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Cytological damage to the red alga *Griffithsia pacifica* from ultraviolet radiation

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Abstract

Continuous exposure for 7–10 days to 60% of ambient levels (sea level at mid-day in December) of UV-A and UV-B radiation caused cytological damage to regenerating fragments of *Griffithsia pacifica* under laboratory conditions. There was high mortality of individual cells and entire fragments in UV treated filaments. Rhizoid initiation was slower and rhizoids grew more slowly following UV treatment. After 7 days, UV radiated thalli showed chloroplast and nuclear degeneration. In addition, filaments tended to disarticulate so that single or groups of apparently healthy cells were common in the medium. These data suggest that the subtidal habitat of *G. pacifica* is based in part on lack of tolerance to UV radiation, and that UV protection mechanisms are not inducible or insufficient to prevent the accumulation of damage in this species.

Introduction

Increasing levels of ultraviolet B radiation (UVBR) reaching the earth's surface as a consequence of atmospheric ozone depletion is a demonstrated hazard for biological systems (Caldwell et al., 1995; Madronich et al., 1995). Many organisms, especially those in aerial habitats, have constitutive protection mechanisms, whereas other organisms have inducible mechanisms that provide protection. Subtidal organisms (e.g., attached seaweeds) may be especially prone to the negative impacts from UVR (Häder & Figueroa, 1997) because their habitats have been subjected to very low levels of UVR over evolutionary time scales. In addition, organisms in habitats where UVR has been low historically may not have evolved inducible protection mechanisms to escape from, or to repair damage caused by increases in UVR. Most research on effects of UVR on macroalgae describe biochemical or physiological impacts, especially those associated with photosynthesis (Franklin & Forster, 1997; Häder & Figueroa, 1997; Franklin et al., 1998) or damage to DNA (Pakker & Breeman, 1997). Here we examine developmental and cytological changes induced by UVR on regenerating fragments of *Griffithsia pacifica* Kylin. Preliminary experiments identified UV dosages such that several days of exposure resulted in sublethal effects.

Material and methods

Algal culture

Stock culture of single clonal individual of *Griffithsia pacifica* from San Juan Island, Washington (ca. 49° N) was maintained for several months in an indoor running seawater table at Friday Harbor Laboratories. The plant was grown under natural light from south facing windows supplemented during working hours with room lighting provided by fluorescent lights. During experiments fragments from a single thallus were grown under continuous light provided by cool-white fluorescent tubes with PAR (photosynthetically active radiation) irradiance of 7–8 μmol m^{-2} s^{-1} as measured with a LI-COR 185 meter. PAR was supplemented with UV radiation as described below. Plants were grown at 13 °C using a modified von Stosch

medium (Guiry & Cunningham, 1984) supplemented with 5 mg l^{-1} GeO_2 to inhibit diatoms.

Ultraviolet radiation and filters

A pair of UVA-340 fluorescent lamps (Q-Panel, Cleveland, Ohio) was used to provide UV radiation. The UV lamps have the same emission spectrum as sunlight between 295 and 365 nm, and there is no emission below 295 nm. Cultures were exposed to 130–168 μW cm^{-2} UV-A and 85-112 μW cm^{-2} UV-B as measured with a newly calibrated UVX Digital Radiometer (UVP, San Gabriel, CA). Total UV radiance (250–400 nm) was 1.7× greater in zenith sky on a sunny day (December 3, 1997) relative to the culture bench, and was equivalent to radiance at 50–60 cm water depth at the dock of Friday Harbor Laboratories. UVR treatment is considerably higher than in nature because of the continuous nature of the laboratory illumination. Radiance was measured with an Ocean Optics radiometer (Dunedin, Florida). Culture dishes were covered with a cellulose acetate filter or a lexan filter. Cellulose acetate is almost transparent to PAR and has high transmission of UV-A and UV-B radiation, (i.e., between 290 and 400 nm). Lexan has a sharp transmission cut off at about 390 nm, and has the same transmission spectrum of cellulose acetate with respect to PAR. Absorption spectra for filters were determined at the beginning and end of each experiment, and there was no change in filter transmission.

Experimental design

In each experiment, 3–7 celled apical fragments were excised from a large plant and placed singly in each well of 6-well, polystyrene, tissue culture plates (FALCON 3224) in 10 ml of medium (10 mm depth). Above each well the lid had been removed and replaced with the appropriate filter (either cellulose acetate or lexan). Two experiments were carried out. In experiment 1, a UV well was paired with a control well, and three fragments were placed in each well and grown for seven days (n=18). In experiment 2, wells were randomly assigned filter type and only a single fragment was placed in each dish (n=10). Rhizoid length and number were determined each day from day 3 to day 10. At day 10 each fragment was assessed for rhizoid health and erect cell health on a five point scale (0=dead, 5=no different from best regenerates in controls). If two rhizoids were present only the longest was measured. Cytological changes in nuclei, chloroplasts and cell walls resulting from exposure to UVR were determined using light and epifluorescence microscopy, and DAPI to stain nuclei (Garbary & McDonald, 1998).

Table 1. Griffithsia pacifica. Quantitative changes in regenerating fragments after 10 days in UV-exposed (cellulose acetate filter) and UV-protected fragments (lexan filter) (experiment 2). Figures indicate $x \pm$S.E.

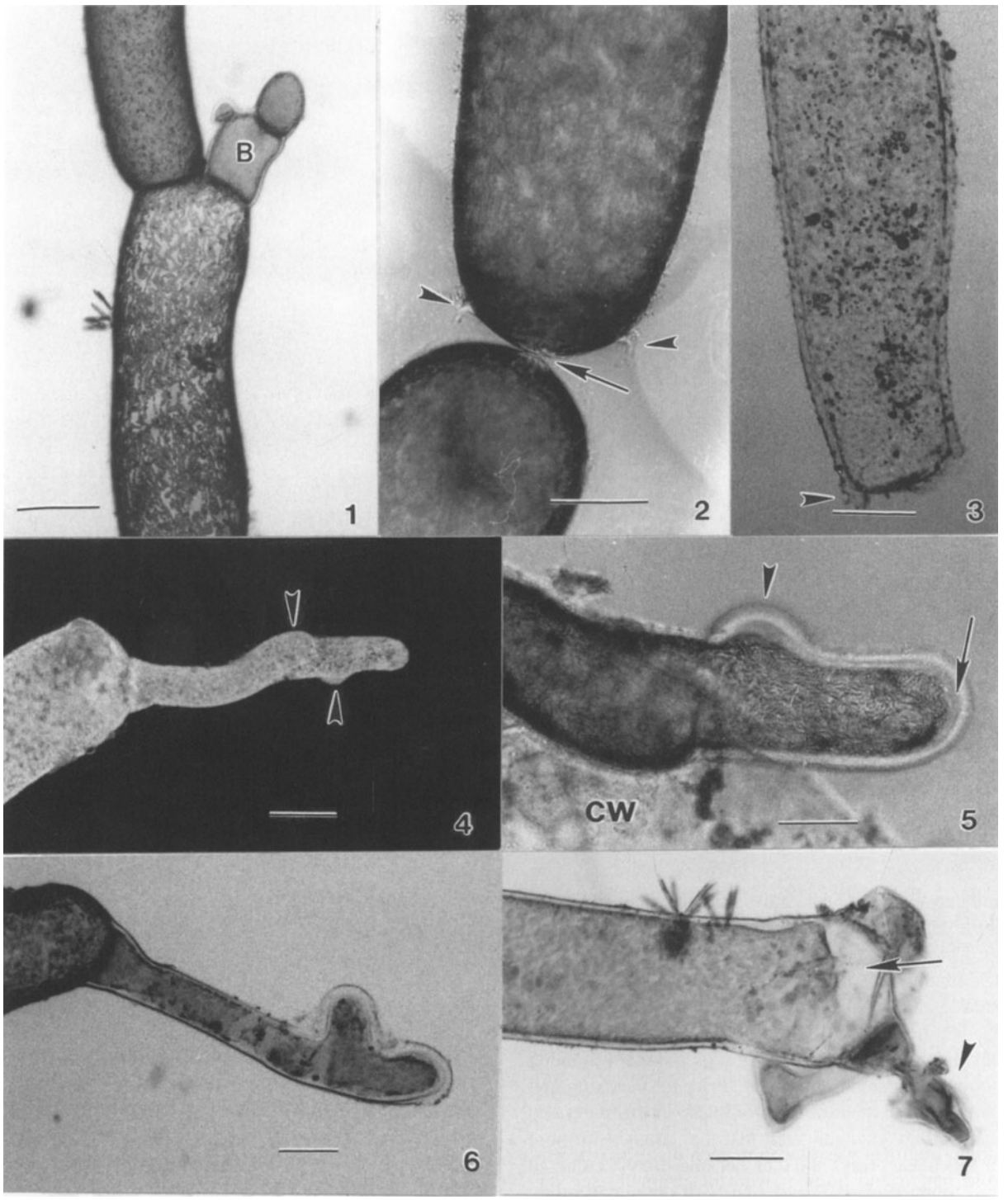
Character	UV-exposed	UV-protected	Significance (p)
Rhizoid length (μm)	407 ± 80	1053 ± 91	<0.01
Rhizoid number	0.8 ± 0.1	1.8 ± 0.2	<0.01
Erect filament health	2.2 ± 0.42	4.9 ± 0.08	<0.01
Rhizoid health	2. 2± 0.37	4.5 ± 0.20	<0.01
Number of living cells	2.7 ± 0.66	5.6 ± 0.23	<0.01

Results

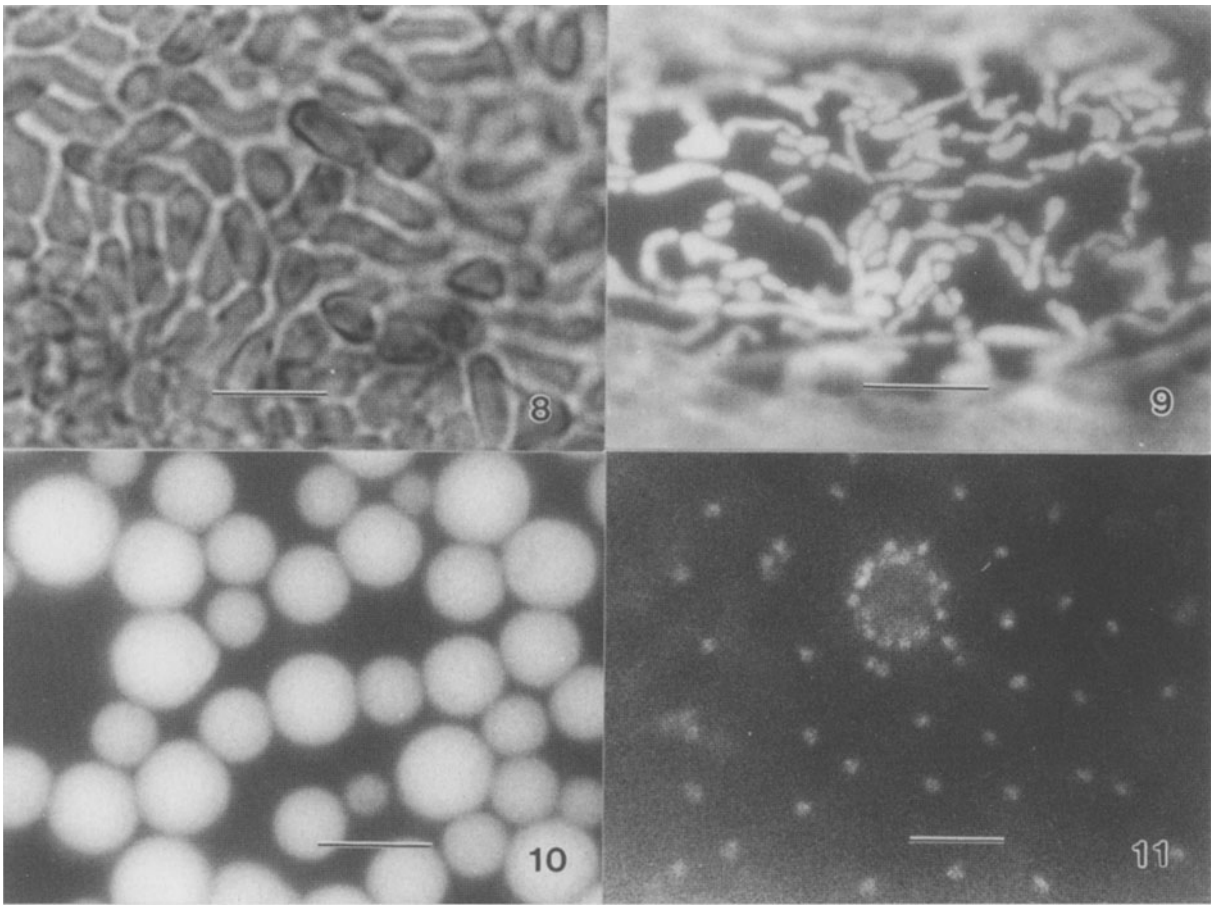
UV effects on erect axes

The UV exposure caused 10–25% mortality among fragments. Qualitative cytological changes were induced in cells of upright axes by UVR (Figs 1–11). In the UV-exposed fragments considerable cell death occurred (Figs 1 and 3). This occurred as complete mortality of a single fragment before or after rhizoid initiation. Cell death occurred as a more or less uniform bleaching through each fragment or as the bleaching of individual cells (Fig. 1). In some cells, deterioration began at one end of a cell and progressed to the other. At the end of the 10 days there were only half as many living (i.e., unbleached) cells in UV treated regenerates as controls (Table 1) and some cells were conspicuously plasmolysed (Fig. 7). Following bleaching neither chloroplasts (Fig. 1) nor nuclei (after staining with DAPI) was visible within cells. Where differential bleaching occurred within a fragment, it was typically the apical cells that retained pigmentation the longest.

A conspicuous effect of UV-radiation was the disarticulation of filaments (Figs 2 and 3). This preceded cell death and in some cases single, apparently living (i.e., pigmented) cells were present as free-floating cells. This breakup is partly reflected in the smaller number of living cells in the axes, although cell death and disarticulation may also be independent events. When disarticulation occurs there is an initial break in the cell walls (Fig. 2). This leaves the cells attached at the pit connection that also breaks. The detached



Figures 1–7. Griffithsia pacifica following UV radiation. **Figure 1**. Fragment with small bleached cell (B) and large cell with chloroplasts in initial stages of degradation. Scale bar=100 μm. **Figure 2**. Cells remaining attached at pit connection (arrow) and with remnants of adjoining cell walls (arrow heads). Scale bar=50 μm. **Figure 3**. Bleached cell with only cytoplasmic remnants showing and with deteriorated end walls (arrow head). Scale bar=50 μm. **Figure 4**. Rhizoid with two lateral swellings. Scale bar=100 μm. **Figure 5**. Tip of rhizoid without apical dome and with remnants of cell wall (CW) of excised cell. Scale bar=50 μm. **Figure 6**. Irregular rhizoid with lateral branch. Scale bar=100 μm. **Figure 7**. Regenerating rhizoid with highly irregular outline (arrow head) and with partially plasmolysed supporting cell (arrow). Scale bar=100 μm.



Figures 8–11. Griffithsia pacifica. **Figure 8**. Portion of control cell with densely packed discoid to cylindrical chloroplasts. Scale bar=5 μm. **Figure 9**. UV radiated cell with chloroplasts assuming irregular shapes and forming linear arrays. Scale bar=10 μm. **Figure 10**. UV treated cell with enlarged, globular chloroplasts prior to bleaching and cell death. Scale bar=5 μm. **Figure 11**. DAPI stained cell with irregular cluster of nuclei and more normal dispersed nuclei. Scale bar=20 μm.

cell can appear to be healthy, although regeneration of these cells was not observed.

Chloroplast degeneration was an evident effect of UV treatment. Chloroplasts in controls were densely packed and ovoid, with bright red pigmentation (Fig. 8). UV treated thalli showed conspicuous bleaching beginning at day 3, with many cells and thalli showing complete bleaching by day 7. Prior to bleaching and complete disintegration, chloroplast arrangement and morphology changed such that they aligned in more or less linear arrays and then became slightly enlarged and rounded (Figs 9 and 10), with continual fading occurring throughout this process.

DAPI staining for nuclei was unsuccessful in bleached cells and they were presumably degraded. Nuclei of unbleached cells in UV treatments were often clumped rather than having a regular spatial distribution (Fig. 11). This was apparent in both erect axes and rhizoids.

Rhizoid initiation and growth

Among remaining regenerates rhizoid initiation was slowed. Rhizoid initiation was delayed 24–48 h in UV treated thalli. Within 24 h control fragments formed localized swelling at the base of the fragment with a concentration of chloroplasts in preparation for extension of the rhizoid which formed within 48 h. After 2 d UV treated thalli showed only slight swelling and little concentration of cytoplasm. At day 3 only 50% of the UV-treated fragments had initiated rhizoids whereas 100% of control fragments did. At day 4, rhizoids in control plants were over twice as long, and by 7–10 d they were 2.5–3 times as long (Fig. 1, Table 1).

Growth rates peaked in both conditions in days 3–4 with rates of ca. 100 and 400 μm d^{-1} in the UV-treated and control thalli, respectively. In addition to slower rhizoid initiation and growth, UV treated fragments formed fewer rhizoids (0.8 vs. 2.4 and 0.8 vs. 1.8 rhizoids per regenerate in experiments 1 and 2, respectively), and the overall health of the rhizoids was diminished (Table 1).

Rhizoids produced in the UV treatment often had irregular lobes or swellings (Figs 4–7) and typically had no apical dome (i.e., an area at the tip devoid of chloroplasts). The chloroplast bleaching, irregular nuclear distribution and nuclear degeneration were also apparent in rhizoids.

Discussion

In these experiments *G. pacifica* was exposed to continuous UVR equivalent to those at 0.5 m depth based on mid-day on a clear day in early December. This resulted in considerable negative effects on thallus growth, cytology and survival. UV delayed rhizoid initiation, suggesting that damage occurred within the first 24 h. Even after rhizoid formation, elongation rates were slowed by factors of two to six. The slowing of the rhizoidal extension rate after day four is considered natural regulation (i.e., rhizoids may be approaching a maximum size for the size of the thallus), whereas it is interpreted as a sign of damage in the UV condition.

Algae have several mechanisms for protection from UVR. These include the production of mycosporine-like amino acids (MAAs) (Karentz et al., 1991; Karentz, 1994; Karsten et al., 1998), and deposition of sporopollenin (Xiong et al., 1997) and phlorotannins (Pavia et al., 1997). Some of these are inducible (e.g., MAAs, phlorotannins) whereas others are constituent (e.g., sporopollenin). Subtidal red algae, such as *Delesseria sanguinea* (Hudson) Lamouroux, *Polyneura hilliae* Greville (Kylin) and *G. pacifica* are easily damaged by UVR (Dring et al., 1996; Pakker & Breeman, 1997, this study) and may not have the protection mechanisms cited above. Avoidance from UVR may be the mechanism for survival.

The rhizoid apex of *G. pacifica* is comparable to many other tip-growing plant systems (Heath, 1990) in that there is a radiating pattern of microfilaments (MF) in a chloroplast-free zone at the rhizoid tip (Garbary et al., 1992). The fact that UV exposure disrupts the apical dome and is associated with reduced growth is consistent with an effect of UVR on rhizoid MF. The reduction in growth is consistent with an effect on microfilaments especially since chloroplast movement in *G. pacifica* is mediated through MF (Russell et al., 1996). In some fungi, ultraviolet microbeams effect microtubules and nuclear movement in tips of fungal hyphae (McKerracher & Heath, 1986). The nuclear clumping in erect axes is consistent with this phenomenon as Garbary et al. (1992) showed that the nuclei in *G. pacifica* are associated with a meshwork of microtubules.

Morphological changes in chloroplasts associated with UV damage are similar to those observed by Koslowsky & Waaland (1987) in *G. pacifica* following somatic cell fusion of incompatible strains. Although we did not observe chloroplast fusion during the degradation process, this would explain the presence of enlarged spherical chloroplasts prior to final bleaching and cell death. The wave of chloroplast destruction that we observed in some cells from one end of the cell to the other was also a feature of the incompatibility reaction (Koslowsky & Waaland, 1984) and may reflect equivalent damage to DNA, albeit from different sources.

Here we describe the effects of UVR on regenerating fragments of *G. pacifica*. The negative effects we demonstrate suggest that the ability to grow intertidally and in shallow subtidal areas may reflect not only desiccation and high light and temperature intolerance, but also a restricted tolerance for UVR. Indeed *G. pacifica* is typically found at depths of 3–5 m or more in the San Juan Islands. The sensitivity of *G. pacifica* is such that environmental increases in UVR may further limit distribution of the species. Although *G. pacifica* does not seem to produce UV absorbing substances (e.g., MAAs) that can protect it from UVR, other substances in the environment may play a similar role. Elsewhere we (Garbary et al., in preparation) show that phlorotannins derived from Laminariales can function as UV absorbing substances and protect *G. pacifica* from ambient levels of UVR. Thus it is difficult to generalize the particular effects of a single variable such as UVR without considering additional phenomena in the natural environment.

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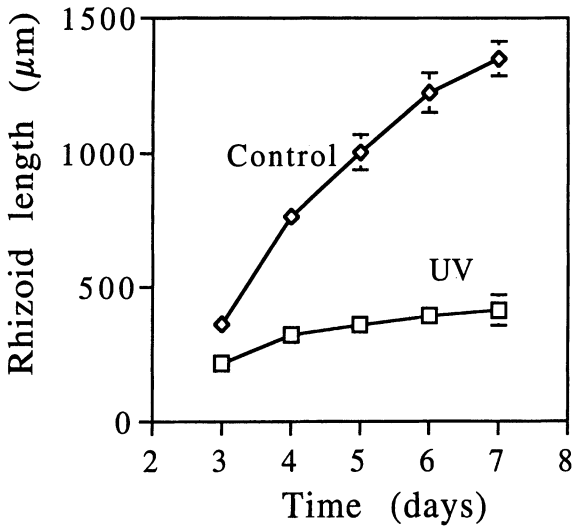

Figure 12. Griffithsia pacifica. Growth of rhizoids under UV-exposed (UV) and UV-protected (Control) conditions over 7 days (experiment 1). Note: data included only regenerates that initiated rhizoids (n=12–18).

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