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# Effects of UV-B on motility and photoorientation in the cyanobacterium, *Phormidium uncinatum*

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**Abstract.** UV-B irradiation has a detrimental effect on the survival of populations of the filamentous cyanobacterium, *Phormidium uncinatum*, at levels slightly higher than those currently measured at the surface of the earth. The organisms are not damaged or killed by UV-B radiation at $\leqq 300$ nm of 200 $\mu Wm^{-2}$ for up to 20 h; but slightly increased levels of UV-B irradiation (2 h of 200 $\mu Wm^{-2}$ at $\leqq 300$ nm) drastically impair motility, phototactic orientation and photophobic responses. These photosynthetic organisms require a narrow light intensity range for growth so that any decrease in their ability to actively search for and move into areas of favorable light conditions is bound to affect the survival of a population. The fluorescence yield of both phycobilins and chlorophyll is not altered even after 20 h of UV-B irradiation (200 $\mu Wm^{-2}$ at 270 nm) indicating that UV-B at that dose does not affect the photosynthetic apparatus. The organisms are killed either by too bright intensities which bleach the photosynthetic pigments or by the lack of energy when they are unable to avoid moving into dark areas.

**Key words:** Cyanobacteria (blue-green algae) – Fluorescence – Motility – *Phormidium uncinatum* – Photomovement – Photophobic response – Phototaxis – UV-B

Recent investigations and modelling indicate a measurable decrease in the stratospheric ozone layer due to the production and emission of manmade chlorofluoromethanes and other pollutants (Schippnick and Green 1982; Steed et al. 1982; Green 1983). This effect results in an increased UV-B impact on the surface of the earth and an extension to shorter wavelengths (Baker et al. 1980; Green et al. 1980; Berger and Urbach 1982).

Increased levels of UV-B irradiation have been shown to affect the growth of higher plants (Biggs et al. 1981; Tevini et al. 1981; Teramura 1983) and to impair the photosynthetic activity (Vu et al. 1982; Iwanzik et al. 1983; Kulandaivelu and Noorudeen 1983). Electron microscopic investigations have demonstrated damages of the epidermal layer of leaves (Tevini and Iwanzik 1983). Some microorganisms have been shown to be affected by increased UV-B levels, such as bacteria (Ascenzi and Jagger 1979), slime molds (Nozu et al. 1980; Ohnishi et al. 1982a, b; Häder 1983) or marine phytoplankton (Worrest et al. 1981). Cyanobacteria are promising candidates as a bioassay for increased UV-B irradiation both because of their sensitivity and their ecological and economical importance due to their ability to fix atmospheric nitrogen.

The filamentous blue-green alga, *Phormidium uncinatum*, glides with a yet not completely understood mechanism when in contact with a substratum. As a photosynthetic organism, it depends on the availability of solar radiation. However, too bright light intensities bleach the photosynthetic pigments and damage the organisms within a short time. The filaments adjust their position in the changing photoenvironment by means of two photobehavior mechanisms (Häder 1979): They move towards the light source by means of a phototactic orientation (Nultsch 1961) and avoid gliding into shaded areas by means of a photophobic response (Nultsch 1962; Häder 1979). Recently it has been demonstrated that the filaments avoid very bright areas using a step-up phobic reversal (Häder and Burkart 1982a, b). Thus, the organisms accumulate in areas of suitable irradiation by means of a delicate balance between several basic photomovement responses. A disturbance of this balance by increased levels of UV-B irradiation is bound to have detrimental effects on the survival of the population.

## Materials and methods

The cyanobacterium, *Phormidium uncinatum*, grew in static culture as described previously (Nultsch and Häder 1974). The filaments were harvested, cut into fragments about 100 cells long and suspended in 0.3% water agar (Häder 1982) at 40° C. After solidification of the agar in plastic petri dishes (10 cm in diameter) the organisms were predarkened over night and then subjected to the irradiation procedures using both population techniques and an individual organism video analysis.

Photophobic responses were assayed using a modified light trap technique (Häder 1979) with a supplementary UV-B irradiation entering the preparation from the top through openings in the plastic petri dishes covered with cut-off filters with a transmittance of $>0.84$ in the UV-B band (WG 230, 1 mm thick, Schott & Gen., Mainz, FRG). The UV-B radiation was produced with 1 kW xenon arc lamps (Kratos, type 102). The infrared component was removed by inserting a cuvette with quartz windows and 145 mm path length of boiled distilled water. The UV-B wavelength was selected using an Ebert-type monochromator (Kratos GM 250) with a grating with 1,180 lines per mm blazed at 300 nm. All optical components were made of quartz. Fluence rates were measured with a radiometer

(IL 1500) with a UV sensitive photomultiplier (PM 271F) and a power supply (IL 760) from International Light. The fluence was adjusted by regulating the xenon lamp current and/or the exposure time. The actinic white light was produced with Leitz Prado quartz halogen slide projectors (250 W), adjusted by inserting neutral densitiy filters (to guarantee a uniform color temperature between experiments) and measured with a luxmeter (Lange, Berlin).

The number of organisms which had accumulated within 8 h was estimated densitometrically (Häder and Burkart 1978). The phototactic orientation of the population was determined using a similar experimental set-up, with the actinic light impinging from the side.

The video analysis of the photoresponses of individual filaments was carried out with an inverted microscope (Zeiss ICM 405) and an infrared-sensitive video camera (National WV 1350E). The monitoring light was produced by the built-in microscope light source combined with an infrared passing cut-off filter (>780 nm, Schott & Gen.). The video signal was recorded on a 1/2 inch timelapse videorecorder (National NV 8030E) which allowed to observe the rather slow gliding movement of *Phormidium* up to 80 times faster. Photophobic responses were observed at light/dark boundaries produced by projecting a white light field into the preparation using a half-silvered front surface mirror. The additional UV-B irradiation was produced with a 300 W xenon arc lamp (Kratos L140) and a monochromator (Kratos GM 252).

The absorption spectra were measured at room temperature in a spectrophotometer (Shimadzu MPS 5000) after white light or UV-B irradiation. The organisms were mixed with 0.2% water agar to keep them in suspension during the measurement. The fluorescence spectra were measured using a computer controlled fluorometer consisting of a xenon arc light source (Kratos type 102), two monochromators (Kratos GM 525-1) operated by a stepper motor under computer control (Elzet, Z80 microcomputer) and a photomultiplier (Hamamatsu R 1104) connected to a lock-in amplifier (EG & G Brookdeal type 9503) and a 12 bit A/D converter (Kontron). The data were stored in computer memory and plotted on a digital plotter (Hiplot, Houston Instruments).

## Results

### *1. Motility*

The motility of a population consists of two components: the percentage of motile individuals and the average speed of the organisms. The number of motile filaments decreased drastically with increasing exposure times to UV-B irradiation (Fig. 1). Especially shorter wavelengths $\leq 300$ nm reduced the percentage of motile organisms drastically even after only 60 min of exposure. Longer wavelengths had only a moderate effect as compared to the control. The second component of motility, the average speed, was not as drastically influenced (Fig. 2). The speed was measured as distance a colony had spread out from the central inoculation spot within 24 h. The spreading speed of a colony is about 10% of the speed of an individual due to the autonomous periodic reversal of movement of the filaments. Even after 2 h of UV-B exposure at the beginning of a 24 h movement period the speed of the population (i.e. the motile fraction thereof) was reduced only by about 15% at 280 nm. There was no obvious recovery from the UV-B treatment during the following 24 h.

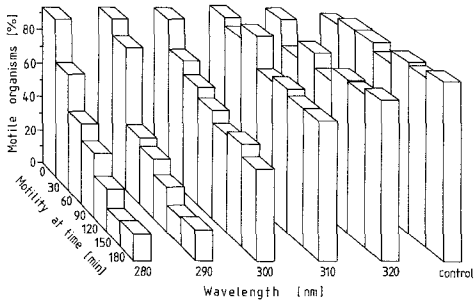


**Fig. 1.** Percentage of motile filaments in a population measured immediately after UV-B exposure of various wavelengths for various times with a fluence rate of 200 $\mu Wm^{-2}$. Number of organisms per data point >100

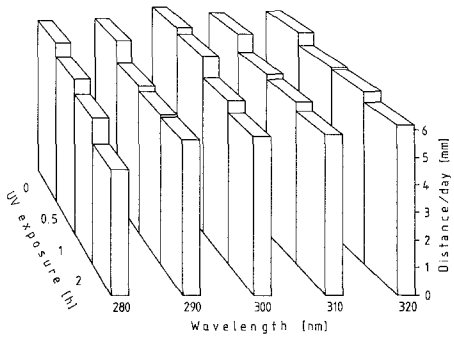


**Fig. 2.** Speed of movement of a population measured in terms of distance the organisms have spread from the inoculation spot within 24 h determined immediately after an initial UV-B exposure of various wavelengths for 0 to 2 h with a fluence rate of 200 $\mu Wm^{-2}$

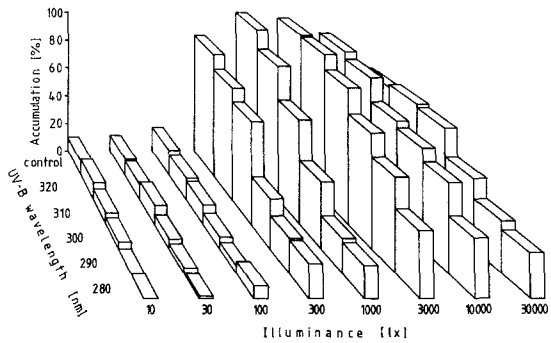


**Fig. 3.** Effect of 30 min UV-B exposure (200 $\mu Wm^{-2}$) of various wavelengths on the density of *Phormidium uncinatum* accumulating in light traps of various illuminances during 12 h. Abscissa as a percentage of the highest value

### *2. Photophobic responses*

The density of photophobic accumulations in a light field strongly depends on the light intensity. Under the experimental conditions used, the optimum was found at 1,000 lx which for this specific light source corresponds to about 4.2 $Wm^{-2}$ (Fig. 3). A 30 min exposure to UV-B irradiation of 0.2 $mWm^{-2}$ drastically reduced the accumulation density especially at short wavelengths. The inhibitory effect was comparable at all intensities of the white actinic light

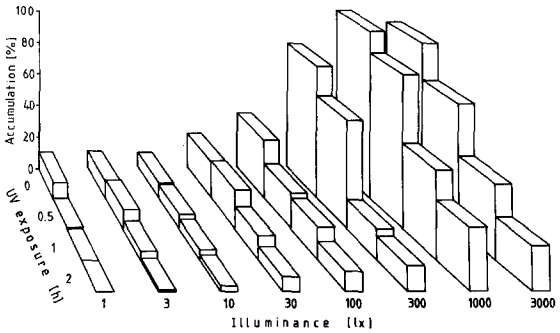


**Fig. 4.** Accumulation density in light fields of various illuminances in per cent of the highest value after UV-B preirradiation (280 nm, 200 $\mu Wm^{-2}$) for various exposure times at the beginning of the 12 h actinic irradiation

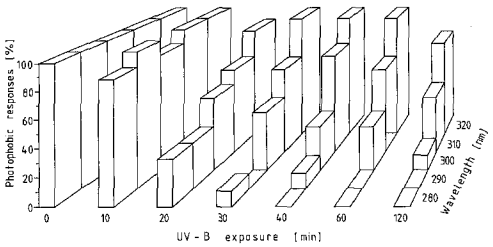


**Fig. 5.** Effect of UV-B irradiation (200 $\mu Wm^{-2}$) of various wavelengths on the number of organisms responding photophobically at a light (1,000 lx) /dark boundary (in percent of the uninhibited control) determined immediately after various UV-B exposure times. Number of organisms per data point >100

field. The inhibition strongly depended on the exposure time (Fig. 4).

In order to study the behavior of individual organisms, photophobic responses at a light/dark boundary were followed using a video system. Almost all filaments reversed the direction of movement when leaving a 1,000 lx white light field and entering a dark field (Fig. 5). After a short exposure time to UV-B irradiation, the percentage of responding filaments decreased drastically again especially in wavelengths $\leq$300 nm. At 280 nm a dose of 2.4 $Jm^{-2}$ reduced the percentage of responding organisms to about 30% and at 5 $Jm^{-2}$ the photophobic response was totally inhibited.

In addition to the step-down photophobic response (when an organism leaves a light field) there is a step-up photophobic response (elicited by an increase in light intensity). This response is negligible in low actinic light intensities and most prominent in high intensities (Fig. 6). Additional UV-B irradiation drastically changes the quantitative distribution between the two responses. At low light intensities (30 lx) the number of step-up responses increases from 2.7% to 35.2% when a supplementary UV-B wavelength of 280 nm is irradiated. At high actinic intensities (30,000 lx) step-up responses are suppressed: 2.3% at 280 nm vs 33.9% in the control.

### *3. Phototactic orientation*

*Phormidium uncinatum* shows positive phototaxis with an optimum at about 300 lx. When irradiated for 1 h with a fluence rate of 200 $\mu Wm^{-2}$ at the beginning of a 24 h exposure to lateral actinic white light the phototactic orientation decreases drastically with decreasing wavelengths (Fig. 7). The relative effect is similar for all actinic white light intensities tested. The decrease in phototactic activity is more pronounced after longer UV-B exposure times. (Fig. 8). The orientation in a population is almost negligible after 2 h of exposure to 280 nm.

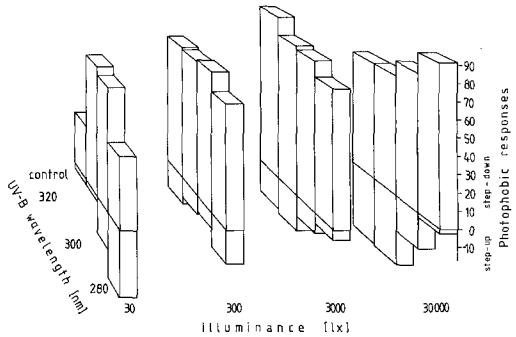


**Fig. 6.** Percentage of organisms which show a step-up or step-down photophobic response at a light/dark boundary at various illuminances in the control and immediately after 30 min UV-B irradiation (200 $\mu Wm^{-2}$) of various wavelengths. Number of organisms per data point >100

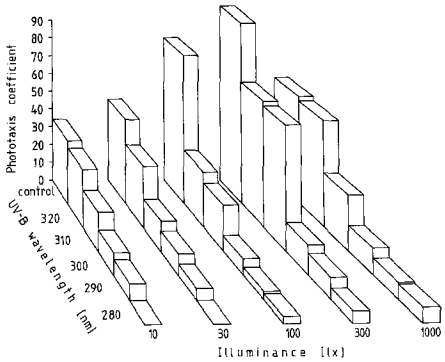


**Fig. 7.** Effect of 1 h UV-B irradiation (200 $\mu Wm^{-2}$) of various wavelengths on the phototactic orientation of *Phormidium* during 24 h to lateral actinic white light of various illuminances, immediately following the UV-B pretreatment

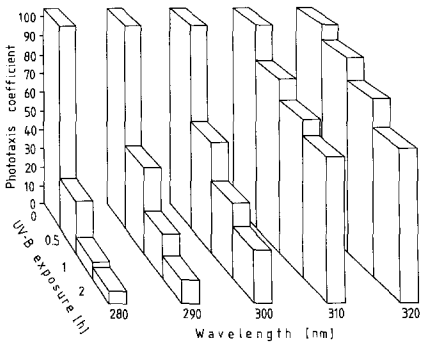


**Fig. 8.** Effect of various UV-B exposure times (200 $\mu Wm^{-2}$) of various wavelengths on the phototactic orientation to actinic lateral white light of 300 lx. The UV-B irradiation was given at the beginning of the 24 h white light period

### *4. Pigment content*

The absorption spectrum in vivo at room temperature indicates the presence of chlorophyll a, carotenoids and the

**Table 1.** Effect of UV-B irradiation of various duration on absorption measured 24 h after the onset of the UV-B exposure (280 nm, 200 $\mu Wm^{-2}$) and fluorescence measured 20 h after the onset of the UV-B exposure (270 nm, 200 $\mu Wm^{-2}$) normalized to the highest value

| Excitation [nm] | Absorption | Fluorescence at | |
|---|---|---|---|
| | | 440 nm | 530 nm |
| UV exposure [h] | | | |
| 0 | 1.00 | 1.00 | 1.00 |
| 0.5 | 1.02 | 1.01 | 0.99 |
| 1 | 1.07 | 1.02 | 0.99 |
| 2 | 1.11 | 1.00 | 1.00 |
| 4 | 1.13 | 0.98 | 1.00 |
| 6 | 1.16 | 1.01 | 1.01 |
| 8 | 1.24 | – | – |
| 20 | – | 1.02 | 1.01 |

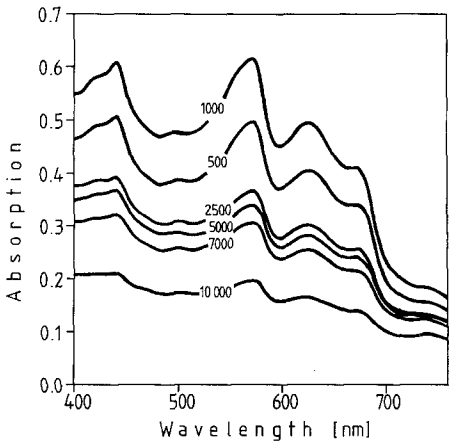


**Fig. 9.** Absorption spectra of *Phormidium uncinatum* measured after 24 h exposure to white light of various illuminances (in lx)

phycobilins, C-phycoerythrin and C-phycocyanin (Table 1). Exposure to a fluence rate of 200 $\mu Wm^{-2}$ at a wavelength of 280 nm increased the absorption of the filaments when measured 24 h after the beginning of the UV-B irradiation. The absoprtion increase was similar in all measured spectral regions.

The absorption changes due to white light irradiation were far more pronounced than after UV-B irradiation (Fig. 9). The highest absorption was found when the organisms were kept at 1,000 lx white light for 24 h. Both lower and higher illuminances decreased the absorption. The decrease at high light intensities was not restricted to certain wavelength ranges, but rather due to a general photobleaching. In high light intensities, the cell density did not decrease during the exposure period; but the smaller absorbance at 500 lx and lower intensities (data not shown) is due to a retarded growth rate rather than a bleaching effect.

*5. Fluorescence*

The fluorescence spectra were measured at room temperature. The organisms were kept in a 0.2% agar solution to prevent the filaments from settling during the measurement. The samples were preirradiated with UV-B (200 $\mu Wm^{-2}$) for various exposure times and the spectra were recorded 20 h after the onset of the UV-B irradiation. When the excitation wavelength was set at 530 nm, fluorescence maxima were observed at 582, 659 and 693 nm. The organism density was the same in all measurements and the fluorescence intensity was not affected by the UV-B irradiation (Table 1). With an excitation wavelength of 440 nm the fluorescence maximum was found at 689 nm. Also in this case the fluorescence yield was not altered by the UV-B irradiation.

## Discussion

Blue-green algae depend on the availability of photosynthetically utilizable radiation in a narrow range of intensities. A deviation to lower intensities causes a reduced growth rate, while an increase above the optimal intensity causes a significant bleaching of the photosynthetic pigments. The organisms actively move into areas of optimal light intensities and avoid unfavorable light conditions using positive phototaxis and step-up and step-down photophobic responses. In order to simulate the natural conditions the UV-B irradiation was administered simultaneously with the actinic white light.

In addition to impairing motility, increased levels of UV-B irradiation inhibit photoorientation. The inhibition of photomovement is independent of and not a result of the decreased motility. The step-down photophobic response induces a reversal of movement each time the organism moves into a shaded area. Based on the expected UV-B fluence for a clear summer day at medium latitudes, the measured data predict a considerable inhibition of the photophobic responses even at current ozone levels (Green 1983): The compilation of expected UV-B fluences by Gerstl et al. (1983) predicts for Marburg (50° north) for a summer month (June) at noon (solar zenith angle = 30°) a downward solar UV flux of $7.4 \times 10^{-7}$ $Wm^{-2}nm^{-1}$ at 290 nm, $3.1 \times 10^{-4}$ $Wm^{-2}nm^{-1}$ at 295 nm and $7.3 \times 10^{-3}$ $Wm^{-2}nm^{-1}$ at 300 nm for 0% ozone depletion. At these UV levels the experiments predict a ca. 70% reduction in the accumulation density. Any further increase in the UV-B level would drastically reduce the ability to avoid moving into unfavorable conditions.

In addition, UV-B has a quantitatively different effect on the two photophobic responses. The addition of ultraviolet radiation to low intensity actinic white light dramatically increases step-up photophobic responses, while the step-down response is not markedly affected. Thus, organisms are prevented from entering light fields from a dark surrounding area, which explains the poor accumulations observed in low light intensity fields in the presence of UV-B. In high intensity white light fields, the individual organism analysis shows a slight increase in step-down photophobic responses and a drastic decrease in step-up responses. Therefore the filaments are no longer prevented from entering hazardous high light intensity fields.

There is an apparent contradiction to the population experiments where UV-B decreased the density of photoaccumulations in high light intensity fields. The reason for this discrepancy is the UV-B impaired phototaxis. Organisms outside light fields with high intensities are attracted by the light scattered from organisms or particles inside the field (Burkart and Häder 1980). This phototactic movement from the surrounding areas into light traps contributes significantly to the accumulation density. It even can-

cels the step-up photophobic response in high light intensities. Since the phototactic orientation is drastically impaired by UV-B radiation, the accumulation density in light fields is reduced.

The molecular UV-B target is not known. A mechanism due to a DNA damage is unlikely, since a decrease in the photophobic response could be observed in the video analysis a few minutes after the onset of the UV-B radiation. Therefore a direct effect on proteins or a specific receptor is to be expected. The photophobic response in *Phormidium* is mediated by the photosynthetic pigments. Since the absorption of the filaments is not impaired even by long UV-B irradiation an effect on the photoreceptor pigments can be excluded as well. Similarly, a pigment damage would have resulted in a fluorescence decrease. UV-B has been reported to affect the electron transport chain between photosystems II and I (Iwanzik et al. 1983). An impaired electron transport results in a fluorescence increase, since the excitation irradiation cannot be utilized photochemically. Since the fluorescence yield was not affected by an even prolonged UV-B irradiation neither a destruction of bulk chlorophyll and phycobilins nor an effect on the electron transport chain has occured at the UV doses used. These fluorescence measurements do not exclude, though, that the reaction centers have been damaged. This could be investigated by measuring the fluorescence induction (Iwanzik et al. 1983, Kulandaivelu and Noorudeen 1983).

The amplification of the photophobic signal is based on gated ionic currents (Murvanidze et al. 1982a, b). These currents eventually alter the electrical potential between the front and rear ends of the filaments (Murvanidze and Glagolev 1981, 1982). The electrical potential changes involved in the reversal of movement are a possible target for UV-B irradiation (Milotic and Solic 1983).

In summary, the results show that the filamentous cyanobacterium, *Phormidium uncinatum* is not directly damaged or killed by slightly enhanced UV-B levels, expected as a result of a decreased stratospheric ozone layer due to the production and emission of manmade gaseous pollutants. However, a dramatic indirect effect is to be expected because of the impaired motility, phototactic orientation and photophobic responses, which reduces the ability of the organisms to orient themselves in their photoenvironment. Any failure to quickly respond to changes in the light intensity is bound to cause the death of the organisms either due to photobleaching of the pigments in high light intensities or the lack of photosynthetically produced energy in shaded areas.

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## References

Ascenzi JM, Jagger J (1979) Ultraviolet action spectrum (238–405 nm) for inhibition of glycine uptake in *E. coli*. Photochem Photobiol 30:661–666

Baker KS, Smith RC, Green AES (1980) Middle ultraviolet radiation reaching the ocean surface. Photochem Photobiol 32:367–374

Berger DS, Urbach F (1982) A climatology of sunburning ultraviolet radiation. Photochem Photobiol 35:187–192

Biggs RH, Kossuth SV, Teramura AH (1981) Response of 19 cultivars of soybeans to ultraviolet-B irradiance. Physiol Plant 53:19–26

Burkart U, Häder D-P (1980) Phototactic attraction in light trap experiments: a mathematical model. J Math Biol 10:257–269

Gerstl SAW, Zardecki A, Wiser HL (1983) UV-B Handbook Vol I. Los Alamos National Laboratory New Mexiko

Green AES (1983) The penetration of ultraviolet radiation to the ground. Physiol Plant 58:351–359

Green AES, Cross KR, Smith LA (1980) Improved analytic characterization of ultraviolet skylight. Photochem Photobiol 31:59–65

Häder D-P (1979) Photomovement. In: Haupt W, Feinleib ME (eds) Encyclopedia of plant physiology. vol. VII. Physiology of movements. Springer, Berlin Heidelberg New York, pp 268–309

Häder D-P (1982) Gated ion fluxes involved in photophobic responses of the blue-green alga, *Phormidium uncinatum*. Arch Microbiol 131:77–80

Häder D-P (1983) Effects of UV-B irradiation on sorocarp development of *Dictyostelium discoideum*. Photochem Photobiol 38:551–555

Häder D-P, Burkart U (1978) Mathematical model for photophobic accumulations of blue-green algae in light traps. J Math Biol 5:293–304

Häder D-P, Burkart U (1982a) Enhanced model for photophobic responses of the blue-green alga, *Phormidium uncinatum*. Plant Cell Physiol 23:1391–1400

Häder D-P, Burkart U (1982b) Mathematical simulation of photophobic responses in blue-green algae. Math Biosci 58:1–17

Iwanzik W, Tevini M, Dohnt G, Voss M, Weiss W, Gräber P, Renger G (1983) Action of UV-B radiation on photosynthetic primary reactions in spinach chloroplasts. Physiol Plant 58:401–407

Kulandaivelu G, Noorudeen AM (1983) Comparative study of the action of ultraviolet-C and ultraviolet-B radiation on photosynthetic electron transport. Physiol Plant 58:389–394

Milotic B, Solic F (1983) Effect of ultraviolet radiation on the biopotential of the cells. Periodicum Biologorum 85:142–144

Murvanidze GV, Glagolev AN (1981) Calcium ions regulate reverse motion in phototactically active *Phormidium uncinatum* and *Halobacterium halobium*. FEMS Microbiol Lett 12:3–6

Murvanidze GV, Glagolev AN (1982) Electrical nature of the taxis signal in cyanobacteria. J Bacteriol 150:239–244

Murvanidze GV, Gabai VL, Glagolev AN (1982a) The role of membrane potential in the photophobic response and certain aspects of chemotaxis in the cyanobacterium *Phormidium uncinatum*. Microbiologia 51:240–246

Murvanidze GV, Gabai VL, Glagolev AN (1982b) Taxis responses in *Phormidium uncinatum*. J Gen Microbiol 128:1623–1630

Nozu K, Ohnishi T, Okaichi K (1980) Viability of the spores formed after UV irradiation in *Dictyostelium discoideum*. Photochem Photobiol 32:261–263

Nultsch W (1961) Der Einfluß des Lichtes auf die Bewegung der Cyanophyceen. 1. Mitteilung: Phototopotaxis von *Phormidium autumnale*. Planta 56:632–647

Nultsch W (1962) Der Einfluß des Lichtes auf die Bewegung der Cyanophyceen. III. Mitteilung: Photophobotaxis bei *Phormidium uncinatum*. Planta 58:647–663

Nultsch W, Häder D-P (1974) Über die Rolle der beiden Photosysteme in der Photo-phobotaxis von *Phormidium uncinatum*. Ber Dtsch Bot Ges 87:83–92

Ohnishi T, Eimoto H, Okaichi K (1982a) Enhancement of ultraviolet or N-methyl-N'nitro-N-nitrosoguanidine sensitivity of *Dictyostelium discoideum* by 3-aminobenzamide. Photochem Photobiol 35:515–519

Ohnishi T, Hazama M, Okaichi K, Nozu K (1982b) Formation of nonviable spores of *Dictyostelium discoideum* by UV-irradiation and caffeine. Photochem Photobiol 36:355–358

Schippnick PF, Green AES (1982) Analytical characterization of spectral actinic flux and spectral irradiance in the middle ultraviolet. Photochem Photobiol 35:89–101

Steed JM, Owens AJ, Miller C, Filkin DL, Jesson JP (1982) Two-dimensional modelling of potential ozone perturbation by chlorofluorocarbons. Nature (London) 295:308–311

Teramura AH (1983) Effects of ultraviolet-B radiation on the growth and yield of crop plants. Physiol Plant 58:415–427

Tevini M Iwanzik W (1983) Inhibition of photosynthetic activity by UV-B radiation in radish seedlings. Physiol Plant 58:395–400

Tevini M, Iwanzik W, Thoma U (1981) Some effects of enhanced UV-B irradiation on the growth and composition of plants. Planta 153:388–394

Vu CV, Allen Jr. LH, Garrard LA (1982) Effects of supplemental UV-B radiation on primary photosynthetic carboxylating enzymes and soluble proteins in leaves of $C_3$ and $C_4$ crop plants. Physiol Plant 55:11–16

Worrest RC, Wolniakowski KU, Scott JD, Brooker DL, Thompson BE, van Dyke H (1981) Sensitivity of marine phytoplankton to UV-B radiation: impact upon a model ecosystem. Photochem Photobiol 33:223–227

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