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Fluridone Effects on Fanwort and Water Marigold

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ABSTRACT

Growth chamber studies were conducted to evaluate the effectiveness of the aquatic herbicide fluridone for control of fanwort, and to assess the impact of treatment on the nontarget plant, water marigold. Treatments included static exposures of 0, 5, 7.5, 10, 15, 20, and 30 g fluridone L^{-1}. Changes in pigment concentrations within the plant (phytoene, β-carotene, and chlorophyll) and biomass were used to appraise treatment efficacy. For fanwort, phytoene levels increased 82% and β-carotene decreased 88% when exposed to 5 g fluridone L^{-1} for 14 days. Effects on these two pigments persisted through 84 days after treatment (DAT) for fanwort. In contrast, phytoene content was not affected in water marigold however, plants exposed to ≥10 µg fluridone L^{-1} showed reduced β-carotene 84 DAT. Leaf chlorophyll decreased with increasing fluridone concentration in fanwort whereas in water marigold, decreased chlorophyll was observed in plants treated with rates of 7.5 g L^{-1} and higher. Despite these observed differences in pigment response, all fluridone treatments significantly reduced shoot dry weight biomass. Overall, the data showed that biomass and pigment levels of water marigold were minimally impacted following treatment of 5 g L^{-1} fluridone. However, the dose of fluridone required to control fanwort by >80%, severely inhibited water marigold growth. We conclude that there is limited potential for selectively controlling nuisance fanwort populations with fluridone where water marigold must be protected.

Key words: Aquatic herbicide, β-carotene, *Cabomba caroliniana*, chlorophyll, *Megalodonta beckii*, phytoene.

INTRODUCTION

Fanwort (*Cabomba caroliniana* Gray) is a rooted, submersed, perennial dicot commonly found in stagnant to slow-flowing waters including ponds, lakes and small rivers. Fanwort is native to the subtropic-temperate regions of eastern North and South America (Ørgaard 1991). Distribution in the U.S. extends westward into Texas and northward to Illinois and Michigan with naturalized populations ranging from Virginia to southern New England (Fassett 1953, Muenscher 1944, Godfrey and Wooten 1981). Fanwort has recently expanded to many lakes in the Pacific Northwest (Gibbons et al. 1994, Hamel and Parsons 2001).

Once introduced, fanwort spreads primarily by stem fragments or rhizomes (Ørgaard 1991). The rhizomes are fragile and easily broken thereby facilitating spread and transport of plant fragments. Seed production has been documented (Tarver and Sanders 1977, Sanders 1979), however little is known concerning seed viability or their importance as an effective means of plant dispersal. Fanwort can be persistent and competitive and will often dominate submersed plant communities. In Australia, fanwort is considered an aggressive invader of freshwater systems; producing monocultures that outcompete native aquatic vegetation and threaten habitat biodiversity (Faithfull and Gunasekera 1999). Riemer and Inicki (1968) also reported that under suitable environmental conditions, fanwort can form dense stands and displace pre-

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viously well-established plants in the U.S. Populations reaching nuisance levels have been documented in many lakes in New England (G. Bugbee and G. Smith, pers. comm.), several of the Gulf Coast states (Tarver and Sanders 1977, Sanders 1979, Leslie 1986, Hanlon 1990) and in the Pacific Northwest (Gibbons et al. 1993). In Connecticut, heavy fanwort infestations threaten the survival of water marigold (*Megalodonta beckii* (Torr.) Greene; a.k.a. *Bidens beckii*) a state-listed, threatened plant that shares the same habitat. As a result of water marigold's protected status, aquatic plant managers in Connecticut are faced with the difficult task of identifying management strategies for controlling nuisance fanwort infestations which will minimally impact water marigold.

Like fanwort, water marigold is a native, rooted, submersed plant with similar habitat requirements. A unique, identifiable feature of water marigold is the presence of dimorphic leaves. Submersed leaves are highly segmented and filiform in shape whereas emergent leaves are simple with a lanceolate shape (Roberts 1985). Water marigold is distributed mostly in glaciated eastern North America however, indigenous populations also exist in the Pacific Northwest (Roberts 1985). Dispersal mechanisms include stem fragmentation and the production of underwater rhizomes and turions (Roberts 1985). Seed production in natural populations is limited, presumably due to the paucity of effective pollinators (Roberts 1985). In addition to its threatened status in Connecticut, water marigold is listed as a state endangered species or a species of concern in Illinois, Pennsylvania, New Hampshire, Ohio, and New York.

Although Westerdahl and Getsinger (1988) reported that several aquatic herbicides including diquat (6,7-dihydrodipyrido[1,2-α:2',1'-*c*]pyrazinediium ion), endothall (7-oxabicyclo [2.2.1] heptane-2,3-dicarboxylic acid), fluridone (1-methyl-3-phenyl-5-[3-trifluoromethyl)phenyl]-4(1*H*)-pyridinone), and 2,4-D ((2,4-dichlorophenoxy)acetic acid), will provide fair to excellent control of fanwort, there is little information in the literature documenting herbicide efficacy on this species. Early studies by Hiltibran (1974) showed that fanwort populations in central Illinois were not susceptible to treatment with endothall (granular and liquid formulations), 2,4-D, 2,4,5-T ((2,4,5-trichlorophenoxy)acetic acid), silvex (2-(2,4,5-trichlorophenoxy)propionic acid), or dichloroprop (2-(2,4-dichlorophenoxy)propanoic acid). Further investigations by Hiltibran (1977) reported inconsistent results when treating fanwort with diquat, endothall (dipotassium salt formulation), or diquat mixed with copper (triethanolamine complex of copper). Laboratory studies conducted by Reimer and Trout (1980) demonstrated that the herbicide terbutryn (2-(tert-butylamino)-4-(ethylamino)-6-(methyl-thio)-*s*-triazine) was effective for reducing the growth and vigor of fanwort, however terbutryn was never further developed for use as an aquatic herbicide in the U.S. Leslie (1986) reported that aquatic plant managers in Florida also had difficulty controlling fanwort with herbicides and that re-treatment was often necessary. Published information concerning non-target herbicide effects on water marigold is non-existent.

Currently, the most widely used aquatic herbicides in Connecticut are fluridone, diquat, and 2,4-D (G. Bugbee, pers. comm.). Of these products, fluridone shows the most promise as a selective treatment for control of fanwort. This is based on studies by Netherland et al. (1997) and Getsinger et al. (2001) which demonstrated aquatic plant selectivity using fluridone. Netherland et al. (1997) showed in an outdoor mesocosm study that 60- and 90-day exposures of 5 g fluridone L^{-1} were sufficient to reduce Eurasian watermilfoil (*Myriophyllum spicatum* L.) biomass with no effect on biomass production of several non-target species including elodea (*Elodea canadensis* Michx.), American pondweed (*Potamogeton nodosus* Poiret), sago pondweed (*Potamogeton pectinatus* L.), and wild celery (*Vallisneria americana* Michx). Based on field studies at several Michigan lakes, Getsinger et al. (2001) determined that maintaining a whole-lake fluridone concentration of 5 µg L^{-1} for more than 60 days provided 90 to 100% control of Eurasian watermilfoil without significantly impacting the diversity of the native plant community. Although we found no mention in the literature regarding fluridone efficacy on fanwort, a report by Leslie (1986) did reference a study conducted by the Florida Department of Natural Resources in which a single, fall application of fluridone eliminated another *Cabomba* species, *C. pulcherrima*, with only minor impacts on marginal plant species. Unfortunately, neither the rate of fluridone application nor the identity of the non-target species were given in this report. It is clear that up-to-date information describing the response of fanwort to fluridone treatment is needed.

The objectives of these studies were to evaluate the potential use of fluridone for selective control of fanwort, and to determine the effect of treatment on the non-target plant, water marigold.

MATERIALS AND METHODS

Studies were conducted in 55-L aquariums located in a large, walk-in growth chamber at the U.S. Engineer Research and Development Center, Waterways Experiment Station, Vicksburg, MS. The growth chamber was maintained at 24 ± 1 C with a 14:10-hr light:dark photoperiod. Overhead lighting (high-pressure sodium and metal halide lamps) provided a mean photosynthetic photon flux density of 520 ± 70 µmol photons m^{-2} s^{-1} at the water surface of each aquarium.

Fanwort and water marigold were collected from Lake Quonnipaug, Guilford, CT and Long Lake, Littleton, MA, respectively. Water marigold was collected in MA where it was not listed as a protected species. For each plant species, four apical stem cuttings (approximately 15 cm in length) were planted 5-cm deep into sediment-filled, plastic pots (14.5 cm tall by 10 cm in diameter). After planting, a thin layer of silica sand was added to the sediment surface to prevent sediment and nutrient dispersion into the water column. The sediment was collected from Brown's Lake, Vicksburg, MS and amended with ammonium chloride at a rate of 200 mg NH_4CL L^{-1} of sediment. Four pots of plants were placed in each aquaria filled with 51 L of Smart and Barko (1985) culture solution. Air was gently bubbled through air stones placed in each aquaria to provide circulation of the culture solution. Once a day, CO_2 was bubbled into each aquaria for 5 minutes to provide plants with an additional dissolved carbon source. We determined in a previous study that daily CO_2 was required to sustain active growth of these two plant species under our experimental conditions. The culture so-

lution was replaced in each aquaria twice weekly to minimize nuisance algal growth. Plants were established under these conditions for 21 days prior to treatment.

Immediately before fluridone treatment, four aquariums of each plant species were randomly selected and harvested to determine pretreatment biomass. For each pot of plants (four pots per aquarium), shoot biomass was clipped at the sediment surface and dried for 72 hours at 70C. The mean pretreatment biomass reported as g dry weight per pot ±1 S.D. was 1.33 ± 0.37 for fanwort and 0.63 ± 0.21 for water marigold. Analysis of variance tests showed that for each plant species, mean biomass was not statistically different among experimental units at the time of treatment.

A concentrated fluridone stock solution was prepared by dissolving the aqueous suspension formulation Sonar™ AS[4] into glass-distilled water. The stock solution was mixed using a stir plate and magnetic stir bar and was prepared approximately 1.0 hour prior to treatment. Calculated volumes of the fluridone stock solution were added (via pipette) to each aquarium to provide the following treatment concentrations: 0, 5, 7.5, 10, 15, 20, and 30 µg fluridone L^{-1}. The final concentrations of all fluridone treatments were verified via direct injection high performance liquid chromatography (HPLC) analysis in accordance with Lilly Research Laboratory Method AM-AA-CA-R005-AC-755.[5]

Since the mechanism of action for fluridone is inhibition of carotenoid synthesis, elevated levels of phytoene (carotene precursor) and decreased levels of β-carotene can be used as diagnostic tools for fluridone activity (Sprecher et al. 1998). The concentrations of three plant pigments (phytoene, β-carotene, and chlorophyll) were monitored in plant tissues at 14, 42, and 84 days after treatment (DAT) as an indicator of fluridone effect. Changes in chlorophyll content were monitored as a secondary effect to reduced carotenoid production. Carotenoid pigments protect chlorophyll from photooxidation (destruction by light), therefore reduced carotenoid synthesis will also result in decreased chlorophyll levels in fluridone-sensitive plant tissues.

Analytical procedures used for determining phytoene and β-carotene in plant tissues were based on techniques developed by Sandmann and Böger (1983) and Duke et al. (1985). Approximately 0.25 g fresh, apical shoot tissue was clipped from plants in each aquaria and homogenized with 6% (w/v) KOH in MeOH. The homogenate was centrifuged and the supernatant (pellet discarded) mixed vigorously with petroleum benzin. The resulting organic epiphase was transferred to UV cuvettes and absorbance measured spectrophotometrically (Beckman DU 640, Fullerton, CA) at wavelengths of 287 nm for phytoene and 445 nm for β-carotene. Pigment concentrations are reported as µg g^{-1} fresh weight using equations and extinction coefficients specified by Sandmann and Böger (1983).

Chlorophyll analysis was conducted on fresh leaf tissue (5-cm stem tip) using a dimethyl-sulfoxide (DMSO) extraction method (Hiscox and Israelstam 1979). A 3-ml sample of chlorophyll extract was transferred to a cuvette and the absorbance was quantified at 645 and 663 nm using a spectrophotometer against a DMSO blank. Chlorophyll content was calculated following equations used by Arnon (1949) and is expressed as mg total chlorophyll (chlorophyll *a* and *b*) g^{-1} fresh weight.

Weekly visual evaluations of plant health and appearance of herbicide-induced chlorosis were recorded. At the conclusion of the study (84 DAT), living shoot biomass was clipped at the sediment surface, dried for 72 hours at 70C and is reported as g dry weight per pot.

Treatments were arranged in a completely randomized design and were replicated three times. Experiments were conducted at different times for each plant species due to the availability of plant material from field sites. Therefore the data for each plant species were statistically analyzed and presented separately. The data were subjected to analysis of variance and regression procedures ($P \leq 0.05$) using SigmaStat software (Jandel Scientific, San Rafael, CA). Reductions in shoot biomass were observed to fit a non-linear regression model, $f(x) = ae^{-bx}$. Comparison of individual treatments with associated untreated controls were made using Dunnett's two-tailed *t* test. For all data, the equality of variance and normality assumptions were met.

RESULTS AND DISCUSSION

The growth response of fanwort and water marigold after 84 days exposure to varying concentrations of fluridone is shown in Figures 1 and 2, respectively. For both plant species, the shoot dry weight biomass response to fluridone was best described by an exponential curve. Compared with untreated plants, all fluridone rates significantly reduced shoot weight of fanwort and water marigold. Although all of the treatments significantly reduced shoot biomass, the data indicated that water marigold was less sensitive than fanwort

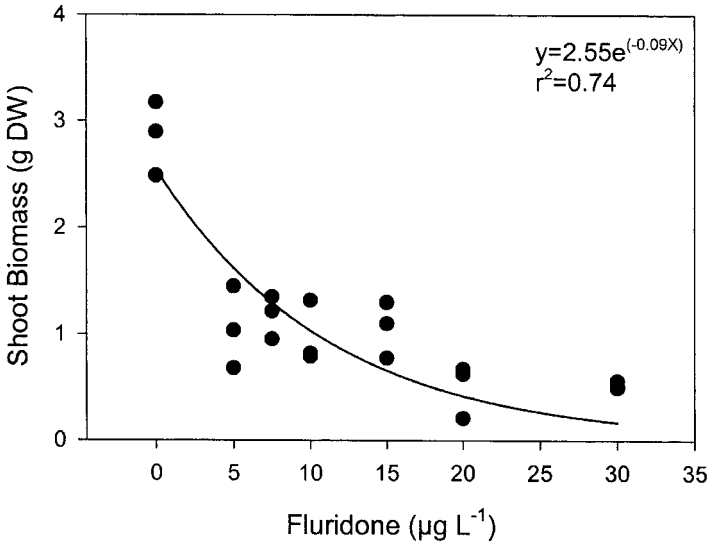

Figure 1. Effect of fluridone on shoot dry weight (DW) biomass of fanwort 84 days after treatment. Each symbol represents a mean of four weights. Shoot biomass (g DW ± 1 S.D.) at the time of treatment was 1.33 ± 0.37.

[4]Citation of trade names does not constitute an official endorsement or approval of the use of such commercial products.

[5]Lilly Research Laboratory. 1980. Method AM-AA-CA-R005-AC-755. Determination of fluridone in water by direct injection high pressure liquid chromatography. Eli Lilly and Co., Greenfield, IN. 4 pp.

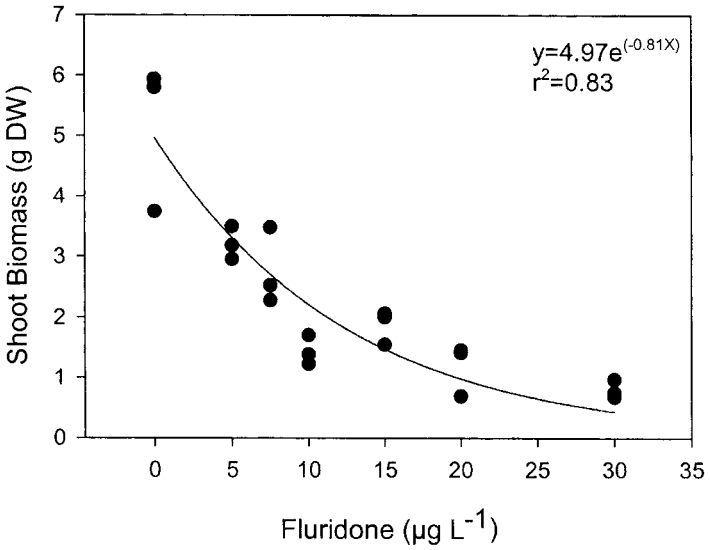

Figure 2. Effect of fluridone on shoot dry weight (DW) biomass of water marigold 84 days after treatment. Each symbol represents a mean of four weights. Shoot biomass (g DW ± 1 S.D.) at the time of treatment was 0.63 ± 0.21.

when exposed to low rates (5 µg L^{-1}) of fluridone. Water marigold biomass was reduced by 38% following static exposure to 5 µg fluridone L^{-1} compared to a 63% reduction for fanwort. A rate of 20 µg fluridone L^{-1} was required to reduce fanwort biomass by more than 80%, which in terms of management, can be considered a successful field application. However, at these high rates water marigold growth was inhibited by 77%, indicating little potential for selectively and successfully controlling fanwort with fluridone where water marigold resides.

Fluridone was applied to actively growing plants in these studies as indicated by an increase in biomass of untreated plants from pre- to post-treatment. For many herbicides including fluridone, rapid plant growth is essential for maximum herbicidal activity. Moreover, the Sonar™ AS specimen label states that best results will occur when fluridone is applied prior to initiation of weed growth or when weeds begin active growth. We observed this effect of application timing in an earlier fluridone trial on fanwort and water marigold. Results of this initial study (data not shown) demonstrated that rates as high as 30 µg fluridone L^{-1} did not affect fanwort or water marigold growth. However, little change in biomass of untreated plants from pre- to post-treatment occurred in this study, indicating growth had slowed or ceased shortly after treatment. Growth cessation was attributed to the fact that a dissolved carbon source (CO_2) was not supplied to plants for the duration of the study. The lack of active growth ultimately lowered susceptibility to fluridone. In follow-up studies we found that daily dosing plants with CO_2 was required to maintain active growth under our experimental conditions. The results emphasize the importance of timing fluridone application with active plant growth as well as stress the importance of interpreting and understanding experimental results.

Changes in plant pigment concentrations following fluridone treatment varied for each plant species and are shown in Figure 3 and Table 1. For fanwort, phytoene increased sig-

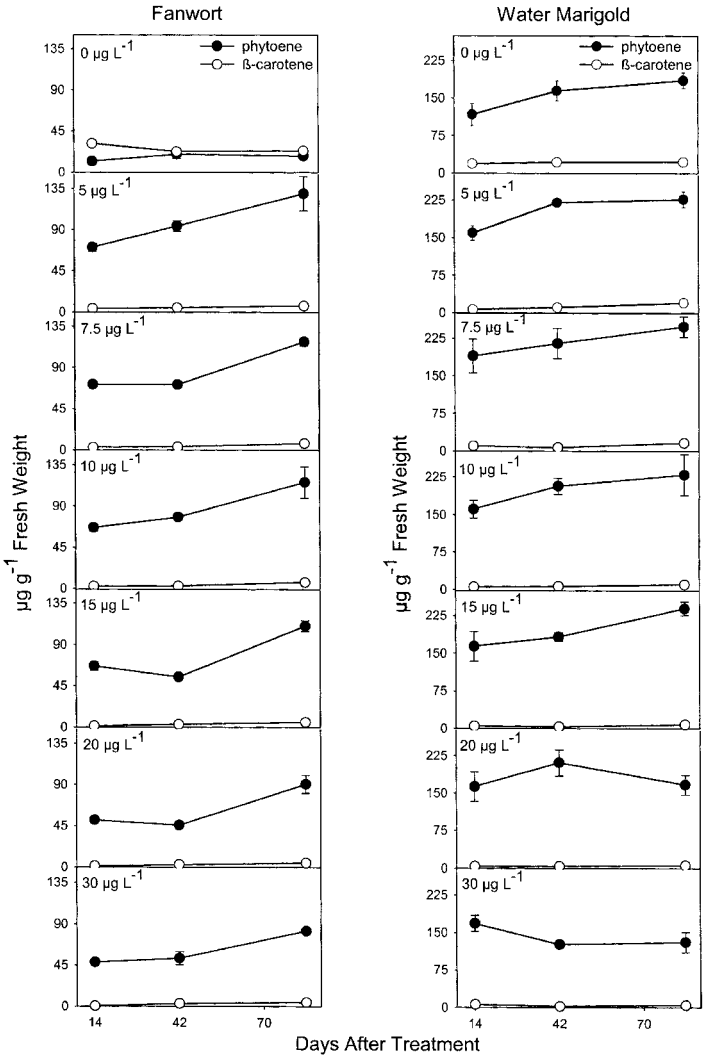

Figure 3. Concentrations of phytoene and β-carotene in fanwort and water marigold shoot tissues sampled 14, 42, and 84 days after exposure to 0, 5, 7.5, 10, 15, 20, and 30 g fluridone L^{-1}. Each data point represents a mean and standard error (bars) of three replicates (some error bars are obscured by the data symbol).

nificantly with fluridone treatment, while β-carotene decreased significantly (Figure 3). After14 days exposure to 5 µg fluridone L^{-1}, concentrations of phytoene and β-carotene were six times higher and eight times lower respectively, than concentrations observed in untreated plants. The effect on these two pigments (phytoene accumulation and β-carotene suppression) was noted through 84 DAT. At the final sampling period, phytoene was 86% higher and β-carotene was 71% lower in plants treated with 5 µg fluridone L^{-1} when compared with levels measured in untreated plants. Concentrations of both pigments remained stable with time in untreated plants. Exposure to fluridone also affected fanwort leaf chlorophyll content (Table 1). Chlorophyll was significantly reduced and decreased linearly over time for all treatment rates. Reductions in chlorophyll ranged from 49 to 85% compared with untreated plants at 84 DAT. Visible symptoms of chlorophyll destruction were also noted. The characteristic white coloration or "bleaching" of new growth

TABLE 1. TOTAL CHLOROPHYLL (*A* AND *B*) CONTENT (MG G^{-1} FRESH WEIGHT) IN FANWORT AND WATER MARIGOLD LEAF TISSUES SAMPLED 14, 42, AND 84 DAYS AFTER EXPOSURE TO 0, 5, 7.5, 10, 15, 20, AND 30 G FLURIDONE L^{-1}.

Fluridone	Days after treatment			Linear response[1]
	14	42	84	
Fanwort				
0	0.82	1.07	0.74	NS
5	0.26*	0.34*	0.38*	0.05
7.5	0.21*	0.24*	0.35*	0.02
10	0.17*	0.16*	0.28*	0.04
15	0.11*	0.08*	0.18*	0.02
20	0.06*	0.09*	0.16*	<0.00
30	0.06*	0.06*	0.11*	0.002
Water marigold				
0	0.46	0.50	0.70	NS
5	0.29	0.32*	0.69	0.01
7.5	0.27*	0.15*	0.40*	NS
10	0.14*	0.15*	0.26*	0.01
15	0.13*	0.09*	0.22*	NS
20	0.13*	0.09*	0.15*	NS
30	0.10*	0.05*	0.12*	NS

[1]Test for linear response of chlorophyll content over exposure time (14, 42, and 84 days after treatment) within each fluridone treatment rate and for each plant species. NS = not significant, $P > 0.05$.
[2]Asterisk (*) indicates significant difference from the untreated control within each sampling time based on Dunnett's two-tailed *t* test, $P \leq 0.05$.

(from stem apices and root crowns) was observed as early as 7 days after treatment in all fluridone-treated fanwort. The response on these plant pigments (increased phytoene, decreased β-carotene and chlorophyll) is typical following fluridone exposure and has been documented in many submersed plant species (Doong et al. 1993, Netherland and Getsinger 1995, Netherland et al. 1997, Sprecher et al. 1998).

The pigment response measured for water marigold was different than that observed for fanwort. One notable difference was that the background phytoene concentrations found in untreated water marigold were 8 to 10 times the phytoene concentrations found in untreated fanwort at all sampling times (Figure 3). Sprecher et al. (1998) found that phytoene in water marigold tissues was 7 to 10 times more concentrated than was measured for several other submersed plant species including coontail (*Ceratophyllum demersum* L.), water stargrass (*Heteranthera dubia* (Jacq.) MacMill.), egeria (*Egeria densa* Planch.), hydrilla (*Hydrilla verticillata* (L.f.) Royle), and Eurasian watermilfoil. The physiological significance of concentrated levels of phytoene in water marigold is unknown, however it is possible that phytoene is also a precursor for biosynthetic pathways other than carotenoids in this species. In addition to exhibiting normally high concentrations of phytoene, our data also showed that at each sampling period, differences in phytoene levels between fluridone-treated and untreated water marigold were not statistically significant (based on results of Dunnett's *t* test). These data further suggest that phytoene is utilized in other biosynthetic pathways in water marigold.

Although phytoene levels in water marigold were not typical of the fluridone-induced response shown in fanwort and other plants, β-carotene levels were affected by fluridone treatment (Figure 3). At 14 and 42 DAT, all fluridone treatments significantly reduced β-carotene concentrations compared with untreated plants. Reductions in β-carotene averaged 68 and 74% across all rates for samples collected 14 and 42 DAT, respectively. By 84 DAT, only plants treated with 10 µg fluridone L^{-1} and higher showed significantly decreased β-carotene. Exposure to fluridone concentrations of 7.5 µg L^{-1} and higher, decreased chlorophyll content in water marigold tissues (Table 1). Similar to results on β-carotene, chlorophyll content of plants treated with 5 µg fluridone L^{-1} increased with sampling time and was not significantly different than levels found in untreated plants at 84 DAT (Table 1). The fact that levels of these plant pigments (chlorophyll and β-carotene) recovered over time while the plants were subjected to static exposures of fluridone, indicates that water marigold can tolerate low fluridone concentrations.

In conclusion, the data from these studies showed that under these experimental conditions, the dose of fluridone required to substantially reduce fanwort growth (20 µg L^{-1} reduced biomass >80%), also severely impacted water marigold growth. As a result, it is unlikely that fluridone can be used successfully to manage fanwort in plant stands mixed with water marigold. However, the data also demonstrated that water marigold could tolerate 84 days of sustained exposure to 5 g L^{-1} fluridone with only minor effects on biomass. Water marigold growth was reduced by 38% at this rate, but plant pigments (phytoene, β-carotene, or chlorophyll) were not affected by this low-dose treatment. It is well documented in laboratory and field studies, that a long exposure (60 to 90 days) to 5 µg L^{-1} fluridone will control another nuisance submersed species, Eurasian watermilfoil (Netherland et al. 1997, Getsinger et al. 2001). Eurasian watermilfoil also commonly co-exists with water marigold. While the data presented here show there is limited potential for selective use of fluridone where fanwort and water marigold cohabit, the control of nearby Eurasian watermilfoil populations with minimal injury to water marigold may be feasible.

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