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The interactive effects of UV-B radiation and a herbicide on uptake and allocation of carbon in two strains of the green alga *Scenedesmus*

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Abstract

We examined the effects of UV-B radiation on ^{14}C-uptake rates and carbon assimilation into the major end-products of photosynthesis of the green alga *Scenedesmus* in the presence and absence of the triazine herbicide simetryn. Experiments were conducted using both a herbicide-susceptible and herbicide-tolerant strains of *Scenedesmus*. Three different UV-B dose rates were used as well as a light control. The lowest dose rate was almost the same level as in subsurface of ponds and lakes, while the other two were slightly lower and higher than natural sunlight on the surface of ponds and lakes, respectively. Total uptake rates of ^{14}C were not reduced by the UV-B irradiation alone even at the highest dose rate. However, in the presence of the herbicide, uptake rates were clearly reduced by the highest dose rate of UV-B concomitant with increasing herbicide concentrations in the herbicide-susceptible strain. On the other hand, the proportion of lipid fraction was slightly reduced by all the UV-B treatments in the herbicide-susceptible strain even in the absence of the herbicide. In the herbicide-tolerant strain, uptake rates were not affected by UV-B radiation or by the herbicide. These facts indicated that UV-B effects could be smaller than predicted. It may be important to examine combined effects of UV-B and other anthropogenic and/or natural stresses for assessing actual UV-B effects in the field.

Abbreviations: HS – Herbicide Susceptible; HT – Herbicide Tolerant; PAR – Photosynthetically Active Radiation (400–700 nm); UV-B – Ultraviolet radiation ranging from 280–320 nm; UV-A – Ultraviolet radiation ranging from 320–400 nm.

Introduction

Algal communities have been exposed to various natural and/or anthropogenic stresses, and have adapted to such stresses during their evolutionary history. One of these stresses, on which much attention is now focused, is ultraviolet radiation. Substantial evidence now exists which demonstrates that current levels of ultraviolet radiation can adversely affect phytoplankton production (Lorenzen, 1979; Smith, 1989; Helbling et al., 1992; Häder, 1993; Karentz et al., 1994). Furthermore, the large variation in species-specific tolerance to UV radiation (Karentz et al., 1991; Xiong et al., 1996, 1997) has been shown to affect species composition (Worrest et al., 1978). Moreover, various environmental changes such as the effects of acid precipitation can result in reduced dissolved organic carbon (DOC) concentrations in the water producing enhanced ultraviolet environments, especially in freshwater ecosystems (Schindler et al., 1996). This effect is in addition to the enhanced UV-B radiation expected as a consequence of ozone depletion (Smith et al., 1980, 1992; Madronich, 1994; Madronich et al., 1995; Häder et al., 1995).

Phytoplankton communities may simultaneously be exposed to various natural and/or anthropogenic stressors. Lakes and ponds surrounded by agricultural regions are often exposed to enhanced loadings of nutrients and pesticides in addition to UV radiation. Among those stressors herbicides directly

inhibit algal growth. Thus, the standing crop of phytoplankton in herbicide-affected communities is usually lowered (deNoyelles et al., 1982), and the species and/or genetic composition of communities may subsequently change from susceptible to tolerant species or strains (deNoyelles et al., 1982; Kasai & Hanazato 1995). Generally, resistant strains are inferior competitors to susceptible ones in the absence of the stress, because they need more energy to maintain their resistance mechanisms (Hoffmann & Parsons, 1991). Therefore, it is reasonable to assume that stress-resistant strains will respond differently compared with susceptible strains to other environmental stresses such as UV radiation. In support of this, the suggestion has been made that clonal adaptation to UV radiation can occur in nature (Karentz et al., 1994).

Research, which focuses on the interaction effects of ultraviolet radiation and other anthropogenic and/or natural stresses, is comparatively rare (e.g. with salinity, Döhler, 1984; with copper, Rai et al., 1995). Therefore, we undertook a study to examine the interactive effects of UV-B radiation and herbicides on photosynthesis (as ^{14}C uptake), using two strains of *Scenedesmus* that differed in their susceptibility to the triazine herbicide simetryn.

We focused on UV-B effects because this wavelength region will be enhanced in surface waters following both acidification and/or ozone depletion events. The projected increases in UV-B radiation are expected to occur in the absence of any change in UV-A or PAR; wavelengths which offer energy essential for repairing damaged DNA and/or the photosynthetic apparatus (Smith & Baker, 1989). Moreover, we examined interaction effects on carbon allocation into major end-products of photosynthesis, because any alteration in biochemical composition (Arts & Rai, in press) may change nutritional values of foods consumed by grazers, thus influencing energy flow throughout the food web (cf. Karentz et al., 1994).

Materials and methods

Algal strains. We used two strains of *Scenedesmus gutwinskii* var. *heterospina* Bodrogk., which were either susceptible (Herbicide Susceptible = HS) or tolerant (Herbicide Tolerant = HT) to the herbicide simetryn [a photosynthetic inhibitor: 2,4-*bis*(ethylamino)-6-methylthio-1,3,5-triazine]. These strains were originally isolated from pond phytoplankton exposed to simetryn (Kasai & Hanazato, 1995). Effective concentrations of simetryn that caused a 50% reduction in population growth (EC 50's) after 7 d were 15–22 $\mu g\ l^{-1}$ and 590–850 $\mu g\ l^{-1}$ for HS and HT strains, respectively.

Preculture in chemostats. Alga was grown in chemostats containing 2 l defined medium (18 mg l^{-1} $NaNO_3$, 3 mg l^{-1} β-Na_2 glycerophosphate, 40 mg l^{-1} $MgSO_4 \cdot 7H_2O$, 50 mg l^{-1} $CaCl_2 \cdot 2H_2O$, 70 mg l^{-1} KCl, 0.5 g l^{-1} Tris (hydroxymethyl) aminomethane and trace metals (Ichimura, 1983)) by the flow rate of 1 l per day.

Chemostats were illuminated by four white fluorescent lamps (Durotest® 20 W; 80–100 $\mu mol\ m^{-2}\ s^{-1}$ measured at the outer surface of the chemostat walls) set on 12h:12h light-dark photo regime (light period = 8.00–20.00 h). Room temperature was kept at 21–22 °C.

When cells were harvested for the experiments, cell densities in chemostats were $2.20 \pm 0.21 \times 10^6$ (mean ± S.D.) and $1.91 \pm 0.16 \times 10^6$ cells ml^{-1} for the HS and HT strains, respectively.

Exposure to the herbicide. Exponentially growing algal cells were harvested from the chemostats into 1 L Ehrenmeyer flasks. Cell density was adjusted to 1×10^6 cells ml^{-1} by adding freshly prepared media (same as chemostat culture). Next, concentrated herbicide stock solutions were added to achieve final herbicide concentrations of 5, 10 and 20 $\mu g\ l^{-1}$.

The flasks (3 herbicide treatments and 1 control for each strain) were shaken at 20–25 °C under white fluorescent lamps (80–100 $\mu mol\ m^{-2}\ s^{-1}$ and a 12h:12h light and dark photo regime; light period = 8:00–20:00 h) for 2 d before the uptake experiments.

Uptake experiments. After the 2 d incubation period, 500 ml of each herbicide-treated or control culture was spiked with 34 $\mu Ci\ NaH^{14}CO_3$ to obtain a specific activity of 1.5×10^5 DPM ml^{-1}. After thorough mixing, two 1 ml aliquots were transferred to scintillation vials to verify the concentration of ^{14}C added to the cell suspension. Forty five ml aliquots were then dispensed quickly into glass culture vessels (4 cm diam., 8.5 cm height) which were placed in a photosynthesizer (see below). The incubations were always initiated between 10:00 and 11:00 h.

We measured total uptake of ^{14}C and incorporation of carbon into major end-products of photosynthesis following 4 h incubations using a photosynthesizer, in which temperature, visible light and UV levels

Table 1. A summary of the filter settings to create a light control and three different UV-B dose rates

Treatment	Cutoff filter		Metal sieve		Black cap[3]
	WG 305[1]	Mylar D[2]	27% penetration	44% penetration	
Light control	–	–	–	–	+
Dose 1	+	+	+	–	–
Dose 2	+	–	–	+	–
Dose 3	+	–	–	–	–

[1] Schott WG 305: Cutoff <305 nm; 50% cutoff at 305 nm.
[2] Perforated Mylar D film: Cutoff <320 nm; 70% cutoff at 320 nm; 22% open area for Mylar film.
[3] Black cap: No UV penetration.

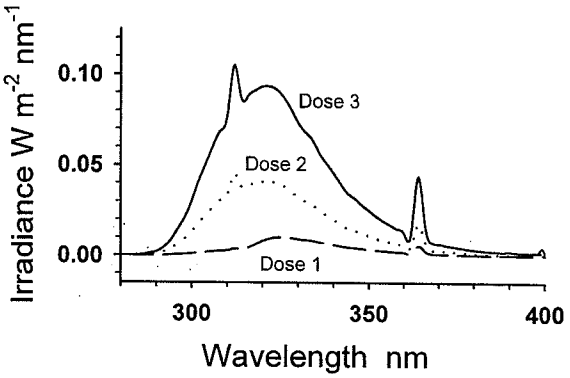

Figure 1. Irradiance spectra of three different dose rates. Irradiance was measured at the surface of the cell suspension in the incubation vessels.

were carefully controlled. The photosynthesizer, modified from Rai and Krambeck (1992), was equipped with temperature controlled aluminum block with 36 ports, into which the glass incubation vessels could be inserted. PAR was provided by halogen lamps (Philips Model 6434 FR, 12 V, 20 W, 18°) mounted underneath the incubation vessels. The incubation ports were arrayed in 4 banks of 8 lamps and the voltages for each bank could be independently controlled by a computer. Four additional ports (sealed at the bottom) were used for dark measurements. PAR levels were adjusted to either 70 (low PAR) or 300 μmol m^{-2} s^{-1} (high PAR) using perforated metal sieves with different mesh sizes and/or neutral density filters (Schott NG #'s 4, 5 or 11) mounted in a tray held between the halogen lamps and incubation vessels. These PAR levels corresponded to subsaturated and saturated PAR intensities for photosynthesis of *Scenedesmus* cells, respectively. PAR was measured by a quantum scalar meter (Biospherical Model QSL-100) at the bottom of the cell suspension in the incubation vessels. Temperature in the cell suspension was 17–20 °C depending on the PAR level.

Ultraviolet irradiation was supplied by 8 UV-B bulbs (Q-panel® 40 W; peak at 313 nm) mounted at 77 cm height above the surface of the culture vessels. UV-C and shorter wavelengths of UV-B irradiation emitted by the UV-B bulbs was screened out using Schott WG 305 filters (Figure 1). Three different dose rates of UV irradiation (Dose 1 to 3) were created using combinations of metal sieves and perforated Mylar-D film (Figure 1, Table 1). PAR only controls (Light controls) were created by covering incubation vessels with blackened glass caps. Dose 1 was not used for the low PAR treatment.

UV radiation levels in the incubation vessels were measured with a spectroradiometer (Optronic Laboratories Inc., Model OL-754) equipped with a cosine receptor (Optronic Laboratories Inc., Model OL IS-670). To simulate surface irradiation values to a cell suspension in the incubation vessels, UV irradiation was measured through the same filter(s) that were used during the experiments (e.g. Schott WG 305, Mylar-D, etc.). This was done by placing the filters over top of a cylinder that was temporarily mounted over the entrance hole in the cosine receptor. The cylinder was made with copper and glass to mimic an incubation vessel inserted in the aluminum block of the photosynthesizer. Mean dose rates for 4 h exposure of UV irradiation were calculated from these measured values (Table 2).

During the incubation period the vessels in the aluminum block were shaken gently to avoid settling of the algal cells. After 4 h incubation under UV irradiation and PAR, 5 and duplicate 10 ml aliquots were taken to measure total uptake and incorporation of ^{14}C into each end-product of photosynthesis, respectively. The cell suspension was immediately filtered through pre-combusted glass-fiber filters (Whatman GF/F) and the collected cells were then rinsed three times using Milli-Q water. For total uptake, filters with labeled cells

Table 2. Unweighted and biologically weighted dose rates of UV irradiation measured at surface of the cell suspension

	UV-B ($W\ m^{-2}$)			UV-A ($W\ m^{-2}$)			UV-B + UV-A ($W\ m^{-2}$)		
	Dose 1	Dose 2	Dose 3	Dose 1	Dose 2	Dose 3	Dose 1	Dose 2	Dose 3
Unweighted dose rate	0.06	0.59	1.33	0.25	0.87	1.96	0.32	1.45	3.29
Biologically weighted dose rate									
Jones and Kok chloroplast (1966; normalized at 275 nm)	0.02	0.25	0.55	0.06	0.21	0.47	0.08	0.45	1.02
Jones and Kok chloroplast (300 nm)	0.04	0.44	0.99	0.10	0.37	0.83	0.15	0.81	1.82
Cullen et al. *Phaeodactylum* sp. (1992; 300 nm)	0.02	0.26	0.56	0.02	0.05	0.13	0.04	0.31	0.69
Cullen et al. *Prorocentrum micans* (1992; 300 nm)	0.02	0.24	0.53	0.01	0.04	0.08	0.03	0.27	0.61
Setlow DNA (1974; 300 nm)	0.02	0.18	0.41	0.00	0.00	0.00	0.02	0.18	0.41
Caldwell Plant (1974; 300 nm)	0.02	0.18	0.40	0.00	0.00	0.00	0.02	0.18	0.40

were placed into scintillation vials and 3.5 ml scintillation cocktail (Filter Count, Packard Instruments) was added. ^{14}C in the samples was measured by a liquid scintillation counter (Canberra-Packard Tri-Carb 1900 CA, Packard Instruments). The GF/F filters containing the algal cells were stored at −70 °C until the analysis of macromolecular end-products.

^{14}C uptake rates were calculated according to Rai (1982) based on the values taken by single measurements for different combination of UV dose rates and herbicide concentrations.

Analysis of macromolecular end products of photosynthesis. The distribution of ^{14}C into major macromolecular fractions was determined according to Rai et al. (in press). This method yields an aqueous methanol-soluble fraction (Low Molecular Weight metabolites including individual amino acids, fatty acids, sugars, nucleotides, etc. = LMW), a chloroform-soluble fraction (lipids), a hot 5% trichloroacetic acid (TCA)-soluble fraction (polysaccharides including nucleic acids) and a hot TCA-insoluble fraction (protein).

A 1 ml aliquot each of the methanol-, chloroform- and TCA-soluble fractions was pipetted individually into scintillation vials and dried overnight. TCA-insoluble fractions on filters were directly placed in scintillation vials and also dried overnight. The next day, the dried TCA-soluble residue was suspended in 1 ml of Milli-Q water and then 3.5 ml of scintillation cocktail (Instagel with 5% Carb-sorb, Packard Instruments) was added. Scintillation cocktail (3.5 ml) was added to each of the other fractions and then the ^{14}C in the samples was measured.

Analysis of chlorophyll a *and dissolved inorganic carbon.* Just prior to the uptake experiment, duplicate 10 ml aliquots of the cell suspension were filtered through GF/F filters. Filters were placed in small tubes and stored at −20 °C until analysis. Chlorophyll *a* was extracted from the filter in boiling 90% ethanol and measured fluorometrically using a Turner Designs (Model 10-AU) fluorometer (Waiser & Robarts, 1995).

Dissolved inorganic carbon available to the alga was estimated from total alkalinity measured by titration (Robarts et al., 1992).

Calculation of biologically weighted dose rates. We calculated biologically weighted dose rates using several different weighting functions (Table 2). They are: (1) an action spectrum for inhibition of electron trans-

port in isolated chloroplasts normalized to 1 at 275 nm (Jones & Kok, 1966); (2) the same action spectrum as (1) but normalized at 300 nm; (3 & 4) an action spectrum each for photosynthesis in the marine diatom *Phaeodactylum* sp. and the marine dinoflagellate *Prorocentrum micans*, respectively (Cullen et al., 1992); (5) the DNA action spectrum (Setlow, 1974 cited in Smith et al., 1980), and; (6) a generalized action spectrum for plants (Caldwell, 1971 cited in Smith et al., 1980). The functions from (3) to (6) were normalized to 1 at 300 nm.

Results and discussion

Biologically weighted dose rates. Herbicide-treated and untreated cells of *Scenedesmus* grown at 80–100 μmol m^{-2} s^{-1} were exposed to three different UV-B irradiances, 0.06, 0.59 and 1.33 W m^{-2} (Table 2), both under high (saturated = 300 μmol m^{-2} s^{-1}) and low (subsaturated = 70 μmol m^{-2} s^{-1}) PAR conditions. The UV-B lamps used in our experiments were skewed towards shorter wavelengths of UV-B and emitted smaller amounts of UV-A radiation (Figure 1) compared to natural sunlight. The very short wavelengths (i.e. <305 nm) of UV-B were eliminated by the Schott WG 305 filters. Therefore, to allow better comparison of these experimental dose rates to natural UV radiation, we calculated biologically weighted dose rates using several biological weighting functions (Smith et al., 1980, 1992; Cullen et al., 1992; Cullen & Neale, 1997).

Despite the growing number of laboratory and field studies on the effects of UV radiation on freshwater and marine biota, actual spectra of UV irradiation are rarely provided. Furthermore, in order to compare the dose rates used experimentally with those in nature it is imperative that weighting factors should be applied to the UV spectra to provide more biologically meaningful dose rates (Cullen et al., 1992; Cullen & Neale, 1997). In order to facilitate comparison between natural sunlight and our experimental UV-B lamps we refer, in the discussion which follows, to comparisons between measurements taken on the rooftop of NHRI and at the subsurface of ponds and lakes in the vicinity of Saskatchewan, Canada (M. T. Arts et al. unpublished data) and the biologically-weighted dose rates of our UV-B lamps, using the biological weighting functions derived from the action spectrum of photoinhibition in *Phaeodactylum* sp. and *Prorocetrum micans* (Cullen et al., 1992) (Table 3).

In Dose 1, the lowest dose rate, a large part of UV-B region was eliminated by the Mylar-D film, thus the biologically weighted dose rate of UV-B (Table 2) was quite similar to weighted values of natural underwater sunlight at subsurface levels of prairie ponds and lakes (Table 3). Weighted dose rates in Dose 2 and 3 were higher than the weighted values of underwater UV-B irradiation in ponds and lakes. In Dose 2, however, the weighted values were slightly lower than UV-B irradiation of natural sunlight measured on the rooftop, while those of Dose 3 were considerably higher than UV-B irradiation of natural sunlight (Tables 2 and 3).

The weighted values of UV-A region were considerably lower than the weighted values of ambient UV-A irradiation even at the highest dose rate, because the biological weighting function of UV-A region was extremely low (Smith et al., 1980; Cullen et al., 1992). Thus, given the output spectra of the Q-panel UV-B 313 lamps and the subsequent effects of the weighting functions we employed (Cullen et al., 1992), we conclude that the effects observed in the present study are principally due to UV-B radiation.

Total uptake of ^{14}C. Chlorophyll-specific ^{14}C uptake rates were compared among different herbicide treatments, different UV-B dose rates and different PAR intensities both in the HS and HT strains. These results are based on the single measurements. However, the variation in the measurements seemed to be small, because we have found very nearly the same trends in our preliminary experiments.

In the HS strain, total uptake rates decreased with increasing herbicide concentration in all UV-B treatments and a light control both under high (Figure 2a) and low PAR conditions (Figure 2b). In the absence of the herbicide, however, uptake rates were almost the same levels between different UV-B treatments under a high PAR condition (mean ± S.D. = 12.1 ± 0.19 μgC μg$Chla^{-1}$ h^{-1}, n = 4) as well as under a low PAR condition (12.1 ± 0.10 μgC μg $Chla^{-1}$ h^{-1}, n = 3) (Figures 2a, b: blank bars).

On the other hand, the uptake rates were not affected by either the herbicide and/or UV-B irradiation in the HT strain (Figures 3a, b). The uptake rates (mean ± S.D.) under different UV treatments and no herbicide addition were 11.5±0.41 μgC μg$Chla^{-1}$ h^{-1} (n = 4) and 11.4 ± 0.17 μgC μg$Chla^{-1}$ h^{-1} (n = 3) under high and low PAR conditions, respectively (Figures 3a, b: blank bars). These results indicated that photosynthetic rates of the *Scenedesmus* strains were not adversely influenced by UV-B irradiation itself in

Table 3. Mean values of unweighted and biologically weighted irradiance of natural sunlight measured on the rooftop of NHRI and at subsurface of 20 ponds and lakes in vicinity of Saskatchewan, Canada

	UV-B (W m^{-2})		UV-A (W m^{-2})		UV-B + UV-A (W m^{-2})	
	Ponds and lakes[2]	Rooftop[3]	Ponds and lakes	Rooftop	Ponds and lakes	Rooftop
Unweighted dose rate	0.24 ± 0.17[4]	1.48 ± 0.53	11.4 ± 5.56	37.8 ± 9.50	11.6 ± 5.72	39.2 ± 10.0
Biologically weighted dose rate[1]						
Phaeodactylum sp.	0.05 ± 0.03	0.32 ± 0.13	0.34 ± 0.18	1.22 ± 0.34	0.38 ± 0.21	1.53 ± 0.46
Prorocentrum micans	0.04 ± 0.03	0.27 ± 0.11	0.15 ± 0.09	0.60 ± 0.17	0.19 ± 0.12	0.87 ± 0.28

[1] According to Cullen et al. (1992) normalized at 300 nm.
[2] Average values of subsurface irradiance of ponds and lakes measured at each time during 10:00–16:00 from July 22 to October 4, 1996.
[3] Average values of natural sunlight measured at 13:00 on June 6, July 3, August 12 and October 17, 1996, using an air sensor directed to the sun. Thus, surface irradiance should be lower than these values, because angles of incident radiation to the water surface are smaller than those directed to the sun.
[4] Mean ± S.D.

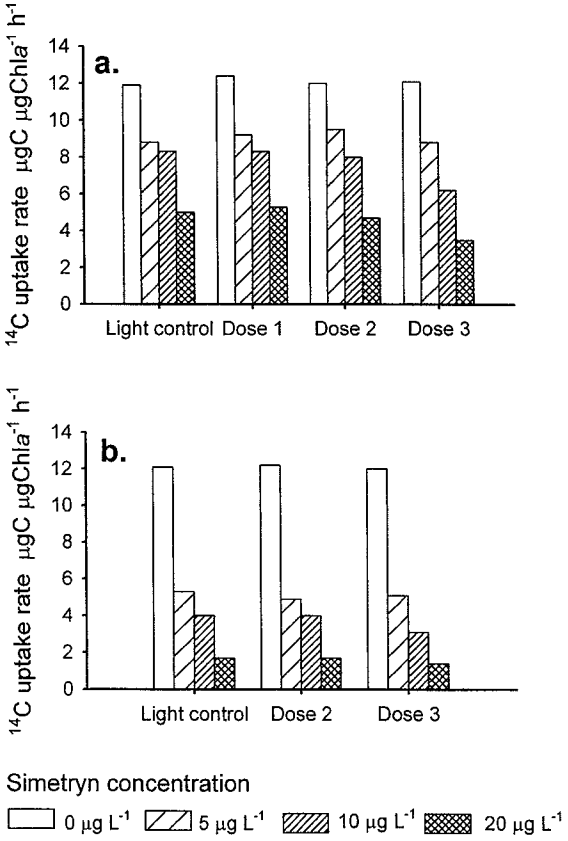

Figure 2. Chlorophyll-specific ^{14}C uptake rates in the herbicide-susceptible strain of *Scenedesmus*. (a) Under 300 μmol m^{-2} s^{-1} of PAR; (b) Under 70 μmol m^{-2} s^{-1} of PAR.

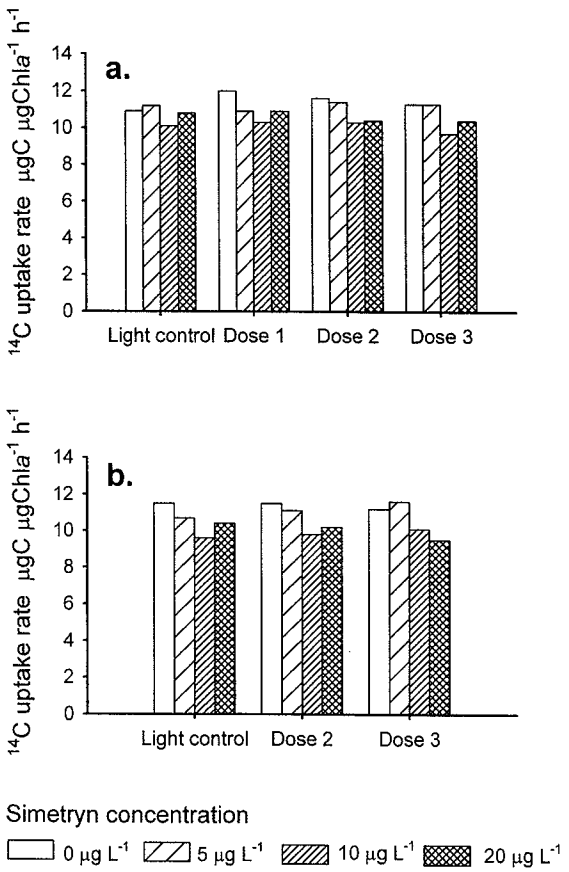

Figure 3. Chlorophyll-specific ^{14}C uptake rates in the herbicide-tolerant strain of *Scenedesmus*. (a) Under 300 μmol m^{-2} s^{-1} of PAR; (b) Under 70 μmol m^{-2} s^{-1} of PAR.

the absence of herbicide even at levels higher than those normally encountered at the surface of ponds and lakes.

Helbling et al. (1992) proposed a threshold of total solar UV radiation (UV-B + UV-A) ranging from 5

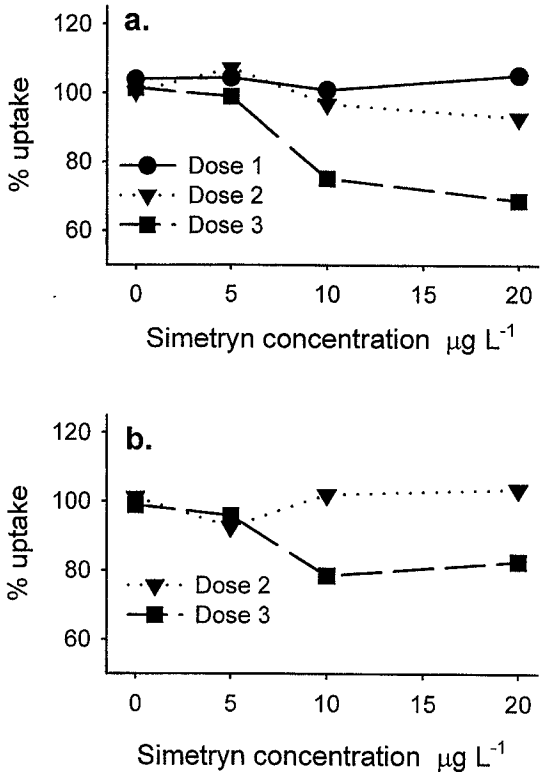

Figure 4. Comparison of UV-B effects on ^{14}C uptake rates with different herbicide concentrations in the herbicide-susceptible strain. % uptake = (uptake rate under each UV-B irradiation)/(uptake rate under a light control) $\times 100$. (a) Under 300 μmol m^{-2} s^{-1} of PAR; (b) Under 70 μmol m^{-2} s^{-1} of PAR.

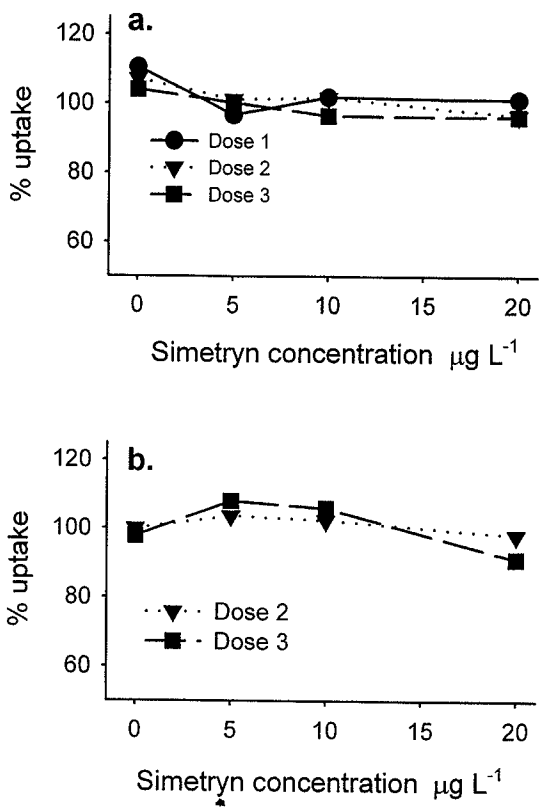

Figure 5. Comparison of UV-B effects on ^{14}C uptake rates with different herbicide concentrations in the herbicide-tolerant strain. % uptake was calculated by the same way as in Figure 4. (a) Under 300 μmol m^{-2} s^{-1} of PAR; (b) Under 70 μmol m^{-2} s^{-1} of PAR.

to 10 W m^{-2} from their experiments using natural phytoplankton communities, below which little or no enhancement of photosynthetic rate was observed. The total UV irradiation level used in the present study was only 3.29 W m^{-2} in the highest dose rate (Table 2). Thus, in the light of the threshold described by Helbling et al. (1992), it is plausible that this amount of UV irradiation did not reduce photosynthetic rates in the absence of the herbicide, even if UV-B wavelengths dominated total UV irradiation.

As previously mentioned, a large variation in UV tolerance has been demonstrated among phytoplankton species. In addition, species inhabiting high UV environments, either seasonally or spatially, seemed to be more UV resistant (Gala & Giesy, 1991; Montecino & Pisarro, 1995; Villafañe et al., 1995; Lesser, 1996). The two strains of *Scenedesmus* used in the present study were isolated from shallow and low DOC ponds in mid-June (in central Japan, Kasai & Hanazato, 1995). Thus, we may be able to ascribe the observed UV tolerance of these strains in the absence of the herbicide as being characteristics of species inhabiting high UV environments.

In addition, contrary to our initial assumption, these two strains (HS and HT strains) did not show any clear differences in their response to UV-B irradiation when the herbicide was absent. Moreover, ^{14}C uptake rates in the absence of both the herbicide and UV stresses did not differ between the HS and HT strains at least under the experimental conditions we used. In higher plants, resistant strains are generally considered to have less ecological fitness in the absence of the stress because of energy requirement to maintain resistant mechanisms and structures (Hoffman & Parsons, 1991). Herbicide-resistant weeds usually showed reduced photosynthetic rates and biomass production in the absence of herbicide stress (Conard & Radsevich, 1979). The two algal strains that we used in our experiments did not show such a phenomenon.

To further unravel the UV effects, we considered % uptake; defined as the percentage of the uptake rates under individual UV-B irradiances compared to those under the light control without UV irradiation (Figures 4, 5). Thus, in this context, % uptake shows the real effects of UV-B under individual herbicide concentrations. In the HS strain, % uptake decreased at the 10 and 20 $\mu g\ l^{-1}$ herbicide concentrations at the highest UV-B dose rate (Dose 3) (Figure 4). Percentage uptake at other UV-B dose rates, however, did not change as herbicide concentrations increased both under high and low PAR conditions (Figures 4a, b). Thus, in the presence of the herbicide, UV-B effects were clearly revealed in the HS strain.

Simetryn is a photosystem II (PS II) inhibiting herbicide. The target site for these types of herbicides is the D1 protein of the PS II complex in the chloroplast membrane (cf. Gronwald, 1994). The herbicidal activity is thus considered to be due to the oxidative stress generated when the photosynthetic electron transport system is blocked (Gronwald, 1994). On the other hand, PS II also seems to be the most sensitive target site for UV-B radiation (Eichhorn et al., 1993; Vass et al., 1996). Vass et al. (1996) have shown that UV-B radiation inhibits the electron transport, when the D1 and D2 proteins of the PS II complex are destroyed. At this point the potential exists for reactive oxygen to form (see Malanga & Puntaluro, 1995). Therefore, the mechanisms that exert harmful effects on photosynthesis in algae seem to be very similar for both UV-B radiation and PS II inhibiting herbicides.

In the HT strain, it may be reasonable to conclude that % uptake was not affected by UV-B irradiation under both high and low PAR conditions, because the HT strain was never affected by the herbicide alone (Figures 5a, b). The UV-B effects became evident when 10–20 $\mu g\ l^{-1}$ of simetryn was used. The herbicide simetryn has been used in restricted areas in the world for weed control in paddy fields. However, similar types of herbicides in terms of their mode of action (e.g. atrazine and simazine) are being used worldwide (cf. Solomon et al., 1996). The concentrations that we used were slightly higher than values commonly observed in streams and rivers adjacent to agricultural regions during spraying periods in Japan (usually $<$5–10 $\mu g\ l^{-1}$, Hatakeyama et al., 1994). However, the portion of photoinhibition due to UV radiation may be enhanced in the field situation because natural UV radiation includes large amounts of energy in the UV-A region as well as in the UV-B region and, in some cases, a majority of UV photoinhibition could be ascribed to UV-A radiation (Karentz et al., 1994; Kim & Watanabe, 1993).

Figure 6 shows the comparison of the uptake rates between under high and low PAR conditions (the percentage of the uptake rates under low PAR condition compared to those under high PAR condition). Low PAR intensity clearly reduced total uptake rates of the HS strain compared to high PAR condition even at the lowest concentration of the herbicide examined (Figure 6a), while the percentage reduction was nearly identical between UV-B treatments. Thus, high PAR seemed to compensate for the harmful effects of the herbicide in the HS strain. In contrast low PAR did not reduce total uptake rates of the HT strain (Figure 6b). In the low PAR condition PS II may be regulated by the mechanism which is suppressed by the herbicide, but not in the high PAR condition. Another possibility may be that higher energy supply under high PAR conditions enhanced the repair and/or synthesis of the photosynthetic apparatus which were damaged by the herbicide in the HS strain.

Allocation of carbon into macro-molecular end-products. In the HS strain, the incorporation rate of ^{14}C into individual fractions was reduced as a function of herbicide concentration under high PAR condition (Figure 7, Light control) as shown for total uptake. This trend was the same in the presence of UV-B irradiation (Figure 7, Dose 1–3), and also under low PAR condition (Figure 8). UV-B irradiation, especially at highest dose rate, seemed to reduce individual incorporation rates into lipids, polysaccharides and protein fractions slightly in accordance with increased herbicide concentration.

In contrast to the absolute incorporation rates, the proportion of each fraction did not change much with herbicide concentration. Under high PAR condition, however, the proportion of the lipid fraction seemed to be slightly lowered by all the UV-B treatments without the herbicide in the HS strain. This phenomenon, however, was not clearly observed under the low PAR conditions. The ranges of the proportion of each fraction shown in all herbicide treatments and a control were: LMW = 1–4%, lipids = 19–35%, polysaccharide = 23–41% and protein = 36–48% under high PAR condition. LMW = 2–9%, lipids = 20–28%, polysaccharide = 23–28% and protein = 43–50% under low PAR condition.

The incorporation rates were not affected by the herbicide in the HT strain, both under high (Figure 9) and low PAR (Figure 10) conditions. The proportion

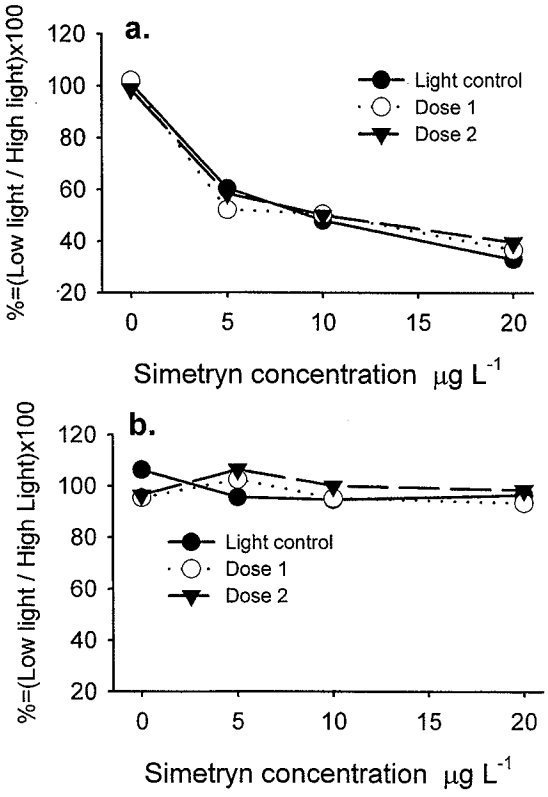

Figure 6. Comparison of ^{14}C uptake rates between low and high PAR conditions (70 μmol m^{-2} s^{-1} vs. 300 μmol m^{-2} s^{-1}) under different UV-B irradiation. % = (uptake rate under low PAR)/(uptake rate under high PAR) × 100. (a) Herbicide-susceptible strain; (b) Herbicide-tolerant strain.

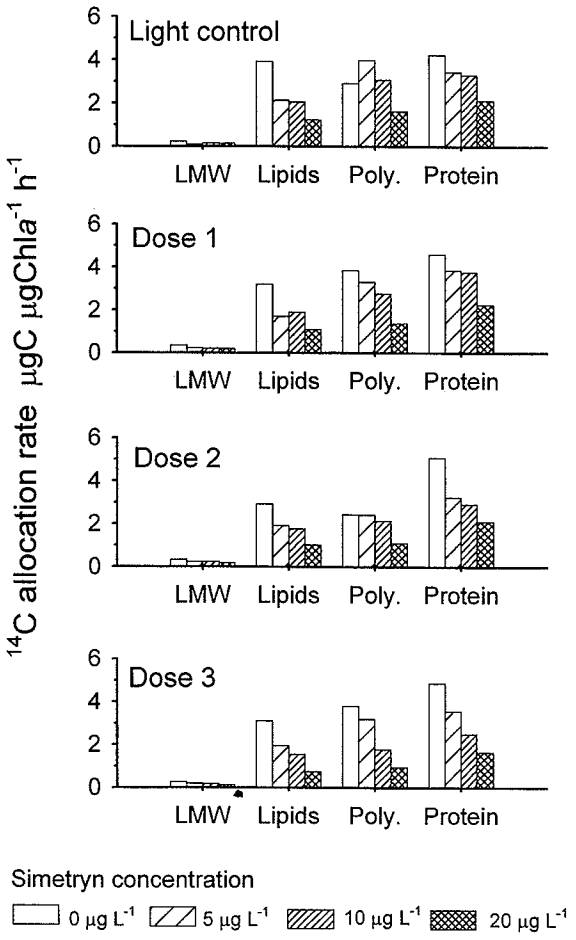

Figure 7. ^{14}C incorporation rates into each macromolecular end product of photosynthesis in the herbicide-susceptible strain under 300 μmol m^{-2} s^{-1} of PAR only (light control) and PAR + three different UV-B dose rates after 4 h.

of each fraction also did not change. The ranges of the proportion of each fraction were: LMW = 2–8%, lipid = 30–42%, polysaccharide = 16–26%, protein = 33–42% under high PAR condition, and LMW = 3–7%, lipid = 23–31%, polysaccharide = 27–42%, protein = 29–43% under low PAR condition. We observed one interesting difference between the two strains in that the proportion of carbon fixed as polysaccharide was generally higher in the HS strain (30% versus 20% in the HT strain) under high PAR condition.

Smith et al. (1987) reported that the proportion of the protein fraction became maximized when photosynthetic rate was high in natural populations. In the present study, the proportion of the protein fraction was generally high in all the treatments. We used exponentially growing cells in the nutritionally sufficient state to examine ^{14}C incorporation. The high proportion of the protein fraction may reflect the high growth rate in the chemostats, although the distribution of ^{14}C also depends on differences between species and other environmental factors (Cuhel & Lean, 1987).

Morris et al. (1974) demonstrated that, in short term experiments, changes in ^{14}C allocation can be observed even if total ^{14}C uptake remains unchanged. In the present study, in contrast with the total uptake rate small changes in proportion of assimilated ^{14}C, as shown in the lipid fraction in the HS strain, occurred under all UV-B treatments without herbicide. Small difference in short term exposure may be amplified during longer periods, and may result in changes in nutritional status of phytoplankton species and ultimately phytoplankton assemblages.

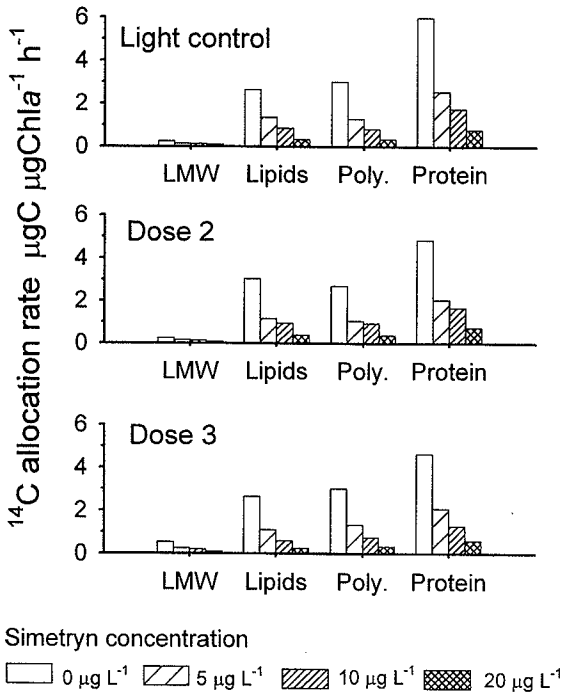

Figure 8. ^{14}C incorporation rates into each macromolecular end product of photosynthesis in the herbicide-susceptible strain under 70 μmol m^{-2} s^{-1} of PAR only (light control) and PAR + two different UV-B dose rates after 4 h.

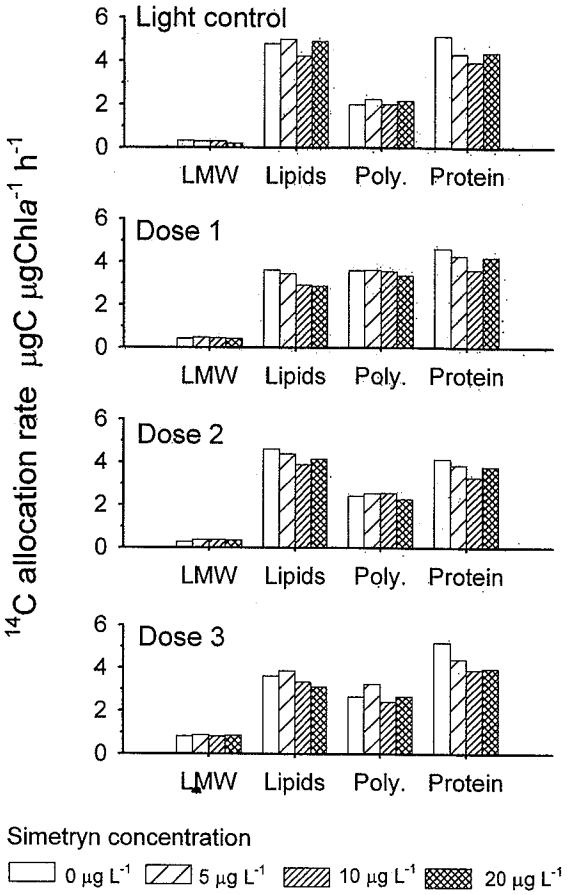

Figure 9. ^{14}C incorporation rates into each macromolecular end product of photosynthesis in the herbicide-tolerant strain under 300 μmol m^{-2} s^{-1} of PAR only (light control) and PAR + three different UV-B dose rates after 4 h.

Conclusion

The results of the present study indicated that the UV-B irradiation higher than in natural sunlight did not appear to affect photosynthetic rates in the *Scenedesmus* strains in the absence of herbicide stress. This fact suggests that harmful effects of UV-B region may be quite smaller than predicted. However, the *Scenedesmus* strains that we used may be relatively tolerant to UV-B irradiation compared to other algal groups (cf. Xiong et al., 1996, 1997).

On the other hand, the results also clearly demonstrated that the UV-B reduced photosynthetic rates in the presence of the herbicide. The herbicide concentrations exhibiting a combined effect with UV-B irradiation were higher than ambient levels commonly observed. However, it is probable that natural sunlight could enhance the reduction of phytoplankton photosynthesis even with much lower concentration of herbicides than examined here, because natural sunlight contains larger amount of UV-A radiation as well as UV-B region.

It is thus crucial to know more detailed information on combined effects of UV radiation and other natural and/or anthropogenic stresses for assessing actual UV-B effects in the field.

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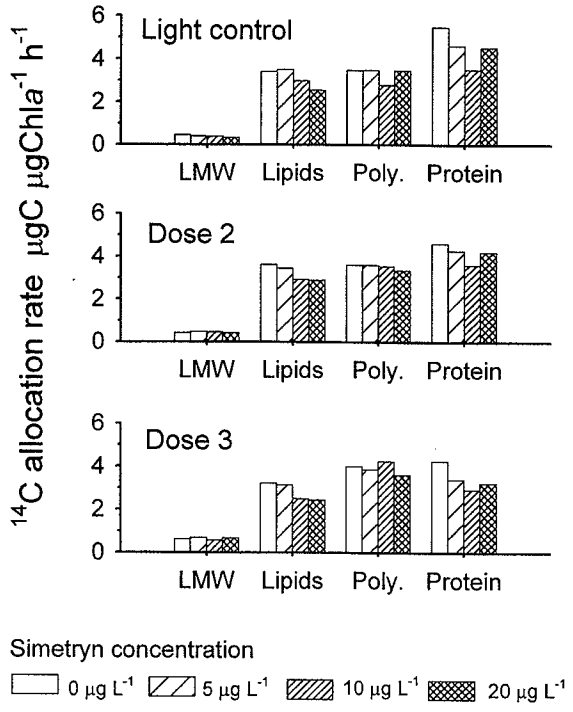

Figure 10. ^{14}C incorporation rates into each macromolecular end product of photosynthesis in the herbicide-tolerant strain under 70 µmol m^{-2} s^{-1} of PAR only (light control) and PAR + two different UV-B dose rates after 4 h.

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