Effects of aerially-applied imazamox on southern cattail and non-target emergent vegetation in a eutrophic sawgrass marsh

LEROY RODGERS AND DAVID BLACK

ABSTRACT

Southern cattail (Typha domingensis Pers.) is a native species that invades disturbed wetlands, forming dense stands that interfere with restoration of wetlands, including parts of the Florida Everglades. Use of herbicides to control southern cattail has been limited by non-target damage from previously available herbicides. Registration of the selective herbicide imazamox for use in aquatic ecosystems provides a potential tool for selective chemical control of cattail. This study is an attempt to determine a foliar application rate for imazamox that controls southern cattail without injuring desirable Everglades vegetation. Imazamox was applied at rates expressed as acid equivalents (ae) of 0.28, 0.14, 0.07, or 0 (control) kg ae ha\(^{-1}\)(32, 16, 8, and 0 oz acre\(^{-1}\)). Data collected 12 months after treatment (MAT) indicated that imazamox at a rate of 0.28 kg ae ha\(^{-1}\) provided excellent (99%) control of southern cattail with minimal damage to desirable emergent macrophytes. Moderate control was observed at 0.14 kg ae ha\(^{-1}\), suggesting the minimum effective rate lies between these values. Change in percent cover between pre-treatment and 12 MAT did not significantly vary between treatments for sawgrass (Cladium jamaicense Crantz), fragrant water lily (Nymphaea odorata Aiton), pickerelweed, (Pontederia cordata L.), bog smartweed (Polygonum setaceum Baldwin) and duck potato (Sagittaria lancifolia L.), suggesting that the dominant emergent macrophytes in this study are not sensitive to imazamox at 0.28 kg ae ha\(^{-1}\). Prior to herbicide treatments, species richness estimates ranged from 9.2 to 10.2 species 0.09 ha\(^{-1}\). Estimates were very similar at 12 MAT, ranging from 9.2 to 10.0 species 0.09 ha\(^{-1}\) with no significant difference between treatments.

Key words: herbicide, Typha domingensis, Cladium jamaicense, Everglades, wetland restoration, selectivity

INTRODUCTION

The proliferation of southern cattail (Typha domingensis Pers.) in the Florida Everglades has been attributed to increased phosphorus levels in the soil and increased water depth and duration of flooding (Newman et al. 1998). Mono-specific stands of southern cattail have replaced the historic sawgrass (Cladium jamaicense Crantz) marsh ridge and slough landscape over nearly 12,500 ha in the Everglades (Rutchev et al. 2011). Everglades restoration has primarily focused on

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*South Florida Water Management District, 3301 Gun Club Road, MS#5230, West Palm Beach, FL 33406. Corresponding authors address: lrogers@sfwmd.gov. Received for publication January 11, 2012 and in revised form May 30, 2012.
fects of imazamox, even at the highest doses prescribed by EPA protocols. Additionally, the chemical properties of imazamox indicate that significant bioaccumulation is highly unlikely (SERA, 2010).

The environmental effect most likely to cause problems would be injury to non-target plants. Fortunately, imazamox is neither highly persistent nor very mobile in the environment. The half-life of imazamox in water ranges from 5 to 15 days, with the length of persistence dependent upon water clarity, depth, and available sunlight. Dilution and photolytic breakdown are the primary means of dissipation in water (SERA 2010). To date, there are limited publications addressing control of freshwater aquatic species using imazamox (Koschnick et al. 2007, Wersal and Madsen 2007, Mateos-Naranjo 2009). Imazamox is recommended for control of cattail at a rate of 32 to 64 oz ac⁻¹ [0.28 to 0.56 kg acid equivalent (ae) ha⁻¹]. Initial field evaluations conducted by the South Florida Water Management District in 2008 indicated that aerial applications of imazamox at a rate of 0.28 kg ae ha⁻¹ resulted in substantial control of cattail with little or no damage observed on other emergent macrophytes. These results suggested low use rates of imazamox can be used for selective control of cattail in Everglades marsh habitats. The objective of this study was to determine the dose response of cattail and other common emergent species to aerially-applied imazamox in a marginally invaded cattail-sawgrass marsh.

**MATERIALS AND METHODS**

Field test plots were established and treated by helicopter in September 2009. The plots were located in Water Conservation Area 3A, south of Alligator Alley (US Interstate 75) in a 75-ha area centered at 26.1386°, -80.5694°. This is a region of nutrient-enriched Everglades and Loxahatchee peats (Gleason and Stone 1994, Bruland et al. 2007) where southern cattail commonly grows taller than two meters. Treatment plots were set up as a randomized complete block. Five experimental blocks were established along a “cattail expansion zone” where southern cattail was co-dominant in a sawgrass marsh ridge and slough mosaic. Each block was divided into four 0.40-ha treatment plots (40 by 100 meters), which were randomly assigned an imazamox application rate of 0.28, 0.14, 0.07, or 0 (control) kg ae ha⁻¹. These rates align with Clearcast™ rates of 32, 16, 8 and 0 oz/ac, respectively. The herbicide was applied in water at an equivalent of 187 L water ha⁻¹, and two adjuvants (0.15 L DLZ™ ha⁻¹ and 0.05 L NU-FILM-IR® ha⁻¹) were added to enhance herbicide activity and reduce spray drift. The herbicide treatment was applied evenly over each plot, with no attempt to avoid application to non-target species.

Percent cover of emergent plant species and species richness were measured in each treatment plot three weeks prior to and 12 months after treatment (MAT). Species richness was measured in two 5 by 90 meter belt transects established along the long axis of each plot, five meters from the plot edges. Presence of all emergent and submersed plant species were recorded in these belt transects. Species cover was measured in six 2 by 1 m quadrats randomly placed in each treatment plot. To minimize effects from surrounding plots, random quadrat coordinates within five meters of the plot boundary were discarded. Quadrats were delineated using a pvc pipe frame. Plant species cover was independently estimated by two observers using cover classes (<1, 1-5, 6-25, 26-50, 51-75, >75%). Standing dead biomass was not included in species cover estimates, but total aboveground necromass was estimated for each quadrat. For each quadrat, the independent cover estimates of each species were averaged. Percent cover estimates for each of the six quadrats (sub-plots) were then averaged to obtain a cover estimate for each treatment plot replicate (n = 5). The change in cover for each species was calculated as the difference between post- and pre-treatment percent cover. Since the absolute cover of plant species at pre- and post-treatment was not of primary interest from a management perspective, proportional change in cover was chosen as the variable of interest in the analysis. Proportional change in cover was obtained by dividing the change in cover by the initial cover. The responses of southern cattail and five common emergent native species to treatments were examined with a one-factor randomized block analysis of variance with multiple comparisons. A log transformation was applied when data were not normally distributed or variances not homogenous. Where the treatment effect was significant, individual treatments were compared using Tukey’s honest significance test. Interaction of block and treatment were tested before examining treatment main effects.

**RESULTS AND DISCUSSION**

Prior to herbicide treatments, the species with the highest percent cover (± 95% CI), averaged across all plots, included sawgrass (12.5 ± 3.7%), southern cattail (8.0 ± 3.6%), fragment water lily (Nymphaea odorata Aiton) (6.3 ± 6.0%), pickrelweed (Pontederia cordata L.) (1.5 ± 1.3%), bog smartweed (Polygonum setaceum Baldwin) (0.9 ± 0.7%), and duck potato (Sagittaria lancifolia L.) (0.3 ± 0.3%). Other detected species with mean percent cover <0.1% included buttonbush (Cephalanthus occidentalis L), muskgrass, Gulf Coast spikerush (Elcocharis cellulosa Torr.), Egyptian panicgrass (Paspalidium geminatum Forsk. Staph.), bog rosemallow (Hibiscus grandiflorus Michx.), Virginia saltmarsh mallow [Kosteletzkya pentacarpos (L.) Ledeb.], marsh mermaidweed (Proserpinaca palustris L.), big floatingheart [Nymphoides aquatica (J.F. Gmel) Kuntze], maidencane (Panicum hemitomon Schult.), green arrow arum [Peltandra virginica (L.)Schott], Carolina willow (Salix carolina Michx.), bladderworts (Utricularia purpurea Walter and U. fo-liosa L.), American cupseed [Sacciolepis striata (L.) Nash], and southern cutgrass (Leersia hexandra Sw.).

At 12 MAT, southern cattail cover decreased 99%, 81%, and 61% in the 0.28, 0.14, and 0.07 kg ae ha⁻¹ treatments, respectively, and southern cattail cover increased 52% in the control plots (Figure 1). There was a block by treatment interaction (p < 0.007) for proportional change in cover of southern cattail. All imazamox treatment levels differed significantly (p < 0.0001) from the control plots (Figure 1). As the 99% cover decrease suggests, the 0.28 kg ae ha⁻¹ treatments transformed the plots. Living southern cattail was hard to find and the marsh canopy was much more open, allowing additional light to the water column. Some follow-up treatment will presumably be necessary to treat occasional surviving southern cattails.

There was considerable observed plant injury to southern cattail in the 0.14 kg ae ha⁻¹ plots. Slight chlorosis and necrosis were commonly observed on southern cattail in the
0.07 kg ae ha\(^{-1}\) treatment plots, but most plants exhibited new growth with no herbicide symptoms at 12 MAT. The moderately high control of southern cattail observed in the 0.14 plots argues for evaluating rates between 0.14 and 0.28 kg ae ha\(^{-1}\) in hope of finding an effective rate less than 0.28 kg ae ha\(^{-1}\), which would reduce herbicide input into the Everglades. However, this consideration must be weighed against the dangers of using too low a dose, which include not just failure to...
control the target species, but also an increased chance of developing herbicide resistance (Manalil et al. 2011). Change in percent cover did not vary between treatments for sawgrass, fragrant water lily, pickerelweed, bog smartweed, and duck potato (Figure 1). This data suggests that the dominant emergent macrophytes in this study are tolerant of imazamox at rates ≤0.28 kg ae ha⁻¹. Occasional herbicide damage was noted on sawgrass and pickerelweed in the 0.28 kg ae ha⁻¹ plots, but the damage appeared to be associated with areas where herbicide spray overlapped between plots. Some minor leaf spotting and chlorosis was occasionally observed at 12 MAT on sawgrass in the 0.28 kg ae ha⁻¹ treatment, but new growth from the basal meristem was frequently observed and no evidence of herbicide damage in the new tissue was noted. Imazamox can exert growth regulation on some plant species at lower rates, but no such effects were detected in this experiment. Koschnick et al. (2007) reported that pickerelweed is highly susceptible to aqueous applications of 150 to 300 µg a.i. L⁻³ of imazamox, but there was no indication of any impact of the foliar treatments on pickerelweed growth in this study. However, the fate of individual plants was not tracked, so we are unable to confirm if the pickerelweed plants evaluated 12 MAT were the same as pre-treatment or if these plants are of a new generation.

While other plant species sampled in cover plots were too infrequent to conduct statistical analyses, field observations suggested differential sensitivity among species to imazamox at 0.28 and 0.14 kg ae ha⁻¹. At these higher rates, Carolina willow and buttonbush frequently exhibited herbicide injury symptoms, although plants remained alive and produced some new growth with minor expression of herbicide activity. In contrast, Gulf Coast sphurikush and maidencane showed no evidence of herbicide injury at 12 MAT. Koschnick et al. (2007) also reported that maidencane was fairly tolerant of imazamox compared to other non-target emergent plant species. Bladderworts and muskrass also remained common in the slough portions of the plots. Prior to herbicide treatments, species richness estimates ranged from 9.2 to 10.2 species 0.09 ha⁻¹. Species richness estimates were very similar at 12 MAT, ranging from 9.2 and 10.0 species 0.09 ha⁻¹. There was no significant difference in species richness estimates among treatments.

The single aerial application of imazamox at 0.28 kg ae ha⁻¹ provided excellent control of southern cattail in marginally-infested marsh ridge and slough habitat with only minimal damage to desirable emergent macrophytes. The apparent selectivity of imazamox for southern cattail control is a promising development in ongoing efforts to manage this species in impacted regions of the Everglades. Specifically, selective control of southern cattail in marginally-infested marsh ridge and slough mosaic could serve to slow the rate of invasion. While control of southern cattail in a nutrient-enriched and hydrologically-altered wetland addresses a symptom of disturbance and improves habitat quality, restoration of the Everglades ultimately relies on the reversal of widespread eutrophication of a formerly oligotrophic landscape. In fact, it is likely that southern cattail will recolonize treated areas unless successful reductions in soil phosphorus concentrations and restoration of pre-disturbance hydrologic regimes occur. Nonetheless, judicious use of imazamox may be an effective tool to reduce southern cattail dominance in lightly to moderately infested areas. Imazamox may also be a preferred alternative to glyphosate/imazapyr treatments in dense southern cattail stands, since increased herbicide selectivity may result in accelerated colonization of desirable species.

Before large-scale herbicide applications are implemented, additional study is recommended to determine imazamox sensitivity of additional plant species commonly occurring in sawgrass-dominated Everglades marsh. Numerous species that are frequently present in this plant community type were either absent or very sparse in this study (e.g., maidencane). Additionally, variation in environmental conditions (e.g., soil pH, hydrology) across the Everglades could play an important role in plant community responses to imazamox treatments. A more complete understanding of imazamox selectivity among the larger complex of native plant taxa under different environmental conditions would allow for more informed decision making with regard to plant community impacts and alterations. In addition, information on southern cattail recolonization rates following imazamox treatments is needed to determine minimum retreatment intervals.

The accelerated rehabilitation of southern cattail-impact areas is a recent focus of scientists and land managers engaged in Everglades restoration. Specifically, active southern cattail management through combinations of herbicides and prescribed fire are being evaluated as potential restoration tools to shift southern cattail-dominated marsh to alternative native plant communities (Newman et al. 2011). To date, available herbicides for cattail control (e.g., glyphosate and imazapyr) have only been feasible in dense cattail stands, where impacts to other emergent macrophytes are of little concern. Findings in this study suggest that imazamox is highly efficacious against southern cattail at moderate to low aerial application rates with little to no herbicide damage to many common emergent macrophytes of the Everglades marsh ridge and slough mosaic. In fact, most plant species except for southern cattail and bog smartweed increased in cover. There is no evidence that decrease in cover of bog smartweed was caused by herbicide, since there was comparable decrease in control plots. The selectivity of imazamox represents a significant enhancement in herbicidal control of southern cattail and will likely increase options for herbicide control in other southern cattail management scenarios such as in marginally-infested areas.

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LITERATURE CITED

Abbreviations and Acronyms

ALS: Acetolactate synthase

biolog: Biological control

EPIC: Environmental Protection Information Clearinghouse

FWRI: Florida Fish and Wildlife Research Institute

IR: Irrigation

PGR: Plant growth regulator

PSP: Pesticide Survey Program

SDA: Status and Distribution Assessment

USEPA: United States Environmental Protection Agency

FDEP: Florida Department of Environmental Protection

SERA: Syracruse Environmental Research Associates, Inc.

U.S. Army Corps of Engineers, Vicksburg, MS

Abstract

Plant growth regulators (PGRs), such as flurprimidol ([α-(1-methylthyl)-α-[4-( trifluoromethoxy) phenyl]-5-pyrimidinemethanol]), and herbicides with growth regulating properties, such as imazamox (2-[4.5]dihydro-4-methyl-4-(1-methylthyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl) 3-pyridinecarboxylic acid-3-pyridinecarboxylic acid), and bensulfuron-methyl (methyl 2-[[[4,6-dimethoxy-2-pyrimidinyl] aminomethyl] benzamide 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 reduced hydrilla shoot biomass by an average of 68%. Habitat complexity was reduced in all treatments by an average of 95%. 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 hinder

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Growth regulating hydrilla and subsequent effects on habitat complexity

HEATHER J. THEEL, LINDA S. NELSON, AND CHRISTOPHER R. MUDGE*
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 ($I_v$), 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 non-native-dominated submerged 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 $I_v$ 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 $I_v$ 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 paconbutrazol ([R*,R*]-(+/-)-β-[4-Chlorophenyl]methyl]-α-[1,1dimehylethyl]-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-2-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl)-5-(methoxymethyl) 3-pyridinecarboxylic acid-3-pyrinedicarboxylic 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 $I_v$ 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 submerged 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$_4$Cl (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 pmol 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 aquarium 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-
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 by 46 to 69% by all treatments 12 WAT compared to nontreated plants remaining at 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-
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
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 $I_{n}$ 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
hydriella 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 hydriella-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 hydriella 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 hydriella growth regulation should not be preferred over a native aquatic plant community, it may be a viable tool for select systems.

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LITERATURE CITED


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 hydriella spatial complexity (Ihv) from 4, 6, and 8 weeks after treatment (WAT) using a repeated measures analysis. Different letters within a graph differ significantly (P < 0.05).
Evaluating fluridone sensitivity of multiple hybrid and Eurasian watermilfoil accessions under mesocosm conditions

SARAH T. BERGER, MICHAEL D. NETHERLAND, AND GREGORY E. MACDONALD*

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(1H)-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 the increased fluridone tolerance by this hybrid population.

Key words: 1-methyl-3-phenyl-5-3-(trifluoromethyl)phenyl-4H-pyridinone, aquatic plant management, herbicide tolerance, hybridity, resistance

INTRODUCTION

Eurasian watermilfoil (EWM; Myriophyllum spicatum L.) and hybrid watermilfoils are problematic submerged weeds
in many waterbodies throughout the northern tier of the United States. Invasive submerged plants, such as watermilfoil species that form dense surface canopies, can displace native vegetation (Madsen et al. 1991), alter water quality with resultant fluctuations in pH and dissolved oxygen (Bowes et al. 1979), and obstruct numerous recreational uses of waterways. Aquatic plant managers often rely on registered aquatic herbicides to address problems caused by invasive watermilfoils both at a site-specific and on a whole-lake basis.

Numerous populations previously described as invasive Eurasian watermilfoil were found, through nuclear ribosomal DNA analysis, to be hybrids from the parental species Eurasian and northern watermilfoil (M. sibiricum Kom.; Moody and Les 2002). Hybrid watermilfoils may present unique challenges for management due to inherited traits such as hybrid vigor or reduced sensitivity to herbicides. For example, the hybrid genotype of watermilfoil from Otter Lake in Minnesota formed apical turions (L.A. Glomski, USAERDC, Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX, pers. comm.), a trait normally restricted to the native northern watermilfoil. The acquisition of a trait associated with overwintering and enhanced survival could result in hybrid watermilfoils showing greater tolerance to management efforts. Because numerous hybrid watermilfoil populations have arisen independently, traits associated with hybrids from one lake may be quite different when compared with those from another lake (Sturtevant et al. 2011). Repeated hybridization as well as back-crossing has been documented in the field, again showing the need to evaluate each hybrid population independently (Moody and Les 2002).

Fluridone1 (1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl]-4(1H)-pyridinone) has been used by many aquatic managers for whole-lake treatments of watermilfoils due to its low use rates, native plant selectivity, ability to target all watermilfoil in the waterbody, and potential for obtaining more than one season of control from a single treatment (Getsinger et al. 2001, 2002a, 2002b). The confirmation of fluridone resistance via somatic mutation by the submersed invasive plant hydrilla (Hydrilla verticillata L.f. Royle; Michel et al. 2004) demonstrates that some aquatic plants may develop resistance to and or be tolerant of fluridone and other aquatic herbicides.

Although there have been anecdotal claims of reduced herbicide response by hybrid watermilfoils, published information to substantiate these claims is limited. Eurasian and northern watermilfoil are considered highly susceptible to low use rates used in whole-lake fluridone applications (Crowell et al. 2006). Triclopyr [(3,5,6-trichloro-2-pyridinyl)oxy acetic acid], 2,4-D amine [(2,4-dichlorophenoxy) acetic acid] and fluridone were found to have similar impacts on a single Eurasian and hybrid watermilfoil accession when the plants were exposed to typical use rates (Poovey et al. 2007, Slade et al. 2007). In contrast, differences between a Eurasian watermilfoil and hybrid watermilfoil population were recently documented following exposure to low continuous concentrations of 2,4-D (Glomski and Netherland 2010).

Laboratory studies showed that a watermilfoil hybrid growing in Townline Lake, Michigan, demonstrated an increased tolerance to fluridone when compared to multiple accessions of Eurasian watermilfoil (Berger 2012, Thum et al. 2012). The observation that a hybrid watermilfoil showed an increased tolerance to fluridone has fueled more speculation on the nature of hybrids and their response to herbicides. Because fluridone is typically used to treat an entire lake, failure to perform is particularly notable due to costs, exposure of native plants (some quite sensitive to fluridone) through the entire system, potential selection for an increased population of fluridone-tolerant watermilfoil, and subsequent requests to provide additional herbicide treatments for relief from the watermilfoil infestation. Further mesocosm studies are needed to compare a purported fluridone-tolerant population of watermilfoil to other watermilfoil populations when exposed to fluridone.

Several methods have been used to determine the response of plants to fluridone; biomass analysis, biochemical analysis, and pigment analysis have been widely used (Netherland and Getsinger 1995, Netherland et al. 1997, Puri et al. 2006). There is a need for nondestructive and repeatable methods of analysis on the same tissue source. Pulse-amplitude modulated (PAM) fluorometry can provide information on chlorophyll functionality by measuring chlorophyll fluorescence. A PAM fluorometer works by focusing a saturating beam of light on the desired region of the plant. By measuring the re-radiation, or 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-Nordenkampf et al. 1989).

PAM fluorometry has been used to study irradiance stress (Ralph et al. 1998), salinity stress (Kammermans et al. 1999), and shoot-to-landscape differences in photosynthesis in sea grasses (Durako and Kunzelman 2002). This technique is useful because it is a nondestructive method of evaluating the activity of chlorophyll and has also been used to evaluate herbicidal effects on plants. Ireland et al. (1986) used fluorometry to document decreased fluorescence in wheat (Triticum spp.) 30 minutes after exposure to glyphosate (N-[phosphonomethyl] glycine) herbicide. The herbicide diuron (N’-[3,4-dichlorophenyl]-N,N-dimethylurea), a photosystem II inhibitor, was shown to reduce fluorescence yield ratio in sea grasses 2 h after exposure as measured with a diving-PAM (Haynes et al. 2000). Ferrell et al. (2003) utilized fluorescence to analyze the response of johnsongrass (Sorghum halepense L.) to several herbicides. Recently, Elmore et al. (2011) measured fluorescence in bermudagrass (Cynodon dactylon [L.] Pers.) treated with pigment synthesis-inhibiting herbicides. Using a PAM fluorometer to detect the effects of pigment synthesis-inhibiting herbicides such as fluridone has not been documented in aquatic plants but is a potential nondestructive method for evaluating fluridone activity in the plant.

To determine if a hybrid watermilfoil population reported to demonstrate increased tolerance to fluridone shows a unique response compared to other watermilfoil accessions, we conducted a series of mesocosm studies. Based on laboratory evaluations (Berger 2012, Thum et al. 2012), we developed mesocosm studies to relate laboratory results to the mesocosm level and to evaluate use of the PAM fluorometer.
in determining tolerance to fluridone. The objective of this research was to evaluate the response to a range of fluridone concentrations to determine variation across populations of both hybrid and Eurasian watermilfoils.

**MATERIALS AND METHODS**

**Study 1**

Study 1 was conducted to compare the suspected fluridone-tolerant Townline population to a greater geographical range of invasive watermilfoils collected from several states. This study was conducted in a greenhouse at the Center for Aquatic and Invasive Plants (CAIP) in Gainesville, Florida, starting in November 2010 and continuing to February 2011. The greenhouse was supplemented with artificial light to achieve a photoperiod of 14h:10h light:dark.

Townline plants were harvested from outdoor stock cultures for the study. Plant material collected from three populations of suspected fluridone susceptible watermilfoils was obtained for the study (Table 1). All populations' genotypes were confirmed as hybrid or EWM through Internal Transcribed Spacer (ITS) analysis (R. Thum, Grand Valley State University, Anis Water Resources Institute, Muskegon, MI, pers. comm.).

A single apical shoot (10 to 15 cm in length) was planted in a 164 mL cone-tainer (3.8 cm width, 21 cm depth) containing topsoil amended with slow-release fertilizer (15-9-12) at a rate of 1 g kg⁻¹ of soil and capped with sand. Cone-tainer design allowed for ease of separation of watermilfoil populations within each tank while still allowing adequate space for root growth due to perforations in the bottom of the cone-tainer. One cone-tainer of each population was placed in a 4.5 inch square pot containing topsoil amended with slow release fertilizer and again capped with sand prior to planting. Each pot contained one cone-tainer from each population. Two square pots (one per harvest) were placed in a 95 L tank filled with water. Water temperature in the tanks for the duration of the study ranged from 22 to 24 C.

Plants were allowed to establish for 2 weeks prior to treatment. Plants were actively growing and beginning to reach the water surface at the end of the establishment period. At this time, six replications of each treatment (0, 5, 10, and 20 µg L⁻¹) of fluridone were added to the appropriate tanks for a static exposure. The experiment was concluded at 11 weeks after treatment (WAT).

Herbicide response was determined by fluorescence of shoot tips and was measured with a PAM fluorometer² and above ground biomass at 7 and 11 WAT. All above ground biomass was harvested, dried for 3 d at 70 C, and weighed.

This study was conducted using a completely randomized design. There were six replicates for each treatment. Data from each harvest were calculated as percent of untreated control to allow ease of presentation and comparison between tolerant and susceptible populations, and then analyzed with Analysis of variance (ANOVA). Means were separated with Fisher's Protected LSD (α = 0.05) to determine significant differences between the combined susceptible populations and the Townline population. Both fluorescence yield and biomass data were combined across all susceptible populations at each harvest because no significant differences were found between these populations.

**Study 2**

This study was conducted in outdoor mesocosms at CAIP from March to June 2011. Four populations of watermilfoils were harvested from outdoor stock cultures of plants used in Study 1 (Table 1). An additional population used in this study was field-collected from Indian Lake in Michigan, which is located near Townline Lake. Both Townline and Indian Lake plant populations are hybrid, genetically similar, and are suspected to be tolerant to fluridone. An additional population of EWM was obtained from research ponds in North Carolina. Populations were again genetically confirmed as hybrid or EWM through ITS analysis (R. Thum, Grand Valley State University, Anis Water Resources Institute, Muskegon, MI, pers. comm.).

Apical shoots were planted in an identical manner to that of Study 1. Three pots, each containing a full complement of the different watermilfoil populations, were added to each mesocosm. Plants were allowed to grow for 3 weeks prior to treatment. At this time, plants were actively growing and beginning to reach the water surface. Treatments for this study included an untreated control and 3, 6, 9, 12, 18, and 36 µg L⁻¹ of fluridone. Due to the potential photodegradation of fluridone herbicide in outdoor mesocosms, water samples were collected every 2 d to determine the half-life of the herbicide in the mesocosms. Samples were analyzed at CAIP using enzyme-linked immunosorbent assay (ELISA), and half-life was determined to be 10 d using an exponential decay model (Figure 1). Therefore, every 10 d for the duration of the study, each mesocosm was treated with a half concentration of the appropriate initial treatment to sustain target fluridone concentrations.

Herbicide response was determined by fluorescence (PAM yield) and above-ground biomass data were collected at 6 WAT as in the previous experiment. An additional harvest of above-ground biomass was collected 8 WAT. This study was completely randomized with 4 replicates per treatment. Fluorescence yield data and biomass data from each harvest were

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Table 1. Invasive watermilfoil (*Myriophyllum* spp.) populations used in Studies 1 and 2. Hybrid watermilfoil is a cross between northern watermilfoil (M. *sibiricum*) and Eurasian watermilfoil (EWM) (M. *spicatum*).

<table>
<thead>
<tr>
<th>Population</th>
<th>Species</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Townline</td>
<td>hybrid</td>
<td>Michigan</td>
</tr>
<tr>
<td>Frog</td>
<td>hybrid</td>
<td>Wisconsin</td>
</tr>
<tr>
<td>Auburn</td>
<td>EWM</td>
<td>Minnesota</td>
</tr>
<tr>
<td>Texas</td>
<td>EWM</td>
<td>Texas</td>
</tr>
<tr>
<td>Study 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Townline</td>
<td>hybrid</td>
<td>Michigan</td>
</tr>
<tr>
<td>Indiana</td>
<td>hybrid</td>
<td>Michigan</td>
</tr>
<tr>
<td>Auburn</td>
<td>EWM</td>
<td>Minnesota</td>
</tr>
<tr>
<td>Texas</td>
<td>EWM</td>
<td>Texas</td>
</tr>
<tr>
<td>North Carolina</td>
<td>EWM</td>
<td>North Carolina</td>
</tr>
</tbody>
</table>
analyzed using ANOVA to detect differences between Townline and the combined susceptible populations within each concentration. Data from all susceptible populations were combined because no significant differences were found between the populations when using Fisher’s Protected LSD (α = 0.05). Nonlinear regression was fitted to the data. Data are presented as percent of the untreated control to allow ease of presentation and comparison between tolerant and susceptible populations.

RESULTS AND DISCUSSION

Study 1

Differences in fluorescence yield and biomass were observed at 5 and 10 µg L⁻¹ between Townline and the combined susceptible milfoil populations at both 7 and 11 WAT (Tables 2 and 3, respectively). At 7 WAT, fluorescence yield decreased by more than half in the susceptible milfoil populations at 5 µg L⁻¹ while Townline showed minimal decrease in fluorescence (Table 2). At 11 WAT, fluorescence yield was decreased to <20% of untreated plants at 5 µg L⁻¹ in susceptible populations while Townline showed increased fluorescence from untreated controls (Table 2). By 11 WAT, no green plant tissue remained at 20 µg L⁻¹ to measure fluorescence.

Above-ground biomass of susceptible populations decreased to <10% of untreated control by 7 WAT at the lowest concentration (5 µg L⁻¹), and was <5% of untreated controls at 11 WAT (Table 3). The hybrid watermilfoil from Townline was >80% of untreated control at 5 and 10 µg L⁻¹ at 7 and 11 WAT. Although differences in fluorescence and biomass between the Townline and susceptible watermilfoils were noted at 5 and 10 µg L⁻¹, the similar response noted at 20 µg L⁻¹ treatment suggests that the Townline population remains susceptible to fluridone at concentrations well below the maximum label use rate of 150 µg L⁻¹ (Tables 2 and 3).

Table 2. Fluorescence yield, represented as percent of the untreated control, in three combined populations of watermilfoil (susceptible) and the Townline population 7 and 11 weeks after treatment (WAT) when treated with fluridone concentrations (5, 10, 20 µg L⁻¹).

<table>
<thead>
<tr>
<th>Fluridone (µg L⁻¹)</th>
<th>7 WAT</th>
<th>11 WAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible %</td>
<td>Townline %</td>
</tr>
<tr>
<td>5</td>
<td>44.9 ± 10.0*bc</td>
<td>98.1 ± 2.2</td>
</tr>
<tr>
<td>10</td>
<td>13.9 ± 6.9 *</td>
<td>98.4 ± 4.0</td>
</tr>
<tr>
<td>20</td>
<td>5.3 ± 3.9</td>
<td>23.5 ± 14.9</td>
</tr>
</tbody>
</table>

*Susceptible populations indicate three combined populations of invasive watermilfoils originating from Minnesota, Texas, and Wisconsin.

Values indicate means with standard error (n = 6).

Asterisks indicate differences between populations within each fluridone concentration. Differences found using Fisher’s Protected LSD (α = 0.05) are marked with an asterisk.

Table 3. Above-ground biomass, represented as percent of the untreated control, in three combined populations of watermilfoil (susceptible) and the Townline population 7 and 11 weeks after treatment (WAT) when treated with fluridone concentrations (5, 10, 20 µg L⁻¹).

<table>
<thead>
<tr>
<th>Fluridone (µg L⁻¹)</th>
<th>7 WAT</th>
<th>11 WAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible %</td>
<td>Townline %</td>
</tr>
<tr>
<td>5</td>
<td>9.3 ± 3.3 *bc</td>
<td>111.2 ± 11.3</td>
</tr>
<tr>
<td>10</td>
<td>2.0 ± 1.6 *</td>
<td>92.3 ± 16.1</td>
</tr>
<tr>
<td>20</td>
<td>2.7 ± 2.6</td>
<td>10.5 ± 7.3</td>
</tr>
</tbody>
</table>

*Susceptible populations indicate 3 combined populations of invasive watermilfoils originating from Minnesota, Texas, and Wisconsin.

Values indicate means with standard error (n = 6).

Asterisks indicate differences between populations within each fluridone concentration. Differences found using Fisher’s Protected LSD (α = 0.05) are marked with an asterisk.
Study 2

In the absence of fluridone pressure, the Townline population produced the lowest biomass values at both sample times (Table 4). Subsequent data are presented as percent of untreated control of each population. Fluorescence yield differences were found at all fluridone concentrations (Figure 2a). Biomass differences existed at 3, 6, 9, and 12 µg L⁻¹ for the susceptible populations when compared to Townline (Figure 2b). At 6 WAT, biomass of Townline plants was approximately 150 to 240% of the untreated control when exposed from 3 to 12 µg L⁻¹ fluridone. This suggests that Townline watermilfoil did not demonstrate enhanced growth properties when competing with the other populations when no herbicide was present in the control mesocosms; however, when fluridone decreased growth of other populations, the Townline plants were capable of rapidly filling the mesocosm tanks (visual observation). Nonlinear regression fitted to the response of Townline and susceptible populations contrast the high sensitivity of susceptible populations to fluridone with the lack of sensitivity exhibited by the Townline plants (Figure 2). In essence, this trial demonstrates how a selection pressure such as fluridone could result in a more tolerant genotype becoming dominant through the course of a long-term exposure.

By 8 WAT, apical shoots were not present on many of the susceptible watermilfoil accessions, so PAM yield data were not collected for comparison. Townline plant biomass continued to increase to approximately 400% of untreated mesocosms at the lowest concentrations of fluridone (Figure 3). The susceptible populations were different from Townline at all concentrations (Figure 3). Fluridone concentrations of 18 and 36 µg L⁻¹ resulted in a large reduction in biomass of the Townline plants when compared to lower rate applications. The 4-fold difference in biomass accumulation between the 12 and 18 µg L⁻¹ treatments suggest a rate-based tolerance to fluridone.

Using the PAM fluorometer was a successful, nondestructive method to document tolerance to pigment synthesis-inhibiting herbicides in this study. Elmore et al. (2011) also used this method in turfgrass to document herbicide response. Fluorescence analysis is a less time consuming and relatively noncomplex method to document fluridone response in plants.

The Townline watermilfoil population did not respond to fluridone in a similar manner as the susceptible populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Biomass (g) 6 WAT</th>
<th>Biomass (g) 8 WAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Townline</td>
<td>1.025</td>
<td>0.565</td>
</tr>
<tr>
<td>Indian</td>
<td>1.923</td>
<td>1.518</td>
</tr>
<tr>
<td>Auburn</td>
<td>2.011</td>
<td>1.915</td>
</tr>
<tr>
<td>Texas</td>
<td>1.838</td>
<td>2.388</td>
</tr>
<tr>
<td>North Carolina</td>
<td>2.679</td>
<td>3.333</td>
</tr>
</tbody>
</table>

Values represent mean of four replications.
Letters within each column represent differences between populations at each sampling date found using Fisher’s Protected LSD (α = 0.05).

While the hybrid Indian Lake plants are genetically similar to Townline, their response was significantly different, suggesting the fluridone tolerance by Townline is unique to this population. The ability to control watermilfoils with fluridone using whole-lake treatment recommendations of 5 to 6 µg L⁻¹ is predicated on all Eurasian and hybrid watermilfoils having a high level of susceptibility. The results of the Townline hybrid watermilfoil demonstrate that this population has an ability to withstand long-term exposures to normally lethal concentrations of fluridone.

The similar response to fluridone by the five other watermilfoil populations evaluated in these trials suggests that screening for tolerant populations should not be confounded by a wide variation in comparative sensitivity of watermilfoil populations.
Figure 3. Dry biomass of suspected fluridone tolerant Townline hybrid watermilfoil and four combined populations of susceptible invasive watermilfoils 8 weeks after treatment with fluridone. Data are presented as percent of the untreated control as means ± standard error (n = 4). Curves represent nonlinear regression. Significant differences within each concentration between Townline and the combined susceptible populations, found using Fisher’s Protected LSD (α = 0.05), are marked with an asterisk.

Herbicide resistance is defined as “the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type” (WSSA 1998). Herbicide tolerance “implies that there was no selection or genetic manipulation to make the plant tolerant; it is naturally tolerant” (WSSA 1998). Because there is no “wild type” hybrid watermilfoil, the differing response to fluridone of hybrid populations, specifically Townline population, cannot be referred to as herbicide resistance. These populations of plants do, however, exhibit an increased tolerance to fluridone. It is unknown if the Townline population was selected for by previous use of fluridone herbicide or if this population developed tolerance for the herbicide during hybridization (Thum et al. 2012).

Now that a fluridone tolerant population of hybrid watermilfoil has been confirmed both in the laboratory (Berger et al. 2010) and in mesocosm studies, resource managers must take necessary steps to prevent the spread of this unique population to neighboring lakes. Townline Lake is located in central Michigan in the near vicinity of numerous other bodies of water. The potential for spread of watermilfoils to other water bodies is highest in close proximity to the originally infested lake (Roley and Newman 2008). Resource managers should consider monitoring neighboring lakes to detect any possible movement of this unique hybrid watermilfoil. Rotation of herbicide mode of action has been well-documented to limit the development of tolerance or resistance in terrestrial species (Gressel and Segal 1990, Jasieniuk et al. 1996) and should also be utilized to control invasive watermilfoils.

SOURCE OF MATERIALS

1Fluridone (SONAR)- 480 g L⁻¹ suspension concentrate liquid formulation Sonar A.S. TM (SePRO Corporation, Carmel, IN).
2PAM Fluorometer - Mini-PAM, Walz, Effetrich, Germany.

ACKNOWLEDGMENTS

We thank the Florida Fish and Wildlife Conservation Commission, Invasive Plant Management Section, and the Aquatic Ecosystem Restoration Foundation for supporting a graduate student stipend for this project. Partial funding for this project was also provided by the US Army Engineer Research and Development Center, Aquatic Plant Control Research Program. Additional product and technical support was provided by the SePRO Corporation. Confirmation of milfoil hybridity was provided by Dr. Ryan Thum, Grand Valley State University, MI. Assistance in study setup, harvesting and processing plant samples was provided by Erin Canter and Anna Greis. Permission was granted by the Chief of Engineers to publish this information. Citation of trade names does not constitute endorsements or approval of the use of such commercial products.

LITERATURE CITED


Field and laboratory documentation of reduced fluridone sensitivity of a hybrid watermilfoil biotype (*Myriophyllum spicatum × Myriophyllum sibiricum*)

RYAN A. THUM, MARK A. HEILMAN, PAUL J. HAUSLER, LISA E. HUBERTY, PAMELA TYNING, DUSTIN J. WCISEL, MATTHEW P. ZUELLIG, SARAH T. BERGER, LEEANN M. GLOMSKI, MICHAEL D. NETHERLAND

ABSTRACT

Since receiving US Environmental Protection Agency registration in 1986, the aquatic herbicide fluridone has been successfully used for selective, low dose (<10 µg L⁻¹) control of many Eurasian watermilfoil (*Myriophyllum spicatum*) populations and, in some states, continues to be a common chemical management option for larger infestations of this invasive aquatic plant. The discovery of fluridone resistance in several Florida strains of hydrilla in the late 1990s has increased awareness of potential shifts in fluridone susceptibility in managed Eurasian watermilfoil populations; however, reports of fluridone tolerance by watermilfoils remain anecdotal. We present detailed field and laboratory data that document reduced fluridone sensitivity by a strain of hybrid watermilfoil (*M. spicatum × M. sibiricum*) from a central Michigan lake. Overall, watermilfoil was more abundant 60 days after fluridone application at a target rate of 6 µg L⁻¹ than before the application, and significantly more sites had watermilfoil post-treatment than expected under a model of at least 80% dieback. Laboratory comparisons of fluridone sensitivity of the central Michigan hybrid strain demonstrated that it grew through concentrations up to 12 µg L⁻¹ whereas one Eurasian watermilfoil strain and a second hybrid watermilfoil strain were highly impacted at concentrations of 3 to 4 µg L⁻¹. This first confirmation of a fluridone-tolerant population of watermilfoil supports the value of pretreatment screening of herbicide sensitivity as part of invasive watermilfoil management. Although the tolerant watermilfoil strain was a hybrid biotype, a second tested strain of hybrid watermilfoil exhibited typical fluridone resistance in the laboratory.

*First, sixth, and seventh authors: Assistant Professor, Research Associate, Graduate Student, Amnis Water Resources Institute, Grand Valley State University, Muskegon, MI 49441; Second author: Aquatics Technology Leader, SePRO Corporation, Carmel, IN 46032; Third and fifth authors: Senior Water Resources Specialist, Progressive Architecture Engineering, Grand Rapids, MI 49525; Fourth author: Aquatic Nuisance Control Program, Water Resources Division, Michigan Department of Environmental Quality – Water Resources Division; Ninth and Tenth authors: Graduate Student and US Army ERDC Research Biologist, University of Florida Center for Aquatic and Invasive Plants, Gainesville, FL 32653. Current address of seventh author: Department of Genetics, Fred C. Davison Life Sciences Complex, University of Georgia, Athens, GA 30602. Corresponding author’s Email: thumr@gvsu.edu. Received for publication September 29, 2011 and in revised form March 26, 2012.
done sensitivity, indicating that hybridity does not necessarily confer fluridone tolerance. Thus, the factors contributing to fluridone tolerance are unknown and warrant further research.

**Key words:** 1-methyl-3-phenyl-5,3-(trifluoromethyl)phenyl-4H-pyridinone, aquatic herbicide, hybridity, resistance, tolerance, watermilfoil, fluridone

**INTRODUCTION**

Eurasian watermilfoil (*Myriophyllum spicatum* L.; EWM) is one of North America’s most common and problematic invasive aquatic weeds, especially in the northern tier of the United States. In addition, a large number of invasive populations of watermilfoil have been identified as hybrids between EWM and the closely related native northern watermilfoil (*Myriophyllum sibiricum* Komarov; NWM; Moody and Les 2002, 2007, Sturtevant et al. 2009; Zuellig and Thum, unpublished data). Both EWM and hybrids are frequently managed with similar methods, including chemical (e.g., Hamel et al. 2001; 2,4-D; Madsen et al. 2002: fluridone; Poovey et al. 2007: triclopyr), biological (Newman et al. 1996: watermilfoil weevil [*Eurychiopsis lecontei*], and mechanical controls (Unmuth et al. 1998: close-cut mechanical harvesting). In many instances, however, lake managers are unaware that they are managing hybrids because the hybrids are difficult to distinguish from EWM on the basis of morphology and require molecular identifications (Moody and Les 2007).

Since being registered by the US Environmental Protection Agency in 1986, fluridone has been effectively used to selectively control EWM and hybrid watermilfoils. In the state of Michigan, fluridone has been used since 1987 to manage EWM. From 1987 to 2003 fluridone was applied to Michigan lakes at rates estimated from 5 to 46 µg L⁻¹. After considerable investigation, the Michigan Department of Environmental Quality (the state agency responsible for approving aquatic plant management permits) concluded that fluridone concentrations between 5 and 8 µg L⁻¹ were effective in controlling EWM with minimal impacts to native plant species, and that retreatment within 10 to 14 days maintained the required concentration–exposure time (MESB Sonar Investigative Panel 1999, Getsinger et al. 2001, 2002). This work culminated in a statewide standard in Michigan of whole-lake treatments at a target concentration of 6 µg L⁻¹, with retreatment 2 weeks later to raise the ambient fluridone concentration back up to 6 µg L⁻¹ (known as the “6-bump-6” treatment protocol and referred to as such hereafter).

Over the past several years, anecdotal accounts of tolerance to fluridone treatment in invasive watermilfoil populations in Michigan have increased. Several Florida populations of the submersed plant hydrida (*Hydrilla verticillata* L.f. Royle) are resistant to fluridone (Michel et al. 2004, Arias et al. 2005), reinforcing the value of sound stewardship of fluridone use for watermilfoil control. No quantitative, peer-reviewed studies have confirmed reduced fluridone response in invasive watermilfoils. The purpose of this study was to present field and laboratory data that document reduced fluridone response by a hybrid watermilfoil population in a central Michigan lake.

**MATERIALS AND METHODS**

**Study lake.** Townline Lake is a 116 ha lake located in the central portion of Michigan’s Lower Peninsula. Mean depth is 3.6 m, and approximately half of the bottomland is shallow enough to support macrophyte growth. Townline Lake has been infested with invasive watermilfoil since at least 1974 (EDI Inc. 1978). Townline Lake was treated with fluridone in 1996 at an estimated concentration of 8 µg L⁻¹ and in 2000 at 6-bump-6. Survey data from Michigan’s Aquatic Vegetation Assessment Sites (AVAS) protocol indicated that the 2000 treatment was not completely successful; however, it is unclear whether those results reflected an insufficient dose and exposure or some difference in herbicide susceptibility of the lake’s watermilfoil. Various other aquatic herbicides were used in the following years, but EWM pressure continued, and in 2009 fluridone was again considered for EWM control.

The decision to treat the Townline Lake milfoil population with fluridone led to a formal pretreatment screen of fluridone susceptibility in fall 2009 using a proprietary commercial assay offered by SePRO Corporation termed the PlanTEST™, a modified analysis of fluridone biochemical injury with methods similar to Sprecher et al. (1998). This initial screen indicated a 3- to 4-fold fluridone tolerance in Townline Lake watermilfoil and triggered additional genetic and susceptibility testing to confirm that this response of watermilfoil occurred in the lake.

Genetic identifications of plants from Townline Lake indicated that the watermilfoil population consisted of hybrids. In 2009, we sampled several scattered locations throughout the lake for genetic analysis and processed 15 plants for genetic analysis. In 2010, we obtained additional samples for genetic analysis from 10 locations in our grid surveys conducted in late April (pretreatment) and 60 days after the fluridone application from the same 10 pretreatment grid points. We identified each individual as EWM or hybrid using established protocols for ITS DNA sequences (Moody and Les 2002, Thum et al. 2006, 2011, Sturtevant et al. 2009, Zuellig and Thum unpublished data). Briefly, we compared our sequences with previously published Eurasian, northern, and hybrid watermilfoil accessions (FJ426346-FJ426357 from Sturtevant et al. 2009). EWM and NWM are separated by four fixed polymorphisms over the directly sequenced stretch of ITS DNA, and hybrids can be identified by obvious biparental sequence polymorphisms at these four sites (Moody and Les 2002, 2007, Sturtevant et al. 2009).

**Laboratory herbicide screens.** Study 1. In March 2010 a greenhouse study was conducted at the US Army Engineer Research and Development Center, Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, Texas. The study was conducted with the hybrid watermilfoil from Townline Lake and EWM obtained from an LAERF pond. Two apical tips of watermilfoil (15 cm) were planted in plastic pots (750 mL) filled with LAERF pond sediment amended with 3 g L⁻¹ Osmocote fertilizer (16-8-12). Pots were topped with a 1 cm layer of sand, and four pots were placed in each aquarium (66 L) on 30 March 2010. Aquariums were filled with alum-treated Lake Lewisville water and were situated in 1000 L fiberglass tanks filled with water. Water temperatures in the aquariums were maintained at 24 C by either aquarium heat-
ers or by circulating water through a Pacific Coast Imports C-1000 chiller.

Study 2. In May 2010 a greenhouse study was conducted at the University of Florida Center for Aquatic and Invasive Plants (CAIP), in Gainesville, Florida, with the hybrid watermilfoil from Townline Lake and a separate hybrid watermilfoil collected from Otter Lake, Minnesota. Plants were established on 27 April 2010 in 95 L tanks as described above with the exception that a commercial potting soil amended with Osmocote was used as the sediment source. The greenhouse was covered in 50% shade cloth, and temperatures were allowed to fluctuate with ambient outdoor conditions. Minimum water temperature was 19 C in late April with maximum temperatures recorded at 28 C in early July.

In both studies, plants were given a 20-day pretreatment growth period and then treated with fluridone (Stock solutions were prepared using Sonar™ A.S.) at concentrations of 1.5, 3, 6, and 12 µg L⁻¹ (Study 1) and at 2, 4, 6, 8, 12, and 24 µg L⁻¹ (Study 2). Water samples were collected at 1, 7, and 21 days after treatment (DAT), and residues were analyzed via enzyme-linked immunosorbent assay (ELISA) technique. Following a 60-day static exposure to fluridone, plants were harvested, and viable shoot biomass was dried to a constant weight at 65 C for 72 h. Each treatment was replicated (4 replicates for Study 1 and 5 replicates for Study 2); shoot biomass data are presented as means ±95% confidence intervals (C.I.). Nonlinear regression analysis was also performed to describe a fluridone treatment rate effect.

Field study of herbicide response. Fluridone was permitted in 2010 for hybrid watermilfoil control on Townline Lake under the 6-bump-6 treatment protocol. Fluridone was applied to Townline Lake on 28 April 2010 at a target concentration of 6 µg L⁻¹. Water samples were taken for estimation of fluridone concentration using FaSTEST (an internal SePRO liquid chromatographic method) on 30 April 11 May, 27 May, and 24 June. Based on estimated concentrations on 11 May, a repeat application (“bump”) of 3.3 µg L⁻¹ fluridone was applied to increase the concentration to the target 6 µg L⁻¹ (Table 1).

We monitored watermilfoil distribution and abundance within Townline Lake before and after treatment and conducted surveys on the day of treatment (28 Apr; the same day of initial fluridone application, but considered as before treatment), 3 weeks after treatment (18 May), and at the end of the summer (13 Aug). Our sampling methods are similar to those described by Hauxwell et al. (2010). Using a geographic information system, a 91 m grid was plotted on the Townline Lake bathymetric map over locations where water depth was 4.5 m or less, creating 93 sampling stations at the

<table>
<thead>
<tr>
<th>Date</th>
<th>30 April 2010</th>
<th>11 May 2010</th>
<th>27 May 2010</th>
<th>24 June 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Days after initial treatment)</td>
<td>(2 DAT)</td>
<td>(13 DAT)</td>
<td>(29 DAT)</td>
<td>(57 DAT)</td>
</tr>
<tr>
<td>µg L⁻¹Fluridone</td>
<td>4.2 ± 1.1</td>
<td>2.7 ± 1.3</td>
<td>5.3 ± 0.2</td>
<td>3.7 ± 0.2</td>
</tr>
</tbody>
</table>

Error is ± 1 standard deviation (n = 4). DAT is days after initial treatment.

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RESULTS

We demonstrated for the first time reduced susceptibility to fluridone in a Eurasian watermilfoil hybrid population. While reduced fluridone susceptibility by watermilfoil has been qualitatively noted in earlier reports to lake boards, no quantitative studies have confirmed its presence in both the laboratory and field. Reduced fluridone sensitivity by the Townline Lake hybrid watermilfoil population was evident in both the field and the laboratory.

Laboratory herbicide screens. As a percentage of untreated control, hybrid watermilfoil collected from Townline Lake attained greater biomass than the LAERF EWM population at 60 days after exposure to 3, 6, and 12 µg L⁻¹ fluridone (Figure 1). Even at the highest test rate, Townline watermilfoil maintained >50% of the untreated control biomass through the 60-day exposure and formed an extensive surface canopy in the presence of fluridone concentrations ranging from 3 to 12 µg L⁻¹. In contrast, the LAERF plants were barely visible in the water column and in poor condition at the time of harvest. The distinct visual differences were confirmed by the biomass data. The results of Study 2 were similar to those for Study 1. The Townline watermilfoil attained >40% of the untreated control biomass despite constant exposure...
to fluridone, while the hybrid watermilfoil from Otter Lake at concentrations of 4 µg L and greater was barely visible, and biomass was reduced to near 0% of the untreated control (Figure 2).

**Field study of herbicide response.** Typically, a 6-bump-6 fluridone treatment in Michigan waterbodies removes watermilfoil biomass from the water column completely or nearly so during the year of treatment. The response of Townline Lake watermilfoil to the 2010 6-bump-6 fluridone application was strongly atypical. Overall, watermilfoil was more abundant 60 days after the herbicide application than it was before the application (Figure 3). Of 82 sampling points with watermilfoil present during our study, 46 (56%) had a higher average rake-toss index post-treatment compared to pretreatment (average increase in rake index of 1.33 per site), and 13 sampling points (16%) exhibited no change pre-versus post-treatment. Twenty-three sampling points (28%) did exhibit reductions in rake toss index pre-versus post-treatment (average decrease in rake index of 1.27 per site), indicating some possible growth regulation at some points within the lake; however, only 3 of these 23 locations had post-treatment rake-toss indices of zero. Under the scenario of at least 80% die-back of watermilfoil following fluridone treatment, we expected that 63 of the 79 sites with watermilfoil in the pretreatment survey would not have watermilfoil in the post-treatment sampling; however, only three sites with watermilfoil in the pretreatment sample did not have watermilfoil in the post-treatment sample ($\chi^2, 1$ d.f. = 59.7, $p < 0.0001$). Thus, the herbicide application clearly did not produce the expected reduction of watermilfoil in our study lake.

Fluridone concentrations varied among the four locations where water samples were collected for analysis (Table 1), and the target concentration of 6 µg L$^{-1}$ was never actually reached. During the first 2 weeks, fluridone concentrations varied from 2.7 to 5.4 µg L$^{-1}$ two days after the initial application (28 Apr 2010) and varied from 1.5 to 4.1 µg L$^{-1}$ two weeks following the application. After the bump on 18 May 2010, fluridone concentrations at the four locations where we collected water samples were much more consistent. At 9 days after the bump, fluridone residues ranged from 5.0 to 5.4 µg L$^{-1}$, and at 37 days after the bump fluridone residues ranged from 3.5 to 3.9 µg L$^{-1}$. Note that the 6-bump-6 protocol in Michigan calls for a calculation of the fluridone amount based on the volume of the top 10 feet of the water column. This practice could lead to under-dosing the 6 µg L$^{-1}$ target if the thermocline is deeper than 10 feet. Although the measured fluridone residues indicate that the 6 µg L$^{-1}$ fluridone concentration was not achieved, watermilfoil control is achieved in other waterbodies with similar fluridone residue measurements, and the laboratory comparisons to two other strains of watermilfoil confirm the reduced fluridone response by the Townline Lake hybrid watermilfoil.

**Implications and future research.** Fluridone resistance has been documented in another major invasive aquatic weed, hydrilla (*Hydrilla verticillata*), due to a single amino acid substitution in the phytoene desaturase gene (PDS; Michel et al. 2004, Arias et al. 2005). In the case of our focal watermilfoil population, it is unknown whether reduced fluridone sensitivity results from mutation(s) in the PDS gene as in hydrilla. Similarly, it is unknown whether reduced fluridone sensitivity represents natural tolerance of this particular lineage or an evolved resistance in response to its previous treatment history with fluridone.

Our study population is composed of hybrid watermilfoil (*M. spicatum × M. sibiricum*), but whether the reduced fluridone sensitivity in our study population is related to its hybridization history is not clear. Evolutionary biologists widely accept that hybridization can lead to rapid adaptive evolutionary change in a wide variety of traits (Anderson and Stebbins 1954, Barton 2001, Rieseberg et al. 2003, Kim et al.)
2008, Arnold and Martin 2010), including the evolution of invasiveness (Ellstrand and Schierenbeck 2000), but whether hybridization can confer increased tolerance to fluridone is unknown. Other waterbodies in Michigan with hybrid watermilfoil have been treated successfully with 6-bump-6 fluridone. Our laboratory study demonstrates that hybrid genotypes may not necessarily exhibit fluridone tolerance; hybrid genotypes from a second lake (Otter Lake, MN) exhibited normal sensitivity to fluridone. Genetic studies of hybrid watermilfoil populations demonstrate that hybrid watermilfoils are composed of distinct genotypes (Zuellig and Thum, unpublished data), and whether different hybrid genotypes will exhibit different levels of fluridone sensitivity warrants further research.

Due to a lack of complete information about the genetic identification of watermilfoils, susceptibility to fluridone, and field responses to fluridone treatments, it is unclear how many other tolerant strains of watermilfoil exist. The widespread development of fluridone tolerance would be a highly undesirable outcome for non-native aquatic invasive species management, especially regarding economics and selective control. Certainly, fluridone is frequently effective for watermilfoil control in the northern United States; however, we currently do not have a quantitative estimate of the number of water bodies that may have fluridone tolerant strains of watermilfoil. Our clear documentation of one fluridone tolerant population of watermilfoil indicates that further screening of fluridone tolerant strains should be conducted and that further research on factors that may contribute to increased tolerance is warranted.

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