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Growth regulating hydrilla and subsequent effects on habitat complexity

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ABSTRACT

Plant growth regulators (PGRs), such as flurprimidol ([α-(1-methylethyl)-α-[4-(trifluoromethoxy) phenyl]-5 pyrimidinemethanol]), and herbicides with growth regulating properties, such as imazamox (2-[4.5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-(methoxymethyl) 3-pyridinecarboxylic acid-3-pyridinecarboxylic acid), and bensulfuron-methyl (methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]methyl]benzoate) have been reported to control or suppress hydrilla (*Hydrilla verticillata* [L.f.] Royle) growth while maintaining the vegetative structure important for fish and invertebrates. This change in vegetative structure created by the use of PGRs and herbicides with growth-regulating properties has not been quantified in terms of habitat complexity. Therefore, we investigated the effects of a static exposure of flurprimidol (active ingredient 150 and 300 µg ai L^{-1}) and bensulfuron-methyl (5 µg ai L^{-1}), as well as a 14-day exposure of imazamox (50 and 100 µg ai L^{-1}) on hydrilla growth and aquatic habitat complexity. Results at 12 weeks after treatment indicate that flurprimidol, imazamox, and bensulfuron-methyl reduced hydrilla shoot length 46 to 69%. Imazamox (50 and 100 µg ai L^{-1}) and bensulfuron-methyl (5 µg ai L^{-1}) reduced hydrilla shoot biomass by an average of 68%. Habitat complexity was reduced in all treatments by an average of 93%. These results indicate that plant growth regulation may be a viable tool to decrease hydrilla's "weediness" while maintaining habitat complexity beneficial for fish and other aquatic fauna.

Key words: bensulfuron-methyl, flurprimidol, habitat complexity, *Hydrilla verticillata*, imazamox, plant growth regulation

INTRODUCTION

Hydrilla (*Hydrilla verticillata* [L. f.] Royle) infestations provide unique challenges for biologists and aquatic plant managers, especially in Florida. In 2007, hydrilla was present in more than 50,585 ha of Florida's public waters and was identified by the Florida Department of Environment Protection (FDEP) as the state's most expensive aquatic invasive plant to manage (FDEP 2007); approximately $16 million was needed to control hydrilla in Florida in fiscal year 2007 (FDEP 2007). Hydrilla's prolific growth and dense canopy hinders

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Received for publication September 29, 2011 and in revised form March 1, 2012.

industrial, commercial, and recreational water uses, as well as disrupts the native aquatic habitat. It forms a dense surface canopy that often displaces native plant species (Haller 1978), initiating subsequent changes in macrophyte-dependent fish and invertebrate communities (Dibble et al. 1996b, Theel and Dibble 2008, Theel et al. 2008).

Traditionally, habitat complexity was defined as an abundance metric (i.e., stem density and/or plant biomass), in which increased stem densities were synonymous with more complex habitats (Crowder and Cooper 1982, Savino and Stein 1982, Gotceitas and Colgan 1989). Complexity has been documented to differ among aquatic plant species, suggesting each species provides a unique contribution to the waterscape (Dibble et al. 1996a, 2006). Plant-specific architecture has been quantified as spatial complexity (I_{hv}), a relationship between the frequency and arrangement of interstitial spaces between stems and leaves, for individual plant species (Dibble et al. 1996a, 2006) and for native and nonnative-dominated submersed plant communities (Valley and Bremigan 2002, Theel and Dibble 2008). Theory predicts predator feeding rates are greatest at intermediate levels of structure in lakes (Crowder and Cooper 1979) and is further supported by experiments investigating effects of plant abundance on fish foraging (Crowder and Cooper 1982, Savino and Stein 1982). In addition to metrics such as stem densities, Valley and Bremigan (2002) also documented the importance of evenly partitioned vegetative complexity throughout the water column for successful largemouth bass foraging. Additional experiments have documented declines in fish foraging efficiency as I_{hv} increases in a simulated hydrilla invasion (Perret 2007, Theel and Dibble 2008). Therefore, hydrilla not only infests public waters causing severe economic losses to industry, recreation, and property values, but also alters habitat structure critical to fish foraging, growth, and ultimately survival.

An optimal level of I_{hv} for fish and invertebrates has not been determined; however, fish are most successful with even vertical partitioning of complexity, characteristic of a heterogeneous native plant assemblage (Valley and Bremigan 2002, Theel and Dibble 2008). This optimal state is difficult to maintain, especially with the establishment of an invasive plant such as hydrilla. In laboratory studies, plant growth regulators (PGRs) have been reported to achieve this balance by controlling nuisance aquatic plant growth while maintaining some level of vegetated structure (Lembi and Chand 1992, Netherland and Lembi 1992, Nelson 1996, Nelson 1997). PGRs such as flurprimidol ([α-(1-methylethyl)-α-[4-(trifluoromethoxy) phenyl]-5 pyrimidinemethanol]) and paclobutrazol [(R*,R*)-(+/-)-β-[(4-Chlorophenyl)methyl]-α-(1,1dimethylethyl)-1H-1,2,4-triazole-1-ethanol] inhibit gibberellin synthesis in plants, which is needed for stem elongation and other developmental processes (Jones 1973). Although PGRs were originally developed for the turf and ornamental vegetation management industry, their use in aquatic systems has been investigated at lab and mesocosm scales (Lembi and Chand 1992, Netherland and Lembi 1992, Nelson 1996, 1997).

The new, federally registered aquatic herbicide imazamox (2-[4.5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-(methoxymethyl) 3-pyridinecarboxylic acid-3-pyridinecarboxylic acid) can also be used to suppress and growth regulate hydrilla below nuisance levels (BASF 2008). In addition, bensulfuron-methyl (methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]methyl] benzoate) has demonstrated growth-regulating properties at low use rates (Anderson 1988). Both bensulfuron-methyl and imazamox are herbicides that inhibit acetolactate synthase (ALS), a key enzyme necessary to produce amino acids (Weed Science Society of America 2007). The change in vegetative structure created by the use of PGRs and herbicides with growth-regulating properties has not yet been quantified in terms of habitat complexity; therefore, it is necessary to determine if aquatic plant management strategies such as the application of PGRs or herbicides with PGR properties influence habitat I_{hv} while providing adequate control or suppression of the target weed species.

Aquatic plant managers rely on relatively few herbicides to meet weed control goals. Continued use of a limited number of herbicides will increase the threat of developing herbicide resistance (Richardson 2008), which has been recently documented in Florida with the herbicide fluridone (MacDonald et al. 2001, Michel et al. 2004). Development and evaluation of new herbicides and PGRs are critical to mitigate resistance to repeated use of the same few active ingredients. The use of PGRs and herbicides that have growth-regulating properties could be a viable tool for controlling invasive aquatic plants by reducing their "weediness" while maintaining habitat complexity beneficial for fish and other aquatic fauna. Therefore, our objectives were to (1) compare the growth regulating properties of flurprimidol, imazamox, and bensulfuron-methyl against the submersed invasive plant hydrilla, and (2) investigate the effect of flurprimidol, imazamox, and bensulfuron-methyl on habitat complexity of a hydrilla plant bed.

MATERIALS AND METHODS

The study was conducted in aquaria (55 L) housed within an indoor environmental growth chamber at the US Army Engineer Research and Development Center (USAERDC) in Vicksburg, Mississippi. All aquaria were filled with a growth water solution (Smart and Barko 1985) to a constant volume (48 L). Hydrilla was field collected from the Rainbow River near Dunnellon, Florida. Four apical stem segments (15 cm) were planted per sediment-filled pot (0.926 L), and 4 pots were placed in each aquarium. Sediment was collected from Brown's Lake, Vicksburg, and amended with NH_4Cl (200 mg L^{-1}) and Osmocote 19-6-12 fertilizer (2.1 g per pot). Photoperiod (14:10 h light:dark) and light intensity (588 ± 91 µmol m^{-2} s^{-1}) were maintained for optimal hydrilla growth. Temperature (21.8 ± 0.6 C) was set to mimic early-spring field conditions in Florida. The plants acclimated for 2 weeks prior to treatment (25 Sep 2008). At the time of treatment, plants were actively growing and had established root systems.

Individual stock solutions were prepared for each herbicide or PGR and pipetted into respective aquaria at concentrations of 5 µg active ingredient per liter (ai L^{-1}) bensulfuron-methyl, 150 and 300 µg ai L^{-1} flurprimidol, and 50 and 100 µg ai L^{-1} imazamox, totaling six treatments including an untreated reference. Bensulfuron-methyl and flurprimidol treatments were static exposures, and the imazamox treatment was a 14-day exposure designed to mimic field condi-

tions (MD Netherland, pers. comm.). All aquaria treated with imazamox were drained and filled three times and replaced with growth culture solution 14 days after treatment (DAT) to remove imazamox residues. Treatments were randomly assigned to aquaria and replicated four times within two blocks. Aquaria in block one were used for harvest, and aquaria in block two were utilized for I_{hv} digital photography calculations.

To determine the efficacy of flurprimidol, imazamox, and bensulfuron-methyl on hydrilla stem length, stem density, and shoot biomass, one pot was removed from each aquaria at 0 (pretreatment), 4, 8, and 12 weeks after treatment (WAT). Viable shoots (main, lateral, and stolons) were enumerated to estimate stem density (stem number per pot) and main stem length per pot was measured (cm) from the sediment surface to the top of the longest stem. All viable plant material above the soil surface was removed, dried (70 C for 72 h), and weighed (g) to obtain biomass.

To determine the physiological response of treatment, two apical stem tips (3 cm) per aquaria were harvested 4, 8, and 12 WAT for chlorophyll content analysis using methods by Hiscox and Israelstam (1979). Chlorophyll was determined with a Beckman spectrophotometer at wavelengths of 645 and 663 nm. Chlorophyll content was calculated using equations by Arnon (1949) and expressed as milligrams chlorophyll per gram of fresh weight (mg chl g^{-1} $fwgt^{-1}$).

Aquaria were digitally photographed 4, 6, 8, and 12 WAT to quantify spatial complexity; the water column and associated plant material represented the experimental unit. All images were captured at an equal distance (0.5 m) from the front of each aquarium. No plants were removed from the block of treated aquaria set aside for photography purposes until the end of the experiment (12 WAT) to prevent interference with any potential temporal effects on spatial complexity. Using Adobe Photoshop (version CS2) software, two horizontal (h) and vertical (v) line transects were superimposed onto each aquaria's image, spanning the width and depth of the water column. Initially, the location of each transect was randomly placed and then remained constant for each image. Length in cm (l) and frequency of interstices per meter (f) were calculated for each h and v line to obtain an index of spatial complexity ($I_{hv;}$ Dibble et al. 1996a, Theel and Dibble 2008), where $I_{hv} = f_h/l_h + f_v/l_v$. Mean spatial complexity was calculated based on two replicate transects per aquaria. The 12 WAT complexity measurements were removed from analysis due to extreme algae growth that concealed the interstitial spaces. Algal growth was minimized by wiping the inside walls of aquaria with a paper towel weekly throughout the study.

Hydrilla shoot length, density, biomass, and chlorophyll concentrations were analyzed using analysis of variance (ANOVA; SAS 2003). Time periods, 0 (pretreatment), 4, 8, and 12 WAT were analyzed separately. Treatment differences were detected at an alpha (α) of 0.05, and a Student-Newman-Keuls (SNK) adjustment was used for multiple comparisons. Normality assumptions were assessed for all response variables. Base-10 log transformations were used when normality assumptions were not met, and respective means were back transformed for graphical depictions.

Repeated measures analysis using the Proc Mixed procedure (SAS 2003) evaluated mean spatial complexity differences between treatments using $\alpha = 0.05$. Information criterion (Akaike 1973) was used to choose the best fit covariance structure, which was first-order autoregressive. A Tukey-Kramer adjustment was used for multiple comparisons. Transformations of I_{hv} (base-10 log) were needed to meet normality and variance assumptions, and means were back transformed for graphical purposes.

RESULTS AND DISCUSSION

Visual differences between treatments were apparent as early as 7 DAT and remained consistent throughout the study. Overall, hydrilla growth (stem length and lateral stem production) was severely inhibited, and stem tips displayed red pigmentation (anthocyanin accumulation) by 7 DAT with bensulfuron-methyl and imazamox treatments. Shoot elongation rapidly decreased and lateral stem production increased in flurprimidol treated hydrilla compared to the untreated reference, similar to previous research (Lembi and Chand 1992, Netherland and Lembi 1992, Nelson 1997). New stem tip formation and rapid shoot elongation was visible in the imazamox-treated (50 µg ai L^{-1}) hydrilla as early as 6 WAT; however, new plant tissues were deformed, and numerous shoots grew from individual internodes creating a "witch's broom," a symptom commonly associated with plants treated with ALS herbicides (Wersal and Madsen 2007). Hydrilla treated with imazamox (100 µg ai L^{-1}) had less consistent regrowth patterns. Only three of the eight aquaria treated with imazamox exhibited abnormal new plant tissue by 12 WAT; no regrowth was observed in the other five replicates. No regrowth was observed for hydrilla treated with the static treatment of bensulfuran methyl (5 µg aiL^{-1}) by 12 WAT.

Main stem length of the untreated control remained essentially the same from 4 to 12 WAT (Figure 1), where stem density (Fgure 2) and shoot biomass (Figure 3) increased through the duration of the study. This is consistent with hydrilla's growth habit in natural settings, where once hydrilla reaches the water surface it branches profusely to efficiently compete for sunlight, forming a dense canopy (Haller and Sutton 1975). Compared to the pretreatment levels, the untreated hydrilla elongated rapidly to reach the water surface by 4 WAT and then continued to produce lateral stems up to 12 WAT.

Flurprimidol, imazamox, and bensulfuron-methyl consistently regulated hydrilla height irrespective of concentration ($P < 0.01$; Figure 1), compared to the control, which increased several-fold compared to pretreatment levels. Throughout the course of the study, hydrilla growth responded similarly regardless of an increase in PGR or herbicide concentration. The only difference between treatments, with respect to stem length, was observed 4 WAT, where bensulfuron-methyl–treated (5 µg ai L^{-1}) hydrilla was significantly shorter than flurprimidol-treated hydrilla. Main shoot length was reduced 46 to 69% by all treatments 12 WAT compared to nontreated plants remaining near pretreatment levels, with no difference between herbicide treatments.

While all bensulfuron-methyl and imazamox treatments reduced stem length, only hydrilla exposed to flurprimidol produced a greater number of stems per pot 4 and 8 WAT ($P < 0.05$; Figure 2). At the conclusion of the study, flurprimidol-

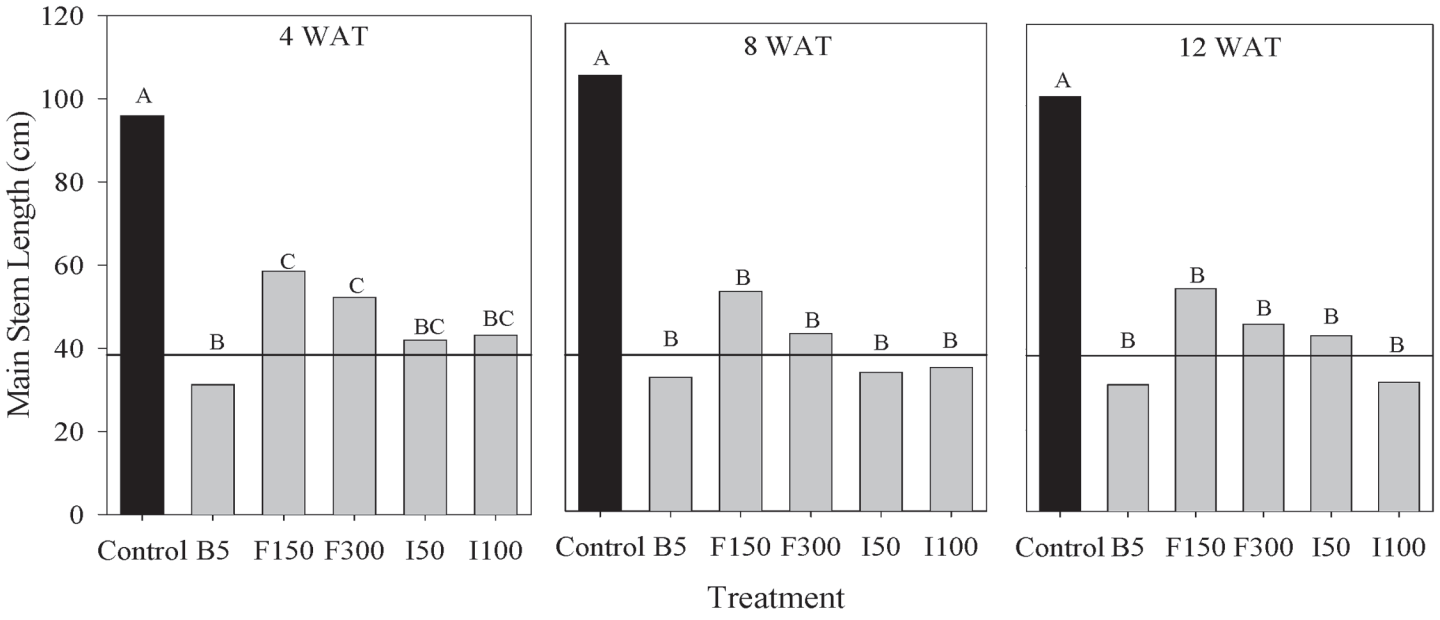

Figure 1. The effect of bensulfuron-methyl at 5 µg ai L^{-1} (B5), flurprimidol at 150 and 300 µg aiL^{-1} (F150 and F300), and imazamox at 50 and 100 µg ai L^{-1} (I50 and I100) on hydrilla main stem length (cm) 4, 8, and 12 weeks after treatment (WAT). Different letters within a graph differ significantly ($P < 0.05$). Line represents pretreatment main stem length.

treated hydrilla produced a similar quantity of stems as the control but in approximately half the volume. This dense, stoloniferous growth habit is a distinguishing characteristic of flurprimidol-treated plants (Netherland and Lembi 1992, Lembi and Chand 1992, Nelson 1996, Nelson 1997). Although approximately 95% of flurprimidol treated hydrilla stems were located in the lower half of the aquaria, shoot biomass remained similar to the control throughout the study (Figure 3). In outdoor mesocosms, Nelson (1997) reported a 50% reduction in hydrilla shoot biomass 6 and 12 WAT following a one-time flurprimidol application of 100 and 200 µg ai L^{-1} with a 28-day exposure and a split application of 100 µg ai L^{-1}. Lembi and Chand (1992) also reported reduced hydrilla dry weight as flurprimidol concentrations and exposure time increased, but noted hydrilla stem length was more sensitive to flurprimidol than hydrilla biomass.

Hydrilla treated with bensulfuron-methyl and imazamox responded differently than hydrilla exposed to flurprimidol with regard to stem density (Figure 2), shoot biomass (Figure 3), and chlorophyll content (Figure 4). Increasing the

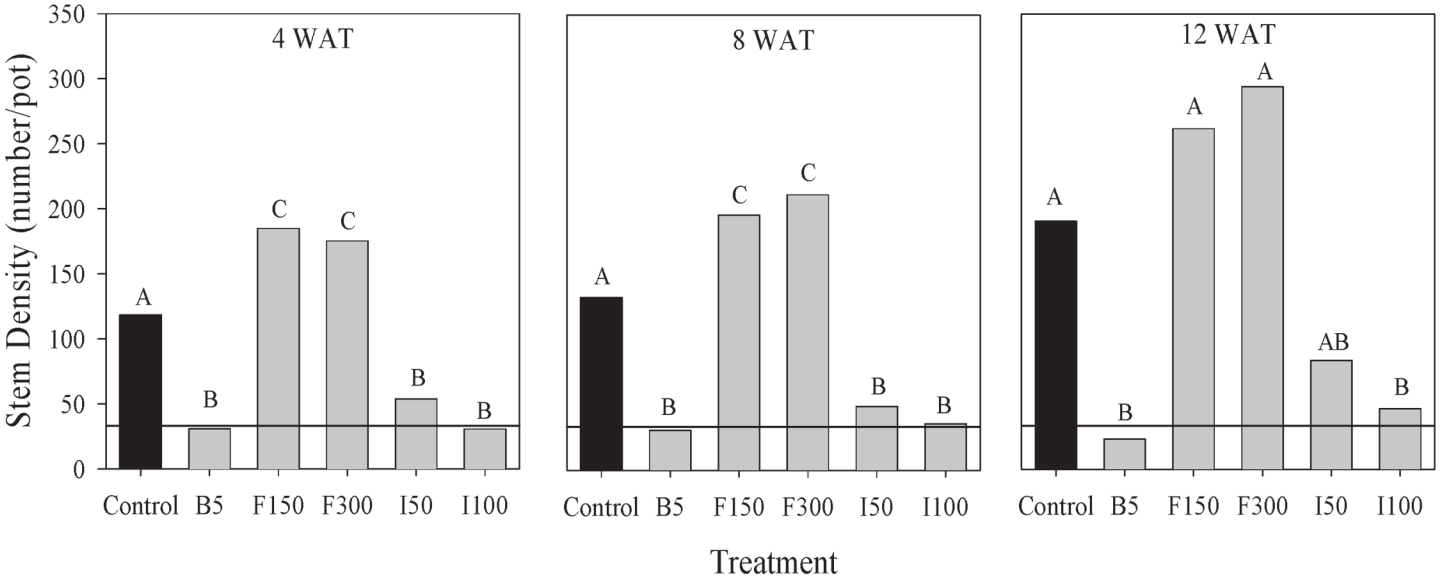

Figure 2. The effect of bensulfuron-methyl at 5 µg ai L^{-1} (B5), flurprimidol 150 and 300 µg ai L^{-1} (F150 and F300), and imazamox at 50 and 100 µg ai L^{-1} (I50 and I100) on hydrilla stem density (number/pot) 4, 8, and 12 weeks after treatment (WAT). Different letters within a graph differ significantly ($P < 0.05$). Line represents pretreatment stem density.

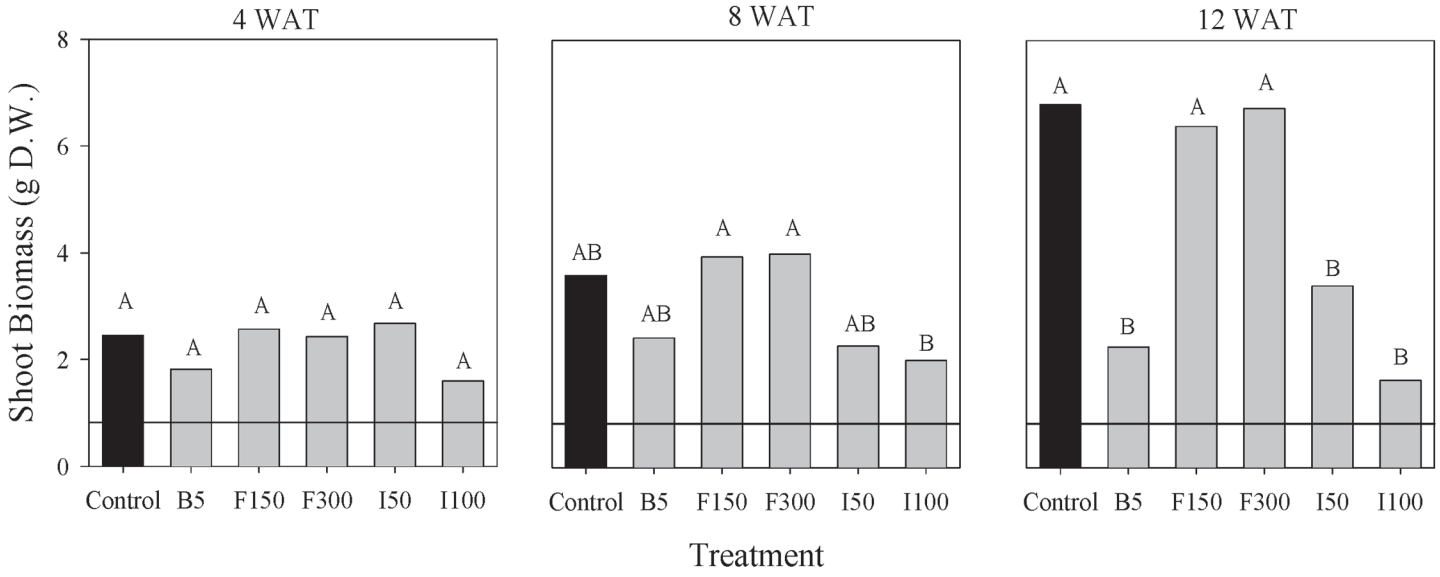

Figure 3. The effect of bensulfuron-methyl at 5 µg ai L^{-1} (B5), flurprimidol 150 and 300 µg ai L^{-1} (F150 and F300), and imazamox at 50 and 100 µg ai L^{-1} (I50 and I100) on hydrilla shoot biomass (g dry weight [D.W.]) 4, 8, and 12 weeks after treatment (WAT). Different letters within a graph differ significantly ($P < 0.05$). Line represents pretreatment shoot biomass.

concentration of imazamox did not increase the response intensity of hydrilla, except for stem density at 12 WAT. Overall, bensulfuron-methyl and imazamox reduced hydrilla stem density, shoot biomass, and chlorophyll content by an average of 71, 64, and 73%, respectively, compared to the control ($P < 0.05$). Anderson (1988) found a similar shoot biomass response to low concentrations of bensulfuron-methyl. No differences were observed between bensulfuron-methyl and either rate of imazamox, although exposure times were drastically different. Bensulfuron-methyl and imazamox concentration–exposure time relationships with hydrilla have not been extensively researched and should be further investigated.

Mean I_{hv} was reduced by all treatments compared to the control ($P < 0.01$; Figure 5). Although visual structural differences were observed following application with a plant growth regulator (flurprimidol) and herbicides with growth-regulating properties (bensulfuron-methyl and imazamox), spatial complexity did not differ between treatments ($P < 0.01$; Figure 5). Complexity ranged from 4.5 for the 100 µg ai L^{-1} imazamox treatment to 104 for the untreated control. Hydrilla bed complexity in the untreated reference was 16 times greater than all treatments. Theel and Dibble (2008) found a monotypic hydrilla bed had greater complexity (7-fold) and poorer bluegill foraging efficiency compared to a native plant bed with a lower complexity value. Due to the highly complex

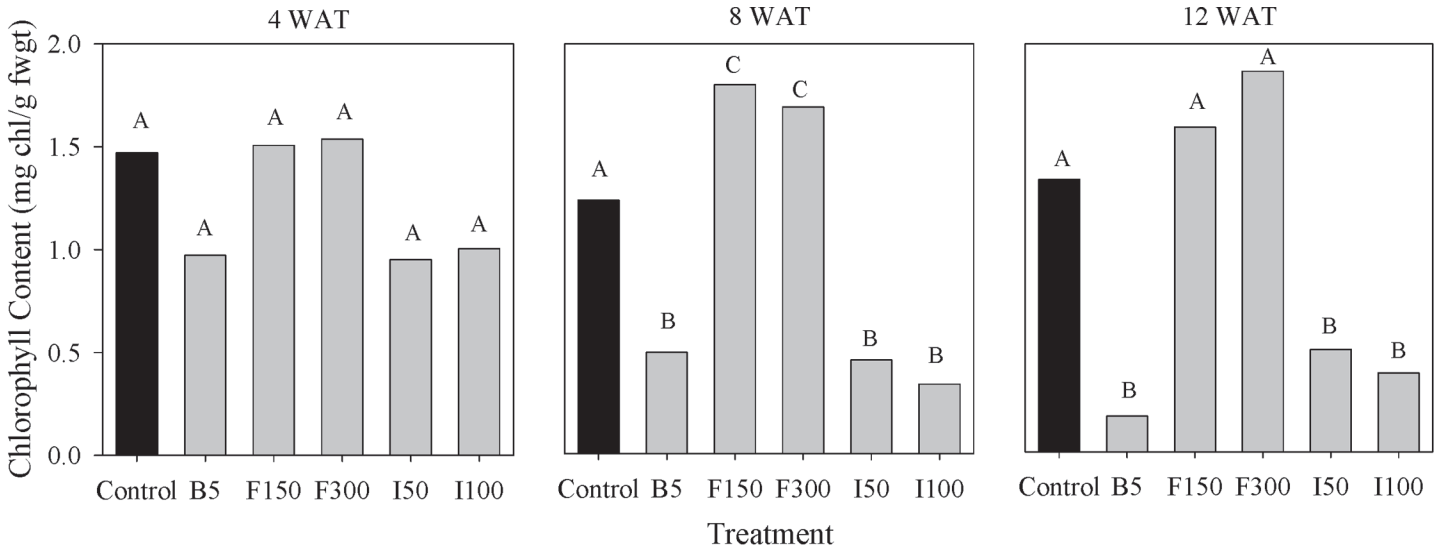

Figure 4. Effects of bensulfuron-methyl at 5 µg ai L^{-1} (B5), flurprimidol 150 and 300 µg ai L^{-1} (F150 and F300), and imazamox at 50 and 100 µg ai L^{-1} (I50 and I100) on chlorophyll content (mg chl/g fwgt) of hydrilla 4, 8, and 12 weeks after treatment (WAT). Different letters within a graph differ significantly ($P < 0.05$).

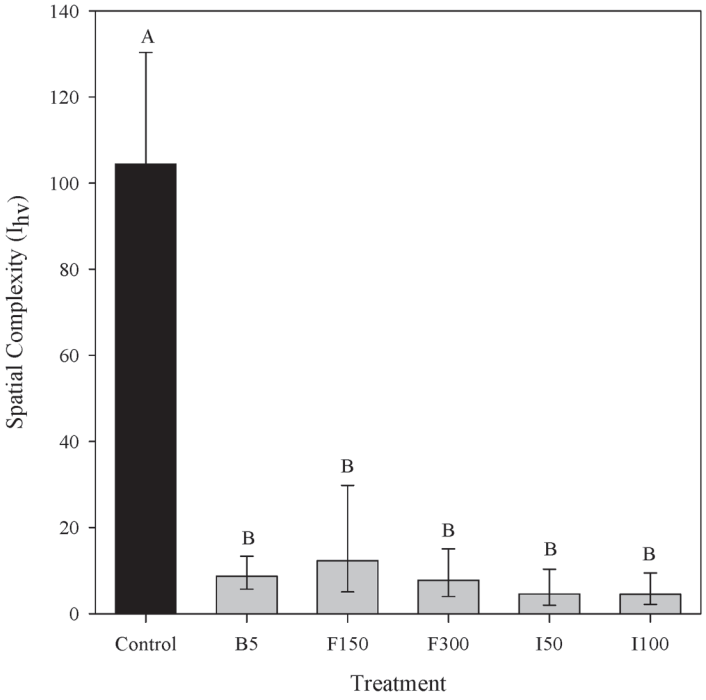

Figure 5. The effects of bensulfuron-methyl at 5 µg ai L^{-1} (B5), flurprimidol 150 and 300 µg ai L^{-1} (F150 and F300), and imazamox at 50 and 100 µg ai L^{-1} (I50 and I100) on mean hydrilla spatial complexity (I_{hv}) from 4, 6, and 8 weeks after treatment (WAT) using a repeated measures analysis. Different letters within a graph differ significantly ($P < 0.05$)

hydrilla habitat, bluegill spent more time foraging with less successful bouts (Theel and Dibble 2008). Largemouth bass have also demonstrated a similar response to high densities of complex invasive macrophytes (Valley and Bremigan 2002, Perret 2007). Lack of complexity differences between treatments could be due to the scale used for analysis. Complexity is highly scale-dependent and varies within a plant species (Dibble et al. 2006); therefore, we investigated complexity differences at the habitat or aquatic bed level to account for this inherent variability. Habitat-level analyses may be used to determine mechanistic effects within a population and/or community level. Greater understanding may be gained by investigating complexity at a fractal dimension, a spatial scale relative to fish and/or invertebrate perception (Dibble and Thomaz 2009).

The importance of macrophyte structure to aquatic communities is well documented. As structural complexity increases from an optimal range, foraging efficiency of fish declines (Savino and Stein 1982, Diehl 1988, Dibble and Harrel 1997, Valley and Bremigan 2002), specifically for a hydrilla-dominated habitat (Theel and Dibble 2008). Results from this study support further research efforts to use herbicides with growth regulating properties as a tool to suppress hydrilla growth and reduce habitat complexity. Growth regulation may be a viable alternative to plant death in systems that would benefit from some level of vegetative structure. Utilizing sublethal or growth-regulating herbicide concentrations requires integrating resistance management into management decisions. Additional herbicides with growth regulation properties should be evaluated, and fish response to habitat structure manipulations using a growth regulator or growth regulating concentrations should be investigated. Although hydrilla growth regulation should not be preferred over a native aquatic plant community, it may be a viable tool for select systems.

ACKNOWLEDGMENTS

The authors thank Mike Netherland and Kurt Getsinger for critical review of an earlier version of this manuscript. Permission was granted by the Chief of Engineers to publish this information. Citation of trade or generic chemical names does not constitute an official endorsement or approval of such commercial products. This research was funded by the Florida Fish and Wildlife Conservation Commission's Invasive Plant Management Section, and conducted under the US Army Corps of Engineers Aquatic Plant Control Research Program, Environmental Laboratory, US Army Research and Development Center. Technical assistance was provided by J. Namanny and C. Grodowitz.

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Evaluating fluridone sensitivity of multiple hybrid and Eurasian watermilfoil accessions under mesocosm conditions

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ABSTRACT

The recent confirmation of widespread watermilfoil hybridity throughout the northern tier states has led some aquatic plant managers to suggest these invasive hybrids have increased tolerance to various management efforts, including the use of fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl]-4(1*H*)-pyridinone) for whole-lake management. In this study we evaluated a hybrid watermilfoil (*Myriophyllum spicatum* × *M. sibiricum*) population from Townline Lake in Michigan that has been putatively identified as fluridone tolerant. We compared this plant to three separate populations of Eurasian watermilfoil (*M. spicatum* L.) and two distinct populations of hybrid watermilfoil. All watermilfoil populations were grown together in mesocosms and exposed to static fluridone treatments ranging from 3 to 36 µg L^{-1}. Fluorescence yield was measured on apical shoots over time and plant biomass was harvested to compare herbicide response between watermilfoil populations. All Eurasian watermilfoil and hybrid watermilfoil populations, except Townline, responded similarly to fluridone. In contrast, the Townline hybrid showed increased fluorescence yield and biomass when compared to other watermilfoil populations at fluridone concentrations between 3 and 12 µg L^{-1}, confirming an increased tolerance to low concentrations of fluridone. The current mechanism for the increased fluridone tolerance by this hybrid population is not yet understood. These results also illustrate that not all hybrids show an increased tolerance to fluridone. Because many states allow only 5 to 15 µg L^{-1} of fluridone for control of watermilfoil, the elevated tolerance of the Townline population at these fluridone rates has implications for regulation of aquatic herbicide applications. Documentation of a fluridone-tolerant population suggests that further sampling and testing is warranted to determine if other fluridone-tolerant watermilfoil populations exist in different waterbodies, especially those near Townline Lake.

Key words: 1-methyl-3-phenyl-5-3-(trifluoromethyl)phenyl-41H-pyridinone, aquatic plant management, herbicide tolerance, hybridity, resistance

INTRODUCTION

Eurasian watermilfoil (EWM; *Myriophyllum spicatum* L.) and hybrid watermilfoils are problematic submersed weeds

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