Attachment 5

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Aquatic Botany 66 (2000) 141–152

Aquatic botany

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Herbicide toxicity of *Halophila ovalis* assessed by chlorophyll *a* fluorescence

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Received 24 August 1998; received in revised form 8 February 1999; accepted 21 April 1999

Abstract

Coastal habitats are increasingly being exposed to herbicide contamination from urban and agricultural catchments. The response of a seagrass, *Halophila ovalis* (R. Br.) Hook. *f.* to four herbicides was assessed using chlorophyll *a* fluorescence. The herbicides tested were atrazine, simazine, DCMU (10, 100 $\mu g\ l^{-1}$ and 1 $mg\ l^{-1}$) and glyphosate (1, 10 and 100 $mg\ l^{-1}$). Atrazine, simazine and DCMU all had substantial impacts on the chlorophyll *a* fluorescence responses, whereas glyphosate at concentrations two orders of magnitude higher than the other herbicides, showed no significant effect. These herbicides affected photosynthesis by reducing electron transport of *H. ovalis* as follows: DCMU > atrazine > simazine > glyphosate (from greatest to least inhibition). Up to 100 $mg\ l^{-1}$ glyphosate did not significantly affect the photosynthetic capacity. Photosynthetic pigment analysis suggested that the photosystems may have been disrupted. ©2000 Elsevier Science B.V. All rights reserved.

Keywords: Seagrass; Stress; Chlorophyll *a* fluorescence; Herbicide

1. Introduction

Herbicide contamination of an estuarine ecosystem can occur through overspray, groundwater leachate and runoff when broad-spectrum herbicides are applied to agricultural crops. Extensive research over the past 40 years has focussed on the effects of commercial herbicides on the photosynthesis, reproduction, morphology and leaf/stem development of

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PII: S0304-3770(99)00024-8

non-target macrophytes (Correll and Wu, 1982; Christopher and Bird, 1992). The dramatic decline of seagrasses in Chesapeake Bay, one of the world's largest estuaries, was originally thought to be linked to increased runoff of the agricultural herbicide atrazine (Correll and Wu, 1982; Bowmer, 1986); however, Schwarzschild et al. (1994) demonstrated that the decline in *Zostera marina* L. abundance was not the result of atrazine phytotoxicity. Atrazine concentration in US rivers have reached over 80 $\mu g\,l^{-1}$, although it is mostly found below 10 $\mu g\,l^{-1}$ (Huber, 1993). Organic toxicants have contaminated Australian seagrass habitats to the extent that both atrazine and DCMU have recently been detected in seagrass tissue and sediment of northern Queensland (Haynes et al., 1998). For effective management of the remaining seagrass meadows, techniques must be developed to rapidly identify stress responses to anthropogenic contamination. The present study investigated the chlorophyll *a* fluorescence and pigment responses of laboratory-cultured *Halophila ovalis* (R. Br.) Hook. f. to four common commercial herbicides.

Atrazine [6-chloro-*N*-ethyl-*N*′-(1-methylethyl)-1,3,5-triazine-2,4-diamine] is a selective systemic triazine-based herbicide absorbed through the roots and foliage, which is accumulated in apical meristems and leaves (Tomlin, 1994). Atrazine affects photosynthesis by binding to the second electron acceptor (Q_b) protein, inhibiting electron transport. This causes an increase in maximum fluorescence (F_m), and a decline in the maximum quantum yield (F_v/F_m ratio) (Conrad et al., 1993). While there are significant effects of micro-molar (μM) concentrations of atrazine on photosynthesis, growth is only affected at milli-molar (mM) concentrations (Delistraty and Hershner, 1984; Schwarzschild et al., 1994).

Simazine [2-chloro-4, 6-bis (ethylamino)-*s*-triazine] is another triazine derivative, with similar modes of action and toxicity to atrazine, used to control germinating annual grasses and broad-leaved weeds (Tomlin, 1994). Chlorophyll *a* fluorescence has previously been used to assess the toxicity of simazine to soybean (*Glycine max* L. Merr.) and barley (*Hordeum vulgare* L.) seedlings (Judy et al., 1991). Both species showed a strong increase in minimum fluorescence (F_o), and a decrease in maximum quantum yield, as well as the effective quantum yield.

DCMU or Diuron [3-(3′,4′-dichlorophenyl)-1,1-dimethylurea] is a phenylurea herbicide which inhibits the photoreduction side of PSII (Conrad et al., 1993). DCMU strongly blocks the reoxidation of the primary electron acceptor (Q_a), and the magnitude of the minimum fluorescence emission increases considerably, causing a decrease in variable fluorescence (F_v) (Judy et al., 1991). The toxicological impact of DCMU on non-target species is poorly understood, although it is extensively used in the selective control of broad-leaved weeds and mosses (Tomlin, 1994). No known toxicological study has assessed the effect of DCMU on seagrass photosynthesis.

Glyphosate [*N*-(phosphonomethyl)-glycine] is the most widely used non-selective systemic herbicide in the world. Its mode of action is absorption through the foliage and subsequent translocation (Tomlin, 1994). Glyphosate acts on various enzyme systems throughout the plant, and interferes with amino acid formation. Glyphosate is classified as relatively non-toxic to aquatic plants (Bowmer, 1986). Several macrophyte trials with glyphosate have demonstrated a limited toxicity to non-target species (Fleming et al., 1991; Christopher and Bird, 1992). No known investigations of glyphosate have been performed on seagrasses.

2. Methods and materials

All glassware, sample containers and experimental chambers were cleaned with an anionic detergent, then soaked in a 2 M HNO_3 acid-bath for 24 h, and finally rinsed in reverse osmosis Type I water. All experiments were performed in 5 l polypropylene chambers to minimise adsorption of herbicides. Final concentrations of the herbicides were not determined analytically, and nominally based on the initial dosages. Tests were conducted under static conditions, where herbicide exposure was a single dose addition at the beginning of the experiment.

The concentration ranges of atrazine, simazine and DCMU used in these experiments were 0, 10, 100 $\mu g\,l^{-1}$ and 1 $mg\,l^{-1}$. Due to the limited toxicity observed in preliminary trials, the concentration range for glyphosate was two orders of magnitude higher: 0, 1, 10 and 100 $mg\,l^{-1}$. Standard solutions were prepared from technical grade atrazine (99.0% purity, Ciba-Geigy), simazine (99.0% pure, Ciba-Geigy), and DCMU (98% pure, Sigma), while glyphosate was supplied as a liquid 45.8% (w/w) IBA glyphosate salts (Monsanto). Simazine was dissolved in acetone, then diluted in 100 ml seawater, and gently heated to volatilise the acetone. All simazine controls were adjusted to contain 1 $ml\,l^{-1}$ acetone, which was volatilised (Schwarzschild et al., 1994). Stock glyphosate solution (100 $mg\,l^{-1}$) required 0.22 ml IBA glyphosate solution/l seawater.

2.1. Plant material

Original stock of *H. ovalis* was collected from Taylor's Bay, Sydney Harbour (151°15′E, 33°50′S). Specimens were cultured under laboratory conditions for at least 3 months prior to experimentation. Growth conditions were 35 ppt filtered seawater, 120 to 150 μmol quanta $m^{-2}\,s^{-1}$, 25°C, photoperiod 16 h : 8 h (L : D), planted in terrestrial sandy loam sediment in a recirculating flow-through system (Ralph, 1997). Each culture tub (11.5 cm × 5.5 cm × 17 cm) contained at least five individual plants with >5 leaf pairs per plant, of which two plants were sampled from two duplicate tubs. The sample leaves were from the second nodal position behind the meristem.

2.2. Chlorophyll *a* fluorescence

A PAM-2000 fluorometer (Walz, Germany) was used to determine F_o, F_m, maximum quantum yield (F_v/F_m ratio) and effective quantum yield ($*F/F_{m'}$) (Schreiber et al., 1994). Chlorophyll *a* fluorescence analysis was performed underwater, using dark-adaptation clips (DLC-8) to ensure a constant distance between fibre optic head and the leaf sample. The fluorescence signal was sampled at a standard position on the leaf, approximately in the middle of the adaxial surface. Preliminary investigations demonstrated that the reduced dark-adaptation period (5 min) produced reproducible results that were not significantly different from those after 15 min dark-adaptation (Ralph, 1997). Fluorescence measurements were collected over a discontinuous time-scale, where, after the initial exposure to herbicide, specimens were measured at hourly intervals for 5 h, then daily (ca. 10:00 hours) for the following 4 days.

2.3. Photosynthetic pigment analysis

Leaf samples were manually cleaned of any attached epiphytes, and the stem was removed. The wet weight of the tissue was measured after the specimens had been hand-dried on absorbent paper towel. Surface area was measured digitally using a Li–Cor Portable Leaf Area Meter (LI-3000A, USA). *N, N′*-dimethylformamide (DMF), a water-miscible solvent, removes all pigments from soft-leafed species such as *H. ovalis* without the additional processes of maceration, centrifugation or filtration. Single leaves were finely sliced with stainless steel scissors, to increase the surface area of tissue exposed to the extractant, and then placed in a 15 ml dark-glass (light-proof) screw-capped bottle containing 5 ml of DMF. The bottles were placed in a darkened container in a refrigerator (4°C) for 3 days, prior to spectrophotometric determination (LKB Ultrospec II UV/Vis, model 4050). Preliminary investigations showed that a single 3-day extraction had an efficiency of 95 to 97%, compared with a subsequent 3-day extraction in fresh DMF. Wellburn's extinction coefficient equations were used to calculate pigment content (Wellburn, 1994).

2.4. Statistical analyses

Samples from the tubs were pooled, provided that a two-way ANOVA found no significant difference between the experimental tanks. Homogeneity of variance was assessed before performing ANOVA, by examining a stem-and-leaf plot of the studentised residuals. A two-way ANOVA was performed on all chlorophyll *a* fluorescence data to determine the significance of the various treatments, exposure period and interaction between these factors. Photosynthetic pigment concentrations were compared by a one-way ANOVA. When the ANOVA identified a significant difference for a main effect ($P < 0.05$), a post-hoc pairwise comparison of the sample means was performed with the 'Tukey's honestly significant difference' (HSD) test.

3. Results

3.1. Atrazine

Minimum fluorescence (Fig. 1(a)) increased for all atrazine treatments, with the signal for the 1 mg l^{-1} treatment increasing up to four times that of the control. Maximum fluorescence (Fig. 1(b)) similarly increased for all atrazine treatments, with the 1 mg l^{-1} treatment almost doubling the control response. After the first hour of exposure, the effective quantum yield for the 1 mg l^{-1} atrazine treatment was approximately halved (Fig. 1(c)). The effective quantum yield of the 100 $\mu g\, l^{-1}$ atrazine treatment declined over the first 5 h, to a similar level to the 1 mg l^{-1} treatment. The 10 $\mu g\, l^{-1}$ treatment was significantly lower than the control, though not as low as the two higher concentrations. The maximum quantum yield (Fig. 1(d)) followed a similar series of response curves to the

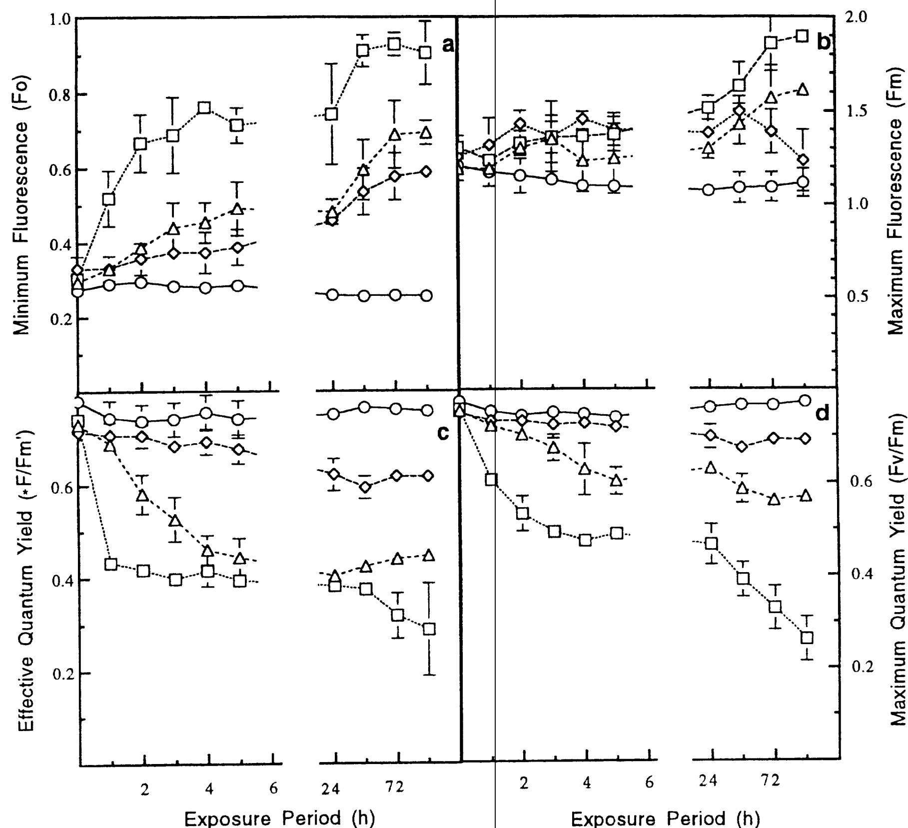

Fig. 1. *Halophila ovalis*. Time course of the (a) minimum fluorescence (F_o), (b) maximum fluorescence (F_m), (c) effective quantum yield ($*F/F_{m'}$), (d) maximum quantum yield (F_v/F_m) of atrazine treatments; 1 mg l^{-1} (□), 100 μg l^{-1} (△), 10 μg l^{-1} (◇) and control (○), as a function of exposure time up to 96 h. Error bars indicate the 95% confidence interval of the mean ($n = 4$). The units of minimum and maximum fluorescence are *V*; maximum and effective quantum yield are relative.

effective quantum yield, although the response was not as sensitive. The two-way ANOVA models for all of the herbicide exposures (except the F_v/F_m ratio for glyphosate) found a significant interaction ($P < 0.05$) between the period of exposure and the treatment. The significant interaction term suggests that these response curves for each treatment were following different patterns with respect to exposure time; and therefore the treatments were found to be different.

Photosynthetic pigment analysis after 5 h exposure to atrazine showed that the chlorophyll *a*/*b* ratios were elevated for all atrazine treatments (Table 1). Similarly, all atrazine treatments had a greater content of carotenoid pigments, which resulted in a significant reduction in the chlorophyll/carotenoid ratio.

Table 1
Photosynthetic pigment (mean values ± standard error μg cm^{-2}) after 5 h exposure of *H. ovalis* leaves ($n = 4$) to atrazine, simazine, DCMU and glyphosate. Treatment means with identical letters are not significantly different (one-way ANOVA, Tukey's HSD multiple comparison [$P > 0.05$] test) within each group of concentrations

Parameter	Chl *a*	Chl *b*	Chl *a/b* ratio	Carotenoids	Chl/carot ratio
Atrazine 10 μg l^{-1}	9.2±0.1	6.4±0.1	1.4±0.1^a	2.8±0.1^a	5.6±0.1^a
Atrazine 100 μg l^{-1}	8.7±0.4	5.7±0.6	1.5±0.1^a	2.7±0.1^a	5.3±0.4^a
Atrazine 1 mg l^{-1}	11.6±2.8	7.8±1.8	1.5±0.1^a	3.0±0.4^a	6.5±0.7^a
Control	10.3±1.4	8.2±0.9	1.2±0.1^b	2.1±0.2^b	9.1±0.5^b
ANOVA *F* ratio	2.1	3.7	12.6***	9.6***	38.5***
Simazine 10 μg l^{-1}	12.0±1.2	7.4±0.6^b	1.6±0.1^a	2.6±0.3	7.5±0.2b
Simazine 100 μg l^{-1}	8.5±2.6	5.7±1.8^a	1.5±0.1^b	2.5±0.4	5.5±1.0a
Simazine 1 mg l^{-1}	7.0±1.1	5.1±0.7^a	1.4±0.1^c	2.7±0.3	4.5±0.2^a
Control	10.3±1.4	8.3±0.9^b	1.2±0.1^d	2.0±0.2	9.2±0.5^c
ANOVA *F* ratio	4.9	5.5***	43.9***	3.1	39.4***
DCMU 10 μg l^{-1}	9.4±1.1^a	6.1±0.8^a	1.6±0.1^a	2.3±0.3^b	6.9±0.9^a
DCMU 100 μg l^{-1}	8.6±0.8^a	6.3±0.5^a	1.4±0.1^a	2.8±0.1^a	5.4±0.6^a
DCMU 1 mg l^{-1}	12.3±0.8^b	8.2±0.3^b	1.5±0.1^a	3.1±0.2^a	6.6±0.6^a
Control	11.4±0.6^b	8.7±0.7^b	1.3±0.1^b	2.3±0.2^b	9.0±0.5^b
ANOVA *F* ratio	11.8***	14.3***	10.3***	11.8***	14.6***
Glyphosate 1 mg l^{-1}	7.7±0.7^a	5.3±0.6^a	1.4±0.1^a	2.7±0.1	4.9±0.4^a
Glyphosate 10 mg l^{-1}	7.4±0.5a	5.2±0.1^a	1.4±0.1^a	2.4±0.1	5.3±0.3^a
Glyphosate 100 mg l^{-1}	11.2±0.6^b	7.3±0.2^b	1.5±0.1^a	2.7±0.3	6.9±1.1^b
Control	11.7±0.3^b	9.0±0.4^c	1.3±0.1^b	2.3±0.2	8.9±0.6^c
ANOVA *F* ratio	49.6***	66.9***	18.0***	2.2	23.8***

*** = significant difference at $P < 0.05$.

3.2. Simazine

Simazine produced similar chlorophyll *a* fluorescence results to those obtained with atrazine. Minimum fluorescence (Fig. 2(a)) increased for all simazine treatments, with the 1 mg l^{-1} treatment increasing up to three times the pre-exposure F_0 signal. Maximum fluorescence (Fig. 2(b)) similarly increased for all simazine treatments. After the first hour of exposure, the effective quantum yield for the 1 mg l^{-1} simazine treatment was approximately half the control response (Fig. 2(c)). After this substantial decline, the 1 mg l^{-1} treatment remained stable for the remainder of the experiment. Effective quantum yield for the 100 μg l^{-1} simazine treatment declined over the initial 5 h to a similar level to the 1 mg l^{-1} treatment. The 10 μg l^{-1} treatment was similar to the control. The maximum quantum yield followed a similar series of response curves to the effective quantum yield, although the F_v/F_m ratio was not as sensitive (Fig. 2(d)).

Chlorophyll *a* levels were not significantly different for all simazine treatments (Table 1). Chlorophyll *b* in the higher simazine treatments was significantly lower than the control. The reduction of chlorophyll *b* resulted in an overall increase in the chlorophyll *a/b* ratio for all of the simazine treatments. Carotenoid concentrations were similar between treatments and the controls, but the chlorophyll/carotenoid ratio was significantly lower for all simazine concentrations.

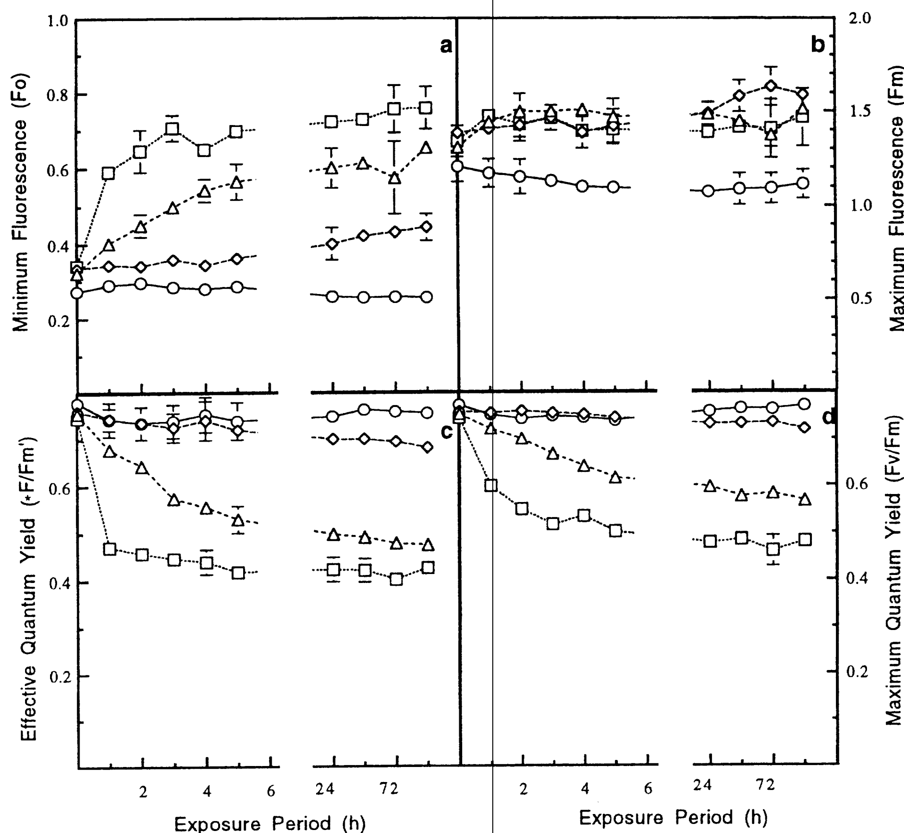

Fig. 2. *Halophila ovalis*. Time course of the (a) minimum fluorescence (F_o), (b) maximum fluorescence (F_m), (c) effective quantum yield ($*F/F_{m'}$), (d) maximum quantum yield (F_v/F_m) of simazine treatment; $1\ mg\ l^{-1}$ (□), $100\ \mu g\ l^{-1}$ (△), $10\ \mu g\ l^{-1}$ (◇) and control (○), as a function of exposure time up to 96 h. Error bars and units are the same as Fig. 1.

3.3. DCMU

Treatment with DCMU has been used extensively in photosynthetic research as a means of rapidly closing PSII reaction centres. Treatment with $10\ \mu g\ l^{-1}$ to $1\ mg\ l^{-1}$ of DCMU resulted in a substantial effect on the fluorescence response. Minimum fluorescence (Fig. 3(a)) increased with DCMU exposure and concentration, where the $1\ mg\ l^{-1}$ treatment doubled the F_o signal within 1 h and the $100\ \mu g\ l^{-1}$ doubled within 5 h. The $10\ \mu g\ l^{-1}$ treatment was slower to respond, though after 24 h the minimum fluorescence was 190% of the pre-exposure signal. Maximum fluorescence (Fig. 3(b)) for all treatments was elevated above the control response after 1 h and remained elevated up to 96 h, with the exception of the $1\ mg\ l^{-1}$ treatment. The maximum fluorescence for the $1\ mg\ l^{-1}$ treatment declined by

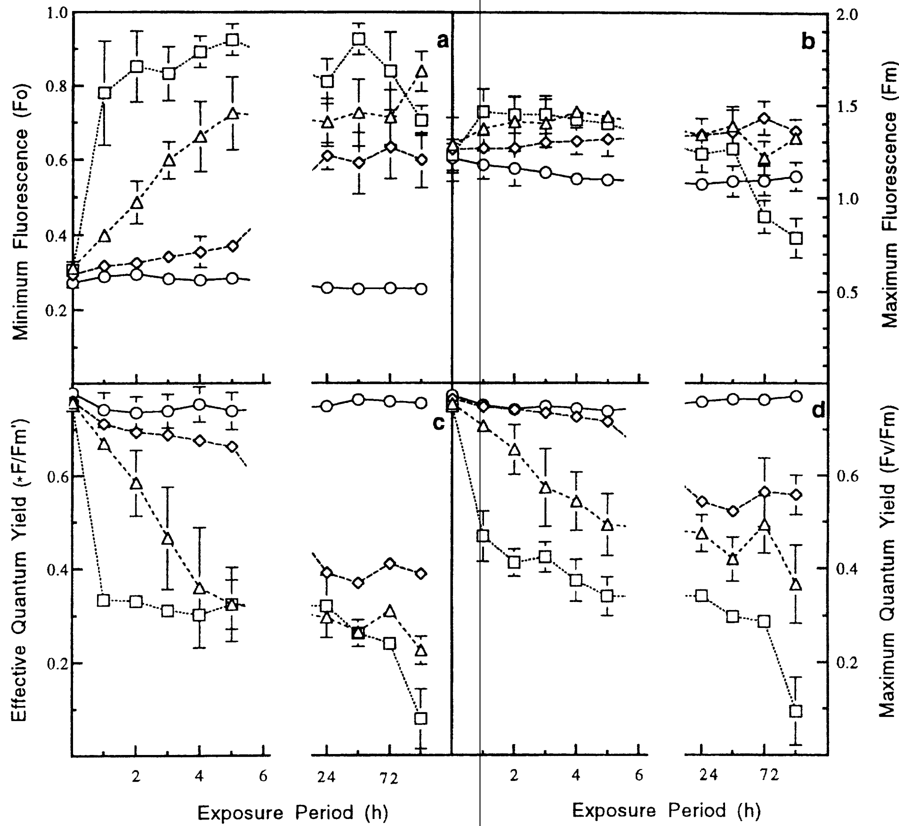

Fig. 3. *Halophila ovalis*. Time course of the (a) minimum fluorescence (Fo), (b) maximum fluorescence (F_m), (c) effective quantum yield ($*F/F_{m'}$), (d) maximum quantum yield (F_v/F_m) of DCMU treatment; 1 mg l^{-1} (□), 100 μg l^{-1} (△), 10 μg l^{-1} (◇) and control (○), as a function of exposure time up to 96 h. Error bars and units are the same as Fig. 1.

approximately 30% after 48 h. Effective quantum yield (Fig. 3(c)) illustrated a significant effect of DCMU on all treatments; the 1 mg l^{-1} declined by over 55% after the first hour, whilst the 100 μg l^{-1} treatment declined to a similar level after 5 h and the 10 μg l^{-1} after 24 h. The F_v/F_m ratio for 1 mg l^{-1} treatment remained steady at approximately 0.3, until 96 h where it declined to less than 0.1; the other treatments remained at around 0.3 for the remainder of the exposure period. The maximum quantum yield (Fig. 3(d)) showed a similar relationship to the effective quantum yield, but the signal was less sensitive with less acute declines and slower response times.

Pigment analysis showed several ambiguous effects of the DCMU treatments. Chlorophyll *a* and *b* content (Table 1) were significantly reduced in the 10 and 100 μg l^{-1} treatments, whilst the 1 mg l^{-1} was similar to the control. All DCMU treatments had a higher chlorophyll *a*/*b* ratio than the control. The two lower DCMU treatments (10 and 100 μg l^{-1})

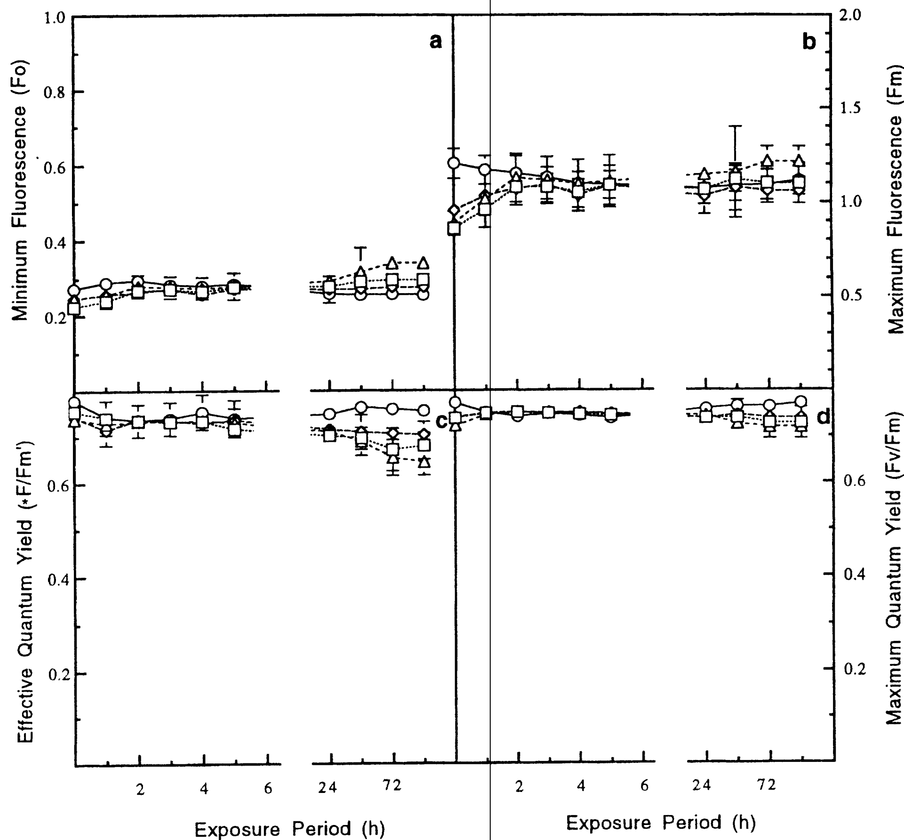

Fig. 4. *Halophila ovalis*. Time course of the (a) minimum fluorescence (F_o), (b) maximum fluorescence (F_m), (c) effective quantum yield ($*F/F_{m'}$), (d) maximum quantum yield (F_v/F_m) of glyphosate treatment; 100 mg l^{-1} (□), 10 mg l^{-1} (△), 1 mg L-1 (◇) and control (○), as a function of exposure time up to 96 h. Error bars and units are the same as Fig. 1.

had elevated carotenoid concentrations. All of the DCMU treatments had lower chlorophyll/carotenoid ratios, than the control.

3.4. Glyphosate

Glyphosate showed no significant impact on the fluorescence signals, even though the concentration range was 100-fold higher than for the other herbicides tested. Minimum fluorescence (Fig. 4(a)) showed only a minor increase in the glyphosate treated samples after 48 h. Maximum fluorescence (Fig. 4(b)) showed no overall effect of this herbicide exposure. Effective quantum yield (Fig. 4(c)) declined slightly after 24 h exposure, to less than 0.7. The maximum quantum yield (Fig. 4(d)) demonstrated no significant effect of glyphosate exposure.

Photosynthetic pigments, however, were affected by exposure to glyphosate (Table 1). The 1 and $10\,mg\,l^{-1}$ glyphosate treatments were significantly lower in chlorophyll *a* and *b* than the control and the $100\,mg\,l^{-1}$ treatment. All glyphosate treatments had elevated chlorophyll *a*/*b* ratios. No significant effect was detected on the carotenoid concentration; however, since the total chlorophyll pigment contents were different, the chlorophyll/carotenoid ratios were also different.

4. Discussion and conclusion

The herbicides tested were found to have a range of toxicities to *H. ovalis* as follows: DCMU > atrazine > simazine > glyphosate. Many xenobiotics impact upon the primary electron acceptor (Q_a), or the water-splitting reaction, while others affect enzyme function. The variation in impact sites, uptake rates and whether the photosynthetic effect is direct or indirect, will influence the relative chlorophyll *a* fluorescence response. The extremely low level of toxicity of glyphosate suggests that only minor impacts on photosynthesis would occur as a result of municipal and agricultural applications of this herbicide. The triazine herbicides (atrazine and simazine), as well as to DCMU significantly increased the F_o and F_m signals. This response would be associated with blocking of the electron transport from the primary to secondary plastiquinone (Q_a to Q_b), resulting in an increase in fluorescence emission (Conrad et al., 1993). Since these herbicides block the reoxidation of Q_a, absorbed energy cannot be used in photochemistry, and so it must be non-photochemically dissipated, so that the F_o signal increases and approaches the F_m level (Judy et al., 1991; Merz et al., 1996). The delay in the F_o increase and decline in effective quantum yield of the $10\,\mu g\,l^{-1}$DCMU treatment could be related to the time required for the thylakoid membrane to become permeable (Lurie et al., 1994). The impact of the DCMU on all fluorescence parameters was substantially greater than for either of the triazine compounds at comparable concentrations.

Atrazine increased F_o and F_m to a greater degree than simazine for each comparable concentration, causing a greater decline in both the effective and maximum quantum yield. *H. ovalis* showed a 50% reduction in the effective quantum yield upon exposure to $1\,mg\,l^{-1}$ atrazine over 5 days (Fig. 3(c)). A similar toxic response to $1\,mg\,l^{-1}$ atrazine has been demonstrated with *Z. marina*, where leaf growth rate was significantly reduced (Delistraty and Hershner, 1984). Walsh et al. (1982) found that $1\,mg\,l^{-1}$ atrazine over 40 h severely affected the photosynthesis of *Thalassia testudinum* Banks ex Konig. Maximum fluorescence increased consistently, such as with simazine exposure, indicating an increase in the pH gradient and a more reduced electron transport chain (Merz et al., 1996). The reduction in electron transport was reflected in the steady decline in both maximum and effective quantum yield (10 and $100\,mg\,l^{-1}$ simazine).

Glyphosate, at concentrations 100-fold higher than the other three herbicides, still produced no significant photosynthetic stress response. The impact of glyphosate on PSII needs further investigation; however, it appears to enhance electron transport (or increase PSI activity) possibly linked to reduced chlorophyll pigment concentration, as indicated by elevated effective and maximum quantum yield. Glyphosate does not directly affect electron transport of PSII, and therefore chlorophyll *a* fluorescence may be less sensitive

to the primary effects of this herbicide. Photosynthetic pigments were significantly more affected by glyphosate, than was PSII activity. To further understand the effect of herbicides, longer-term exposure and assessment of the plants' capacity for recovery are needed.

All herbicide treatments had reduced chlorophyll/carotenoid ratios, mainly due to increased carotenoids. This increase in carotenoids would be associated with an increased need for energy dissipation. Although simazine and atrazine performed similarly with respect to the chlorophyll *a* fluorescence response, these two herbicides had substantially different effects on the photosynthetic pigments. Atrazine had no effect on the chlorophyll pigments after 5 h exposure, while the carotenoid concentration increased significantly. Schwarzschild et al. (1994) found after 40 days exposure to atrazine (up to $2.46\,mg\,l^{-1}$) *Z. marina* showed no significant difference in total chlorophyll or chlorophyll *a*/*b* ratio compared to the control specimens. They suggested that *Z. marina* may be capable of atrazine detoxification. Simazine preferentially reduced the chlorophyll *b* concentration, by comparison to chlorophyll *a*, and did not increase the carotenoid concentration to the same degree as atrazine. Pigment analysis of DCMU exposure was inconclusive, with several anomalies. The chlorophyll pigment of the $1\,mg\,l^{-1}$ DCMU treatment were similar to the control, whilst the lower two treatments had reduced chlorophyll concentrations. Glyphosate exposure affected the pigment analysis, where the lower glyphosate concentrations had lower chlorophyll pigment content, yet carotenoid concentration remained stable. Further research is needed to confirm these pigment results over longer exposure periods.

Toxicological responses of several herbicides have been described, some causing complete inhibition of photosynthesis, while others showed a limited impact. Correll and Wu (1982) suggested that herbicides in combination can have synergistic effects; this type of investigation needs further research. The results discussed in this paper provide a useful addition to our knowledge of the phytotoxic impact of herbicides on non-target species. These results also provide guidance for the control of nuisance aquatic macrophytes (Bowmer, 1986). A characteristic of PSII inhibitor herbicide action on aquatic macrophytes is a rapid recovery after the exposed specimen is returned to uncontaminated water (Bowmer, 1986). The results of these static tests provide an indication of the maximum impact for a given dosage; however, additional experiments are required to understand the response of pulses of herbicides in flowing water as well as the complexing action of turbidity. The maximum quantum yield was less sensitive to herbicide impact than the effective quantum yield, and therefore would be less effective as an early-warning system to monitor toxic herbicide exposure. Given that few comparable investigations have been performed with herbicides, chlorophyll *a* fluorescence and seagrasses, the findings demonstrate that such data can provide a foundation for further detailed investigations into the physiological effect and subsequent decline of seagrasses as a result of herbicide exposure.

Acknowledgements

I would like to acknowledge my supervisors Professor Margaret Burchett and Dr Lou De Filippis. I thank Dr David Morrison and the reviewers for editorial comments on the

manuscript. I would like to thank Monsanto Australia Limited and Ciba-Geigy for supplying technical herbicides; and the NSW National Parks and Wildlife Service for allowing me to collect seagrasses from within several conservation areas.

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