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Atrazine Uptake, Photosynthetic Inhibition, and Short-Term Recovery for the Submersed Vascular Plant, *Potamogeton perfoliatus* L.

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Abstract. The processes of atrazine (2-chloro-4-[ethylamino]-6-[isopropylamino-]-*s*-triazine) uptake and release in the submersed vascular plant, *Potamogeton perfoliatus* L., were rapid, approaching equilibrium with the surrounding environment within one hr. The ratio of internal atrazine concentration to external concentration was approximately 10 at the point of maximum photosynthetic inhibition and rapidly increased at lower external atrazine concentrations. The I_{50} (the concentration inhibiting photosynthesis by 50%) for atrazine in solution was 80 μg/L with the maximum observed photosynthetic reduction (87%) at a solution concentration of 650 μg/L. Initial photosynthetic recovery of *P. perfoliatus* following exposure to atrazine was rapid with oxygen evolution from treated plants (5, 25, and 100 μg/L) being statistically indistinguishable from control plants after two hr of atrazine-free wash. However, there was an indication of residual photosynthetic depression in dosed plants, even after a 77 hr recovery period. In Chesapeake Bay, potential long-term exposure of submersed plants to concentrations of atrazine greater than 10 μg/L is doubtful so that reduction of *P. perfoliatus* photosynthesis under such conditions would be minimal and reversible.

A small percentage of the herbicides used for agricultural weed control is lost from croplands with some eventually entering contiguous watercourses (Muir *et al.* 1978; Triplett *et al.* 1978; Wu 1980; Glotfelty *et al.* 1984). If significant concentrations of these herbicides occur in adjacent aquatic systems, a potential stress exists for the indigenous plant community including the submersed vascular plants. One of the most commonly used herbicides is atrazine (2-chloro-4-[ethylamino]-6-[isopropylamino]-*s*-triazine), a compound observed recently in the waters of a large North American estuary, the Chesapeake Bay (Wu 1980; Stevenson *et al.* 1978; Glotfelty *et al.* 1984). Numerous species of submersed vascular plants have been important in this estuarine ecosystem until recent years, when their abundance declined (Stevenson and Confer 1978; Bayley *et al.* 1978; Orth and Moore 1983). Thus, the effect of herbicides such as atrazine on the growth and production of vascular plants in Chesapeake Bay is a matter of some concern.

Several recent studies have examined the effect of atrazine on various submersed vascular plants in Chesapeake Bay and other aquatic ecosystems (Forney and Davis 1981; Correll and Wu 1982; Cunningham *et al.* 1984; Jones and Estes 1984; Jones and Winchell 1984; Delistraty and Hershner 1984; Kemp *et al.* 1985). In each of these studies, significant reductions in growth and production of these plants were observed consistently at atrazine concentrations ≥50–100 μg/L. Concentrations of <50 μg/L occasionally resulted in significant levels of inhibition in some experiments. In small coves surrounded by agricultural watershed, these submersed plants may be exposed periodically to atrazine concentrations of the order of 5–50 μg/L for brief periods during runoff events (Kemp *et al.* 1982). However, dilution, adsorption, and degradation of the herbicide tend to reduce concentrations in the water phase to <5 μg/L within 6–24 hr Correll *et al.* 1978; Jones *et al.* 1982; Kemp *et al.* 1982; Glotfelty *et al.* 1984). Since exposure of submersed plants to phytotoxic herbicide concentrations tends to be short-term, it is important in assessing potential herbicide effects to know: how rapidly the com-

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pounds are taken-up by plants; how uptake and plant response are related, and whether effects persist after the herbicide concentration in the water phase is reduced.

The purpose of this study was to examine the kinetic relationships between uptake of atrazine and photosynthetic inhibition for a common submersed angiosperm. Experiments were conducted with the submersed plant, *Potamogeton perfoliatus* L., an important species in upper Chesapeake Bay (Stevenson and Confer 1978). Specific questions investigated include: 1) the rate of atrazine uptake; 2) the relative affinity of roots vs shoots for atrazine uptake; 3) the relation between uptake and reduction in carbon assimilation; and 4) the time required for photosynthetic recovery following brief (2 hr) exposure to atrazine.

Materials and Methods

Collection of Experimental Materials

Plants were collected from shallow coves (0.5 to 1.5 m) near the mouth of the Choptank River estuary, a tributary of Chesapeake Bay during July 1981 and 1982. Plants were obtained one day prior to experimental incubations by gently removing roots and rhizomes from their substrate and washing leaves and roots free of sediments and epiphytic material. Plants were held overnight in filtered (1 μm) estuarine water (10% salinity) at 25°C under constant fluorescent illumination of 150 μEin m^{-2} s^{-1}. All experiments were conducted either in a Percival environmental chamber or 55 L glass aquaria.

Atrazine Uptake and Phytotoxicity

Matched sets of plant material (5 g wet wt) were selected for use in two parallel series of treatments. The first treatment series employed uniformly ring-labelled [^{14}C]-atrazine (1.85 MBq/mg, CIBA-Geigy Corp) dissolved in methanol to ascertain atrazine uptake, while the other series used [^{14}C]sodium bicarbonate (24.86 MBq/mg) plus non-labelled atrazine (99.7%, CIBA-Geigy Corp) dissolved in methanol to determine the amount of CO_2-fixation at a given herbicide dosage. Plants were incubated in 2-L Erlenmeyer flasks (with 1 L of treatment solution) at atrazine concentrations of 10, 50, 100, 450, and 650 μg/L. Controls consisted of flasks containing [^{14}C]-sodium bicarbonate only and flasks containing [^{14}C]-sodium bicarbonate and methanol. All flasks were simultaneously incubated in a Percival Environmental chamber at 25°C and 150 μEin m^{-2} s^{-1} for four hr with periodic (hourly) mixing by hand swirling. At the end of the experiment, triplicate plants from each flask were radioassayed (Jones and Estes 1984).

Time-Course Atrazine Uptake

Plants (apical 20 cm of shoot) were placed in 2-L Erlenmeyer flasks containing either 20, 50, or 100 μg/L ^{14}C-atrazine (ring-labelled, 1.85 MBq/mg) dissolved in 1.0 L of filtered (GF/C) estuarine water. The flasks were incubated for a total of 24 hr at constant temperature and light as described above. Plants were removed from the flasks at 0, 0.25, 0.50, 0.75, 1, 2, 4, 8, 12, and 24 hr from the initial treatment, washed, blotted, weighed, and processed for radioassay as described above. Measurements of blotted wet weight (w.w.) were converted to dry weight (d.w.) using relations obtained on a subsample of 25 plants, and ratios of (w.w.):(d.w.) were 10.3 ± 1.2 (s.d.) for shoots and 8.1 ± 0.6 for roots.

Photosynthetic Recovery

Individually tagged shoots (apical 20 cm) were placed in a treatment series (5 replicates each) consisting of a control plus atrazine concentrations of 5, 25 and 100 μg/L. Bottles (300 ml BOD) containing plants and treated water were suspended in 55 L glass aquaria with the surrounding water agitated gently by submersible pumps. Apparent photosynthesis (P_a) was estimated by changes in dissolved oxygen (DO) in each bottle following 2-hr incubations. Dissolved oxygen measurements were made with a polarographic oxygen electrode (Orbisphere Model 2709), air-calibrated prior to the experiment. The incubation temperature was 25°C, and the light intensity at the water surface was 150 μEin m^{-2} s^{-1}.

Two related experimental designs were used to investigate photosynthetic recovery of these plants. In the first of these, herbicide-dosed plants (5 replicate plants having been exposed to 5, 25 and 100 μg/L atrazine for 2 hr in BOD bottles) were transferred to another series of BOD bottles containing filtered, atrazine-free estuarine water; P_a was measured again following a 2 hr incubation. This procedure was repeated in two more sequences, after which the plants were removed from the bottles and placed in 20 L of atrazine-free filtered estuarine water, where they were maintained in the light for 18 hr. A final 2 hr incubation in BOD bottles was performed to estimate P_a (as above) on each plant at 24 hr after the initial dose period.

The second experiment to examine photosynthetic recovery was identical to that described above in terms of BOD bottle incubations for measurement of P_a; however, it differed in the length of the recovery period and volume of water used to wash the plants. Here, plants were removed from the atrazine-containing BOD bottles following the initial 2 hr incubation and placed directly into aquaria containing 20 L of atrazine-free water and allowed to "wash" for 3 hr before being incubated again in BOD bottles to monitor photosynthesis. The plants were returned to their respective aquaria following this 2-hr incubation (the water in each aquaria was replaced between P_a measurements). P_a was again determined as above for individual plants at 29 and 77 hr after initial exposure.

Atrazine Release

^{14}C-atrazine release was monitored for previously dosed (100 μg/L atrazine) plants in the first of the photosynthetic recovery experiments described above. A parallel set of 10 plants were exposed to 100 μg/L ring-labelled ^{14}C-atrazine (1.85 MBq/mg) for 2 hr. Five of these plants were immediately processed for radioassay. The other five plants were placed in BOD bottles and apparent photosynthesis measured polarographically as above. At the end of the each incubation in atrazine-free water, triplicate 1

ml aliquots were taken from each BOD bottle and placed in 10 ml of Aquasol-2 (New England Nuclear). At the end of the entire series of incubations and washes, the plants were removed and dried for analysis of remaining ^{14}C content.

The release of ^{14}C-atrazine was also measured in another experiment using a wash series of various solvents. Whole plants, incubated for 2 hr in 10, 50, 100, 450 and 650 μg/L ^{14}C-atrazine, were subjected to a 4-step wash series in the following order: (1) 500 ml of filtered estuarine water, (2) 0.1% Tween 80® (surfactant) in 500 ml of estuarine water, (3) 500 ml of estuarine water, and (4) 500 ml of a 2% HCl solution in estuarine water. Triplicate 1-ml aliquots from each beaker were placed into 10 ml Aquasol-2 for radioassay.

Results and Discussion

Plant Uptake of Atrazine

The uptake of atrazine by *P. perfoliatus* shoot tissue was rapid, reaching equilibrium with the surrounding solution within 15 min (Figures 1a, b). There was no significant change in atrazine concentrations ($p > 0.05$, ANOVA, SNK) in the plants after the first 15 min. of incubation through the duration of the experiment, 24 hr later (Figure 1b). Furthermore, varying the solution concentrations of atrazine from 20 to 50 μg/L had little or no effect on the time required to achieve equilibrium.

Although results of similar time-course atrazine uptake studies are not available for other submersed macrophytes, experiments involving single-celled algae have been reported. Vallentine and Bingham (1976) found that for several species of freshwater algae rapid atrazine uptake occurred within the first minute of incubation, with cellular concentrations remaining relatively constant over the experimental period (24 hr). Bohm and Muller (1976), working with *Scenedesmus* (another single-celled algae) found that cellular atrazine uptake reached equilibrium with the culture solution in 4–8 hr.

The uptake of ^{14}C-atrazine by rhizome/roots (roots) of *P. perfoliatus* (per gdw of tissue) was lower than shoot uptake (per gdw of tissue), with root uptake at a given concentration of atrazine ranging from 16–40% of shoot uptake (Table 1). Although roots and shoots were not separated in these experiments, the likelihood of significant translocation of atrazine occurring during these short-term (2 hr) incubations is minimal (Funderburk and Lawrence 1963). Typical root: shoot biomass ratios for *P. perfoliatus* populations in Chesapeake Bay are 0.2–0.3 (Kemp *et al.* 1984), so that root uptake of atrazine would only constitute a small fraction of total plant uptake if both plant

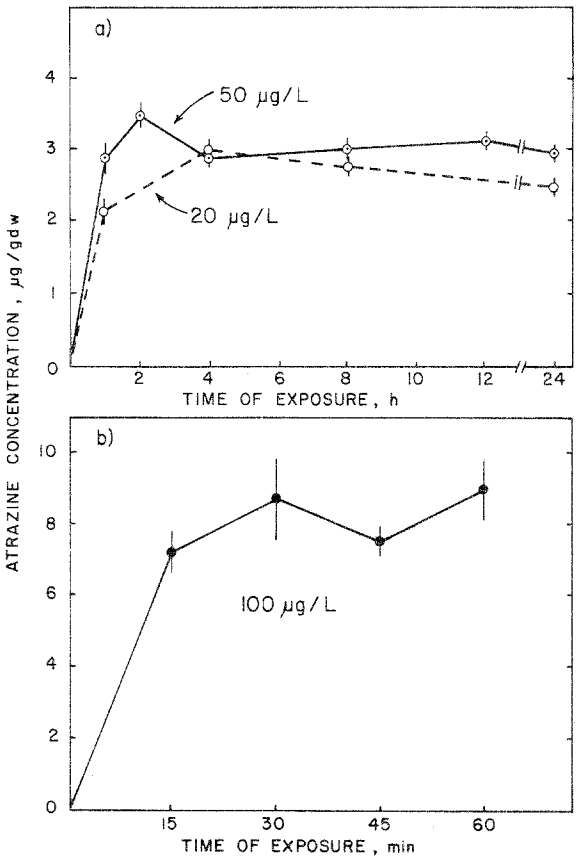

Fig. 1. Atrazine uptake by *P. perfoliatus* shoots over periods of: (a) 24 hr at both 20 and 50 μg/L; and (b) 1 hr at 100 μgL (X ± S.E.)

sections were exposed to the same herbicide concentrations. The ratio of root to shoot uptake on a whole plant basis was considerably lower in these experiments than on a per gram dry weight of tissue basis ranging from .04 to .16 (Table 1). There are, however, few if any reliable data on pore-water concentrations of atrazine in estuaries, making it difficult to extrapolate these results to conditions in nature. Forney and Davis (1981) also concluded from another approach that root uptake of atrazine was relatively unimportant for both *P. perfoliatus* and *Elodea canadensis*.

Uptake of atrazine by *P. perfoliatus* shoots (Figure 2a) was positively correlated with increasing solution concentrations over the concentrations tested reaching 7.10 μg atrazine/gdw shoot at 650 μg/L aqueous concentration. Rhizome/root (root) uptake was also positively correlated but the uptake per gdw was less with the internal concentration reaching 2.75 μg atrazine/gdw in a treatment of 650 μg/L atrazine (Table 1). However, the partitioning of atrazine between the shoots of *P. perfoliatus* and the incubation solution was not constant with increasing solution concentrations while it was

Table 1. Root:shoot ratio of ^{14}C-atrazine uptake in *P. perfoliatus* (4 hr incubation)

Atrazine solution concentration (μg/L)	Atrazine uptake *Roots*	*Shoots*	Partition Coefficient[a] *Roots*	*Shoots*	Ratio of weight-specific uptake by roots:shoots[b]	Ratio of root:shoot uptake for whole plant[c]
10	0.19 ± .04[d]	0.85 ± .05	.0190	.0850	0.22	0.04
50	0.22 ± .06	1.30 ± .07	.0044	.0260	0.17	0.07
100	0.32 ± .07	2.00 ± .07	.0032	.0220	0.16	0.07
450	1.68 ± .38	4.20 ± .51	.0037	.0093	0.40	0.16
650	2.75 ± .62	7.10 ± .62	.0041	.0109	0.39	0.06

[a] Tissue concentration divided by solution concentration (μg/gdw:μg/L)
[b] Ratio expressed per gdw of root or shoot, respectively
[c] Ratio expressed per gdw of total plant
[d] μg/gdw ± S.E.

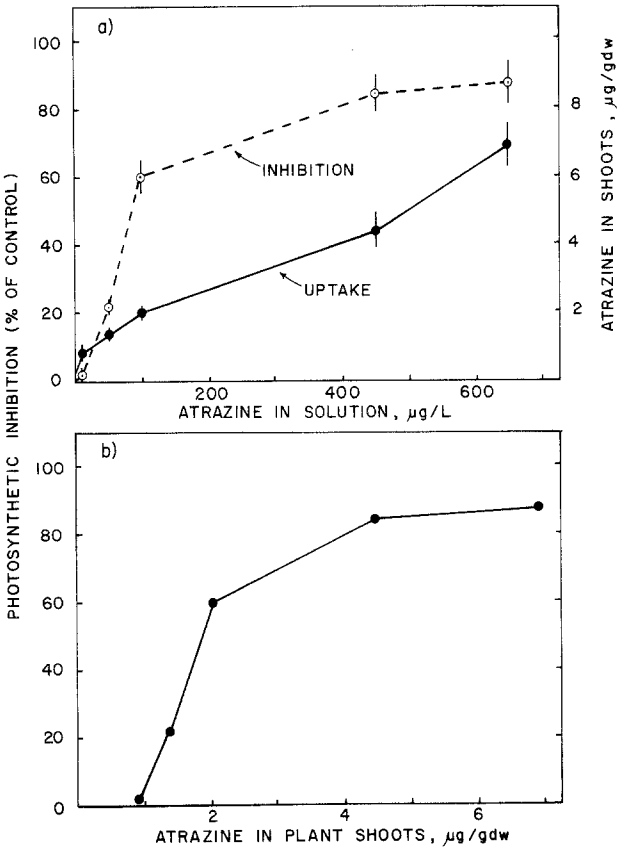

Fig. 2. (a) Atrazine uptake and associated photosynthetic inhibition for *P. perfoliatus* shoots at external solution concentrations of atrazine from 10 to 650 μg/L (b) Internal atrazine concentration and associated photosynthetic inhibition in *P. perfoliatus*

relatively constant for root tissue (Table 1). This is discussed in more detail below.

Atrazine Phytotoxicity

Inhibition of photosynthesis with increasing atrazine concentrations follows Michaelis-Menton kinetics with the maximum inhibitions observed (87 and 88%) occurring at atrazine concentrations of 450 and 650 μg/L, respectively (Figure 2a). Dark CO_2 uptake for these plants was determined to be 2% of the total CO_2 fixed in the light, while uptake of atrazine was the same in the light as in the dark (this insensitivity to light for atrazine uptake has also been reported by Vallentine and Bingham (1976) working with the alga *Scenedesmus*).

The I_{50} (atrazine concentration at which photosynthesis is inhibited by 50%) for *P. perfoliatus* in these experiments was 80 μg/L. Kemp *et al.* (1985), also working with *P. perfoliatus*, reported a 50% reduction in apparent photosynthesis (P_a) at an atrazine concentration of 130 μg/L. Of interest is the fact that their experiments were long term (5 wks) and used oxygen evolution to assess photosynthesis as compared to the shorter incubations (4 hr) and $H^{14}CO_3$-incorporation methodology used in the present study. In another study where P_a was measured using oxygen evolution Jones and Winchell (1984) found an average I_{50} for four species of submerged macrophytes to be 95 μg/L with the I_{50} for *P. perfoliatus* being 75 μg/L.

Forney and Davis (1981) observed 50% reductions in growth of *P. perfoliatus* and *Elodea canadensis* at atrazine concentrations of 30 μg/L and 125 μg/L atrazine, respectively. Correll and Wu (1982) reported various effects on submersed macrophyte production resulting from exposure to 75 μg/L atrazine, ranging from stimulation for *P. pectinatus* to reductions of 50% and 20% for *Zannichellia palustrus* and *Vallisneria americana*, respectively. Thus, it appears that 50% inhibition of photosynthesis and growth of many submersed macrophytes occurs at atrazine concentrations of from 50–150 μg/L.

Relationship between Atrazine Uptake and Toxicity

The relationship of internal atrazine concentration in *P. perfoliatus* shoots to photosynthetic inhibition

suggests that maximum photosynthetic inhibition occurs at external concentrations >450 μg/L corresponding to internal concentrations >5 μg/gdw (Figure 2b). At these external concentrations associated with maximum photosynthetic inhibition, an equilibrium is achieved between internal and external levels of atrazine. The ratio of internal (shoot) to external solution concentrations of atrazine approached a constant level of 10 (μg/kg)/(μg/L) at this equilibrium point, while it increased markedly with lower solution concentrations (Figure 3).

Similar patterns of accumulation of atrazine at low concentrations have been observed in algae by direct measurements of tissue concentrations (Bohm and Muller 1976) and indirectly by observations on the % atrazine remaining in solution (Vallentine and Bingham 1976). Atrazine is known to have a high binding affinity for organic matter (Weber *et al.* 1969). The acute accumulation of plant-bound atrazine at low atrazine concentrations could result from the fact that the ratio of plant organic matter to solution atrazine concentration increases as the external solution atrazine concentration decreases. In support of this is the report by Geller (1979), who found a good correlation between bacterial surface area and atrazine accumulation, *i.e.*, increased surface area yielded higher atrazine accumulation.

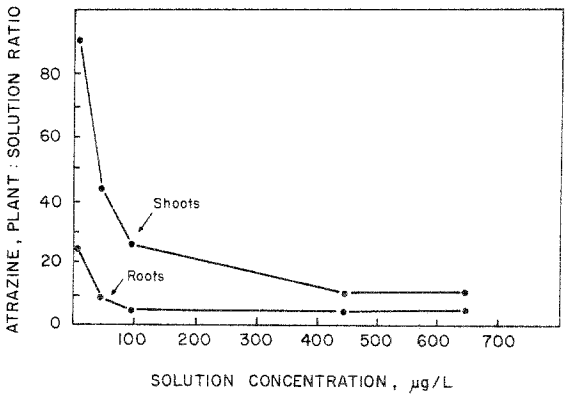

Fig. 3. Ratio of atrazine uptake in *P. perfoliatus* to atrazine concentration in external medium

Table 2. ^{14}C-atrazine released from treated plants (100 μg atrazine/L) with repeated washes

Washing period[a]	^{14}C-atrazine contained in plant material per bottle	^{14}C-atrazine released per bottle
Initial 2-hr treatment	1.12 ± .15[b]	0
1	N.D.[c]	0.49 ± .03
2	N.D.	0.18 ± .01
3	N.D.	0.18 ± .01
4	0.13 ± .02	0.04 ± .01

[a] Periods 1–3 are consecutive 2-hr wash periods in BOD bottles followed by an 18-hr wash period in a 55-L aquarium and then another 2 hr wash in BOD bottles (period 4)
[b] μg ^{14}C-atrazine ± S.E.
[c] N.D. = not determined

Atrazine Release

The release of atrazine from *P. perfoliatus* shoot tissue originally exposed to 100 μg/L for 2 hr was examined in a series of washes involving 2-hr incubations of the plants in BOD bottles containing atrazine-free estuarine water (Table 2). Approximately 45% of the initial ^{14}C-atrazine bound to the plant material was released after its return to atrazine free solution (2 hr). The rate of release slowed markedly over the next washings, and by the third wash, 76% of the initially bound atrazine had been released.

Bohm and Muller (1976) observed an atrazine desorption time for the algal species, *Scenedesmus*, of 4–8 hr and found that about 10% of the sorbed atrazine was irreversibly bound. An atrazine adsorption/desorption study on three aquatic bacteria by Geller (1979) showed that these organisms reached equilibrium with their atrazine-containing solution within 30 min; desorption was also complete within 30 min. It was also observed that 10% of the atrazine sorbed by the bacteria could not be desorbed as was found for *Scenedesmus* by Bohm and Muller (1976). This observation of irreversibly bound atrazine to plant tissue has been reported elsewhere (Izawa and Good 1965).

The strength of atrazine binding to shoot tissue of *P. perfoliatus* exposed to ^{14}C-atrazine for 4 hr was investigated using a washing sequence of progressively stronger solvents (Table 3). It was assumed that the first wash of filtered ambient water would remove the loosely associated surface film containing the ^{14}C-atrazine due to the sharp difference in atrazine concentration between the plant surface film and the wash water, while the surfactant in the second wash would remove the physically bound lipophilic atrazine. The third wash with filtered ambient water removed the surfactant from the plant. The final wash, a 2% HCl solution, was intended to shock the membrane electrical potential allowing materials in the cytoplasm to flow out and/or detach ionically-bound ^{14}C-atrazine.

The first immersion in water removed considerable ^{14}C-atrazine. The surfactant wash and third reimmersion in water removed very little additional heribcide. However, the acid wash did remove a substantial amount of ^{14}C-atrazine. The results in-

Table 3. The quantity of ^{14}C-atrazine removed from *P. perfoliatus* by various washes following a four-hour incubation period

Atrazine concentration (μg/L)	#1 Water	#2 Surfactant	#2 Water	#4 2% HCl
10	0.14[a]	0.01 (3)[b]	0.00 (0)	0.02 (5)
50	0.10	0.03 (5)	0.00 (0)	0.18 (29)
100	0.26	0.09 (11)	0.00 (8)	0.29 (37)
450	1.14	0.38 (16)	0.19 (8)	0.45 (19)
650	1.54	0.41 (13)	0.20 (8)	0.60 (19)

[a] μg ^{14}C-atrazine

[b] Numbers in parentheses are the percent of total plant ^{14}C-atrazine removed. Water wash #1 not reported due to variability in the amount of water carried-over from incubation solutions

dicate that a very low percentage of the sorbed atrazine is "loosely" bound to external plant surfaces.

The exact physio-chemical mechanisms of atrazine removal effected by the acid wash and the location of the atrazine within the plant material are open questions. Complications such as atrazine on the plant surfaces being hydrolyzed to hydroxyatrazine (Jones *et al.* 1982) by the lowered pH of the acid wash, hence, facilitating the dissolution of this relatively more polar compound cannot be discounted.

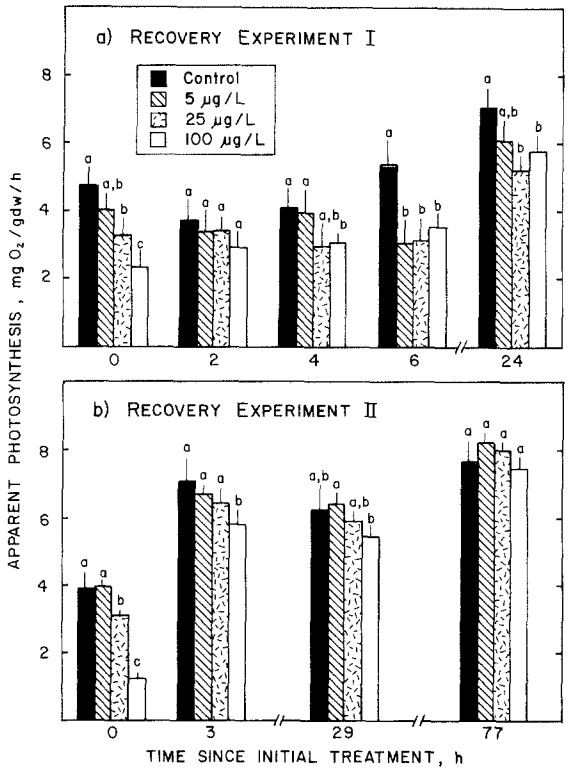

Fig. 4. Photosynthetic recovery by *P. perfoliatus* following a 2 hr exposure period with 5, 25, and 100 μg/L atrazine determined over (a) 24 hr and (b) 77 hr ($\bar{X} \pm$ S.E.). Statistically different groups ($P < .05$) indicated by differing letters (ANOVA, Student-Newman-Kuels test)

Photosynthetic Recovery

The data indicate that photosynthetic recovery of *P. perfoliatus* occurred rapidly. In both experimental procedures, the plants recovered to greater than 75% of their maximum photosynthetic potential within 2–3 hr (Figures 4a, b). A slower recovery occurred over the next 10–80 hr. Although a t-test indicated no significant difference ($p > 0.05$) between the means of the control and treatment plants after 2–3 hr, a slow but steady increase in photosynthesis was evident (the reason for the drop in photosynthesis seen in the 25 μg/L treatment (Figure 4a) is unknown). Even after 77 hr of flushing in the large volume experiment, mean photosynthesis was still less than control plants in the 100 μg/L treated plants (Figure 4b).

Analysis of the plants in the first recovery procedure exposed to 100 μg/L of ^{14}C-atrazine determined that the plants contained approximately 10% of the original ^{14}C-atrazine at the end of 24 hr of flushing (Table 2). However, there was no simple linear correlation between atrazine release and photosynthetic recovery of plants after 6 hr of wash. The initial 100 μg/L treatment resulted in a 60% reduction in photosynthesis, and after 6 hr of recovery and wash, photosynthesis had only recovered to 75% of the control, only a 50% increase in photosynthesis compared to a 76% reduction in internal atrazine.

Cunningham *et al.* (1984) and Kemp *et al.* (1985) observed a partial recovery of photosynthesis by *P. perfoliatus* growing in laboratory aquaria (microcosms) within 1–2 wk after treatment with ≤100 μg/L atrazine. In these experiments, there was a continual exposure to elevated concentrations of atrazine, which remained at 80–85% of initial levels at 4 wk after treatment.

The results indicate that uptake and release of atrazine in *Potamogeton perfoliatus* was rapid, reaching equilibrium with surrounding solution within one hour. Photosynthesis in this plant is relatively sensitive to atrazine exposure at least under estuarine (10%) conditions with an estimated 50% loss of photosynthesis occurring at 80 μg/L aqueous atrazine. Root affinity for uptake of atrazine was relatively low compared to shoot uptake. When *P. perfoliatus* was exposed to a range of atrazine concentrations followed by incubation in atrazine-free water, photosynthetic recovery oc-

curred rapidly, with no significant treatment effects evident after 29 hr. Thus, although this species of submersed macrophyte was significantly inhibited by atrazine concentrations between 10 and 50 μg/L, recovery occurred within one hour after atrazine was removed.

In Chesapeake Bay, potential exposure of submersed plants to concentrations of atrazine greater than 10 μg/L generally persist in the water column for less than 1 day (Wu *et al.* 1980; Glotfelty *et al.* 1984; Kemp *et al.* 1984), so that the total reduction of *P. perfoliatus* photosynthesis under such conditions would be minimal and reversible. Although more information is necessary to evaluate rhizosphere effects, the initial findings show that roots and rhizomes have lowered potentials for uptake, again suggesting very high sediment concentrations of atrazine would be necessary for inhibition of macrophyte productivities. Furthermore we question to what extent the translocation of atrazine to chloroplasts can occur if substantial amounts of this herbicide does enter the roots.

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