INVESTIGATIONS OF THE EFFECTS OF TWO TRIAZINE HERBICIDES ON PERIPHYTIC ALGAL COMMUNITIES IN THE DELTA MARSH, MANITOBA

by

Sharon Elizabeth Gurney

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INTRODUCTION

Triazine herbicides are used in aquatic management primarily to control macrophytes and undesirable phytoplankton populations. Triazines used for terrestrial weed control also enter aquatic systems through runoff, aerial drift or from spillage. This study assesses the impact of two of these triazines, simazine and terbutryn, on the structure, function and biomass of periphytic algae within _in-situ_ littoral enclosures. Secondary effects of treatment on water chemistry were also monitored during an 87 day experimental period.

The second half of this study describes an automated quantitative grain density microautoradiography procedure. The effects of Lugol's iodine on isotope leakage from cells preserved for autoradiography, was demonstrated using three haptobenthic diatom species. The applicabilty of using this technique to evaluate intra and interspecific variation in algal response to triazine exposure _in-situ_ was also discussed.

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LITERATURE REVIEW

1.1 TRIAZINES: MODE OF ACTION

Although atrazine is the most commonly used triazine herbicide, a number of other triazines have been used for aquatic and terrestrial weed control (Fedtke, 1982). All s-triazines consist of a central 6 membered triazine ring (alternating nitrogen and carbon) with alkylamino side chains originating from the 4 and 6 position of the ring. Triazines are further defined by differences in the alkylamino side chains and the substituted constituent on the remaining ring carbon at position 2. The chloro-triazines, simazine, cyanazine, procyazine, trietazine and atrazine, include a chlorine atom at this position. A substituted methylthio group in terbutryn, prometryn, cyanatryn, simetryn and ametryn, classifies these compounds as methylthio-triazines. The third class of s-triazines is referred to as methoxy-triazines. Prometon, atraton and simeton all contain substituted methoxy groups on the triazine ring. The physical and chemical properties of these herbicides are further described in WSSA (1983).

The primary mode of action of all triazines is inhibition of photosysnthesis. Triazines are one of several classes of herbicides which are considered electron transport inhibitors (Moreland, 1980). These herbicides bind noncovalently to a special high-affinity site on PS II, inhibit electron transfer from Q- to B, and effectively prevent electron transfer through PS II (Shipman, 1982). The binding site is located on the thylakoid surface and is proteinaceous in nature (Fedtke, 1982). The binding is believed to lead to a change in the redox potential or an equilibrium shift in the plastoquinone B (Moreland, 1980) which prevents its participation in electron transport. The relationship between triazine structure and level of inhibition has been investigated by Radosevich _et al_. (1979). This research suggests that the inhibition is favored by asymmetric alkylamino substitution at the 4 and 6 position of the triazine ring, and the orientation of the triazine molecule at the active site may be controlled by these alkylamino groups. Of the three groups of s-triazines, Radosevich _et al_. (1979) has found methylthio-s-triazines to be the strongest Hill reaction inhibitors. Thylakoid suspensions were least affected by the methoxy-s-triazines, and chloro-s-triazines were intermediate in effect. This finding was substantiated by Goldsborough and Robinson (1983) as the methylthio-triazine terbutryn was found to be 55x more toxic to periphyton than the chloro-triazine simazine, at a concentration of 0.1 $mg \cdot L^{-1}$. Competitive binding studies have suggested that many of the s-triazines, triazinones, pyridazinones, biscarbamates, phenylureas, N-phenylcarbamates, uracils, acylanilides, cyclic ureas and thiadiazoles interfere at the same electron carrier in the electron transport chain through a similar mechanism (Tischer and Strotmann, 1977; Pallett and Dodge, 1979; Moreland, 1980; Van Assche and Carles, 1982). Pfister _et al_. (1981) and Gardner (1981) identify a 32 kD protein present in PS II which is associated with herbicide binding, however Gressel (1982) suggests that this 32 kD protein may only be adjacent to the thylakoid binding site. Shipman (1982) states that each herbicide molecule must have a flat polar component with hydrophobic substituents to be active. These hydrophobic components serve to partition the molecule into lipid regions of the cell and to fit the hydrophobic region of the binding site. The flat polar region of the herbicide is used for electostatic binding to the polar region of the herbicide binding site.

Although the primary influence of triazine toxicity is the inhibition of electron transport, evidence of secondary effects, have been documented (Fedtke, 1982).

1.2 TRIAZINE RESISTANCE

Triazine resistance and tolerance in higher plants have been well documented. The chloro-s-triazines can be degraded in higher plants by three major metabolic pathways:

hydrolysis at the 2 position of the triazine ring; N-dealkylation of the side chains; or conjugation with glutathione (Jensen, 1982). Corn which is extremely tolerant to atrazine and simazine, utilizes all three degradation pathways. Sorghum, however which is also tolerant to atrazine, primarily utilizes conjugation and to a lesser extent N-dealkylation in detoxification. Sorghum is not able to hydrolyze atrazine since it is deficient in benzoxazinone, the cyclic hydroxamic acid that catalyzes the hyrdolysis of chloro-s-triazines nonenzymatically (Jensen, 1982). Plants which are extremely or moderately suceptible to atrazine (eg. oats, wheat and peas) are devoid of the enzyme glutathione-s-transferase, which catalyzes the conjugation of chloro-s-triazines with glutathione. Less is known about the metabolism of methoxy or methylthio-s-triazines. Plants can degrade terbutryn and prometryn by oxidation of the methylthio group to hydroxy-metabolites and by dealkylation of the side chains, however degradation is slow compared to atrazine in corn (WSSA, 1983).

Many of the triazine resistant weeds do not have the enhanced rate of herbicide degradation such as that found in corn. Instead, these species are resistant because the triazines do not bind to the thylakoids. Triazine tolerance and resistance have evolved differently even within the same species. Gressel (1986) cites an example where one population of Senecio vulgaris slowly increased tolerance to sublethal triazine doses, while another population suddenly evolved plastid resistance to high levels of atrazine. Biotypes which have evolved triazine resistance via a chloroplast membrane alteration, contain a thylakoid component that has a subtle alteration responsible for reduced triazine binding affinity (Arntzen et al., 1982). Nucleotide sequence analysis done by Hirschberg and McIntosh (1983), has demonstrated that the 32 kD protein in a triazine resistant biotype of Amaranthus hybridus differs from the protein of a herbicide susceptible biotype by only one amino acid. A number of studies have focused on the characterization of the chloroplast membranes between triazine-resistant and triazine-susceptible biotypes (Pfister et al., 1979; Holt et al., 1981; Pillai and St. John, 1981; Burke et al., 1982; Ducruet and DePrado, 1982; Oettmeier et al., 1982; Gasquez and Darmency, 1983; Ort et al., 1983; Turcsányi and Faludi-Dániel, 1983; Galloway and Mets, 1984). Pfister and Arntzen (1979) have found that the PS II complex of chloroplasts from several different triazine-resistant weed biotypes share common traits. These include an alteration in the thylakoid membrane which results in very strong resistance to all symmetrical triazines and asymmetrical triazinones, but only slight resistance to ureas or amides. Chloroplasts of both triazine-susceptible and triazine-resistant biotypes of *Amaranthus hybridus* synthesize a 34 kD herbicide binding protein, but the resistant biotypes do not bind the herbicide (Steinback *et al*., 1981). Mattoo *et al*. (1984) found that atrazine adaptation in *Spirodela oligorrhiza* coincided with high proportions of thylakoid polyunsaturated fatty acid constituents which increase membrane fluidity. They suggest that alterations in the lipid environment may mediate conformational, orientational or functional changes of the 32 kD binding protein which could hinder herbicide binding.

A few studies have reported triazine resistance in certain algal species. Using visual observations, Vance and Smith (1969) report simazine to be non-toxic to *Scenedesmus quadricauda* and *Chlamydomonas eugametos*. Four *Chlamydomonas reinhardii* mutants were found to have reduced herbicide binding to thylakoid membranes (Galloway and Mets, 1984). A reduced inhibition of carbon assimilation by phytoplankton from atrazine treated ponds to further atrazine additions, has been considered to be an indication of induced resistance (deNoyelles *et al*., 1982). Goldsborough and Robinson (1987) also found evidence of triazine resistance in periphyton exposed to simazine concentrations over 0.8 $mg \cdot L^{-1}$

1.3 TRIAZINE USE IN AQUATIC MANAGEMENT

Triazines have been used in a number of countries for the management of aquatic macrophytes and algae. Simazine has been used to control phytoplankton (Tucker and Boyd, 1978a), filamentous algae and Chara sp. (Tucker and Boyd, 1978b; Tucker et al., 1983) in catfish ponds in the southern United States, and in the control of undesirable blue-green algal populations in Moriane Lake, New York (Harman, 1978). Simazine, atrazine, propazine and prometone have been used to control aquatic vegetation in fish habitats in Missouri (Walker, 1964). In this study, control of filamentous algae and Chara sp. required a higher simazine concentrations than was required to control other aquatic vegetation. Mauck et al. (1976) and Crawford (1981) both report simazine as being an effective herbicide in the management of aquatic vegetation in ponds. Simazine and terbutryn have been used to control vegetation growing in irrigation canals in California, Colorado and Washington (Anderson et al., 1978; Bowmer et al., 1979). Preliminary investigations into the effectiveness of cyanatryn in controlling aquatic macrophytes and algae in small ponds, suggest that excellent control can be obtained with herbicide concentrations ranging from 50-150 $\mu g \cdot L^{-1}$ (MacKenzie et al., 1985). Murphy et al. (1981) has evaluated the use of terbutryn and cyanatryn for aquatic weed control in navigable canals in Britain UK, and terbutryn has been applied to Lake

Peterborough, Britain UK, in an attempt to control filamentous algal growth (Robson _et al_., 1976). The effects of cyanatryn, on the aquatic vegetation in a drainage channel, has been evaluated by Scorgie (1980). Terbutryn has been applied to Ontario farm ponds and lakes to assess its effectiveness in controlling filamentous algae, submerged macrophytes and emergent aquatics (Mackenzie _et al_., 1983). During this study, best control occurred under static water conditions, and filamentous algae were found to be most sensitive to terbutryn treatment.

1.4 TRIAZINE CONTAMINATION IN NON-TARGET AQUATIC SYSTEMS

Although some of the triazine herbicides have been used in aquatic management, many used for terrestrial weed control incidentally contaminate aquatic systems through aerial drift or runoff. Hall (1974) found that with an atrazine application rate of 2.2 $kg \cdot ha^{-1}$, a level of 2.3 $ug \cdot L^{-1}$ could be detected in runoff water. With a wide range of atrazine application rates (0.6-9.0 $kg \cdot ha^{-1}$) an average of 2.4% of the herbicide was lost in runoff water from corn fields (Hall _et al_., 1972). The quantity and the rate of herbicide loss from agricultural fields may depend on the amount of rainfall, and the cultivation techniques employed. Triplett _et al_. (1978) found that less runoff, containing simazine and atrazine occurred from corn fields planted to no-tillage than to conventionally tilled soil. In that study the greatest quantity of atrazine and simazine transported in runoff was 64 $g \cdot ha^{-1}$ (5.7% of total applied) and 123 $g \cdot ha^{-1}$ (5.4%), respectively. Ridge planting of corn was also found to greatly reduce atrazine losses when compared to surface-contoured planting (Ritter et al., 1974). A storm occurring seven days after atrazine application resulted in approximately 15% loss from surface contoured watersheds, with concentrations of atrazine in the surface water ranging from 1.17-4.91 $mg \cdot L^{-1}$. Quantities of atrazine and N-deethylated atrazine were monitored in five rivers that drained agricultural areas in Quebec (Muir et al., 1978). Atrazine and N-deethylated atrazine residues ranged in concentration from 0.01-26.9 $\mu g \cdot L^{-1}$ and <0.01-1.34 $\mu g \cdot L^{-1}$, respectively, between April and December of 1974 and 1975. The highest levels were observed in July which coincided with herbicide spraying and occasional heavy rainfall. The total discharge of atrazine into these watersheds was found to range from 0.1-2.9% of that applied. A similar study was conducted for 11 agricultural watersheds in southern Ontario (Frank and Sirons, 1979). In this study atrazine and desethylatrazine were detected in 80% of the stream waters at a mean concentration of 1.4 $\mu g \cdot L^{-1}$. Losses of atrazine were found to be greatest on clay and least on sandy soils. Of the total amount lost, 60% was caused by storm runoff, spills accounted for 20% and base flow associated with drainage accounted for the remaining 20%. The quantity of atrazine and simazine that entered the Wye River was reported to depend upon the quantity applied in the watershed and the timing of runoff with respect to application rates (Glotfelty _et al_., 1984). The maximum concentration of atrazine measured in the Wye River estuary was near 15 $\mu g \cdot L^{-1}$, with an average concentration at peak loading of <3 $\mu g \cdot L^{-1}$. Anderson _et al_. (1978) have determined the amount of simazine present in irrigation water after ditchbank treatment for weed control. At application rates of 2.25-7.43 $kg \cdot ha^{-1}$, simazine levels in flowing canal water immediately after herbicide application did not exceed 60 $\mu g \cdot L^{-1}$. In first-flow samples collected 4 to 6 months after application from sites that were dewatered at application, simazine levels peaked at about 250 $\mu g \cdot L^{-1}$ within the treated section but decreased rapidly to <5 $\mu g \cdot L^{-1}$. The heavy use of atrazine in the South Platte River Valley, Colorado initiated a study which monitored groundwater at gradient points above, directly beneath, and below an atrazine treated site (Wilson _et al_., 1987). The maximum concentration recorded in a groundwater well during this study was 2.3 $\mu g \cdot L^{-1}$.

1.5 TRIAZINE PERSISTENCE

The persistence of triazine herbicides within water and sediment has been reported to vary considerably. The rate of herbicide disappearance from a water body is likely related to the flushing rate of the system, the amount of mixing and also rates of biotic and abiotic degradation. In general however, triazine persistence appears to be greater in sediments than in the overlying water. Muir *et al.* (1981) reported a half-life of terbutryn in water ranging from 21 days in a pond that contained heavy growths of *Typha* and *Lemna*, to 30 days in a pond that was free of aquatic macrophytes. With an application rate of 100 $\mu g \cdot L^{-1}$, these two ponds had terbutryn residues in the sediment as high as 1.4 $\mu g \cdot g^{-1}$ (dry wt) and 0.5 $\mu g \cdot g^{-1}$. After 61 weeks, 51% of terbutryn added to the the unvegetated pond, and 35% of that added to the vegetated pond could still be accounted for. MacKenzie *et al.* (1983) have also found considerable variation in terbutryn persistence in both water and sediment from treated ponds and lakes. In a laboratory study, Muir and Yarechewski (1982) found that the rate of terbutryn degradation was dependent on the redox potential. Under anaerobic conditions degradation rates were considerably slower. In another study monitoring the persistence of herbicides in irrigation ditches, three years following treatment, 50% of the simazine added was still present in the soil of the ditch sides and bottoms (Smith *et al.*, 1975). In this study, atrazine was found to be a less persistent compound, with 30% remaining in the soil after this time period. Atrazine was however more persistent than two non-triazine herbicides, monuron and bromacil, which had only 15% and negligible amounts remaining, respectively. Absorption constants for simazine on high organic sediments were found to be linear over a 0.01-1.0 mg·L^{-1} concentration range (Glotfelty et al., 1984). Mauck et al. (1976) found the concentration of simazine applied to pond water in Missouri was directly related to the concentration ending up in the sediment. These concentrations were shown to decline after application, however residues of 0.16 µg·g^{-1} were measured 456 days after the initial 3.0 mg·L^{-1} application. After strong winds, higher concentrations of simazine were found in the water column, suggesting that the herbicide can quite easily dissociate from the sediment with any physical disturbance. Walker (1978) found simazine to be somewhat more persistent than atrazine, however both herbicides demonstrated considerably shorter half-lives, with increases in temperature and moisture content in the soil. Since triazine herbicides are known to adsorb readily to organic matter (Pillay and Tchan, 1972; Nicholls et al, 1984) the reported variation in persistence may largely be due to differences in the organic content of sediment. The persistence of triazines in water overlying marsh sediments has been shown to increase when sediments are excluded from contact with the overlying water (Goldsborough and Robinson, 1985). Sediment adsorption of pesticides from overlying water has also been documented by Gillott et al. (1975). Murphy (1982) reviews the persistence and degradation of the methylthio-triazines: ametryn, cyanatryn and terbutryn in freshwater systems.

1.6 TRIAZINE TOXICITY TO AQUATIC MACROPHYTES AND ALGAE

While the physiological effects of triazine herbicides on terrestrial plants have been well documented, comparatively less research has been conducted on aquatic macrophytes and algae. The absorption and translocation of simazine in *Myriophyllum brasiliense* has been studied by Sutton and Bingham (1969). Root applications greater than 1.0×10^{-7}M inhibited growth and simazine was found to accumulate in the top of the shoot. In a similar study, simazine concentrations of 0.12 -1.0 $mg \cdot L^{-1}$ in cultures of *Lemna minor*, *Elodea canadensis* and *Myriophyllum brasiliense* inhibited oxygen evolution within 24 hr (Sutton *et al*., 1969). Murphy *et al*. (1981) found suppression in the growth of aquatic macrophytes for 3-12 months subsequent to terbutryn treatment, followed by blooms of *Lemna*. A cyanatryn concentration of 0.12 $\mu g \cdot g^{-1}$ was found adequate to eliminate the dominant macrophyte (*Myriophyllum spicatum*) from a drainage channel (Scorgie, 1980) and cyanatryn levels between 50-150 $\mu g \cdot L^{-1}$ were found effective in controlling growth of vascular macrophytes in shallow ponds in Ontario (MacKenzie *et al*., 1985). Dabydeen and Leavitt (1981) conducted a study to evaluate the effects of a 3.0 $mg \cdot L^{-1}$ concentration of atrazine and simazine on cultures of *Elodea* canadensis. Toxic systems were found to develop faster in the simazine treatment, however absorption of atrazine was actually faster. Although simazine is more toxic to Elodea canadensis, both herbicides caused migration of the chloroplasts to the center of the cell. This was followed by loss of pigment and death.

Laboratory investigations of triazine toxicity in algae have focused mainly on short-term primary effects of triazine inhibition of individual species. Prometryn concentrations between 0.1 and 10 µM were used to evaluate effects on growth, photosynthesis and respiration in four species of algae (Hawxby et al., 1977). Levels of inhibition were found to vary, with Lyngbya being most susceptible. Tubea et al. (1981) report that prometryn concentrations of 10 uM drastically reduced growth of Chlorella pyrenoidosa and Lyngbya birgei. Plumley and Davis (1980) report that atrazine concentrations of 2.2 $mg \cdot L^{-1}$ will reduce photosynthetic rate, chlorophyll content, and cell numbers in unialgal cultures of Nitzschia sigma and Thalassiosira fluviatilis isolated from a salt marsh. The EC_{50} values of atrazine and its degradation products were derived for two species of green algae and three species of blue-green algae (Stratton, 1984). Deethylated atrazine was found to be the most toxic breakdown product. Evidence of synergistic, antagonistic, and additive interaction responses were also documented in this study. Synergistic inhibition of chlorophyll production by *Chlorella vulgaris* has been reported for specific concentrations of atrazine and simazine, although other concentrations were found to have stimulatory effects (Torres and O'Flaherty, 1976). Vance and Smith (1969) also report varying degrees of simazine toxicity to three algal species. A study of the effects of simazine on photosynthesis and growth of filamentous algae found that algicidal effects are reduced under low light conditions (O'Neal and Lembi, 1983). Verber *et al*. (1981) found atrazine concentrations of 0.25-5.0 $mg \cdot L^{-1}$ to inhibit growth of *Chlorella vulgaris*. Concentrations of 0.25-12.0 $mg \cdot L^{-1}$ prometryn were evaluated for influence on cell numbers of *Chlamydomonas segnis*, heterocyst frequency in *Anabaena*. and generation time of *Klebsiella pneumoniae* strain M5A1 and *Rhizobium japonicum* strain 61A76 (Weinberger *et al*., 1985). A study on the effects of atrazine on salt marsh edaphic algae indicated that a 2.2 $mg \cdot L^{-1}$ concentration will significantly reduce the rate of photosynthesis, chlorophyll content, and cell numbers in cultures treated in the lab (Plumley and Davis, 1980). In their field investigations, a higher level of atrazine was required to produce equivalent inhibition. Robson *et al*. (1976) have evaluated the effects of various concentrations of terbutryn and cyanatryn on nuisance filamentous algal species. Simazine has been found to induce ultrastructural changes in the thylakoids of Anacystis nidulans (Mehta and Hawxby, 1979). Kruglov and Mikhailova (1975) have shown that algae can actively absorb and accumulate simazine in an amount exceeding its concentration in the surrounding medium by 100-fold. In another laboratory experiment, Verber et al. (1981) found that Chlorella vulgaris under conditions of atrazine inhibition (2.5 $mg \cdot L^{-1}$), was still capable of removing most of the atrazine present in the medium.

Field experiments with triazine exposure have evaluated physiological and compositional changes in algal communities. Goldsborough and Robinson (1983) used varying concentrations of simazine and terbutryn to report their effect on carbon assimilation rate and chlorophyll a accumulation in marsh periphyton. Effects of these herbicides on carbon assimilation, chlorophyll a, periphyton biovolume and community structure were correlated to increased herbicide concentrations (Goldsborough and Robinson, 1986). Hamilton et al. (1987) have evaluated the impact of atrazine on lake periphyton communities. Consistent with the observations of Goldsborough and Robinson (1986), the community structure shifted from a chlorophyte- to a diatom-dominated community. Herman et al., (1986) have also found the diatom community to be least sensitive to atrazine treatment, and report the major impact on periphyton as a decrease in the Chlorophyta. Density of