TOXICITY OF THE HERBICIDE ATRAZINE AT ENVIRONMENTAL CONCENTRATIONS TO *VALLISNERIA GIGANTEA*, ASSESSED USING CHLOROPHYLL FLUORESCENCE

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ABSTRACT

Long-term exposure to low concentrations of contaminants may have greater effects on organisms and ecosystems than short-term exposure to high concentrations. The objective of this experiment was to establish if atrazine contamination of aquatic ecosystems could be contributing to the decline of aquatic macrophytes.

The photosynthetic stress response of the submerged aquatic macrophyte *Vallisneria gigantea* exposed to environmental concentrations of atrazine for 21 days, was assessed using chlorophyll fluorescence measured as optimum quantum yield (Fv/Fm), and the photosynthetic pigments (chlorophyll *a*, *b*, and total carotenoids). After the exposure phase of the experiment, the recovery of optimum quantum yield was investigated. Change in atrazine concentrations in static experimental aquaria was also assessed.

The results of this experiment show atrazine concentrations as low as 30 μg L^{-1} for 21 days can impact on the photosynthetic activity of *V. gigantea*. The photosynthetic response recovered fully within 14 days of the plants being returned to atrazine-free water. Concentrations of chlorophyll *a* and total carotenoids were not significantly affected by exposure to 2, 10 or 30 μg L^{-1} atrazine in static experimental aquaria, whereas exposure to atrazine caused a significant difference in chlorophyll *b in V. gigantea*.

Keywords: atrazine, chlorophyll *a* fluorescence, herbicides, macrophytes, *Vallisneria gigantea.*

INTRODUCTION

Contamination of aquatic ecosystems by herbicides such as atrazine has been postulated as a cause of the decline of submersed aquatic and marine vegetation (Correll and Wu 1982; Fleming *et al.* 1991). In New South Wales (NSW), there has been a reduction in the abundance and diversity of aquatic macrophytes over the last 30 years. The cause of this loss is largely unknown, however increased contamination and river regulation have been widely implicated in the decline years (Sainty and Jacobs 1993). Long-term exposure to low concentrations of contaminants may have greater effects on organisms and ecosystems, than short-term exposure to high concentrations (Depledge 1993; Biernacki *et al.* 1997).

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is a selective, systemic herbicide that provides rapid mortality and residual suppression of weed growth for the control of a range of broad-leaved weeds and grasses (Tomlin 1994). Atrazine uptake is principally through the roots, and it is translocated via the xylem to the leaves and apical meristems, where it accumulates (Tomlin 1994). Atrazine is a potent inhibitor of photosystem II (PS II), as it disrupts electron transport by binding to the second electron acceptor (Q_b) protein (Conrad *et al.* 1993; Tomlin 1994).

Atrazine is one of the most widely used agricultural chemicals in Australia and around the world (Davies *et al.* 1993; NRA 1997). Atrazine is used on a wide variety of crops including: sorghum, sweet corn, maize, canola, sugarcane, and forestry plantations (Davies *et al.* 1993). The transport of atrazine to aquatic ecosystems occurs through runoff water and the attachment to eroded soil particles (Goswami and Green 1971). As a mobile chemical, atrazine has the potential to cause low-level contamination of aquatic ecosystems as a result of its widespread use and environmental properties. The chemical and biological degradation of atrazine is slow (Klaassen and Kadoum 1979), with a half-life of more than two months for freshwater systems (NRA 1997).

Atrazine has been detected in both surface waters and groundwaters of NSW. In groundwaters of the Namoi Valley, atrazine concentrations range from 0.1 to 5.8 μg L^{-1} (Muschal 1997). The highest recorded levels of atrazine contamination in NSW occurred in the Gwydir River basin in March and April 1994 and February 1996, with levels up to 60 μg L^{-1} detected. Surface waters in NSW show slight seasonal patterns with levels of atrazine being higher over summer and early autumn. Atrazine is generally applied during spring, prior to planting of summer crops. However, atrazine is not sprayed over summer, therefore spray drift cannot be responsible for the higher levels detected over summer (Muschal 1997).

Australian levels of atrazine contamination are relatively low compared to international levels, with levels in the US and Canadian surface waters range from 1 to 1000 μg L^{-1} (Dewey 1986). Huber (1993) reported atrazine concentrations up to 80 μg L^{-1}, but generally below 10 mg L^{-1} in US rivers. Atrazine is found in European streams at concentrations ranging from 0.03 to 10.0 μg L^{-1} (Hofmann and Winkler 1990).

There is limited information available on the effects of atrazine on aquatic macrophytes in Australia (Ralph 2000) or outside Australia (Correll and Wu 1982; Fleming *et al.* 1991). The results of these studies vary widely for different macrophyte species and test conditions, with much of the research examining the effects of acute exposures to atrazine. It appears that longer periods of exposure to atrazine result in a greater phytotoxic response. Correll and Wu (1982) found the sensitivity of *Potamogeton pectinatus, Vallisneria americana* and *Zostera marina* at low atrazine concentrations could best be demonstrated after long exposure periods (ca. 30 to 40 days).

Chlorophyll fluorescence can be used to examine plant responses to direct or indirect stressors impacting upon photosynthesis. Changes in the chlorophyll fluorescence yield are related to alterations in the PS II photosynthetic efficiency. Fluorescence yield will increase as a plant is stressed and unable to utilize captured photosynthetic energy. Herbicides, such as atrazine, disrupt electron transport in PS II, by binding to the second electron acceptor (Q_b) protein (Muir *et al.* 1978; Bowmer 1986; Conrad *et al.* 1993; Tomlin 1994) or interfering with the pigment protein apparatus. The absorbed light not utilised in the photosystems or released as heat, is released as fluorescence (Judy *et al.* 1991; Merz *et al.* 1996). Hence alterations in the level of chlorophyll *a* fluorescence can be monitored and used as a measure of reduced photosynthetic activity caused by photoinhibitors (Judy *et al.* 1991; Merz *et al.* 1996).

The main objective of this experiment was to determine if 21-day exposures to environmental concentrations of the herbicide atrazine affect optimum PS II quantum yield (Fv/Fm) of *Vallisneria gigantea* (L.) in static experimental aquaria. Three additional objectives were 1) to examine the recovery of *V. gigantea* after a 21 day exposure to environmental concentrations of atrazine; 2) to examine changes in the concentrations of chlorophyll *a*, *b* and total carotenoid in the leaf tissue of *V. gigantea* during exposure to atrazine for 21 days; and 3) to examine the change in atrazine concentration in static aquaria in a glasshouse over the 21 day exposure period.

MATERIALS AND METHODS

Experimental design

The experimental design was based on four treatments, including the control. The four treatment concentrations were 0 (the control), 2, 10 and 30 µg L^{-1} atrazine. There were four replicate tanks of each treatment (n = 4). In each tank, two individuals of *V. gigantea* were grown and two leaves from each plant were sampled for photosynthetic activity. The average value of the four measurements from each tank (one from each of two separate leaves and two plants) was used for analysis. A Latin square design was used to set the column and row arrangement of the experimental aquaria. Figure 1 illustrates the 4 x 4 Latin square experimental design, where each treatment occurs only once in each row or column. Latin square designs ensure that any treatment effects can be distinguished from any row or column effects. The Latin square design was used to ensure any gradient along or across the glasshouse that could affect photosynthetic activity, pigment concentration, or survival of the plants, such as shading or temperature, could be detected.

Plant stock material

The *Vallisneria gigantea* plant stock used in this experiment was commercially grown in Sydney, NSW. The macrophytes were then acclimatised for 21 days to ensure stable growth, before the aquaria were dosed with atrazine.

Experimental aquaria systems

This experiment was conducted in a glasshouse, exposed to natural light. The aquaria used were standard 10-L glass aquaria. All experimental containers were cleaned with the anionic detergent Teepol, soaked in 10% nitric acid (HNO_3) for 24 hours and then rinsed three times with reverse osmosis water. Aeration of the aquaria was achieved with aquarium air pumps via glass Pasteur pipettes inserted into air tubes. Glass pipettes were used to minimise the binding of atrazine to plastic air tubing.

Two *V. gigantea* plants were grown in each of the 16 2-L Pyrex trays (30 x 20 x 8 cm) and placed in the experimental aquaria. Macrophyte trays were carefully placed into the experimental aquaria, to reduce sediment disturbance. The trays were required to facilitate the removal and transfer of the macrophytes from the exposure aquaria to the atrazine-free aquaria for the recovery period, without disturbing the plants. The plants were grown in a 5-cm layer of heavy topsoil substrate, topped with a layer of fine gravel. The function of the fine gravel was to reduce sediment release and minimise turbidity, thereby reducing nutrient availability to epiphytic algae that may have grown on the leaves of the plants and confounded the measurements of photosynthetic activity. Before use, the heavy topsoil substrate and the fine gravel were analysed for atrazine. Atrazine stock solution (10 mg L^{-1}) was prepared from 98% pure technical grade atrazine powder in reagent grade water.

Chlorophyll fluorescence

Chlorophyll fluorescence was measured as optimum quantum yield (Fv/Fm ratio) as a measure of the photochemical efficiency of PS II. The fluorescence parameters needed to determine Fv/Fm were minimal fluorescence (Fo), maximal fluorescence (Fm), and variable fluorescence (Fv, Fv = Fm - Fo). A PAM-2000 fluorometer (Walz, Effeltrich, Germany) was used to measure the chlorophyll fluorescence variables Fo, Fm, Fv and Fv/Fm.

Two leaves on each plant were sampled for Fv/Fm, making a total of four Fv/Fm measurements from each tank. Treatment replication therefore, is at the tank (treatment) level only. The four Fv/Fm measurements from each tank were used to calculate a mean Fv/Fm value and used to plot the overall Fv/Fm trends over the exposure and recovery periods.

Sample leaves were dark-adapted for 20 minutes before being sampled for Fv/Fm, using dark-adaptation leaf clips (DLC-8 Walz). Dark-adapted Fv/Fm was measured immediately prior to dosing and then 1, 3, 5, 7, 9, 12, 14, 16, 18, 20, and 21 days after dosing. At the conclusion of the 21-day exposure period the macrophytes were transferred to aquaria containing atrazine-free water. During the recovery period, Fv/Fm was measured to examine the recovery of the photosynthetic activity after atrazine exposure. The Fv/Fm was measured 1, 3, 5, 7, 11, 13, and 15 days post-dose, with the same sampling method used during the exposure period. For consistency, Fv/Fm measurements commenced as close as possible to 16:00 h on each day of sampling. Fv/Fm measurements were repeat measures of the same area on the same two leaves, for both

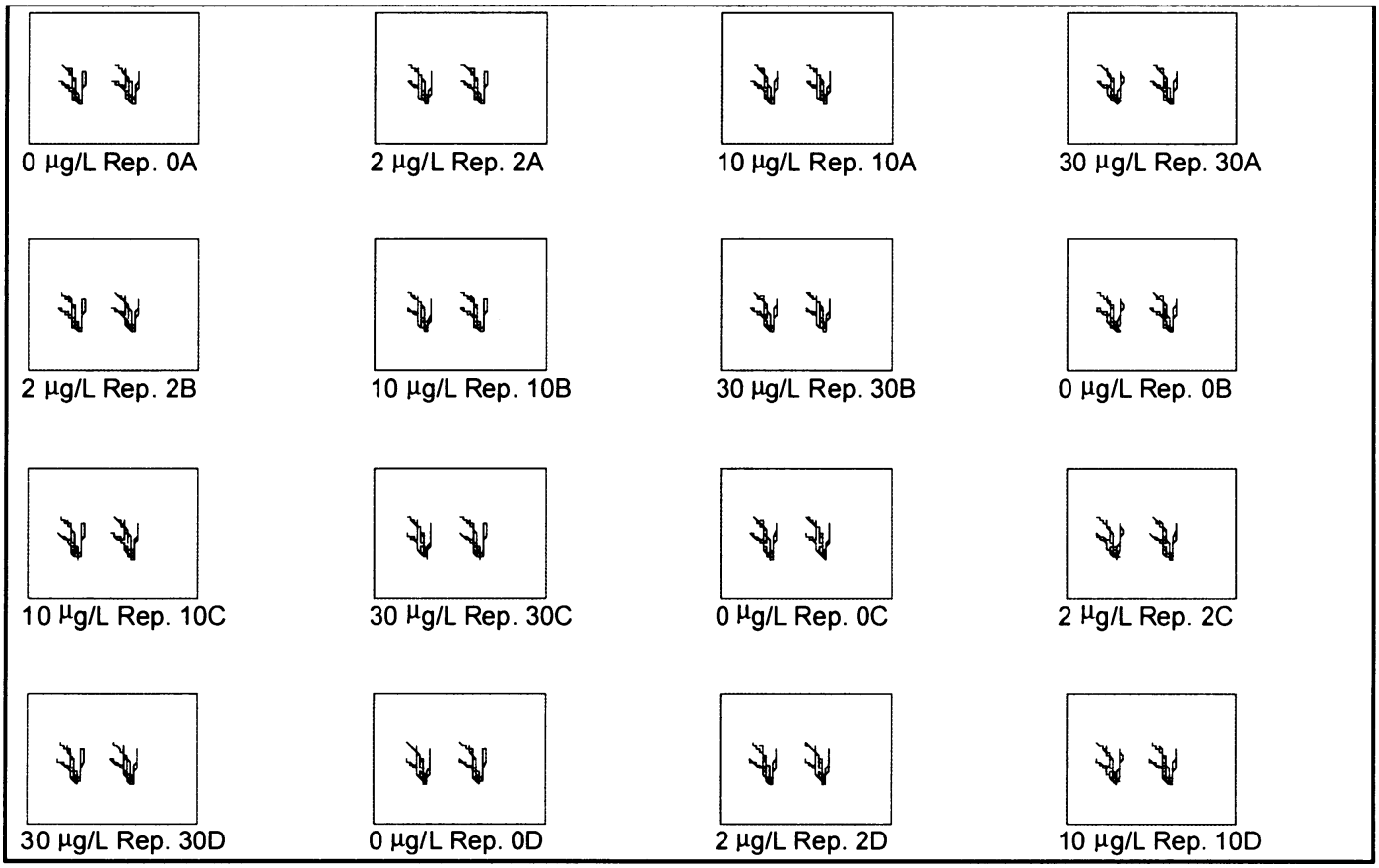

Figure 1. *Schematic representation of the Latin Square experimental design, showing the four treatments (0, 2, 10, 30 mg L^{-1} atrazine), each treatment with four replicates (n = 4) and two V. gigantea individuals per replicate. Each treatment only occurs once in each row or column to test for any gradient effects.*

plants in each tank. This was done to ensure repeated measures analysis could be used to analyse the data.

Photosynthetic pigment analysis

The photosynthetic pigments examined were chlorophyll *a*, chlorophyll *b*, and total carotenoids. Pigment samples were collected at 0, 7, 14, and 21 days during the exposure period, and 7 and 14 days during the recovery period. Pigments were sampled from one leaf from one plant in each tank. Each leaf was trimmed of any necrotic tissue or tissue that looked unrepresentative of the overall leaf before a 1 cm^2 piece of leaf tissue was removed for analysis. Once collected, the 1 cm^2 leaf cutting was weighed, and traced, for later leaf area determination using a leaf area meter (LI 3000A and LI 3050A, Li COR, USA). The leaf tissue samples were placed in 5 mL of dimethyl formamide (DMF), and refrigerated for 7 days at 4°C in the dark (Wellburn 1994). The absorbance of the extract was determined using a spectrophotometer (LKB Biochrom Ultraspec II UV/Visible Spectrophotometer 4050) at 480, 647 and 664 nm wavelengths. The resolution of the spectrophotometer was 0.5 nm. The concentrations of the pigments were then calculated using the equations of Wellburn (1994).

Determination of atrazine concentration

Atrazine concentration was measured immediately after dosing. A 200-mL water sample from each of the 16 experimental aquaria was collected and transported to the laboratory. All water samples for atrazine analysis were extracted within three days of collection. Atrazine concentration was determined by gas chromatography using USEPA Method Number 507 (Graves 1989). The heavy topsoil substrate and fine gravel used in the aquaria were also analysed for atrazine concentrations using this method. The samples were analysed in a NATA certified laboratory and all QA procedures were followed.

Statistical data analyses

The experiment was planned as a repeated measures design, where measurements are made of the same characteristic on the same observational unit, but on more than one occasion (Green 1993). In this experiment, the Fv/Fm and photosynthetic pigments of the plants were observed at different points in time.

The four Fv/Fm measurements from each tank were used to calculate an average Fv/Fm value. Homogeneity of variances was assessed before running the repeated measures ANOVA, using Bartlett's test. The Fv/Fm and photosynthetic pigment (chlorophyll *a*, chlorophyll *b*, and total carotenoids) data were analysed with repeated measures ANOVA, and tested at the $p < 0.05$ level of significance. *Post-hoc* comparisons of the sample means were completed using Tukey's honestly significantly different (HSD) test. All statistical data analyses were performed using Statistica software (Statsoft 1999).

RESULTS

Response of optimum quantum yield (Fv/Fm) to atrazine exposure

The mean Fv/Fm response of *V. gigantea* to exposure to the four treatments is illustrated in Figure 2. Initial Fv/Fm (t = 0) were very similar for all plants in all tanks. On days 1 and 2 there were significant differences between the treatments, where the Fv/Fm response of the 30 μg L^{-1} treatment was significantly lower than the control and other treatments. An instrumentation fault prevented the collection of Fv/Fm data on days 7, 9, and 11, and these results were omitted from the data analysis.

The results of the repeated measures ANOVA of the mean Fv/Fm response during the exposure period are summarised in Table 1. This analysis showed that there were highly significant differences (p = 0.002) in the mean Fv/Fm responses between the four treatments. There were no significant differences between columns (p = 0.732) or between rows (p = 0.652) of the Latin square design, meaning there was no gradient effect on Fv/Fm.

The Tukey's honestly significant difference (HSD) test results for the exposure mean Fv/Fm are summarised in Table 2. This test revealed there were significant differences between the control and the 30 μg L^{-1} treatments, between 2 μg L^{-1} and the 30 μg L^{-1} treatments, and between 10 and 30 μg L^{-1} treatments. There were no significant differences in Fv/Fm between the control and the 2 μg L^{-1} treatments, the control and the 10 μg L^{-1} treatments, or between the 2 and 10 μg L^{-1} treatments.

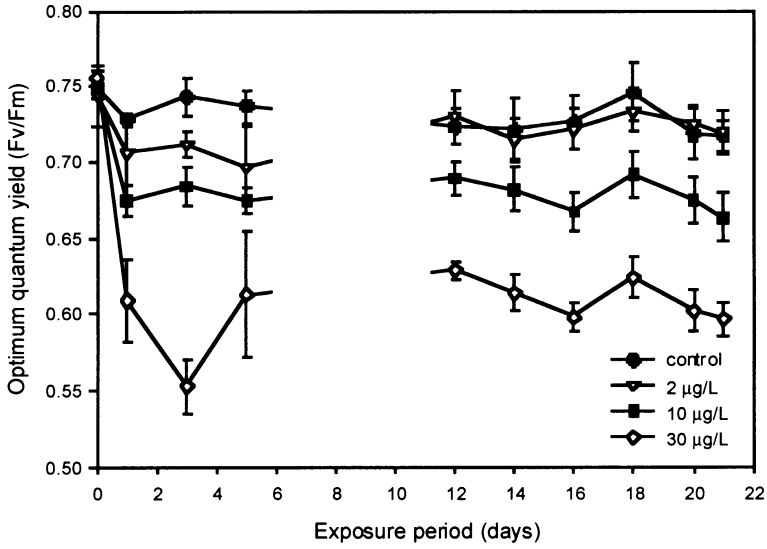

Figure 2. *Treatment mean Fv/Fm (n = 4) response over the 21-day exposure period. Error bars are standard error of mean Fv/Fm. An instrumentation fault prevented the collection of Fv/Fm data on days 7, 9, and 11, and these results were omitted from the data analysis.*

Recovery response of optimum quantum yield (Fv/Fm) after atrazine exposure

The mean Fv/Fm recovery response is illustrated in Figure 3. The response patterns of all treatments were similar to each other over the 14-day recovery period, indicating the recovery from all treatments was complete within this time. The Fv/Fm responses on the final day of the exposure period did not align with the first day of the recovery period because the first Fv/Fm measurements of the recovery period were made 24 hours after the end of exposure period.

The results of the repeated measures ANOVA of the recovery mean Fv/Fm response during the 14-day recovery period are summarised in Table 1. This analysis showed there were no significant differences in mean Fv/Fm response between treatments (p = 0.065). There were no significant differences in mean Fv/Fm response between rows (p = 0.509) during the recovery period. There were significant differences in mean Fv/Fm response between columns (p = 0.031) of the Latin square design.

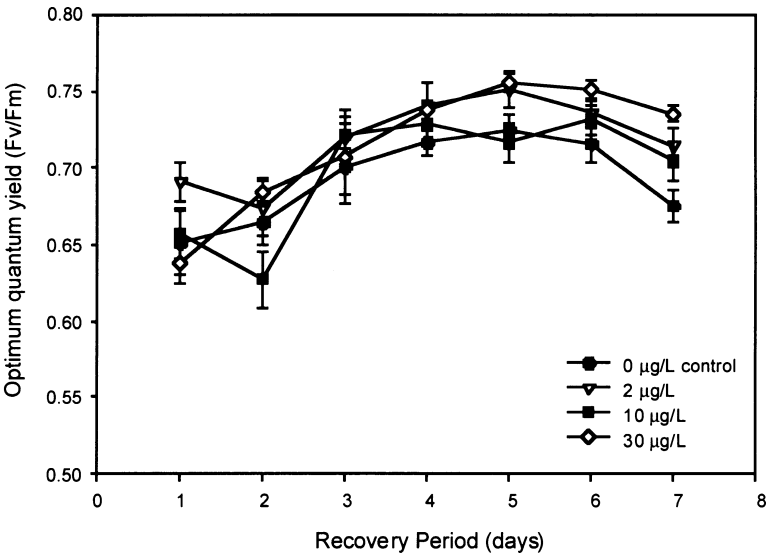

Figure 3. *Treatment mean Fv/Fm (n = 4) response over the 14-day recovery period. Error bars are standard error of mean Fv/Fm.*

Response of photosynthetic pigments to atrazine exposure

The mean (n = 4) chlorophyll *a*, *b* and total carotenoids concentration had large standard errors in all treatments caused by wide variations across replicates, and on all four sampling occasions. The results of the repeated measures ANOVA for the effects of atrazine exposure on chlorophyll *a* concentration are summarised in Table 3. This analysis showed there were no significant differences in mean chlorophyll *a* concentration between treatments (p = 0.206), or between columns (p = 0.277). There were significant differences in chlorophyll *a* between rows (p = 0.043).

The results of the repeated measures ANOVA for the effects of atrazine exposure on chlorophyll *b* concentration are summarised in Table 3. This analysis showed there were significant differences in mean chlorophyll *b* concentration between treatments (p = 0.032), between columns (p = 0.029), and between rows (p = 0.007) of the Latin square design.

The results of the repeated measures ANOVA for effects of atrazine exposure on total carotenoid concentration per gram of leaf tissue are summarised in Table 3. This analysis showed there were no significant differences in mean total carotenoid concentration between treatments (p = 0.172), or between columns (p = 0.068). There were significant differences in mean total carotenoid concentration between rows (p = 0.020) of the Latin square design.

Table 1. *Repeated measures ANOVA results for mean Fv/Fm (n = 4) response to atrazine exposure and recovery at the four treatments. *** is significant at 0.05.*

	Exposure		Recovery	
	F	p	F	p
Column	0.440	0.732	5.940	***0.031
Row	0.580	0.652	0.860	0.509
Treatment	18.930	***0.002	4.170	0.065
Time	10.150	***0.000	35.740	***0.000
Time*column	0.920	0.577	1.110	0.383
Time*row	0.620	0.908	0.960	0.522
Time*treatment	2.600	***0.001	2.260	***0.018

Table 2. *Probability values of the Tukey's honestly significant difference (HSD) test on exposure mean Fv/Fm (n = 4).*

Treatment (Ìg L^{-1})	0	2	10	30
0		0.931	0.119	0.002
2			0.243	0.003
10				0.025
30				

Table 3. *Repeated measures ANOVA results for mean chlorophyll a, chlorophyll b, and total carotenoid (Cx+c) concentration (mg per gram of leaf tissue) (n = 4), in response to exposure to the four atrazine treatments. *** is significant at p = 0.05.*

	Chl *a*		Chl *b*		Cx+c	
	F	p	F	p	F	p
Column	1.64	0.277	6.13	***0.029	4.06	0.068
Row	5.14	***0.043	11.6	***0.007	7.3	***0.02
Treatment	2.07	0.206	5.88	***0.032	2.35	0.172
Time	5.14	***0.01	12.32	***0.000	4.91	***0.012
Time*column	1.06	0.437	1.58	0.195	0.95	0.51
Time*row	0.88	0.56	0.38	0.928	0.92	0.528
Time*treatment	0.73	0.676	0.25	0.98	0.44	0.897

Analysis of change in atrazine concentration

The water sample analyses from each of the 16 experimental aquaria are presented in Table 4. The atrazine concentrations in the experimental tank systems decreased over the 21-day exposure period. The percentage decrease in atrazine concentration across 15 of the experimental aquaria ranged from 0 to 42 percent. The sample from experimental tank 30-D leaked from its sample container whilst in transit to the laboratory and could not be analysed.

DISCUSSION

Response of optimum quantum yield to atrazine exposure

Mean Fv/Fm responses across the 21 day exposure period (Figure 2) indicated the highest atrazine concentration (30 µg L^{-1}), had a significant effect on the mean Fv/Fm ratio compared to the control plants and therefore the significant differences (p = 0.002) in the Fv/Fm response between treatments during the exposure period indicate that atrazine exposure did impact upon the photosynthetic activity of *V. gigantea*. The Tukey's honestly significant difference test indicated there were no significant differences between the controls and the 2 and 10 µg L^{-1} treatments. Although the 30 µg L^{-1} treatment did significantly affect the photosynthetic activity of *V. gigantea*, this concentration did not result in the mortality of

Table 4. *Atrazine concentrations in each of the 16 experimental aquaria on day 0 (immediately after dosing) and on day 21 of the exposure period. * this sample not analysed because of a leakage in the sample container.*

Experimental Tank	Atrazine (µg/L) Day 0 (3.6.98)	Atrazine (µg/L) Day 21 (24.6.98)	% Decrease over 21 days
0-A	0	0	0
0-B	0	0	0
0-C	0	0	0
0-D	0	0	0
2-A	2.1	1.5	29
2-B	2.8	1.7	39
2-C	2.3	1.9	17
2-D	1.7	1.7	0
10-A	8.2	7.4	10
10-B	9.4	7.0	25
10-C	9.1	6.8	25
10-D	8.8	6.7	24
30-A	33.7	23.1	31
30-B	42.2	25.7	39
30-C	36.2	21.0	42
30-D	32.2	n/a*	n/a*

any plants during the 21-day exposure. Other studies suggest that a longer exposure period may have resulted in increased damage and mortality. For example, it has been found that atrazine concentrations of 12 µg L^{-1} resulted in 50% mortality *V. americana* after 47 days exposure in estuarine microcosms. Concentrations of 120 µg L^{-1} resulted in 100% mortality of *V. americana* within 30 days (Correll and Wu 1982). It should be noted that differences in culture methods can cause significantly different responses of macrophytes exposed to herbicides (Fleming *et al.* 1991).

Recovery of optimum quantum yield (Fv/Fm) after atrazine exposure

After the exposure period, plants were transferred to atrazine-free water. The repeated measures analysis of the recovery Fv/Fm results found no significant differences ($p = 0.065$) between the treatments. These results indicate that recovery of Fv/Fm was rapid when the plants were placed in the atrazine-free water. This finding is in agreement with Bowmer (1986) who suggested that photosynthetic function may be restored in plants exposed to inhibitors of PS II, such as atrazine, when they are returned to inhibitor-free water.

The nature of the Fv/Fm recovery is an important aspect of the sensitivity of fluorescence measurements. It was demonstrated that atrazine did impact on the photosynthetic activity of *V. gigantea*, but this reduction in was not permanent. When the plants were returned to fresh water, the toxic effect of atrazine exposure was rapidly removed. This recovery has important implications for understanding the impact of an acute spill in comparison to low-level chronic exposure.

There were no significant differences between the Fv/Fm responses across the rows ($p = 0.509$). However, there was a significant ($p = 0.031$) non-treatment effect detected across the columns of the Latin square. The Fv/Fm values during the recovery period show an overall increase after day 3, which could be attributed to daily changes in light climate and possibly temperature. The exposure period provided more stable growth conditions. Toxic responses of atrazine should be assessed in comparison to the state of the control Fv/Fm ratio.

Response of photosynthetic pigments to atrazine exposure

Chlorophyll *a* fluorescence measured as Fv/Fm is a more sensitive measure of atrazine impact on photosynthesis, compared to changes in concentrations of the photosynthetic pigments, such as chlorophyll *a*, *b*, or total carotenoids (Ralph 2000). There were large standard errors for all pigment concentrations as a result of the variation among the treatment replicates. The only significant difference detected between treatments among the three photosynthetic pigments examined was for chlorophyll *b* concentration ($p = 0.032$). In addition to the significant treatment effect on chlorophyll *b* concentration, there were significant gradient effects on chlorophyll *b* concentration between rows ($p = 0.007$) and columns ($p = 0.032$).

Ralph (2000) found that when the seagrass *Halophila ovalis* was exposed to atrazine concentrations ranging from 1 µg L^{-1} to 1 mg L^{-1}, there were no effects on the chlorophyll *a*, *b*, or total carotenoid content. Similarly, no major difference in fresh weight or total chlorophyll was detected when *Lemna minor* was exposed to atrazine (Kirby and Sheahan 1994). These findings support the conclusion of the present study that photosynthetic pigments are not sensitive measures of the effects of atrazine exposure.

The concentrations of total chlorophyll (chlorophyll *a* + *b*) in *V. gigantea* calculated in this experiment had an average value of 637 µg g^{-1} fresh weight, and were similar to those reported in Barko and Filbin (1983). The total chlorophyll content of *V. americana*, at 16°C was 596 µg g^{-1} fresh weight, while at 20°C it was 997 µg g^{-1} fresh weight over a range of light conditions (Barko and Filbin 1983).

Atrazine concentrations

Atrazine concentrations in the experimental aquaria (Table 4) decreased in all treatment replicates. The magnitude of the decrease did not show any consistent trend. For example, in the four replicate aquaria for the 2 μg L^{-1} treatment, the percent decrease in atrazine concentration ranged from 0 to 39%. The mean percent decrease in atrazine concentration across all experimental aquaria was 26%.

The mechanism responsible for the loss of atrazine in the experimental aquaria is unknown, because neither the plant tissue nor sediment was analysed for atrazine at the end of the experiment. The authors recommend that atrazine uptake and dissipation mechanisms should be examined in future research. Environmental fate of atrazine suggests there are a number of breakdown pathways of atrazine in aquatic systems. The most important factor governing the breakdown of atrazine is the medium in which it is contained (Tomlin 1994). Photolysis of atrazine is not a rapid degradative pathway (Abildt 1995) and is not considered a mechanism contributing to the decrease in atrazine concentration in this experiment.

The results of this experiment only apply in the context of the experimental conditions used, and should be extrapolated cautiously in an environmental context, as the laboratory-based experimental conditions are different from natural river ecosystems. Even though these results are laboratory-derived, they are useful because they provide an indication of the negative effects of atrazine on the photosynthetic activity of *V. gigantea*. Chronic exposure to atrazine at concentrations at 30 μg L^{-1} would have a negative effect on the photosynthetic activity, and therefore the health, of *V. gigantea*. Herbicides other than atrazine, for example diuron, fluometron, metolachlor, prometryn, pendimethalin and glyphosate, are detected in NSW rivers (Muschal 2001) and have the potential to act synergistically or additively with atrazine on macrophytes. Atrazine was found to act additively with alachlor, reducing the biovolume of benthic microalgae (Carder and Hoagland 1998). The consequences of additive or synergistic effects on macrophytes can potentially cause substantial damage to aquatic ecosystems.

In Australia, trigger values have been developed for substances that may impair water quality of degrade aquatic ecosystems (ANZECC and ARMCANZ 2000). The trigger values of atrazine for the protection of 99% and 95% percent of species in freshwater ecosystems are 0.7 μg L^{-1} and 13 μg L^{-1} respectively (ANZECC and ARMCANZ 2000). The trigger value for the protection of 95% of species applies to typical slightly-moderately disturbed systems (ANZECC and ARMCANZ 2000). Rivers that are subject to atrazine contamination occur in agricultural areas (Muschal 2001) and these rivers are not necessarily slightly-moderately disturbed, but substantially disturbed, by altered flow regimes, erosion, and high levels of nutrient and other contamination. The results of the present study indicate that *V. gigantea* is affected by concentrations of atrazine as low as 30 μg L^{-1}, which is only 2.3 times the guideline trigger value. This indicates atrazine is a contaminant that has the potential to adversely affect freshwater macrophytes at concentrations relatively close to guideline trigger values.

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REFERENCES

Abildt, U. 1995. *Estimation of the Environmental Photochemical Half-Life of G30027 in Surface Waters*. Project Report 45/94, Ciba-Geigy, Basle, Switzerland. In *The NRA Review of Atrazine*. Draft for Public Comment. NRA National Registration Authority for Agricultural and Veterinary Chemicals. Canberra, Australia.

ANZECC and ARMCANZ. 2000. *Draft Guidelines for Fresh and Marine Water Quality.* Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand. Canberra, Australia.

Barko JW and Filbin GJ. 1983. Influences of light and temperature on chlorophyll composition in submerged freshwater macrophytes. *Aquat. Bot.* **15**, 249-255.

Biernacki M, Lovett-Doust J and Lovett-Doust L. 1997. Temporal biomonitoring using wild celery, *Vallisneria americana*. *J. Great Lakes Res*. **23**(1), 97-107.

Bowmer KH. 1986. Rapid Biological Assay and Limitations in Macrophyte Ecotoxicology: A Review. *Aust. J. Mar. Freshwater Res.* **37**, 297-308.

Carder JP and Hoagland KD. 1998. Combined effects of alachlor and atrazine on benthic algal communities in artificial streams. *Environ. Toxicol. Chem*. **17**(7), 1415-1420.

Conrad R, Buchel C, Wilhelm C, Arsalane W and Berkaloff C. 1993. Changes in yield of *in-vivo* fluorescence of chlorophyll as a tool for selective herbicide monitoring. *J. App. Phycol.* **5**, 505-516.

Correll DL and Wu TL. 1982. Atrazine toxicity to submersed vascular plants in simulated estuarine microcosms. *Aquat. Bot.* **14**, 151-158.

Davies PE, Cook LJS and Barton JL. 1993. Triazine herbicide contamination of Tasmanian streams: streams, concentrations and effects on biota. *Aust. J. Mar. Freshwater Res.* **45**, 209-226.

Depledge MH. 1993. The rational basis for detection of the early effects of marine pollutants using physiological indicators. *Ambio* **18**, 301-302.

Dewey SL. 1986. Effects of the herbicide atrazine on aquatic insect community structure and emergence. *Ecology* **67**, 148-162.

Fleming WJ, Ailstock MS, Momot J and Norman CM. 1991. Response of Sago Pondweed, a submerged aquatic macrophyte to herbicides in three laboratory culture systems. In *Plants for Toxicity Assessment*, Volume 2. Gorsuch JW, Lower WR, Lewis MA and Wang W. (Eds). ASTM, Philadelphia.

Goswami KP and Green RE. 1971. Microbial degradation of the herbicide atrazine and its 2-hydroxy analog in submerged soils. *Environ. Sci. Technol.* 5(5), 426-429.

Graves RL. 1989. *Determination of nitrogen and phosphorous containing pesticides in water by gas chromatography with a nitrogen-phosphorous detector.* USEPA Method Number 507 (Revision 2.0). USEPA Environmental Monitoring Systems Laboratory, Ohio, USA.

Green RH. 1993. Application of repeated measures designs in environmental impact and monitoring studies. *Aust. J. Ecol.* **18**, 81-98.

Hofmann A and Winkler S. 1990. Effects of Atrazine in environmentally relevant concentrations on submersed macrophytes. *Arch. Hydrobiol.* **118**(1), 69-79.

Huber W. 1993. Ecotoxicological relevance of atrazine in aquatic systems. *Environ. Toxicol. Chem.* **12**, 1865-1881.

Judy BM, Lower WR., Ireland FA and Krause GF. 1991. A Seedling Chlorophyll Fluorescence Toxicity Assay. In *Plants for Toxicity Assessment,* Volume 2. Gorsuch JW, Lower WR, Lewis MA and Wang W. (Eds). ASTM, Philadelphia.

Kirby MF and Sheahan DA. 1994. Effects of atrazine, isoproturon and mecoprop on the macrophyte *Lemna minor* and the alga *Scenedesmus subspicatus. Bull. Environ. Contam. Toxicol.* **53**, 120-126.

Klaassen HE and Kadoum AM. 1979. Distribution and Retention of Atrazine and Carbofuran in Farm Pond Ecosystems. *Arch. Environ. Contam. Toxicol.* **8**, 345-353.

Merz D, Geyer M and Moss DA. 1996. Chlorophyll fluorescence biosensor for the detection of herbicides. *Fresenius J. Anal. Chem.* **354**, 299-305.

Muir DCG, Yoo JY and Baker BE. 1978. Residues of atrazine and N-deethylated atrazine in water from five agricultural watersheds in Quebec. *Arch. Environ. Contam. Toxicol.* **7**, 221-235.

Muschal M. 1997. *Central and North West Regions Water Quality Program. 1996/97 Report on Pesticide Monitoring. Ecosystem Management.* NSW Department of Land and Water Conservation. CNR97.063. Parramatta, NSW, Australia.

Muschal M. 2001. *Central and North West Regions Water Quality Program 1999-2000 Report on Pesticides Monitoring.* NSW Department of Land and Water Conservation, Centre for Natural Resources, CNR2000.067. Parramatta, NSW, Australia.

NRA. 1997. *The NRA Review of Atrazine.* Draft for Public Comment. National Registration Authority for Agricultural and Veterinary Chemicals. Canberra, Australia.

Ralph PJ. 2000. Herbicide toxicity of *Halophila ovalis* assessed by chlorophyll *a* fluorescence. *Aquat. Bot.* **66**, 141-152.

Sainty G and Jacobs SWL. 1993. *Waterplants and bank vegetation in the Upper Barwon, Mole, Dumaresq, Macintyre and Severn Rivers and Macintyre Brook.* Sainty and Associates, Sydney, Australia.

Statsoft. 1999. *Statistica for Windows.* Version 5.5 99 edition. Statsoft, Oklahoma, USA.

Tomlin C. 1994. *The Pesticide Manual: Incorporating the Agrochemicals Handbook.* Tenth Edition. British Crop Protection Council and The Royal Society of Chemistry. Crop Protection Publications.

Wellburn A.R. 1994. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J. Plant Physiol.* **144**, 307-313.