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GROWTH RESPONSE OF *MYRIOPHYLLUM SPICATUM* AND *HYDRILLA VERTICILLATA* WHEN EXPOSED TO CONTINUOUS, LOW CONCENTRATIONS OF FLURIDONE

by

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The objective of this study was to determine the minimum sustained (threshold) concentrations of fluridone required to control the growth of Eurasian watermilfoil (*Myriophyllum spicatum* L.) and hydrilla (*Hydrilla verticillata* Royle). A diluter system was used to deliver five different concentrations of fluridone to five sets of four test aquaria. Another set of four reference aquaria received only filtered well water. Each aquarium

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contained meristematic cuttings of *M. spicatum* and *H. verticillata* planted in beakers containing either a natural, fine-textured, organic substrate or a mixed sand-peat substrate (70:30 volume). Plant injury was assessed after 12 weeks of continuous exposure to the various fluridone concentrations. Results of this study suggest that the threshold fluridone concentration required to control *M. spicatum* growing on both substrates was estimated to be between 10 and 20 µg/ℓ. When root and shoot biomass data and percent injury ratings were considered for *H. verticillata*, the fluridone threshold concentration necessary to provide greater than 50-percent control was estimated to be 20 µg/ℓ. However, 100-percent control of *H. verticillata* was not achieved, based on percent injury and chlorophyll results.

PREFACE

This study was conducted as part of the U. S. Army Corps of Engineers Aquatic Plant Control Research Program (APCRP). Funds for the effort were provided by the Office, Chief of Engineers (OCE), under Department of the Army Appropriation No. 96X3122, Construction General, 902740, through the APCRP at the U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. Mr. Dwight Quarles was OCE Technical Monitor.

The work was initiated in November 1981 under the direct supervision of Mr. Donald L. Robey, Ecosystem Research and Simulation Division (ERSD), Environmental Laboratory (EL). Dr. John Harrison was Chief, EL, and Mr. J. Lewis Decell was Program Manager, APCRP. The principal investigators for this work were Mr. Jerry F. Hall and Dr. Howard E. Westerdahl, Aquatic Processes and Effects Group (APEG), ERSD. Dr. Troy J. Stewart, APEG, assisted in the conduct of the study. The Tennessee Valley Authority, Laboratory Branch, in Chattanooga, Tenn., conducted analysis of fluridone residues in water samples.

Commander and Director of the WES during the study was COL Tilford C. Creel, CE. Technical Director was Mr. F. R. Brown.

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CONTENTS

GROWTH RESPONSE OF *MYRIOPHYLLUM SPICATUM* AND *HYDRILLA VERTICILLATA* WHEN EXPOSED TO CONTINUOUS, LOW CONCENTRATIONS OF FLURIDONE

PART I: INTRODUCTION

Background

1. Fluridone, 1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone, has been shown to be an effective terrestrial and aquatic herbicide (Webster et al. 1977, McCowen et al. 1979, Sanders and Theriot 1979, and West and Parka 1981). For terrestrial plant control, the mode of action of fluridone was suggested by Berard, Rainey, and Lin et al. (1978) to be through disruption of the development and/or stability of newly formed cell pigments. Moreover, Kunert and Böger (1979) showed that fluridone affected *Scenedesmus accutus*, an alga, through inhibition of carotenoid and chlorophyll formation. However, Anderson (1981) concluded that previous studies with fluridone have shown only that the primary mode of action of fluridone is uncertain at present. He suggested that only the synthesis of specific light-induced RNA's is blocked, indicating that fluridone remains active in the plant for only a short time before it is inactivated.

2. Under field conditions, Anderson (1981) suggested that fluridone at 1.0 mg/ℓ will control American pondweed (*Potamogeton nodosus*) and Sago pondweed (*Potamogeton pectinatus*) during sprouting only when sufficient light and a 4- to 6-day contact time is available. Results of other fluridone efficacy and field dissipation studies in lakes and ponds, treated to provide 0.02, 0.03, and 0.3 mg/ℓ fluridone concentrations relative to the total water column, showed that the half-life of fluridone in water averaged 5 days (West, Day, and Burger 1979). One study also showed that fluridone in small quantities (6-14 µg/ℓ) was detected in untreated areas of Gatun Lake, Panama, within 24 hr following treatment, suggesting that the herbicide dispersed out of the treated area (Sanders and Theriot 1979). Fluridone efficacy in the treated and untreated areas suggests that a much lower fluridone concentration in

the water could be effective at controlling aquatic macrophytes. In another study (Marquis, Comes, and Yang 1981) submersed Sago pondweed and Richardson pondweed (*Potamogeton Richardsonii* Rydb.) developed typical fluridone injury symptoms (retarded growth, albescent young leaves, and leaf necrosis) whether they were growing in treated water or emerging from treated sediment. Moreover, less than 1 percent of the ^{14}C fluridone applied (100 µg/ℓ) was absorbed by the roots and shoots over a 14-day period. Though less than 5 percent of the sediment-applied fluridone was translocated to the lower stem and shoots, plant injury was observed. This finding further suggests that a very low fluridone concentration (<0.1 mg/ℓ) may be required to control these submersed aquatic plants. Other studies have reported similar results (Rivera, West, and Perez 1979; Muir et al. 1980; and West and Parka 1981).

3. Based on results of previous studies, it was apparent that fluridone dissipates very rapidly in water through absorption to plants and sediment and dispersion out of the treated area. Moreover, the fluridone concentration required to control a wide variety of submersed aquatic plants appears to be less than 0.1 mg/ℓ. Field representatives for Elanco, Inc., have observed that the effects of treating a specific area of a lake resulted in control of the target aquatic plant in an area from two to five times the actual area treated. Barko and Smart (1983) have reported inhibition of growth in certain submerged species when organic matter was added to sediment substrates.

Purpose

4. The aforementioned investigations prompted the Corps of Engineers' Aquatic Plant Control Research Program (APCRP) to initiate a study to determine the lowest sustained, aqueous fluridone concentration required to control the growth of *Hydrilla verticillata* Royle (hydrilla) and *Myriophyllum spicatum* L. (Eurasian watermilfoil) on sand-peat and natural sediment substrates under laboratory conditions over a 12-week study period. Results of these tests will be used in considering

fluridone for ongoing controlled-release research within the APCRP to develop a formulation that releases fluridone constantly over a defined 6- to 8-week posttreatment period, thereby providing a continuous and constant low-level herbicide exposure to plants within a defined treatment area.

PART II: MATERIALS AND METHODS

Experimental Design

5. The diluter system used for this study delivered different constant-rate concentrations of fluridone to 24 aquaria. A detailed description of this system was provided elsewhere (Westerdahl and Hall 1983). The fluridone concentrations selected were 10, 20, 40, 70, and 90 µg/ℓ. A simple randomized experimental design was used to assign a fluridone concentration to each of four replicate aquaria. The test aquaria were wrapped in black plastic to provide more uniform lighting. Excessive algal growth on the sides of the aquaria was prevented by allowing only light from the overhead light bank to enter the aquaria. Supplemental lighting was provided by a light bank suspended 1.3 m above the aquaria (Westerdahl and Hall 1983). The mean photosynthetically active radiation (PAR) received by the aquaria (Figure 1) was approximately 1600 $\mu E \cdot m^{-2} \cdot sec^{-1}$ which corresponds to 75 percent of solar noon sunlight received at this latitude.

6. The water used for operating the diluter system was uncontaminated tapwater originating from a deep well water supply used by Vicksburg, Miss. This water was passed through activated charcoal and a 0.45-µ cartridge filter. Water temperature was maintained at 25 $\pm$ 2°C throughout the study.

7. Mature plants of watermilfoil and hydrilla were obtained from Lake Seminole near Chattahoochee, Fla. A stock culture of each species was maintained in reconstituted hard water (U. S. Environmental Protection Agency 1975) using a standard substrate containing by volume 70-percent washed sand and 30-percent Michigan peat as a planting medium. Approximately 4 weeks prior to testing, four 15-cm meristematic cuttings of watermilfoil were planted in each 250-ml glass beaker by burying the cut end of the plants approximately 5 cm in the hydrosoil. Meristematic cuttings of watermilfoil were also planted in 250-ml beakers containing a natural substrate obtained from the sublittoral region of Lake Washington in Seattle, Wash. This substrate was predominantly

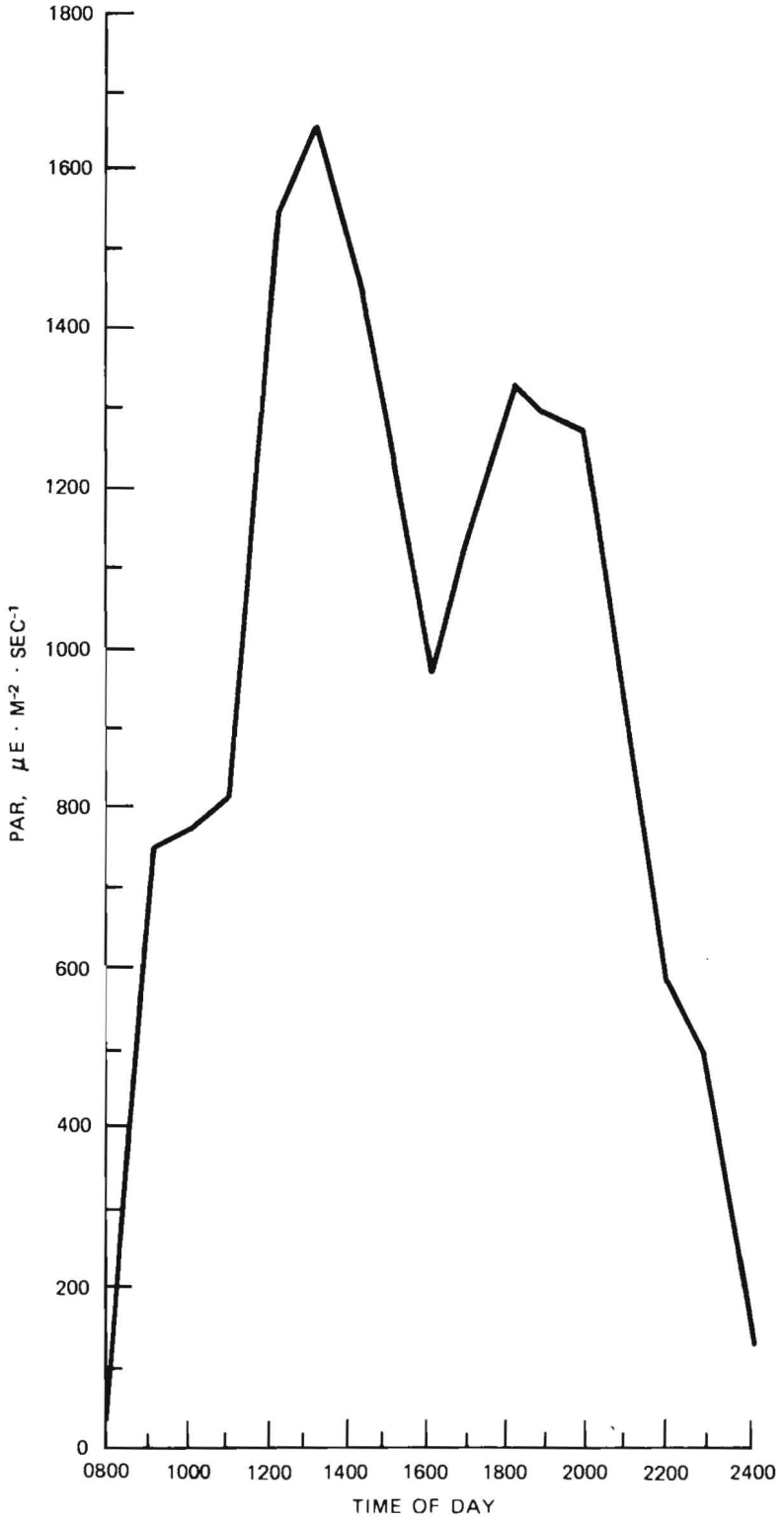

Figure 1. Photosynthetically active radiation received by aquaria. (Note: aquaria were located in a greenhouse covered with 55-percent shade fabric)

fine textured, containing approximately 20-percent sand, 75-percent silt, and 5-percent clay. Nutrient composition of the natural substrate indicated optimal conditions for plant growth (Barko and Smart 1981). A finely sieved, washed sand was placed over the sand-peat and natural substrates to an approximate depth of 2 cm to prevent the substrate from mixing with overlying water during handling.

8. The previously described procedure was also used with meristematic cuttings of hydrilla. The sand-peat and natural substrates were used to determine the effect of substrate type on plant response to a given herbicide concentration.

9. Three beakers each of natural and sand-peat substrate, containing watermilfoil, were placed on the left side of the aquaria. Three beakers each of natural and sand-peat substrate, containing hydrilla, were placed on the right side of the aquaria. During the 4 weeks prior to testing, only water flowed through the aquaria to permit root development of the plants prior to fluridone exposure.

Laboratory Analysis

10. Water samples for fluridone analysis were obtained 3, 7, 14, 21, 28, 35, 43, 51, 59, 71, and 84 days following initiation of herbicide flow through the aquaria. A composite 800-ml sample from each of the 24 aquaria was collected over a 4-hr period, i.e. eight cycles of the diluter system, to provide a representative sample of inflow herbicide concentrations (Hall et al. 1982). During each cycle, a 100-ml sample was collected from each inflow tube.

11. At the end of the 12 weeks, the aquaria were dismantled and the remaining plants were removed from each beaker by washing the substrate with deionized water. Shoots and leaves of watermilfoil and hydrilla were subsampled from each beaker, representing each herbicide concentration and substrate type. Plant tissues were extracted to clarity in vials containing 20 ml of dimethylsulphoxide (DMSO) in accordance with the procedures of Hiscox and Israelstam (1980). Optical densities of chlorophyll in extracts were determined at 645 and 663 nm within 10 hr of completed extraction. All chlorophyll determinations

were made on a Beckman Model 26 dual beam spectrophotometer with an adjusted bandwidth of 0.8 nm. Chlorophyll a and *b* concentrations were calculated using the equations of Arnon (1949). The remaining plants within a beaker were separated into roots and shoots, dried at 70°C for 36 hr, weighed, and recorded (accuracy: ±0.5 mg).

12. The effects of various fluridone concentrations on the growth of watermilfoil and hydrilla were determined using percent plant injury (0 = no control, 100 = total kill) and a set of qualitative factors currently in use at the Aquatic Plant Management Laboratory in Fort Lauderdale, Fla. (Hoeppel and Westerdahl 1981). The qualitative factors included: heavy algal cover; roots evident; absence of meristems on stems and branches; leaf loss; evidence of solarization; stem flaccidity; degree of node or internode decomposition; stem and branch tip decomposition; general decomposition of plants; advanced decomposition (only a few stems remaining intact); complete disintegration of plant material; and subsequent regrowth. The mean shoot and root biomass in natural and sand-peat substrates, expressed as dry weight and representing the four replicate aquaria, were computed for each fluridone concentration and compared to the reference aquaria. Moreover, the mean concentration of total chlorophyll, chlorophyll a, and chlorophyll *b* from plants grown on each substrate, expressed as milligrams of chlorophyll per gram of fresh tissue, were computed for each fluridone concentration and compared to the reference aquaria.

Data Analysis

13. Dunnett's test (Dunnett 1955) was used to compare all experimental root and shoot biomass means and the total chlorophyll, chlorophyll a, and chlorophyll *b* concentration means of plants grown in natural and sand-peat substrates with the reference means. Duncan's test (Duncan 1955) was used to make comparisons among root and shoot biomass means and total chlorophyll, chlorophyll a, and cholorophyll *b* means at each of the fluridone concentrations. All statements of significance refer to the 10-percent level of statistical confidence.

PART III: RESULTS AND DISCUSSION

Fluridone Residues in Water

14. Periodic fluridone analysis of the inflow water to each aquarium permitted evaluation of the fluridone concentration passing into the aquaria. The mean herbicide concentration flowing into each set of four test aquaria was determined for the 12-week study period. The maximum and minimum values and the standard error were calculated for each mean herbicide concentration:

Mean Concentration µg/ℓ	Standard Error of Mean	Minimum Value, µg/ℓ	Maximum Value, µg/ℓ
10	1	0	10
20	2	10	30
40	3	20	60
70	3	50	80
90	5	60	120

Plant Growth Response

15. To evaluate the effect of substrate type on plant growth, a t-test was run comparing root and shoot biomass of watermilfoil and hydrilla grown on a natural substrate, which was conducive to plant growth, with that of a sand-peat substrate in the reference aquaria only. Significant differences were seen between plant biomass grown on the natural substrate and that on sand-peat in both species. The natural substrate supported three times as much hydrilla root and shoot biomass as did the sand-peat substrate. Likewise, the natural substrate supported more than three times as much watermilfoil shoot biomass and twice as much root biomass as did the sand-peat substrate. A t-test was also run comparing total chlorophyll, chlorophyll *a*, and chlorophyll *b* content of hydrilla grown on sand-peat versus that grown on natural substrate. With 14 degrees of freedom, t-values of 1.30, 1.29, and 1.30 were calculated for total chlorophyll, chlorophyll *a*, and chlorophyll *b*,

respectively. The differences in chlorophyll content of natural substrate versus sand-peat were not statistically significant. However, this may be due to the small sample size. The values obtained for total chlorophyll, chlorophyll a, and chlorophyll *b* content of watermilfoil grown on sand-peat and natural substrates were approximately equal, indicating that substrate type had little effect on this species. However, the variation between values obtained for chlorophyll content of hydrilla grown on sand-peat versus natural substrate even though not significant may indicate that hydrilla is more susceptible to the effects of substrate type than is watermilfoil. Barko and Smart (1983) observed greater inhibition of growth in hydrilla than in watermilfoil using various organic amendments.

16. The decreased root and shoot biomass values obtained when both species were grown on the sand-peat substrate may indicate a state of physiological stress induced by nutrient deficiency. The chemically inactive properties of sand make its nutrient-supplying ability essentially nil (Brady 1974). Also, it has been reported that additions of organic matter to substrates resulted in inhibition of growth of submerged plants (Barko and Smart 1983). The inherent chemical inactivity of sand combined with the possible inhibition of plant growth due to the addition of organic matter (peat) could result in physiological stress of plants on the sand-peat substrate due to one or more nutrient deficiencies.

17. Table 1 illustrates the effects of various fluridone concentrations on root and shoot biomass of watermilfoil growing on natural and sand-peat substrates following a 12-week continuous exposure to fluridone. Results of both Dunnett's and Duncan's tests showed that the biomass of roots grown in natural organic substrate at all herbicide concentrations was significantly less than that of the reference. Reductions in root biomass ranged from 53 percent at 10 µg/ℓ to 79 percent at 20 µg/ℓ fluridone. Also, mean shoot biomass at all herbicide concentrations was significantly less than the reference. Reductions in shoot biomass ranged from 68 percent at a fluridone concentration of 10 µg/ℓ to 84 percent at a concentration of 90 µg/ℓ . Dunnett's test showed

significant reduction in mean root biomass of watermilfoil grown in the sand-peat substrate at 20 µg/ℓ . At this concentration, root biomass was reduced by 59 percent. Duncan's test showed significant variation from the reference at 10 and 20 µg/ℓ. At 10 µg/ℓ fluridone, root biomass was reduced by 41 percent. Mean shoot biomass of watermilfoil grown on the sand-peat substrate at 10 µg/ℓ fluridone was significantly less than the reference according to Dunnett's test. At this concentration, shoot biomass was reduced by 62 percent. Duncan's test showed significant variation from the reference at 10 and 90 µg/ℓ. At 90 µg/ℓ fluridone, mean shoot biomass was reduced by 38 percent.

18. Table 1 also illustrates the effects of various fluridone concentrations on root and shoot biomass of hydrilla grown on the natural and sand-peat substrates. Dunnett's test showed significant reductions in mean root biomass of hydrilla in the natural substrate at all fluridone concentrations except 40 µg/ℓ. These reductions ranged from 30 to 48 percent. Duncan's test also showed significant variation from the reference at all concentrations except 40 µg/ℓ. The results of both Dunnett's and Duncan's test showed hydrilla shoot biomass grown on the natural substrate to be significantly less than the reference at all fluridone concentrations. The reductions ranged from 68 to 77 percent. Dunnett's test showed significant reductions in mean root biomass of hydrilla grown on the sand-peat substrate at 10 and 40 µg/ℓ fluridone. Duncan's test also showed significant variation from the reference at these concentrations. Root biomass was reduced by 44 percent. Dunnett's test showed significant reductions in mean shoot biomass of hydrilla grown on the sand-peat substrate at all fluridone concentrations except 40 µg/ℓ. These reductions ranged from 62 to 79 percent. Duncan's test showed significant variation from the reference at all fluridone concentrations.

19. The biomass reductions such as those reported above and other phytotoxic effects, e.g. growth retardation and suppression, have been observed when fluridone was applied to water as a preemergence treatment on established infestations of hydrilla in Florida (Dechoretz and Frank 1978). Reduction in shoot length or stunted growth of American pondweed

and Sago pondweed has also been reported (Anderson 1981). After a 10-day exposure to fluridone, Anderson (1981) observed 87- and 50-percent reductions in length for Sago and American pondweed, respectively. Various degrees of stunting have also been reported when fluridone was applied as a preemergence herbicide to annual grass and broadleaf weeds (Waldrep and Taylor 1976).

20. The loss of chlorophyll has been used as the principal criterion of senescence in plants by many workers (Leopold, Niedergang-Kamien, and Janick 1959; Fletcher 1969; Back and Richmond 1971). Mode of action studies with fluridone have shown that, in treated corn or wheat seedlings, carotenoid content drops dramatically regardless of light intensity (Devlin et al. 1978). This inhibition of carotenoid synthesis causes the accumulation of the colorless carotenoid precursors, phytoene and phytofluene, in wheat seedlings. In the absence of carotenoids, the chloroplast constituents become susceptible to photo-oxidation resulting in white or chlorotic plants (Krinsky 1967, Bartels and Watson 1978). Bleaching may be expressed as an absolute decrease in pigments due to their herbicide-induced destruction and/or an inhibition of pigment formation (Kunert and Böger 1979). Under high light intensity, wheat seedlings treated with fluridone concentrations of 1, 5, and 10 μM showed decreases in total chlorophyll content of 22, 65, and 95 percent, respectively (Devlin et al. 1978). When corn seedlings were treated with 5, 10, and 50 μM fluridone, chlorophyll content diminished 27, 91, and 94 percent, respectively. Fluridone effects on the chlorophyll content of watermilfoil and hydrilla growing in the natural and the sand-peat substrates are shown in Table 2. Total chlorophyll content ($a + b$) for watermilfoil growing on the natural substrate was significantly less than the reference at all fluridone concentrations except 10 μg/ℓ according to Dunnett's test. Reductions in total chlorophyll ranged from 94 to 99 percent less than that of the reference. Duncan's test showed significant reductions in total chlorophyll content at all fluridone concentrations. The total chlorophyll content for watermilfoil growing on the sand-peat substrate was significantly less than the reference at all of the fluridone concentrations except

10 µg/ℓ according to Dunnett's test. Duncan's test showed significant reductions at each fluridone concentration (Table 2). No significant variations in total chlorophyll content of hydrilla growing on the natural substrate were observed based on results of Dunnett's test. Duncan's test showed significant reductions at each of the fluridone concentrations except 10 µg/ℓ. Significant variations in total chlorophyll content of hydrilla growing on the sand-peat substrate were not observed. Although a reduction in total chlorophyll content of 48 percent did occur at the 90-µg/ℓ fluridone treatment, this was not statistically significant according to Dunnett's and Duncan's tests.

21. Chlorophyll a content was determined for watermilfoil and hydrilla grown on the natural and sand-peat substrates. The effects of the five fluridone concentrations on chlorophyll a content are shown in Table 3. Dunnett's test showed significant reductions in chlorophyll a at all fluridone concentrations except 10 µg/ℓ for watermilfoil grown on the natural substrate. Reductions ranged from 94 to 98 percent. Duncan's test showed significant reductions in chlorophyll a at all fluridone concentrations. Dunnett's test showed significant reductions in chlorophyll a at all fluridone concentrations except 10 µg/ℓ for watermilfoil grown on the sand-peat substrate. Reductions ranged from 97 to 98 percent. Duncan's test showed significant reductions in chlorophyll a at all fluridone concentrations. The chlorophyll a content of hydrilla grown on the natural substrate was not significantly reduced at any of the fluridone concentrations according to Dunnett's test. However, Duncan's test showed significant reductions at all of the fluridone concentrations. Reductions ranged from 62 to 77 percent. The chlorophyll a content of hydrilla grown on the sand-peat substrate was not significantly reduced at any of the fluridone concentrations according to Dunnett's test. Duncan's test showed significant reductions at 70 and 90 µg/ℓ fluridone. Reductions at these concentrations were 43 and 53 percent, respectively.

22. Chlorophyll *b* content was determined for watermilfoil and hydrilla grown on the natural and the sand-peat substrates. The effects of the five fluridone concentrations on chlorophyll *b* content are

also illustrated in Table 3. The chlorophyll *b* content of watermilfoil grown on the natural substrate was significantly less than the reference at all fluridone concentrations except 10 µg/ℓ based on the results of Dunnett's and Duncan's tests. Reductions in chlorophyll *b* ranged from 93 percent at 20 µg/ℓ fluridone to 99 percent at 90 µg/ℓ fluridone. The chlorophyll *b* content of watermilfoil grown on the sand-peat substrate was significantly lower than that of the reference at all fluridone concentrations except 10 µg/ℓ, based on the results of Dunnett's and Duncan's tests. Reductions in chlorophyll *b* content ranged from 97 to 99 percent. The chlorophyll *b* content of hydrilla grown on the natural and the sand-peat substrates did not vary significantly from that of the reference at any of the fluridone concentrations based on the results of Dunnett's test. However, Duncan's test showed significant reductions in chlorophyll *b* content at the 70- and 90-µg/ℓ fluridone concentrations. Reductions in chlorophyll content of submerged aquatics following treatment with fluridone have been reported (Anderson 1981). Exposure of American pondweed to 1.0 mg/ℓ fluridone for only 24 hr reduced chlorophyll a content by approximately 75 percent compared to reference plants. Chlorophyll a in Sago pondweed was reduced by 40 percent compared to controls. The difference in chlorophyll a between controls and fluridone-treated plants tended to increase with increasing duration of exposure to fluridone. The chlorophyll *b* content of Sago pondweed and American pondweed was reduced by 76 and 95 percent, respectively, at 10 days posttreatment.

23. The effects of fluridone on the root and shoot biomass of watermilfoil and hydrilla grown on the natural substrate were similar. The only exception was the lack of significant reduction in hydrilla root biomass at 40 µg/ℓ fluridone. The natural variation among individuals in populations may account for this lack of statistical significance.

24. The responses of root and shoot biomass to fluridone were inconsistent when watermilfoil and hydrilla were grown on the sand-peat substrate (Table 1). Also, the lack of significant reductions in total chlorophyll, chlorophyll a, and chlorophyll *b* content of hydrilla was

observed when it was grown on the sand-peat substrate (Tables 2 and 3). This indicates that these species can be subjected to physiological stress as a result of substrate type. The effects of this stress could outweigh the effects of the fluridone treatments. This may account for the inconsistent responses of the watermilfoil biomass to fluridone and the lack of significant reductions in hydrilla chlorophyll content when these species were grown on the sand-peat substrate.

Determination of Threshold Fluridone Concentrations

25. Photos 1-6 show the growth response of watermilfoil and hydrilla before and after exposure to fluridone. The injury ratings for watermilfoil grown on the natural substrate (Figure 2) and on the sand-peat substrate (Figure 3) show that growth was controlled at 10 µg/ℓ fluridone (Photo 2) when compared to the reference aquaria (Photo 6). The injury ratings for hydrilla grown on the natural substrate (Figure 4) and on the sand-peat substrate (Figure 5) show that growth was controlled at 20 µg/ℓ fluridone (Photo 4). Total control, i.e.

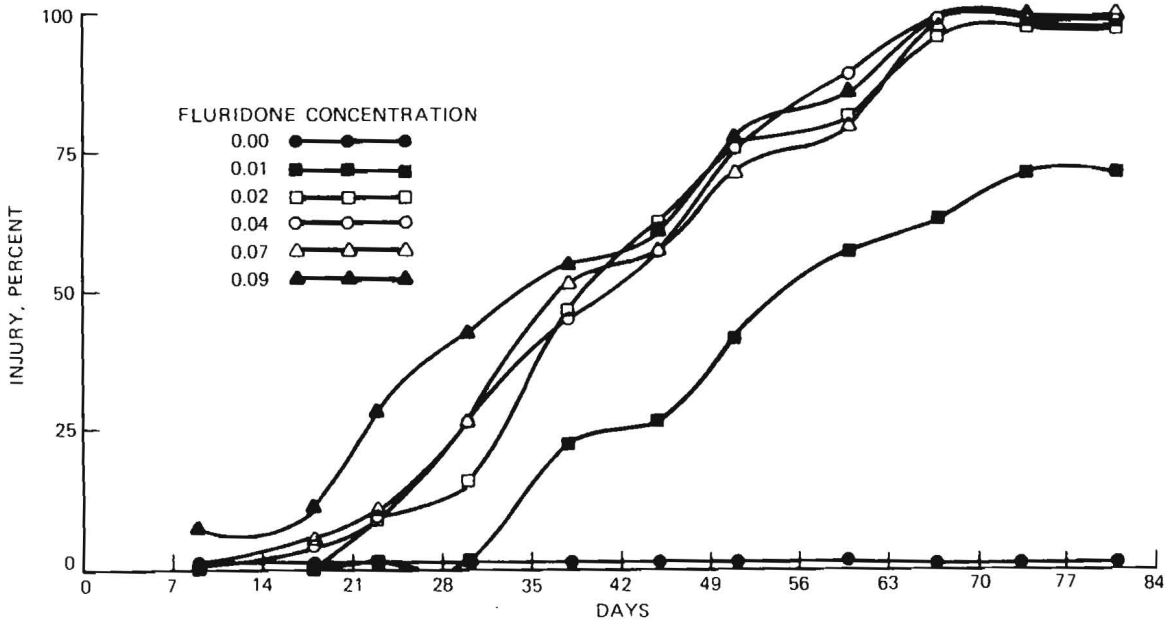

Figure 2. Response of watermilfoil grown on a natural substrate to five fluridone concentrations (and a reference) over a 12-week study period

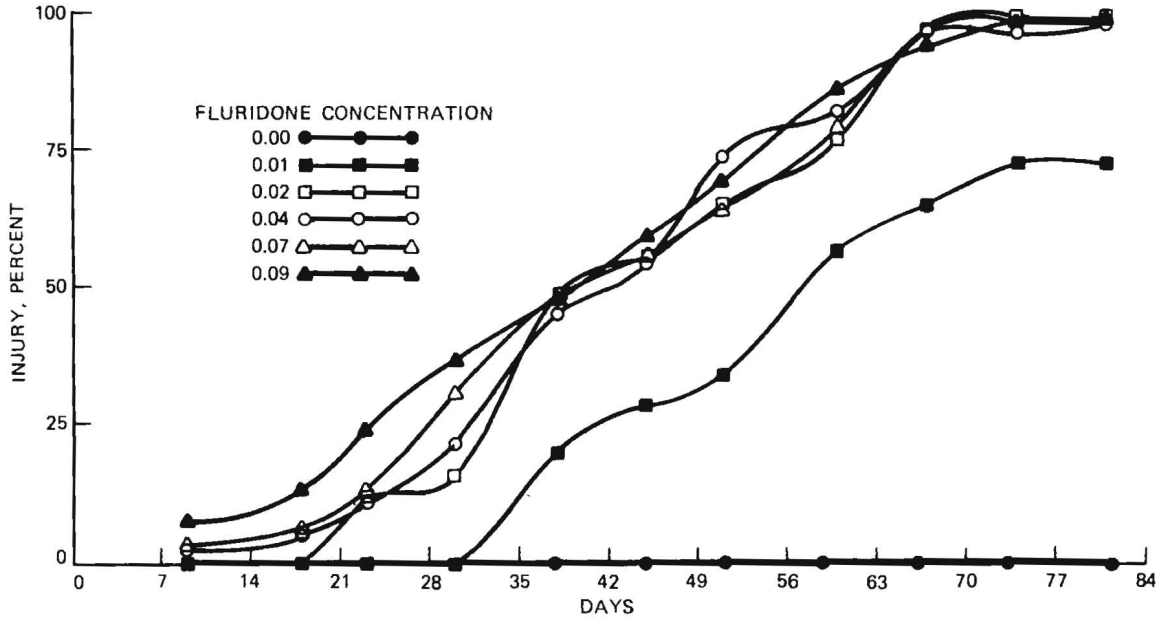

Figure 3. Response of watermilfoil grown on a sand-peat substrate to five fluridone concentrations (and a reference) over a 12-week study period

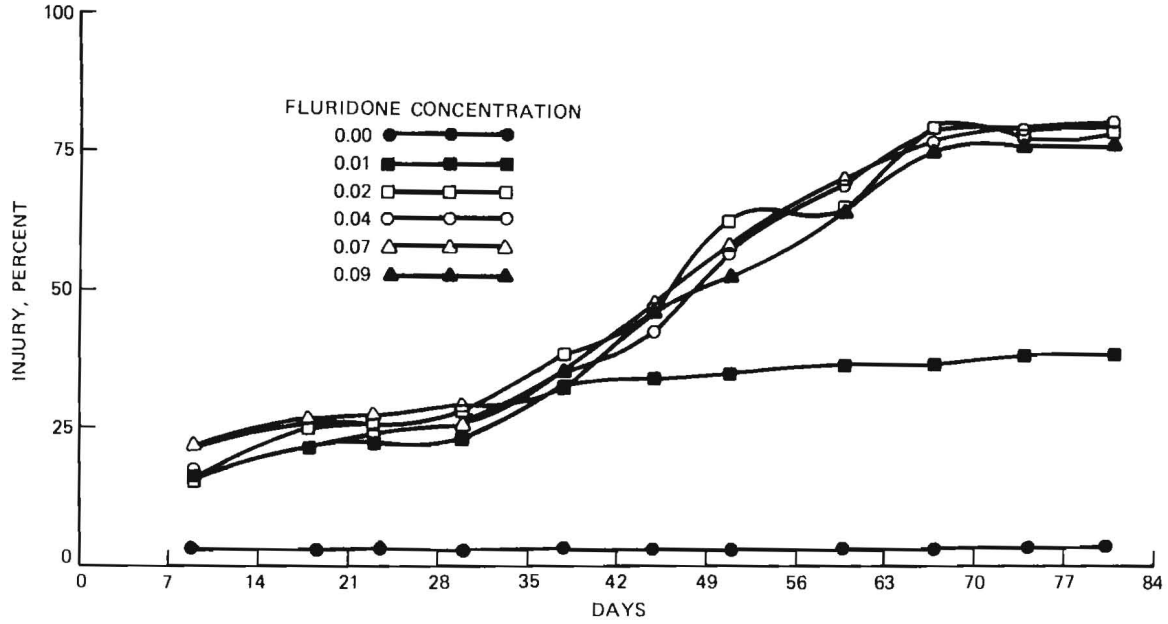

Figure 4. Response of hydrilla grown on a natural substrate to five fluridone concentrations (and a reference) over a 12-week study period

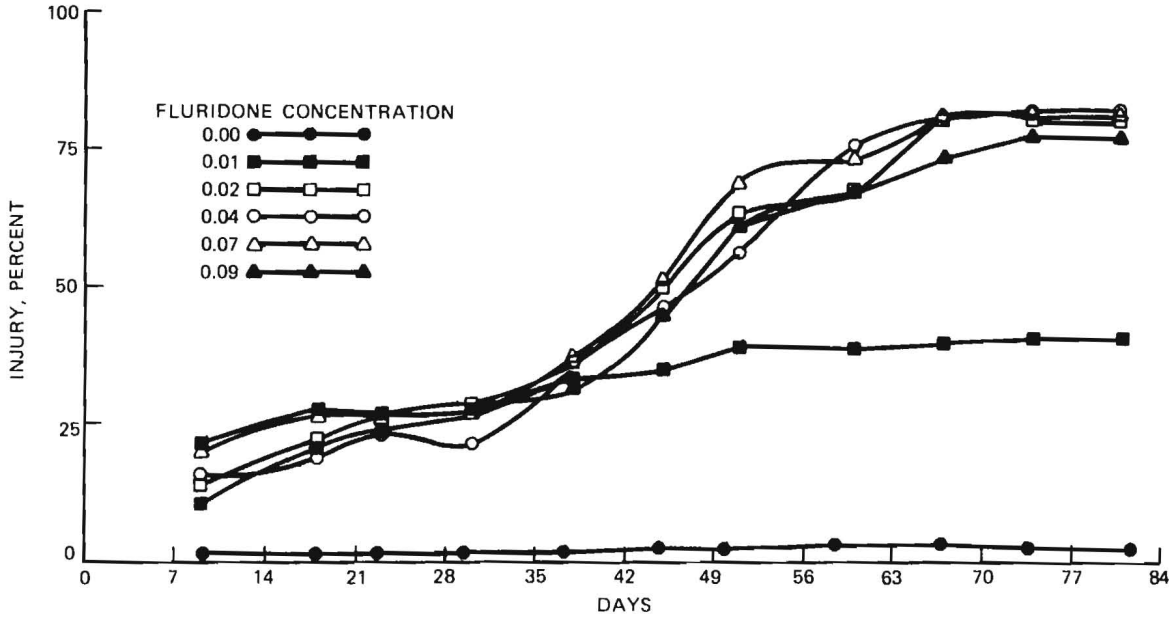

Figure 5. Response of hydrilla grown on a sand-peat substrate to five fluridone concentrations (and a reference) over a 12-week study period

100-percent injury, did not occur at any of the fluridone concentrations.

26. The response of root and shoot biomass and chlorophyll content to the five fluridone concentrations was used along with the injury ratings to determine the threshold concentrations required to control growth of watermilfoil and hydrilla. The threshold fluridone concentration required to control growth of watermilfoil on the natural or the sand-peat substrate was between 10 and 20 µg/ℓ. The threshold fluridone concentration for hydrilla growing on the natural or the sand-peat substrate according to root and shoot biomass data and the injury ratings would appear to be 20 µg/ℓ.

PART IV: CONCLUSIONS AND RECOMMENDATIONS

27. The following is a list of conclusions and recommendations concerning the estimates of the threshold fluridone concentrations for controlling growth of watermilfoil and hydrilla:

a. The influence of substrate type on plant growth and herbicide efficacy varies depending upon the species evaluated. Physiological stress and/or nutrient deficiency affect the response of the target species to a given herbicide.

b. The threshold fluridone concentration required to control growth of watermilfoil on a natural or sand-peat substrate was between 10 and 20 µg/ℓ.

c. The threshold fluridone concentration required to control growth of hydrilla was 20 µg/ℓ.

d. Effective control of hydrilla was achieved on both sand-peat and natural substrates; however, much greater plant damage was evident on hydrilla grown on the natural substrate.

e. Future studies should be conducted to determine how substrate type affects the growth response of hydrilla to fluridone.

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Table 1

Root and Shoot Biomass of Watermilfoil and Hydrilla Grown on Natural and Sand-Peat Substrates Following 12-Week Continuous Exposure to Five Fluridone Concentrations

Fluridone Concentration µg/ℓ	Biomass, Dry Weight, mg*			
	Natural		Sand-Peat	
	Shoots	Roots	Shoots	Roots
		Watermilfoil		
Reference	460.0 ± 40.0a	370.0 ± 10.0a	130.0 ± 10.0ab	170.0 ± 10.0a
10	140.0 ± 20.0b**	170.0 ± 20.0b**	50.0 ± 6.0c**	100.0 ± 4.0b
20	140.0 ± 20.0b**	80.0 ± 1.0c**	170.0 ± 30.0a	70.0 ± 4.0b**
40	110.0 ± 10.0b**	100.0 ± 10.0bc**	100.0 ± 20.0abc	100.0 ± 7.0b
70	110.0 ± 3.0b**	80.0 ± 4.0c**	140.0 ± 10.0ab	100.0 ± 10.0ab
90	70.0 ± 4.0b**	110.0 ± 9.0bc**	80.0 ± 6.0b	120.0 ± 20.0ab
		Hydrilla		
Reference	720.0 ± 70.0a	270.0 ± 10.0a	240.0 ± 10.0a	90.0 ± 7.0ab
10	190.0 ± 20.0b**	190.0 ± 8.0c**	60.0 ± 6.0c**	50.0 ± 4.0c**
20	160.0 ± 8.0b**	140.0 ± 4.0c**	90.0 ± 20.0bc**	70.0 ± 3.0bc
40	170.0 ± 20.0b**	220.0 ± 10.0ab	140.0 ± 30.0b	50.0 ± 2.0c**
70	230.0 ± 20.0b**	150.0 ± 10.0c**	50.0 ± 4.0c**	80.0 ± 6.0abc
90	210.0 ± 20.0b**	190.0 ± 20.0bc**	80.0 ± 8.0bc**	110.0 ± 10.0a

Note: Values in a column followed by the same letter are not statistically different at the 10 percent level as determined by Duncan's multiple range test.

* Values are mean ± standard error, n = 6 for shoots, n = 12 for roots.

** Statistically different from the reference at the 10-percent level as determined by Dunnett's test.

Table 2

Total Chlorophyll Content (a + *b*) for Watermilfoil and Hydrilla Grown on Natural and Sand-Peat Substrates Following 12-Week Continuous Exposure to Five Fluridone Concentrations

Fluridone Concentration µg/ℓ	Total Chlorophyll, mg/g Fresh Tissue*			
	Watermilfoil		Hydrilla	
	Natural	Sand-Peat	Natural	Sand-Peat
Reference	1.62 ± 0.12a	1.54 ± 0.12+a	0.93 ± 0.12a	0.48 ± 0.03a
10	1.07 ± 0.04b	0.88 ± 0.10b	0.41 ± 0.06ab	0.37 ± 0.04a
20	0.09 ± 0.01c**	0.03 ± 0.004c**	0.33 ± 0.03b	0.25 ± 0.03a
40	0.03 ± 0.01c**	0.03 ± 0.003c**	0.29 ± 0.04b	0.38 ± 0.04a
70	0.02 ± 0.003c**	0.02 ± 0.003c**	0.26 ± 0.05b	0.28 ± 0.06a
90	0.02 ± 0.004c**	0.03 ± 0.002c**	0.22 ± 0.03b	0.25 ± 0.01a

Note: Values in a column followed by the same letter are not statistically different at the 10-percent level as determined by Duncan's multiple range test.

* Values are mean ± standard error, n = 4.

** Statistically different from the reference at the 10-percent level as determined by Dunnett's test.

Table 3

Chlorophyll α and β Content for Watermilfoil and Hydrilla Grown on Natural and Sand-Peat Substrates Following 12-Week Continuous Exposure to Five Fluridone Concentrations

Fluridone Concentration µg/ℓ	Chlorophyll, mg/g Fresh Tissue*			
	Watermilfoil		Hydrilla	
	Natural	Sand-Peat	Natural	Sand-Peat
	Chlorophyll *a*			
Reference	1.23 ± 0.08a	1.17 ± 0.01a	0.74 ± 0.09a	0.40 ± 0.02a
10	0.74 ± 0.03b	0.58 ± 0.07b	0.28 ± 0.04b	0.25 ± 0.03ab
20	0.07 ± 0.003c**	0.03 ± 0.003c**	0.26 ± 0.03b	0.21 ± 0.02ab
40	0.03 ± 0.007c**	0.02 ± 0.002c**	0.22 ± 0.03b	0.27 ± 0.02ab
70	0.02 ± 0.014c**	0.02 ± 0.004c**	0.20 ± 0.05b	0.23 ± 0.05b
90	0.02 ± 0.003c**	0.02 ± 0.002c**	0.17 ± 0.02b	0.19 ± 0.01b
	Chlorophyll *b*			
Reference	0.40 ± 0.03a	0.38 ± 0.03a	0.19 ± 0.03a	0.10 ± 0.01a
10	0.34 ± 0.01a	0.31 ± 0.03a	0.13 ± 0.02ab	0.11 ± 0.01a
20	0.03 ± 0.003b**	0.005 ± 0.001b**	0.07 ± 0.01ab	0.04 ± 0.01a
40	0.01 ± 0.004b**	0.01 ± 0.004b**	0.007 ± 0.01ab	0.11 ± 0.02a
70	0.005 ± 0.001b**	0.003 ± 0.001b**	0.06 ± 0.01b	0.05 ± 0.01a
90	0.005 ± 0.001b**	0.01 ± 0.002b**	0.06 ± 0.01b	0.06 ± 0.003a

Note: Values in a column followed by the same letter are not statistically different at the 10-percent level as determined by Duncan's multiple range test.

* Values are mean ± standard error, n = 4.

** Statistically different from the reference at the 10-percent level as determined by Dunnett's test.

Photo 1. Growth response of watermilfoil (left) and hydrilla (right) in test aquaria prior to exposure to 10 µg/ℓ fluridone

Photo 2. Growth response of watermilfoil (left) and hydrilla (right) following 80 days of continuous exposure to 10 µg/ℓ fluridone

Photo 3. Growth response of watermilfoil (left) and hydrilla (right) in test aquaria prior to exposure to 20 µg/ℓ fluridone

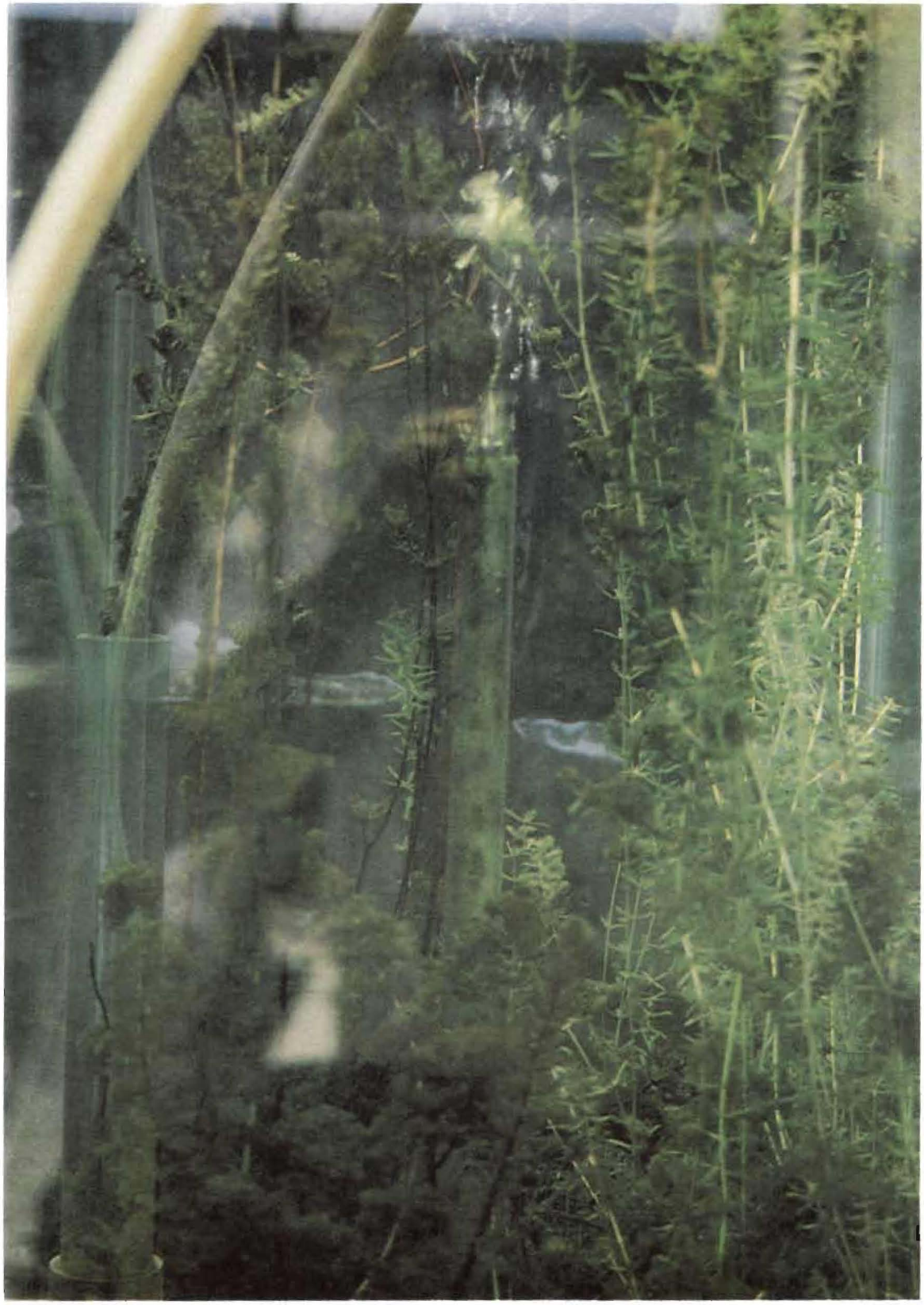

Photo 4. Growth response of watermilfoil (left) and hydrilla (right) following 80 days of continuous exposure to 20 µg/ℓ fluridone

Photo 5. Growth response of watermilfoil (left) and hydrilla (right) in reference aquaria on Day 1

Photo 6. Growth response of watermilfoil (left) and hydrilla (right) in reference aquaria on Day 80