.67	10.49	84.85	4.80
380	10.6	4.31	16.39	79.31	4.49	10.25	85.26	4.47
750	0.0	4.17	16.21	79.63	4.03	10.48	85.49	4.61
750	4.6	4.43	16.63	78.94	4.48	10.48	85.03	4.83
750	7.6	4.51	16.05	79.45	4.44	10.40	85.15	4.79
750	10.6	4.81	16.68	78.52	5.09	10.57	84.34	4.61
CO_2		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
UV-B		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
$CO_2 \times$ UV-B		n.s.	n.s.	n.s.	0.042	n.s.	n.s.	n.s.

transmittance of UV-A light decreased by 78% comparing the same treatments. The decrease in transmittance was not the same for all wavelengths measured. In the UV-B range, the transmittance of light between 280 and 290 nm did not change, whereas the largest decrease was observed between 300 and 320 nm (Figure 1a). In the UV-A range, changes in transmittance were not as pronounced, but still a larger decrease in transmittance towards the longer wavelengths for the UV-B treated plants was observed (Figure 1b). The sensitivity of the leaf for changes in transmittance did not change with wavelength (Figures 1c and 1d). No effects of UV-B radiation were observed on the transmittance of both UV-B and UV-A light after 47 days (Table 3). This can be explained by a decreased transmittance of both the 0 and 4.6 kJ treatments to the same level as the 7.6 kJ treatment. Leaf age was not a factor here since all youngest fully developed leaves used for these measurements had the same leaf number.

The UV-B absorbance of methanolic leaf extracts after 20 days of treatment significantly increased by 16% at the highest UV-B radiation level (Table 4). The highest absorbance however was observed in the 7.6 kJ treatment where the absorbance had increased by 29% compared to the control treatment. CO_2 enrichment did not affect UV-B absorbance of methanolic leaf extracts. At day 47, UV-B radiation still affected the UV-B absorbance, which had increased by 9% at the highest UV-B level (Table 4). At this time the highest UV-B treatment showed the highest UV-B absorbance.

Table 2. The effect of CO_2 and UV-B_{BE} on chlorophyll content. Measurements were conducted on the youngest fully developed leaf, 20 days after the start of the experiment (a), and 47 days after the start of the experiment (b). Values represent means of 5 replicate measurements, n.s. = not significant ($p > 0.05$)

CO_2 (μmol mol^{-1})	UV-B_{BE} (kJ m^{-2} day^{-1})	Chl *a* (mg m^{-2})	Chl *b* (mg m^{-2})	Total Chl (mg m^{-2})	Chl *a*/*b* (ratio)
(a)					
380	0.0	323.8	124.6	448.3	2.61
380	4.6	349.8	139.7	489.5	2.52
380	7.6	389.1	151.7	540.8	2.56
380	10.6	378.7	148.8	527.5	2.54
750	0.0	354.0	134.1	488.1	2.64
750	4.6	382.9	149.7	532.6	2.56
750	7.6	409.8	155.5	565.3	2.64
750	10.6	370.0	154.3	524.4	2.41
CO_2		n.s.	n.s.	n.s.	n.s.
UV-B		0.043	0.011	0.027	n.s.
$CO_2 \times$ UV-B		n.s.	n.s.	n.s.	n.s.
(b)					
380	0.0	366.6	151.3	517.9	2.45
380	4.6	375.3	154.0	529.2	2.46
380	7.6	320.1	122.8	442.9	2.61
380	10.6	362.4	154.9	517.3	2.35
750	0.0	417.9	171.7	589.6	2.47
750	4.6	386.2	158.4	544.5	2.48
750	7.6	340.2	137.4	477.6	2.47
750	10.6	404.9	178.1	583.0	2.29
CO_2		0.045	n.s.	0.045	n.s.
UV-B		0.029	0.030	0.022	n.s.
$CO_2 \times$ UV-B		n.s.	n.s.	n.s.	n.s.

Table 3. The effect of CO_2 and UV-B_{BE} on leaf transmittance of UV-B (280–320 nm) and UV-A (321–400 nm). Measurements were conducted on the youngest fully developed leaf. Values represent means of 5 replicate measurements; n.s. = not significant ($p > 0.05$)

CO_2 (μmol mol^{-1})	UV-B_{BE} (kJ m^{-2} day^{-1})	Leaf transmittance			
		day 20		day 47	
		(%)	(%)	(%)	(%)
380	0.0	0.31	0.56	0.07	0.20
380	4.6	0.14	0.27	0.05	0.11
380	7.6	0.07	0.12	0.06	0.12
750	0.0	0.18	0.39	0.08	0.20
750	4.6	0.14	0.23	0.05	0.08
750	7.6	0.07	0.09	0.05	0.11
CO_2		n.s.	n.s.	n.s.	n.s.
UV-B		0.000	0.000	n.s.	n.s.
$CO_2 \times$ UV-B		n.s.	n.s.	n.s.	n.s.

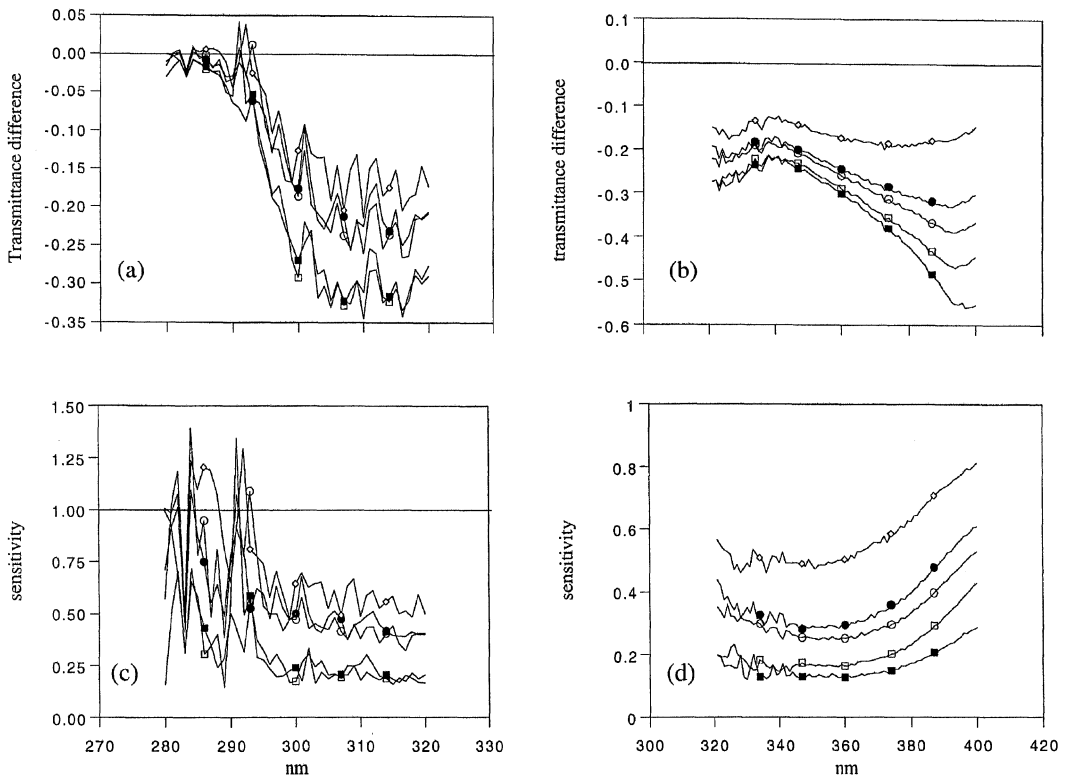

Figure 1. Differences in leaf transmittance of *Vicia faba* leaves for (**a**) UV-B (280–320 nm) and (**b**) UV-A (321–400 nm), and sensitivities of leaf transmittance of *Vicia faba* leaves for (**c**) UV-B and (**d**) UV-A. Differences were calculated by subtracting transmittance of control leaves (380 μmol mol^{-1} CO_2, 0 kJ UV-B_{BE}) from those of leaves of the different treatments. Sensitivities were computed by dividing the transmittance of the different treatments by the transmittance of the control treatment. Symbols do not represent actual measurements but are used to separate curves of different treatments. Closed symbols: ambient CO_2; open symbols: elevated CO_2; 0 kJ UV-B_{BE} (◇); 4.6 kJ UV-B_{BE} (○); 7.6 kJ UV-B_{BE} (□).

Table 4. The effect of CO_2 and UV-B_{BE} on the relative UV-B absorbance of methanolic leaf extracts of *Vicia faba*. The absorbance was calculated by integration of the absorbance of 1 mg fresh leaf per ml extraction solution between 280 and 320 nm. Measurements were conducted on the youngest fully developed leaf. Values represent means of 5 replicate measurements. n.s. = nog significant ($p > 0.05$)

CO_2 (μmol mol^{-1})	UV-B_{BE} (kJ m^{-2} day^{-1})	Relative UV-B absorbance	
		day 20	day 47
380	0.0	11.1	17.8
380	4.6	12.8	17.9
380	7.6	14.5	15.1
380	10.6	13.9	21.4
750	0.0	12.0	19.1
750	4.6	13.5	16.8
750	7.6	15.1	16.9
750	10.6	12.9	18.8
CO_2		n.s.	n.s.
UV-B		0.000	0.001
CO_2 × UV-B		n.s.	n.s.

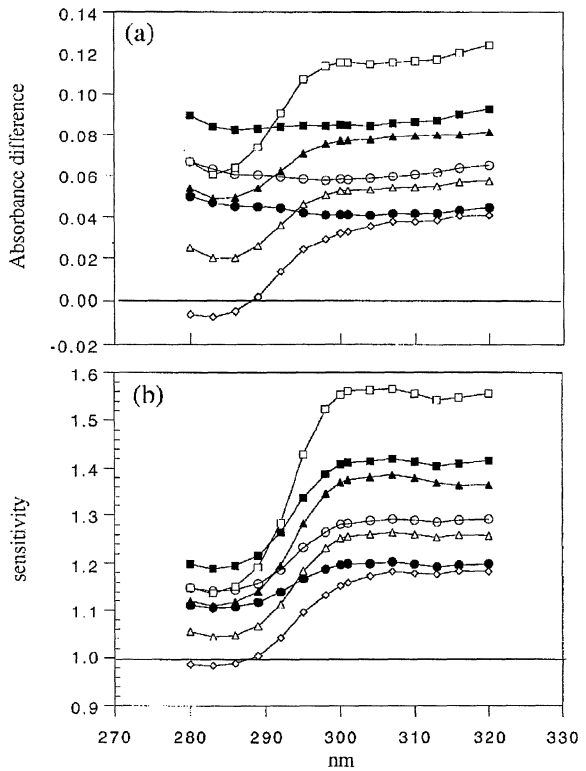

Figure 2. Differences in relative absorbance of methanolic leaf extracts of *Vicia faba* leaves (**a**) and sensitivities of relative absorbance of methanolic leaf extracts of *Vicia faba* leaves (**b**). Differences and sensitivities were computed in the same manner as explained in the legend of Figure 1. 10.6 kJ UV-B_{BE} (△). Other symbols as in Figure 1.

In general the UV-B absorbance at day 47 was higher than the absorbance at day 20. For example the absorbance of the control treatment was higher at day 47 compared to the absorbance of the 10.6 kJ treatment at day 20. The increase in UV-B absorbance was most pronounced in the 300–320 nm range (Figure 2a). This wavelength range showed to be the most sensitive for changes in UV-B fluence (Figure 2b). After 47 days, absorbance did not differ per wavelength nor did the sensitivity change for the different wavelengths (data not shown).

Leaf surface characteristics are presented in Table 5. A clear difference between the response of the adaxial leaf surface and the abaxial leaf surface can be seen. UV-B radiation only affected the abaxial leaf surface, where the total number of epidermal cells per unit leaf area decreased by 21% at the highest UV-B fluence. Because the number of stomata decreased to the same degree, the SI did not change. CO_2 enrichment did not affect the abaxial leaf surface significantly. In contrast, no significant UV-B effects were found on the adaxial leaf surface characteristics. CO_2 enrichment however, increased both the number of epidermal cells (11%) and stomata (14%) resulting in an unchanged SI.

Leaves grown at the 10.6 kJ treatment were strongly curled and showed bronzing (Figure 3).

Discussion

The effects of both CO_2 and UV-B on the leaf optical properties in the PAR range were the largest on the abaxial side. When new leaves of this cultivar emerge, the leaves are folded with the abaxial side oriented towards the light source. Only just before the leaves have reached their mature size, the leaves unfold and the adaxial side gets oriented towards the light source. In this way, the abaxial side experiences most of the UV-B radiation during cell-division.

Effects on PAR absorptance in response to UV-B radiation as found in this study, have been reported by other authors as well and seems to be species specific. In a solar UV-B exclusion experiment (Tosserams et al. 1996), PAR transmitted through intact attached leaves of *Plantago lanceolata* decreased in the presence of UV-B radiation in contrast to the increased transmittance in *Urtica dioica*. Bornman & Vogelmann (1991) observed an increased absorptance in *Brassica campestris*, but an increased transmittance in *Medicago sativa*.

The increase in absorptance in this study can largely be explained by an increase in chlorophyll content in response to UV-B radiation (Table 2a), as indicated by a significant correlation between absorptance and chlorophyll content on a leaf area basis ($r^2 = 0.46$; Figure 4a). UV-B radiation has been shown to decrease the chlorophyll content in some species (Teramura 1983). This decrease may be the result of repressed mRNA levels and gene expression for chloroplast proteins (Jordan et al. 1992, 1994). Although in most cases chlorophyll content decreases, increases in chlorophyll content have been reported as well (Ålenius et al. 1995). Teramura & Sullivan (1994) suggested that increased chlorophyll content expressed on a leaf area basis may partly explain increased photosynthesis as presented in some reports. In this study, this was probably not the case since light saturated photosynthesis decreased with increasing UV-B radiation and the 'apparent' light efficiency was unaffected, measured

Table 5. The effect of CO_2 and UV-B_{BE} on epidermal cell density (CD), stomatal density (SD) and sotmatal index (SI) of *Vicia faba* leaves. Measurements were conducted on the first leaflet of the youngest fully developed leaf, 40 days after the start of the experiment. Values represent means of 4 replicate measurements. n.s. = not significant ($p > 0.05$)

CO_2 (μmol mol^{-1})	UV-B_{BE} (kJ m^{-2} day^{-1})	Abaxial surface			Adaxial surface		
		CD (mm^{-2})	SD (mm^{-2})	SI (mm^{-2})	CD (mm^{-2})	SD (mm^{-2})	SI (mm^{-2})
380	0.0	507	110	22	479	81	17
380	4.6	470	103	22	447	75	17
380	7.6	475	107	23	481	77	16
380	10.6	408	87	22	420	76	18
750	0.0	522	126	24	505	91	18
750	4.6	555	130	24	517	97	19
750	7.6	473	110	23	508	91	18
750	10.6	404	88	22	495	74	15
CO_2		n.s.	n.s.	n.s.	0.039	0.043	n.s.
UV-B		0.001	0.005	n.s.	n.s.	n.s.	n.s.
$CO_2 \times$ UV-B		n.s.	n.s.	n.s.	n.s.	n.s.	0.017

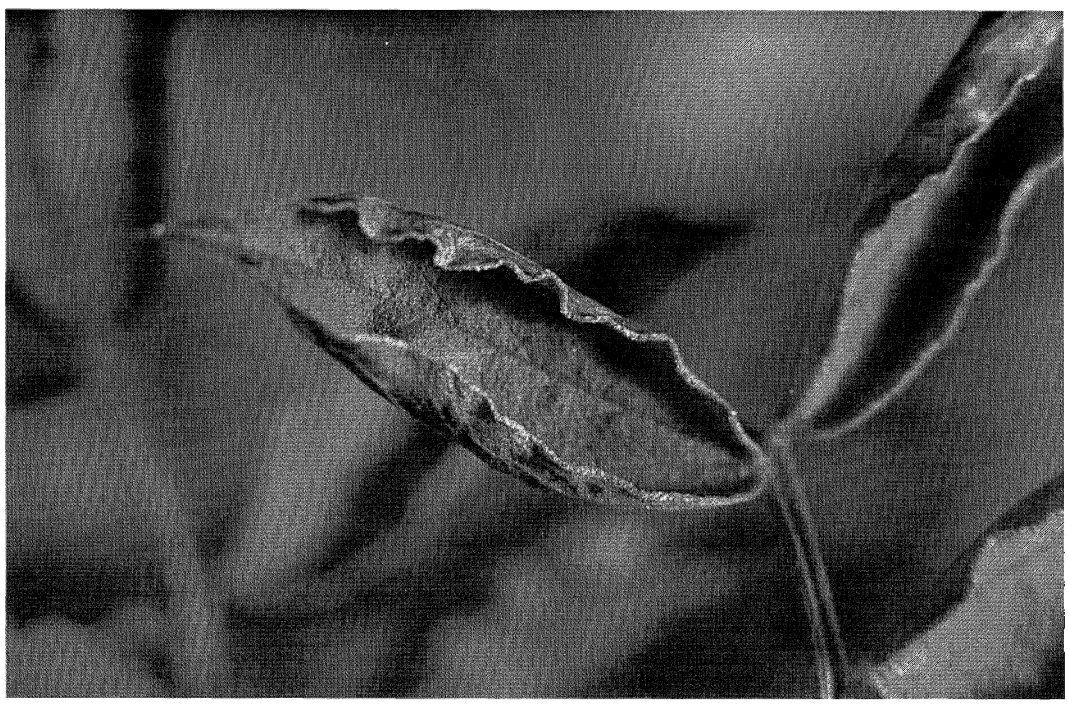

Figure 3. Photograph of the last fully expanded leaf of *Vicia faba* after 20 days of growth at the 10.6 kJ UV-B_{BE} treatment.

14 days after the start of the treatment (Visser et al. 1997). The chlorophyll *a/b* ratio did not differ between treatments in this experiment (Table 2). Effects of UV-B radiation on chlorophyll *a/b* ratio differ between studies, with both reports of increases (Vu et al. 1984) and decreases (Jordan et al. 1994; Vu et al. 1981).

The reflectance of PAR by the abaxial side of the leaf decreased (Table 1a). Reflected light comes either from the cuticle/air interface or from light that enters the mesophyll but is reflected by the numerous air/cell interfaces (Vogelmann 1993). We cannot make a distinction between these two origins of reflected light in this experiment since we measured whole leaf reflect-

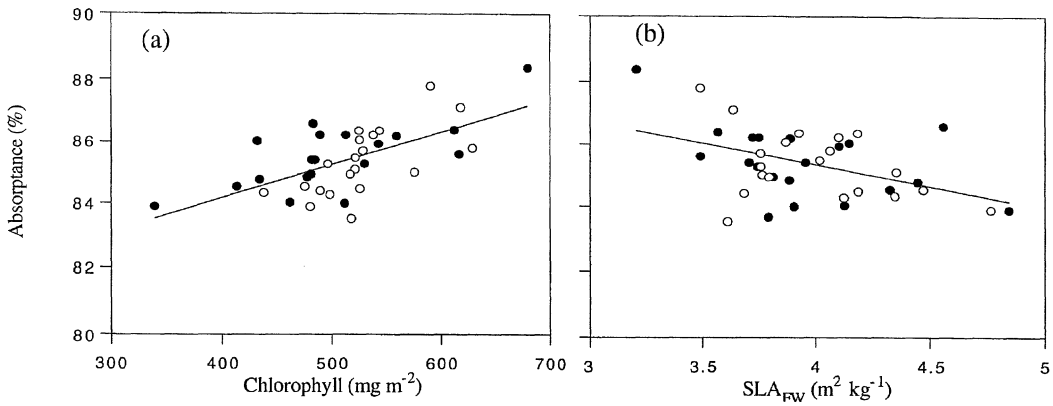

Figure 4. The absorptance of PAR as a function of (**a**) chlorophyll content and (**b**) the specific leaf area (fresh weight basis) of *Vicia faba* leaves. Measurements were performed after 20 days of treatment. The correlation coefficients for the ambient-grown and elevated-grown plants were tested for homogeneity. Since these correlation coefficients were the same, the data were pooled. Closed circles: ambient CO_2; open circles: elevated CO_2.

ance and we do not have information on the epidermal wax layer produced. Still some possible explanations for the decreased reflectance on the abaxial side can be given. Day & Vogelmann (1995) showed that the chlorophyll concentration differed at different depths in the leaves of *Pisum sativum*. In response to UV-B radiation the chlorophyll content was higher towards the abaxial side of the leaf, whereas in control plants the highest chlorophyll content was measured at the adaxial side. If something similar has occurred in this experiment, the lower reflectance at the abaxial surface might be caused by a higher absorptance by chlorophyll in the lower areas of the leaf. In this way simply the chance that light is being reflected by air/cell interfaces is lowered. Another explanation for the decreased reflectance might be the decreased number of cells per area on the abaxial leaf surface (Table 5). This means that cells were larger at the leaf surface in the high UV-B treatments and consequently, the chance that light was reflected by air/cell interfaces is lower. We did not make cross sections of the leaves so we do not know if the thickness of the epidermal layer had changed. The number of cells were counted on day 40, therefore we do not know if cell size on a leaf area basis really was affected at day 20. Besides that, the abaxial reflectance was not significantly altered at day 40 (Table 1b), therefore cell size probably was not important in respect to reflectance.

UV-B radiation significantly decreased the SLA_{fw} (Table 1a) after 20 days of treatment. A decrease in SLA, or an increase in leaf thickness is a common response of most plant species to UV-B radiation. In this manner upper leaf tissue layers might act as anatomical screens or filters to decrease UV-B transmittance to lower cell layers (Teramura 1983). One remark has to be made on the relationship between SLA and leaf thickness; SLA on a fresh weight basis (SLA_{fw}) gives an approximation for leaf thickness. The SLA on a dry weight basis should not be used as an estimate for leaf thickness, since the ratio between the dry weight and the fresh weight of leaves may vary (Dijkstra 1990). In accordance with this, Britz & Adamse (1994) showed that decreases in SLA on a dry weight basis in response to UV-B radiation in cucumber did not represent increases in leaf thickness. Murali et al. (1988) showed that the SLA decreased in the soybean cultivar Williams and not in the cultivar Essex. These differences in response were correlated with differences in sensitivity between these cultivars. However, decreases in SLA in response to UV-B radiation are not always correlated with UV-B radiation resistance as was demonstrated by Biggs & Kossuth (1978, cited in Teramura 1983). Also, the strong response of the SLA of the faba bean cultivar used in this study is not an indication for UV-B tolerance since biomass dramatically decreased with increasing UV-B radiation (Tosserams et al. 1997). In contrast, no effects of reduction of solar UV-B were observed on the SLA using the same cultivar grown in open top chambers (Visser et al. 1996). Bornman & Vogelmann (1991) reported an increase in leaf thickness in *Brassica campestris*. They also showed changes in the amounts and distribution of PAR by the use of fibre optics. In the present study a significant correlation between light absorption and

SLA_{fw} was found although the relationship was not very strong ($r^2 = 0.2$; Figure 4b). That this relationship is not very strong can also be concluded from the fact that the SLA_{fw} increased by 23% comparing the 10.6 kJ treatments of day 20 and day 47, whereas the optical properties at this UV-B radiation level did hardly change.

CO_2 enrichment had little effect on the optical properties of faba bean leaves (Table 1). In a previous study with the same cultivar grown in open top chambers, CO_2 enrichment significantly decreased PAR transmittance of intact attached leaves (Visser et al. 1996). In that study no differences in chlorophyll content on a fresh weight basis were found, but probably on an area basis chlorophyll content increased since the SLA (whole plant leaf area basis) decreased. A reduction in SLA in response to CO_2 enrichment is a common observation in CO_2 research and is often accompanied by increased levels of carbohydrates (Farrar & Williams 1991). In the present study, CO_2 enrichment did not affect the SLA although a small increase in total carbohydrates was found (Visser et al. 1997). One of the differences between the two experiments was the light intensity which was relatively low in the present experiment. Apparently at this relatively low light intensity, all additional carbon gained at elevated CO_2 can be used in growth, whereas in the open top chamber experiment part of the (larger) surplus of carbon is stored in the leaves.

Publications on effects of CO_2 enrichment on optical properties are limited and those available discuss very different species. For example, Carter et al. (1995) showed changes in the optical properties in the PAR range of *Liriodendron tulipifera* with a different sensitivity at individual wavelengths and Nobel et al. (1994) observed an increased reflectance of PAR in the CAM species *Opuntia ficus-indica*.

A small percentage of the incoming UV-B and UV-A light was transmitted through the leaves of this faba bean cultivar (Table 3). Although this transmittance was very low, it means that UV radiation could penetrate into the mesophyll. Not all UV radiation was absorbed inside the leaf which might be explained by the fact that not all the leaf tissues absorb in the UV range. Day et al. (1993) showed by the use of film fluorescence that transmittance of UV-B was much greater in anticlinal cell wall regions compared to chloroplasts. Transmittance was high through stomatal pores and low through guard cells. The epidermal layer in most plant species is an effective screen for the underlying leaf tissues since the epidermis transmits generally less than 10% of the UV radiation (Gausman et al. 1975; Robberecht & Caldwell 1978). Species differ in epidermal transmittance with species originating from the lower latitudes showing a lower transmittance than species originating from higher latitudes (Robberecht et al. 1980). Furthermore, epidermal transmittance varies among different plant species with dicotyledons possessing the highest transmittance and conifers the lowest (Day et al. 1992). Plants treated with additional UV-B radiation generally exhibit decreased epidermal transmittance of UV-B light and increased levels of UV-B absorbing compounds (Caldwell et al. 1983, 1995). An exception on the relationship between UV-B absorbing compounds and transmittance of UV-B light is described by Van de Staaij et al. (1995), who found increased levels of UV-B absorbing compounds at high UV-B radiation but no effects on transmittance of UV-B light in *Silene vulgaris*. The transmittance of both UV-B and UV-A light in this study decreased significantly in response to UV-B radiation 20 days after the start of the treatment (Table 3). Simultaneously the absorbance of methanolic leaf extracts increased at higher UV-B levels (Table 4). The fact that after 47 days of treatment no differences in UV transmittance between treatments were observed (Table 3), can be explained by the increased absorbance of methanolic leaf extracts (Table 4). Apparently, the UV-B absorbing capacity of the leaves of this cultivar increases in time since the absorbance in the 0 kJ treatment increased as well. UV transmittance was hardly influenced by leaf thickness since the SLA_{fw} of the 0 kJ treatment showed only small variation in time and the SLA_{fw} was higher at day 47 at all treatments (Table 1), suggesting thinner leaves. The decrease in UV transmittance in response to UV-B radiation was larger towards the longer wavelengths (Figure 1a). This can easily be explained by the fact that the UV-B radiation in the shorter wavelength range is almost entirely absorbed in the control treatment. The sensitivity of the leaf for UV transmittance shows that the UV transmittance changed to the same extent for all wavelengths (Figure 1c). In the methanolic leaf extracts the same patterns can be found with larger increases in absorption towards the longer wavelengths (Figure 2a). There was a discrepancy in sensitivity between transmittance and absorbance however, since absorbance in the longer wavelengths was proportionately more affected (Figure 2b). This means that UV-B absorbing pigments alone were not responsible for the observed effects of UV-B radiation on transmittance through intact leaves. In the same cultivar grown outdoors in open top chambers, no effects of UV-B radi-

ation on UV transmittance and absorbance were found (Visser et al. 1996). The relative absorbance of methanolic leaf extracts in the open top chamber grown plants was nearly twice as high. Probably the higher light intensity ($\pm$1800 μE m^{-2} s^{-1}) induces flavonoid synthesis and the small differences in UV-B($\pm$3.2 kJ $UV\text{-}B_{BE}$) in that experiment only contributed an insignificant part. Flint et al. (1985) showed that in field grown *Vicia faba* (cv. Broad Windsor), the flavonoid content increased in response to supplemental UV-B radiation, indicating that receptors for flavonoid synthesis are not saturated by solar radiation in the field. The latter is in agreement with results obtained in the OTC experiment since CO_2 enrichment increased UV-B absorbance.

CO_2 enrichment did not affect transmittance and absorbance of UV light in the present experiment. We think that additional flavonoids are formed in response to CO_2 enrichment when all available sinks for growth are saturated. Flavonoid synthesis probably acts as a kind of overflow system when carbon is in excess (Lambers 1993), and explains why under the relatively low light intensity in this experiment, effects of CO_2 enrichment on absorbance were absent.

UV-B radiation decreased both the stomatal and epidermal cell density on the abaxial surface after 20 days of treatment resulting in an unchanged SI (Table 5). Both decreases (Dai et al. 1995; Tevini et al. 1983), and increases (Stewart & Hoddinott 1993) in stomatal density have been reported. Staxen & Bornman (1994) found no changes in the number of stomata, but the SI decreased on the adaxial leaf surface in *Petunia hybrida*. The individual leaf area in the present experiment was significantly lower in the UV-B treated plants (Tosserams et al. 1997) resulting in a decreased total number of stomata and epidermal cells. This means that both the stomatal initiation and the epidermal cell division were negatively affected. The lower cell density indicates that epidermal cells were larger. Possibly because cell division was slowed down, cell expansion could continue for a longer period.

CO_2 effects were opposite to UV-B effects in this study. The increased stomatal and epidermal cell density with unchanged SI together with an increased individual leaf area (Tosserams et al. 1997), indicates positive effects on stomatal initiation and cell division. Effects of CO_2 enrichment on leaf surface characteristics vary greatly between species. Ferris & Taylor (1994) showed for example in four native herbs both increases and decreases in SI, stomatal density, epidermal cell density and size depending on species. Radaglou & Jarvis (1993) on the other hand found no effects of CO_2 on leaf surface characteristics in *Vicia faba*. However, in general it seems that the SI decreases in response to CO_2 enrichment mainly through a reduction in stomatal density as shown by Woodward & Kelly (1995) in a survey of 100 species.

In summary, both CO_2 and UV-B affected leaf (optical) characteristics. Effects of CO_2 and UV-B did not interact, with UV-B effects being more pronounced than CO_2 effects. This experiment shows that UV-B radiation not only affects optical properties in the UV range, but in the PAR range as well. We do not know if the increased PAR absorptance caused by an increased chlorophyll content is still present when the leaves are older and situated lower in the vegetation. If so, especially in this light limiting environment small increases in PAR absorptance in response to UV-B radiation may influence plant growth. Subtle changes in absorbance of UV and PAR light, or in cell size and density in response to UV-B and CO_2 as in this experiment, may affect plant performance in a future high CO_2 and high UV-B world.

Acknowledgements

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Growth chambers covered with UV-transmitting plexiglass cuvettes designed for UV-B, temperature and CO_2 experiments performed in Portugal. By flooding one of the cuvettes with ozone, the ambient UV-B radiation of Portugal was reduced to UV-B levels presently found at Central European latitudes.

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Effects of solar ultraviolet-B radiation, temperature and CO_2 on growth and physiology of sunflower and maize seedlings

U. Mark & M. Tevini*
*Botanical Institute II, University of Karlsruhe, D- 76128 Karlsruhe, Germany (*author for correspondence)*

Key words: CO_2, *Helianthus annuus*, Photosynthesis, Pigments, Temperature, Transpiration, UV-B radiation, *Zea Mays*

Abstract

The effects of solar UV-B radiation, in combination with elevated temperature (4 °C) and CO_2 (680 μL L^{-1}) concentration, on sunflower and maize seedlings were studied from May to August in 1991 at the research station Quinta de São Pedro in Portugal (38.7°N). The ambient solar radiation of Portugal was reduced to levels of Central European latitudes by using the ozone filter technique. This radiation served as control, while the ambient solar radiation of Portugal was to simulate intense UV-B treatment (+30%). All plants were grown up to 18 days in 4 climate controlled growth chambers simulating a daily course of temperature with T_{max}=28 °C or 32 °C, resp., and ambient CO_2 concentrations (340 μL L^{-1}); in one chamber the CO_2 concentration was twice as high (680 μL L^{-1}).

Under intense UV-B and at 28 °C (T_{max}) all growth parameters (height, leaf area, fresh and dry weight, stem elongation rate, relative growth rate) of sunflower and maize seedlings were reduced down to 35% as compared to controls. An increase in growing temperature by 4 °C, alone or in combination with doubled CO_2, compensated or even overcompensated the UV-B effect so that the treated plants were comparable to controls. Chlorophyll content, on a leaf area basis, increased under intense UV-B radiation. This increase was compensated by lower leaf areas, resulting in comparable chlorophyll contents. Similar to growth, also the net photosynthetic rates of sunflower and maize seedlings were reduced down to 29% by intense UV-B calculated on a chlorophyll basis. This reduction was compensated by an increased temperature. Doubling of CO_2 concentration had effects only on sunflower seedlings in which the photosynthetic rates were higher than in the controls. Dark respiration rates of the seedlings were not influenced by any experimental condition. Transpiration and water use efficiency (wue) were not influenced by intense UV-B. Higher temperatures led to higher transpiration rates and lower water use efficiencies, resp.. Doubling of CO_2 reduced the transpiration rate drastically while for wue maximum values were recorded.

Introduction

There is now well documented evidence for the ongoing depletion of the stratospheric ozone layer and for an increase of trace gases responsible for the greenhouse effect (Bojkov 1995). Responsible gases are chlorofluoromethanes and CO_2, respectively. CO_2 concentration in the atmosphere may rise from ambient levels of about 340 μL L^{-1} up to about 700 μL L^{-1} between the years 2030 and 2100. As a consequence temperature increases between 1.5 °C and 5.5 °C are expected depending on different scenarios (Schneider 1992). Generally, an increase of CO_2 can have positive effects on net photosynthesis in C_3-plant species because photorespiration will be suppressed, a process not inherent to C_4-plants. However, long term experiments have shown that doubling of CO_2 increases photosynthesis not as high as short term experiments predict (Bazzaz & Fajer 1992). Elevated temperature increases growth and photosynthesis in all plants as long as their optimal temperatures have not well been exceeded (Warren-Wilson 1966). But there are also plant groups, which cannot adapt to higher temperatures (Berry & Björkmann 1980). Stratospheric ozone depletion will cause an increase of UV-B radiation on the earth's surface. During the antarctic

ozone hole, experiments in that area showed substantial decreases in phytoplankton biomass (Smith et al. 1992). Increased UV-B radiation has also damaging effects on growth and photosynthesis of UV-B sensitive terrestrial plants (Tevini & Teramura 1989). To date only a few studies exist combining elevated CO_2 and UV-B radiation, or CO_2 and temperature, but none in which all three environmental factors were combined (Eamus 1991; Kulandaivelu & Nedunchezhian 1993; Ziska & Teramura 1992). Therefore, this study should examine whether the deleterious effects of intense UV-B radiation on growth and photosynthesis can be ameliorated by elevated CO_2 and temperature.

Materials and methods

Plants and growing conditions

The experiments were carried out in 1991 at the research station Quinta de São Pedro in Portugal, about 10 km south of Lisbon (38.65°N; 9.11°W), where relatively high levels of ambient UV-B radiation are present during the summer season. Seeds of sunflower (*Helianthus annuus* L., cv. Polstar) and maize (*Zea mays* L., cv. Zenit 2000) were sown in trays (15×15 cm) on standard substrate TKS 1, each tray containing 25 seeds.

The trays were placed in 4 identical growth chambers covered with UV-transmitting plexiglass cuvettes. To avoid shadow effects on the plant material plants were taken only from the middle of the chambers with no shadow during the day. The experiments were conducted under solar radiation using the ozone filter cuvette technique. By flooding one of the cuvettes with ozone the ambient UV-B radiation of Portugal was reduced to UV-B levels presently found at central European latitudes (Tevini et al. 1990). Plants growing under the reduced UV-B conditions served as controls (–UV-B), which in the future might be subjected to 'enhanced' levels of UV-B. Plants grown in a 'not filtered' growth chamber received the intense UV-B radiation of Portugal which is, in comparison to the 'control radiation' relatively enhanced (+UV-B) by 30% (Mark & Tevini 1995; Tevini et al. 1990). In both chambers a daily course of air temperature and relative humidity ranging from 13.5 °C to 28 °C (T_{max}) and from 79% to 35%, respectively, were simulated. The additional two growth chambers were adapted to a parallel diurnal temperature and humidity course with a 4 °C increase in temperature resulting in a maximum temperature of 32 °C instead of 28 °C. CO_2 concentration in one of these growth chambers was doubled compared to all others containing ambient levels of about 340 μL L^{-1}. For doubling the CO_2 concentration a specially designed gas mixing system (Fa. Walz, 91090 Effeltrich, Germany) was used. The CO_2 concentration was continuously monitored by a infrared gas analyzer which regulates the pump systems for CO_2 and normal air according to the actual CO_2 concentration inside the chamber.

The radiation conditions, measured with an Optronics 742, are summarized in Table 1 and the spectral energy distribution in the UV-B range (280–320 nm) is given as an example in Figure 1.

Growth analysis

Growth parameters such as seedling height, leaf area, fresh weight, stem elongation, relative growth rate of sunflower and maize were measured after 13 and 18 days on above-ground plant material. Dry weight of this material was taken after drying it at 70 °C until constant weight readings were obtained.

Calculations of the specific growth parameters SE (stem elongation rate) and RGR (relative growth rate) followed the equations by Harper (1977).

Pigment analysis

Chlorophyll was extracted with acetone and calculated according to Lichtenthaler (1987). The chlorophyll content was expressed on a leaf area and a plant basis (all leaves).

Gas exchange measurements

Net photosynthesis was determined based on three different parameters: leaf area, chlorophyll content, and plant, as well as respiration and transpiration, the latter only based on leaf area and plant.

Determination of respiration was carried out at the beginning of each measurement in the dark. Plants were then irradiated with a 75 W cold-light lamp with 2800 μmol m^{-2} s^{-1} (PAR), which was sufficient for both species to reach their light saturation point. Actual measurements were made during the steady state of each plant after approximately 1 h of irradiation. In 13-day old sunflower seedlings the gas exchange measurements were performed with the entire plant (cotyledones and primary leaves), whereas in maize seedlings as well as in older sunflower seedlings only

Table 1. Mean fluence rates at noon and daily fluences inside and outside the growth chambers from May to August, 1991 in the UV-B (280–320 nm), UV-A (320–400 nm) and PAR (400–700 nm) measured at the research station 'Quinta de São Pedro' in Portugal (38.7° N, 9.1° W, appr. 100 m above s.l.)

	–UV-B	+UV-B	%	Outside
Fluence rates				
UV-B [W/m^2]	1.56±0.47	2.08±0.37	+33.3%	2.60±0.47
UV-A [W/m^2]	30.42±3.01	30.42±3.01		36.65±3.56
PAR [W/m^2]	317.6±26.5	317.5±26.5		362.1±31.3
Daily fluence				
UV-B [kJ/m^2]	36.54±11.51	47.14±10.02	+29.0%	61.75±13.64
UV-A [MJ/m^2]	0.91±0.13	0.91±0.13		1.10±0.15
PAR [MJ/m^2]	10.8±0.85	10.8±0.85		12.3±0,95

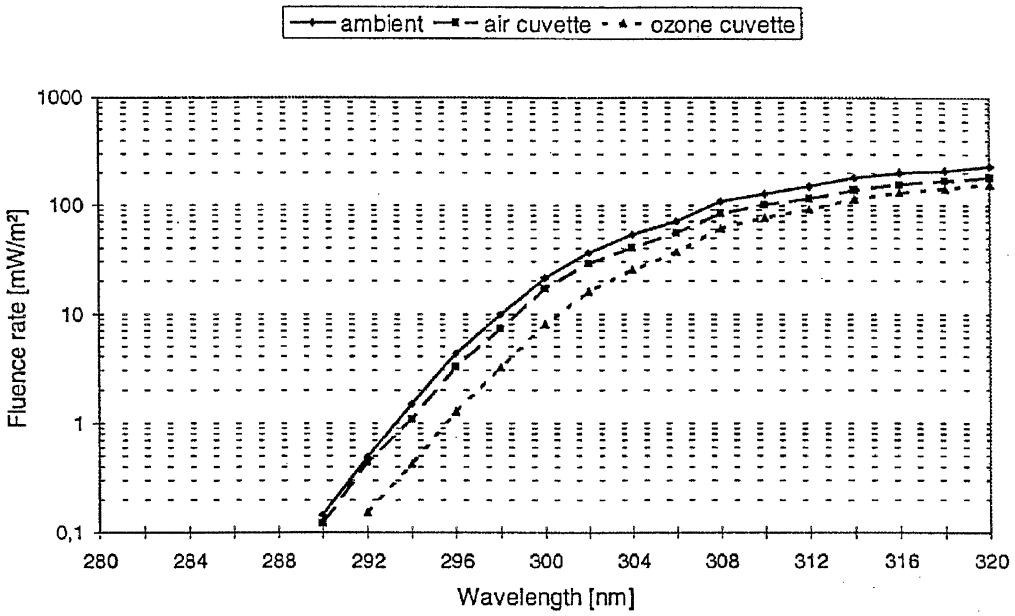

Figure 1. Spectral energy distribution in the UV-B region of ambient and reduced solar radiation in Portugal (38.7°N) measured on the 13th July 1991 at noon.

secondary leaves were used. At each developmental phase the gas exchange parameters were measured at 20 °C, 55% rH and appropriate CO_2-concentrations the plants were grown in.

All measurements were done in a minicuvette system (Fa. Walz, 91090 Effeltrich, Germany) and the results calculated according to von Caemmerer & Farquhar (1981).

Statistical analysis

Each experiment involved several repetitions so that the total number of plants used for estimating one parameter ranged from 40 to 75. The following results represent mean values and their respective standard deviations. An analysis of variance (ANOVA, Model I) was applied to qualify significant (α=5%) differences of the treatments (UV-B, temperature, CO_2). For the quantification of significant differences between two means the Fisher's LSD-test was used. Prior to each test a Kolmogoroff-Smirnov-Adjustment test (KSA)

was made in order to determine if an unknown, given distribution $F(x)$ would correspond with a normal distribution $N(\mu, \delta)$. For all series a correspondence with the normal distribution could be seen; the independence of the series resulted from the experimental design.

Results

Growth

Increased UV-B radiation of about 30% resulted in reductions of dry weight as well as shoot height and leaf area in sunflower and maize seedlings between 10% and 35% compared to controls (Tables 2 and 3). With increasing age the differences in most parameters became smaller between treated and control plants but remained significant.

Increasing the growing temperature by 4 °C overcompensated the UV-B effects and led to an increase of plant weight compared to controls. The shoot height increased only in maize seedlings whereas it remained unaffected in sunflower (Tables 2 and 3).

A doubling of CO_2 in addition to higher temperature did not further increase growth parameters except for biomass of sunflower seedlings (Tables 2 and 3).

Pigments

Chlorophyll content based on leaf area increased in both plant species irradiated with intense UV-B, whereas based on the entire plant (all leaves) no significant changes were measured (Tables 4a and 4b).

An elevation of temperature and CO_2 had no major effects on chlorophyll content based on leaf area. Furthermore, young seedlings (13 days old) exhibited enhanced chlorophyll contents per plant at these environmental growing conditions (Tables 4a and 4b).

Net photosynthesis

Net photosynthetic rates of sunflower and maize seedlings grown under enhanced UV-B were similar or slightly enhanced (12%) when based on leaf area and compared to controls. Since chlorophyll content did not change under intense UV-B, this parameter was used to demonstrate environmental effects on photosynthetic function. Net photosynthetic rates measured at light saturation decreased under enhanced UV-B by 8% to 29% depending on plant age and species when based on chlorophyll. These results were also confirmed by the net photosynthetic rates when expressed on a plant basis (Tables 5 and 6).

By elevating the daily maximum temperature from 28 °C to 32 °C photosynthetic rates increased compared to those in plants grown under intense UV-B at lower temperature and exceeded those of the control plants (Tables 5 and 6).

As expected a doubling of CO_2 concentration had no further stimulating effects on net photosynthesis of maize seedlings. In contrast the net photosynthetic rates of sunflower seedlings were increased up to 32% compared to seedlings grown under enhanced temperature, and up to 21% compared to controls (Tables 5 and 6).

Dark respiration, transpiration and water use efficiency (wue)

Dark respiration rates of sunflower and maize seedlings showed no significant differences at all growing conditions (data not shown) when calculated on a plant basis. On a leaf area basis only older plants grown under the intense UV-B level exhibited higher respiration rates than the control plants at lower UV-B (Tables 7 and 8).

Transpiration and wue of sunflower and maize seedlings were not affected by intense UV-B radiation, but were higher or lower, respectively, at elevated temperature. Doubling of CO_2 simultaneously applied with elevated UV-B and temperature led to lower transpiration rates based on leaf area, but higher rates based on plant. The wue was also increased under these conditions (Tables 7 and 8).

Discussion

The results clearly show that damaging effects of intense solar UV-B radiation on sunflower and maize seedlings grown at diurnal temperature courses with T_{max} of 28 °C can be compensated or surpassed by enhanced temperatures (T_{max} = 32°C) and/or doubling of CO_2 concentration (680 μL L^{-1}) in respect to growth, chlorophyll content and gas exchange.

Although these data were obtained with seedlings, they can be used as an indication about the possible results of adult plants. This is confirmed by experiments with *Zea mays* cv. Zenit 2000 grown until harvest in greenhouses under different UV-B regimes as well as in the above mentioned growth chambers (Mark et al. 1996). Despite the different growing conditions and

Table 2. Growth parameters of sunflower seedlings of different age grown at ambient and elevated levels of UV-B, temperature and CO_2. Means in the same row with different letters are significantly ($\alpha \leq 0.05$) different. A: +UV, T_{max}=28 °C, ambient CO_2; B: –UV, T_{max}=28 °C, ambient CO_2; C: +UV, T_{max}=32 °C, CO_2=680 μL L^{-1}; D: +UV, T_{max}=32 °C, ambient CO_2; RGR = relative growth rate; SE = stem elongation rate.

	A	B	C	D
13 days:				
dry weight (mg)	116.7a ±15.5	136.7b ±23.9	203.7c ±30.2	160.6d ±29.1
shoot height (cm)	6.26a ±0.85	8.64b ±1.74	8.32b ±1.19	8.42b ±1.82
leaf area (cm^2)	24.67a ±3.51	29.78b ±4.26	33.06c ±4.69	34.82c ±6.47
RGR (ln mg day^{-1})	0.37a ±0.01	0.38b ±0.01	0.41c ±0.01	0.39d ±0.01
SE (cm day^{-1})	0.48a ±0.07	0.66b ±0.13	0.64b ±0.09	0.65b ±0.14
18 days:				
dry weight (mg)	236.4a ±50.2	277.2b ±48.7	328.6c ±58.1	290.4bc ±59.3
shoot height (cm)	13.23a ±1.78	16.77b ±2.11	16.15b ±1.43	16.53b ±1.66
leaf area (cm^2)	31.00a ±8.07	34.34b ±6.69	35.42bc ±6.63	38.97c ±7.19
RGR (ln mg day^{-1})	0.14a ±0.04	0.14a ±0.04	0.09b ±0.05	0.12ab ±0.04
SE (cm day^{-1})	1.39a ±0.40	1.63b ±0.50	1.60b ±0.43	1.66b ±0.49

Table 3. Growth parameters of maize seedlings of different age grown at ambient and elevated levels of UV-B, temperature and CO_2. Means in the same row with different letters are significantly ($\alpha \leq 0.05$) different. A: +UV, T_{max}=28 °C, ambient CO_2; B: –UV, T_{max}=28 °C, ambient CO_2; C: +UV, T_{max}=32 °C, CO_2=680 μL L^{-1}; D: +UV, T_{max}=32 °C, ambient CO_2.

	A	B	C	D
13 days:				
dry weight (mg)	102.2a ±18.8	156.9b ±32.7	191.0c ±27.5	171.2bc ±45.1
shoot height (cm)	17.60a ±2.42	22.66b ±2.31	27.54c ±2.87	27.38c ±4.00
leaf area (cm^2)	33.94a ±7.19	47.82b ±8.89	60.03c ±13.07	58.74c ±14.33
RGR (ln mg day^{-1})	0.35a ±0.01	0.39b ±0.02	0.25c ±0.04	0.29d ±0.06
SE (cm day^{-1})	1.35a ±0.19	1.74b ±0.18	2.16c ±0.82	2.17c ±0.89
18 days:				
dry weight (mg)	221.0a ±38.6	289.1b ±67.6	382.3c ±96.7	379.1c ±101.5
shoot height (cm)	28.30a ±3.82	33.51b ±3.47	37.78c ±4.43	41.26d ±4.66
leaf area (cm^2)	65.01a ±12.53	83.42b ±13.33	87.76b ±18.16	88.70b ±20.90
RGR (ln mg day^{-1})	0.25a ±0.04	0.29b ±0.06	0.15c ±0.05	0.12c ±0.06
SE (cm day^{-1})	2.11a ±0.92	2.80b ±1.17	1.51c ±0.61	1.62c ±0.41

age the maize plants grown in greenhouses also exhibited growth reductions under intense UV-B, the extent, however, depends on the difference of UV-B scenarios simulated (Mark et al. 1996).

Growth

Sunflower and maize seedlings grown up to 18 d under 30% higher solar UV-B radiation at T_{max} of 28 °C exhibited significant reductions in shoot height and leaf area. Growth reductions are often found in UV-sensitive plant species in greenhouses under enhanced UV-B radiation when artificial UV-B sources were used, or in the field when artificial UV-B radiation supplemented solar UV-B (Sullivan & Teramura 1988, 1990; Barnes et al. 1988; Teramura et al. 1990a, b). The mechanism for the reduction in stem elongation might be due to changes in the phytohormon levels, especially of indole acetic acid (IAA) which participates in elongation processes. In sunflower seedlings grown under artificial UV-B radiation and low white-light conditions a photooxidation of IAA was found

Table 4. Chlorophyll content (Chl.) of sunflower (a) and maize (b) seedlings of different age grown at ambient and elevated levels of UV-B, temperature and CO_2. Means in the same row with different letters are significantly ($\alpha \leq 0.05$) different. A: +UV, T_{max}=28 °C, ambient CO_2; B: –UV, T_{max}=28 °C, ambient CO_2; C: +UV, T_{max}=32 °C, CO_2=680 μL L^{-1}; D: +UV, T_{max}=32 °C, ambient CO_2.

(a)	A	B	C	D
13 days:				
Chl. (μg cm^{-2})	30.89a ±3.39	27.96b ±3.17	26.94b ±3.34	31.33a ±2.49
Chl. (mg $Plant^{-1}$)	720.0a ±123.4	787.1a ±182.3	815.1a ±205.2	971.6b ±254.0
18 days:				
Chl. (μg cm^{-2})	27.47a ±4.46	23.12b ±3.64	23.17b ±3.49	25.30ab ±1.79
Chl. (mg $Plant^{-1}$)	846.6a ±262.7	775.8a ±230.3	843.5a ±221.6	955.3a ±213.6
(b)				
13 days:				
Chl. (μg cm^{-2})	24.37a ±4.80	18.87b ±3.57	18.16b ±3.94	21.30ab ±3.76
Chl. (mg $Plant^{-1}$)	857.9a ±222.9	909.5a ±188.1	1173.1b ±416.5	1276.3b ±349.2
18 days:				
Chl. (μg cm^{-2})	17.13a ±2.23	14.82b ±3.28	13.36b ±3.24	15.32ab ±2.40
Chl. (mg $Plant^{-1}$)	1055.5a ±216.6	1046.3a ±324.5	984.8a ±191.8	1066.6a ±206.5

Table 5. Net photosynthetic (NP) rates of sunflower seedlings of different age grown at ambient and elevated levels of UV-B, temperature and CO_2. Means in the same row with different letters are significantly ($\alpha \leq 0.05$) different. A: +UV, T_{max}=28 °C, ambient CO_2; B: –UV, T_{max}=28 °C, ambient CO_2 C: +UV, T_{max}=32 °C, CO_2=680 μL L^{-1}; D: +UV, T_{max}=32 °C, ambient CO_2.

	A	B	C	D
13 days:				
NP (nmol CO_2 $cm^{-2}s^{-1}$)	1.79a ±0.27	1.92a ±0.32	1.89a ±0.20	1.78a ±0.21
NP (nmol CO_2 $Plant^{-1}$ s^{-1})	41.78a ±9.42	55.01b ±11.16	66.38c ±12.25	54.04b ±9.08
NP (μmol CO_2 g^{-1} $Chl.^{-1}$ s^{-1})	54.29a ±10.54	62.87b±8.63	75.67c ±10.00	57.27ab ±7.84
18 days:				
NP (nmol CO_2 cm^{-2} s^{-1})	1.73a ±0.30	1.55b ±0.21	1.54b ±0.12	1.46b ±0.16
NP (nmol CO_2 $Plant^{-1}$ s^{-1})	46.06a ±6.86	50.98b ±3.14	55.63b ±5.59	50.64ab ±7.97
NP (μmol CO_2 g^{-1} $Chl.^{-1}$ s^{-1})	57.93a ±10.96	71.98b±7.16	72.30b ±9.63	55.91a ±6.71

(Ros & Tevini 1995). Additionally, the UV-induced activation of peroxidases may oxidize IAA (Ros 1990). It is supposed that the same mechanisms take place also under solar UV-B radiation. The reduction in leaf area is also caused by a reduction in cell length (Wolf 1988) and in addition by a change in leaf structure to smaller, but thicker leaves (Tevini et al. 1983). Since net photosynthesis of plant seedlings is reduced under higher UV-B, lower supply of sugars for cell wall growth might also be involved in growth reductions.

In both plant species the temperature increase of 4 °C resulted in higher absolute values of growth parameters, such as shoot height, dry matter and leaf area. However, in maize seedlings the temperature effect was more pronounced than in sunflower seedlings. Growth increases by rising temperature up to the optimum for photosynthesis are well known in C_3 and C_4 species (Auld et al. 1978; Johnson & Thornley 1985). Tollenaar (1989) observed a 300% (200%) increase of growth rate (net photosynthetic rate) in maize. Similar stimulations by temperature are known for potato and ryegrass (Wooledge & Parsons 1986). Generally, growth stimulation is being discussed as an effect of increased photosynthetic rates according to Q_{10} values, which are

Table 6. Net photosynthetic rates (NP) of maize seedlings of different age grown at ambient and elevated levels of UV-B, temperature and CO_2. Means in the same row with different letters are significantly ($\alpha \leq 0.05$) different. A: +UV, T_{max}=28 °C, ambient CO_2; B: -UV, T_{max}=28 °C, ambient CO_2; C: +UV, T_{max}=32 °C, CO_2=680 μL L^{-1}; D: +UV, T_{max}=32 °C, ambient CO_2.

	A	B	C	D
13 days:				
NP (nmol CO_2 cm^{-2} s^{-1})	1.52a ±0.35	1.42a ±0.24	1.15b ±0.26	1.40ab ±0.26
NP (nmol CO_2 Plant s^{-1})	46.57a ±7.35	53.85b ±8.25	71.62c ±11.74	71.53c ±11.42
NP (μmol CO_2 g^{-1} Chl.$^{-1}$ s^{-1})	60.18a ±5.28	65.42b ±3.48	61.97ab ±9.17	61.47ab ±6.19
18 days:				
NP (nmol CO_2 cm^{-2} s^{-1})	0.90a ±0.22	0.94a ±0.20	0.62b ±0.21	0.76ab ±0.21
NP (nmol CO_2 Plant^{-1} s^{-1})	57.33a ±10.48	72.29b ±21.55	68.38b ±13.96	70.55b ±8.64
NP (μmol CO_2 g^{-1} Chl.$^{-1}$ s^{-1})	50.45a ±8.98	70.96b ±13.30	71.12b ±13.27	67.86b ±9.28

Table 7. Respiration (RR), transpiration (TR) rates and water use efficiency (wue) of sunflower seedlings of different age grown at ambient and elevated levels of UV-B, temperature and CO_2. Means in the same row with different letters are significantly ($\alpha \leq 0.05$) different. A: +UV, T_{max}=28 °C, ambient CO_2; B: -UV, T_{max}=28 °C, ambient CO_2; C: +UV, T_{max}=32 °C, CO_2=680 μL L^{-1}; D: +UV, T_{max}=32 °C, ambient CO_2.

	A	B	C	D
13 days:				
RR (nmol CO_2 cm^{-2} s^{-1})	0.15a ±0.03	0.14a ±0.02	0.15a ±0.02	0.10b ±0.02
TR (μmol H_2O cm^{-2} s^{-1})	0.26ab ±0.03	0.25ab ±0.05	0.23a ±0.04	0.29b ±0.03
TR (mmol H_2O Plant^{-1} s^{-1})	6.23a ±1.80	7.46ab ±2.60	8.14b ±2.13	8.67b ±1.11
wue	7.21a ±2.29	7.92ab ±2.14	8.36b ±1.06	6.22c ±0.59
18 days:				
RR (nmol CO_2 cm^{-2} s^{-1})	0.11a ±0.02	0.09b ±0.02	0.09b ±0.02	0.09b ±0.02
TR (μmol H_2O cm^{-2} s^{-1})	0.26a ±0.06	0.23ab ±0.03	0.19b ±0.06	0.21b ±0.10
TR (mmol H_2O Plant^{-1} s^{-1})	7.15a ±2.27	7.65a ±0.78	6.87a ±2.27	7.16a ±1.56
wue	7.01a ±2.37	6.77a ±2.63	10.03b ±3.19	7.54a ±2.62

Table 8. Respiration (RR), transpiration (TR) rates and water use efficiency (wue) of maize seedlings of different age grown at ambient and elevated levels of UV-B, temperature and CO_2. Means in the same row with different letters are significantly ($\alpha \leq 0.05$) different. A: +UV, T_{max}=28 °C, ambient CO_2; B: –UV, T_{max}=28 °C, ambient CO_2; C: +UV, T_{max}=32 °C, CO_2=680 μL L^{-1}; D: +UV, T_{max}=32 °C, ambient CO_2.

	A	B	C	D
13 days:				
RR (nmol CO_2 cm^{-2} s^{-1})	0.08a ±0.03	0.08ab ±0.03	0.06b ±0.04	0.07ab ±0.02
TR (μmol H_2O cm^{-2} s^{-1})	0.14a ±0.04	0.13a ±0.02	0.08b ±0.03	0.18c ±0.05
TR (mmol H_2O Plant^{-1} s^{-1})	4.28a ±0.93	4.92a ±1.46	5.27a ±1.67	9.45b ±3.09
wue	11.33a ±3.16	11.48a ±2.41	14.81b ±4.88	8.24c ±2.57
18 days:				
RR (nmol CO_2 cm^{-2} s^{-1})	0.07a ±0.02	0.06b ±0.01	0.05b ±0.01	0.06ab ±0.03
TR (μmol H_2O cm^{-2} s^{-1})	0.10a ±0.02	0.10a ±0.02	0.05b ±0.02	0.12c ±0.04
TR (mmol H_2O Plant^{-1} s^{-1})	6.22a ±1.12	7.78a ±1.47	4.90b ±2.04	11.38c ±3.02
wue	9.44a ±1.84	9.49a ±1.56	14.74b ±5.98	6.56c ±1.82

commonly 2 to 3 over the range from 0 °C to 30 °C. This temperature dependent increase of photosynthetic rates was also demonstrated in this study.

Photorespiration in C_3-plants, such as sunflower, is reduced by enhancing CO_2 concentration and the net photosynthetic rates are increased. Therefore, further growth enhancement was expected at higher CO_2 levels, but this was not the case. Only the biomass accumulation and the net photosynthetic rates of sunflower seedlings were increased. This leads to the conclusion that an enhancement of CO_2 might have no effects on shoot height and leaf area corresponding to earlier findings of Nijs et al. (1988 a, b).

Dry above-ground biomass was suppressed by enhanced UV-B. The mechanisms for biomass reductions are not yet fully understood. On the one hand, decreases in overall photosynthesis were observed (Brandle et al. 1977; Sisson & Caldwell 1976) which is supported by our results, on the other hand, no effects on photosynthetic characteristics were found, e.g. in wheat (Beyschlag et al. 1988). In that case, morphological changes in the canopy structure with different light interception had been made responsible for biomass changes (Caldwell & Flint 1994). In sunflower and maize seedlings investigated here, the net photosynthesis per plant was substantially reduced under enhanced UV-B, which is virtually due to a lower total leaf area, since the net photosynthesis per leaf area had not significantly decreased.

Sunflower and maize seedlings grown under enhanced temperature regimes singly or in combination with doubled CO_2 concentration compensated or surpassed (sunflower) the reducing effects of UV-B. Since the net photosynthetic rates were simultaneously increased these findings indicate that the biomass accumulation is directly correlated to the photosynthetic capacity. Furthermore, the higher wue supports the biomass production. This was shown for wheat, radish, soybean and clover (Sionit et al. 1980; Idso 1988).

Chlorophyll content

The total chlorophyll content per plant was reduced, although the leaf area of sunflower and maize was smaller under intense UV-B. In contrast, the plants showed a higher chlorophyll content when based on leaf area. The same effect in combination with a thickening of the leaves was observed in different crop plants (Tevini et al. 1983). A significant influence of temperature and CO_2 on the chlorophyll content was not observed.

Gas exchange

As discussed earlier (Mark & Tevini 1995) the interpretation of UV-B effects on gas exchange is dependent on the parameter to which the data are related. Regarding the net photosynthetic rate based only on leaf area one would predict a stimulation of photosynthesis in sunflower, but not in maize. This would correspond to results cited in the literature (Beyschlag et al. 1988; Teramura et al. 1990a, b). However, comparing photosynthetic rates on a chlorophyll and plant basis the results indicate a major impact of UV-B on photosynthesis. This obvious contradiction is probably due to the different structure of the plant leaves grown under normal and enhanced UV-B (Tevini et al. 1983). The UV-B dependent decrease of photosynthesis may be attributed to the lower enzyme activities of ribulose bisphosphate carboxylase and phosphoenolpyruvat carboxylase and/or lower photosystem II activity. Both were found in different plant species (Vu et al. 1982; Bornman et al. 1984; Tevini & Pfister 1985), and especially in sunflower and maize under enhanced artificial UV-B radiation (Mark & Tevini 1996). An indirect effect of UV-B through an influence on transpiration, and therefore on internal CO_2 concentration was not observed in plants grown at higher UV-B.

Concerning the effects of increased temperature it was found that the net photosynthetic rates were mainly higher in sunflower and maize, which may have been caused by a general temperature induced stimulation of photosynthesis (Q_{10} values), which has not yet been fully investigated. Nevertheless, the UV-B effect was compensated in both plant species. This could be also due to the photorepair mechanism (Teramura 1980) and an increased accumulation of flavonoids, which are able to filter and reduce UV-B radiation (Tevini et al. 1991).

Increases in photosynthetic capacity as found here under enhanced CO_2 are well documented in the literature (Azcón-Bieto et al. 1994; Laisk & Sumberg 1994; Wullschleger et al. 1994; Ziska & Bunce 1994).

Major effects of UV-B radiation on respiration and transpiration have not been observed. In correspondence with previous results (Mark & Tevini 1995; Mirecki & Teramura 1984; Teramura et al. 1980) it is suggested that respiration and transpiration of sunflower and maize are insensitive to enhanced UV-B radiation. However, Teramura et al. (1983) found UV-effects on stomatal conductance of cucumber seedlings.

An increase of the diurnal temperature of 4 °C leads to an increase in transpiration, and this resulted

in a decrease of the wue. On the one hand, this might be due to a direct effect of temperature on stomatal opening. On the other hand, well watered plants regulate the stomate opening according to the relation of internal and external CO_2 which is held constant (von Caemmerer & Farquhar 1981). Since plants grown at enhanced diurnal temperature exhibit higher photosynthetic rates, the CO_2 uptake will be increased and this, in turn, decreases the stomatal resistance. Therefore, increasing transpiration consequently lowers the wue.

Doubling of the CO_2 concentration has contrasting effects. The transpiration rate is lowered, and the wue is higher compared to controls. Since the external CO_2 concentration is doubled the seedlings are able to increase the stomatal resistance without decreasing the internal CO_2 concentration to such an extent that the CO_2 concentration limits the photosynthetic capacity. Similar effects were found by Rozema et al. (1991) in *Scirpus olneyi* and *Spartina patens*.

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Combined effects of enhanced UV-B radiation and nitrogen deficiency on the growth, composition and photosynthesis of rye (*Secale cereale*)

Gaby Deckmyn & Ivan Impens
Laboratory of Plant Ecology, University of Antwerpen, Wilrijk, Belgium

Key words: Growth, Nitrogen deficiency, Photosynthesis, Pigmentation, Rye, UV-B Radiation

Abstract

The interactive effects of N-deficiency and enhanced UV-B radiation on growth, photosynthesis and pigmentation of rye were studied. The plants were grown for 5 weeks in growth chambers with high (700 μmol m^{-2} s^{-2}) irradiance levels. A 30% difference in UV-B at plant level was achieved by using different thicknesses of UV-B transparent Plexiglass. One half of the plants received optimal N nutrition, while the other received half of this dose. Both enhanced UV-B and N deficiency strongly decreased production (from 24–33%). The combined effect was additive (no interaction) on most parameters, including total dry weight production which was 52% lower than in the control series. Significant interaction was found on the root/shoot ratio. While reduced N supply induced an increase in the ratio at normal UV-B irradiation, under the increased UV-B, N deficiency had no effect on the root/shoot ratio. The reduced biomass due to UV-B was clearly correlated to a reduction in photosynthesis. At optimal N supply the plants increased the production of protective pigments in response to UV-B, but at reduced N supply this response was lacking. The increased N content of the high UV-B/high N plants could be a result of increased flavonoid production as well as changes in light penetration in the canopy.

Introduction

The destruction of the ozone layer by man-made chlorofluorocarbons, and the predicted increase in UV-B (280–320 nm, Blumthaler & Ambach 1990; Kerr 1993) at the earth/s surface have raised many questions concerning the effects on human health and plant growth. Many studies have proved the sensitivity of a wide variety of crop and wild species (reviews : Caldwell et al. 1989; Strid et al. 1994; Teramura & Sullivan 1994). Photosynthesis and biomass production are often reduced by enhanced UV-B levels. Some plants increase their resistance by an increased production of protective pigments (mainly flavonoids) and the development of thicker leaves. Low levels of photosynthetic active radiation (PAR) strongly increase the sensitivity of plants, which is important when interpreting growth chamber results.

Although N is a major limiting factor to plant growth, interactive effects of increased UV-B and N deficiency have not been studied before. As UV-B is known to influence the root/shoot ratio of plants there are some indications for interactive effects.

This study was conducted to investigate the effects of a 30% increase in weighted UV-B, under conditions of optimal and 50% reduced N supply. To ensure a high PAR in the growth chambers, high pressure metal halogenide lamps were used.

Materials and methods

Plant material and growth conditions

Rye plants (*Secale cereale* cv. Akkord) were grown from seed in 1.1L pots filled with normal potting soil and watered daily until harvest at 35 days of age. Every group contained 12 pots in which 10 seeds were planted and thinned to four plants per pot after 1 week. The pots were placed in a growth chamber with constant temperature (20 °C).

The light was provided by high pressure metal halogenide lamps (Höhnle SOL 500) in combination with Plexiglass filters of 3 and 5 mm (Imatex), resulting in a 30 % difference in UV-B_{BE} (weighted with the general plant action spectrum, Caldwell 1971, irradiation up to 313 nm). The plants received a daily dose of 417.2 J m^{-2} day^{-1} and 284.0 J m^{-2} day^{-1}, for the high and the normal UV-B treatment. The normal dose is comparable to the daily Dose in spring (Belgium, 50° N) and was measured with an Optronics OL-753 spectroradiometer. The PAR dose was 30.2 mol m^{-2} day^{-1}, for both UV-B treatments. The plants were irradiated 12 hours a day.

Fertiliser was added weekly. Half the plants received optimal N nutrition (8.33 g m^{-2} NH_4OH), the other half 50% of the optimal dose, but the same amount of P (6.25 g m^{-2} $-P_2O_5$) and K (9.75 g m^{-2} K_2O).

Measurements

Photosynthesis-light response curves were made with a portable infra-red gas analyser in an open circuit (ADC-LCA3) at 30 days of age from sowing, at 10–15 cm height from the pot. Net photosynthesis was recorded at 7 PAR irradiances (from 0 to 1400 μmol m^{-2} s^{-1}) and the relationship between net photosynthesis PN and PAR fitted to a rectangular hyperbolic curve (Ceulemans et al. 1980) resulting in 4 parameters (dark respiration DR, light compensation point I_0, apparent quantum efficiency QE and maximum photosynthesis P_{max}).

On the same leaves pigment concentrations were determined. Chlorophyll was extracted from 1 cm^2 sections (10–16 samples per group) in N,N-dimethylformamide and the concentration on a unit area basis of chlorophyll a and b calculated from the absorbance of the extract at 664 and 647 nm (Moran 1982).

UV-B absorbing pigments were extracted likewise from 1 cm^2 sections in a 1/79/20 mixture of HCl, water and methanol (Caldwell 1968). As the absorbence showed the largest difference at 300 nm, this wavelength was used as a measure for the protection offered by the pigments.

At 35 days of age the plants (48 in each treatment) were harvested. After measuring the total plant height, the plants were cut in 5 cm layers, and leaf area (measured with a portable area metre (LI 6000) and dry and fresh weight of the leaf parts from each layer recorded. The roots were washed and dried (48 h, 60 °C) and dry weight recorded. From these data specific leaf weight (SLW), leaf area ratio (LAR), dry/fresh weight ratio(D/F) and root/shoot ratio(R/S) were calculated.

All dried material was later used for N determinations. The dried material was finely ground and 3 samples of 50 mg from the mixed leaf layers and roots of each treatment (material from 48 mixed together) were analysed on total (organic + inorganic) N-content following the micro-Kjeldahl method (destruction with H_2SO_4, H_2O_2, $LiSO_4$ and Na-Selenite; spectrophotometric measurement at 410 nm after addition of Nesslers reagent, Isaac & Johnson 1976). From these results total N content of the plants and average leaf concentrations on an area basis were calculated.

All results were analysed with multiple analyses of variance (Manova, two factors : UV-B and N) and the significance of the single factors as well as the interaction between the factors calculated. To obtain information about the variance in N content between plants from the same treatment 4 series of measurements on samples from 6 different plants of the same treatment were analysed. The average standard error on the N determinations on a leaf area basis was 0.043.

Results

UV-B effects

Increased UV-B significantly reduced the growth of the rye plants (Table 1). Total dry weight was reduced by 24 (high N supply) and 25% (low N supply) respectively. Leaf area and dry weight were similarly reduced, so SLW was unaffected. The higher UV-B level resulted in shorter plants with an increased allocation to the lower leaf levels (Figures 1, 2). The R/S ratio was unaffected by UV-B at optimal N nutrition but reduced under N deficiency. LAR and D/F ratio were unaffected.

Maximum photosynthesis was significantly lower for the high UV-B irradiated plants, but QE, I_0 and dark respiration were unaffected (Table 2). Although there was no effect on total chlorophyll concentration, the ratio chlorophyll a/b was slightly, but not significantly, increased in response to UV-B (Table 3). Under optimal N supply, rye plants increased their flavonoid production with increasing UV-B, but this response was lacking in the plants suffering N deficiency (Table 3).

The average leaf N content (leaf area basis) of the high UV-B treated plants was higher: 1.1283 and 0.8748 mg cm^{-2} for the control and reduced N treat-

Table 1. Effects of enhanced UV-B and N deficiency on growth of rye plants. Averages of 48 plants are given and significant effects of the two factors as well as of the interaction (Manova, $p < 0.05$) are indicated in the last columns ($p < 0.05^*$, $p < 0.01^{**}$, n.s. = not significant)

	Control UV-B		High UV-B		UV	N	UV × N
	Control N	Low N	Control N	Low N			
Leaf area, cm^2	157.5	111.6	119.7	78.1	**	**	n.s.
Leaf fresh weight, g	4.712	3.163	3.509	2.243	**	**	n.s.
Leaf dry weight, g	1.042	0.708	0.774	0.543	**	**	n.s.
Root dry weight, g	1.112	0.763	0.756	0.563	**	**	n.s.
Total dry weight, g	2.144	1.467	1.530	1.022	**	**	n.s.
Dry/fresh weight	0.219	0.223	0.221	0.239	n.s.	n.s.	n.s.
Root/shoot weight	1.016	1.123	0.981	0.904	n.s.	*	**
SLW, g cm^{-2}	0.00664	0.00673	0.00654	0.00692	n.s.	n.s.	n.s.
LAR, cm^2 g^{-1}	74.67	77.82	78.38	76.84	n.s.	n.s.	n.s.
Height, cm	26.04	24.19	22.05	21.14	**	**	n.s.

Table 2. Effects of enhanced UV-B and N defficiency on parameters of the photosynthesis-light response curve. The data (6 series per treatment) were fitted by non-linear regression to a rectangular- hyperbolic curve and the correlation coefficient is given (no statistical difference in correlation coefficient between the treatments)

	Control UV-B		High UV-B	
	Control N	Low N	Control N	Low N
P_{max}, μmol CO_2 m^{-2} s^{-1}	41.61	25.77	36.50	26.72
I_0, μmol CO_2 m^{-2} s^{-1}	6.853	4.824	4.978	3.040
QE	0.113	0.104	0.109	0.117
R, μmol CO_2 m^{-2} s^{-1}	1.071	0.936	1.321	1.021
r^2	0.894	0.857	0.942	0.840

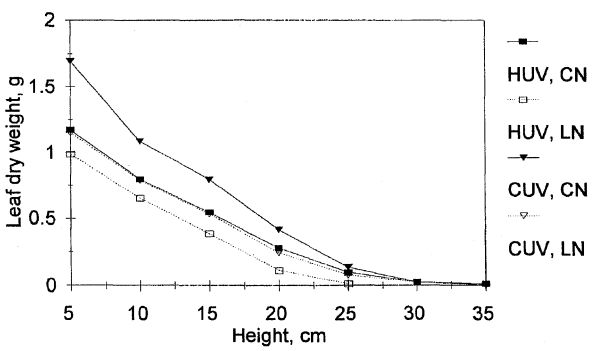

Figure 1. Effects of enhanced UV-B (Control UV-B: CUV; high UV-B: HUV) and N deficiency (control N: CN; low N: LN) on the leaf dry weight distribution in function of the height (5 cm layers) of rye. All data are means of 48 replicates.

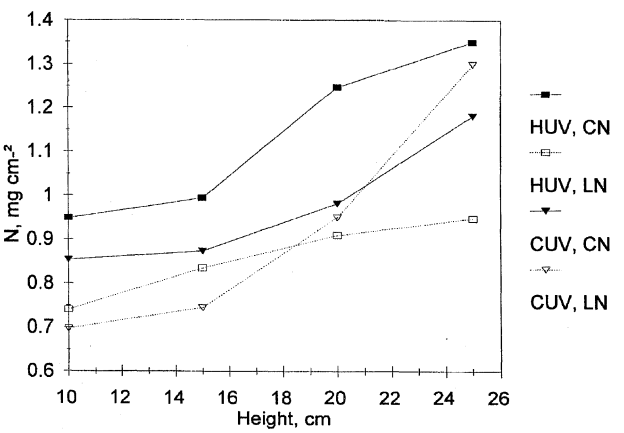

Figure 2. Effects of enhanced UV-B (Control UV-B: CUV; high UV-B: HUV) and N deficiency (control N: CN; low N: LN) on the N distribution in function of the height (leaf area basis).

ment respectively compared to 1.0091 and 0.8237 for the control UV-B series. For both treatments N content increased with height in the canopy (Figure 3).

N effects

Nitrogen deficiency reduced total biomass by 32 (normal UV-B) and 33% (enhanced UV-B) respectively.

Table 3. Effects of enhanced UV-B and N defficiency on chlorophyll and flavonoid content of rye leaves.Averages of 10 samples from each treatment are given and significant differences between the treatments as well as of the interaction (Manova) are indicated in the last columns ($p < 0.05^*$, $p < 0.01^{**}$, n.s. = not significant)

	Control UV-B		High UV-B		UV	N	UV × N
	Control N	Low N	Control N	Low N			
Chlorophyll a, mg cm^{-2}	20.23	15.51	20.96	14.40	n.s.	**	n.s.
Chlorophyll b, mg cm^{-2}	6.47	4.93	6.15	4.57	n.s.	**	n.s.
Chlorophyll a/b	3.15	3.13	3.37	3.33	n.s.	n.s.	n.s.
Chlorophyll a+b, mg cm^{-2}	27.70	20.44	27.01	18.97	n.s.	**	n.s.
Flavonoids, OD	311	491	445	486	*	**	**

Height, leaf area and leaf dry weight were decreased proportionally, so SLW was unaffected. The R/S ratio was increased in response to N shortage only in the control UV-B group. In this group LAR was also reduced.

Maximum photosynthesis was significantly reduced by N deficiency, I_0, quantum efficiency and dark respiration were not significantly altered. The total chlorophyll content of the N deficient plants was significantly lower, but the flavonoid content was increased.

The average leaf N content (leaf area basis) was slightly lower at the reduced N availability.

Interaction

Only on R/S ratio and flavonoid content statistically significant interaction between the N and UV-B effects was found. All other effects were additive.

Discussion

UV-B effects

Many plants show reduced biomass production in response to enhanced UV-B radiation (reviews : Caldwell et al. 1989; Teramura & Sullivan 1994). Generally, plants are less sensitive in the field. The increased sensitivity of plants in growth chamber experiments is often the result of interaction with the low PAR levels (Teramura 1980a, b; Cen & Bornman 1990). However, in our experiment, the used PAR irradiance was similar to field values, as was the used UV-B dose (values recorded during summer in Belgium, 50° NL). The used rye variety appears to be extremely sensitive to UV-B, although the same cultivar was much less sensitive in a field study (unpublished data).In many cases, morphology is more sensitive than biomass production. Often increased SLW and changes in R/S ratio are found, even when photosynthesis and total dry weight are unaffected. In this experiment however, there were very few morphological effects. Although the high UV-B irradiated plants were shorter, this was probably only the result of the reduced biomass. Only under N stress did UV-B induce a decrease in R/S ratio.

The reduced production was clearly correlated to a strong reduction in photosynthesis, a result found in many studies on sensitive plants (Iwanzik et al. 1983; Kulandaivelu et al. 1989; Teramura 1980a). Although the exact nature of the damage can not be derived from these results, it was clearly not a result of chlorophyll degradation.

Several authors have reported changes in chlorophyll a/b ratio after exposure to enhanced levels of UV-B. Increases in the ratio could be the result of the higher sensitivity of photosystem II (to which chlorophyll b is mainly associated) to UV-B (Adamse & Britz 1992; Deckmyn et al. 1994). UV-B induced changes in leaf anatomy (including SLW) could change the light environment within the leaves and thus lead to increases as well as decreases in a/b ratio (Bornman & Vogelmann 1991). Since in our experiment we found no chlorophyll degradation and no change in SLW, no significant change in the chlorophyll a/b ratio was to be expected.

Many studies concerning the induction of flavonoid synthesis have been made. As flavonoids offer protection from UV-B to the underlying tissue, induction of the production by UV-B is a useful protective response, found in many species (Murali & Teramura 1985; Tevini et al. 1991; Warner & Caldwell 1983).The results appear to be very species dependent. In some cases, high PAR levels are necessary to allow stimulation of the production by UV-B (Cen & Bornman 1990; Lingakumar & Kulandaivelu 1993). In our study UV-B increased flavonoid levels, but only under optimal N availability. This was probably because of the increased

flavonoid content of the N-deficient plants (to levels as high as in the high UV-B irradiated plants), possibly indicating a maximum concentration of the leaves. The increased N content of the high UV-B irradiated plants could not be explained by increased flavonoid production (found only under optimal N availability). Possibly the reduced leaf area in creased light penetration in the canopy and thus optimal N content of the leaves. Hirose & Werger (1987) have found a close relationship between the light environment and the N content of leaves, explained by the close relationship between N-content and photosynthesis (Evans 1989). In our study, the largest differences in N content were found at the top of the canopy, while root N content was unaffected by UV-B, a further indication for this hypothesis.

N-effects

As expected N deficiency resulted in smaller plants, with reduced biomass. Generally, the R/S ratio of plants grown in N poor soils is higher, but in our experiment this was only the case under normal UV-B levels. The effect of UV-B on the R/S ratio completely overshadowed this effect. As the plants were grown in pots, the lack of this response did not increase the sensitivity of the plants to N deficiency at high UV-B levels. However, in the field plants could be more sensitive to N stress at increased UV-B irradiation. We have no explanation for the increased flavonoid production of the N deficient plants, but as the concentration was not further increased by UV-B, this response offered no supplemental protection to UV-B. Generally, plants attempt to keep their C/N ratio constant, even at low nutrient levels, by decreasing the growth rate (Ingestadt & Ågren 1988, 1992). Only when the N stress increases in time, and plants are unable to adjust themselves does the average leaf N content decrease, as was the case in our study.

Interactive effects

In conclusion, it appears that N-deficiency and UV-B have mainly additive effects. This resulted in a total reduction of biomass production by 52% under the combined stresses. Only on R/S ratio and flavonoid production was significant interaction found. Possibly under field conditions this could lead to increased sensitivity of N deficient plants to UV-B radiation.

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V. UV-B and terrestrial ecosystems

UV-B supplementation system and CO_2 open top chambers in the sub-arctic forest heath ecosystem at Abisko, North Sweden. (Photograph: Nils Åke Andersson)

Plant Ecology **128:** 242–249, 1997.
J. Rozema, W. W. C. Gieskes, S. C. van de Geijn, C. Nolan and H. de Boois (eds), UV-B and Biosphere.

Effects of enhanced UV-B radiation and elevated carbon dioxide concentrations on a sub-arctic forest heath ecosystem

D. Gwynn-Jones, J. A. Lee & T. V. Callaghan
Department of Animal and Plant Sciences, The University of Sheffield, UK

Key words: Berries, Branching, Dwarf shrubs, Ecosystem, Elevated CO_2, Enhanced UV-B radiation, Growth, Herbivory, Leaf thickness, Ozone, Reproduction, Sub-Arctic

Abstract

An experiment is described which studies the effects of enhanced UV-B radiation (simulating a 15% reduction in the Ozone layer) and elevated atmospheric concentrations of CO_2 (600 ppm) on the dwarf shrub layer of a sub-arctic forest heath ecosystem at Abisko, North Sweden. The experimental treatments were first applied in 1993, and have covered most of the snow-free season (late May to early September) 1993–1995. Effects of the treatments on the four dwarf shrub species have been recorded largely using non-destructive measures (*Vaccinium uliginosum, Vaccinium myrtillus* – deciduous species and *Vaccinium vitis-idaea* and *Empetrum hermaphroditum* – evergreen species). Effects of the treatments on stem growth and leaf thickness have so far been small, although CO_2 treatments initially stimulated stem extension in *Vaccinium myrtillus* 1993 and depressed growth in *V. vitis idaea* in 1994 and *E. hermaphroditum* during 1995. UV-B treatments stimulated fruit production in *V. myrtillus* in both 1994 and 1995, but there was no effect on reproductive phenology. There were also marked effects of UV-B treatments on insect herbivory in the deciduous dwarf shrubs; with leaf area loss being greater than the control in the UV-B treatment in *V. myrtillus* and less in *V. uliginosum*. The results point to the possibility of important effects of the treatments on physiological and chemical processes within the plants. The ecological results of such effects may not be immediately apparent, but may be far reaching, pointing to the need for long-term *in situ* experimentation in predicting the effects of these global change variables.

Introduction

Global climatic change may involve alterations in a number of potentially interacting variables which could have major effects on the structure and function of ecosystems. Depletion of the Ozone layer resulting in the increase in ultra violet-B (UV-B, 280–320 nm) reaching the surface of the Earth (Frederick & Snell 1988; Gleason et al. 1993; Proffitt et al. 1990) is one such factor. Another is the increasing atmospheric CO_2 concentration which is predicted approximately to double the pre-industrial concentration (ca. 270 ppm) by the middle of the next century (IPCC 1990).

A wide range of biochemical, physiological, morphological, anatomical and growth responses have been reported to enhanced UV-B radiation (see, e.g. Tevini 1993), but generally the last mentioned of these has been negative. Growth reductions have often been attributed to decreases in photosynthesis (see, e.g. He et al. 1993; Teramura et al. 1983; Ziska et al. 1993), and differential growth reductions among species may be of great ecological importance. In contrast, elevated CO_2 concentrations have generally been observed either to stimulate plant growth or to have little effect (see, e.g. Hunt et al. 1991). It might be hypothesized therefore that any damaging effect of enhanced UV-B radiation on photosynthesis might at least in part be off-set by elevated atmospheric concentrations of CO_2.

There has been a dearth of whole ecosystem studies of both the ecological effects of enhanced UV-B radiation and of elevated CO_2 concentrations. Examples of the latter include sustained increases in the productivity of coastal salt marsh (Arp & Drake 1991) and only small, transient effects on wet tussock tundra species

(Tissue & Oechel 1987). Arctic vegetation is naturally exposed to low levels of UV-B radiation, and perhaps, therefore, may be particularly susceptible to increases in this form of radiation. Evidence suggests that the Ozone layer above the Arctic may in recent years be thinning at ca. 1% per annum (Hoffman & Deshler 1991) thereby exposing arctic ecosystems and peoples to increasing risk.

This paper describes a large scale field experiment designed to investigate the effects of elevated CO_2 concentrations and enhanced UV-B radiation on important ecosystem processes in a sub- arctic forest heath.

Methods

Experimental design

An experiment designed to investigate the effects of elevated CO_2 and enhanced UV-B radiation was established at Abisko, Swedish Lapland (68°35′ N, 18°82′ W, 360 m a.s.l.). The vegetation is the *Empetrum-Vaccinium myrtillus* variant of sub-arctic forest with an open canopy of *Betula pubescens* ssp. *tortuosa*, and a dense dwarf shrub layer including only scattered herbs and grasses. The vegetation also contains a prominent ground layer of mosses and lichens. The major dwarf shrub species are *Empetrum hermaphroditum* Hagerup, *Vaccinium myrtillus* L., *Vaccinium uliginosum* L. and *Vaccinium vitis-idaea* L. A detailed description of the vegetation is given by Sonesson & Lundberg (1974). The experiment utilized open areas in the birch forest with little shading from the small trees (up to 4 m), and concentrated on the effects of the treatments on the dwarf shrub and ground layers.

The experiment was established in the summer of 1992, and has so far run for three growing seasons (1993–1995). Enhanced UV-B radiation was supplied from metal frames (2.5 × 1.3 × 1.5 m high) each with 6 fluorescent lamps (Q-PANEL UVB–313, Cleveland, OH, USA). The middle 70 cm of the 2 central lamps in each frame was covered with aluminium foil to give an even radiation distribution at canopy height (Johanson et al. 1995a). A stable output from the lamps was achieved by pre-burning them for 100 h before field use. Control frames had an identical design except that window glass excluded all UV-B emission from the lamps. UV-B treatment lamps had in place of the window glass, UV-transmitting Plexiglass (Röhm GmbH, Darmstadt, Germany) holding a cellulose diacetate filter (0.13 mm, Courtaulds, Derby, UK) to exclude UV-C radiation (<280 nm). All plots received the natural UV-B radiation with the exception of some small shading from the frames. Enhanced CO_2 (600 ppm) was supplied to the vegetation below the frames in 0.73 m^2 area open top chambers. The open top chambers were 0.50 m high and had no frustrum. They were constructed of UV-transmitting Plexiglas (Röhm 2458, Röhm GmbH, Darmstadt, Germany) and air was introduced to the base of each chamber by a 10 cm diameter external skirt. Air was passed through each chamber by a fan (model TD350/125, Soler and Palau, Spain), and in the CO_2-treated chambers, CO_2 was bled into the air supply to give 600 ppm CO_2. The system maintained a concentration of 600 ± 50 ppm CO_2 at canopy height in the centre of the chambers. Control chambers received ambient air from ca. 50 cm above the dwarf shrub canopy. Concentrations of CO_2 in the chambers were monitored using an infra-red gas analyser (Series 2000, ADC, UK).

Sensors were placed within the chambers to measure photosynthetically active radiation (PAR sensor, Licor, USA), relative humidity (Rotronic, Germany), and air temperature (thermocouples) linked to a datalogger (CR10, Campbell, USA). Relative humidity was reduced in the chamber by up to 8% when humidities were low (40–60%) but differences were negligible when ambient relative humidity was above 70% (data not shown). The mid-day chamber temperature was up to 3 °C higher than outside (data not shown), whilst the mean daily air temperature within the chambers was 0.93 °C, 1.08 and 0.97 °C higher than outside respectively during the 1993, 1994 and 1995 seasons (see, e.g. Figure 1).

Both elevated and ambient CO_2 open top chambers were placed centrally and randomnly beneath the UV-B frames. Smoke-bomb tests and CO_2 measurements revealed that there was little or no contamination of surrounding vegetation from the chambers. There were 16 frames and 16 chambers giving a four fold replication of each of the following treatments: $-$UV-B $-CO_2$ (control), $+$UV-B $-CO_2$, $-$UV-B $+CO_2$, and $+$UV-B $+CO_2$. The UV-B treatment simulated a 15% ozone depletion.

The CO_2treatments began at snow melt in late May (the UV-B treatments in early May), and both treatments continued until early September when the leaves of deciduous species had senesced, i.e. throughout the growing season. Additional UV-B radiation was supplied daily around noon, and was controlled by timers that switched on 3 lamps at a time to give a step-

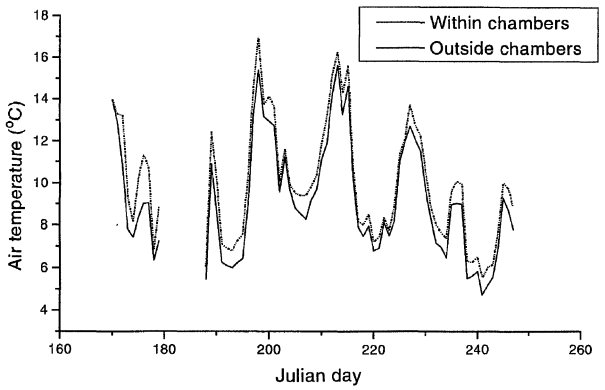

Figure 1. Mean air temperature at a height of 15 cm, inside and outside the open top chambers during the 1995 growing season. The values are a mean of two separate measurements.

wise 'square wave' increase. The daily exposure time was changed every second week to follow the seasonal change in natural UV-B radiation which is measured continuously at Abisko. Cellulose diacetate filters were pre-solarized to reach a stable transmission which was approximately constant for 50 h of field irradiation. Thus, filters were changed every 50 h of irradiation and spectral irradiance on the plots was measured with an Optronics 742 spectroradiometer (Orlando, FL, USA) interfaced with a Hewlett Packard 85 Computer.

The model developed by Björn & Murphy (1985) and Björn & Teramura (1993) was used to calculate the daily increase in UV-B radiation resulting from a 15% ozone depletion under clear skies.

Plant growth

The experiment was designed to extend for 6 years. The small size of the chambers and plots dictated that in the early years of the experiment, the assessment of plant performance should largely be confined to non-destructive measurements.

Stem growth measurements on dwarf shrubs were made using a slide-caliper. Ten (15 in 1995) shoots per species per plot were chosen at random in each plot and growth measured as described by Johanson et al. (1995b).

The thickness of the leaves produced in 1995 was measured during August 1995 at peak leaf thickness. A minimum of 6 leaves per plot per species was measured at the widest part of the leaf using a caliper (Mitutoyo 102–217) applied to the middle of the leaf.

Leaf herbivory was measured in August 1995. The 10 shoots were selected at random in each plot. The leaf outlines were drawn on paper, and the leaf area consumed was determined, using a computerised scanning system. No estimatate of herbivory was performed on the dwarf shrub *Empetrum hermaphroditum* as it has small scelerphyllous needle like leaves which are unsuitable for these measurements.

Reproductive phenological measurements were made in 1993. Ten shoots per plot of *Vaccinium myrtillus* were marked in late May, and development was assigned to one of five ranks as follows: 1, flower bud visible; 2, flower bud bursting; 3, flower fully developed; 4, flower senescence; 5, berry visible. The shoots were measured on 5 occasions during the growing season.

Total numbers of berries produced in each treatment were recorded in each of the three years of the experiment.

Data were transformed where appropriate and were analysed by analysis of variance (SPSS).

Results

Growth as measured by shoot length extension in the growing season is shown in Figures 2 and 3. Although small differences in the shoot lengths of *Empetrum hermaphroditum* and *Vaccinium myrtillus* occurred in the pre-treatment season (1992), presumably reflecting small differences in microclimate or soils, these were not significant. Over the three years of treatment, there was no significant effect of the UV-B treatment on growth. There was a tendency for CO_2 treatments to increase the growth of *V. myrtillus* which was significant in 1993. However, CO_2 significantly decreased the growth of *E. hermaphroditum* in 1995, and of *V. vitis-idea* in 1994 (data not shown).

There was no effect of treatment on leaf thickness in 1995 on both the evergreen *V. vitis-idaea* and the deciduous *V. myrtillus* (Figure 4). There was a slight tendency in both species for UV-B-treated plants to have thicker leaves.

There were significant effects of UV-B treatments on herbivory in *V. myrtillus* and *V. uliginosum.* In *V. myrtillus* leaf surface area lost to insect herbivory was enhanced by UV-B treatment, whereas in *V. uliginosum* there was less insect damage compared to the control in the UV-B treatment (Figures 5 and 6). There was no effect of CO_2 on herbivory in these species, and there was no effect of any treatment on herbivory in *V. vitis-idaea.* Levels of herbivory in each of the three

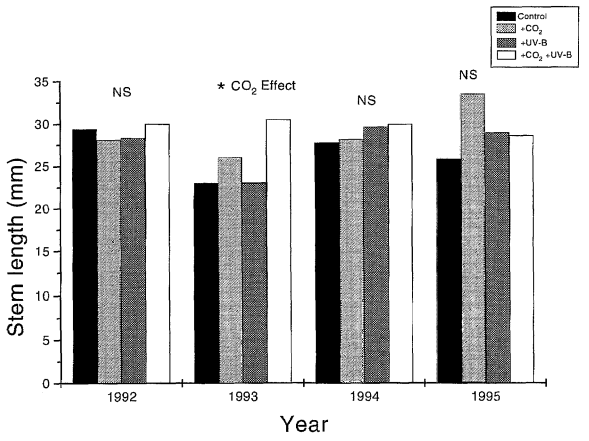

Figure 2. Stem length of *V. myrtillus* before the treatments (1992), and following three years (1993–1995) exposure to the treatments. The values represent a mean from four replicate plots which involve 10–15 shoots per plot (where $* p < 0.05$ significance).

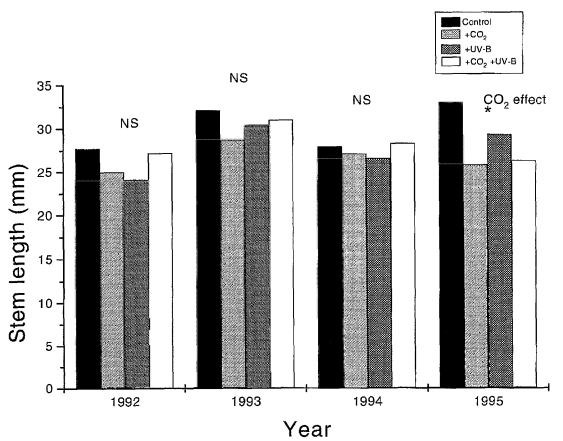

Figure 3. Stem length of *E. hermaphroditum* before the treatments (1992), and following three years (1993–1995) exposure to the treatments. The values represent a mean from four replicate plots which involve 10–15 shoots per plot (where $* p < 0.05$ significance).

species signified up to a 60% reduction in total leaf surface area.

There was no effect of treatment on reproductive phenology in *V. myrtillus* during 1993 (Figure 7), and there was no evidence of major treatment effects in either 1994 or 1995 (data not shown). However, there was a significant effect of UV-B treatment on berry production in this species (Figure 8). Numbers of berries increased on control and treatment plots during the period of observations, but the largest numbers were observed in the UV-B treatments (the UV-B treatment in 1994 and the UV-B + CO_2 treatment in 1995).

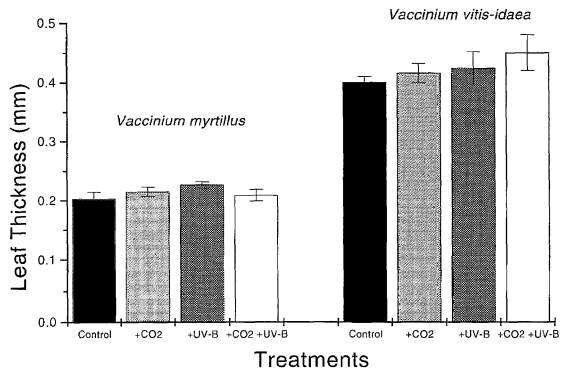

Figure 4. The thickness of *V. myrtillus and V. vitis-idaea* leaves produced in 1995, measured during August 1995 at peak leaf thickness. The values represent a mean from four replicate plots with 10 leaves measured per species per plot. There were no significant differences using an LSD test at $p = 0.05$.

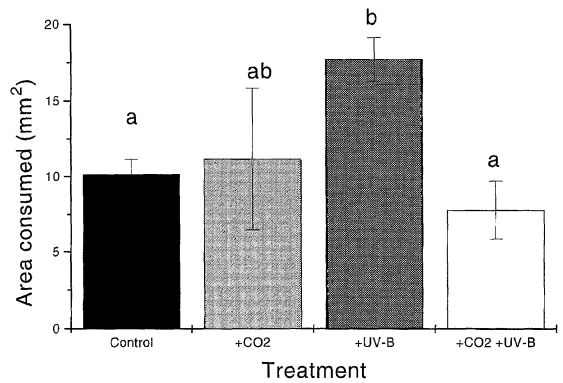

Figure 5. Leaf herbivory measured on *V. myrtillus* during August 1995. The values represent a mean consumption rate on leaves from four replicate plots involving 10 random measurements. Columns with different letters represent significant differences (LSD test) at $p < 0.05$.

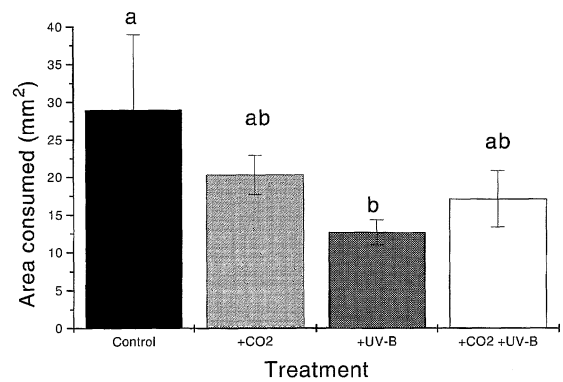

Figure 6. Leaf herbivory measured on *V. uliginosum* during August 1995. The values represent a mean consumption rate on leaves from 3–4 replicate plots involving 10 random measurements. Columns with different letters represent significant differences (LSD test) at $p < 0.05$.

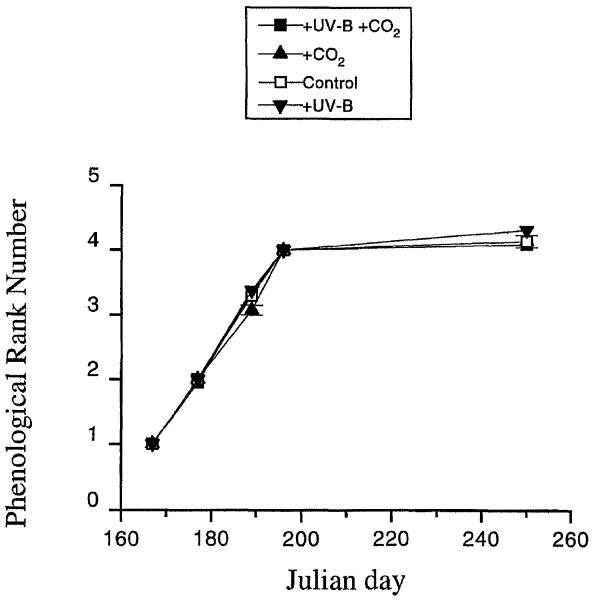

Figure 7. Measurements of reproductive phenology made in 1993 on *V. myrtillus*. Ten shoots per plot were marked in late May, and development was assigned to one of five ranks as follows: 1, flower bud visible; 2, flower bud bursting; 3, flower fully developed; 4, flower senescence; 5, berry visible. The shoots were measured on 5 occasions during the growing season.

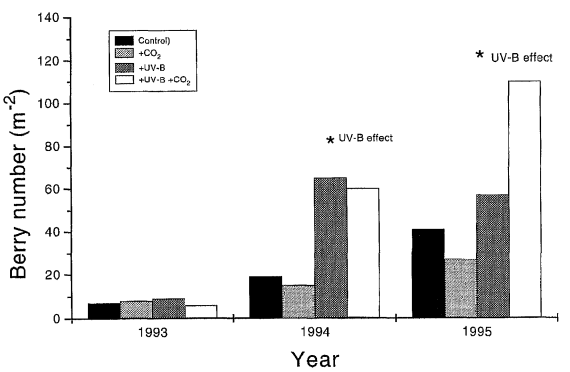

Figure 8. The total number of *V. myrtillus* berries per m^2 collected during three years (1993–1995) exposure to the treatments. The values are a mean from four replicate treatment plots (where $* p <$ 0.05 significance).

Discussion

This experiment involves the establishment of treatments on near-pristine vegetation. Sub-arctic heathlands are dominated by long-lived woody plants, and their longevity and size must provide considerable buffering to small changes in global climate. The experimental treatments employed here were chosen to be realistic of climate change scenarios over the next century, and as such were unlikely to cause major detrimental effects on plant growth in the short-term. This expectation is borne out by the results. There is no effect of UV-B treatment on stem growth, and this to some extent contrasts with the results of Johanson et al. (1995b) from a slightly more xeric site at Abisko. These workers did find small effects of UV-B treatment (simulating a 15% reduction in the Ozone layer) on absolute stem growth in the dwarf shrubs. Growth of *V. myrtillus* was reduced in both years of treatment, whereas growth of *V. vitis idaea* was stimulated in the first and reduced in the second. *Vaccinium uliginosum* and *E. hermaphroditum* were unaffected. The significant effects observed may relate to the fact that the site used by Johanson and his co-workers has less snow lie, and the plants may therefore be exposed longer to the irradiation treatment. Physiological responses early in the growing season may also contribute to the small changes in leaf thickness observed by Johanson et al. (1995b), but not in the present study.

Significant effects of CO_2 treatments were observed. Enhanced CO_2 increased the growth of *V. myrtillus* in the first year of treatment (Figure 2), and significantly decreased the growth of *E. hermaphroditum* in 1995 (Figure 3) and *V. vitis idaea* in 1994. (Growth of the latter species was also lower, though not significantly, in 1995). The absence of any marked fertilizer response to CO_2 in this system (apart from the transient initial stimulation in *V. myrtillus*, Figure 2) is not surprising given that these soils are strongly nutrient (nitrogen) limited (see, e.g. Parsons et al. 1994). These data are consistent with the only other *in situ* arctic ecosystem study where Tissue & Oechel (1987) observed only small increases to CO_2 in the productivity of the wet tussock tundra species *Eriophorum vaginatum*. Studies where no plant growth response to elevated CO_2 has been shown demonstrate a down- regulation of photosynthesis through reductions in the amount and activity of Rubisco (see, e.g. Besford et al. 1990).

Stem extension is only one measure of plant growth. It may be particularly important in closed canopies, such as in the dwarf-shrub layer, because any changes may influence the position of leaves within the canopy and hence overall photosynthetic gain. Branching patterns will also very much influence this, and there is evidence from the present study of a significant UV-B $\times$ CO_2 interaction in the number of branches produced per shoot in *E. hermaphroditum* in one of the three years (data not shown). Thus small changes in shoot

extension and branching pattern may eventually result in major ecosystem responses. A full analysis of above and below ground growth responses to the treatments will have to await destructive harvests at the end of the experiment.

Evidence that profound changes are occurring in this ecosystem in response to UV-B treatments comes from the effects of herbivory (Figures 5 and 6). Surprisingly, there were no significant effects of CO_2 on herbivory *in situ*, despite the fact that these treatments probably caused an increase in the leaf C:N ratio (see Fajer et al. 1989). UV-B treatments significantly reduced herbivory in *V. uliginosum*, increased it in *V. myrtillus*, and had no effect in *V. vitis idaea*. No simple analysis of these observations are possible at present since the effects may result either from changes in the chemistry and physiology of the plants (Hatcher & Paul 1994; McCloud & Berenbaum 1994), or from direct effects of the treatments on the life cycles of the insects involved, or from direct effects on insect predators or diseases (Bornman & Teramura 1993; Killick & Warden 1991) or from a combination of all these factors. A simple interpretation of these data might suggest an expansion of *V. uliginosum* in the heathland at the expense of *V. myrtillus* in response to enhanced UV-B radiation. Insect herbivory plays a major role in the dynamics of sub-arctic birch forests (see, e.g. Tenow 1972), and the effects observed in 1995 may represent a major ecosystem response to enhanced UV-B radiation.

The stimulation of berry production in *V. myrtillus* by UV-B radiation may also represent an important ecosystem response (Figure 8). Berries represent a major food source for vertebrates (including humans, see Anderson 1985) in the sub-arctic. If this stimulation of berry production is sustained, and is also found in other species, then there may be profound ecosystem effects. At the physiological level, it is difficult to explain the mechanism by which UV-B stimulates flowering and fruit production in *V. myrtillus*. Presumably this, in part at least, results from hormonal (e.g. Witztum et al. 1978; or Tevini et al. 1991) and other physiological changes within the plant. Evidence that positive effects of UV-B radiation on plant growth can occur is increasing (see, e.g. Barnes et al. 1990; Gwynn-Jones and Johanson, 1996).

During the first three years of this experiment, the treatment effects have been rather subtle, and only one interaction (in branching) has been observed between enhanced UV-B and elevated CO_2. The experiment is beginning to demonstrate the potential for major ecosystem change. The results demonstrate the importance of long-term experimentation in the field in order to predict successfully ecosystem responses to major global change variables.

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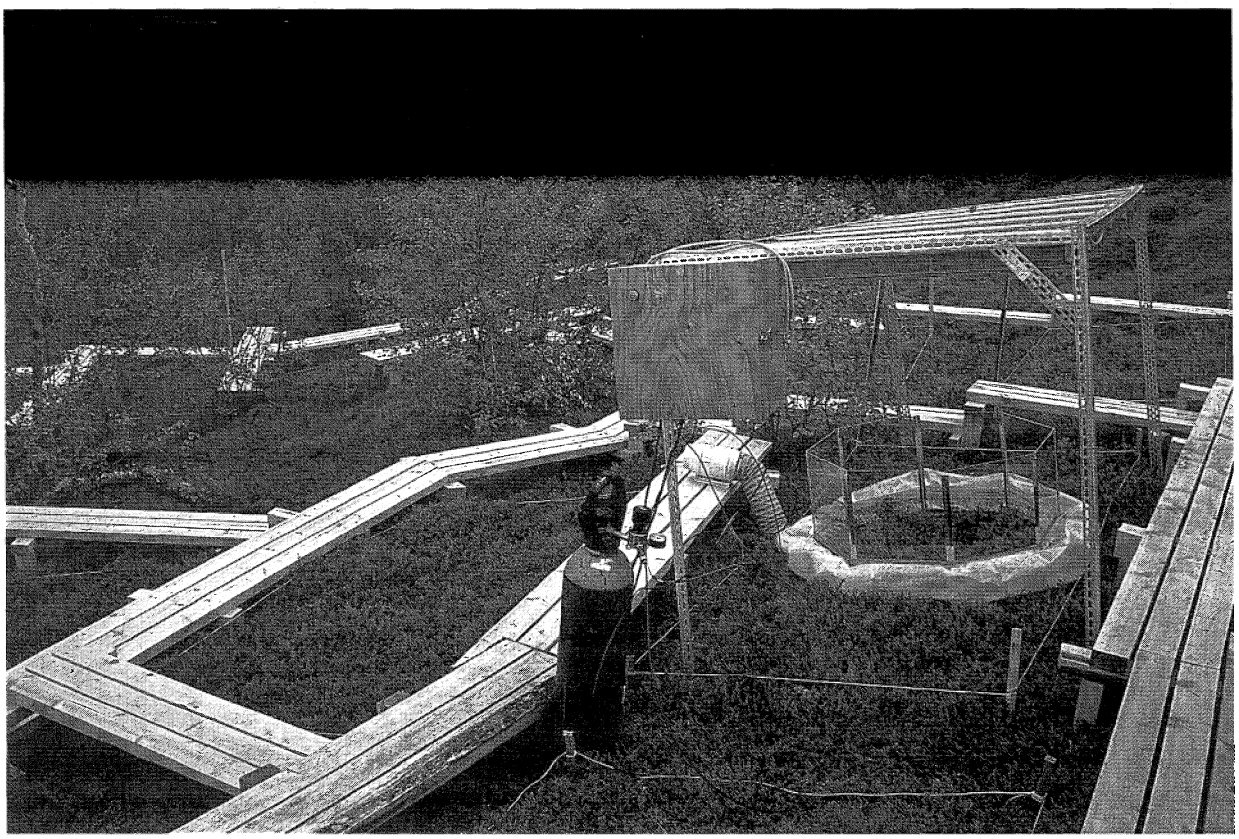

Open top chambers for CO_2 enrichment and frames with tubes for enhanced UVB radiation at the subarctic dwarf shrub vegetation, Abisko, Sweden. (Photograph: J. A. Lee)

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J. Rozema, W. W. C. Gieskes, S. C. van de Geijn, C. Nolan and H. de Boois (eds), UV-B and Biosphere.

The effects of UV-B radiation on European heathland species

L. O. Björn[1], T. V. Callaghan[2], I. Johnsen[3], J. A. Lee[2], Y. Manetas[4], N. D. Paul[5], M. Sonesson[6], A. R. Wellburn[5], D. Coop[5], H. S. Heide-Jørgensen[3], C. Gehrke[7], D. Gwynn-Jones[2], U. Johanson[1], A. Kyparissis[4], E. Levizou[4], D. Nikolopoulos[4], Y. Petropoulou[4] & M. Stephanou[4]

[1]*Department of Plant Physiology, University of Lund, S-221 00 Lund, Sweden;* [2]*Department of Animal & Plant Sciences, The University of Sheffield, Sheffield S10 2TN, UK;* [3]*Department of Plant Ecology, University of Copenhagen, Øster Farimagsgade 2D, DK-1353 Copenhagen, Denmark;* [4]*Laboratory of Plant Physiology, University of Patras, Patras, Greece;* [5]*Biology Division, University of Lancaster, Bailrigg, Lancaster LA1 4YQ, UK;* [6]*Abisko Scientific Research Station, S-98107, Abisko, Sweden;* [7]*Department of Plant Ecology, Lund University, S-22362 Lund, Sweden*

Key words: Ecophysiological responses, Elevated CO_2, Growth and phenology, Heathland vegetation, Precipitation, UV-B radiation

Abstract

The effects of enhanced UV-B radiation on three examples of European shrub-dominated vegetation were studied *in situ*. The experiments were in High Arctic Greenland, northern Sweden and Greece, and at all sites investigated the interaction of enhanced UV-B radiation (simulating a 15% reduction in the ozone layer) with artificially increased precipitation. The Swedish experiment also involved a study of the interaction between enhanced UV-B radiation and elevated CO_2 (600 ppm). These field studies were supported by an outdoor controlled environment study in the United Kingdom involving modulated enhancement of UV-B radiation in combination with elevated CO_2 (700 ppm). Effects of the treatments on plant growth, morphology, phenology and physiology were measured. The effects observed were species specific, and included both positive and negative responses to the treatments. In general the negative responses to UV-B treatments of up to three growing seasons were small, but included reductions in shoot growth and premature leaf senescence. Positive responses included a marked increase in flowering in some species and a stimulation of some photosynthetic processes. UV-B treatment enhanced the drought tolerance of *Pinus pinea* and *Pinus halepensis* by increasing leaf cuticle thickness. In general, there were few interactions between the elevated CO_2 and enhanced UV-B treatments. There was evidence to suggest that although the negative responses to the treatments were small, damage may be increasing with time in some long-lived woody perennials. There was also evidence in the third year of treatments for effects of UV-B on insect herbivory in *Vaccinium* species. The experiments point to the necessity for long-term field investigations to predict the likely ecological consequences of increasing UV-B radiation.

Introduction

Dwarf shrub heaths represent a major component of vegetation of arctic regions, north of the boreal forest. Here, the vegetation is relatively little disturbed by human activity. Further south, heathland communities are markedly affected by, and largely owe their existence to, human influence. For example, the deforestation of the Mediterranean basin has resulted in large areas of shrub-dominated ecosystems on uncultivated soils. In northern and western Europe, heathlands on poor acidic soils have been maintained by a combination of fire, grazing and turf cutting, and also occupy extensive areas, particularly in upland regions. Despite the fact that many European shrub-dominated ecosystems rely on human activity for their existence, they

can have high conservation value. They can also be of considerable importance to the local economies of particular regions e.g. the *Calluna* heathlands of upland Britain. Other examples are the semi-natural heathlands north of the arctic circle which are of considerable economic importance to Saami reindeer herding communities although still little disturbed by man.

There is growing awareness of the potential biological importance of stratospheric ozone depletion as part of global climatic change. Although most attention so far has been given to the phenomenon in the Antarctic, over the period 1979–1993 the decrease in stratospheric ozone at 65° N corresponds to a ten percent increase in annual DNA-weighted UV-B radiation (Madronich et al. 1995). Further, in the spring the relative increase is greater than the annual average. Thus the potential for large increases in UV-B radiation reaching vegetation canopies exists at high northern latitudes, even though it may currently be offset to some extent by sulphate aerosols and tropospheric ozone (UNEP, 1994). Although less conspicuous, reducing trends in stratospheric ozone have also been reported for lower latitudes (see e.g. Stolarski et al. 1992). This potential increase in UV-B radiation is also expected to be combined with other changes in the global climate, including a marked increase in atmospheric carbon dioxide concentrations, increases in mean annual temperatures and changes in precipitation patterns (IPCC 1990). Thus, any effects of increased UV-B radiation may be influenced and modified by other climatic factors.

The vast majority of studies of the effects of UV-B radiation on plants have been performed on fast growing crop species under laboratory conditions (see e.g. Teramura & Sullivan 1994). Results of such studies may be difficult to extrapolate to natural vegetation, which is mostly composed of long-lived perennial plants frequently growing in nutrient-limiting soils. To partly offset this situation, a programme was established by the European Community in 1992 to examine the effects of UV-B radiation on shrub-dominated ecosystems. A central thrust of the study was not only to study the effects of UV-B radiation *per se*, but in particular to examine the interaction between UV-B radiation and other environmental variables, notably elevated carbon dioxide concentrations and increased precipitation. This paper summarises the findings from the first three field seasons (1993–1995). Field investigations were supported by a number of controlled environment experiments aimed at more detailed studies, e.g. of physiological or below-ground processes, than was possible in the field.

Study Areas

There were three main centres of field investigations: Qaanaaq, Greenland (77° N 68° W), Abisko, North Sweden (68.3° N 18.8° E) and Patras, Greece (38.3° N, 29.5° E). In addition, a semi-controlled environment study was undertaken at Lancaster, UK (54.0° N 2.8° W). The vegetation at the three field sites corresponded to *Cassiope tetragona* heath (Qaanaaq), *Betula pubescens* ssp *tortuosa* forest heath (Abisko), and phrygana and maquis, dominated by drought semi-deciduous and evergreen sclerophyllous shrubs respectively (Patras). Further details of the vegetation and sites can be found in Heide-Jørgensen & Johnsen (1995), Johanson et al. (1995a) and Nikolopoulos et al. (1995).

Experimental Treatments

At Abisko, UV-B was generated by 40 W fluorescent lamps (Q-Panel UVB-313) arranged in parallel in groups of six, 1.5 m above the ground. The centres of the two central lamps were covered with aluminium foil to obtain a more uniform distribution of radiation (Björn & Teramura 1993). The lamps were connected to time-switches in such a way that half the lamps in an array were first switched on, then the rest, then the first half were switched off, and then the rest. In this way a step-wise change of irradiance was obtained over the day. Half of the lamp arrays were equipped with cellulose acetate filters that transmit UV-A and UV-B (but stop UV-C) radiation to provide treatments. The other (control) arrays were equipped with window glass that stops UV-B (and UV-C) but transmits most of the UV-A.

To compute the daily UV-B addition required, the UV-B model mentioned above was used, in combination with a weighting function devised by Thimijan, Carns & Campbell (1978), that resembles the Caldwell generalised plant action spectrum (Caldwell 1971). The mathematical function used for the spectrum is $EXP(-(((265-L)/21)^2))/EXP(-((35/21)^2))$, where L is the wavelength in nm, and every nm and every hour of the day using the program, was weighted and added up to yield the daily exposure by daylight. The radiation from the lamps was measured using an Optronic model 742 spectroradiometer, and weighted in a similar fashion. Occasional spectral measurements were taken also of ambient UV-B.

It should be recognized that the weighting function used is somewhat arbitrary, and several experiments have been done, also by us, to determine action spectra for UV effects on plants (Bornman, et al. 1984; Negash & Björn 1986; Negash 1987; Cen & Björn 1994). However, it is assumed that the errors due to inappropriate weighting are not of such a magnitude as to invalidate the results, since the spectrum of the administered artificial radiation is rather similar to the difference spectrum between 'depleted' and 'normal' daylight (see Björn 1995).

There were small differences in the UV-B exposure systems at Patras and Qaanaaq (see Heide-Jørgensen & Johnsen, 1995; Nikolopoulos et al. 1995). However, experimental treatments simulated a 15% ozone reduction at all three sites assuming cloudless skies. In addition, at Qaanaaq a treatment simulating a 30% reduction was included.

A number of other experimental treatments was established under the lamp arrays. At each site, precipitation was increased by weekly watering events during the experimental periods. These involved a 200% increase in mean summer precipitation at Qaanaaq, 80% at Abisko, and up to 315% at Patras. At Abisko, an open-top chamber system under the tube arrays was used to provide an elevated CO_2 treatment (600 ppm) during the snow-free period (see Gwynn-Jones et al. this volume). Control chambers received ambient air.

At Lancaster, a closed chamber system was employed to allow closer control of CO_2 concentrations. This was combined with the development of a modulated UV-B exposure system. The experiments here employed two important dwarf shrubs of UK heathlands, *Calluna vulgaris* (L.) Hull and *Vaccinium myrtillus* L. grown in mesh bags on moorland soil (pH 3.9). The latter species is a major component of the vegetation at Abisko (see Johanson et al. 1995a).

Closed chamber construction

Experiments were conducted in chambers glazed using teflon film (0.05 mm FEP film, SW Plastics) which, unlike glass and other conventional glazing materials, has approximately equal transmission (> 95%) of wavelengths between 300 nm and 800 nm and which transmits > 90% between 290 and 300 nm. Thus, the spectral distribution of daylight within the chambers is comparable to that in the field, allowing modulated UV-B treatments (see below) to be made against a realistic background of longer wavelengths.

Lamp modulation (Lancaster experiment)

Modulated treatments, which continuously vary UV-B additions in proportion to incident radiation, are increasingly considered to be the method of choice for studies of plant responses to UV-B (e.g. Caldwell et al. 1994; Fiscus & Booker 1995). During the course of this project a modulated UV-B lamp system was developed, which has now been used for almost two years (see Mepsted et al. 1996 for full details).

The UV-B supplement was controlled using BW100-UVB sensors (Vital Technologies) to measure radiation under a control and treatment array. Over the course of the experiment, sensors were calibrated every 7–14 days against PAS300 in both daylight and under lamps, using a double monochromator spectroradiometer (SR991-PC, Macam Photometrics). The sensor calibration under lamps is constant but, as expected, that in daylight varies over the course of the year with changing solar spectrum. Therefore, this changing calibration was included in weekly calculations of the percentage increase in sensor signal required to achieve the required percentage supplement to incident PAS300.

Sensor signals were used to regulate the output from UV-B fluorescent tubes (Philips TL40/12RS), using adjustable ballasts (A40T LCR, Transtar) and thyristor dimmers (QU20-RM, Quantran Systems Ltd.). To remove radiation below 290 nm, each active lamp was tightly wrapped with a cellulose diacetate sheet. Since lamp output was automatically corrected for reduced transmission as filters aged, filters were only replaced when lamps could no longer achieve the necessary maximum output.

Sensor signals were monitored and lamp output was controlled by a data acquisition and control program (LanDACS, Lancaster University). The level of supplement required to simulate a 15% reduction in the Ozone Layer for each week of the experiment was calculated using the modified 'daylight' model of Björn and Murphy (1985) and Caldwell's generalised plant action spectrum (PAS300) normalised to unity at 300 nm (Caldwell 1971; Caldwell et al. 1986).

Ventilation and CO_2

Chambers were force-ventilated and CO_2 was added at a constant rate to increase ambient CO_2 concentrations by 350 ppm, i.e. the elevated concentration was approximately 700 ppm compared with the ambient of approximately 350 ppm.

Summary results

Lichen growth and performance

Qaanaaq
Historical collections of the lichen *Cladonia mitis* Sandst. from Greenland since 1889 revealed that a dark, crust-like surface structure of the podetia has become increasingly common since 1970 which may be correlated with increasing UV-B (Heide-Jørgensen & Johnsen 1995). This lichen is an important component of the *Cassiope tetragona* heaths of High-Arctic Greenland. These workers also demonstrated that in laboratory and field experiments, this crust-like surface increased with UV-B irradiance (Heide-Jørgensen & Johnsen, in preparation). Scanning electron microscopy revealed that the superficial hyphae were absent from these areas, exposing the stereome (the inner compact layer of the the podetia). Transmission electron microscopy showed that increased UV-B radiation caused a reduction in starch formation, deterioration of the thylakoid structure and an increase in the number of cytoplasmic lipid bodies (Heide-Jørgensen & Johnsen, in preparation). There was apparently no interaction between increased UV-B radiation and increased summer precipitation, despite the fact that in a general survey of the distribution of the crust-like damage, the effects seemed to be greatest in soils of high humidity.

Abisko
No morphological damage was observed in the major lichen species (*Nephroma arcticum* L. (Torss) on *Peltigera apthosa* L. (Torss.)) in the field treatments. A supporting laboratory study of the interaction between enhanced UV-B radiation and elevated CO_2 (600 and 1000 ppm) demonstrated that enhanced UV-B increased the photochemical quantum yield of photosystem II as measured with pulse modulated fluorimetry at 350 and 600 ppm CO_2 (Sonesson et al. 1995). This effect was greater in collections of lichen from Abisko than from southern Sweden. This study gains further support from the fact that there have been no adverse effects on lichens of enhanced UV-B radiation simulating a 15% reduction in the ozone layer in the UV-B × CO_2 and UV-B × increased summer precipitation field treatments during the first three years of the study.

Mosses

Mosses are important components of sub-arctic vegetation. Major species within the experimental treatments at Abisko include *Hylocomium splendens* (Hedw.) B. & S., *Pleurozium schreberi* (Brid.) Mitt. and *Polytrichum commune* Hedw. These experiments have demonstrated a marked interaction between increased summer precipitaton and enhanced UV-B radiation (Gehrke et al. 1996). For example the growth (as measured by stem extension) of *Hylocomium splendens* was strongly stimulated by enhanced UV-B radiation (by 15%, 31% and 27% in the 1993, 1994 and 1995 growing seasons respectively), but only in the treatment receiving increased summer precipitation. In contrast, the treatment receiving enhanced UV-B radiation alone showed either no effect (1993) or an inhibition in growth (25% in 1994 and 18% in 1995).

Dwarf Shrubs

CO_2 × UV-B studies: controlled environments
The major results from the Lancaster controlled environment closed chamber system are as follows:

Vaccinium myrtillus
Growth. There were no statistically significant interactions between UV-B and CO_2 in either growing season. In the first growing season (1994), elevated CO_2 significantly increased the dry weights of both above- and below-ground organs (25% and 30% respectively) but the root:shoot (R:S) ratio was little changed (Figure 1). In the second year (1995) elevated CO_2 increased biomass only in below-ground organs (by approximately 16%), resulting in a significant increase in R:S under elevated CO_2. Clearly, the effects of CO_2 on plant biomass in *Vaccinium* growth were not cumulative, being far less after two seasons than one season (+13% and +27%, respectively).

Supplemental UV-B had little effect on either above-ground or below-ground biomass of *Vaccinium* in the first season (Figure 1). In the second year UV-B reduced below-ground biomass by 15% while that of the shoots tended to increase slightly (5%), so that R:S was significantly decreased (Figure 1). The effects of UV-B were cumulative over the two seasons, although even at the final harvest, elevated UV-B had reduced plant biomass by only 5%. In 1995 plants grown under elevated UV-B produced 18% more reproductive structures, although this increase was not significant.

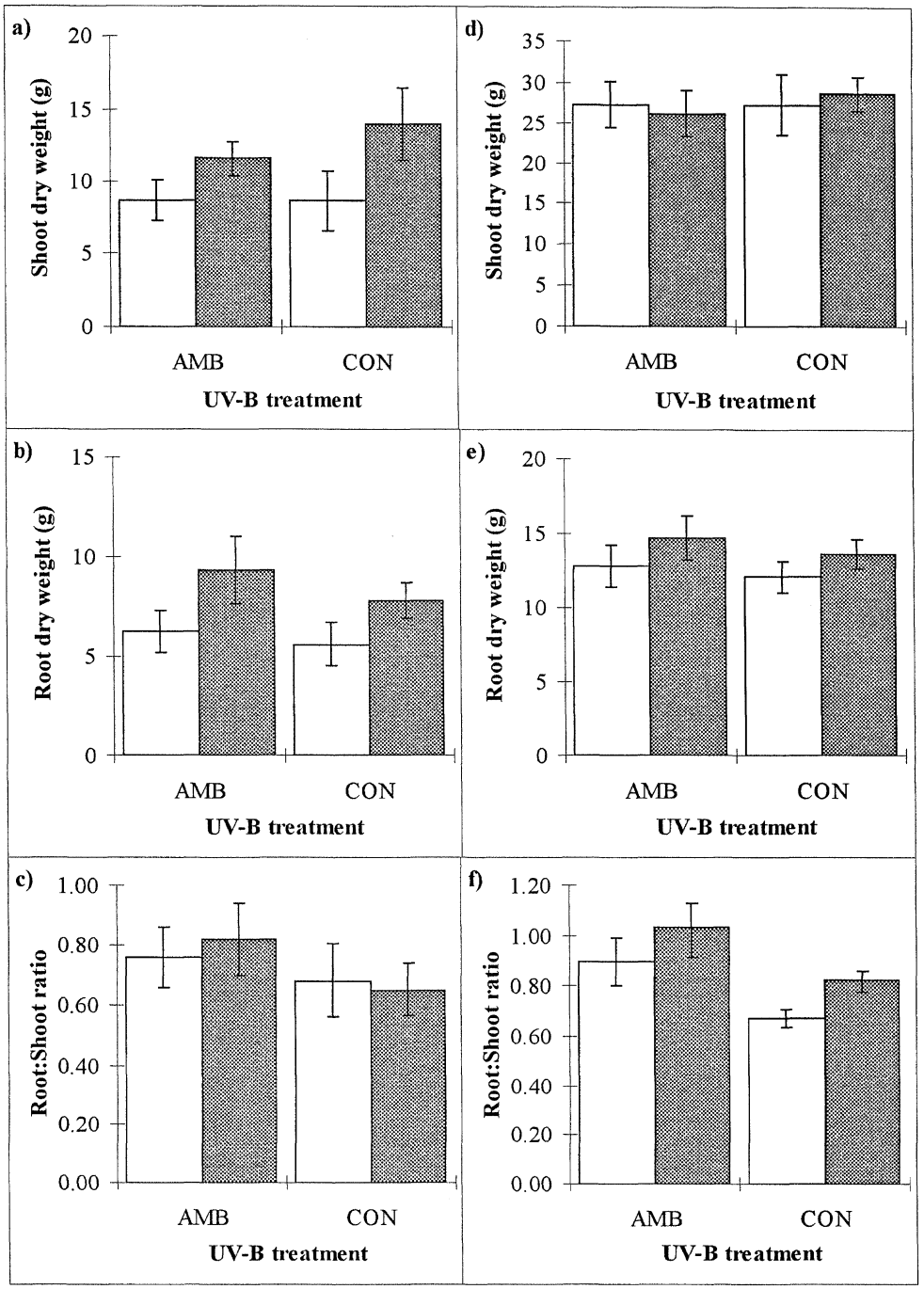

Figure 1. Shoot (a,d), and root (b,e) dry weight and root:shoot ratio (c,f) of *Vaccinium myrtillus* at ambient and elevated CO_2 (open and shaded columns respectively) and ambient (AMB) and elevated (CON) UV-B in September 1994 (a–c) and September 1995 (d–f). Means ± standard deviation.

Phenology. Neither elevated CO_2 nor UV-B significantly altered the time of initial bud burst in spring but the flush lasted about 5–6 days longer at elevated than at ambient CO_2.

Pigments. In 1994, the concentrations of chlorophyll a and b and of carotenoids all decreased significantly ($p < 0.001$) over the course of the season and were significantly ($p < 0.001$) lower at elevated than ambient CO_2. There was no significant change in the chlorophyll a:b ratio, nor did elevated UV-B have any significant effect on any of the pigments measured. Neither UV-B nor CO_2 resulted in any consistent change in UV-B absorbing compounds in either growing season.

C:N ratio. Elevated CO_2 significantly decreased shoot nitrogen concentration and increased C:N ratio, but increased UV-B had no significant effect.

Calluna vulgaris

Growth. There were no statistically significant interactions between UV-B and CO_2 in either growing season. In 1994 increased CO_2 tended to slightly increase the dry weight of *Calluna*, both roots and shoots (Figure 2). Elevated CO_2 also resulted in increased partitioning to the roots. In the second year, growth responses to increased CO_2, were small and by the end of the experiment, increased CO_2 at ambient UV-B had resulted in little change in total biomass of *Calluna*. R:S ratio tended to be rather higher under elevated than ambient CO_2.

Supplemental UV-B tended to reduce shoot and root growth in both seasons and the effect of UV-B was relatively greater at high CO_2. In 1995, UV-B reduced shoot weight more than root weight so that R:S increased. The effects of UV-B were cumulative over the two seasons, especially for shoot growth. At the final harvest, elevated UV-B had reduced shoot weight by 17%, root weight by 7% and plant weight by 11%.

Phenology. *Calluna* started flowering in mid-late July, comparable to natural plants in the field, but in both growing seasons, plants grown at elevated CO_2 flowered approximately one week earlier than controls. UV-B treatments had no effect on flowering phenology.

Pigments. The concentration of chlorophyll a and b and of carotenoids all increased significantly ($p < 0.001$) over the course of the season and were signficantly ($p < 0.001$) lower at elevated than ambient CO_2. There was no significant change in the chlorophyll a:b ratio, nor did elevated UV-B have any significant effect on any of the pigments measured.

Neither UV-B nor CO_2 resulted in any significant change in UV-B absorbing compounds in current year growth, but in second season growth (1995) UV-B absorbing pigments were significantly increased by elevated UV-B. The effect of UV-B was greater under ambient than elevated CO_2, although overall elevated CO_2 had no significant effect on pigments. The percentage induction of UV-B pigments by UV-B under ambient CO_2 was twice as large as that under elevated CO_2.

C:N ratio. Elevated CO_2 significantly decreased shoot nitrogen concentration and increased C:N ratio, but increased UV-B had no significant effect.

CO_2 × UV-B studies: field investigations Abisko

A more detailed analysis of this open top chamber experiment can be found in Gwynn-Jones et al. (this volume). A summary of the major findings is provided here.

Growth. There were few effects of the treatments on the growth of *Empetrum hermaphroditum* Hagerup, *Vaccinium myrtillus* L., *Vaccinium uliginosum* L. or *Vaccinium vitis-idaea* L. over the three years of the experiment, and only one UV-B × CO_2 interaction (on shoot branching rate in *Empetrum hermaphroditum*). In general, the effects of elevated carbon dioxide on growth were greater than those of enhanced UV-B radiation. No attempts have yet been made to investigate the effects of the treatments on below-ground processes.

Phenology and flowering. There was no effect of treatments on phenology. However, there was a marked stimulation of flowering and berry production in *Vaccinium myrtillus* in both 1994 and 1995 by the enhanced UV-B treatment. There was no effect of elevated CO_2 concentration and no UV-B × CO_2 interaction.

Physiology. The major response was a stimulation of leaf photosynthetic capacity in *Vaccinium myrtillus* by the elevated CO_2 treatment in 1993. This effect was not observed in 1994 or 1995, and presumably relates to a rapid down regulation of photosynthesis as the result

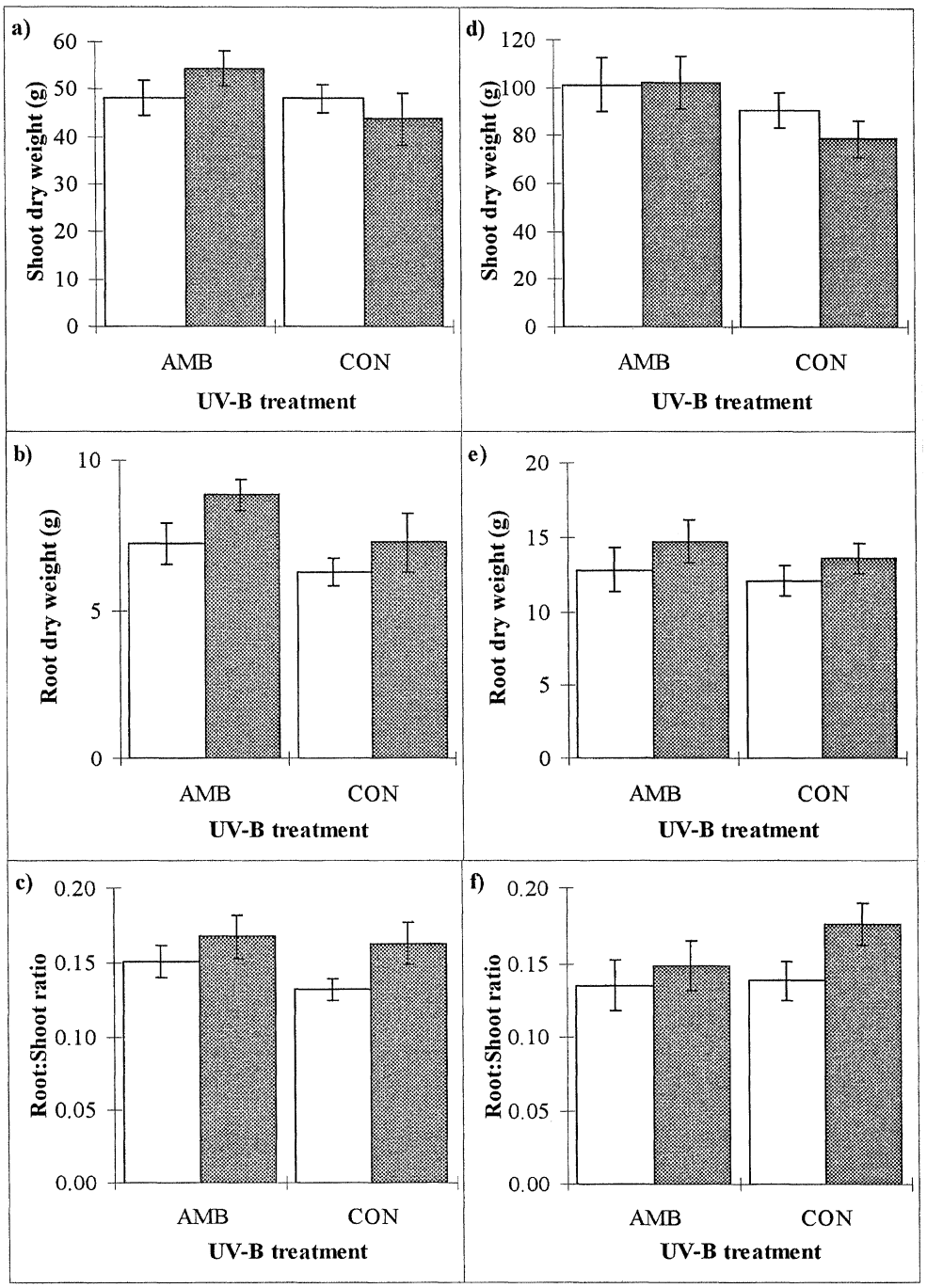

Figure 2. Shoot (a,d), and root (b,e) dry weight and root:shoot ratio (c,f) of *Calluna vulgaris* at ambient and elevated CO_2 (open and shaded columns respectively) and ambient (AMB) and elevated (CON) UV-B in September 1994 (a–c) and September 1995 (d–f). Means ± standard deviation.

of nutrient deficiency. These soils are strongly nitrogen limited (Gwynn-Jones et al. this volume).

Herbivory. In 1995, there were marked effects of UV-B treatment on insect herbivory. Herbivory on *Vaccinium myrtillus* was stimulated by enhanced UV-B radiation, whereas that on *Vaccinium uliginosum* was decreased. Surprisingly, given the likely change in leaf C:N ratio (see above), there was no effect of elevated CO_2 on herbivory.

Related studies. An earlier investigation of enhanced UV-B radiation alone (simulating a 15% reduction in the ozone layer) at another Abisko site showed some effects of the treatment on the growth and morphology of dwarf shrubs (Johanson et al. 1995b). The leaves of *Vaccinium vitis-idaea* became thicker (by 4–9% depending on year), those of the deciduous dwarf shrubs thinner (by 4–10% depending on year and species) as a consequence of UV-B enhancement. There were significant effects on leaf dry weight and leaf area only for *Vaccinium uliginosum*, for which enhanced UV-B caused increases by 29% for both parameters.

The relative longitudinal shoot growth (i.e. shoot growth under enhanced UV-B as divided by growth of the same shoot during the year before application of enhanced UV-B) was reduced in *Empetrum hermaphroditum* by 14% after one year and by 33% after two years. For the *Vaccinium* species no significant effects on relative longitudinal growth of shoots were found after one year of irradiation, but after two years there was a reduction by 27% for *V. vitis-idaea*, and in the two deciduous species, *V. myrtillus* and *V. uliginosum* the decrease ws 11% and 10% respectively (Johanson et al. 1995b).

Mediterranean shrubs and trees

A summary of the effects of UV-B enhancement on the species together with species characteristics is shown in Table 1.

Phlomis fruticosa L.
No UV-B radiation effects on leaf number and total leaf area were observed during the wet period of the year, up to mid-spring. However, an inhibition of new leaf development during mid to late spring and a premature fall of the older leaves during early summer, resulted in considerable decreases in leaf numbers and total leaf areas of the plants under supplemental UV-B radiation (Nikolopoulos et al. 1995). Photosynthetic rates were not affected and, in fact, the inhibitory UV-B radiation effects on morphology were observed at the period of maximum photosynthesis. In spite of considerable seasonal fluctuations in diffusive resistance to water vapour, relative water content, specific leaf mass, photochemical efficiency of PS II (F_v/F_m) and UV-B absorbing compounds, the differences in these variables between control and UV-B treated plants were negligible. However, an increase in the carotenoid to chlorophyll ratio was observed during the summer under UV-B supplementation, indicating a possible protective role for carotenoids. At final harvest, leaf, stem and root dry weights were considerably decreased by UV-B supplementation.

A glass house experiment confirmed the inhibitory effects of increased UV-B radiation but, in addition, a considerable suppression of growth in the absence of UV-B was also observed (Petropoulou et al. 1995a).

Although UV-B radiation may be important for normal growth of *P. fruticosa*, above ambient levels are strongly inhibitory. Since photosynthesis and stomatal function were not influenced, we may suggest a morphogenetic role for UV-B radiation.

Cistus creticus L.
UV-B radiation had no effects on leaf number, F_v/F_m, photosynthetic pigments, photosynthetic rates and relative water content but it caused a slight increase in internode length. Concerning UV-B absorbing compounds, a strong seasonal fluctuation was observed with peaks during the summer period of high UV-B irradiances. This seasonal pattern was followed by both external (epicuticular) and internal compounds. Supplemental UV-B radiation caused an additional increase in UV-B absorbing compounds during the first year of the study, but the effect was abolished during the following year.

Cistus creticus matures very early and this gave us the opportunity to study its reproductive effort during both of the years. Although the details of the reproductive behaviour differed from year to year, the final result was a UV-B radiation induced increase in the number of seeds per plant. During the first year, the number of pollinated flowers per plant were the same in the control and UV-B plots. However, the seed mass and seed number were considerably increased by supplemental UV-B radiation. During the second year, the seed mass was not affected, but the number of pollinated flowers and the seed number per plant were increased. *Cistus*

Table 1. A summary of enhanced UV-B radiation effects on vegetative growth of selected Mediterranean species.

Species used	Species characteristics	Duration of the experiment	Effects on the growth
Phlomis fruticosa	Malacophyllous, drought semi-deciduous shrub	1 year	Negative
Cistus creticus	Malacophyllous, drought semi-deciduous shrub	2 years	None
Nerium oleander	Evergreen sclerophyllous	10 months	None (watered) Slightly negative (water-stressed)
Pinus pinea	Sclerophyllous conifer	1 year	None (watered) Positive (water-stressed)
Pinus halepensis	Sclerophyllous conifer	1 year	None (watered) Positive (water-stressed)
Dittrichia viscosa	Malacophyllous, evergreen, ruderal	80 days (glasshouse)	None

creticus is entomogamous and bees are the principal pollinators. It is not known whether the supplemental UV-B radiation affected the plant *per se* or the behaviour of the bees. The optical properties of flower petals in the UV and visible region of the spectrum were not changed by supplemental UV-B radiation.

Germination of seeds of *C. creticus* is very low (ca 4%), but it can be considerably improved by breaking the seed dormancy at high temperatures (100 °C for 20 min). This may be related to the fact that *C. creticus* dominates recently burnt areas. It was found that the rates of seed germination after high temperature treatment did not differ between seeds from control or UV-B irradiated plants. We may conclude that although supplemental UV-B radiation has no effect on growth and photosynthesis in *C. creticus*, the effects on reproductive effort may have considerable ecological consequences.

Nerium oleander L. *(UV-B × increased summer precipitation)*
UV-B radiation had no effects on photosynthetic pigments, UV-B absorbing compounds, F_v/F_m and specific leaf mass. In plants receiving additional water, UV-B radiation had negligible effects on leaf number, total leaf area and total biomass. An inhibition of growth was, however, observed in water stressed plants during the summer. Under both water regimes, UV-B radiation induced an increase in the root/shoot ratio and in the cuticle thickness.

The inhibitory effects of UV-B radiation depend on the extent of the summer dry period. However, the changes in the root/shoot ratio and in the cuticle thickness may counter-balance the inhibitory effects of UV-B radiation and be critical for the survival of the plants during the Mediterranean summer with extremely limited water availability and high evaporative demand.

Pinus pinea L. and *Pinus halepensis* (Mill.) *(UV-B × increased summer precipitation)* No UV-B radiation effects on any measured parameter (including growth) were observed in plants receiving additional watering during the summer. It seems therefore that Mediterranean pines, under these conditions, are very resistant to enhanced UV-B radiation. Plants responded to water stress during the summer with extensive needle loss and reduced F_v/F_m, apparent quantum yield for O_2 evolution and photosynthetic capacity of the remaining needles. These effects were, however, less pronounced in plants receiving supplemental UV-B radiation. All the above parameters recovered to normal values for both control and UV-B plants after the autumn rains. The transient superiority of the UV-B treated plants during the summer, however, led to a considerable increase in their total biomass accumulation on a yearly basis (Petropoulou et al. 1995b). Repeated measurements of needle relative water content during the summer showed that it was consistently higher under UV-B supplementation, indicating improved water relations. Stomata were tightly closed during the dry period and, accordingly, we can not ascribe the improved water content of the needles to a UV-B effect on stomatal closure. Cuticle thickness, however, was considerably (ca 80%) increased under the combined effects

of UV-B radiation and water stress (Manetas et al. this volume). We may argue that when stomata are closed during a long, dry period, cuticular transpiration becomes extremely important and any restriction of its rate could be critical for the maintenance of adequate water content in the mesophyll.

Thus enhanced UV-B radiation may be beneficial for Mediterranean pines, alleviating the adverse effects of summer drought through a restriction in cuticular transpiration.

Dittrichia viscosa (L.) Greuter

This was a comparatively short glass house experiment with plants growing either in the absence of UV-B radiation or in the presence of UV-B corresponding to the daily doses received by the plants in their natural environment on May 5 under clear sky and normal column ozone thickness and 10 July under clear sky and 10% ozone depletion. *Dittrichia viscosa* was chosen as a test plant because it secretes an epicuticular material rich in flavonoids on the leaf surfaces. This material is water soluble, it is drained to the soil by rain and it is strongly allelopathic (Stephanou & Manetas 1995). However, UV-B radiation caused only a slight increase in the epicuticular UV-B absorbing compounds, while their allelopathic potential was not affected. Generally, *D. viscosa* was shown to be very resistant to UV-B radiation and the only observed effects were an increase in the carotenoid to chlorophyll ratio and a selective allocation of photosynthate to the production of assimilative surfaces at the expense of leaf thickness, with no net change in leaf biomass (Stephanou & Manetas 1996, this volume).

Discussion

The results from these studies employing an enhancement of UV-B radiation equivalent to a 15% reduction in the ozone layer suggest that the direct effects are not always and perhaps not usually detrimental to plant growth and physiology. Both positive and negative responses to enhanced UV-B in lichens, mosses, dwarf shrubs and trees have been reported, although some of these effects depend on interactions with other variables, notably summer precipitation. It is quite clear that the responses observed here are species specific, and may not be readily generalisable to plant functional types.

A caution on the interpretation of these data is that the longest experiments reported here are of three years duration. Whilst this is long in terms of crop physiological experimentation, it is extremely short in terms of the life spans of dwarf shrubs and trees. There is evidence that UV-B damage may have a cumulative effect. Johanson et al. (1995b) showed that the relative longitudinal growth (i.e. shoot growth under enhanced UV-B as divided by the growth of the same shoot during the year before application of UV-B) in *Empetrum hermaphroditum* was reduced by 14% after one year and by 33% after two years of treatment. Similarly, under controlled conditions reported here, although the effects on growth of *Calluna vulgaris* and *Vaccinium myrtillus* were generally small, they were cumulative over two years. These and other observations point at the necessity for long term field experimentation if the ecological importance of enhanced UV-B radiation is to be fully understood.

There is little evidence from the present study to support the hypothesis that because arctic vegetation has existed for at least tens of thousands of years in a low UV-B environment, it is peculiarly susceptible to any enhancement of this radiation as the result of ozone depletion. With the possible exception of the Greenland *Cladonia mitis* study (Heide-Jørgensen & Johnsen, 1995), the short-term effects (up to 3 years) of enhancement are rather small. Indeed, the comparison of *Vaccinium myrtillus* plants from the UK and from sub-arctic Sweden suggests similar responses.

A common thread running through these studies is that UV-B radiation enhancement may stimulate plant growth, development and physiological parameters. Thus flowering was stimulated in field studies of *Cistus creticus* and *Vaccinium myrtillus* and there was also a trend to increased flowering in *V. myrtillus* in controlled environment experimentation. Stimulation of physiological processes, e.g. the photochemical quantum yield of photosystem II in lichens, has also been observed. The mechanisms involved in these stimulations by UV-B radiation are far from clear at the present, but the end result particularly in the case of flowering and seed production may be of considerable ecological importance. Positive responses resulting from the interaction between UV-B radiation and water stress are perhaps the easiest to explain. The large increase in cuticle thickness as the result of enhanced UV-B radiation in Mediterranean *Pinus* species when combined with stomatal closure during drought ensures an improved needle water content.

Changes in biomass allocation as the result of UV-B treatment are also potentially of far reaching ecological importance. Thus the trend to a reduction in below-ground and an increase in above ground biomass in *Vaccinium myrtillus* as observed in controlled conditions in this study may have profound effects on the nutrient and water relations of this species if repeated in the field. This will be a future focus of field experimentation at Abisko. Increases in the R:S ratio as observed here in *Nerium oleander* may help at least to offset water deficits, and influence the effects of water stress on plant community composition.

Long-term field experimentation is essential if the ecological effects of enhanced UV-B radiation are to be fully evaluated. Although the interactive effects of enhanced UV-B radiation and elevated CO_2 appear rather small in the current investigation, this may represent the length of observations. For example changes in leaf quality as reflected in C:N ratios may be perturbed relatively quickly (see e.g. the Lancaster study), however, this may take several years to have any influence on decomposition processes and nutrient cycling. In arctic soils where nitrogen supply strongly limits growth (see Gwynn Jones et al. this volume) this could have far reaching effects. Gehrke et al. (1995) showed that exposure of *Vaccinium uliginosum* leaf litter to enhanced UV-B decreases the cellulose content, increases tannins and decreases the cellulose:lignin ratio. These workers also showed that UV-B exposure during decomposition decreased fungal colonization and total microbial respiration. Of three fungal species investigated, *Mucor hiemalis* and *Truncatella truncata* were more UV-B sensitive than was *Penicillium brevicompactum*. Changes in leaf chemistry as the result of elevated CO_2 exposure could markedly interact with UV-B effects on litter quality and decomposition processes (Rozema et al. 1996). Some support for the need for long-term experimentation comes from the fact that the insect herbivory changes as the result of increased UV-B radiation only became observable in the third year of the UV-B $\times$ CO_2 field experiment, and it is currently too early to predict with confidence its ecological significance.

The present experiments spanning more than 38° of latitude in Europe (including Greenland) suggest that dramatic and rapid effects of any thinning of the ozone layer on European vegetation are likely to be rare. Rather there will be subtle species-specific responses which may be markedly influenced by other climatic factors leading to slow but important interactions between species, and perhaps impacts on ecosystem structure and function far greater than on the component species.

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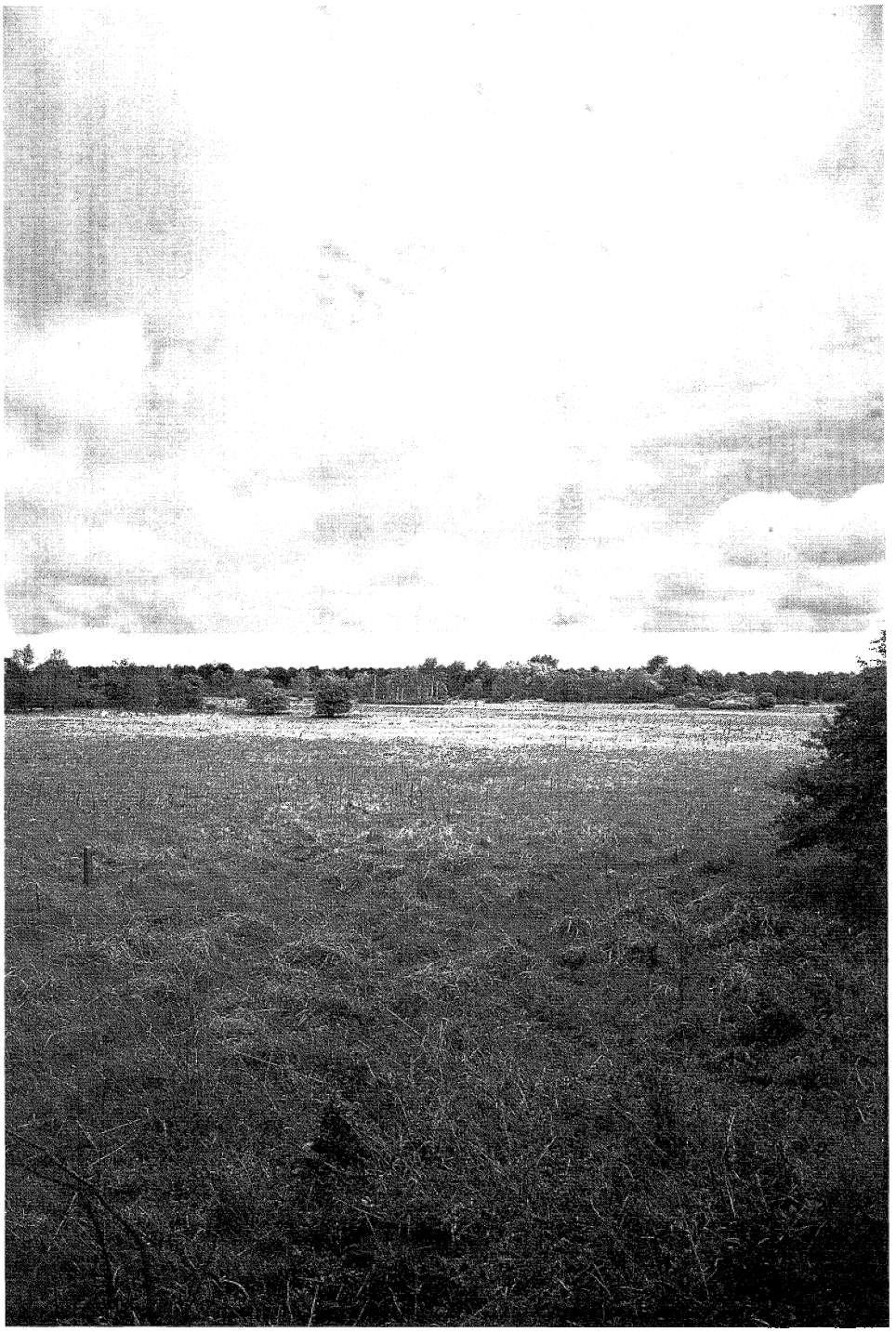

The dune grassland at Heemskerk, dominated by the grass species *Calamagrostis epigeios*. (Photograph: J. Rozema)

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Differential effects of elevated ultraviolet-B radiation on plant species of a dune grassland ecosystem

Marcel Tosserams, Erwin Magendans & Jelte Rozema
Department of Ecology and Ecotoxicology, Faculty of Biology, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

Key words: Dune grassland ecosystem, Plant growth, Ozone depletion, Solar radiation, UV-absorbing pigments, UV-B

Abstract

In a greenhouse study, plants of three monocotyledonous and five dicotyledonous species, which occur in a Dutch dune grassland, were exposed to four levels of ultraviolet-B (UV-B) radiation. UV-B levels simulated up to 30% reduction of the stratospheric ozone column during summertime in The Netherlands. Six of the plant species studied in the greenhouse were also exposed to enhanced UV-B irradiance in an experimental field study. In the field experiment plants either received the ambient UV-B irradiance (control) or an enhanced UV-B level simulating 15–20% ozone depletion during summertime in The Netherlands. The purpose of both experiments was to determine the response of the plant species to UV-B radiation and to compare results obtained in the greenhouse with results of the field experiment. Large intraspecific differences in UV-B sensitivity were observed in the greenhouse study. Total dry matter accumulation of monocotyledons was increased, while dry matter accumulation of dicotyledons remained unaffected or decreased. The increase in biomass production of monocotyledons at elevated UV-B was not related to the rate of photosynthesis but to alterations in leaf orientation. In the greenhouse study, UV-B radiation also affected morphological characteristics. Shoot height or maximum leaf length of five out of eight species was reduced. In the field study only one species showed a significantly decreased maximum leaf length at enhanced UV-B. Possible reasons for this discrepancy are discussed. The absorbance of methanolic leaf extracts also differed between species. UV absorbance of field-grown plants was higher than greenhouse-grown plants. In the greenhouse study, the highest UV-B level increased UV-B absorbance of some species. In the field study however, this stimulation of UV absorbance was not observed. In general, results obtained in the greenhouse study were similar to results obtained in the field study. Difficulties in extrapolating results of UV-B experiments conducted in the greenhouse to the field situation are discussed.

Introduction

Reductions in stratospheric ozone column thickness result in an increase of solar ultraviolet-B (UV-B) radiation at the earth's surface (Blumthaler & Ambach 1990, WMO 1994). Relative to 1960 the maximal ozone reductions expected at northern mid-latitudes are 13% in winter and spring and 7% in summer and fall. The reduction at southern latitudes will be approximately 11% on a year-round basis (WMO 1994). With full compliance to the Montreal Protocol and its amendments, these values will be reach around the year 1998, with a slow recovery of stratospheric ozone over the subsequent 50 years (WMO 1994).

The mentioned ozone reduction values imply an increase of the biologically effective UV-B (UV-B_{BE}) radiation for plant damage (generalised plant action spectrum, Caldwell 1971) of 15% (June) and 37% (December) at 52° N latitude. Since physiological and developmental processes of plants may already be affected by present-day sunlight (Caldwell et al. 1995; Tosserams et al. 1996; Visser et al. in press), a further increase of UV-B radiation at the earth's surface might

have considerable effects on agricultural crops as well as natural vegetation.

Since the first reports of potential stratospheric ozone reduction, UV-B effects on higher plants have been the subject of a considerable amount of research (Caldwell et al. 1995). Results obtained till now clearly show that plant species exhibit differential UV-B sensitivity. Negative as well as neutral and positive effects on plant performance have been reported (Krupa & Kickert 1989; Sullivan et al. 1992; Tezuka et al. 1993; Tosserams & Rozema 1995). The existing data also suggest that ecotypic differentiation may have developed. Natural plant species originating from high UV-B irradiance environments (low latitude, high altitude) may be less susceptible to elevated UV-B compared to plants from low UV-B irradiance environments (Robberecht et al. 1980; Sullivan et al. 1992; Rozema et al. in press). However, other studies showed no correlation between the origin of the cultivar and its UV-B sensitivity (Barnes et al. 1993; Dai et al. 1994). In addition, it has become evident that indoor studies compared to field experiments tend to exaggerate UV-B effects on plants due to experimental shortcomings, like low Photon Flux Density (PFD) limiting photorepair of UV-B damage and unrealistic levels of enhanced UV-B radiation (Caldwell & Flint 1994).

Although some generalisations can be made, it is as yet not possible to predict the UV-B response of plant species let alone ecosystems. Furthermore, only a small percentage of the studies performed so far, deal with plants from forests and non-agricultural systems (Teramura & Sullivan 1994). To provide a realistic assessment of the potential impact of increased solar UV-B irradiance on natural ecosystems, it is necessary to enlarge the knowledge of the UV-B sensitivity among natural plant species of different ecosystems. Recent field studies at ecosystem level suggest that photomorphogenic effects of UV-B rather than direct damage and decreased biomass production are important. Protective mechanisms (e.g. UV-B absorbing compounds, DNA damage repair and radical scavengers) are apparently efficient in mitigating direct UV-B damage under these conditions (Rozema et al. 1997). Results of a field study conducted in a natural dwarf shrub tundra in northern Sweden suggest that enhanced UV-B radiation over an extended time could result in species composition changes (Johanson et al. 1995). In addition, results of the latter field study (Gehrke et al. 1995) and a study in a dune grassland vegetation (Rozema et al. in press) both demonstrated that increased solar UV-B irradiance promotes litter decomposition by a direct photodegradative effect.

While field experiments are necessary for a more realistic assessment of the impact of enhanced UV-B irradiation on vegetation, greenhouse experiments offer information on mechanisms of UV-B action and can help to identify potentially UV-B sensitive plant species.

It is the objective of the present study to compare the effects of elevated UV-B radiation on the performance of several common monocotyledonous and dicotyledonous plant species of a Dutch dune grassland. In addition, a comparison of the UV-B response of plant species obtained in greenhouse experiments and the UV-B response of field grown plants is presented.

Materials and methods

Plant growth. Seeds of all plant species (Tables 1 and 2) were collected in a dune grassland area near Heemskerk (52° 30′ N, 4° 40′ E, Noord-Holland, The Netherlands). For both greenhouse and field experiments, seedlings were initially cultivated inside the greenhouse. A random sample of the collected seeds was sown on plastics trays (45 × 30 × 8 cm), containing commercial potting soil (Jongkind BV, Aalsmeer, The Netherlands). After germination individual seedlings were transplanted to pots (2.6 l), containing commercial potting soil and 3g l^{-1} soil Osmocote controlled release fertiliser (13-13-13-3, N-P-K-MgO, Grace Sierra International B.V., Heerlen, the Netherlands). After one week experiments were started. During the experiment plants were watered regularly.

Greenhouse experiments. In the greenhouse experiments eight plant species were studied (Table 1). Plants were grown at four UV-B levels. UV radiation was supplied by Philips 40W/12 fluorescent tubes in a square-wave fashion. UV-B tubes were attached at both sides of a 400W Philips HPI/T lamp that provided at least 200 μmol m^{-2} s^{-1} (Table 1) of photosynthetically active radiation (PAR), 16 hours daily. Maximal PAR received by the plants at clear sky days, comprising solar PAR transmitted through the greenhouse glazing and the supplemental PAR from lamps, was approximately 1000 μmol m^{-2} s^{-1}. Mean daily temperatures (day/night) and relative humidity data during the experimental periods are presented in Table 1. For the UV-B treatments, the radiation emitted by the 40W/12 fluorescent lamps was filtered with cellulose diacet-

Table 1. Overview of plant species and experimental conditions in the greenhouse experiment. RH: relative humidity; PAR: Photosynthetically Active Radiation. Temperature data represent the average minimum (night) and maximum (day) temperature ± SD during the UV treatment period. RH data shown, represent extremes measured during the UV treatment period.

Plant species	UV treatment (days)	Mean temperature (°C)		RH (%)		PAR (μmol m^{-2} s^{-1})
		min.	max.	min.	max.	
Monocotyledons						
Bromus hordeaceus L.	80	12.6±1.2	22.4±1.4	55	98	250
Bromus sterilis L.	45	16.6±1.0	22.9±0.9	24	97	200
Calamagrostis epigeios L. Roth	59	17.6±0.2	23.7±1.8	65	99	300
Dicotyledons						
Oenothera biennis L.	38	18.4±1.1	24.5±1.2	65	99	300
Plantago lanceolata L.	52	14.1±0.3	26.1±3.0	72	95	300
Rumex obtusifolius L.	37	12.4±1.7	23.5±2.6	33	97	200
Senecio jacobaea L.	59	13.1±1.3	23.4±3.0	30	96	200
Verbascum thapsus L.	43	15.1±1.2	30.3±3.0	60	99	300

ate (0.10 mm thick; Tamboer & CO. Chemie B.V., Haarlem, The Netherlands), which absorbs radiation below 290 nm (UV-B treated plants). For the control treatment, polyester foil (Mylar 0.13 mm thick), which absorbs all radiation below 313 nm was used instead. Because of photodegradation the cellulose diacetate was replaced twice a week and the Mylar foil once a week. All treatments were carried out in duplo. Plants were rotated between and within duplicate treatments every three days, to minimise site effects within the greenhouse compartment.

The spectral irradiance of the UV-B lamps was measured with a double-monochromator spectroradiometer (Optronic Model OL 752, Orlando, FL, USA). The spectroradiometer was calibrated for absolute responsivity against a 200 W tungsten–halogen standard lamp (Optronic Model OL 752–10, Orlando, FL, USA). Before the measurements, wavelength accuracy was checked with a dual calibration and gain check source module (Optronic Model OL 752–150, Orlando, FL, USA), by scanning a low-pressure mercury vapour lamp with a known peak emission at 312.9 nm.

The generalised plant action spectrum (Caldwell 1971), normalised at 300 nm, was used to determine the biologically effective UV-B dose (UV-B_{BE}). Weighted daily UV-B_{BE} dose was 0 for control plants and 4.6, 7.6 and 10.6 kJ m^{-2} day^{-1} for UV-B treated plants. Plants were irradiated from 1000 to 1600 h daily. The different UV-B treatments were obtained by adjusting the height of the UV-B lamps above the top of the plants. As plants grew, the height of the lamps was adjusted in such a way that UV-B levels remained the same during the course of the experiment. This was checked using a portable UVX radiometer equipped with a UVX 31 sensor (San Gabriel, CA, USA).

According to the empirical model of Green et al. (1980), the UV-B_{BE} dose of the UV treated plants simulate 0 (approximately ambient), 15 and 30% ozone reduction respectively, during clear sky conditions on June 21 in Amsterdam (52° N latitude).The long-term (1971-1993) mean stratospheric ozone thickness measured in June at the KMI in Ukkel (Belgium, 51° N latitude) and the RIVM in Bilthoven (The Netherlands; 52° N latitude), was used for the model calculations.

Field experiments. For these experiments a total of six plant species were used (Table 2). After initial cultivation in the greenhouse, seedlings were transferred to the experimental field. The pots were placed in small containers (3 l) that were dug into the soil. In this way it was possible to reduce evaporation from the soil on sunny days, while it remained possible to rotate plants to minimise site effects. Pots were rotated once every two weeks. Growth periods, duration of UV-B treatment and minimum and maximum temperatures during the experiment are presented in Table 2. The daily global irradiance (290–3000 nm) during the course of the field experiments is plotted in Figure 1.

As in the greenhouse experiments, plants were irradiated with additional UV-B emitted by Philips 40W/12 fluorescent tubes that were suspended above the plants. UV-B irradiance was provided in a square wave fashion. Fluorescent tubes operated from 1130

Table 2. Overview of plant species, growth period and experimental conditions in the field experiment. Temperature data represent the average minimum (night) and maximum (day) temperature ± SD during the UV treatment period.

Plant species	Field growth period	Mean temperature (°C)		UV treatment (days)
		minimum	maximum	
Monocotyledons				
Bromus hordeaceus L.	20-07 - 01-09 1993	11.1±2.4	19.4±2.0	44
Bromus sterilis L.	02-06 - 21-07 1994	11.6±2.9	22.0±4.3	43
Dicotyledons				
Oenothera biennis L.	27-05 - 29-07 1994	12.3±3.3	22.9±4.7	52
Plantago lanceolata L.	24-06 - 12-08 1993	11.9±2.2	19.7±1.7	35
Rumex obtusifolius L.	27-05 - 04-08 1994	12.7±3.4	23.5±4.9	58
Verbascum thapsus L.	24-06 - 25-08 1993	11.4±2.3	19.6±2.0	48

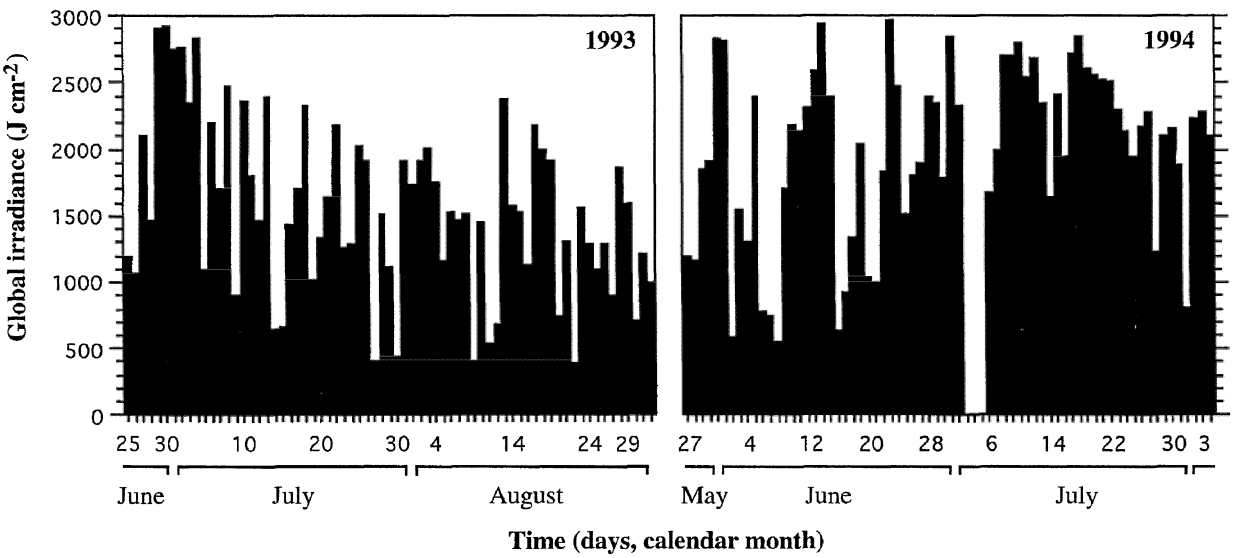

Figure 1. Daily global irradiance (290–3000 nm) during the course of the field experiments of 1993 and 1994 measured at 'Schiphol' airport meteorological station (Amsterdam, The Netherlands).

to 1530 h daily. Before the experiments started, the spectral irradiance of the lamps was measured with the OL model 752 spectroradiometer. In the field experiments two UV-B treatments were applied. Control plants received the ambient solar UV-B fluence and supplemental radiation from polyester foil (0.13 mm thick Mylar) filtered UV-B tubes. The UV-B treated plants received the solar UV-B fluence supplemented with 2.5 kJ m^{-2} day^{-1} UV-B_{BE} (generalised plant action spectrum normalised at 300 nm). According to the model of Green et al. (1980), the maximal UV-B_{BE} dose of the UV treated plants simulated between 15 and 20% ozone reduction (Figure 2), during the total experimental period assuming clear sky conditions. As plants grew, the height of the lamps was adjusted to ascertain similar UV-B levels during the course of the experiment.

Growth analysis. After the treatment period (Tables 1 and 2), plants were harvested. Dry weights of leaves (leaf blades and leaf sheaths for monocotyledons), stems, roots and reproductive tissues (if present) were measured after drying for at least 48 hours at 70 °C. The number of shoots, leaves and inflorescence per plant as well as plant height were determined. Leaf area was

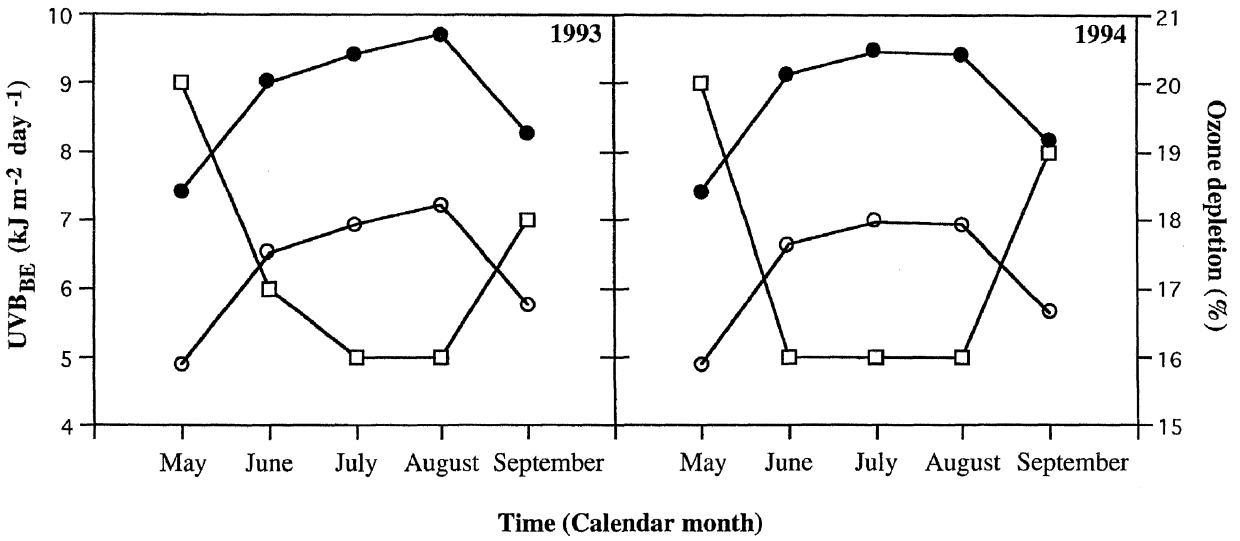

Figure 2. Ambient (○) and elevated (●) biologically effective UV-B (UV-B_{BE}) levels and the simulated percentage stratospheric ozone reduction (□) during the field experiment of 1993 and 1994, assuming clear sky conditions throughout the experiment. Ambient and elevated UVB_{BE} levels were calculated using the model of Green et al. (1980) and the generalised plant action spectrum (Caldwell 1971) normalised at 300 nm. Model calculations were performed using the average monthly ozone column thickness during the experimental period. Ozone column data were provided by the RIVM in Bilthoven (The Netherlands, 52° N).

measured with a Licor 3100 area meter (Li-Cor Inc., Lincoln, Nebraska, USA).

Pigment analysis and net photosynthesis. During harvest, leaf samples for chlorophyll and UV absorbing pigment determination were taken. Chlorophyll contents of young fully expanded leaves were estimated using the method of Arnon (1949). Chlorophyll samples were either measured immediately or stored at –20 °C. UV absorbing pigments were extracted from fresh leaf samples of the youngest fully developed leaf with a methanol:water:hydrochloric acid solution (79:20:1 v/v). Samples were used immediately or stored overnight (8 °C). All samples were heated (90 °C) for one hour, after which the absorbance of the solution at 300 nm was recorded using a Perkin-Elmer Lambda 15 UV/VIS scanning spectrophotometer.

Net photosynthesis of the youngest fully developed leaf of all plant species was measured during the last week of the treatment period. Measurements were conducted in a climate room using LCA3 equipment (Analytical Development Co., Hoddesdon, Herts, UK).

Statistical analysis. All data were subjected to a one-way analysis of variance (ANOVA; Sokal & Rohlf 1981). Dry weight and leaf area data were transformed to their natural logarithms.

Results

Greenhouse experiments

The effect of UV-B radiation on total biomass accumulation and leaf area parameters varied among plant species (Table 3). Total dry weight production of the monocotyledonous species was increased by UV-B radiation. For *B. hordeaceus* this increase was most pronounced for plants grown at 7.6 kJ m^{-2} day^{-1} UV-B_{BE}. The relative increase was similar for below and aboveground plant parts, as shown by an unaffected SRR. Total leaf area of control plants was significantly less compared to UV-B treated plants (Table 3). A significant increase of total biomass was also observed in *B. sterilis* at 4.6 kJ m^{-2} day^{-1} UV-B_{BE}. However, SRR declined because at this UV-B_{BE} level root dry weight was stimulated by 24%, while shoot dry weight was only raised by 4%. Although total leaf area remained unaffected, area per leaf was reduced. This is in accordance with the observed increase in the number of leaves for this species (Table 4). In addition, SLA as well as the LAR were reduced at 4.6 kJ m^{-2} day^{-1} UV-B_{BE}. Although not affected significantly, the largest increase in biomass was observed for *C. epigeios* (20% at the highest UV-B_{BE} treatment). As for *B. sterilis* root biomass increased relatively more than shoot

Table 3. The effect of four different levels of biologically effective UV-B radiation (UV-B_{BE}) on growth characteristics of plant species in the greenhouse experiment. Of *O. biennis* only the dry weight of the tap root and primary roots was determined. The percentage change indicates the change in total dry biomass. SRR: Shoot to Root Ratio; SLA: Specific Leaf Area; LAR: Leaf Area Ratio. Values represent means of 18 replicates. Means marked with different letters are significantly different at $p \leq 0.05$.

Plant species	UVB_{BE}	Dry biomass (g)			Change	SRR	Total leaf area	Area $leaf^{-1}$	SLA	LAR
	(kJ m^{-2} day^{-1})	Root	Shoot	Total	(%)		(cm^2)	(cm^2)	(m^2 kg^{-1})	(m^2 kg^{-1})
B. hordeaceus	0	4.47	13.7	18.2a	–	3.29	3,081a	15.2	36.1	17.0ab
	4.6	4.61	14.9	19.5ab	+7	3.35	3,388b	16.3	36.5	17.6ab
	7.6	5.31	15.0	20.4b	+12	2.94	3,374b	17.0	35.5	16.7a
	10.6	4.49	14.0	18.4ab	+1	3.24	3,391b	16.0	37.4	18.5b
B. sterilis	0	3.48a	9.30ab	12.8a	–	2.71a	2,851	16.0a	47.5a	22.3ac
	4.6	4.33b	9.63a	14.0b	+9	2.24b	2,892	14.5b	45.5b	20.8b
	7.6	3.95ab	8.89b	12.8a	0	2.30b	2,760	14.2b	46.1ab	21.5ab
	10.6	3.59a	9.15ab	12.7a	0	2.60a	2,904	14.6ab	47.5a	22.9c
C. epigeios	0	1.38	3.56	4.95	–	2.78	607	10.2	25.4	12.2
	4.6	1.63	3.63	5.26	+6	2.47	603	9.6	24.4	11.3
	7.6	1.76	3.95	5.71	+15	2.34	646	10.1	24.2	11.4
	10.6	1.87	4.06	5.93	+20	2.24	645	9.1	23.3	11.0
O. biennis	0	2.42	12.5	14.9	–	5.29	2,301	46.9	18.4	15.5
	4.6	2.40	12.6	15.1	+1	5.36	2,290	45.7	18.6	15.6
	7.6	2.42	12.1	14.5	–3	5.15	2,311	43.4	19.2	15.9
	10.6	2.32	12.0	14.3	–4	5.39	2,233	42.5	18.7	15.7
P. lanceolata	0	1.78	7.03	8.81	–	4.31	1,337ab	15.8	21.4	15.4
	4.6	1.86	7.16	9.02	+2	4.17	1,347a	15.7	20.4	15.2
	7.6	1.54	6.30	7.84	–11	4.48	1,194ab	14.0	21.2	15.6
	10.6	1.40	5.61	7.01	–20	4.35	1,050b	13.3	20.9	15.0
R. obtusifolius	0	3.23	9.41	12.6	–	2.98a	2,859a	66.0	43.2	22.6
	4.6	3.76	8.86	12.6	0	2.43b	2,669ab	64.9	41.6	21.1
	7.6	3.26	8.39	11.6	–8	2.66ab	2,403b	61.7	40.6	20.7
	10.6	3.85	8.71	12.6	–1	2.33b	2,575ab	60.9	41.2	20.5
S. jacobaea	0	4.21a	7.34a	11.6a	–	1.80	1,647a	31.3	21.9	14.2
	4.6	3.38b	6.92a	10.3a	–13	2.11	1,515ab	30.5	21.8	14.7
	7.6	3.08b	5.68b	8.8b	–24	1.92	1,272bc	29.5	22.6	14.6
	10.6	2.95b	5.45b	8.4b	–27	1.93	1,188c	30.5	21.8	14.2
V. thapsus	0	2.22	10.2	12.5	–	4.77	3,092	110	34.3	25.2a
	4.6	2.76	11.2	14.0	+12	4.27	3,118	108	32.2	22.7b
	7.6	2.39	9.8	12.2	–2	4.32	2,984	110	35.4	25.0ab
	10.6	2.29	9.7	12.0	–4	4.34	2,847	105	34.1	24.1ab

biomass (36% and 14% respectively). All monocotyledons allocated less biomass to the leaf sheath at enhanced UV-B_{BE} (data not shown).

Of the studied dicotyledons only biomass production of *S. jacobaea* was significantly affected by UV-B. At the highest UV-B_{BE} dose a decrease in total biomass accumulation of 27% was observed for this species. SRR remained unaffected because root and shoot biomass decreased to the same extent. In addition, leaf area was reduced up to 28% by enhanced

Table 4. The effect of four different levels of biologically effective UV-B radiation (UV-B_{BE}) on morphological characteristics of plant species in the greenhouse experiment. Length should be read as shoot length for monocotyledons and as maximal leaf length for dicotyledons. Values represent means of 16-18 replicates. Means marked with different letters are significantly different at $p \leq 0.05$. nm=not measured.

Plant species	UV-B_{BE} (kJ $m^{-2}day^{-1}$)	Total number of			Length (cm)
		Leaves	Shoots	Inflorescence	
B. hordeaceus	0	206	43.6	–	68.5
	4.6	212	46.2	–	70.8
	7.6	204	45.5	–	69.5
	10.6	217	45.4	–	69.7
B. sterilis	0	180a	50.4	–	59.0a
	4.6	200b	54.0	–	50.7b
	7.6	195ab	51.5	–	48.8b
	10.6	201b	53.9	–	50.5b
C. epigeios	0	59	19.0	–	71.9
	4.6	63	20.4	–	68.7
	7.6	66	21.8	–	72.9
	10.6	72	24.9	–	71.7
O. biennis	0	50	12.6	–	36.2
	4.6	51	11.7	–	36.8
	7.6	54	12.7	–	36.7
	10.6	53	12.4	–	36.7
P. lanceolata	0	90	nm	16.2	32.2ab
	4.6	91	nm	14.7	33.3a
	7.6	89	nm	12.8	31.8ab
	10.6	82	nm	12.5	29.3b
R. obtusifolius	0	43	8.5	–	41.1a
	4.6	41	8.4	–	37.9b
	7.6	40	8.3	–	36.2b
	10.6	43	8.7	–	35.9b
S. jacobaea	0	55	8.4	–	25.8a
	4.6	52	8.4	–	25.3ab
	7.6	47	7.7	–	23.5b
	10.6	42	6.9	–	23.6b
V. thapsus	0	28	–	–	44.4a
	4.6	29	–	–	42.8ab
	7.6	27	–	–	42.8ab
	10.6	27	–	–	41.8b

UV-B irradiance. Although total biomass production of *P. lanceolata* was reduced by 20% at the highest UV-B_{BE} level, differences between groups were not significant (p=0.07). However, when comparing plants grown at 4.6 kJ m^{-2} day^{-1} with plants of the highest UV-B_{BE} level, leaf dry weight as well as total leaf area were significantly reduced (p=0.02 and p=0.05 respectively). Although biomass accumulation was not

affected by enhanced UV-B radiation, UV-B irradiated *R. obtusifolius* plants showed a decreased petiole weight (p=0.02, data not shown) as well as a decreased total leaf area when compared to control (no UV-B) plants. In addition, more biomass was allocated to the root system as indicated by a decrease of the SRR. *O. biennis* and *V. thapsus* remained largely unaffected by UV-B irradiance. However, shoot dry weight of *V. thapsus* plants grown at 4.6 kJ m^{-2} day^{-1} tended to be higher compared to the other UV-B treatments (p=0.054). Although not all significantly, it should be noted that four out of eight species tested reacted with a slight total biomass increase at the lowest UV-B_{BE} irradiance level when compared to control plants.

Morphological changes as a result of UV-B enhancement were limited to an increase in the number of leaves for *B. sterilis* (Table 4). Although the increase in the number of leaves for *C. epigeios* and the decrease for *S. jacobaea*, resulting from an increase of the UV-B_{BE} level were relatively high (23%), these differences were not found to be significant. The monocotyledon *B. sterilis* and all dicotyledons except *O. biennis* reacted to enhanced UV-B radiation with a reduced maximal shoot length and reduced leaf length (Table 4). It was observed that the growth form of control plants of *C. epigeios* and *B. hordeaceus* was more erect than the appearance of UV-B treated plants (Figure 3).

Net photosynthetic rate of all species was not affected by UV-B enhancement (data not shown). In addition, chlorophyll content (w:w) of all species except for *B. hordeaceus* and *R. obtusifolius* remained unaffected (data not shown). *B. hordeaceus* plants grown at 4.6 kJ m^{-2} day^{-1} UV-B_{BE} had a higher chlorophyll content than plants grown at 7.6 kJ m^{-2} day^{-1} UV-B_{BE}. The highest UV-B treatment raised the chlorophyll content by 19%, which was largely the result of an increase in chlorophyll-a content (data not shown).

UV absorbance of control plants varied among species (Figure 4). As a result of enhanced UV-B levels, the UV absorbance at 300 nm (Figure 4) was increased for *B. hordeaceus*, *B. sterilis* and *V. thapsus* and a trend towards an increased was also observed for *P.lanceolata* (p=0.06). Significant differences however, were only obtained between control plants and plants grown at 10.6 kJ m^{-2} day^{-1}. Furthermore, it should be noted that absorbance at 310 nm was always higher compared to the absorbance at 300 and 320 nm (data not shown).

Field experiments

Biomass accumulation of all species except *P. lanceolata* remained unaffected by an enhancement of the ambient UV-B_{BE} level with 2.5 kJ m^{-2} day^{-1} (Table 5). *P. lanceolata* plants grown at enhanced UV-B radiation had a smaller total shoot biomass (including reproductive tissue) compared to control plants receiving the ambient UV-B level. Total biomass production showed a tendency to a decrease too (p=0.07). However, SRR was not significantly altered by UV-B enhancement. Furthermore, total leaf area was reduced by 23%, which was caused by a reduction in area per leaf (25%) rather than a decrease in the number of leaves (Table 6). *B. hordeaceus* plants grown at elevated UV-B, allocated relatively more biomass to the root system as indicated by the reduced SRR.

As in the greenhouse experiments, morphological characteristics of the studied species remained largely unaffected by UV-B enhancement (Table 6). Only maximal leaf length of *P. lanceolata* was reduced by elevated UV-B radiation. Moreover, a reduction of 22% (p=0.007) in the maximal length of the inflorescence was also observed at elevated UV-B (data not shown). Leaf length of *B. sterilis* also tended to be reduced (p=0.08).

Net photosynthetic rates were not influenced by an enhancement of UV-B. Similar to the greenhouse experiments only the chlorophyll content of *B. hordeaceus* and *R. obtusifolius* was affected by enhanced UV-B (data not shown). Chlorophyll content of *B. hordeaceus* plants grown at enhanced UV-B was reduced by 8%. For *R. obtusifolius* in contrast to results obtained in the greenhouse, a reduction in total chlorophyll content of 13% was observed (data not shown).

Although UV absorbance of methanolic leaf extracts varied among species (Figure 5), no effect of UV-B enhancement was found. UV absorbance of *O. biennis* plants was two to three times higher than the absorbance of the other species. In addition, for all species except *B. hordeaceus* UV absorbance of field grown plants was higher than the absorbance of the same species in the greenhouse experiments (Figure 4).

Discussion

The results of the greenhouse experiments clearly show a considerable degree of variability in UV-B sensitivity

Table 5. The effect of UV-B radiation on growth characteristics of plant species in the field experiment. Of *O. biennis* only the dry weight of the tap root and primary roots was determined. The percentage change indicates the change in total dry biomass. SRR: Shoot to Root Ratio; SLA: Specific Leaf Area; LAR: Leaf Area Ratio. Values represent means of 16–20 replicates. For *B. sterilis* the number of replicates is 12 for leaf area related parameters. Means marked with different letters are significantly different at $p \leq 0.05$.

Plant species	UV-B treatment	Dry biomass (g)			Change	SRR	Total leaf area	Area leaf^{-1}	SLA	LAR
		Root	Shoot	Total	(%)		(cm^2)	(cm^2)	($m^2\ kg^{-1}$)	($m^2\ kg^{-1}$)
B. hordeaceus	ambient	2.43	5.56	7.99	–	2.34a	1025	9.09	30.4	12.8
	elevated	2.61	5.01	7.62	–5	1.95b	967	8.58	32.2	12.7
B. sterilis	ambient	9.88	12.3	23.3	–	1.29	2249	7.12	31.5	9.72
	elevated	8.98	12.1	22.1	–5	1.37	2059	6.52	30.4	9.67
O. biennis	ambient	2.49	23.3	26.2	–	9.37	2117	10.8	13.7	8.13
	elevated	2.60	22.9	25.9	–1	8.85	2191	11.6	13.8	8.53
P. lanceolata	ambient	3.17	8.56a	11.7	–	2.79	1132a	15.9a	16.7	9.72
	elevated	2.86	6.92b	9.8	–16	2.49	876b	12.0b	15.4	9.21
R. obtusifolius	ambient	29.6	11.5	41.7	–	0.41	1090	53.7	23.7	2.60
	elevated	28.1	12.7	40.8	–2	0.46	1169	49.5	22.5	2.85
V. thapsus	ambient	5.40	12.0	17.4	–	2.35	1380	53.3	13.3	8.2
	elevated	5.43	11.4	16.9	–3	2.27	1315	52.9	12.8	7.9

Table 6. The effect UV-B radiation on morphological characteristics of plant species in the field experiment. Length should be read as shoot length for monocotyledons and as maximal leaf length for dicotyledons. Values represent means of 16-20 replicates. Means marked with different letters are significantly different at $p \leq 0.05$.

Plant species	UV-B treatment	Total number of			Length
		Leaves	Shoots	Inflorescence	(cm)
B. hordeaceus	ambient	117	40.7	–	43.5
	elevated	114	38.5	–	42.7
B. sterilis	ambient	314	75.4	–	38.4
	elevated	325	76.5	–	36.4
O. biennis	ambient	202	–	60	68.5
	elevated	209	–	59	65.4
P. lanceolata	ambient	75	–	23	23.2a
	elevated	75	–	20	21.1b
R. obtusifolius	ambient	20	5.7	–	45.2
	elevated	23	6.0	–	43.7
V. thapsus	ambient	26	–	–	26.2
	elevated	25	–	–	25.7

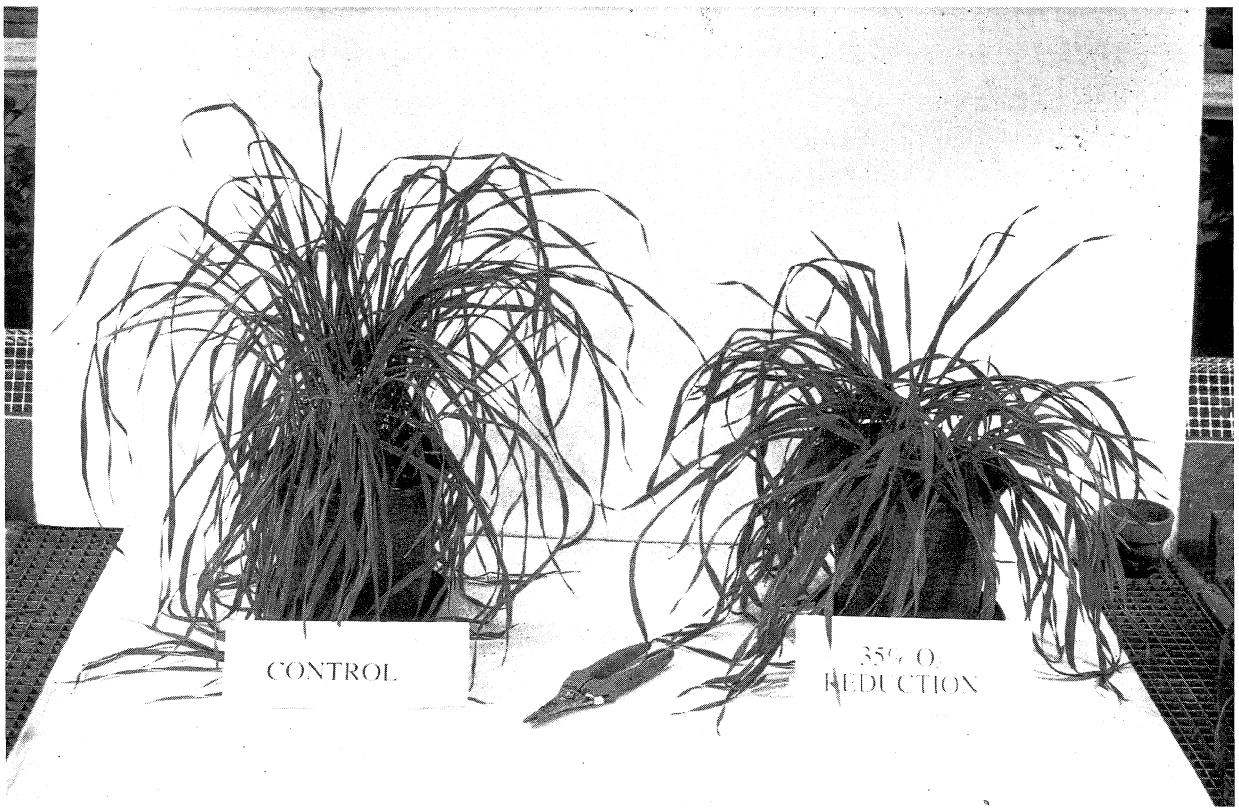

Figure 3. The effect of UV-B radiation on leaf orientation in *Bromus* species. Left: more erectophilous leaf inclination at 0 kJ m^{-2} day^{-1} $UV\text{-}B_{BE}$. Right: more planophilous leaves at 10.6 kJ m^{-2} day^{-1} $UV\text{-}B_{BE}$.

among the plant species that were studied. Interspecific variability has been reported in many studies and reviews (Teramura 1983; van de Staaij et al. 1990; Bornman & Teramura 1993; Musil 1995). Moreover, sizeable intraspecific response differences among populations and cultivars of a single species were also observed frequently (Biggs et al. 1981; Teramura & Murali 1986; Barnes et al. 1993; Dai et al. 1994). Total biomass accumulation of the monocotyledons was increased by enhanced UV-B radiation. However, no clear dose-response relationship was observed. An increase of total plant biomass due to UV-B radiation has been reported by Dai et al. (1994) for several rice cultivars and by Tezuka et al. (1993) for tomato and radish plants. Tosserams & Rozema (1995), reported an increased biomass of 33% for *C. epigeios* plants exposed to enhanced UV-B. The same trend was observed in the present experiment. Although the mechanism behind a positive response to enhanced UV-B is still unclear, results of the experiment conducted by Tosserams & Rozema (1995), suggested that the more planophilous leaves at elevated UV-B, allowed the plants to use the available PAR emitted by the HPI/T lamps more efficiently for growth than plants receiving no UV-B with more erectophilous leaves. Bornman & Teramura (1993) suggested that differences in leaf behaviour as a result of UV-B irradiance may be correlated to the differential UV-B sensitivity of soybean cultivars. In addition, Tendel & Häder (1995) recently showed that UV-B impaired phototropic and gravitropic reactions of shoots of *Triticum aestivum*. The present experiment also shows that UV-B influences leaf inclination of the studied monocotyledons. Leaves of control plants receiving no UV-B were oriented more vertically compared to irradiated plants. As a consequence, more PAR (emitted by the HPI/T lamps) was intercepted by the leaves of UV-B irradiated plants. As photosynthesis and plant growth of the monocotyledonous species was not negatively affected by the different UV-B treatments in the present experiment, total plant biomass of these species might have increased due to an increase in plant carbon fixation.

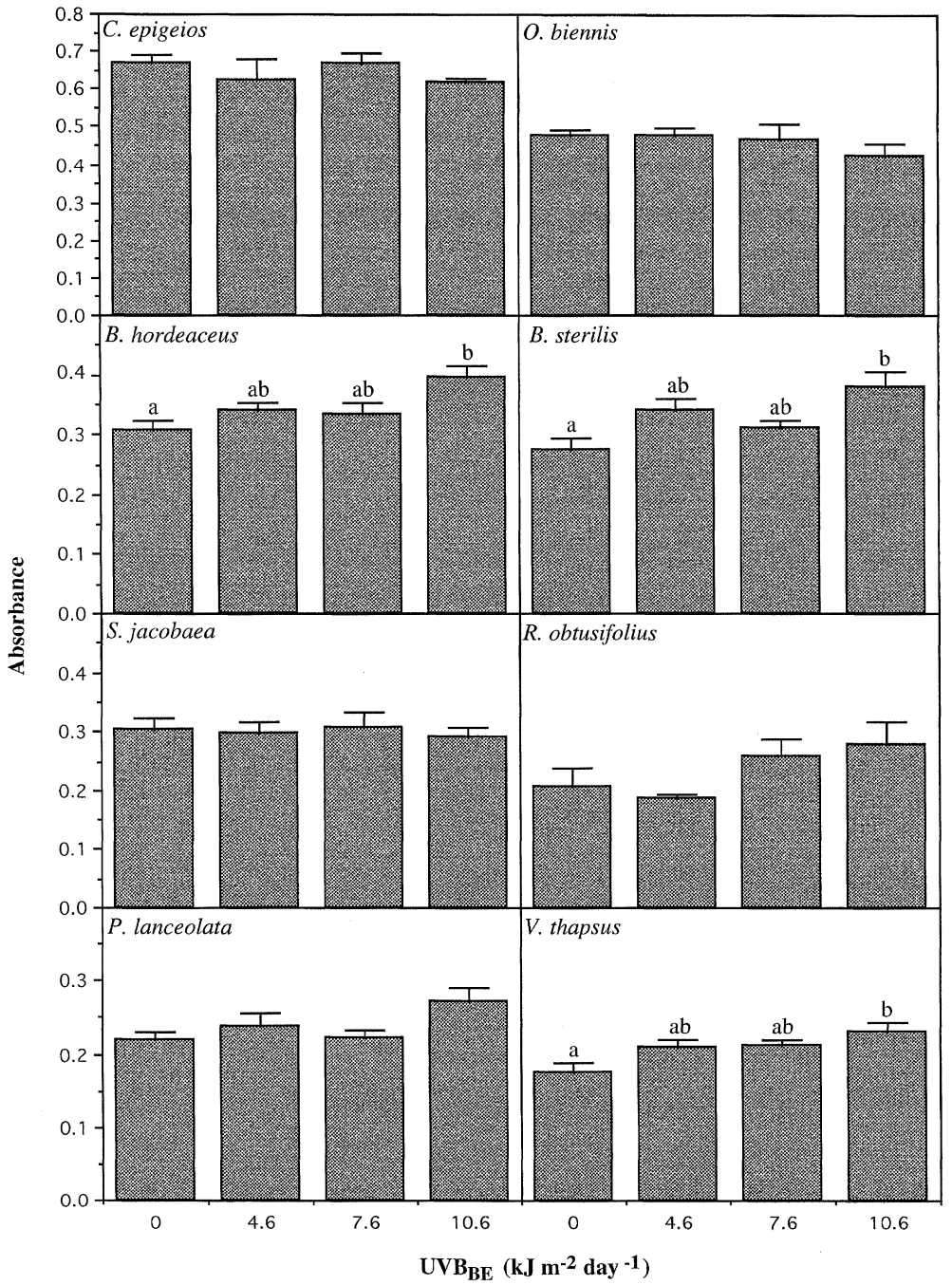

Figure 4. The effect of four levels of biologically effective UV-B (UV-B_{BE}) on UV absorbance of methanolic leaf extracts of eight dune grassland plant species studied in the greenhouse experiment. Absorbance was measured at 300 nm and recalculated to the absorbance of 1 mg fresh leaf per ml extract. Values are means of 5–6 replicates ± SEM. Bars marked with different letters are significantly different at $p \leq 0.05$.

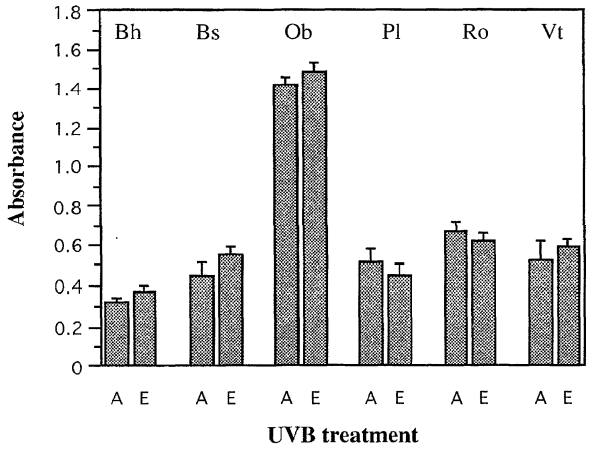

Figure 5. The effect of UV-B radiation on UV absorbance of methanolic leaf extracts of six dune grassland plant species studied in the field experiment. Absorbance was measured at 300 nm and recalculated to the absorbance of 1 mg fresh leaf per ml extract. Ambient UV-B (A); Elevated UV-B (E). *Bromus hordeaceus* (Bh); *Bromus sterilis* (Bs); *Oenothera biennis* (Ob); *Plantago lanceolata* (Pl); *Rumex obtusifolius* (Ro); *Verbascum thapsus* (Vt). Values are means of 6 replicates (*V. thapsus* ambient UV-B = 4 replicates) ± SEM.

In the outdoor experiments this phototropic response as well as the increase of total plant biomass was not observed. However, an important difference compared to the greenhouse experiments is that control plants in the field experiments received the ambient solar UV-B radiation. This possibly explains the absence of a visible phototropic response in the field as the ambient UV-B level might be sufficient to trigger this phototropic response. Indeed, during the greenhouse experiments it was observed that plants receiving the lowest UV-B treatment (comparable to the ambient UV-B level) had a similar leaf orientation than plants receiving the highest UV-B level. In addition, the outdoor PAR level was significantly higher than the PAR level in the greenhouse. As a consequence, differences in total plant photosynthesis are expected to be less between treatments, further reducing the ultimate effect on growth due to potential UV-B induced changes in leaf orientation.

Negative effects of UV-B on biomass accumulation were observed for *S. jacobaea*. This species also exhibits a reduced germination rate as well as a reduced percentage of germinated seeds when exposed to enhanced UV-B radiation (Tosserams et al. 1997). Unfortunately this species was not included in the field experiment. Therefore, further experimentation in the dune grassland ecosystem is necessary to elucidate potential UV-B sensitivity of this species in its natural environment, for it is known that other environmental factors like nutrient deficiency or water shortage (Bornman & Teramura 1993) may alter or ameliorate the UV-B response of plant species.

UV-B radiation is known to cause damage to most components of the photosynthetic machinery. However, in general this inhibition is mostly observed in greenhouse or growth chamber studies with low PAR and unrealistic UV-B_{BE} levels (Fiscus & Booker 1995). In general, negative effects of UV-B on plant growth and physiology are more pronounced when PAR intensity is low (Teramura 1980; Cen & Bornman 1990; Caldwell et al. 1994), most probably because only limited photoprotection and/or photorepair of UV-B induced damage is possible in this situation.

The present study indicates that morphological alterations as well as growth promotion or reduction occurred without any apparent alteration of leaf carbon assimilation. This is in accordance with findings of Barnes et al. (1990), who observed a similar response in several monocotyledonous and dicotyledonous plant species. In addition, they argued that plant morphology is a more sensitive indicator of realistic UV-B radiation exposure than either leaf photosynthesis or total biomass production at least under conditions of relatively high PAR, which is in agreement with the present greenhouse experiment. UV-B irradiated *B. sterilis* plants produced more smaller leaves. UV-B treated *C. epigeios* plants also tended to develop more leaves and shoots, which is in agreement with earlier findings (Tosserams & Rozema 1995). In addition, a tendency towards reduced leaf production in *S. jacobaea* was also observed. However, shoot height (monocotyledons) and maximal leaf length (dicotyledons) showed the largest response to UV-B radiation. Barnes et al. (1990), showed that monocotyledons, compared to dicotyledons, were more morphologically responsive to UV-B. In contrast to these findings, dicotyledons appeared to be more responsive in the present experiment.

The observed reductions in leaf length and shoot height, which did not appear to be related to biomass accumulation, may reflect specific photomorphogenic responses of plants to UV-B mediated by UV-B photoreceptors (Lercari et al. 1990; Ballaré et al. 1991; Ballaré et al. 1995). Results of Ballaré et al. (1995) suggest the involvement of a flavin chromophore in the elicitation of inhibition of hypocotyl elongation in tomato plants grown at enhanced UV-B. Another potential mechanism for UV-B induced growth inhibition may be a reduction or destruction of auxin (Kulandaivelu

et al. 1989; Ziska et al. 1993; Ros & Tevini 1995). Ros & Tevini (1995) demonstrated that at low white light irradiances, the inhibition of elongation growth in seedlings and hypocotyl segments of sunflower could be explained by a reduction of indole-3-acetic acid (IAA) concentration and by the formation of growth inhibiting IAA photoproducts.

The importance of UV absorbing pigments in protecting plants against deleterious effects of UV-B radiation has been reported extensively (Caldwell et al. 1983; Li et al. 1993; Day et al. 1994; Lois & Buchanan 1994; van de Staaij et al. 1995). After being exposed to UV-B radiation, many plants species show increased amounts of UV absorbing pigments in epidermal cells, thereby reducing the amount of UV reaching underlying tissues. Stimulation of the biosynthesis of these compounds however, may differ widely among species (Bornman & Teramura 1993). This differential stimulation of UV absorbance was also observed in the greenhouse experiment. UV absorbance of leaf extracts was increased only in both *Bromus* species and *V. thapsus*. In the field experiment however, no differences between the ambient control and enhanced UV-B treatment were observed. The maximal absorbance at 300 nm also differed considerably among species. This is in accordance with findings of Lovelock et al. (1992) and Wand (1995). In addition, UV absorbance in the field experiment was generally higher than in the greenhouse indicating the importance of the UV-A and PAR waveband for the biosynthesis of UV absorbing compounds (Cen & Bornman 1990; Wand 1995).

Because of the controlled conditions in the greenhouse and the fact that control plants did not receive any UV-B radiation, it is difficult to compare the results of the greenhouse and field experiment. Trying to do so, it is most realistic to regard the lowest UV-B_{BE} level (4.6 kJ m^{-2} day^{-1}) applied in the greenhouse, as the control treatment and 7.6 kJ m^{-2} day^{-1} as the enhanced UV-B treatment. Taking this into account, the results of the greenhouse experiment are similar to the results obtained in the field experiment. *P. lanceolata*, which was sensitive to UV-B radiation in the greenhouse, also showed sensitivity in the field. However, the present experiment further stresses the importance of including a UV-B treatment similar to the ambient UV-B_{BE} level in greenhouse studies. Tosserams & Rozema (1995) pointed out that using the 0 kJ m^{-2} day^{-1} UV-B_{BE} treatment as a control may lead to overestimation of UV-B effects when trying to extrapolate results to a field situation. For example, five out of eight species showed reduced shoot height or leaf length. Using the ambient level of 4.6 kJ m^{-2} day^{-1} UV-B_{BE} instead of the 0 kJ m^{-2} day^{-1} UV-B_{BE} treatment as a control, only one species (*P. lanceolata*) would have a significantly reduced leaf length at enhanced UV-B. This also holds for most of the measured growth parameters and for the UV absorbance of leaf abstracts. Therefore, using 0 kJ m^{-2} day^{-1} UV-B_{BE} as the control treatment in greenhouses studies is not recommended when experimental results are used for the formulation of expectations of future UV-B effects on a plant community or ecosystem level. To obtain realistic data concerning the consequences of elevated UV-B levels on plant performance and competition in natural ecosystems, long term field experiments are a necessity. Greenhouse and experimental field studies using plants in containers can assist in the identification of sensitive plant species, for a better understanding of UV-B action on plant physiology and the elucidation of adaptive mechanisms.

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UV-B supplementation system in the dune grassland. Heemskerk, The Netherlands. (Photograph: J. Rozema)

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Stratospheric ozone reduction and ecosystem processes: enhanced UV-B radiation affects chemical quality and decomposition of leaves of the dune grassland species *Calamagrostis epigeios*

J. Rozema, M. Tosserams, H. J. M. Nelissen, L. van Heerwaarden, R. A. Broekman & N. Flierman
Department of Ecology & Ecotoxicology, Vrije Universiteit, Amsterdam, The Netherlands

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Abstract

This study reports changes in the plant's chemical composition and the decomposition of this plant material under enhanced solar UV-B radiation. *Calamagrostis epigeios*, a dominant grass species in the dune grassland in The Netherlands, was grown outdoor on an experimental field under ambient and enhanced solar UV-B (5 and 7.5 kJ m^{-2} day^{-1} $UV\text{-}B_{BE}$, respectively), corresponding to about 15% stratospheric ozone depletion. After one growing season aerial plant parts were harvested. The decomposition of this harvested leaf material was studied in a dune grassland and on the above mentioned experimental field under ambient (5 kJ m^{-2} day^{-1} $UV\text{-}B_{BE}$) and enhanced (7.5 kJ m^{-2} day^{-1} $UV\text{-}B_{BE}$) radiation, using litter bags. The chemical quality of the leaves grown under enhanced solar UV-B changed. There was an increase in the leaf content of lignin, while no significant changes occurred for the content of α-cellulose, hemicellulose and tannins under enhanced UV-B. In the field, the rate of decomposition of leaf material grown under enhanced UV-B (with an increased content of lignin) was reduced. The content of lignin of the decomposing leaf material increased, but less under exposure to enhanced UV-B. The latter may be explained by photodegradation of the lignin. The consequences of enhanced UV-B radiation for carbon fluxes in the dune grassland ecosystem are discussed.

Introduction

Anthropogenic emissions of chlorofluorocarbons and halons cause reduction of ozone in the stratosphere. Reduced thickness of the ozone column has not only been observed above the antarctic and arctic regions but also for the mid latitudes (WMO 1994). As a result of stratospheric ozone depletion, increasing levels of solar UV-B radiation (280–320 nm) reach the surface of the earth. Damage by UV-B radiation to DNA and membrane functions may lead to reduced plant growth. Yet, primary targets of damaging UV-B in the metabolism of plants have not been generally indentified. Repair of DNA damage by the enzyme photolyase is induced by photosynthetically active radiation (PAR, 400–700 nm) (Caldwell & Flint 1994). For many plants it is unknown how much of the biomass decrease or yield reduction by enhanced UV-B relates to DNA damage not repaired by reactivating photorepair (Caldwell et al. 1995; Rozema et al. 1995). The net rate of photosynthesis and transpiration of *Calamagrostis epigeios,* a dune grassland species, was not significantly affected with a weighted daily $UV\text{-}B_{BE}$ exposure increasing from 0, 6.7, 10 and 14.9 kJ m^{-2} day^{-1}. The latter three values simulate about 10, 28 and 44% stratospheric ozone reduction respectively (Rozema et al. 1995; Tosserams & Rozema, 1995). Apart from negative effects on biomass production, UV-B radiation may affect plant growth and morphology in a more subtle way. Such changes relate to altered biomass allocation, timing of plant development and senescence, branching, leaf and canopy architecture (Caldwell et al. 1995; Gwynn-Jones et al. 1997). These plant morphogenetic changes will alter competitive relationships between

plant species. In addition, there is growing evidence that UV-B radiation influences the pathways of secondary metabolites. UV-B radiation induces phenylalanine ammonia lyase (PAL). This PAL enzyme supports the first stage in the metabolism of phenylpropanoid. Also other end-products of the phenylpropanoid metabolism may be stimulated by UV-B radiation. This comprises flavonoids (Beggs & Wellmann 1994) and other polyphenolic compounds affecting plant pathogens, and compounds such as tannins reducing palatability to herbivores (Gehrke et al. 1995; Gwynn-Jones et al. 1997), and reducing degradation by micro-organisms (lignin). Flavonoids and related phenolic compounds tend to increase with raised ultraviolet B radiation (van de Staaij et al. 1995). Since flavonoids and phenolics show absorbance in the UV-B wavelength band, such an increase of UV-B absorbing secondary metabolites may be functional in screening UV-B radiation. Increased synthesis of tannins and lignin with enhanced UV-B radiation may have consequences for important ecosystem processes: decomposition of plant material and plant animal relations. Paul et al. (1997) discuss changes in the interactions between trophic levels related to UV-B radiation. Tannins consist of polymers of phenolic acids and lignin consists of complex polymers of phenolic compounds. An increased content in plants of these phenolic polymers will decrease the microbial degradability (Aber & Melillo 1982; Fox et al. 1990; Hartley & Jones 1977; Kuiters 1987; Lewis & Yamamoto 1990; Nicolai 1988). Studies of effects of UV-B radiation at the ecosystem level are sparse (Caldwell et al. 1995). Gehrke et al. (1995), studying deciduous dwarf shrubs of the subarctic tundra in North Sweden, report effects of UV-B radiation on the decomposition of *Vaccinium* leaf litter. Rozema et al. (1995) study effects of enhanced UV-B radiation on the species of a dune grassland ecosystems. Although enhanced UV-B compared to ambient UV-B, does not affect growth and photosynthesis of *C. epigeios* (Tosserams & Rozema 1995), there are differences in the growth and physiological responses of other dune grassland species to enhanced UV-B (Tosserams et al. 1997). Enhanced UV-B may affect decomposition of leaf litter directly or indirectly. A direct effect of enhanced UV-B may be the photochemical breakdown of litter (photodegradative effect of UV-B). UV-B may also directly affect decomposer organisms, when these become exposed to solar UV-B. Indirect effects of enhanced UV-B may relate to changes in the plant's secondary metabolism, which may alter leaf litter decomposition.

The present paper aims at assessing both direct and indirect effects of enhanced UV-B on leaf litter decomposition. We report on the effects of enhanced UV-B radiation on the leaf content of organic compounds such as (hemi)cellulose, tannins and lignin and the consequences of the (altered) chemical composition of plants, due to UV-B radiation, for the process of decomposition of leaf material of *C. epigeios*.

Material and methods

Cultivation of Calamagrostis epigeios *under ambient and enhanced UV-B on the experimental field*

C. epigeios was grown from seeds on commercial potting soil (Jongkind, Aalsmeer). Plants were grown for three weeks in a greenhouse and then transferred to an experimental field, at the University Campus. The soil of the experimental field consisted of a mixture of dune sand with some potting soil. *Calamagrostis epigeios* was cultivated in monoculture stands on the outdoor experimental field under ambient (about 5 kJ m^{-2} day^{-1} UV-B_{BE}) and enhanced UV-B (7.5 kJ m^{-2} day^{-1} UV-B_{BE}). The ground area of the monocultures was 1.20 × 1.20 m. Enhanced UV-B radiation was obtained with Philips 40 W/12 lamps 2 m above the soil surface. UV-B radiation was filtered through a 0.10 mm thick cellulose acetate foil (Tamboer & Co Chemie, Haarlem, The Netherlands), absorbing UV radiation below 290 nm. To avoid different levels of UV-A between the UV-B treatments, Philips 40 W/12 UV-lamps burned as well in the ambient UV-B treatment, but were wrapped in Mylar foil, type S, 0.13 mm thick, transmitting radiation with a wavelength > 313 nm. Mylar foil excludes about 90% of the UV-B (280–320 nm) (Tosserams et al. 1996). More details of the UV-B supplementation system at the experimental field are given in Rozema et al. (1995) and in Tosserams et al. (1997). According to the empirical model of Green et al. (1980), it was calculated that the UV-B_{BE} exposure of the enhanced UV-B radiation treatment simulates a 15% reduction of stratospheric ozone.

At the end of the growing season, when shoots of *C. epigeios* had formed a dense sward, all aerial parts of *C. epigeios* were harvested from the monoculture stands and dried in a oven for 48 hours at 40 °C. Dried material was stored in envelopes at room temperature in the dark before it was used for decomposition studies. Dried leaf material from plants grown under

ambient solar UV-B is indicated as '−UV-B litter' and leaf material from plants grown under enhanced UV-B as '+UV-B litter'. This dried leaf material has been analysed for the content of organic compounds and C and N ('Before decomposition' in Tables 1 and 2).

Decomposition experiments

Decomposition experiments were performed in the dune grassland and on an experimental field.

Aerial parts of the dried dune grassland grass *C. epigeios* were cut into pieces of about 5 cm. Litter bags used were made of 8.5 cm × 8.5 cm gauze closures. Mesh size was 1 mm on the lower side (preventing loss of plant material) and 3 mm on the upperside, allowing the entrance of micro-organisms and mesofauna. The litter bags were filled with 2 g of the dried plant material. Coded and closed litter bags were loosely fixed with wooden pins about 3 cm above the sandy soil within the canopy of the dune grassland vegetation in Heemskerk (cf. Van Beckhoven 1995). From above the canopy the litterbags were visible and remained visible during the decomposition experiments, indicating that the leaf litter in the bags intercepted at least part of the UV-B radiation.

Alternatively, litter bags were placed on the bare soil surface on the experimental field where the litter was more directly exposed to ambient (5 kJ m^{-2} day^{-1}) or enhanced (7.5 kJ m^{-2} day^{-1}) UV-B, during a period of 60 days (autumn 1994).We assume that in this case a photodegradative effect of UV-B will be more pronounced than in the dune grassland decomposition experiments.

In the first experiment, litter bags were placed in the dune grassland vegetation on August 18, 1994 and collected October 17, 1994 (60 days). In a second experiment litter bags were placed in the dune grassland vegetation on September 9, 1995 and removed October 25, 1995 (50 days). Leaf material in the litter bags was exposed to ambient solar UV-B radiation (5 kJ m^{-2} day^{-1}) or to enhanced UV-B radiation (7.5 kJ $m^{-2}day^{-1}$), in the dune grassland in Heemskerk (52°30′ N, 4°40′ E). Litter bags with leaf material were placed in the center of plots of 450 × 150 cm. UV-B radiation was supplied by Philips 40 W/12 TL UV lamps, 150 cm above the soil surface. For ambient UV-B, lamps were wrapped in Mylar foil and for enhanced UV-B in cellulose acetate foil. Foil was renewed weekly. Distance between the lamps above the plots was 40 cm. At the end of a decomposition period, collected litter bags were ovendried at 40 °C (48 h) and weighed. The percentage of mass loss was determined based on the dry weight of remaining plant material at the end and the dry weight of the plant material at the start.

Chemical analysis

Ovendried plant material was homogenised in a mill (Retsch PM 4). Carbohydrates, cellulose, hemicellulose, tannins and lignin in plant material were determined basically according to Allen (1989).

Total carbon and nitrogen

The content of carbon and nitrogen of plant material was determined in 3 mg samples using gas chromatography (Perkin Elmer 2400 CHNSO Analyser series II).

Total soluble carbohydrates and starch

40 mg of dried and homogenised plant material was extracted with 80% ethanol and centrifuged at 1900 *g*. The carbohydrate content of the extract was determined using the Anthrone reagent (Allen 1989). For the determination of starch, the pellet was hydrolyzed with 5 ml H_2O and 2.5 ml 8 *M* HCl for 1 h at 100 °C. After centrifugation at 1900 *g*, 2 ml of the extract was mixed with 0.66 ml 8 *M* NaOH and made up to 10 ml. The starch content was determined in this solution using the Anthrone reagent, glucose was used as a standard.

α-cellulose and hemicellulose

For determination of cellulose 100 mg of dried and grounded plant material was delignified using sodium chlorite ($NaClO_3$) and 10% acetic acid. The suspension was filtered through a teflon (durapor) filter (0.22 μm). The ovendried (105 °C, 30 min) residue was weighed, representing a total polysaccharide fraction indicated as holocellulose. This holocelluose fraction was further treated with a 24% KOH solution, resulting in a relatively pure form of α-cellulose. The suspension was filtered through a 0.22 μm teflon (durapor) filter. The residue was washed with water until all alkali was removed with 0.5 ml 5% acetic acid and with acetone. The residue (α-cellulose) was dried for 30 min at 105 °C and weighed. The filtrate was collected and after neutralisation and addition of excess alcohol (industrial spirit 95% v/v) the precipitated hemi-

Table 1. Organic compounds (% DW) in leaf litter material grown under ambient and enhanced UV-B ('−UV-B' and '+UV-B' litter quality), before and after decomposition (decomposition), under ambient and enhanced UV-B radiation, on a dune grassland (autumn 1995) . Average values and standard error of the mean of 6 replications. Statistical significance of effects of litter quality, decomposition and photodegradation. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; n.s. = not significant. First, the significance of litter quality was tested with a one-way anova comparing '−UV-B' and '+UV-B' data for litter 'before decomposition'. The significance of a photodegradative effect was then tested with a two-way anova, analysing data from 'Ambient UV-B'/'Enhanced UV-B' and '−UV-B'/'+UV-B'. The effect of decomposition on the content of organic compounds was tested with a two-way anova comparing the data 'before decomposition'/'after decomposition' ('Ambient UV-B') and '−UV-B'/'+UV-B'. In both two-way analyses, neither the litter quality effect (−UV-B/+UV-B), nor the interaction term was significant for any of the compounds

	Litter quality	Before decomposition	After decomposition	
			Ambient UV-B	Enhanced UV-B
Soluble carbohydrates	−UV-B	1.65 ± 0.25	1.12 ± 0.13	0.86 ± 0.13
	+UV-B	1.48 ± 0.35	1.08 ± 0.16	1.24 ± 0.23
Starch	−UV-B	3.62 ± 0.37	3.29 ± 0.32	3.02 ± 0.22
	+UV-B	4.04 ± 0.52	3.13 ± 0.22	3.86 ± 0.45
α-Cellulose	−UV-B	36.7 ± 1.2	24.6 ± 2.1	20.5 ± 2.7
	+UV-B	41.3 ± 3.3	25.4 ± 1.5	23.2 ± 1.6
Hemicellulose	−UV-B	28.9 ± 3.3	26.1 ± 3.8	14.4 ± 3.6
	+UV-B	23.0 ± 1.6	18.9 ± 2.3	13.1 ± 4.0
Tannins	−UV-B	5.36 ± 0.18	3.41 ± 0.19	3.28 ± 0.20
	+UV-B	4.76 ± 0.47	2.93 ± 0.11	3.57 ±0.27
Lignin	−UV-B	5.7 ±0.5	11.7 ±1.5	9.6 ±0.6
	+UV-B	8.9 ±0.1	10.9 ±1.5	10.0±0.9

	Litter quality	Decomposition	Photodegradation
Soluble carbohydrates	n.s.	***	n.s.
Starch	n.s.	*	n.s.
α-cellulose	*	***	n.s.
Hemicellulose	n.s.	*	*
Tannins	n.s.	**	n.s.
Lignin	*	*	*

cellulose was filtered, dried and weighed (30 min at 105 °C).

Tannins (water soluble)

10 mg of plant material was boiled in 5 ml H_2O (1 h), and filtered. The content of tannins in the filtrate was determined using the Folin-Ciocalteus reagent. After colour development (20 min, 25 °C) absorption was measured at 760 nm. Tannic acid was used as a standard (Allen 1989; Kuiters 1990; Tolsma 1989).

Lignin

For quantitative determination of the lignin fraction, 100 mg of dried and homogenised plant material was treated with ether using a soxhlett apparatus to remove lipids, with demineralized water to remove soluble carbohydrates and dried (105 °C, 30 min). This dried material was hydrolysed with 72% sulphuric acid, breaking down the cellulose complex. Thereafter, the concentration of sulphuric acid was reduced to 3%, the remaining lignin was filtered through a teflon filter (durapor, 22 μm mesh size), dried (105 °C, 30 min) and weighed. The content of cellulose and lignin of the plant material was corrected for the protein content

(nitrogen content of the subsamples × 6.25) and ash content of the plant samples.

Statistical analysis

Homogeneity of variances was tested with Bartlett's test. Effects of litter quality and UV-B radiation on mass loss of plant material were analysed with a two-way analysis of variance according to Sokal & Rohlf (1981). Differences in the content of organic compounds, and the total carbon and nitrogen content of the plant material were tested with one-way or two-way analysis of variance and the Tukey HSD multiple comparisons test, using Systat 5.2.1. If necessary, percentages were transformed before analysis to obtain homogeneity of variance.

Results

Indirect and direct UV-B effects on decomposition (mass loss) of leaf material

Mass loss of leaf material grown under ambient and enhanced UV-B and decomposing under ambient and enhanced solar UV-B for 60 days in August, September and October 1994 and for 50 days in September and October 1995 is given in Figures 1a and 1b. Decomposition of leaf material grown under enhanced UV-B ('+UV-B' litter) is significantly reduced both in the fall of 1994 and 1995. The mass loss of leaf material decomposing under enhanced solar UV-B in the dune grassland is higher than under ambient UV-B. This direct effect of UV-B radiation (photodegradation) was apparent both in 1994 and 1995. The percentage weight loss of the leaf material in 1994 is slightly higher than in 1995, this probably relates to the longer decomposition period (60 days) in 1994, compared to 50 days in 1995.

Decomposition of *Calamagrostis* plant material was also studied on an experimental field at the university campus. '+UV-B' litter of *C. epigeios* decomposed more slowly than '−UV-B' litter (Figure 1c). Like in the dune grassland, exposure of the litter bags to enhanced UV-B led to increased mass loss. The photodegradative effect of UV-B was most pronounced for the decomposition experiment on the experimental field (1c, $p = 0.005$), compared to the dune grassland experiments (1a, $p = 0.017$; 1b, $p = 0.047$).

An 'overall' two-way anova, pooling mass loss data of the three separate experiments indicated a significant

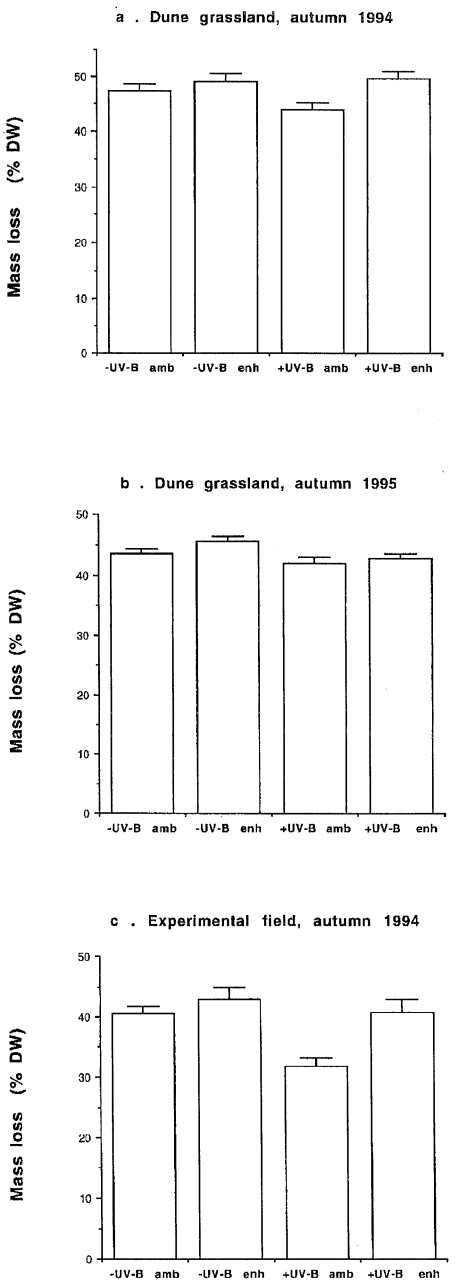

Figure 1. Mass loss (% dw) of leaf material of *Calamagrostis epigeios*, grown under ambient and enhanced UV-B ('−UV-B' and '+UV-B' litter quality), decomposing in the dune grassland under ambient (amb) and enhanced (enh) UV-B radiation for 60 days, autumn 1994. (**a**) for 50 days, autumn 1995 (**b**) or for 60 days on the experimental field, autumn 1994 (**c**). Average values of 10 replications and standard error of the mean. Effect of litter quality ($p = 0.047$ (a), $p = 0.001$ (b), $p = 0.006$ (c)) and UV-B radiation (photodegradation) ($p = 0.017$ (a), $p = 0.049$ (b) and $p = 0.005$ (c)) statistically significant. Interaction not significant (two-way anova).

effect of litter quality ($p = 0.003$) and a significant photodegradative effect of UV-B ($p = 0.038$) on mass loss. The interaction term was not significant ($p = 0.107$).

The decomposition of leaf material of *C. epigeios* in the dune grassland shows both an indirect effect of UV-B: '+UV-B' leaf litter is decomposing slowly, and a direct effect of UV-B: increased mass loss of leaf litter material decomposing under enhanced UV-B.

Chemical composition of leaf material before and after decomposition

Leaf litter material of *C. epigeios*, grown under ambient or elevated solar UV-B, used for the second decomposition experiment in the dune grassland, presented in Figure 1b, was chemically analysed for the content of organic compounds before decomposition and at the end of the decomposition period (autumn 1995, dune grassland decomposition experiment) (Table 1).

a. Effect of UV-B on content of organic compounds in leaf material

Neither the concentrations of soluble carbohydrates nor the starch concentration were signicantly different for '−UV-B' and '+UV-B' litter. The percentage α-cellulose of leaves grown at enhanced UV-B was significantly increased. Hemicellulose was not significantly affected. The concentration of tannins is not significantly changed with enhanced UV-B. The concentration of lignin of leaves grown under enhanced UV-B was significantly increased: lignin was 56% higher than in leaves grown under ambient UV-B (Table 1).

b. Effect of decomposition on content of organic compounds in leaf itter

The concentration of all organic compounds, except lignin, in the litter, decreased during decomposition. This was most pronounced for soluble carbohydrates and α-cellulose.The concentration of lignin of the leaf material remaining after decomposition increased (Table 1).

c. Photodegrative effect of UV-B on content of organic compounds in leaf litter

Evidence for a direct photodegradative effect of enhanced UV-B was found for hemicellulose and lignin. The concentration of hemicellulose and lignin in leaf material decomposing under elevated UV-B in the field was reduced compared with leaf material decomposing under ambient solar UV-B (Table 1).

Carbon and nitrogen content of leaf material

Neither the total carbon content nor the total nitrogen content changed in leaf material of plants grown under enhanced UV-B . The total content of carbon of the leaf material was not changed during the process of decomposition. There was a reduction of the total N-content during decomposition, particularly when decomposing leaf material was exposed to enhanced UV-B radiation. The C/N ratio of decomposing leaf material increased (Table 2).

Discussion

There is growing evidence that enhanced solar UV-B radiation, rather than affecting photosynthesis and primary production, causes plant morphogenetic changes, which will alter temporal and spatial characteristics of ecosystems. UV-B induced morphogenetic effects concern leaf morphology, leaf angle, biomass allocation, branching and timing of plant development and senescence. Changes in the competitive relationships between plant species may be more directly related to plant morphogenetic effects of UV-B rather than to differences in sensitivity to UV-B between plant species (Caldwell et al. 1995; Rozema et al. 1997). In addition to UV-B induced plant morphogenetic effects, enhanced solar UV-B affects the secondary productivity of plants. As a consequence, changes in flavonoids, phenolics and polymers of phenolics may affect the plant-animal relationships, other trophic relationships (Paul et al. 1997) and decomposition processes (Zepp et al. 1995).

UV-B radiation, secondary plant metabolism and litter decomposition

There is evidence that UV-B radiation is stimulating the phenylpropanoid metabolism. The aromatic aminoacid L-phenylalanine is being produced by the shikimate pathway. The enzyme phenylalanine ammonia-lyase (PAL) catalyses the first of a sequence of reactions, leading to flavonoids and to polymeric phenolic compounds such as tannins and lignin. Chalcone synthase (CHS) is involved and is a key enzyme of the flavonoid biosynthesis (Beggs & Wellman 1994).

Table 2. Total carbon and nitrogen (% DW) and C/N ratio of leaf material of *Calamagrostis epigeios* grown under ambient and enhanced UV-B, before and after decomposing under ambient and enhanced UV-B in the dune grassland (autumn 1995). Average values of eight replications and standard error of the mean , are shown together with significances due to litter quality, decomposition and photodegradation. * = $p < 0.05$; *** = $p < 0.01$; *** = $p < 0.001$; n.s. = not significant

	C	N	C/N
Leaf material grown under ambient UV-B, before decomposition ('−UV-B)	45.1 ± 0.3	2.81 ± 0.12	15.9 ± 2.2
Decomposing under ambient UV-B	46.1 ± 0.5	1.94 ± 0.08	23.9 ±1.3
Decomposing under enhanced UV-B	45.5 ± 0.4	1.84 ± 0.18	24.8 ± 2.1
Leaf material grown under enhanced UV-B, before decomposition ('+UV-B')	45.0 ± 0.2	2.80 ± 0.1	16.1 ± 2.1
Decomposing under ambient UV-B	45.2 ± 0.4	1.84 ± 0.11	24.9 ± 2.1
Decomposing under enhanced UV-B	45.1 ± 0.4	1.71 ± 0.21	26.7 ± 2.8
Litter quality	n.s.	n.s.	n.s.
Decomposition	n.s.	**	***
UV-B radiation (photodegradation)	n.s.	*	*

There was a significantly increased content of lignin in the leaves of *Calamagrostis epigeios*, grown under enhanced solar UV-B (Table 1).

The increase of the lignin content of the leaf material remaining after the two months period of decomposition in the dune grassland confirms the limited microbial degradability of lignin. On the other hand, leaf material decomposing under enhanced solar UV-B showed a reduced lignin content indicating photodegradation by UV-B (Table 1).

The increased lignin content of leaf material of *Calamagrostis epigeios* grown under increased solar UV-B may relate to the reduced mass loss of the '+UV-B 'litter compared to '−UV-B' litter (Figure 1, Table 1). The aerial plants parts of *Calamagrostis epigeios* grown outdoor under ambient and enhanced solar UV-B, harvested after one growing season, had not fully senesced. Therefore, this plant material we used for the decomposition experiments cannot fully be denoted as litter. In completely senesced leaves retranslocation of nutrients and other compounds will have taken place. Since young and old leaves in the field will differ in time of senescence, natural litter may be heterogeneous with respect to the content of inorganic and organic plant compounds. Van Beckhoven (1995) studied decomposition of dune slack species, collecting litter from sites in the dunes. The total N-content of this natural litter (% DW) of *Calamagrostis epigeios* is about 1.0, which is lower than the N-content of the plant material we used (2.8% N). According to the higher N-content in the plant material we used for decomposition, the mass loss percentage was about twice the value reported by an Beckhoven (1995) for decomposition of *Calamagrostis* litter in the autumn. The standard error of the mass loss of this natural litter was much higher than values in our decomposition experiments. This confirms that our harvested plant material was more homogeneous in chemical composition. This may have helped in detecting effects of '−UV-B' and '+UV-B' leaf quality as well as photodegradation effects of UV-B on the rate of litter mass loss. In addition to decomposition experiments of *Calamagrostis* plant material in the fall of 1994 and 1995 in the field, we conducted similar experiments in the winter and summer period. For the winter period 1995–1996 with frost and snow, reduced rates of mass loss were found. Mass loss values in the warm and dry summer (July–September) of 1995 were comparable to the autumn values for 1994 and 1995 reported in this paper.

The results of our studies of UV-B and leaf decomposition agree well with the findings of Gehrke et al. (1995). Gehrke and colleagues report that litter from *Vaccinium uliginosum* leaves, grown under enhanced UV-B in the field, showed reduced mass loss in a decomposition experiment in the field. A direct photodegrading effect of enhanced UV-B increasing mass loss compared to no UV-B was indicated, but was not statistically significant. Our studies in relation to UV-B demonstrate not only that enhanced solar UV-B increased the lignin percentage in leaves in outdoor grown dune grassland plants, but also that decompos-

ition of such plants material in the *field* was *indirectly* and *directly* affected by UV-B radiation simulating about 15% stratospheric ozone reduction. The direct UV-B effect reported by Gehrke et al. (1995) relates to a laboratory experiment with a 10 kJ m^{-2} day^{-1} UV-B dose compared with a no UV-B treatment. The content of lignin and tannins in *Vaccinium* leaves grown under ambient and enhanced UV-B in the field did not significantly differ. Lignin of *Vaccinium* litter remaining after decomposition was reduced under UV-B compared with no UV-B. The leaf content of tannins was increased under UV-B. During the decomposition of plant material soluble and non soluble carbohydrates are being decomposed easier and earlier than α-cellulose and hemicelluose. Lignin is being degraded even later (Berg et al. 1984; Swift et al. 1979). This explains why the concentration of lignin increased during the early stages of the decomposition process we studied (Table 1). The increased concentration of lignin in the decomposing plant material may reduce the rate of litter turnover in later stages of decomposition (Berendse et al. 1987; McLaugherty & Berg 1987). Since lignin and the polymer carbohydrates α-cellulose and hemicellulose are physically associated, decomposition rates in later stages are difficult to predict and may also differ among litter types (Berg & Staaf 1981). Anyway, the mass loss of decomposing material being cultivated under enhanced solar UV-B and with an increased lignin content was significantly reduced, during the 50 and 60 days of exposure to ambient or enhanced solar UV-B in the dune grassland compared to leaf material cultivated under ambient UV-B. In addition, decomposition of plant material in the dune grassland was increased under enhanced UV-B probably as a result of photodegradation. We do not know whether enhanced UV-B radiation also directly inhibits growth and activity of decomposing fungi and bacteria as has been indicated by Gehrke et al. (1995).

Enhanced UV-B and plant animal relationships

The content of tannins was not significantly increased in leaves of *Calamagrostis epigeios* grown under enhanced solar UV-B (Table 1). Tannins are well-known to affect palatability of plants to herbivores (Harborne 1993). Generally, increased tannin concentrations in the plant will reduce feeding of plants by animals. Gwynn-Jones et al. (1997) report marked UV-B effects on insect herbivory in subarctic dwarf shrubs. Herbivory increased in *Vaccinium myrtillus* and decreased in *V. ulignosum* with enhanced solar UV-B. Total phenolics increased in *Pisum sativum* with enhanced UV-B, but herbivory by *Autographa gamma* caterpillar was not changed by this. Reduced feeding of the moth caterpillar on the plant grown under enhanced UV-B was linked with an increased nitrogen content (Hatcher & Paul 1994).

Apart from tannins, many other secondary metabolites may affect plant animal relationships. Synthesis of furano coumarins is increased in *Citrus jambhiri* with enhanced UV-B, and makes the citrus plant toxic to a caterpillar, in particular in a high UV-B environment (McCloud & Berenbaum 1994). Effects of UV-B on herbivory may be species-specific and need careful experimentation and field analysis. No doubt that changed herbivory by UV-B may affect the structure and processes of ecosystems (Paul et al. 1997).

Like increasing UV-B, increasing atmospheric CO_2 is likely to affect plant-animal relationships, since it may also influence the plant content of secondary metabolites (Lambers 1993). Therefore, such UV- B and CO_2 effects should be studied not only in single factor mode, but also in combination (Rozema et al. 1997).

Enhanced solar UV-B, soil carbon storage and carbon cycling

Our field studies of a dune grassland species have demonstrated two significant but opposite effects of enhanced UV-B radiation on decomposition of plant material. Decreased decomposition related to an increased lignin content and increased decomposition by photodegradation of enhanced solar UV-B. It is difficult to predict the outcome of the opposing processes for the later stages of the decomposition process. Although there was a marked difference in lignin in plant material grown under ambient or enhanced UV-B, (Table 1) this difference in lignin content tends to decrease during decomposition. Also it is likely that decomposing plant material will be less affected by photodegrading influence of UV-B during later stages of decomposition when remaining litter will be gradually covered by newly fallen litter.

Modelling will help to predict the long-term effects of increased solar UV-B on carbon storage in natural ecosystems (Moorhead & Callaghan 1994).

In many ecosystems with limited nutrient availability, plants will produce more secondary metabolites including polymeric phenolics (Lambers 1993). This may also decrease the degradability of plant material.

The effects of UV-B radiation on decomposition we demonstrated may influence carbon storage and carbon fluxes in ecosystems (Zepp et al. 1995). Under the current process of climate change, atmospheric CO_2 enrichment co-occurs with increased solar UV-B radiation (Rozema et al. 1990). The combined effects of increasing atmospheric CO_2 and enhanced solar UV-B on plants and ecosystems are largely unknown. As far as biomass increment and other growth parameters were concerned, Rozema et al. (1990) found additive effects of CO_2 and UV-B and few interactions (Rozema et al. 1996). Unlike UV-B, atmospheric CO_2 enrichment generally stimulates (at least short-term) plant growth and plant photosynthesis (Tissue & Oechel 1987; Rozema 1993; Rozema 1995). Increased primary productivity may induce secondary productivity, as suggested by Lambers (1993). As a result, this may reduce the rate of decomposition of plant material grown in a CO_2 enriched atmosphere. In addition, the dilution of nutrients in plants growing under elevated atmospheric CO_2, and an increased ratio of C/N may reduce the decomposition rate of such litter (Couteaux et al. 1991).

Currently we have started modelling the direct and indirect effects of enhanced UV-B and atmospheric CO_2 on carbon fluxes in ecosystems.

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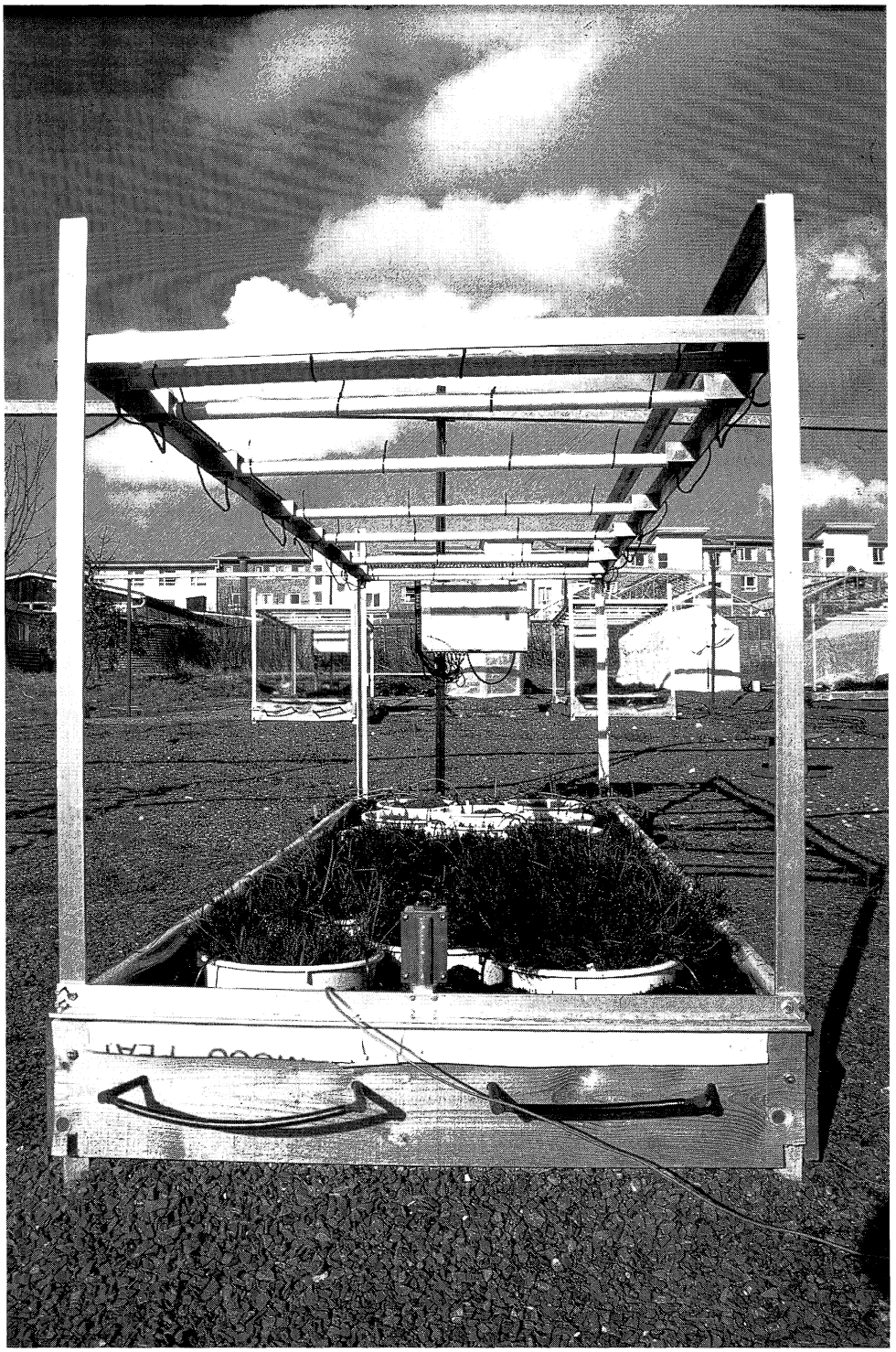

A modulated UV-B field array at Lancaster. The system illustrated here is being used to grow *Calluna vulgaris* and *Rubus chamaemorus* for studies of decomposition. The same system has been used at other sites to investigate plant-pathogen interactions.

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The role of interactions between trophic levels in determining the effects of UV-B on terrestrial ecosystems

Nigel D. Paul, Sharima Rasanayagam, Sandra A. Moody, Paul E. Hatcher & Peter G. Ayres
Biology Division, Institute of Environmental and Biological Sciences, Lancaster University, Bailrigg, Lancashire, LA1 4YQ, UK

Key words: Decomposers, Herbivores, Higher plants, Micro-organisms, Pathogens, Ultraviolet-B radiation (UV-B)

Abstract

Understanding the potential impact of ozone depletion on terrestrial ecosystems is constrained by lack of information on the effects of environmentally realistic UV-B doses on terrestrial organisms other than higher plants. Increasing UV-B may alter interactions between plants and consumers through direct effects on consumer organisms (herbivores, phytopathogens, decomposers, etc.). The effects of increasing UV-B on arthropods are not known. Significant UV-B effects on fungi have been reported, and may be either negative (inhibition of spore germination and mycelial growth) or positive (increased growth, induction of reproductive development and sporulation). However, in many cases consumers are unlikely to be directly exposed to UV-B in the field. In addition, UV action spectra for fungi suggest that this major group may be less sensitive to the effects of ozone depletion than higher plants. Host mediated effects of UV-B on consumers may include alterations in plant chemistry. While secondary metabolites such as phenolics may increase under increased UV-B, this is not invariably the case and evidence that such changes have significant effects on consumers is limited. In particular, there is no evidence that increased UV-B increases resistance of higher plants to fungal pathogens. Indeed, increased UV-B prior to inoculation results in no significant effect or increased disease. Such responses may be attributable to UV-B effects on host surface properties or on compounds other than phenolics. However, such changes are poorly known, and their potential effects on phytopathogens, herbivores or decomposers cannot be assessed. Understanding the effects of UV-B on terrestrial ecosystems is further limited since virtually nothing is known of possible impacts on higher trophic levels, i.e. predators, parasites or pathogens.

Introduction

With the use of increasingly conservative UV-B treatments, both in controlled environments and in the field, there has been a marked shift in the perception of what constitutes a 'typical plant response' to UV-B (Caldwell et al. 1995). For example, it is becoming apparent that few species of higher plants suffer gross damage to photosynthetic processes from UV-B doses within the range expected given realistic predictions of stratospheric ozone depletion (Fiscus & Booker 1995). Even these authors concede that conservative UV-B treatments can result in morphological and chemical changes, but doubt that such changes have significant effects on plant growth or crop productivity. Nonetheless, UV-B induced changes in host morphology and chemistry may be of considerable significance if they modify interactions between plants and higher trophic levels; consumers such as herbivores, pathogens and saprotrophs, including decomposers. However, while the effects of UV-B on plant consumers have been the subject of speculation for a number of years (Caldwell et al. 1989), data remain very limited.

In considering the potential role of higher trophic levels in determining the overall effects of UV-B on terrestrial ecosystems, we will consider three elements.
(1) The risk of exposure of higher trophic levels to UV-B in the field environment.
(2) UV-B effects on the host that have secondary effects on consumers.

(3) Direct UV-B effects on consumers.

The risk of exposure to UV-B in the field environment

Above-ground herbivores

Many arthropods exhibit behaviour that, although presumably selected for by factors such as predation, is likely to reduce the potential for direct exposure to UV-B in the field. Above all, few invertebrate herbivores feed on exposed leaf surfaces during daylight. Animals that feed during the day on the lower surface are unlikely to experience significant UV-B since little UV-B penetrates through the leaf (Day et al., 1992) and little UV-B radiation is reflected back from lower leaves (Brown et al. 1994). Casual observations suggest a few exceptions in which significant exposure may occur. For example colonies of some aphid species are not uncommon on exposed parts of stems, while many leaf miners may be protected only by the epidermis and cuticle.

Above-ground micro-organisms

At least some micro-organisms are certainly exposed to incident UV-B in the field. The phylloplane community consists of a wide range of bacteria, yeasts and filamentous fungi, both saprotrophs and pathogens. This community is potentially directly exposed to UV-B, although clearly, even within a plant, actual exposure may differ widely between leaves high and low within the canopy, between upper and lower leaf surfaces, etc. There may also be small scale variation in exposure, brought about by both host characteristics, and features of the micro-organism, including *inter alia*, the production of extracellular polysaccharides, and whether they grow in isolation or as part of microbial aggregates (I. Thompson, pers comm.). Although not part of the phylloplane community, other micro-organisms may be exposed on leaf surfaces, for example insect viruses prior to ingestion by their host (Killick & Warden, 1991).

Among phytopathogens, powdery mildews (Erysiphales) which grow epiphytically on both upper and lower leaf surfaces may be especially vulnerable. Fungal pathogens that grow beneath the cuticle (i.e. *Diplocarpon roseum*) may also be outside most of the protection conferred by strongly absorbing host plant tissues. However, the rapid attenuation of UV-B within the host plant (Day et al. 1992) may protect most pathogens through much of their development. Except for vector-borne organisms, any pathogen attacking the above-ground parts of plants is potentially exposed to UV-B at particular phases of their life cycle. Germinating spores, germ-tubes or infection structures are at risk at the time of infection, as are reproductive tissues at sporulation and spores during dispersal. However, in many species, infection and sporulation are coupled to environmental cues, and so largely confined to periods of darkness or rainfall (Lacey 1986). Such patterns may be a result of selection pressure by environmental factors other than UV-B (temperature and, especially, moisture) but provide an effective means of UV-B avoidance. Not all fungi have dispersal patterns which reduce minimise UV-B exposure. Some release spores only after a lag period of several hours after rain and in others the maximum airborne concentration of spores coincides with the midday maximum in UV-B (Lacey 1986).

Decomposers

The exposure of the decomposer fauna and flora is likely to be highly variable, both in time and space. In open canopies, or where plant material decomposes *in situ* as "standing dead", exposure may be comparable to that in the growing plant. In closed canopies, UV-B penetration to the litter layer will be very small, but in deciduous systems litter may be exposed for considerable periods between autumn and spring (Brown et al. 1994). While absolute irradiances during this period are low, relative increases due to ozone depletion may be large, especially in late winter and spring (Madronich et al. 1995). Even where litter is exposed, UV-B penetration would be expected to be limited to the uppermost layer. Therefore, exposure will be sporadic, as litter is disturbed by wind, rain or the action of animals.

UV-B effects on the host plant that have secondary effects on consumers.

Changes in host chemistry

It has often been argued that increasing UV-B will alter plant-consumer interactions due to changes in secondary plant metabolism. For example, Caldwell et al. (1995) state that 'it has been shown repeatedly that

flavonoids and related phenolic compounds increase when plants are exposed to increased UV-B' and that 'these compounds are important for plants in deterring insects and other herbivores from consuming plant tissues and they play a role in resistance to pathogens'. While we do not necessarily question the validity of either statement in general terms, they must be applied cautiously in any assessment of the possible effects of UV-B on host-consumer interactions.

Does increased UV-B necessarily result in accumulation of secondary metabolites?

Specific measurements of secondary compounds considered to play a specific role in resistance to pests or pathogens remain infrequent. Elevated UV-B increased concentrations of cannabinoids in *Cannabis sativa* (Lydon et al. 1987), furanocoumarins in *Pastinacea sativa* (Zangler & Berenbaum 1987) and *Citrus jambhiri* (McCloud et al. 1992 but also see Asthana et al. 1993), total soluble phenolics in pea (Hatcher & Paul, 1994), and some ferulic acid derivatives in wheat (Liu et al. 1995). By contrast, increased UV-B reduced alkaloids in *Aquilegia caerulea* (Larson et al. 1990) and furanocoumarins in *Citrus jambhiri* (Asthana et al. 1993). In the field, Rozema et al. (1996) found an increased concentration of lignin in the aerial parts of *Calamagrostis epigeios* exposed to enhanced UV-B but concentrations of tannins and lignin in leaf litter of *Vaccinium* spp. were unchanged by UV-B irradiation prior to leaf fall (Gehrke et al. 1995). We have found a similar lack of UV-B response in the concentration of tannins in green leaf tissue and fresh litter of *Calluna vulgaris* and *Rubus chamaemorus* (S.A. Moody, unpublished). Thus, evidence that increased UV-B results in increased phenolics is largely based on measurements intended to quantify changes in the UV-B absorption of plant tissues. Such methods may also provide an indication of changes in compounds directly related to plant resistance to consumer organisms, but this is not necessarily the case. The effects of UV-B on resistance to consumers in a specific host will depend on both its particular 'defense' compounds and the specific mechanism by which UV-B stimulates the phenylpropanoid pathway. UV-B results in up-regulation of enzymes involved in various steps of the phenylpropanoid pathway (Jordan 1992). UV-B induction of phenylalanine ammonium lyase (PAL), which catalyses the initial step in phenylpropanoid metabolism, could potentially increase all its end-products, including both UV-B absorbing flavonoids and anti-consumer compounds. By contrast, induction of chalcone synthase, which catalyses the first step specific to flavonoid synthesis (Hahlbrock & Scheel 1989), might result in increased UV-B absorbing compounds but, through competition for substrate, inhibit 'defense' chemicals derived from other branches of the phenylpropanoid pathway. Interestingly, increases in the concentration of flavonoids would not have predicted changes in ferulic acid in wheat (Liu et al. 1995) or alkaloids in *Aquilegia caerulea* (Larson et al. 1990). It is also worth noting that UV-B is but one of several environmental factors, both abiotic and biotic, influencing the phenolic of plants.

Do increases in secondary metabolitesnecessarily affect consumers?

If secondary metabolites accumulate under elevated UV-B, and regardless of the underlying mechanism(s), what are the consequences for consumers? Given the complexity of host-consumer interactions, are any generalisations possible?

(a) Arthropods

Host plant resistance to arthropod herbivores generally results from many characteristics, chemical and non-chemical (Fritz & Simms 1992), even within the same host species. Different herbivores may respond to different resistance characteristics. For example, secondary compounds that have major effects on some herbivores may have little effect on others, due to variation in the metabolic inactivation compounds or behavioural avoidance of tissues were they are accumulated. This complexity can be illustrated in one of the best studied systems *Pastinaca sativa,* in which the presence of UV-B increases concentrations of three (bergapten, isopimpinellin and xanthotoxin) of five furanocoumarins (Zangler & Berenbaum 1987). However, in *P. sativa* resistance to parsnip budworm (*Depressaria pastinacella*), a major specialist herbivore, was poorly correlated to total furanocoumarin content (Berenbaum et al. 1986). Furthermore, when incorporated in artificial diets, the growth of *D. pastinacella* was inhibited by bergapten but stimulated by xanthotoxin (Berenbaum et al. 1989; Berenbaum & Zangler 1992). By contrast, xanthotoxin was toxic to generalist herbivores, especially in the presence of UV-B (Berenbaum 1978; Berenbaum & Zangler 1988). The only report where increases in secondary compounds (furanocoumarins

in *Citrus jambhiri*) brought about by realistic increases in UV-B have been shown to be deleterious to a herbivore also deals with a non-specialist herbivore, larvae of the noctuid moth *Trichoplusia ni* (McCloud & Berenbaum 1994). Contrasting effects on different herbivores may also result from the localisation of chemical defenses within the host plant. In the shoots of *P. sativa*, furanocoumarins are highly localised in specific tissues within the leaf veins, which are not consumed by sap-sucking herbivores, or even by chewing insects when they avoid feeding on the major leaf veins (Berenbaum & Zangler 1992). It is not clear how such localisation of chemical resistance may interact with the effects of UV-B on secondary metabolism, which may also be confined to particular tissues, notably the epidermis (Hahlbrock & Scheel, 1989). Localised increases in surface tissues might have particularly significance for those secondary metabolites that play a role in host location (Visser 1986) or as feeding stimulants (Bernays & Chapman 1994). Such effects of secondary metabolites cannot be overlooked when considering the possible impacts of UV-B on herbivory. Interpreting UV-B induced increases in secondary metabolites is further complicated since it is possible that increased phenolics in the host plant might increase the fecundity of insect herbivores, by reducing the susceptibility of larvae to viral infections (Schultz et al. 1990).

In many systems, herbivory is determined not only by secondary metabolites but by the nutritional quality of host tissues, in terms of water, carbohydrate or nitrogen contents. The possible effects of UV-B on these compounds should not be ignored. Herbivores may compensate for reduced tissue quality by increasing the amount they consume (e.g. Simpson & Simpson 1990). Increased consumption of nutrient-poor tissues may also increase intake of secondary compounds to significant levels (Slansky & Wheeler 1992), so that resistance to herbivores may best be seen as the product of interactions between nutrients and secondary compounds. For the host plant, increased consumption of nutrient-poor tissues may result in increased damage. Elevated UV-B may increase leaf carbohydrate content (Britz & Adamse 1995) but the content of proteins (see Jordan 1992) or total nitrogen may increase or decrease. Reduction in carbohydrate, protein or especially the carbohydrate:nitrogen ratio would tend to inhibit many herbivores (Dadd, 1985). The significance of such changes was evident in our own work on herbivory of pea by the silver-Y moth, *Autographa gamma* L. (Hatcher & Paul, 1994). Larvae of this moth showed a greater response to the increase in leaf nitrogen content induced by UV-B than to the concurrent increase in bulk phenolics. As with secondary metabolism, the effect of UV-B on nutrient content will be expected to have different consequences for different herbivores. For example, changes in bulk leaf C:N may be highly significant for chewers, but would be of little significance for sap-sucking insects, unless expressed in phloem concentrations (Dadd 1985).

(b) Phytopathogens

Variation in tissue nitrogen, carbohydrates, etc. may also affect plant pathogens. Responses to nitrogen are complex and variable, but increasing concentration stimulates many foliar pathogens (Paul 1989). Carbohydrates may also influence pathogens but, in contrast to a recent review (Manning & Teidemann 1995), we consider this less likely to apply to biotrophs, such as rusts and mildews, than to necrotrophs, such as *Botrytis cinerea*. There is no doubt that phenolics also play a key role in resistance to plant pathogens but, as with herbivores, they do not act alone. For example, Carver et al. (1995) studying the resistance of cereals to powdery mildew concluded that '...phenolic synthesis may be one of multiple additive or interacting factors contributing to penetration resistance, or it may simply back-up other resistance mechanisms'. The diversity of interacting mechanisms that contribute to resistance is also increasingly recognised by the rapidly developing awareness of systemic induced resistance to pathogens (Kuc 1995). From the point of view of ozone depletion, it is not known whether a quantitative increase in particular components of chemical resistance, phytoalexins for example (Beggs & Wellman 1994), resulting from environmentally realistic increases in UV-B would have any significant effect on pathogens. It is worth noting that studies of the effects of UV-B before inoculation with a pathogen have generally shown either no significant effect or an increase in infection (Orth et al. 1990; Finckh et al. 1992; Rasanayagam et al. 1996). In addition, Asthana et al. (1993) reported that when *C. jambhiri* was grown under elevated UV-B, leaf extracts were less fungitoxic to a number of necrotrophic pathogens. Therefore, the hypothesis that increased UV-B will increase plant resistance to pathogens is not supported by the existing data from the literature.

(c) Decomposers

As noted above, elevated UV-B may result in changes in decomposition which may be related to alterations

in the chemical composition of litter, for example increased lignin in *C. epigeios* (Rozema et al. 1996). In considering the possible effects of UV-B on decomposition, the responses of the decomposer fauna and micro-organisms share much in common with those of herbivores and pathogens. Thus, nitrogen and lignin contents, their ratio and C:N ratio are considered key determinants of litter decomposition (Berg et al. 1982; Taylor et al. 1989). Some insight into the responses of decomposers to UV-B may be gained by analogy with the effects of elevated CO_2 (Cotrufo et al. 1994; Cotrufo & Ineson, 1995; Couteaux et al. 1991). Litter grown at high CO_2 initially decomposed more slowly than control litter due to increased C:N ratio and, interestingly, decreased water holding capacity, itself a function of leaf structure (Couteaux et al. 1991). However, the initial inhibition of decomposition in 'high CO_2' may not predict changes in the long term. In the presence of complex decomposer communities decomposition was stimulated in litter from high CO_2 (Couteaux et al. 1991) due to increased diversity of decomposers, especially the stimulation of white-rot fungi.

In summary, for all groups of consumers, behaviour cannot be assumed to change as the result of modified host secondary metabolism. Different consumers respond differently to the secondary chemistry of their hosts, and the effects of secondary metabolites must be seen in the context of other host plant characteristics, such as water or nitrogen content. Where significant changes in host chemistry occur, effects on consumers are likely to be greater for generalists than for host-specific organisms. Especially for decomposition, changes in consumer communities must be considered, and these may be poorly predicted from the responses of specific organisms.

Indirect effects of UV-B on consumer mediated by mechanisms other than host chemistry

Compared with changes in host plant chemistry, the possible effects of UV-B on plant-consumer interactions mediated via changes in plant morphology and development have generally been ignored, although Orth et al. (1990) proposed that increases in pathogen infection in cucumber might result from changed leaf surface properties. Manning & Teidemann (1995) have speculated that infection by many pathogens might be increased by changes in canopy microclimate, resulting from the stunting of plant growth typical of increased UV-B, even given realistic treatments (Deckmyn et al. 1994). However, while UV-B induced reductions in plant growth may be clear-cut, effects on canopy structure and microclimate have not been studied. Other relatively well-characterised responses to UV-B remain uninvestigated in terms of their effects on consumers. For example, in some species UV-B may reduce leaf surface wax and alter its chemical composition (Barnes et al. 1994, 1996; Tevini & Steinmuller, 1987). Changes in leaf surface waxes may have a range of consequences for herbivores (Bernays & Chapman, 1994) and pathogens (Hammer & Evensen, 1994; Podila et al. 1993). In tobacco we found altered surface wax was associated with a significant increase in leaf wettability (Barnes et al. 1996) that was sufficient to expect substantial effects on spore retention of splash-dispersed pathogens (Ayres et al. 1996), but this has yet to be confirmed in the field. Elevated UV-B may change leaf surface properties, perhaps including the ultrastructure of leaf surface (Tevini et al. 1981) and stomatal aperture (Negesh & Bjorn, 1986), which may be important for certain consumers, but this also remains uninvestigated. One problem in investigating such effects is that many are unlikely to be apparent in short-term controlled environment studies. Especially for pathogens, proper evaluation of UV-B effects which would act on epidemiological processes, those of canopy structure on infection, or of leaf properties on spore-dispersal, probably requires full-scale field experiments. An illustration of the possible role of non-chemical changes in the field is provided by our own observations of *Calluna vulgaris* grown under ambient UV-B or modulated supplement based on a 15% ozone depletion. We found no measurable change in tannins, C:N ratio, or amino acid content. However, over the course of two growing seasons, natural populations of the sap-sucking psyllid *Strophingia ericae* were significantly reduced under elevated UV-B. The mechanisms underlying this response are unclear. However, given (a) the lack of change in host chemistry and (b) that this insect occupies sites at the leaf axil which in *C. vulgaris* are well-protected from direct exposure to UV-B, a change in shoot morphology affecting the number or suitability of feeding sites is one possibility (S.A. Moody, unpublished data).

Direct UV-B effects on consumers

Arthropods

The effects of UV-B on arthropods are poorly characterised. Compared with micro-organisms, many arthropods may be less prone to UV-B damage due to behavioural responses that are likely to minimise exposure to UV-B in the field (see above) or the possession of thick, heavily pigmented cuticles. However, in many arthropods, various aspects of behaviour that rely on visual stimuli might be sensitive to increasing UV-B, since many insects are capable of perceiving both UV-B and UV-A wavelengths (Tovée 1995). It is clear that the response of the insect eye to broad-band ultraviolet varies significantly between species (Tovée 1995), and even between males and females of the same species (Miall 1978). In studies of electrocutor traps, the maximum response of the house fly (*Musca domestica* L.) to flickering UV occurred at 330–360 nm, although lamps with peak emission around 310–315 nm were almost as effective (Roberts et al. 1992). This suggests that any response in the field will be dominated by UV-A, and hence that increasing UV-B may have minimal effects. However, flickering light, such as fluorescent tubes powered by conventional control equipment, are especially effective (e.g. Roberts et al. 1992; Syms and Goodman 1987). Therefore, insect attraction by flickering UV-B should not be overlooked as a potential problem in field experiments using standard UV tubes and control gear (McLeod 1996).

Micro-organisms

There is no doubt that many micro-organisms are vulnerable to damage by UV-B. With respect to organisms occurring in terrestrial habitats, UV-B sensitivity has been shown directly, or implied from responses to sunlight, in viruses (Killick & Warden 1992; Semeniuk & Goth 1980), bacteria, yeasts and filamentous fungi (see reviews by Ayres et al. 1996; Manning & Teidemann 1995). Sensitive fungi include entomopathogens (Ignoffo & Garcia 1992; Moore et al. 1993), phytopathogens (Bashi & Aylor 1983; Caesar & Pearson 1983; Maddison and Manners 1973; Rotem & Aust 1991; Rotem et al. 1985; Semeniuk & Stewart 1981) and decomposers (Moody et al. 1997). However, it is clear that there is tremendous variation in sensitivity to UV-B damage both between species (Maddison and Manners 1973; Rasanayagam et al. 1997; Rotem et al. 1985) and between isolates within species (see below). Variation in response can be attributed in part to pigmentation (Asthana & Tuveson 1992; Rotem et al. 1991). In at least some cases, UV-B responses are such that significant effects of stratospheric ozone depletion would be expected. For example, from our own studies of fungal decomposers, we would predict that an increase in UV-B expected for a 15% ozone depletion might reduce spore germination by as much as 40% in some species. Changes in the rate of mycelial expansion, although still significant, were far smaller in magnitude, and perhaps of greater significance were changes in the morphology of mycelia. For example, in some species denser, more compact mycelia were produced under elevated UV-B. Variation in growth and morphology in response to UV-B could have profound effects on microbial communities. Indeed, Gehrke et al. (1995) showed that increased UV-B resulted in marked changes in the relative abundance of three decomposer fungi.

In a few cases, the sensitivity of a pathogenic micro-organism has been shown to be reflected in reduced infection of the host (Semeniuk & Stewart 1981; and see below). By contrast, most studies of plant pathogens have shown that, if anything, increasing UV-B results in increased infection (see Manning & Teidemann 1995). Increased severity of fungal diseases may be due to the stimulation of sporulation by UV-B, which has been found for both sexual and asexual reproduction in a range of fungi. This response is sufficient for the use of UV-B opaque plastics to be considered as a means of limiting disease in protected crops. For example, the use of a film that did not transmit wavelengths below approximately 290 nm caused a 70–80% reduction in the production of *Sclerotinia sclerotiorum* ascocarps and in crop infection (Honda & Yunoki 1977). It is notable that this reduction in infection occurred even though the ascospores of *S. sclerotiorum* are injured by solar ultraviolet (Caesar & Pearson 1983). However, as Manning & Teidemann (1995) pointed-out, results from selective filters may bear little relation to the far smaller changes in solar UV-B resulting from ozone depletion, especially if induction is brought about by longer UV wavelengths. Ultraviolet action spectra have been published primarily for fungal photomorphogenesis (Honda & Yunoki 1978; Leach 1972; Leach & Trione 1965; 1966; Sproston 1971) but also for inhibitory responses (Maddison & Manners 1973). Not all studies have considered the UV-B waveband and, given the range of species and methods used, it is not surprising that there are clearly differences between the various action spectra. However, the overall response to UV-B

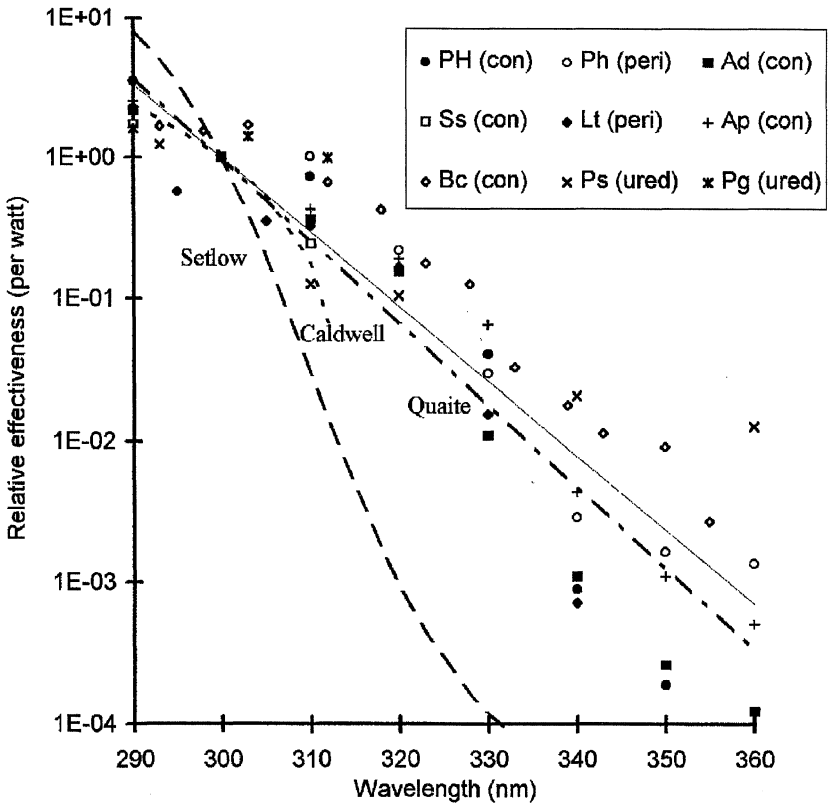

Figure 1. UV action spectra for fungi. Data are taken from published results for a range of species and responses, as follows: Ph (con) Stimulation of conidiogenesis in *Pleospora herbarum,* Ph (peri) Stimulation of perithecial formation in *P.herbarum,* Ad (con) Stimulation of conidiogenesis in *Alternaria dauci,* all from Leach & Trione (1966). Ss (con) Stimulation of conidiogenesis in *Stemphylium solani* (Sproston 1971). Lt (peri) Stimulation of perithecial formation in *Leptosphaeria trifolii* (Leach 1972). Bc Stimulation of conidiogenesis in *Botrytis cinerea* (Honda & Yunoko 1978). Ps Inhibition of germination of *Puccinia striiformis* uredospores, Pg Inhibition of germination of *Puccinia graminis* uredospores, both from Maddison & Manners (1973). Published action spectrum expressed on a per quantum basis, data have been re-calculated on an energy basis. The solid line is the best log linear fit (r^2=0.898, p<0.001) to the above data, using geometric means when different action spectra coincide at the same wavelength, and is given by: effect (per unit energy)=10 (15.71-(0.0525 × wavelength)). Caldwell's generalised plant action spectrum (Caldwell et al 1986:- - - - -) and the DNA action spectra of Setlow (1974: – – –) and Quaite et al. (1992: – - – -) are shown for comparison.

and UV-A wavelengths is relatively consistent (Figure 1). With respect to changes in UV-B resulting from ozone depletion, the key aspect of this fungal response is that its logarithmic slope against wavelength is relatively shallow. The slope is closer to the action spectrum for DNA damage in intact alfafa seedlings (Quaite et al. 1992) than either Setlow's DNA action spectrum (Setlow 1974) or the commonly used generalised plant action spectrum (Caldwell et al. 1986). When the consequences of this spectral response were considered (using the model of Bjorn & Murphy (1985) as software developed by E. L. Fiscus & F. L. Booker, N. Carolina State University), the calculated radiation amplification factor for mid-temperate latitudes was approx. 0.8. Therefore, on grounds of spectral response, fungi seem as likely to be affected by effects on ozone depletion on UV-B as many other organisms and processes (Madronich et al. 1995). However, this possible action spectrum for fungal responses to UV-B suggests that pathogens may be less affected than their hosts, given the most widely used plant action spectrum (Caldwell 1971; Caldwell et al. 1986).

Interactions: what are the overall impacts of elevated UV-B for plant-consumer relationships?

Given that both host plants and consumers may be affected by UV-B, but that responses may be inhibitory or stimulatory, it is clear that an enormous range

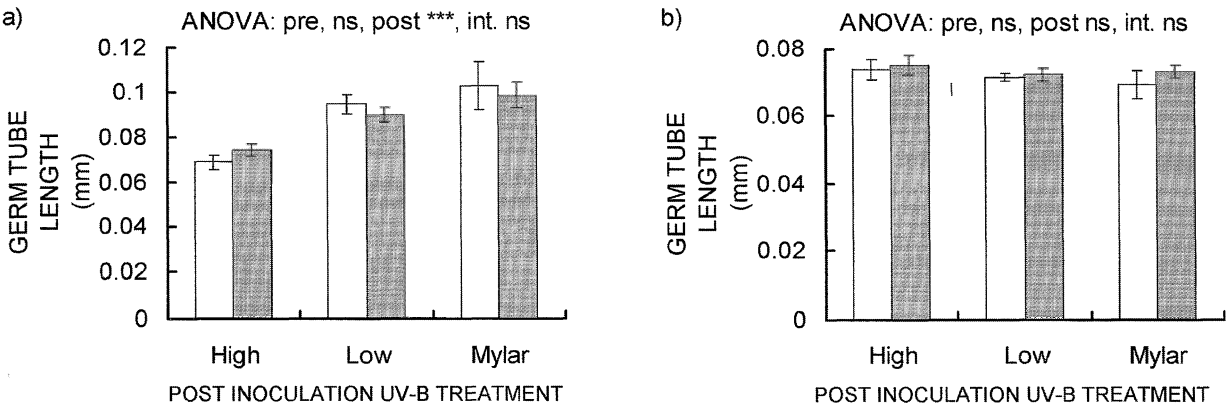

Figure 2. Germ tube growth of (a) a UK isolate and (b) a Tunisian isolate of *Septoria tritici* on the leaf surfaces of wheat (cv Riband) exposed to high or low UV-B before ('pre') or after ('post') inoculation. High (1.9 kJ m^{-2} d^{-1} UV-B_{DNA}) and low (1.3 kJ m^{-2} d^{-1} UV-B_{DNA}) UV-B treatments (weighted according to Setlow, 1974) were provided from cellulose acetate filtered Philips TL40 lamps, the post- inoculation treatments also included mylar filtered lamps. Data are means of 12 replicates ± standard error. The results of analysis of variance are shown *, **, *** equal significance at $p<0.05$, 0.01 and 0.001 respectively, n.s.=not significant.

of effects are possible in response to ozone depletion. We have studied in detail the effects of UV-B on the interaction between cultivated wheat (*Triticum aestivum*) and fungal pathogens of the imperfect genus Septoria. *Septoria tritici* (perfect stage *Mycosphaerella graminicola*), and *S. nodorum* (perfect stage *Leptosphaeria nodorum*) are the causal agents of, respectively, leaf blotch and glume blotch, both diseases causing significant yield loss in commercial wheat crops. In a UK isolate of *S. tritici*, the germination of conidia and germ tube growth are strongly inhibited by UV-B, but not UV-A, both *in vitro* (Rasanayagam et al. 1995) and on leaf surfaces (Figure 2a). There was no evidence that this UV-B response was modified by background illumination with white light (Rasanayagam et al. 1995) or UV-A (Rasanayagam, unpubl). Wheat was grown in controlled environment chambers at either 1.4 kJ m^{-2} d^{-1} UV-B_{DNA} (comparable to modelled 'ambient' UV-B for summer at 55 °N under clear-sky conditions) or 2.0 kJ m^{-2} d^{-1} (equivalent to an ozone depletion of approximately 15%) before and/or after inoculation with *S. tritici*. UV-B prior to inoculation had no effect on subsequent infection (or on concentrations of UV-B absorbing compounds) but high UV-B post-inoculation resulted in a highly significant inhibition of infection (Rasanayagam et al. 1997). Similarly, during spring in the field, irradiation (using a simple square treatment to increase ambient UV-B_{DNA} by 40%) significantly reduced the number of *S. tritici* lesions that developed following artificial inoculation (Rasanayagam et al. 1997).

The wheat-*S. tritici* system thus appears to be a relatively straightforward example of how UV-B may affect disease. Changes in infection are dependent only on the response of the pathogen: changes in the host, if they occur, have no overall effect. However, closer examination reveals increasing complexity. The responses just described are of a genotype of *S. tritici* (ST91) isolated from a commercial wheat crop in the UK. However, an isolate obtained from Tunisia proved insensitive to UV-B *in vitro* and *in planta* in controlled environment studies (Figure 2b: Rasanayagam et al. 1995, 1997). In a larger screen of isolates from lower latitudes (using the same methods as described by Rasanayagam et al. 1995) none were inhibited by UV-B *in vitro* (Figure 3a): in some isolates germination and germ tube growth were stimulated. This lack of response might be expected if there is significant selection for UV-B tolerance in high UV-B climates. However, further investigation revealed very marked variation in isolates collected from a small area of southern England (Figure 3b). We do not know whether this variation is the result of selection by incident UV-B, or simply random variation. More 'fine scale' screening of isolates has not been possible. However, given the known diversity of *S. tritici* populations (Ahmed et al. 1995), we do not exclude the possibility that comparable variation in UV-B response may occur in isolates from a single field, perhaps even from a single ascocarp. Clearly, variation in UV-B response within pathogen populations has profound implications for overall responses to ozone depletion.

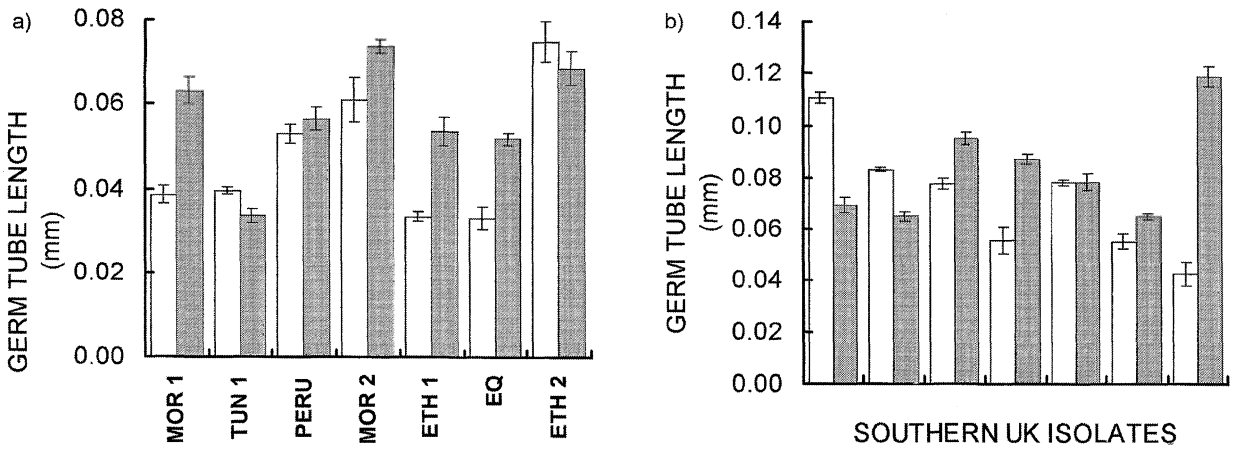

Figure 3. In vitro germ tube growth of (a) sub-tropical and tropical isolates and (b) UK isolates of *S.tritici* exposed to 1.9 kJ m^{-2} d^{-1} $UV\text{-}B_{DNA}$ or darkness (no UV-B: shaded bars) and subsequently incubated for 24 h. Data are means of 12 replicate plates ± standard error (germ tube length was measured for 20 conidia per plate). The origin of isolates was as follows: (a) MOR1 & MOR2 from Morocco, TUN1 from Tunisia: PERU from Peru, ETH1 & ETH2 from Ethiopia, EQ from Equador. (b) all UK isolates were collected from SW England or S.Wales.

It is also clear that the effects of UV-B on leaf blotch disease caused by *S. tritici* do not predict the responses of other diseases of wheat. For example, *S. nodorum*, the cause of glume blotch, was more sensitive to UV-B *in vitro* than *S. tritici* ST91 (Rasanayagam et al. 1995), and germ tube growth on the leaf surface was also inhibited (Rasanayagam, unpubl). In controlled environments there was also a tendency for infection by *S. nodorum* to be reduced by high UV-B post inoculation, but high UV-B before inoculation pre-disposed plants to infection (Rasanayagam et al. 1997). In the field, increasing UV-B above ambient had no effect on *S. nodorum* infection (Rasanayagam et al. 1997). We do not know whether these responses determined with a single isolate are representative of *S. nodorum* in general, although Biggs et al. (1986) found no effect of field supplementation on natural infections of this pathogen. Another unknown is the effect of host cultivar. We have studied the effects of *Septoria* diseases only in the highly susceptible cultivar Riband, currently the dominant winter wheat used in the UK. However, we clearly cannot assume that comparable responses to UV-B would be found in other wheat cultivars, especially as infection of wheat by stem rust *(Puccinia recondita)* was increased by UV-B, but only in a rust-susceptible cultivar (Biggs et al. 1986). Similar variation in interactions between UV-B and pathogens has been observed in cucumber, where effects varied between different cultivars and between true leaves and cotyledons (Orth et al. 1990).

Since wheat is generally insensitive to the direct effects of UV-B on growth or development, the overall effects of UV-B on interactions between wheat and *Septoria* spp. will be determined by changes in infection. However, in many host plants, the direct effects of UV-B on host growth will interact with those resulting from altered consumer activity. Arguably, while changes in the severity of infection may be of interest, the critical outcome of interactions between UV-B and consumers is their consequences for the growth of the host. In sugar beet, damage caused by UV-B and *Cercospora beticola* was greater than additive (Panagopoulos et al. 1992). The effect of increased UV-B on infection of rice by *Pyricularia grisea*, the cause of blast disease, was very variable (Finckh et al. 1992). However, under increased UV-B the host suffered greater reductions in growth from a given level of infection, i.e. UV-B decreased tolerance to disease. Given that tolerance to both pathogens (Clarke 1986) and herbivores (Rosenthal & Kotanen 1994) is partly a function of host characteristics such as assimilate partitioning, which are known to be affected by increased UV-B, it is unfortunate that this has not been considered in other systems.

Conclusions

Our existing understanding of the effects of UV-B on higher plant-consumer interactions allow only very tentative conclusions to be made. On the grounds

of their greater exposure and, perhaps, their spectral response, plants may be more likely to show significant responses to increased UV-B than consumers. However, a considerable range of host responses to UV-B may affect consumer organisms or the outcome of their activity, and changes may be positive or negative. The direct effects of UV-B on consumers are also either positive or negative. However, such effects may be of limited significance in the field due to characteristics of many consumers that limit their exposure, and also the relative importance of UV-A compared with UV-B in inducing responses. Furthermore, given that many consumers, especially micro- organisms, are genetically diverse and reproduce rapidly, there is clearly significant scope for rapid selection of UV-B tolerant genotypes, and so adaptation to a changing UV-B environment. Overall, it seems likely that the effects of increased UV-B on interactions between different hosts and consumers will differ in nature and magnitude. In the context of ozone depletion, we currently know too little to make even preliminary assessments of how, if at all, such diverse responses to UV-B may combine to alter consumer communities and their impact on the host plant.

Finally, we should note that we have considered the effects of UV-B only on interactions between higher plants and primary consumers, i.e. only a very limited subset of interactions between trophic levels. The effects of UV-B on higher trophic levels, predators, parasites etc. are essentially unknown and seem rarely to have been considered. It is intriguing that insect pathogens, both viral (Killick & Warden 1992) and fungal (Ignoffo & Garcia 1992; Moore et al. 1993) may be vulnerable to environmental UV-B, while insect susceptibility to viral pathogens might be reduced by UV-B increases in phenolics (Schultz 1990). The lack of information on the effects of UV-B on higher trophic levels is a further limit on any consideration of the overall impacts of UV-B on terrestrial ecosystems.

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