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1982 (19th) Making Space Work For Mankind

Apr 1st, 8:00 AM

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ASSESSMENT OF THE IMPACT OF INCREASED SOLAR-ULTRAVIOLET RADIATION ON SEAGRASSES

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ABSTRACT

An early concern in the planning of the Space Shuttle Program was the loss of atmospheric ozone due to repeated Shuttle flights and a resultant increase in penetrant ultraviolet radiation.

Our research group has examined the effects of an increase in this radiation (UV-B, 290-315 nm) on three marine angiosperms (<u>Halophila engelmannii</u> Aschers, <u>Halodule</u> wrightii Aschers, and Syringodium <u>filiforme</u> Kutz) important to shallow marine and estuarine ecosystems. The photosynthetic tolerance of each seagrass to UV-B and mechanisms which might prevent or reverse damage were investigated.

The data show little effect by current environmental levels of UV-B and suggest the capacity to adapt to an increased UV-B flux by various mechanisms in the different species: photorepair, flavonoid synthesis, chloroplast clumping, and epiphytic shielding.

INTRODUCTION

The depletion of the stratospheric ozone layer by anthropogenic agents (1,2,3,4) such as chlorofluoromethanes and NO_X has been studied for over a decade. A decrease in the atmospheric concentration of ozone will result in a shift in penetrant ultraviolet wavelengths from approximately 295 in biological effects (6,7). Early in the Shuttle Program concern was expressed that use of the Shuttle would contribute significantly to the ozone loss. A number of studies were therefore initiated to expand the current body of literature examining UV-B effects. This study examines the impact of ambient UV-B levels and enhanced irradiation (using the portion of the UV-B spectrum from 290 to 315 nm) on photosynthesis by three seagrass species. The photorepair capabilities of these species and the role of epiphytic growth as shielding from UV-B were directly evaluated. The three seagrasses were selected for study due to their natural distribution along a gradient of UV-B and visible radiation intensities and their ecological importance. A reduction in abundance of these seagrasses in estuarine systems could have a significant effect on many trophic levels resulting in both environmental and economic loss.

METHODOLOGY

Intact samples of <u>H. erightii</u> Aschers, <u>5. filiforme</u> Kutz and <u>H. engelmannii</u> Aschers were collected from the Indian River lagoonal system (near Melbourne, FL). Leaf tissue samples of about 75 mg were excised, cleaned of epiphytes, fresh weights determined, and placed in Petri plates of filter sterilized seawater for irradiation. The bicarbonate concentration of the seawater was determined according to Strickland and Parsons (8) and the salinity calculated from the refractive index.

The leaf tissues were irradiated at room temperature (21-25 C) with simulated solar UV-B from 290 to 315 nm to reflect moderate atmospheric ozone loss. Details of experimental design and irradiation rationale are presented in Trocine et al., 1981 (9). The leaf samples were divided into three sets: test samples to receive the experimental irradiation, irradiation controls filtered to remove UV-B, and dark controls receiving no irradiation at all. Photorepair evaluation was conducted by exposing seagrass leaves to UV-B and photosynthetically active radiation (PAR, 400-700 nm) simultaneously. The PAR intensity used was 700 μ E.m², s⁻¹ selected from preliminary studies to be photosynthetically saturating yet insufficient to cause photooxidation.

As the experimental irradiation does not match the solar spectrum exactly and not all wavelengths of UV are equally effective at inhibiting photosynthesis, the relative biological efficiency of the irradiation regime was determined. The photoinhibition action spectrum of Jones and Kok (10) as normalized by Smith et al. (11) was selected as the most appropriate weighing system for calculation of biologically effective irradiance. The resultant weighted irradiance (UV-Bpy) takes into account the wavelength dependency of photosynthetic inhibition. Biologically effective dose rates and total doses of UV-B are presented as w.m-2pt and kJ.m⁻²PI respectively.

After irradiation, the net photosynthetic rate of the leaf tissues was measured according to the modified procedure of. Bassham and Calvin (12) using a PAR in-tensity of 700 µE.m⁻².s⁻¹ at the leaf surface and an optimal temperature of 30 C. The seagrass samples were equilibrated at these conditions in 20 ml fresh filtersterilized seawater for 10 min and then transferred to an additional 5 ml of ster-ilized seawater containing 15 $\mu l~(^{14}\text{C})$ bicarbonate (1 mCi.ml-1, 50 mCi.mM-1). Following an incorporation period of 15 min, each sample was homogenized with methanol and then water. The chlorophyll was extracted with ether and total amount determined according to the method of Strain and Svec (13). Radioactive incorporation was determined by liquid scintillation counting and the photosynthetic rate calculated as by Goldman et al. (14). Inhibition of photosynthesis is expressed as the per cent decrease in the rate of (14C) uptake per unit chlorophyll by the test samples exposed to UV-B or UV-B and PAR as compared to the control tissues.

To evaluate the ability of epiphytic growth on seagrass blacks to function as shielding against UV-B, samples of leaf tissue were drradiated with their epiphytic covering intact. After irradiation, the leaves were quickly stripped of epiphytes, each fraction being weighed, and the photosynthetic rate of the leaf tissue determined. These results were then compared to the photosynthetic rates of samples stripped of epiphytes prior to

irradiation.

Scanning electron micrographs of seagrass blade cross-sections were taken to examine gross physical differences in epidermal leaf structure of the seagrasses and relate them (if possible) to photosynthetic senitivities to UV-B.

RESULTS

The intrinsic sensitivitity of seagrass photosynthesis to UV-B was determined by exposing each species to increasing dose rates and dosages of UV-B. All applications of UV-B to Halophila resulted in significant photosynthetic inhibition (Figure 1). A linear relationship between UV-B dosage and per cent inhibition of photosynthesis was seen to approximately 14.7 kJ.m-2PT, or 50% inhibition. At the dose rates used. UV-B dose rate had no statistically significant effect on photosynthetic inhibition. Total dosage of UV-B determined the extent of inhibition. The maximum UV-B experienced by <u>Halophila</u> at the collection site is approximately 3.7 kJ.m⁻²day⁻¹p_I at dose rates approaching a maximum of 0.40 w.m-2pt. Simulation of these peak solar conditions resulted in 10% inhibition of photosynthesis.

Unlike the effect of UV-B on <u>Halophila</u>, photosynthesis by <u>Halodule</u> is clearly influenced by the rate of UV-B exposure as well as the total amount applied (Figure 2). Increasing either variable enhanced photosynthetic inhibition. Peak envirommental levels of UV-B seen at the mean blade depth of Balodule were 9.2 kJ.m⁻². day⁻¹pr at 0.46 w.m⁻²pr. Laboratory recreation of this irradiance resulted in less than 2% photosynthetic inhibition. Increasing thg average dose rates to 0.88 and 1.16 w.m⁻²pt UV-B resulted in inhibition of about 3 and 15% respectively at the ambient total dose of 9.2 kJ.m⁻²pt.

Syringodium required extensive UV-B irradiation (55.0 kl.m⁻²pr) to induce 50% inhibition of photosynthesis (Figure 3); approximately four times the dosage required to cause the same degree of inhibition in <u>Halophila</u>. As in <u>Halophila</u> however, reciprocity of dose rates at a given dosage was observed. The inhibition obtained was a function of the total UV-B dosage applied. The rapid initial increase in photosynthetic inhibition at UV-B dosages reflecting peak natural conditions for <u>Syringodium</u> (7.4 kl.m⁻².day⁻¹pr] indicates a <u>low intrinsic</u> tolerance to UV-B.

During the chlorophyll extraction procedure

interesting observations were made regarding test samples receiving high dosages and/ or dose rates of UV-B. After intense exposure to UV-B the methanol-water fraction obtained in the chlorophyll extraction of <u>Halophila</u> showed an accumulation of red pigment while <u>Halodus</u> and <u>Syringodium</u> methanol-water fractions contained a yellow substance(3). In all controls (dark and irradiation) and short UV-B exposures, che methanol-water fractions obtained after chlorophyll extraction were colorless. These data suggest the synthesis of anthocyanins and other flavonoids in response to UV-B irradiation.

The possibility of UV-B induced, photosynthetic inhibition being subject to photorepair was examined in each of the seagrasses. In these experiments, where 700 ukm⁻².s⁻¹ PAR was combined with UV-B, <u>Halophila</u> failed to show a statistically significant photorepair response (Figure 1). However a slight and general reduction in the mean value of photosynthetic inhibition was obtained. No correlation between UV-B dose rate and photorepair efficiency (if present) was observed.

No UV-B photorepair capability was apparent in <u>Syringodium</u>. The combined effect of PAR and UV-B irradiation seemed to increase this species' sensitivity to UV-B (Figure 3). The increase in photosynthetic inhibition was not due to photoxidation by PAR and the photosensitization response was observed with all UV-B dose rates applied. Fifty percent inhibition was reached with only 10.0 kJ.m⁻²pI, one-fifth the dosage required to produce the inhibition by UV-B irradiation alone. This photosensitization response is examined in Trocine et al., 1982 (15).

<u>Halodule</u> was the only seagrass species to give clear evidence of a photorepair mechanism able to reverse or attentuate UV-B induced, photosynthetic inhibition (Figure 2). The addition of PAR to the UV-B irradiation held the level of inhibition to approximately 10% regardless of UV-B dose rate or dosage. This response was seen up to 20.0 kJ.m⁻²p₁, twice the maximum environmental dosage seen by this species.

The attenuation of UV-B by epiphytic growth on the leaf surfaces was found to reduce photosynthetic inhibition in the underlying tissues. An example of the results, representing an epiphytic bloom in the system, is seen in Figure 4. The shielding effect appears to correspond well to the dry weight of epiphytes present during UV-B exposure, but is only significant when the growth is dense. The scanning electron micrographs taken of seagrass blade epidermis provided useful information regarding photosynthetic sensitivity to UV-B. The epidermal cell layers of <u>Syringodium</u> (Figure 5A) and fialodule (Figure 5B) are composed of many small, thick walled cells. Micrographs of <u>Halophila</u> the most sensitive seagrass to UV-B, show the leaf to be only two or three cells thick. These cells are large and thinly walled (Figure 5C) in comparison to <u>Halodule</u> and <u>Syringodium</u> and may be a significant factor in <u>Halophila</u>'s high sensitivity to UV-B.

DISCUSSION

Photosynthesis by all of the seagrasses examined in this study was, to some degree. sensitive to UV-B. Although Halodule was able to tolerate environmental levels of IIV-B, higher dose rates and dosages (used to simulate atmospheric ozone loss) were inhibitory. The sensitivity of Halodule appears to be a function of UV-B dose rate rather than total dose. The limit of effective physiological resistance (the dose rate above which photosynthetic inhibition increases with UV-B dose) seems to be a dose rate of approximately 0.9 w.m⁻²pI. <u>Halodule</u> has the capacity to endure at least a doubling of the environmental UV-B flux at mean blade depth before 10% photosynthetic inhibition may be expected, Halophila and Syringodium, while more sensitive to UV-B than Halodule, are exposed to significantly lower dose rates and total dosages in the environment. These two seagrasses are most abundant in greater depths or more turbid conditions than Halodule, and thus their higher intrinsic sensitivity to UV-B does not subject them to increased stress under present environmental conditions.

A variety of mechanisms, both active and passive, were implicated in the attenuation or reversal of UV-B induced, photosynthetic inhibition. Two were directly examined: photorepair (seen only in <u>Halodule</u>) and epiphytic shielding (effective for all species). Other processes suggested by the data include flavonoid synthesis, chloroplast clumping, and the passive feature of epidermal thickness.

Biosynthesis of UV-absorbing substances in response to UV-B irradiation has been reported by Caldwell (6), Wellman (16) and Lindoo and Caldwell (17). Evidence from the chlorophyll extractions suggest the production of these substances as an adaptive response to long periods of UV-B exposure. Observations correlating physical appearance, chlorophyll content, and PAR intensity at <u>Halophila</u> collection sites imply that chloroplast clumping (18,19,20) occurs in this species. In this process chloroplasts clump together in response to high intensities of visible light reducing photosynthetic inhibition by photooxidation. Leaf tissues exhibiting such clumping appear pale but are not photosynthetically inhibited (20). As high PAR intensities usually corresponded to high UV-B dose rates in this very shallow water environment, chloroplast clumping due to PAR may also reduce in part the effective UV-B dosage. This response may be responsible for the slight, but general reduction in photosynthetic inhibition when UV-B irradiation was combined with 700 $\mu\text{E.m}^{-2}.\,\text{s}^{-1}$ PAR (Figure 1).

The scanning electron micrographs suggest that thickness of the epidermal cell wall plays a significant role in the attenuation of UV-B irradiation. Of the two seagrasses without measurable photorepair, Syringodium with it's thicker epidermal cell wall was far less sensitive at a given UV-B dosage than Halophila. Robberecht and Caldwell (21) have shown epidermal attenuation to be primarily due to the structural components of the epidermis, although flavonoids and related pigments contribute. The lack of a variable response to different dose rates of UV-B and it's greatly reduced sensitivity relative to Halophila suggests the structural thickness of Syringodium's epidermal cell wall is the species' principle protective mechanism.

On the basis of the photosynthetic sensitivities to UV-B displayed by the seagrasses, and the direct and indirect evidence that these species may adapt to increased UV-B irradiation, it seems unlikely that the small reduction in atmospheric zone expected to result from the Shuttle Program would impose undue stress on seagrass communities. This is particularly true as increased environmental awareness has begun to decrease the destruction of the zone layer by other sources, such as chlorofluoromethanes.

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Fig. 1. Photosynchetic inhibition in H_ engelmanni by UV-B, and UV-B in the presence of PAR. Dose rates of UV-B in the absence of PAR (\bigcirc) were 0.37 to 1.4 w.m² pr. Dose rates of UV-B in the presence_of PAR (\bigcirc) were 0.42 to 1.2 w.m² pr.



Fig. 2. Photosynthetic inhibition in <u>H</u>: wrighti by UV-B, and by UV-B in the presence of PAR. Dose rates of UV-B in the absence of PAR ranged as follows: 0.37 to 0.51 v.m⁻²pI (\bigcirc); 0.74 to 0.97 v.m⁻²pT (\bigcirc); 1.1 to 1.2 v.m⁻²pI (\bigcirc). Dose rates of UV-B in the presence of PAR ($\triangle - \neg \triangle$) were from 0.40 to 0.97 v.m⁻²pI.





Fig. 4. Attenuation of photosynthetic inhibition by epiphytic shielding. Epi-phytes present (\boxtimes), epiphytes removed (\square). Results based on duplicate samples exposed to 0.94 w^{-m}- $_{\rm PI}$, total dose 20.4 kJ.m²p_I. Average dry weight of epiphytes (ng epiphyte/g leaf): <u>H. engelmanni</u>, 100.3 mg; <u>H. wrightij</u>, <u>22.2 mg; S. filiforme</u>, 75.5 mg.

Seagrasses



Fig. 5A. Scanning electron micrograph of <u>S. filiforme</u> blade cross-section (X900). Epidermal cell wall (ECW), Chloroplast (C). Bar equals 10 µm. Average cell wall thickness (leaf-water interface), 1.5 ± 0.5 µm.



Fig. 5B. Scanning electron micrograph of <u>H. wrightii</u> blade cross-section (X1900). Epidermal cell wall (ECW). Bar equals 10 µm. Average cell wall thickness (leaf-water interface), 1.0 ± 0.5 µm.



Fig. 5C. Scaning electron micrograph of <u>H. engelmanili</u> blade cross-section (X600). Epidermal cell vall (ECW), Mitochondria (M), Chloroplast (C). Bar equals 10 µm. Average cell vall thickness (leafwater interface), 0.5 ± 0.2 µm.

ACKNOWLEDGEMENTS

The authors wish to thank John D. Rice, William C. Aspden, and Kathy S. Austin for their technical assistance in the lab and field, and Mr. Joe Giardano for his assistance with the SEM.

This work was funded by the National Aeronautics and Space Administration contract NAS9-15516, National Science Foundation grant SER77-06567, and a grant from the Dean's Research Fund, Florida Institute of Technology.