

Laboratory and greenhouse response of monoecious hydrilla to fluridone

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ABSTRACT

Spread of the monoecious biotype of hydrilla (*Hydrilla verticillata* L.f. Royle) into natural lakes and streams of northern-tier states is of concern to resource managers. In response, multiple eradication programs relying on fluridone have been initiated. Although fluridone controls monoecious hydrilla, limited quantitative information exists on effective concentrations and exposures. I conducted growth-chamber and mesocosm studies to determine sensitivity of hydrilla to various concentrations and exposures of fluridone. Sprouted hydrilla tubers were exposed to fluridone at concentrations ranging from 1.5 to 48 $\mu\text{g L}^{-1}$ and chlorophyll fluorescence yield of apical shoots measured via a pulse amplitude-modulated fluorometer was reduced by over 85% at fluridone concentrations $> 3 \mu\text{g L}^{-1}$. Hydrilla was also exposed to fluridone for intermittent periods and compared with plants that received continuous fluridone exposures. Removal of treated plants from fluridone for periods of 3 and 6 d followed by placing plants back in fluridone-treated water produced similar results through a 35-d period. Data confirm that a significant lag period exists between removal from fluridone exposure and recovery of photosynthetic pigments. I evaluated the response of sprouting tubers in greenhouse trials to fluridone at 1.5 to 12 $\mu\text{g L}^{-1}$. Fluridone at 6 and 12 $\mu\text{g L}^{-1}$ prevented hydrilla from emerging, whereas concentrations of 1.5 and 3 $\mu\text{g L}^{-1}$ reduced biomass by 84 to 96%. I also evaluated the response of established hydrilla to fluridone at concentrations of 3 to 48 $\mu\text{g L}^{-1}$. Although chlorophyll fluorescence yield of apical shoots from established plants was similar to that observed in the lab trials, the reduction in biomass was much slower over a 70-d period and was concentration dependent. Monoecious hydrilla is highly sensitive to fluridone and results suggest that control can be achieved via maintenance of low fluridone concentrations. Application of fluridone early in the growing season before shoot emergence or before accumulation of significant biomass is recommended to reduce overall exposure requirements.

Key words: aquatic herbicide, aquatic plant management, PAM fluorometer, photosynthesis, submersed invasive plant.

INTRODUCTION

Hydrilla is a submersed invasive plant that has been called the “perfect aquatic weed” because of multiple

physiological and reproductive characteristics that allow for aggressive growth and expansion across a wide variety of aquatic systems (Langeland 1996). There are two unique biotypes of hydrilla found in the United States (Madeira et al. 2004), and although the biology and management literature is dominated by research on the dioecious biotype of hydrilla, the continued northward spread of monoecious hydrilla suggests that additional focus on this biotype is needed. A literature review specific to monoecious hydrilla was recently completed (Richardson 2013) and this information will help serve as a valuable resource on prior research. Moreover, a recent report that recommends establishing new research priorities for this biotype of hydrilla should also serve as a future resource (Netherland and Greer 2014). Differences in growth, physiology, and reproductive biology between the dioecious and monoecious strains of hydrilla have been documented by various investigators (Harlan et al. 1985, Spencer and Anderson 1986, Carter et al. 1987, McFarland and Barko 1987, 1999, Steward and Van 1987, Van 1989, Van and Steward 1990, Sutton et al. 1992, Spencer et al. 1994, Spencer and Ksander 2001, Owens et al. 2012). There are still many unknowns associated with the biology, invasion ecology, and competitive interactions of monoecious hydrilla in the northern-tier states. Studies on competitive interactions of monoecious hydrilla in the United States remain limited (Spencer and Ksander 2000, Chadwell and Englehardt 2008). Cook and Luond (1982) noted that hydrilla has a wide range of tolerance for varying water chemistries and trophic levels, and the authors attributed the somewhat global distribution of the plant to this broad plasticity. Several authors have used plant biology information to predict the invasiveness of hydrilla in the northern-tier states; however, results have been mixed with no clear consensus (Spencer and Ksander 2001, Peterson et al. 2003, Maki and Galatowitsch 2008).

The provenance of the monoecious biotype of hydrilla is reported as Korea (Madeira et al. 2004) and the first descriptions of monoecious hydrilla occur during the early 1980s in Delaware, the Potomac River near Washington, DC, and several sites in North Carolina (Harlan et al. 1985). Monoecious hydrilla has since spread up and down the Atlantic seaboard from Georgia to Maine. Although the two biotypes are reported as different in appearance, hydrilla morphology can be highly plastic and genetic tests are necessary to distinguish between the biotypes (Ryan et al. 1991; Madeira et al. 2004; Rybicki et al. 2013). Introduction of monoecious hydrilla into numerous reservoirs in the mid-Atlantic states has resulted in heavy reliance on triploid grass carp and herbicides for management (Manuel et al. 2013). Monoecious hydrilla has also been introduced into

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California, Washington, and multiple Midwestern states (Indiana, Missouri, Ohio, and Wisconsin) and eradication efforts have been implemented in many of these sites. Two recent high-profile findings in the inlet of Lake Cayuga, NY and the Erie Canal, NY have heightened concerns regarding the spread of monoecious hydrilla throughout New York and the northern-tier regions of the country. The movement of monoecious hydrilla into the Northeast and Upper Midwest is of particular concern because of the vast number of natural lakes and streams that support native submersed aquatic vegetation and the unknown impact of hydrilla on these ecosystems. In addition to widespread establishment in reservoirs, the presence of monoecious hydrilla in numerous rivers (e.g., Ohio, Potomac, Croton, Eno) has managers concerned about limited management options in these sites and further establishment and spread via flowing waters (Netherland and Greer 2014).

The potential threat posed by monoecious hydrilla in the Northeast has led to several eradication projects that have relied on the herbicide fluridone. Fluridone is widely used for whole-lake or large-scale treatments because of low use rates, potential for species-selective control, cost-effectiveness, and limited restrictions on the use of the water posttreatment (Netherland et al. 1997, Getsinger et al. 2002). Despite a heavy reliance on fluridone for numerous hydrilla eradication programs, there is no published literature describing the response of the monoecious biotype to fluridone. Prior fluridone research on dioecious hydrilla established a high level of sensitivity by this biotype to concentrations as low as $5 \mu\text{g L}^{-1}$ (this is only 3.3% of the maximum label rate of $150 \mu\text{g L}^{-1}$) provided an extended exposure period was maintained (Netherland et al. 1993, Netherland and Getsinger 1995a, 1995b). Given the current reliance on fluridone for monoecious hydrilla eradication and control projects, development of data to quantify the concentration and exposure time relationship is warranted. To date, literature on management using herbicides on monoecious hydrilla is very limited, with single publications on diquat, bensulfuron methyl, and endothall (Van et al. 1987, Van and Vandiver 1992, Poovey and Getsinger 2010).

Prior research on fluridone and multiple genotypes of watermilfoil (*Myriophyllum spicatum* L.) have demonstrated the utility of a pulse amplitude-modulated (PAM) fluorometer to determine photosynthetic yield of plants exposed to different concentrations of fluridone (Berger et al. 2012, 2014). This nondestructive technique allows for multiple readings through time, and it is particularly useful in quantifying the response of plants to herbicides that cause bleaching of new growth such as fluridone, topramazine, diquat, and diuron (Elmore et al. 2011). Whereas prior fluridone research on dioecious hydrilla focused on measurement of chlorophyll and β -carotene to quantify a change in pigment concentrations (Netherland and Getsinger 1995a,b), the use of a PAM fluorometer provides a nondestructive method for measuring fluridone injury on the basis of the intensity of bleaching of target apical shoot tissue (Berger et al. 2012, 2014). A PAM fluorometer works by focusing a saturated beam of light on the desired region of the plant, and by measuring reradiation (fluorescence), a yield ratio is calculated by the instrument. A higher

fluorescence yield ratio indicates highly functioning chlorophyll, whereas a lower yield ratio indicates damaged or nonfunctioning chlorophyll (Bolhar-Nordenkamp et al. 1989). A comparison of photosynthetic yield data generated via the PAM fluorometer was strongly correlated to chlorophyll and β -carotene concentrations after exposure of hybrid watermilfoil and dioecious hydrilla to various concentrations of fluridone (Berger 2012, Berger et al. 2014).

To assess the sensitivity of monoecious hydrilla to fluridone, a series of growth-chamber and greenhouse trials was established to evaluate the following: 1) photosynthetic yield response of newly sprouted tubers to static fluridone concentrations under laboratory conditions; 2) photosynthetic yield response of monoecious hydrilla to intermittent exposures of fluridone under laboratory conditions; 3) photosynthetic yield and biomass response of sprouting tubers to fluridone under greenhouse conditions; and 4) photosynthetic yield and biomass response of established monoecious hydrilla to fluridone under greenhouse conditions.

MATERIALS AND METHODS

All laboratory and greenhouse studies were conducted at the University of Florida, Center for Aquatic and Invasive Plants (UF CAIP), Gainesville, FL between August 2012 and September 2013. A fluridone stock solution ($10 \text{ ng } \mu\text{L}^{-1}$) was created using technical-grade fluridone¹ (99.7% fluridone) dissolved in methanol. Analyses to confirm fluridone concentrations were conducted at the UF CAIP using an enzyme-linked immunoassay kit² capable of quantifying fluridone to $0.2 \mu\text{g L}^{-1}$. Water was sampled for fluridone concentrations from selected treatments at the beginning and conclusion of each study described below. A PAM fluorometer³ was used to measure the chlorophyll fluorescence yield by removing the plants from treatment flasks and focusing a saturated beam of light on apical shoot tissue (top 3 mm) and measuring fluorescence (Berger et al. 2012, 2014). The probe producing the saturating beam of light was placed in a clip that provided a 4-mm distance between the tip of the probe and apical plant tissue. This setting remained consistent across all studies and times. Plant dry weights were determined by placing hydrilla in a forced-air drying oven set to 70 C for a minimum of 48 h.

Response of hydrilla to fluridone in growth chambers

A monoecious hydrilla culture established from Lake Gaston, NC was utilized for laboratory assays. Tubers were removed from the sediment, placed on a tray to dry for 90 min, and then placed in 12-L containers with well water. Containers were transferred to the greenhouse and by 18 d, sprouted tubers that had formed a minimum of three new shoot meristems were collected for trials. Percival E-36L growth chambers⁴ were set to $25 \text{ C} \pm 1 \text{ C}$ and a photoperiod of 16 light (L) : 8 dark (D). Light intensity measured at five sites in the chambers using a Li-Cor 250 light meter ranged from 319 to $347 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The sprouted tubers were transferred to 1-L containers with well water and 2%

Hoagland solution and treated with fluridone at 0, 1.5, 3, 6, 12, and 48 $\mu\text{g L}^{-1}$. All treatments were conducted as static exposures for a 35-d period. To avoid any confounding issues with algal growth, well water and Hoagland's solution were exchanged at 21 d after treatment (DAT) and containers were cleaned and retreated with target fluridone concentrations.

At 3, 7, 14, 21, 28, and 35 DAT, plants were removed from the containers and the apical tips were carefully blotted and chlorophyll fluorescence yield was measured on two apical shoots using a PAM fluorometer (Berger et al. 2012). Each treatment was replicated four times, and studies were conducted in September through October 2012 and April through May 2013. ANOVA indicated no difference in response to fluridone between the two studies. Therefore, data were pooled for analysis. Chlorophyll fluorescence yield data are presented as mean values \pm 95% confidence intervals.

Response of hydrilla to intermittent fluridone exposures in growth chambers

A monoecious hydrilla culture established from J. Strom Thurmond Lake (Georgia/South Carolina border) was utilized for these laboratory assays. Tubers were removed from the sediment, placed on a tray to dry for 90 min, and then placed in 12-L containers with well water. Containers were transferred to the greenhouse and by 18 d sprouted tubers had formed a minimum of three shoot meristems. The growth chambers were set to 25 C \pm 1 C with a photoperiod of 16L : 8D. Light intensity was measured as described above and ranged from 313 to 370 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Single sprouted tubers were placed into a series of 1-L containers filled with well water and 2% Hoagland's solution. Treatments consisted of static exposures to 0, 6, and 12 $\mu\text{g L}^{-1}$ fluridone and a series of intermittent exposures to 6 $\mu\text{g L}^{-1}$ fluridone. Intermittent exposures were conducted by removing hydrilla from fluridone-treated containers, thoroughly rinsing the plants, and moving them to untreated water plus 2% Hoagland's solution. Intermittent exposures to fluridone were conducted over a 35-d period and treatment cycles included 3 d treated and 3 d untreated, 3 d treated and 6 d untreated, 3 d treated and 12 d untreated. At 3, 7, 14, 21, 28, and 35 DAT, all hydrilla was removed from containers and the apical tips were carefully blotted dry and chlorophyll fluorescence yield was measured using a PAM fluorometer as described above. Each treatment was replicated four times, and studies were conducted from November to December 2012 and February to March 2013. ANOVA indicated no difference in response to fluridone between the two studies and data were combined for analysis. Chlorophyll fluorescence yield data are presented as mean values \pm 95% confidence intervals.

Greenhouse response of sprouting tubers to fluridone

In the late summer of 2012, a series of 10-cm² containers were planted with monoecious hydrilla from Smith Mountain Lake, VA and allowed to form tubers from August through December under ambient conditions. This hydrilla was planted in containers that were filled with Margo Professional Topsoil⁵ (92% sand, 4% silt, 4% clay) amended

with fertilizer (Osmocote[®] 15-9-12)² at 1 g kg⁻¹ of soil. In March 2013, containers were removed from culture tanks and sediments were allowed to dry for a 20-d period (these pots have small slits at the bottom that allowed for rapid draining). Ten of the containers were harvested and the mean tuber number was 13.2 \pm 2.7 tubers/pot. Harvest confirmed that all tubers were quiescent when removed from the sediment in March 2013. After the 20-d drying period, three containers with sediments and *in situ* tubers were placed in each of a series of 95-L tanks in the greenhouse. The submersion of dried sediments containing hydrilla tubers is a strong cue for rapid sprouting (Netherlands 1997). The greenhouse was set to allow a minimum temperature of 15 C and we otherwise relied on ambient light and photoperiod. Temperatures ranged from 15 C to 27 C during the 70-d trial. The 95-L tanks were immediately treated with fluridone at concentrations of 0, 1.5, 3, 6, and 12 $\mu\text{g L}^{-1}$ and water was sampled at day 1, 35, and 70 to confirm herbicide concentration.

At 25, 45, and 70 d, two apical shoots formed from sprouting tubers were sampled from tanks and a PAM fluorometer was used to measure chlorophyll fluorescence yield. Shoot biomass was harvested at 70 d and dry weights were determined by placing plant tissue in a forced-air oven at 70 C for a period of 48 h. After harvest of shoot biomass, sediments were sorted to determine the percentage of sprouted tubers. Each treatment was replicated four times.

Greenhouse response of established hydrilla to fluridone

In May 2013, 10-cm² containers were filled with potting soil and amended with Osmocote as described above. Three sprouted monoecious hydrilla tubers from Lake Gaston were placed in each container and moved to a greenhouse containing 95-L treatment tanks. Hydrilla was given a 25-d pretreatment growth period. By 25 d, hydrilla had produced multiple shoots and the dense growth was just below the water surface at the time of application. Five pots were harvested to provide an estimate of pretreatment biomass. The 95-L tanks were treated with fluridone at 0, 3, 6, 12, and 48 $\mu\text{g L}^{-1}$. The study was conducted using ambient light and photoperiod and greenhouse temperatures ranged from 20 C to 28 C during the 60-d trial.

At 15, 30, 45, and 60 d, two apical shoots were sampled from each tank and a PAM fluorometer was used to measure chlorophyll fluorescence yield. Sediments were sorted to determine if any tubers were being formed during the course of the study. Shoot biomass was harvested at 70 DAT and dry weights were determined by placing plant tissue in a forced-air oven at 70 C for a period of 48 h. Each treatment was replicated four times, and data are presented as mean shoot biomass \pm 95% confidence intervals.

RESULTS AND DISCUSSION

Water samples collected at the time of application and at the completion of the studies indicated that target fluridone concentrations were within \pm 11% of nominal treatment rates for initial and final readings for all laboratory and greenhouse

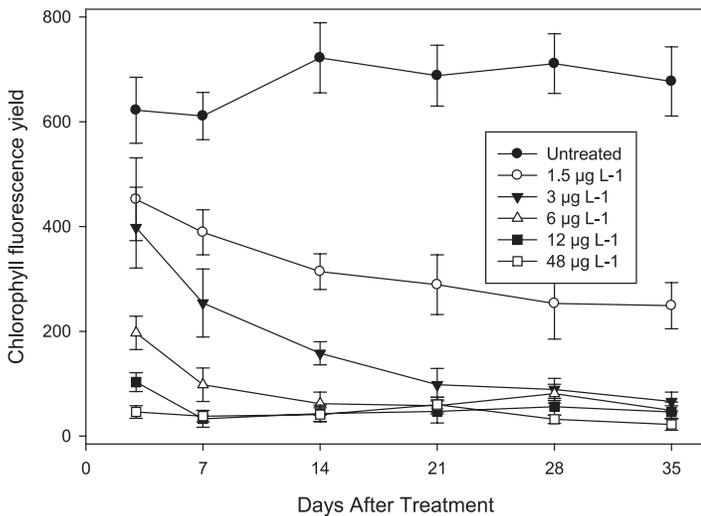


Figure 1. Effect of static fluridone exposure on chlorophyll fluorescence yield measured at six time intervals on monoecious hydrilla over a 35-d period. Each treatment was replicated four times and data are presented as mean values \pm 95% confidence intervals. Data represent the values for two studies that were combined for analysis.

trials. Given the low fluridone concentrations evaluated in this study, an 11% difference between nominal and measured concentrations would be considered negligible. The lack of ultraviolet light in growth chamber and greenhouse facilities precludes photolytic degradation of fluridone, and therefore fluridone tends to remain stable during these short-term laboratory and greenhouse studies (Netherland and Getsinger 1995a). For intermittent trials, water samples were taken at 3 and 6 d in the untreated water to determine if fluridone was potentially being released from the hydrilla tissue. Results indicated that fluridone remained $< 0.5 \mu\text{g L}^{-1}$.

Response of hydrilla to fluridone in growth chambers

All fluridone treatments reduced chlorophyll fluorescence yield of newly sprouted tubers. Whereas the higher treatment concentrations resulted in a more rapid rate of decrease, all fluridone concentrations $\geq 3 \mu\text{g L}^{-1}$ ultimately reduced chlorophyll fluorescence yield between 87 and 92%. (Figure 1). Prior work by Netherland and Getsinger (1995a,b) described a similar concentration threshold whereby the complete disruption of the phytoene desaturase enzyme pathway occurs at a low concentration of fluridone (3 to $5 \mu\text{g L}^{-1}$) and additional fluridone does not further reduce chlorophyll or β -carotene levels. The $1.5 \mu\text{g L}^{-1}$ treatment resulted in a slower initial response as well as a reduced overall response to fluridone compared with the other treatments through both studies. Although the impact was reduced at this low concentration, a 59 to 65% reduction in chlorophyll fluorescence yield was noted at 21 and 35 DAT.

Response of hydrilla to intermittent fluridone exposures in growth chambers

Comparison of sprouted tubers that received continuous fluridone exposures versus those that received intermittent

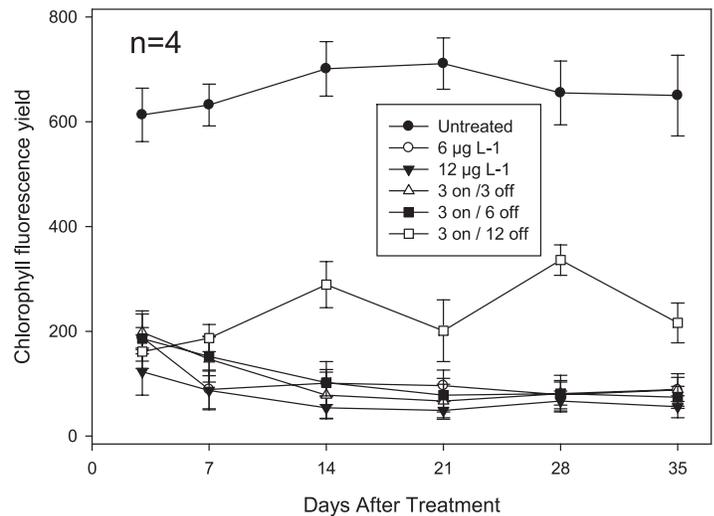


Figure 2. Effect of continuous and intermittent fluridone exposures on chlorophyll fluorescence yield measured at six time intervals on monoecious hydrilla over a 35-d period. Static treatments included fluridone at concentrations of 6 and $12 \mu\text{g L}^{-1}$. Intermittent treatments included exposure of hydrilla to fluridone at $6 \mu\text{g L}^{-1}$ for 3 d on, 3 d off; 3 d on, 6 d off; and 3 d on, 12 d off. Each treatment was replicated four times and data are presented as mean values \pm 95% confidence intervals. Data represent the values for two studies that were combined for analysis.

exposures indicated that removal of fluridone for a period of 3 and 6 d followed by retreatment did not affect chlorophyll fluorescence yield (Figure 2). In contrast, removal of fluridone for 12 d did result in an increase in chlorophyll fluorescence yield compared with the continuous exposures (Figure 2). There was only one concentration of fluridone tested for the intermittent exposure work ($6 \mu\text{g L}^{-1}$) and additional research to determine if a relationship exists between fluridone concentration and the intervals between intermittent exposures is warranted.

There are numerous fluridone applications that rely on injection of the liquid formulation over an extended period of time. In these cases, managers often question the need to continue dripping fluridone during temporary high flow events as this can greatly increase project costs. The present data indicate that once hydrilla is exposed to fluridone at a sufficient concentration, removal of the product for a period of a few days should not affect efficacy provided the manager can bring fluridone concentrations back to target levels. Although these data have clear implications for liquid injection strategies, the results are also informative in situations where a high flow, significant rainfall, or other dilution event may reduce fluridone concentrations in a pond or lake. In these cases, the manager may question whether or not additional liquid or pellet application would build on the initial application. The present data suggest that temporary loss of fluridone should not affect overall efficacy. It is important to note that in addition to the lag noted between removal of fluridone and pigment recovery, an even longer lag between pigment recovery and onset of healthy regrowth has been documented (Netherland et al. 1993, Netherland and Getsinger 1995a, 1995b).

Greenhouse response of sprouting tubers to fluridone

Tubers in the untreated mesocosm tanks emerged within 12 to 16 d of study initiation and once the shoots emerged, hydrilla began to rapidly fill the tanks. In contrast, there was no evidence of hydrilla emergence for tanks treated at 6 and 12 $\mu\text{g L}^{-1}$. Emerging hydrilla shoots were observed after the fluridone treatments at 1.5 and 3 $\mu\text{g L}^{-1}$; however, these plants remained very small and bleaching symptoms were evident.

Chlorophyll fluorescence yield was reduced by 78 to 90% for hydrilla treated at 1.5 and 3 $\mu\text{g L}^{-1}$ (Table 1). Biomass collected at 70 d showed a similar trend to the chlorophyll fluorescence yield. There was no shoot biomass harvested in tanks treated at 6 and 12 $\mu\text{g L}^{-1}$, whereas treatments of 1.5 and 3 $\mu\text{g L}^{-1}$ resulted in biomass reductions of 84 and 96%, respectively (Figure 3). Although hydrilla biomass was harvested, the overall condition of the plants treated with fluridone at 1.5 and 3 $\mu\text{g L}^{-1}$ was poor, with bleaching symptoms notable. Light intensities in the greenhouse would generally be in the range of 700 to 1,400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ or two to four times greater than light intensities in the greenhouse. The 70-d harvest confirmed that all treatment pots had supported tubers (min 8 and max 17) and > 88% of these tubers had sprouted. As noted with the growth-chamber study above, apical shoot tissue from newly sprouted tubers was highly sensitive to fluridone. The untreated control was forming new tubers by the end of the study (multiple rhizomes with swollen terminal buds), but there was no evidence of new tuber formation in any of the treatments.

There has been significant speculation over the years regarding a high level of activity of fluridone on newly sprouted tubers. These data demonstrate the potential for low concentrations of fluridone to prevent the establishment of hydrilla from sprouting tubers. Conditions in the field (light penetration, depth, etc.) will also influence fluridone activity on emerging hydrilla; however, these studies clearly demonstrate a high level of sensitivity of sprouting monoecious hydrilla tubers to low concentrations of fluridone. The potential interaction between fluridone activity and low light conditions was not investigated in this study; however, future studies on the relationship between light intensity and fluridone activity on newly sprouted tubers would be of value.

Greenhouse response of established hydrilla to fluridone

Treatment of established plants resulted in a rapid onset of fluridone symptoms, but a slow reduction of biomass.

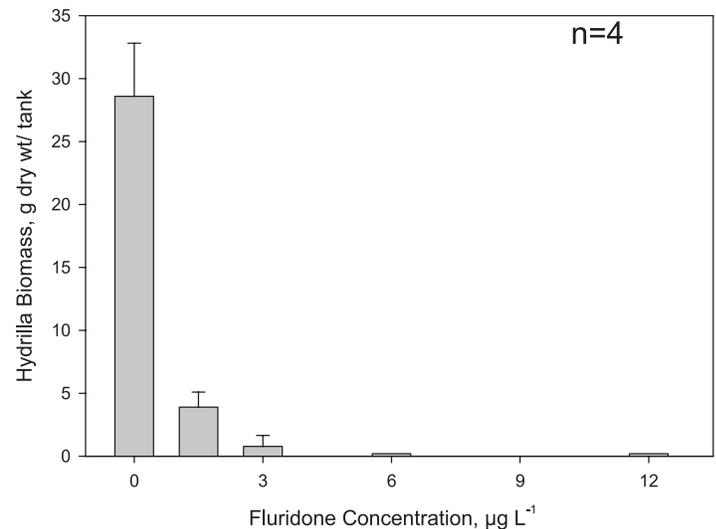


Figure 3. Monoecious hydrilla biomass under greenhouse conditions after a 70-d exposure to fluridone. Pots containing hydrilla tubers had been exposed to drawdown conditions for 20 d to stimulate tuber sprouting when pots were placed in treatment containers. Each treatment was replicated four times and data are presented as mean values \pm 95% confidence intervals.

This observation has been noted in numerous prior laboratory and mesocosm studies with fluridone (Netherlands et al. 1993, Netherlands and Getsinger 1995a and b, Netherlands et al. 1997). The chlorophyll fluorescence yield measurements were generally consistent with those from the laboratory studies and reductions of > 85% were noted at concentrations $\geq 6 \mu\text{g L}^{-1}$ (Figure 4). Although the apical shoots of mature plants were sensitive to fluridone as predicted by the chlorophyll fluorescence yield from the small-scale assays, the change in plant biomass is subtle during the initial weeks of observation. The untreated plants continued to grow; however, the biomass of the fluridone-treated plants remained static during the first 2 to 3 wk posttreatment. The 48 $\mu\text{g L}^{-1}$ treatment resulted in a faster collapse of the plant canopy compared with the 6 and 12 $\mu\text{g L}^{-1}$ treatments. By the harvest date, treatment concentrations of 6 to 48 $\mu\text{g L}^{-1}$ had reduced hydrilla biomass by 87 to 94% compared with the untreated control (but only 61 to 82% of the initial biomass) (Figure 5). Although greenhouse trials are predictive of sensitivity to fluridone, these controlled conditions are fairly benign and they do not necessarily represent the prevailing conditions

TABLE 1. CHLOROPHYLL FLUORESCENCE YIELD OF MONOECIOUS HYDRILLA APICAL SHOOTS OVER A 70-D PERIOD AFTER TREATMENT OF MESOCOSMS CONTAINING NEWLY SPROUTING TUBERS.

Fluridone ($\mu\text{g L}^{-1}$)	Chlorophyll Fluorescence Yield at 25 DAT (Mean \pm 95% CI) ²	Chlorophyll Fluorescence Yield at 45 DAT (Mean \pm 95% CI)	Chlorophyll Fluorescence Yield at 70 DAT (Mean \pm 95% CI)
0	677 (75)	714 (89)	685 (48)
1.5	121 (39)	127 (39)	141 (25)
3.0	84 (24)	71 (14)	53 (21)
6 ¹	51 (12)	-	-
12	-	-	-

¹If no value is provided, there was not sufficient tissue available for sampling.

²CI, confidence interval; DAT, days after treatment.

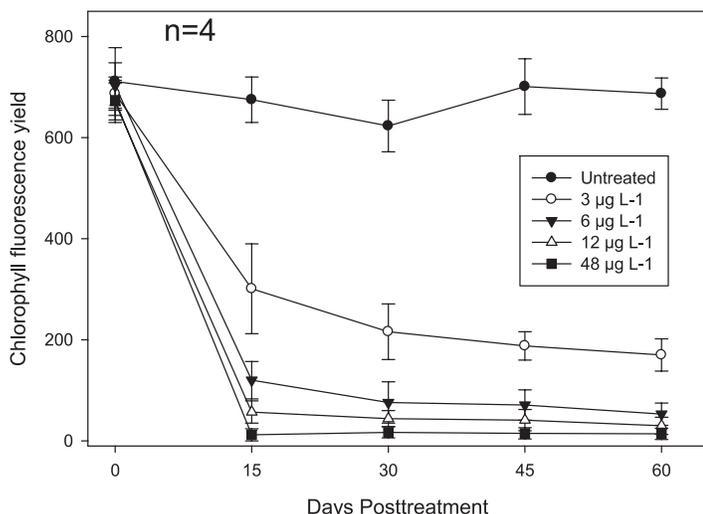


Figure 4. Effect of fluridone exposures on chlorophyll fluorescence yield of established monoecious hydrilla measured at five time intervals over a 60-d period. Each treatment was replicated four times and data are presented as mean values \pm 95% confidence intervals.

in the field (limited light, herbivory, mechanical stress from waves/flow) that may contribute to an enhanced rate of biomass reduction. Although untreated reference plants had formed multiple rhizomes (five to nine rhizomes/pot) at the time of harvest, there was no rhizome formation noted for any of the fluridone treatments.

The results of both the laboratory and greenhouse trials confirm that monoecious hydrilla is highly sensitive to fluridone. The data also provide the first evidence that intermittent exposures to an aquatic herbicide can provide equivalent results to continuous exposures. This finding suggests that managers may have greater flexibility than once thought in terms of treating newly emerging monoecious hydrilla in areas of increased water exchange. Prior chamber studies on dioecious strains of hydrilla proved to be predictive of field requirements for fluridone concentrations and exposures across a broad range of waters (Netherland and Getsinger 1995a, 1995b). Although these current data are some of the first published for fluridone and monoecious hydrilla, additional factors such as water temperature and light intensity should be evaluated to insure that fluridone remains active across a broad range of environmental gradients on newly establishing hydrilla. Overall, these data support ongoing eradication strategies with fluridone for control of monoecious hydrilla and suggest that resource managers may also have greater flexibility than initially thought in terms of concentrations necessary for control. Application of fluridone early in the growing season before shoots emerge or before accumulation of significant biomass is recommended to reduce overall exposure requirements. Additional research on sprouting dynamics of monoecious hydrilla tubers, optimizing fluridone treatment timing, and the interaction between granular fluridone formulations and sprouting monoecious hydrilla tubers is recommended.

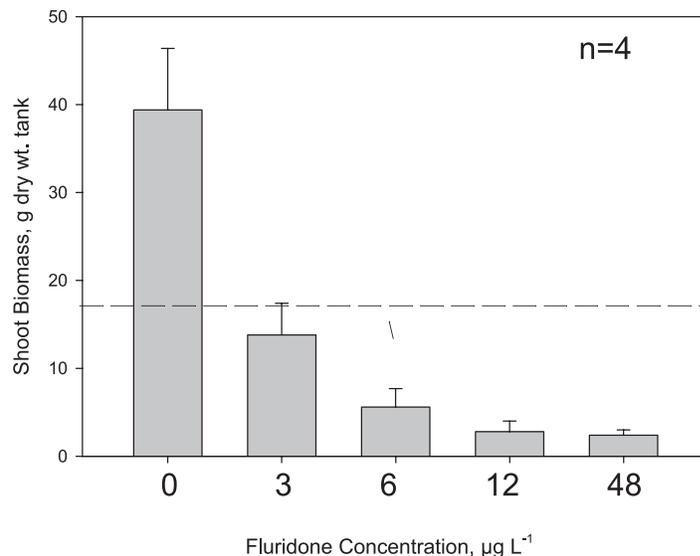


Figure 5. Monoecious hydrilla biomass after a 70-d exposure of established plants to fluridone. The dashed line represents the initial biomass. Each treatment was replicated four times and data are presented as mean values \pm 95% confidence intervals.

SOURCES OF MATERIALS

¹SePRO Corporation, Carmel, IN 46032.

²Fluridone Immunoassay Kit, Modern Water, New Castle, DE 19720.

³PAM Fluorometer—Mini-PAM, Walz, Effetrich, Germany.

⁴Percival E36L Growth Chamber, Boone, IA 50036.

⁵Margo Professional Topsoil, Margo Garden Products, Folkston, GA 31537.

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