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Absorption and Effect of Simazine and Atrazine on *Elodea canadensis*[1]

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Very little work has been done on the effects of herbicides on submerged aquatic plants. This may be a reflection of the belief that these plants do not constitute a serious weed problem. BALDWIN (1963) reported that diquat moves apoplastically in land plants and suggested that extensive movement would not be expected in submerged aquatics which have little, if any, xylem flow. MEHTA & HAWXBY (1979) observed that the thylakoids of *Anacystis nidulans* (Drouet No. 1550), a blue-green algae, "appear empty, distorted, and functionless" after 48 h of treatment with 10^{-5} M simazine. They believed the cells were dead at the time of appearance of these symptoms. DAVIES & SEAMAN (1968) reported that when *Elodea canadensis* (Michx.) with diquat in its tissues was immersed in distilled water only 34% of previously absorbed diquat diffused out into the surrounding water. Diquat was retained in the tissues, even though the plants were dead. They concluded that diquat binds strongly to the organic matter of the plant.

In land plants, ASHTON et al. (1963) reported that atrazine at 10 ppm induced structural changes under light in bean leaf cells. Sixty h after treatment the chloroplasts in the palisade tissue appeared as rounded bodies randomly arranged in the cell. The chloroplasts then became aggregated in the equatorial region of the cell and had a greater affinity for stains. After 90 h the chloroplasts were completely disintegrated. HILL et al. (1968) observed that 5 ppm atrazine caused disintegration of chloroplasts of barnyardgrass [*Echinochloa crusgalli* (L.) Beauv.] after 4 h of treatment in light. Degradation started 2 h after treatment with swelling and derangement of the fret system, swelling of the grana, and subsequent rupturing of the chloroplast envelope. The purpose of this research was to measure the effects of atrazine and simazine on the submerged aquatic *Elodea* and to measure the rate of absorption of both herbicides.

MATERIALS AND METHODS

Experiments were conducted to measure uptake of herbicide and to look for any morphological and structural changes in the leaf cells attributed to the herbicides. Atrazine (98%) and simazine (98%) solutions of 3 ppm were made of technical grade herbicides

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and 50 mL of each were measured into separate beakers. Fifty µL (containing 0.7 µCi) of ^{14}C-simazine (s.a. 7.6 µCi/mg) or 50 µL (containing 0.6 µCi) of ^{14}C-atrazine (s.a. 24.9 µCi/mg) were then added to each beaker. Branches of *Elodea* were dissected into segments 2 cm long and 7 segments were simultaneously placed into a beaker. Immediately, one segment was taken out, thoroughly rinsed in distilled water and placed on filter paper to dry. Similar procedures were done for the other six segments at time intervals of 5, 10, 15, 30, 60 and 120 min. These operations were conducted under a light intensity that approximates the natural requirements for active photosynthesis in *Elodea*. This experiment was repeated three times with three replications per treatment. The segments were then air-dried, weighed and oxidized in a biological oxidizer. ^{14}C content was determined using a liquid scintillation counter. In a similar experiment 5 cm *Elodea* branches were immersed in 5 ppm solutions of both ^{14}C-herbicides for 2 h. Branches were dissected into stems versus leaves both of which were oxidized and counted for ^{14}C as described. Twenty cm *Elodea* branches were immersed in 200 mL of 3 ppm atrazine (containing 0.45 µCi ^{14}C-atrazine) and allowed to equilibrate for 2 h. The branches were then dipped into distilled water and then allowed to desorb atrazine in 200 mL of distilled water for 1 h after which they were transferred to 200 mL fresh distilled water and allowed to desorb another 1 h. Water samples (2 mL) were taken from each rinse at 0.5, 10, 20, 40, and 60 min, placed in a scintillation vial with an aqueous counting solution and desorbed ^{14}C determined.

Actively growing *Elodea* shoots (5 cm long) were placed in four 250 mL Erlenmeyer flasks each containing atrazine solution of 0, 1, 3, and 5 ppm, respectively. The same was done with simazine. The flasks were placed under illumination from a 60 watt bulb with reflector. Microscopic examination of fresh leaves were carried out every 24 h for 72 h by placing leaf pieces in water on a microscope slide illuminated from below. This was possible because *Elodea* leaves are only a few cells thick. Photographs were taken to show any changes that occurred. This experiment was duplicated twice.

RESULTS AND DISCUSSION

The effects of simazine on *Elodea* are detailed in Table 1. No effect was seen from the 1 ppm treatment during the 72 h observation period. The first observable effect on simazine at 3 ppm was a cessation of cyclosis and plasmolysis of the cell. At both the 3 and 5 ppm levels of simazine chloroplasts migrated toward the center of the cells until they were aggregated, chloropyll began to leak into the cytoplasm and the chloroplasts died. Atrazine-treated *Elodea* showed the same symptoms as simazine-treated *Elodea* but symptoms developed at a slower rate. The 48 h atrazine treatment appeared equivalent to the 24 h simazine treatment. These effects are illustrated in Figure 1. The mechanism causing the chloroplast migration and aggregation is unknown, but the loss of pigment from the chloroplast to the cytoplasm is probably the cause of death. It seems doubtful that cell starvation, caused by atrazine blockage of

Table 1. Visible changes in *Elodea canadensis* cells after treatment with simazine.

Time after treatment (h)	ppm	Microscopic Observations
24	1	No visible changes - cells show normal cytoplasmic streaming (cyclosis).
	3	No evidence of cyclosis - cells showing signs of plasmolysis and chloroplasts have initiated a movement towards one area in the middle of the cell.
	5	Chloroplasts aggregate in clumps and start to show signs of disintegration and loss of pigmentation. Chlorophyll is diffusing out around each aggregation.
48	1	No change evident.
	3	Chloroplasts aggregated into clumps which are now yellowish-brown in color.
	5	Clumps have become smaller and are almost brown due to further disintegration of the plastids. At this point it is reasonable to say that the cells are functionally dead.
72	1	No change evident.
	3	Chloroplast clumps have become almost brown and show signs of shrinkage due to progressive destruction. Cells are certainly dead or very near to that stage.
	5	Chloroplasts are almost completely disintegrated only a few "fragments" of clumps remain. Cell walls are the only component remaining prominently visible.

photosynthesis, could cause this progression of symptoms leading to death.

HILL et al. (1968) proposed that a secondary toxic product or free radical formed by the atrazine-light interaction caused the disintegration of barnyardgrass chloroplasts. KLEPPER (1979)

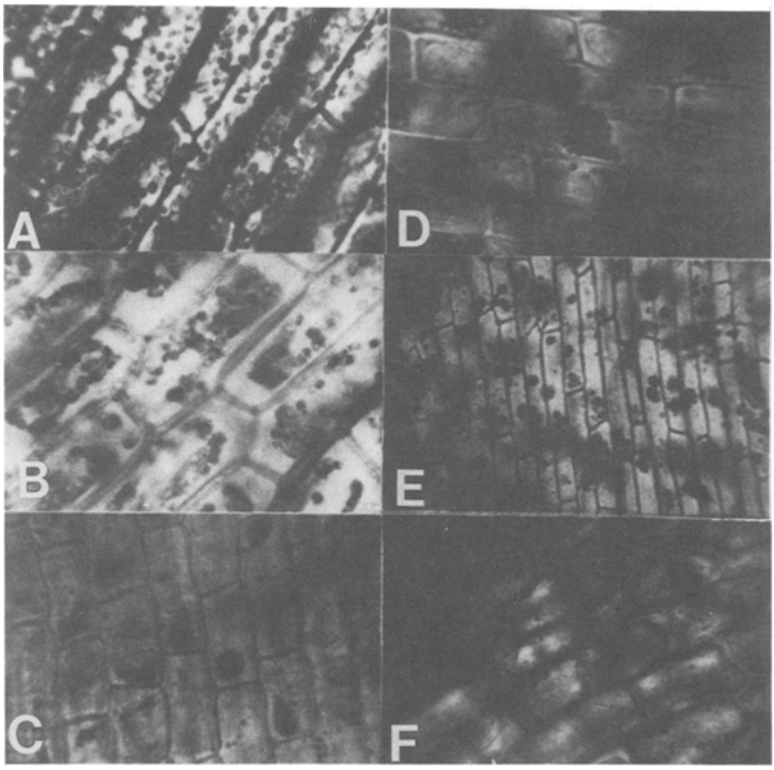

Figure 1.

a. *Elodea canadensis* Michx., untreated control, 24 h after experiment began, magnified 485 x,
b. *Elodea* 24 h after treatment with atrazine 5 ppm - 485 x,
c. *Elodea* 48 h after treatment with atrazine 5 ppm - 485 x,
d. *Elodea* 24 h after treatment with simazine 5 ppm - 485 x,
e. *Elodea* 48 h after treatment with simazine 5 ppm - 215 x,
f. *Elodea* 72 h after treatment with simazine 5 ppm - 485 x.

reported that illumination of excised wheat leaves in the presence of atrazine caused accumulation of toxic nitrite. It is reasonable to conclude that similar mechanisms could be the cause of death in both land plants and submerged aquatics.

Although toxic symptoms developed faster in the simazine treated *Elodea*, atrazine absorption by *Elodea* was actually faster than simazine absorption. After 120 min the *Elodea* had absorbed 33 ng of atrazine/mg dry wt but only 22 ng/mg of simazine (Table 2). This would indicate that simazine is inherently more toxic to *Elodea* than atrazine. It was found that 22% of the ^{14}C from atrazine and simazine was absorbed by the *Elodea* stems in 120 min

Table 2. Absorption of atrazine and simazine by *Elodea*.

Time after treatment (min)	ng herbicide absorbed/mg dry wt	
	Atrazine	Simazine
0	3*a	4 a
5	19 b	14 b
10	22 cde	15 b
20	24 de	17 bc
40	28 ef	19 bcd
60	36 fg	22 cd
120	33 f	22 cde

*Numbers followed by the same letter or letters are not significantly different at the 5% level using Least Significant Difference Test following prior significant F test.

with the remaining 78% absorbed by the leaves. When transferred from 3 ppm atrazine solution to two 1 h distilled water rinses 91% of the atrazine desorbed from the *Elodea*. Fifty-two % of the atrazine desorbed within the first 5 min. This indicates that atrazine was passively absorbed by the *Elodea*.

In summary, simazine is absorbed slower but is more toxic to *Elodea* than is atrazine. The most striking effect of both herbicides on *Elodea* is the migration of the chloroplasts to the center of the cell, their aggregation, loss of pigment and death.

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