

# Potential Control of Hydrilla and Eurasian Watermilfoil Under Various Fluridone Half-life Scenarios

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## ABSTRACT

Fluridone {1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone} efficacy against Eurasian watermilfoil (*Myriophyllum spicatum* L.) and hydrilla (*Hydrilla verticillata* (L.f.) Royle) was evaluated by simulating herbicide degradation/dissipation half-lives ( $t^{1/2}$ ) under controlled-environment conditions. Fluridone treatment rates of 100  $\mu\text{g/L}$  for 7, 10, and 14 d  $t^{1/2}$ , 50  $\mu\text{g/L}$  for 14 and 21 d  $t^{1/2}$ , 25  $\mu\text{g/L}$  for a 28 d  $t^{1/2}$ , and static treatments of 5 and 15  $\mu\text{g/L}$  for 105 d were tested. Chlorophyll content and net photosynthesis

were measured at 7, 28, 56, 77, and 105 d posttreatment, and biomass was collected at 28, 56, 77 or 84, and 105 or 108 d posttreatment to assess efficacy. The 7 and 10 d  $t^{1/2}$  treatments dissipated to 0  $\mu\text{g/L}$  at 42 and 62 d respectively, resulting in rapid recovery of both hydrilla and Eurasian watermilfoil. The 14 d  $t^{1/2}$  resulted in exposures of 82 to 84 d, and although hydrilla biomass remained significantly reduced, physiological recovery indicated the potential for biomass recovery. Eurasian watermilfoil was completely controlled at 84 d posttreatment following both 14 d  $t^{1/2}$  treatment. The 21 and 28 d  $t^{1/2}$  and static treatments maintained low fluridone exposures throughout the 105 d study period. These treatments reduced hydrilla biomass significantly (> 90%), and physiological variables showed no evidence of recovery at completion of the study. These treatments also resulted in near 100% control of milfoil biomass by the 84 d

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harvest. Results indicate that following initial injury at higher treatment levels, extremely low levels of fluridone (1 to 3 µg/L) prevent recovery. Herbicidal activity of these low concentrations may explain the long-term control achieved with fluridone in situations that allow long degradation half-lives (>14 d).

*Key words:* *Hydrilla verticillata*, *Myriophyllum spicatum*, dose response, herbicide half-life, rhodamine dye.

## INTRODUCTION

The herbicide fluridone has been reported to have an aqueous half-life ranging from 5 to 60 days, with an average of 20 days, following treatment of entire aquatic systems (West et al. 1983, Osborne et al. 1989). Although photolysis is the major degradation process (Mossler et al. 1989), in large systems the half-lives within a treatment area are more likely to be significantly influenced by flow-, thermal-, and wind-generated water exchange (Fox et al. 1993, Getsinger et al. 1990, Getsinger et al. 1992). Inconsistent control of submersed species with fluridone has been attributed to the high variability in fluridone dissipation rates (half-lives) following treatment.

Laboratory and mesocosm studies assessing the efficacy of fluridone on several submersed species have been conducted at rates ranging from 0.05 to 1000 µg/L (maximum label rate = 150 µg/L) with exposure periods ranging from 1 hour to several months (Anderson 1981, Hall et al. 1984, Van and Steward 1986, Van and Conant 1988, Spencer and Ksander 1989, Spencer et al. 1989, MacDonald et al. 1993, Netherland et al. 1993, Netherland and Getsinger 1995). Results from these studies suggest that although initial plant injury is more severe at increased treatment rates, fluridone-induced injury remains qualitatively similar over a very broad range of concentrations (10 to 10,000 µg/L) following comparable exposure periods. Field and laboratory studies have shown that maintaining an extended exposure period (10 to 15 weeks) at rates as low as 10 to 25 µg/L provides excellent control of hydrilla and Eurasian watermilfoil (hereafter called milfoil) (Fox et al. 1994, Getsinger 1993, Netherland et al. 1993).

Studies utilizing <sup>14</sup>C-labeled fluridone have shown that the bioconcentration factor (plant tissue conc./H<sub>2</sub>O conc.) continues to increase for up to 21 d posttreatment (Marquis and Comes 1981, Anderson 1981, and Van and Steward, 1986). Continued uptake and concentration of fluridone over extended periods of time suggest a lack of initial herbicidal effects on plant metabolism. The slow uptake of fluridone has led to speculation that at lower concentrations, longer exposure requirements are necessary to provide adequate levels of fluridone to control plants. Recent studies with hydrilla have shown that a treatment of 5 µg/L takes up to 6 weeks longer to manifest fluridone injury symptoms similar to a 50 µg/L treatment (MacDonald et al. 1993). Although initial injury is delayed, evidence also suggests that long-term exposure (90 d) to rates as low as 5 µg/L can provide control similar to that produced by higher treatment rates (Netherland and Shearer 1995).

The delayed onset of fluridone injury at low concentrations has led to speculation that higher initial treatment rates

followed by dissipating concentrations may reduce exposure requirements. Furthermore, fluridone activity at extremely low rates has generated interest in the minimum initial treatment rates and concentrations of fluridone that must be maintained to provide control. Based on potential fluridone half-lives in aquatic systems, it is hypothesized that many half-life scenarios provide prolonged exposure to herbicidally active concentrations of fluridone. It is thought that maintaining these long-term exposures is the key to controlling submersed plants with fluridone. The objectives of this study were to: (a) determine fluridone efficacy on hydrilla and milfoil over a range of half-lives and static concentrations that provide different exposure scenarios; and (b) determine whether the critical factor for long-term control of these two target plants is initial treatment rate or exposure time.

## MATERIALS AND METHODS

Studies were conducted in a controlled-environment growth chamber with a photosynthetic photon flux density (PPFD) of 570 ± 75 µmoles/m<sup>2</sup>/sec, a 14L:10D photoperiod, and temperature of 23 ± 2 C. The chamber contains 52 independently plumbed 55-L glass aquariums (0.9 m tall × 0.09 m<sup>2</sup>), and is described in further detail in Netherland et al. 1991. Hydrilla planting stock was obtained from the Suwannee River, FL and milfoil stock was obtained from the Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX.

Sediment was collected from Brown's Lake at the US Army Engineer Waterways Experiment Station, Vicksburg, MS and amended with NH<sub>4</sub>Cl (150 mg/L) and Osmocote<sup>®</sup> 20:15:15 (1g/L) to decrease the potential for nutrient limitation during the course of the study. Glass beakers (300 ml) were filled with 250 ml of sediment and four, 10 to 15 cm apical shoots of either hydrilla or milfoil were planted in each individual beaker. A 1-cm layer of silica sand was added to the sediment surface to prevent suspension of sediment. Ten beakers containing a single target species were placed in an aquarium containing a water culture solution recommended for submersed macrophytes (Smart and Barko 1984). Water was exchanged via peristaltic pumps every 72 h prior to fluridone treatment.

One beaker was removed from each aquarium following 21 d of plant growth to provide an estimate of pretreatment biomass. Estimated shoot biomass (hydrilla = 121 g dry weight (DW)/m<sup>2</sup> and milfoil = 133 g DW/m<sup>2</sup>) approximates early summer field biomass for hydrilla and milfoil (Grace and Wetzel 1978, Bowes et al. 1979, Harlan et al. 1985).

Fluridone (Sonar<sup>®</sup> AS) and rhodamine WT dye were applied at initial rates and predicted half-lives ( $t^{1/2}$ ) reported in Table 1. Regression of predicted half-life values provided daily target concentrations (Table 1). Based on these target rates, a calculated volume of treated water was replaced with untreated water on a daily basis. Previous chamber studies have shown fluridone and rhodamine WT are negligibly degraded and remain highly correlated ( $r^2 = 0.97$ ) over a 40-d period (Netherland et al. 1993). Dye was measured daily using a Turner Designs<sup>®</sup> fluorometer. Water samples, collected every 2 weeks, were analyzed for fluridone by the TVA Analytical Branch, Chattanooga, TN, using HPLC with a detection limit of 1 µg/L.

The flow-through system was reactivated when rhodamine WT dye levels first reached < 0.1 µg/L (equivalent to 1 µg/L fluridone). This procedure allowed for precise definition of the exposure period and day when fluridone concentrations reached 0 µg/L. Within 24 h of reactivating the flow-through system, neither dye nor fluridone were detected.

Physiological responses to fluridone treatment were measured at 7, 28, 56, 77 or 84, and 105 days after treatment (DAT). Four apical shoots per aquarium were collected at each sample period and placed in a 300 ml BOD bottle filled with culture water. Bottles were placed in a Percival<sup>R</sup> growth chamber under constant environmental conditions of temperature (25 ± 0.5 C) and PPF (250 ± 30 µmoles/m<sup>2</sup>/sec). Net photosynthesis (PTS) was monitored using a digital pH meter equipped with a dissolved oxygen (DO) probe (Netherlands and Lembi 1992). Following an incubation period of 30 to 60 min, final DO readings were taken and fresh weights (fw) recorded. Total chlorophyll (chlorophyll a and b) expressed as mg chlorophyll/g fw. was measured using a DMSO extraction (Hiscox and Israelstam 1979). Biomass was collected by removing 3 beakers from each aquarium at 28, 77, and 105 DAT for hydrilla and 28, 84, and 108 DAT for milfoil. Shoots and roots were separated and oven dried (65 C for 48 h) to a constant DW for biomass determination.

Treatments were replicated three times using a completely randomized design. Biomass and physiological data were subjected to analysis of variance and data at each sampling time were subjected to Dunnett's test (α=.05) to compare treated plants to untreated controls. Regression analysis of physiological variables and biomass was used to test for a linear dose response at each sampling time.

## RESULTS AND DISCUSSION

Regression analyses of daily dye readings showed that actual half-lives were very close to the predicted target half-lives (Table 1). Fluridone concentrations showed nominal variation from predicted concentrations over time (Table 2). Furthermore, fluridone and dye values were highly correlated throughout the study (r<sup>2</sup>=0.98). A summary figure of predicted weekly fluridone dissipation rates based on calculated half-lives is presented in Figure 1. Half-life predictions showed that fluridone residues should reach < 1 µg/L at 46 DAT (7 d t<sup>1/2</sup>), 66 DAT (10 d t<sup>1/2</sup>), and 91 d (14 d t<sup>1/2</sup>). Dye readings were < 0.1 µg/L up to 4 to 7 d earlier than predicted; therefore, actual fluridone exposure was 4 to 7 d less than predicted.

Hydrilla apical tips became albescent (bleached) and manifested a purple color within 4 DAT at treatment rates > 15 µg/L. This change in coloration has been attributed to anthocyanins which are either unmasked after chlorophyll photooxidation or stimulated to increase depending on the plant's physiological stage of development and/or light intensity (Doong et al. 1993).

Hydrilla injury and response to fluridone treatment was generally similar through 28 DAT for all treatment rates, with the exception of the 5 µg/L treatment. Throughout this paper, results are presented in comparison to the untreated reference aquaria. Analyses at 7 DAT showed that all treatments (except 5 µg/L on PTS) significantly reduced both

TABLE 1. COMPARISON OF PREDICTED AND MEASURED FLURIDONE DISSIPATION RATES AND HALF-LIVES BASED ON REGRESSION OF DATA.

Fluridone (dye) Treatment rate (µg/L / half-life)	Dissipation rate based on predicted values <sup>1</sup> (Measured values) <sup>2</sup>	Predicted half-life <sup>3</sup> (Actual half-life) <sup>4</sup> (days)
100 (10)/7 d	lnY=4.59-0.099 * (day #), r <sup>2</sup> =.99 (lnY=4.70-0.101 * (day #), r <sup>2</sup> =.97)	7.0 (6.8)
100 (10)/10 d	lnY=4.59-0.070 * (day #), r <sup>2</sup> =.99 (lnY=4.72-0.073 * (day #), r <sup>2</sup> =.98)	10.0 (9.5)
100 (10)/14 d	lnY=4.59-0.050 * (day #), r <sup>2</sup> =.99 (lnY=4.65-0.052 * (day #), r <sup>2</sup> =.98)	14.0 (13.3)
50 (5)/14 d	lnY=3.89-0.050 * (day #), r <sup>2</sup> =.99 (lnY=3.86-0.051 * (day #), r <sup>2</sup> =.98)	14.0 (13.6)
50 (5)/21 d	lnY=3.89-0.033 * (day #), r <sup>2</sup> =.99 (lnY=3.80-0.030 * (day #), r <sup>2</sup> =.96)	21.0 (23.0)
25 (2.5)/28 d	lnY=3.18-0.025 * (day #), r <sup>2</sup> =.99 (lnY=3.10-0.023 * (day #), r <sup>2</sup> =.98)	28.0 (30.1)
0	static	
5 (0.5)	static	
15 (1.5)	static	

<sup>1</sup>Dissipation rate based on regression of predicted half-life values.

<sup>2</sup>Dissipation rate based on regression of measured dye values. Dye values were multiplied by 10 to provide corresponding fluridone concentrations.

<sup>3</sup>Predicted half-lives were calculated using the equation, t<sup>1/2</sup> = ln 0.5/slope of the regression.

<sup>4</sup>Measured half-lives were calculated using the equation, t<sup>1/2</sup> = ln 0.5/slope of the regression.

chlorophyll (30-97%) and PTS (50-89%) (Tables 3 and 4). Regression analysis of chlorophyll (r<sup>2</sup>=.87) and PTS (r<sup>2</sup>=.81) suggested an initial dose response as fluridone concentrations increased. By 28 DAT, the dose response relationship had begun to weaken as chlorophyll (r<sup>2</sup>=.54) and PTS (r<sup>2</sup>=.47) values from the lower treatment rates (≤25 µg/L) approached the levels of the higher treatments (Tables 3 and 4). The phenomenon of delayed onset of injury symptoms at lower fluridone use rates was also noted by Doong et al. (1993) and MacDonald et al. (1993). Negative PTS readings at 28 DAT indicated that apical tips were under stress as respiration rates exceeded photosynthesis.

TABLE 2. MEASURED AND PREDICTED FLURIDONE VALUES FOLLOWING A WATER REPLACEMENT PROTOCOL TO SIMULATE VARIOUS HALF-LIFE DISSIPATIONS.

Treatment rate/t <sup>1/2</sup> (µg/L)/days	Fluridone values <sup>1</sup> , µg/L (predicted value <sup>2</sup> )				
	Days after treatment				
	7	21	49	70	98
100/7	48 (50)	17 (12)	0 (0.7)	0 (0.1)	0 (0.06)
100/10	53 (60)	25 (23)	4 (3)	0 (0.7)	0 (0.10)
100/14	59 (69)	31 (34)	12 (9)	2 (3)	0 (0.7)
50/14	28 (34)	17 (17)	6 (4)	2 (2)	0 (0.4)
50/21	31 (39)	19 (24)	11 (10)	5 (5)	3 (2)
25/28	16 (20)	11 (14)	6 (7)	6 (4)	3 (2)

<sup>1</sup>Fluridone residue analyses resulted in percent recoveries of 86 to 105%.

<sup>2</sup>Predicted concentrations were obtained from regression equations.

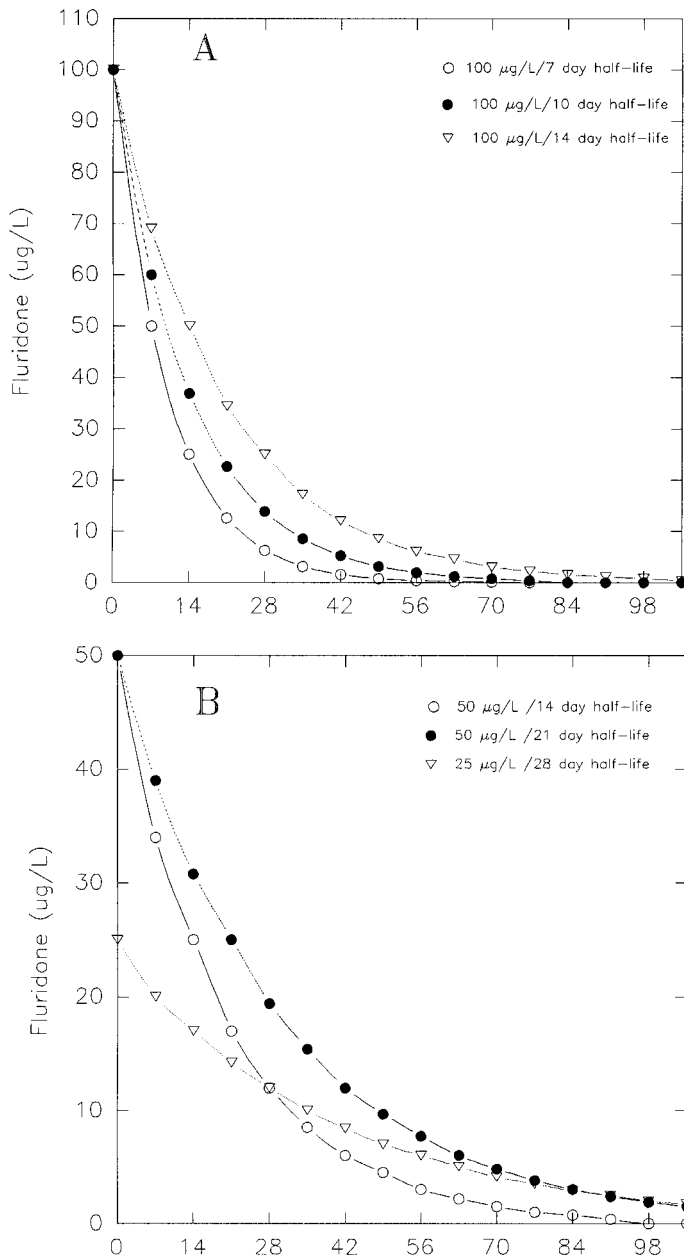


Figure 1A and 1B. Predicted dissipation of fluridone under various half-life scenarios.

Chlorophyll and PTS measures from tissue several nodes below the apical tips showed no differences between fluridone treated and untreated reference plants at 7 and 28 DAT (data not shown). Fluridone symptoms are not readily manifested in mature tissue compared to new growth which is highly susceptible to and immediately manifests fluridone symptoms. Bleaching at the apical tip is caused by the inhibition of carotenoid synthesis (existing carotenoids are not affected) which results in the photodestruction of newly-synthesized chlorophyll molecules (Bartels and Watson 1978). Eventually non-photosynthetic growing tips and loss of metabolic efficiency in mature tissue result in a net demand for carbohydrates with deleterious effects on the plant.

TABLE 3. CHLOROPHYLL CONTENT OF HYDRILLA APICAL SHOOTS SAMPLED AT 7, 28, 56, 77, AND 105 DAYS AFTER INITIAL FLURIDONE TREATMENT.

µg/L / t <sup>1/2</sup> day	Hydrilla chlorophyll content (mg/g fresh weight)				
	Days after treatment <sup>2</sup>				
	7	28	56	77	105
Untreated Control	1.10	1.07	1.15	1.18	1.04
5 (5/105) <sup>1</sup>	0.77*	0.31*	0.19*	0.10*	0.05*
15 (15/105) <sup>1</sup>	0.39*	0.21*	0.13*	0.09*	0.07*
100/7 (0/42) <sup>1</sup>	0.07*	0.06*	1.02	1.09	1.06
100/10 (0/61) <sup>1</sup>	0.06*	0.08*	0.12*	1.11	1.10
100/14 (0/84) <sup>1</sup>	0.09*	0.05*	0.08*	0.10*	0.93
50/14 (0/79) <sup>1</sup>	0.05*	0.08*	0.05*	0.04*	0.74*
50/21 (1.8/105) <sup>1</sup>	0.12*	0.09*	0.10*	0.07*	0.03*
25/28 (2.7/1.05) <sup>1</sup>	0.18*	0.10*	0.11*	0.08*	0.05*

<sup>1</sup>Numbers in parentheses indicate the day at which treatments were confirmed at 0 µg/L or the measured fluridone concentration at the 105 d harvest.

<sup>2</sup>Values followed by a \* are significantly different from the untreated control within each sampling interval (Dunnett's "t" test at the 0.05 level).

Hydrilla shoot biomass was reduced by 25 to 54% following 28 DAT of fluridone exposure (Figure 2). However, in comparison to pretreatment levels, biomass in the 5 µg/L treatment increased (19%) and other treatments resulted in biomass decreases of < 31%. Regression analysis of biomass showed that no significant linear dose response existed as treatment rates were increased.

The gradual reduction of plant biomass over long periods of time (30 to 90 DAT) is typical of fluridone treatment in the field (Elanco, 1986). Therefore the measurement of biomass alone (especially soon after treatment) may be a poor indicator of fluridone efficacy in laboratory/mesocosm studies. Wells et al. (1986) tested fluridone on several submersed species for a 60-d period and erroneously concluded that flu-

TABLE 4. NET PHOTOSYNTHETIC RATES (MEASURED AS OXYGEN EVOLUTION) OF HYDRILLA APICAL SHOOTS SAMPLED AT 7, 28, 56, 77, AND 105 DAYS AFTER INITIAL FLURIDONE TREATMENT.

µg/L / t <sup>1/2</sup> day	Hydrilla net PTS (mg O <sub>2</sub> /gram fr. wt./min)				
	Days after treatment <sup>2</sup>				
	7	28	56	77	105
Untreated Control	0.032	0.027	0.031	0.025	0.021
5 (5/105) <sup>1</sup>	0.021	0.010*	-0.004*	-0.010*	-0.005*
15 (15/105) <sup>1</sup>	0.016*	0.007*	-0.011*	-0.013*	-0.008*
100/7 (0/42) <sup>1</sup>	0.004*	0.004*	0.023	0.021	0.024
100/10 (0/62) <sup>1</sup>	0.013*	-0.003*	-0.006*	0.019	0.021
100/14 (0/82) <sup>1</sup>	0.009*	-0.007*	-0.010*	0.002*	0.016*
50/14 (0/84) <sup>1</sup>	0.006*	0.003*	-0.011*	-0.005*	0.013*
50/21 (1.8/105) <sup>1</sup>	0.010*	-0.008*	-0.004*	-0.003*	-0.008*
25/28 (2.7/105) <sup>1</sup>	0.012*	0.002*	0.000*	-0.008*	-0.005*

<sup>1</sup>Numbers in parentheses indicate the day at which treatments reached 0 µg/L or the measured fluridone concentration at the 105 d harvest.

<sup>2</sup>Values followed by a \* are significantly different from the untreated control within each sampling interval (Dunnett's "t" test at the 0.05 level).

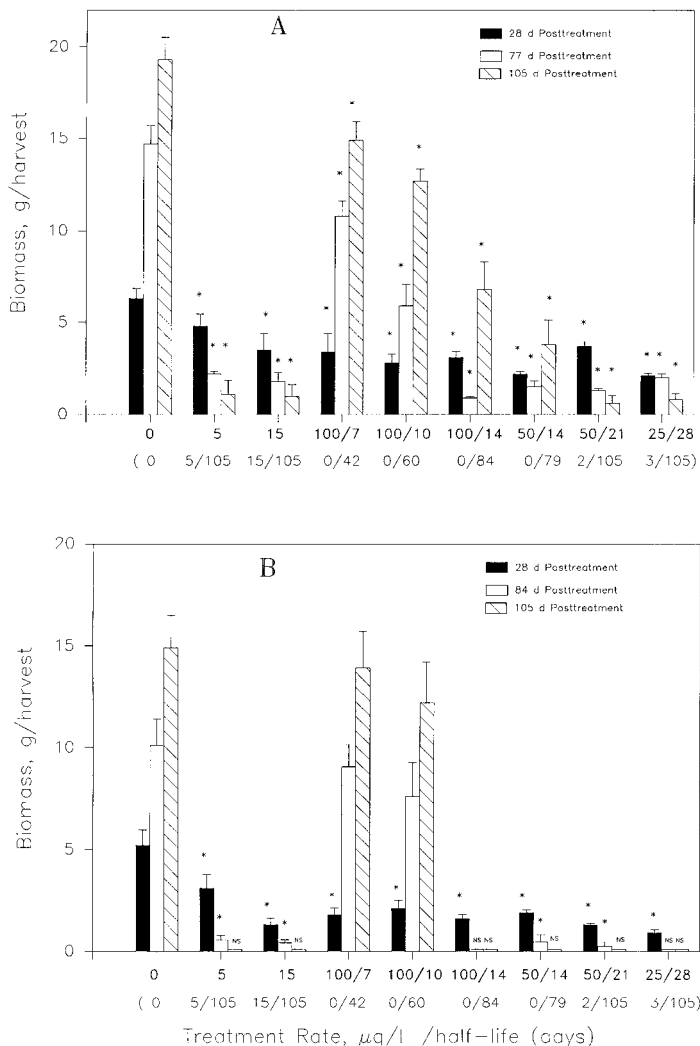


Figure 2. Effects of fluridone treatment on hydrilla (A) and Eurasian water-milfoil (B) biomass over time. Each bar represents the average of three replicates and bars with an \* above them indicate treatments that are significantly different from untreated references within each sampling date according to Dunnet's test ( $\alpha=0.05$ ). NS indicates no sample was taken due to lack of biomass.

ridone was ineffective at rates up to 10,000  $\mu\text{g/L}$  (2 orders of magnitude greater than the maximum label rate). These conclusions were based on the fact that biomass was only reduced by 40 to 80% and treated plants recovered following transfer to untreated water. Other studies clearly showed that conclusions drawn on fluridone efficacy following a 60-d exposure period were premature.

The 7-d  $t^{1/2}$  treatment reached  $< 1 \mu\text{g/L}$  fluridone at 42 DAT, at which point the flow-through system was reactivated. From 56 DAT through the remainder of the study, chlorophyll and PTS were equal to, or exceeded reference treatments (Tables 3 and 4). Although biomass was reduced compared to untreated controls, when compared to pretreatment levels it had increased 4-fold by 77 DAT (Figure 2). The time lag between physiological recovery and biomass recovery has also been noted in previous fluridone studies (Spencer et al. 1989 and Netherland et al. 1993).

Following a 10 d  $t^{1/2}$ , the 100  $\mu\text{g/L}$  treatment was estimated (based on dye readings) at 1.4  $\mu\text{g/L}$  fluridone at 56 d. Chlorophyll and PTS remained significantly reduced and were indistinguishable from treatments that maintained higher fluridone concentrations (Tables 3 and 4). The flow-through was activated at 62 d, and recovery from root crowns was visible within 5 d. Chlorophyll and PTS recovered by 77 DAT and exceeded untreated references by 105 DAT (Tables 3 and 4). Rapid chlorophyll recovery following removal of fluridone has also been observed in laboratory studies following static exposures of 12 to 24  $\mu\text{g/L}$  for 60 d (Netherland et al. 1993). Biomass recovery remained significantly reduced; however, a 4-fold increase over pretreatment levels resulted in a surface canopy at 105 d.

The 50 and 100  $\mu\text{g/L}$  treatments for 14 d  $t^{1/2}$  remained just above 1.0  $\mu\text{g/L}$  at the 77 d harvest. Chlorophyll, PTS, and biomass all remained significantly reduced (Tables 3 and 4, and Figure 2). Both treatments reached  $< 1 \mu\text{g/L}$  at 83 DAT, with visual evidence of recovery noted at 88 DAT.

Although chlorophyll and PTS rates continued to increase, they remained reduced by 11 to 40% at 105 DAT. Biomass was reduced by 69 to 80%; however, it had increased 1.5 to 2-fold over pretreatment levels. Although hydrilla was recovering, plants remained stressed at 25 d post fluridone exposure. It is notable that these treatments were indistinguishable throughout the study even though initial concentrations differed by 50  $\mu\text{g/L}$ .

Chlorophyll and PTS measures taken on mature stem tissue several nodes above the rootcrowns at 77 DAT showed reductions of 38 to 77% in plants that remained exposed to fluridone (14, 21, and 28 d  $t^{1/2}$ , and the 5 and 15  $\mu\text{g/L}$  treatment), whereas no differences were noted in 7 and 10 d  $t^{1/2}$  treatments (data not shown). Results suggest that as carbohydrate stores are depleted, mature tissue is losing metabolic efficiency.

By 105 DAT, the 21 and 28 d  $t^{1/2}$  treatments had fluridone residues of 1.8 and 2.7  $\mu\text{g/L}$  respectively. The 5 and 15  $\mu\text{g/L}$  treatments maintained constant exposures throughout the study. Analyses of these treatments showed reductions in chlorophyll ranging from 91 to 94% and negative PTS readings (Tables 3 and 4). Biomass was reduced by  $>90\%$  compared to untreated references (Figure 2). Hydrilla showed no ability to recover in the presence of these low levels of fluridone. The level of injury produced by these treatments was comparable from 28 DAT through the remainder of the study.

Milfoil response was variable following fluridone treatment. Several apical tips became albescent, whereas many tips never manifested the bleached symptoms but were brown and necrotic by 7 DAT. In contrast to the response of hydrilla, albescent tips did not continue to elongate following fluridone treatment. Analyses at 7 DAT showed that all treatment rates significantly reduced chlorophyll (40 to 92%) and PTS (26-100%) (Tables 5 and 6). Fluridone symptoms were delayed following the 5  $\mu\text{g/L}$  treatment. Regression analysis of chlorophyll ( $r^2=.86$ ) and PTS ( $r^2=.92$ ) suggested a linear dose response in these parameters as initial fluridone concentrations were increased.

By 28 DAT chlorophyll and PTS rates remained significantly reduced by 65 to  $>100\%$  (Tables 5 and 6). Milfoil

TABLE 5. CHLOROPHYLL CONTENT OF EURASIAN WATERMILFOIL APICAL SHOOTS SAMPLED AT 7, 28, 56, 84, AND 105 DAYS AFTER INITIAL FLURIDONE TREATMENT.

$\mu\text{g/L} / \text{t}^{1/2} \text{ day}$	Milfoil chlorophyll content (mg/g fresh weight)				
	Days after treatment <sup>2</sup>				
	7	28	56	84	105
Untreated Control	1.25	1.37	1.28	1.21	1.11
5 (5/105) <sup>1</sup>	0.74*	0.22*	0.15*	0.04*	NA <sup>3</sup>
15 (15/105) <sup>1</sup>	0.48*	0.11*	0.12*	NA	NA
100/7 (0/42) <sup>1</sup>	0.19*	0.05*	1.37	1.13	1.16
100/10 (0/62) <sup>1</sup>	0.10*	0.02*	0.09*	1.11	1.02
100/14 (82) <sup>1</sup>	0.11*	0.09*	0.08*	NA	NA
50/14 (84) <sup>1</sup>	0.21*	0.14*	0.05*	NA	NA
50/21 (1.8/105) <sup>1</sup>	0.17*	0.05*	0.10*	0.02*	NA
25/28 (2.7/105) <sup>1</sup>	0.31*	0.12*	0.11*	0.01*	NA

<sup>1</sup>Numbers in parentheses indicate the day at which treatments were confirmed at 0  $\mu\text{g/L}$  or the measured fluridone concentration at the 105 d harvest.

<sup>2</sup>Values followed by a \* are significantly different from the untreated control within each sampling interval (Dunnett's "t" test at the 0.05 level).

<sup>3</sup>NA indicates that no sample was taken due to lack of intact tissue.

shoot biomass was significantly reduced by 48 to 80% following 28 d of fluridone exposure (Figure 2). The 5  $\mu\text{g/L}$  treatment effects were not as severe as the higher treatment rates; however, no linear dose response was noted as treatment rates were increased.

By 56 DAT the 100  $\mu\text{g/L} / 7 \text{ d t}^{1/2}$  treatment had been free of fluridone exposure for 16 d, whereas the 100  $\mu\text{g/L} / 10 \text{ d t}^{1/2}$  was at 1.4  $\mu\text{g/L}$ . All other treatment concentrations remained above 5  $\mu\text{g/L}$  at 56 DAT. Chlorophyll and PTS measurements showed no differences between untreated references and the 100  $\mu\text{g/L} / 7 \text{ d t}^{1/2}$  treatment (Tables 5 and 6). All other treatments resulted in chlorophyll and PTS reductions of 87 to >100%. The 10 d  $\text{t}^{1/2}$  continued to sup-

TABLE 6. NET PHOTOSYNTHETIC RATES (MEASURED AS OXYGEN EVOLUTION) OF EURASIAN WATERMILFOIL APICAL SHOOTS SAMPLED AT 7, 28, 56, 77, AND 105 DAYS AFTER INITIAL FLURIDONE TREATMENT.

$\mu\text{g/L} / \text{t}^{1/2} \text{ day}$	Milfoil net PTS (mg O <sub>2</sub> /gram fr. wt./min)				
	Days after treatment <sup>2</sup>				
	7	28	56	84	105
Untreated Control	0.041	0.033	0.030	0.029	0.026
5 (5/105) <sup>1</sup>	0.030*	0.014*	0.003*	-0.009*	NA
15 (15/105) <sup>1</sup>	0.021*	0.011*	-0.002*	NA <sup>3</sup>	NA
100/7 (0/42) <sup>1</sup>	0.004*	-0.002*	0.024	0.034	0.024
100/10 (0/62) <sup>1</sup>	0.004*	-0.003*	0.002*	0.027	0.021
100/14 (0/82) <sup>1</sup>	-0.001*	0.001*	-0.013*	-0.009*	NA
50/14 (0/84) <sup>1</sup>	0.006*	-0.008*	-0.003*	NA	NA
50/21 (1.8/105) <sup>1</sup>	0.017*	0.002*	-0.003*	NA	NA
25/28 (2.7/105) <sup>1</sup>	0.012*	0.002*	0.004*	NA	A

<sup>1</sup>Numbers in parentheses indicate the day at which treatments reached 0  $\mu\text{g/L}$  or the measured fluridone concentration at the 105 d harvest.

<sup>2</sup>Values followed by a \* are significantly different from the untreated control within each sampling interval (Dunnett's "t" test at the 0.05 level).

<sup>3</sup>Indicates that no sample was taken due to lack of intact tissue.

press milfoil recovery at rate of 1.4  $\mu\text{g/L}$ , however, after concentrations reached 0  $\mu\text{g/L}$  (62 DAT), healthy regrowth was evident within 5 d.

Chlorophyll, PTS and shoot biomass at 84 and 105 DAT showed that no differences existed between the 7 and 10 d  $\text{t}^{1/2}$ 's and untreated references (Tables 5 and 6, and Figure 2). Efficacy at all other treatment rates prevented harvest of viable tissue for physiological and biomass measurements.

Previous laboratory studies indicated that 90 DAT of exposure to static fluridone concentrations of 12 and 24  $\mu\text{g/L}$  resulted in 95% to 99% milfoil control (Netherland et al. 1993). Furthermore, these studies also showed milfoil to be more susceptible to fluridone than hydrilla under laboratory conditions (Netherland et al. 1993).

The large disparity between milfoil control achieved between the 10 and 14 d  $\text{t}^{1/2}$  demonstrates the importance of the duration of the exposure period. The 100  $\mu\text{g/L} / 10 \text{ d t}^{1/2}$  reached 0  $\mu\text{g/L}$  at 62 days and milfoil fully recovered, whereas the 50  $\mu\text{g/L} / 14 \text{ d t}^{1/2}$  extended the fluridone exposure (at rates below 4  $\mu\text{g/L}$ ) for 20 additional days and resulted in complete milfoil control.

Results from this study suggest that fluridone exposure time was the critical factor for effective control of hydrilla and milfoil, regardless of initial treatment rate. Moreover, extremely low concentrations of fluridone ( $\approx 1 \mu\text{g/L}$ ) actively suppressed hydrilla and milfoil recovery. Evidence from this study suggests that following initial injury from higher treatment rates ( $\geq 25 \mu\text{g/L}$ ), fluridone can suppress and eventually control hydrilla and milfoil at concentrations much lower than previously thought. Treatment with fluridone at rates as low as 1 to 2  $\mu\text{g/L}$  in static laboratory tests resulted in hydrilla and milfoil growth inhibition; however, biomass remained above pretreatment levels by 90 DAT (Netherland and Getsinger 1995).

Suppression of submersed plant growth at these low concentrations in the laboratory can be coupled with potential field dissipation scenarios. Minimum active concentrations were previously believed to range between 5 and 10  $\mu\text{g/L}$ ; however, if growth suppression continues in the range of 1  $\mu\text{g/L}$ , then half-life dissipations must be carried out until fluridone concentrations have essentially reached < 1  $\mu\text{g/L}$  to determine the valid length of exposure. In the case of the 50  $\mu\text{g/L} / 21 \text{ d t}^{1/2}$  and the 25  $\mu\text{g/L} / 28 \text{ d t}^{1/2}$  exposure periods theoretically would reach 120 and 175 d before dropping below 1  $\mu\text{g/L}$ . This study did not take into account the influence of fluridone accumulation in plant tissue or the sediment; however, rapid recovery of apical tips indicated that tissue and sediment concentrations were either low or rapidly depurated following removal of fluridone from the water column. Fluridone activity at these very low concentrations following higher initial treatment rates may help explain the long-term efficacy often noted following treatment of entire aquatic systems with fluridone. The relationship between plant control and fluridone exposure days (FED) under field conditions has been proposed by Fox et al. (1994). Very few studies have correlated plant control and fluridone exposure in the field, and quantification of this relationship is necessary to validate laboratory predictions and to improve guidance for operational control with fluridone.

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