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Laboratory Evaluation of Threshold Fluridone Concentrations Under Static Conditions for Controlling Hydrilla and Eurasian Watermilfoil

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ABSTRACT

Fluridone {1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone} was evaluated against Eurasian watermilfoil (*Myriophyllum spicatum* L.) and hydrilla (*Hydrilla verticillata* (L.f.) Royle) under laboratory conditions at initial treatment rates of 0.0, 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, and 25.0 µg/L for 90 days of exposure. Treatment concentrations < 1 µg/L had no effect on either hydrilla or Eurasian watermilfoil biomass and resulted in only a small reduction (13 to 33%) in hydrilla net photosynthesis (PTS) and chlorophyll at 0.75 µg/L. Both hydrilla and Eurasian watermilfoil growth were inhibited by fluridone concentrations between 1.0 to 3.0 µg/L. Rates of 1.0 and 2.0 µg/L did not reduce hydrilla biomass below pretreatment levels, but did inhibit growth. Biomass remained static from 30 through 90 days after treatment, while chlorophyll and PTS continued to decrease. Eurasian watermilfoil biomass and physiological variables were inhibited as fluridone rates reached 3.0 µg/L. With fluridone concentrations of 4.0, and 25.0 µg/L, hydrilla and Eurasian watermilfoil biomass and physiological variables were reduced (42 to 88%) below pretreatment levels by 30 days after treatment. If PTS readings at shoot apices remained positive following fluridone treatment, shoot biomass increased; whereas negative PTS readings were associated with biomass decreases below pretreatment levels. In general, the appearance of treatment symptoms was delayed as treatment concentrations decreased.

Key words: *Hydrilla verticillata, Myriophyllum spicatum*, dose-response aquatic herbicide.

INTRODUCTION

The ability of the herbicide fluridone to control hydrilla and Eurasian watermilfoil (hereafter called milfoil) at low initial treatment concentrations (8 to 20 µg/L) has been documented in both the laboratory and field (Farone and McNabb 1993, Getsinger 1993, Netherland et al. 1993, Fox et al. 1994, Netherland and Shearer 1995). In addition, results from a laboratory dissipation study showed that following initial exposure to fluridone at rates ≥ 25 µg/L, growth and physiological variables of hydrilla and milfoil remained completely inhibited as fluridone concentrations dissipated to 1 to 3 µg/L (Netherland and Getsinger 1995). Although successful control using fluridone has been linked to extended exposure periods, the minimum initial treatment rate and concentration of fluridone that must be maintained to effectively and/or selectively control these submersed exotic species is not currently known.

Fluridone treatment generally causes a bleached appearance of new growth due to inhibition of the carotenoid synthesis pathway. Insufficient levels of carotenoids result in the photodestruction of chlorophyll molecules in new growth (Bartels and Watson 1978). Although recent evidence from outdoor microcosm studies (Doong et al. 1993, MacDonald et al. 1993) suggested that fluridone at rates as low as 0.5 µg/L inhibits carotenoid and chlorophyll production in both young and mature hydrilla tissue, this inhibition did not translate to a reduction in biomass by 12 weeks after treatment. In these studies biomass reduction did occur at fluridone rates of 5.0 µg/L. However, efficacy information at fluridone treatment rates between 0.5 and 5.0 µg/L currently is lacking for both hydrilla and milfoil.

Previous studies have shown that the onset of fluridone injury symptoms are delayed as treatment concentrations are decreased; however, over time (60 - 90 days) these treatments often provide efficacy comparable to that of higher initial rates (MacDonald et al. 1993, Netherland and Getsinger 1995). The objectives of this laboratory study were to determine the minimum fluridone concentrations that inhibit physiological parameters and growth of newly established hydrilla and milfoil during a 90-day static exposure period.

MATERIALS AND METHODS

Studies were conducted in a controlled-environment growth chamber with a photosynthetic photon flux density of 520 ± 70 µmoles/m²/sec, a 14L:10D photoperiod, and water temperature of 24 ± 1 C. The chamber contained 60 independently plumbed 55-L glass aquaria and is described in detail in Netherland et al. (1991). Hydrilla and milfoil planting stock were obtained from the Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX. Sediment collection and planting procedures are described in Netherland and Getsinger (1995).

Three replicate aquaria (six total) containing either hydrilla or milfoil were harvested following 14 days of growth to provide pretreatment biomass for each species. Fluridone (Sonar® AS) was then applied to remaining aquaria to achieve target concentrations of 0.0, 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, and 25.0 µg/L. Treatments were replicated three

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times using a completely randomized design. Analyses of selected water samples (>1.0 µg/L) showed that fluridone concentrations were maintained within ± 20% of target rates over the course of the 90-day treatment. Previous studies in the environmental chambers have shown fluridone degradation to be negligible over a 98-day exposure (Netherland et al. 1993; Netherland and Getsinger 1995). Water samples were analyzed for fluridone using HPLC with a detection limit of 1.0 µg/L by the Tennessee Valley Authority Analytical Branch, Chattanooga, TN.

Physiological variables and biomass were measured at 30, 60, and 90 days after treatment (DAT). Net photosynthesis (PTS) was monitored on four shoot apices per aquarium using a method described by Netherland and Getsinger (1995). Following an incubation period of 60 minutes, final dissolved oxygen readings were taken and fresh weights (fw) recorded. Chlorophyll content (chlorophyll a and b) expressed as mg chlorophyll/g fw. was measured using a DMSO extraction technique (Hiscox and Israelstam 1979). Biomass samples were collected by removing 3 beakers from each aquarium at 30, 60, and 90 DAT. Shoots and roots were separated and dried to a constant weight for biomass determination.

Data were subjected to analysis of variance (ANOVA) and values within each sampling time were subjected to Dunnet's test (α=.05) to compare each fluridone treatment rate to the untreated controls. ANOVA and regression analysis were used to test for a linear response to each treatment over time.

RESULTS AND DISCUSSION

Fluridone at concentrations of 0.25, and 0.5 µg/L did not affect hydrilla growth compared to untreated references, and biomass increased over the 90-day static exposure period (Table 1). A decrease in chlorophyll and PTS rates was noted in all of these treatments (including the untreated reference) between 60 and 90 DAT (Tables 2 and 3). The reduction in plant vigor was attributed to slow growth rates due to space and/or nutrient limitations. The 0.75 µg/L treatment slightly reduced chlorophyll and PTS rates at 60 and 90 DAT (Tables 2 and 3), and resulted in a small decrease in biomass at the time of the 90-day harvest (Table 1).

With exposure to 1.0 and 2.0 µg/L fluridone, hydrilla biomass increased from pretreatment levels by 30 DAT, but was already reduced compared to untreated references (Table 1). Biomass remained fairly constant from 30 to 90 DAT, whereas, chlorophyll content and PTS rates were reduced at almost all sample periods (Tables 2 and 3). The lack of biom-

TABLE 2. CHLOROPHYLL CONTENT OF HYDRILLA SHOOT APICES SAMPLED AT 30, 60, AND 90 D AFTER INITIAL FLURIDONE APPLICATION.

	Hydrilla chlorophyll content (mg/g fresh weight)			
	Days after treatment			
µg/L	30	60	90	Linear response[2]
Untreated	1.15	1.18	0.91	NS
0.25	1.21	1.29*	0.84	NS
0.50	1.31*	1.15	0.84	NS
0.75	1.21	1.03*	0.72*	.05
1.0	1.10	0.71*	0.65*	NS
2.0	0.86*	0.59*	0.41*	.05
3.0	0.80*	0.24*	0.06*	.05
4.0	0.48*	0.18*	0.10*	.05
25.0	0.16*	0.07*	0.08*	NS

[1]Values followed by a * are significantly different from the untreated control within each sampling interval (Dunnet's "t" test at the 0.05 level).
[2]Test for linear response of chlorophyll content over exposure (30, 60, and 90 d) time within each treatment rate. NS = not significant at the 0.05 level of confidence.

TABLE 1. MEAN BIOMASS OF HYDRILLA SHOOT TISSUE SAMPLED AT 30, 60, AND 90 D AFTER FLURIDONE APPLICATION.

	Hydrilla biomass (g DW/harvest)				
	Days after treatment				
µg/L	0	30	60	90	Linear response[2]
Untreated	3.4	9.3	14.1	20.6	.05
0.25	3.4	8.4	16.2	22.3	.05
0.50	3.4	8.5	14.8	19.9	.05
0.75	3.4	9.0	12.3	16.7*	.05
1.0	3.4	7.4*	6.7*	6.9*	NS
2.0	3.4	5.5*	5.2*	5.0*	NS
3.0	3.4	4.7*	2.5*	1.4*	NS
4.0	3.4	4.3*	3.1*	1.0*	NS
25.0	3.4	2.6*	1.2*	0.5*	.05

[1]Values followed by a * are significantly different from the untreated control within each sampling interval (Dunnet's "t" test at the 0.05 level.)
[2]Test for linear response of biomass over exposure time (0, 30, 60, and 90 d) within each treatment rate. NS = not significant at the 0.05 level of confidence.

TABLE 3. NET PHOTOSYNTHETIC RATES OF HYDRILLA SHOOT APICES SAMPLED AT 30, 60, AND 90 D AFTER FLURIDONE APPLICATION.

	Hydrilla net PTS (mg O_2/g fresh weight/min)			
	Days after treatment			
µg/L	30	60	90	Linear response[2]
Untreated	0.041	0.038	0.025	NS
0.25	0.035	0.044	0.029*	NS
0.50	0.033	0.033	0.022	NS
0.75	0.038	0.029*	0.016*	.05
1.0	0.026*	0.028*	0.018*	NS
2.0	0.020*	0.015*	0.008*	.05
3.0	0.015*	-0.001*	-0.011*	.05
4.0	0.019*	-0.005*	-0.005*	NS
25.0	0.005*	-0.013*	-0.011*	NS

[1]Values followed by a * are significantly different from the untreated control within each sampling interval (Dunnet's "t" test at the 0.05 level).
[2]Test for linear response of PTS rates over exposure time (30, 60, and 90 d) within each treatment rate. NS = not significant at the 0.05 level of confidence.

TABLE 4. MEAN BIOMASS OF EURASIAN WATERMILFOIL SHOOT TISSUE SAMPLED AT 30, 60, AND 90 D AFTER FLURIDONE APPLICATION.

	Milfoil biomass (g DW/harvest) Days after treatment				
µg/L	0	30	60	90	Linear response[2]
Untreated	3.6	6.5	10.3	16.1	.05
0.25	3.6	7.2	11.4	17.4	.05
0.50	3.6	6.4	10.8	16.2	.05
0.75	3.6	7.1	9.8	16.3	.05
1.0	3.6	6.2	9.4	14.9	.05
2.0[3]	3.6	5.4	5.0	4.1	NA
3.0	3.6	3.9*	3.4*	2.0*	NS
4.0	3.6	3.8*	3.1*	1.5*	NS
25.0	3.6	2.6*	1.1*	0.1*	.05

[1]Values followed by a * are significantly different from the untreated control within each sampling interval (Dunnet's "t" test at the 0.05 level).
[2]Test for linear response of biomass over exposure time (0, 30, 60, and 90 d) within each treatment rate. NS = not significant at the 0.05 level of confidence.
[3]Not analyzed due to loss of replicate and high variability.

ass reduction below pretreatment levels may be due to the fact that hydrilla apices maintained the capacity for carbon fixation, as indicated by positive PTS values. Nonetheless, these treatments were clearly growth inhibiting.

Following the 3.0 and 4.0 µg/L treatments, hydrilla biomass initially increased over pretreatment levels at 30 DAT, yet by 60 and 90 DAT biomass had decreased below pretreatment levels (Table 1). The 25.0 µg/L treatment resulted in a linear decrease in biomass (all harvests resulted in biomass below pretreatment levels) (Table 1). Following the 3.0, 4.0, and 25.0 µg/L treatments, both chlorophyll and PTS were reduced at 30 DAT, and PTS readings were negative by 60 DAT (Tables 2 and 3). The decreased ability to fix carbon coupled with respiratory demands of the apical tips most likely combined to cause a decline in biomass over time.

Results from this study compare reasonably well with treatment rates of 0.5 and 5.0 µg/L from outdoor microcosm studies conducted by MacDonald et al. (1993). However, direct comparison of treatment rates between these studies may not be appropriate due to the unknown rate of photolytic degradation that occurred in the outdoor studies.

Fluridone concentrations of 0.25, 0.5, and 0.75, and 1.0 µg/L resulted in a linear increase in milfoil biomass over the 90-day exposure. (Table 4). No significant differences between these treatment rates and untreated references were found at 30, 60, or 90 DAT. Slight reductions in chlorophyll and PTS following the 1.0 µg/L treatment were noted at 30 and 90 days (Tables 5 and 6).

The 2.0 µg/L treatment rate was not subjected to statistical analysis due to the early loss of replicates; however, this treatment produced a growth inhibitory effect over time (Table 4). Chlorophyll content and PTS also were reduced approximately 50% by 60 DAT.

The 3.0, and 4.0 µg/L treatments initially resulted in increased biomass at 30 DAT, but declined to pretreatment levels by 60 DAT (Table 4). Chlorophyll decreased in a linear manner over time following the 3.0 and 4.0 µg/L treatments and negative PTS readings were first recorded at 60 DAT (Tables 5 and 6). As noted for hydrilla, negative PTS readings at the apical tips were associated with biomass reduction below pretreatment levels. The 25 µg/L treatment resulted in a linear decrease in biomass, with significant reductions in chlorophyll and PTS at 30, 60, and 90 DAT (Tables 4, 5, and 6).

Laboratory results have shown that both hydrilla and milfoil growth are inhibited by fluridone concentrations of 1 to 3 µg/L and that appearance of fluridone-induced injury

TABLE 5. CHLOROPHYLL CONTENT OF EURASIAN WATERMILFOIL SHOOT APICES SAMPLED AT 30, 60, AND 90 D AFTER FLURIDONE APPLICATION.

	Milfoil chlorophyll content (mg/g fresh weight) Days after treatment			
µg/L	30	60	90	Linear response[2]
Untreated	1.25	1.27	1.03	NS
0.25	1.35*	1.19	1.17*	NS
0.50	1.33*	1.25	1.08	NS
0.75	1.18	1.21	1.00	NS
1.0	1.10*	1.18	0.79*	NS
2.0[3]	0.68	0.33	0.21	NA
3.0	0.61*	0.15*	0.06*	.05
4.0	0.39*	0.21*	0.11*	.05
25.0	0.10*	0.05*	0.08*	NS

[1]Values followed by a * are significantly different from the untreated control within each sampling interval (Dunnet's "t" test at the 0.05 level).
[2]Test for linear response of chlorophyll content over exposure time (30, 60, and 90 d) within each treatment rate. NS = not significant at the 0.05 level of confidence.
[3]Not analyzed due to loss of replicate and high variability.

TABLE 6. NET PHOTOSYNTHETIC RATES OF EURASIAN WATERMILFOIL SHOOT APICES SAMPLED AT 30, 60, AND 90 D AFTER FLURIDONE APPLICATION.

	Milfoil net photosynthesis (mg O_2/g fresh weight/min) Days after treatment			
µg/L	30	60	90	Linear response[2]
Untreated	0.029	0.034	0.023	NS
0.25	0.037*	0.029	0.021	.05
0.50	0.030	0.034	0.028	NS
0.75	0.027	0.029	0.021	NS
1.0	0.025	0.028	0.019	NS
2.0[3]	0.025	0.013	0.014	NA
3.0	0.020*	-0.002*	-0.004*	NS
4.0	0.018*	-0.007*	-0.004*	NS
25.0	0.008*	-0.013*	-0.009*	NS

[1]Values followed by a * are significantly different from the untreated control within each sampling interval (Dunnet's "t" test at the 0.05 level).
[2]Test for linear response of PTS rates over exposure time (30, 60, and 90 d) within each treatment rate. NS = not significant at the 0.05 level of confidence.
[3]Not analyzed due to loss of replicate and high variability.

symptoms is greatly influenced by initial treatment concentrations (MacDonald et al. 1993, Netherland and Getsinger 1995). Results from this study indicate that hydrilla may be more sensitive to low initial treatment concentrations of fluridone than milfoil. Data from this study also suggest that low initial treatment rates are likely to be very slow-acting with respect to measurable plant response and when maintained may only produce growth inhibitory effects, rather than plant death.

From an operational perspective, the degradation and dissipation of fluridone in the field must be carefully considered prior to choosing low treatment rates (< 10 µg/L). In natural systems, adsorption of fluridone by particulates and organic matter, photolytic degradation, and water exchange could rapidly reduce aqueous target concentrations below threshold injury values. Based on information from this and other studies, a sound treatment strategy may be to apply an initial fluridone rate (> 10 µg/L) that produces more rapid initial injury followed by long-term maintenance (> 60 days) of threshold concentrations.

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