J. Aquat. Plant Manage. 50: 125-129

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 aerially 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). Monospecific 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 (Rutchey et al. 2011). Everglades restoration has primarily focused on reductions in nutrient concentrations and restoration of hydroperiods, but recent efforts have also investigated means to actively reduce southern cattail dominance in severely impacted areas. For example, the Cattail Habitat Improvement Project (CHIP) is investigating methods to rehabilitate cattail-invaded areas in the northern Everglades using combinations of herbicides and fire (Sklar et al. 2008, Newman et al. 2011). Most herbicides approved for aquatic weed control are broad spectrum (non-selective), which are likely to impact desired emergent and floating macrophytes and some submerged aquatic vegetation (SAV). In CHIP, a combination of glyphosate and imazapyr effectively eliminated all emergent vegetation in dense southern cattail stands, creating open water habitat with subsequent colonization by muskgrass (*Chara* spp.), a native macroalga. However, use of these herbicides in areas where southern cattail has yet to establish dense stands may be counterproductive due to non-target impacts to the remnant emergent flora. If herbicidal control of southern cattail is desired along the leading front of invasion, a more selective herbicide, which effectively controls southern cattail without damaging desirable native species, is necessary.

The herbicide imazamox (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid) was registered for aquatic use in 2007. Imazamox is a selective, systemic herbicide that kills plants by binding to and inhibiting activity of the acetolactate synthase (ALS) enzyme, leading to lethal reduction in branched chain amino acid biosynthesis (Shaner et al., 1984). Animals lack acetolactate synthase and obtain branched chain amino acids from their diets so they are not affected by this chemical activity of imazamox (Hamel 2012). Differences in sensitivity to imazamox among plants are due to the ability of certain species to metabolically detoxify the herbicide in addition to variations in the structure of the ALS enzyme that affect binding and inhibition processes and other factors (Délye, C. et al. 2011).

Potential injury to animals and non-target plants are vital concerns when applying herbicides in the Everglades. A thorough human health and ecological risk assessment for imazamox supports the concept that imazamox is a low-risk herbicide appropriate for certain uses in natural areas (SERA, 2010). For example, research in support of EPA registration found that imazamox concentrations three orders of magnitude greater than would be created by the maximum application rate failed to produce toxic effects in fish (rainbow trout), aquatic invertebrates (daphnia), waterfowl (mallard duck), mammals (rabbits and guinea pigs), and honey bees (EPA 1997). It was not possible to detect significant toxic ef-

*South Florida Water Management District, 3301 Gun Club Road, MS#5230, West Palm Beach, FL 33406. Corresponding authors address: lrodgers@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%), fragrant water lily (*Nymphaea odorata* Aiton) (6.3 ± 6.0%), pickerelweed (*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 (*Eleocharis cellulosa* Torr.), Egyptian panicgrass [*Paspalidium geminatum* (Forssk.) 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. foliosa* L.), American cupscale [*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

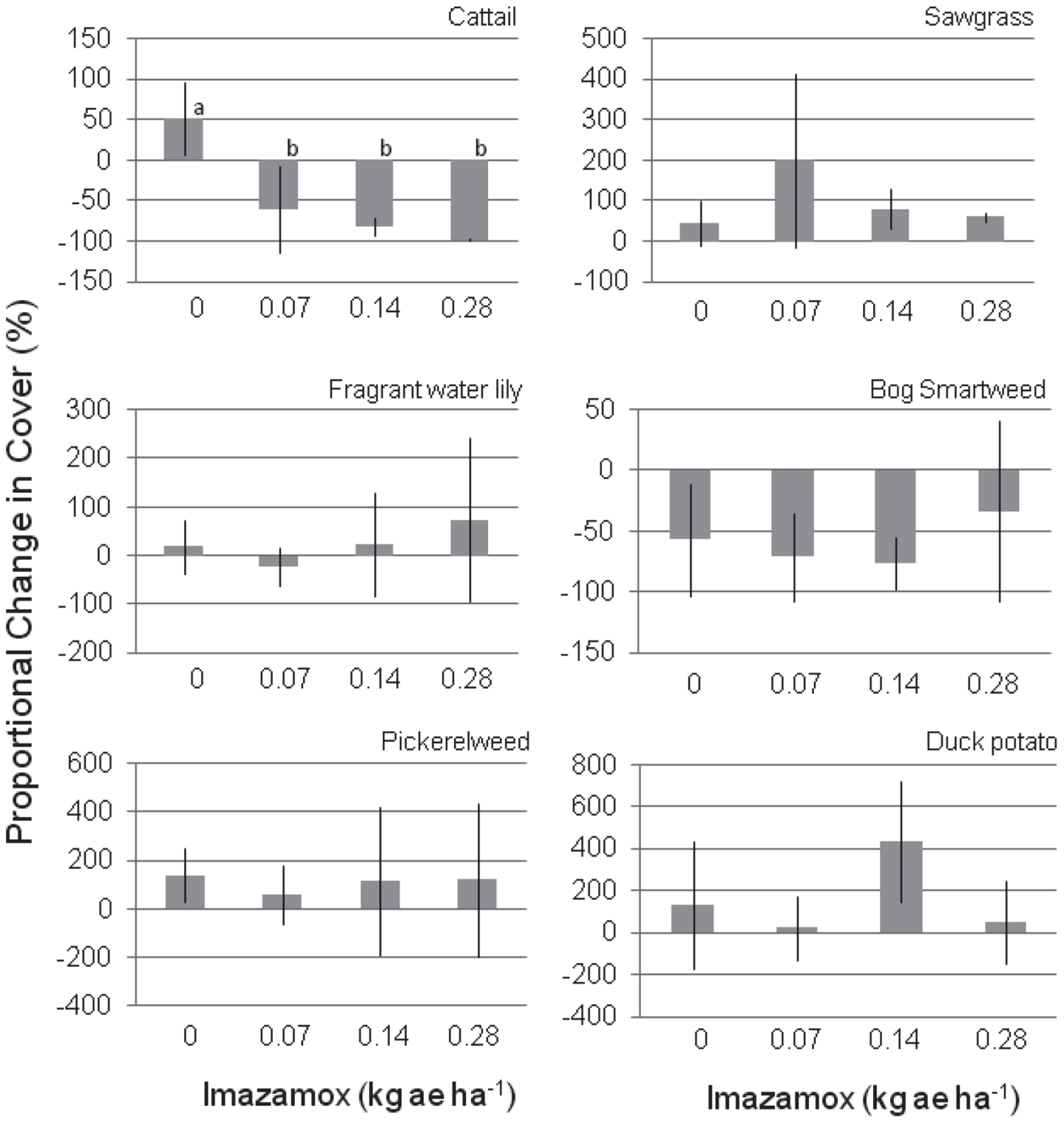

Figure 1. Mean proportional change in cover (12 MAT) for six common macrophytes at four imazamox rates. The vertical lines represent the 95% confidence intervals. Negative values represent decreases in mean cover at 12 MAT. Bars with different letters indicate means are significantly different according to Tukeys HSD ($\alpha = 0.05$).

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^{-1} 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 spikerush 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 muskgrass 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-invaded 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-impacted 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.

ACKNOWLEDGEMENTS

This project was funded by the South Florida Water Management District. We thank Tony Griffin, Jim Galloway, Steve Smith, and Jimmy Hines for field assistance. We also thank Dan Thayer, Francois LaRoche, and Todd Horton for assistance with experimental design and other insight on the project. Mike Page (Helicopter Applicators, Inc.) provided skilled herbicide application. We are also grateful to Lou Toth, Sue Newman, William Haller, and two anonymous reviewers for comments that greatly improved this manuscript.

LITERATURE CITED

Bruland, GL, Osborne TZ, Reddy KR, Grunwald S, Newman S, DeBusk WF. 2007. Recent Changes in Soil Total Phosphorus in the Everglades: Water Conservation Area 3. Environ. Monit. Assess. 129:379–395.

Délye, C, Pernin F, Scarabel L. 2011. Evolution and diversity of the mechanisms endowing resistance to herbicides inhibiting acetolactate-synthase (ALS) in corn poppy (Papaver rhoeas L.). Plant Sci. 180: 333–342.
EPA (Environmental Protection Agency). 1997. Pesticide fact sheet for imazamox. Office of Prevention, Pesticides and Toxic Substances, Arlington, VA. 6 pp. Available online: http://www.epa.gov/opprd001/factsheets/imazamox.pdf. Accessed May 21 2012.
Gleason, PJ, Stone P. 1994. Age, origin, and landscape evolution of the Everglades peatland. Pages 149-197 In Davis, SM, and Ogden, JC (eds.) Everglades: the Ecosystem and its Restoration. Delray Beach, Fla., St. Lucie Press.
Hamel, K. 2012. Environmental Impact Statement for Penoxsulam, Imazamox, Bispyribac-sodium, Flumioxazin, & Carfentrazone-ethyl. Washington St. Dept. Ecol. Publ. 00-10-040Addendum1. 98 pp.
Koschnick, TJ, Netherland MD, Haller WT. 2007. Effects of three ALS-inhibitors on five emergent native plant species in Florida. J. Aquat. Plant Manage. 45:47-51.
Manalil, S, Busi R, Renton M, Powles SB. 2011. Rapid Evolution of Herbicide Resistance by Low Herbicide Dosages. Weed Sci. 59(2):210-217.
Mateos-Naranjo, E, Redondo-Gomez S, Cox L, Cornejo J, Figueroa ME. 2009. Effectiveness of glyphosate and imazamox on the control of the invasive cordgrass Spartina densiflora. Ecotoxicol. Environ. Safety 72(6):1694-1700.
Newman, S, Schuette J, Grace JB, Rutchey K, Fontaine T, Reddy KR, Pietrucha M. 1998. Factors influencing cattail abundance in the northern Everglades, Aquatic Bot. 60(3):265-280.
Newman, S, Miao S, Saunders C, Hagerthey S, Cook M, Thomas C, Manna M, Jacoby M, Bellinger B, Holowecky P, Wheeler K, Kobza RM, Shuford R, Lynch JA, Xu X, Tian H, Childers DL. 2011. Ecosystem Ecology. In: 2011 South Florida Environmental Report – Volume I, Chapter 6. So. Fl. Water Manag. Dist., West Palm Beach, FL.
Rutchey, K, Gu B, Rodgers L, Schall T, Ewe S, Vega J, Li Y. 2011. Chapter 6: Landscape Processes. In: 2011 South Florida Environmental Report – Volume I. . So. Fl. Water Manag. Dist., West Palm Beach, FL.
SERA, 2010 (Syracuse Environmental Research Associates, Inc.). Imazamox Human Health and Ecological Risk Assessment. USDA Forest Service Contract: AG-3187-C-06-0010, Final Report. Accessed at http://www.fs.fed.us/foresthealth/pesticide/pdfs/052-24-02a_Imazamox.pdf
Sklar, FH, Cline E, M Cook, Dreschel T, Coronado C, Gawlik DE, Gu B, Hagerthey S, Lantz S, McVoy C, Miao S, Newman S, Richards J, Rutchey K, Saunders C, Scinto L, Shuford R, Thomas C. 2008. Chapter 6: Ecology of the Everglades Protection Area. In: 2008 South Florida Environmental Report – Volume I. So. Fl. Water Manag. Dist., West Palm Beach, FL.
Shaner, DL, Anderson PC, Stidham MA. 1984. Imidazolinones potent inhibitors of acetohydroxyacid synthase. Plant Physiol. 76:545-546.
Wersal, RM, Madsen JD. 2007. Comparison of imazapyr and imazamox for control of parrotfeather (Myriophyllum aquaticum (Vell.) Verdc.). J. Aquat. Plant Manage. 45:132-136.

J. Aquat. Plant Manage. 50: 129-135

Growth regulating hydrilla and subsequent effects on habitat complexity

HEATHER J. THEEL, LINDA S. NELSON, AND CHRISTOPHER R. MUDGE*

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

*First, second, and third authors: Research Biologist, Assistant Technical Director, and Research Biologist, US Army Engineer Research and Development Center, Environmental Laboratory, 3909 Halls Ferry Rd., Vicksburg, MS 39180. Corresponding author email: Heather.J.Theel@usace.army.mil.
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-

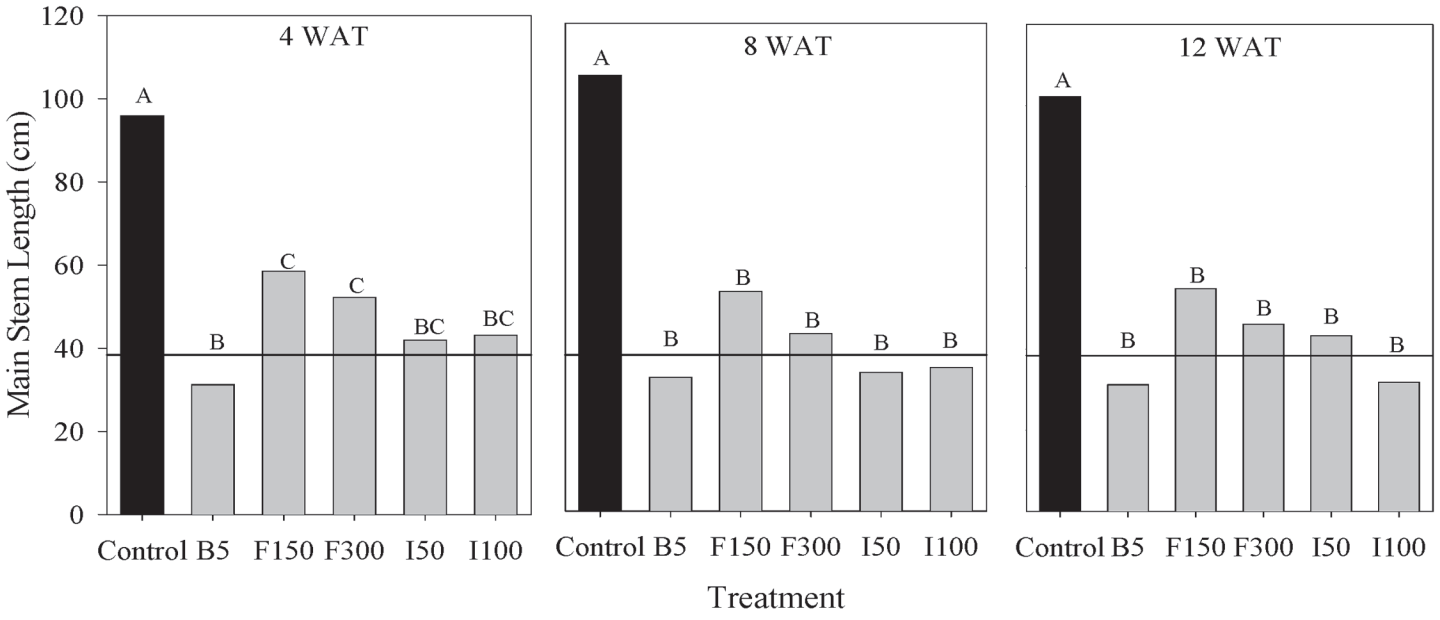

Figure 1. The effect of bensulfuron-methyl at 5 µg ai L^{-1} (B5), flurprimidol at 150 and 300 µg aiL^{-1} (F150 and F300), and imazamox at 50 and 100 µg ai L^{-1} (I50 and I100) on hydrilla main stem length (cm) 4, 8, and 12 weeks after treatment (WAT). Different letters within a graph differ significantly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

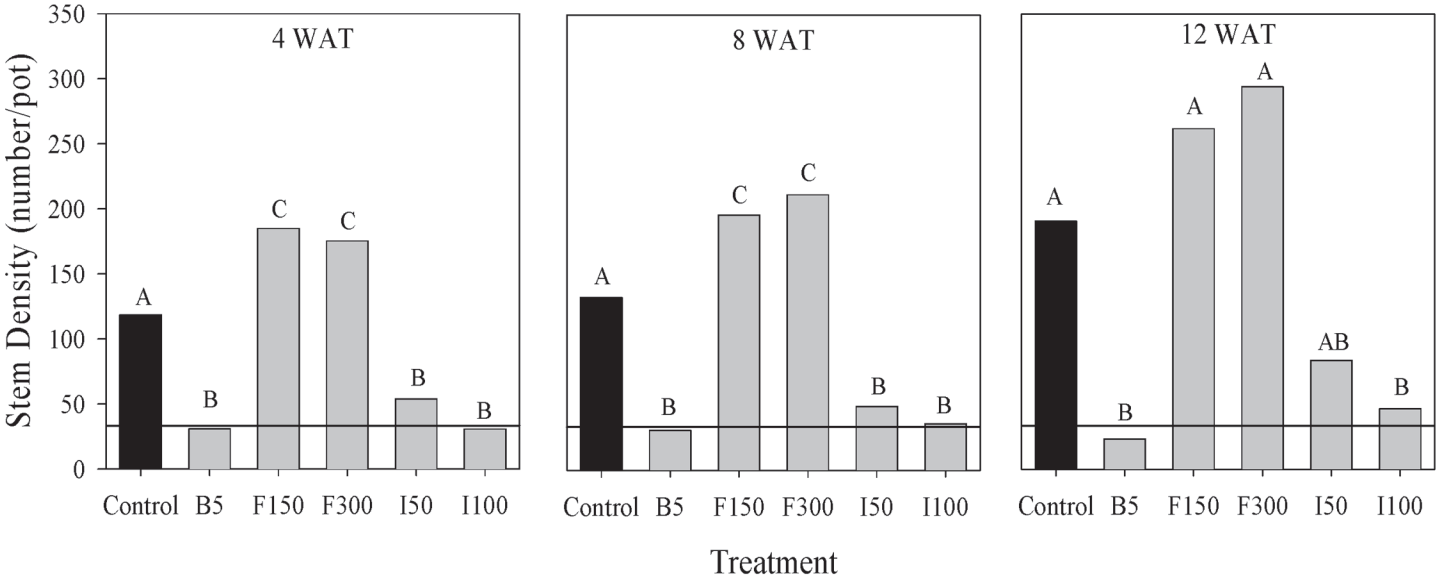

Figure 2. The effect of bensulfuron-methyl at 5 µg ai L^{-1} (B5), flurprimidol 150 and 300 µg ai L^{-1} (F150 and F300), and imazamox at 50 and 100 µg ai L^{-1} (I50 and I100) on hydrilla stem density (number/pot) 4, 8, and 12 weeks after treatment (WAT). Different letters within a graph differ significantly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

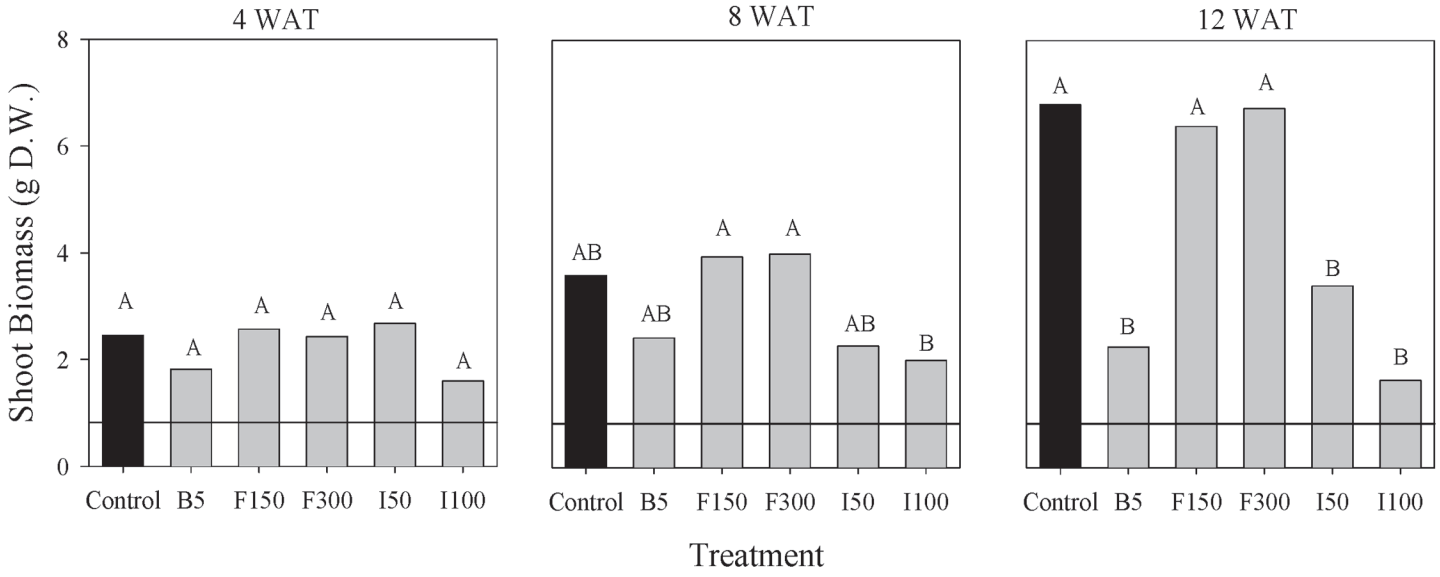

Figure 3. The effect of bensulfuron-methyl at 5 µg ai L^{-1} (B5), flurprimidol 150 and 300 µg ai L^{-1} (F150 and F300), and imazamox at 50 and 100 µg ai L^{-1} (I50 and I100) on hydrilla shoot biomass (g dry weight [D.W.]) 4, 8, and 12 weeks after treatment (WAT). Different letters within a graph differ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

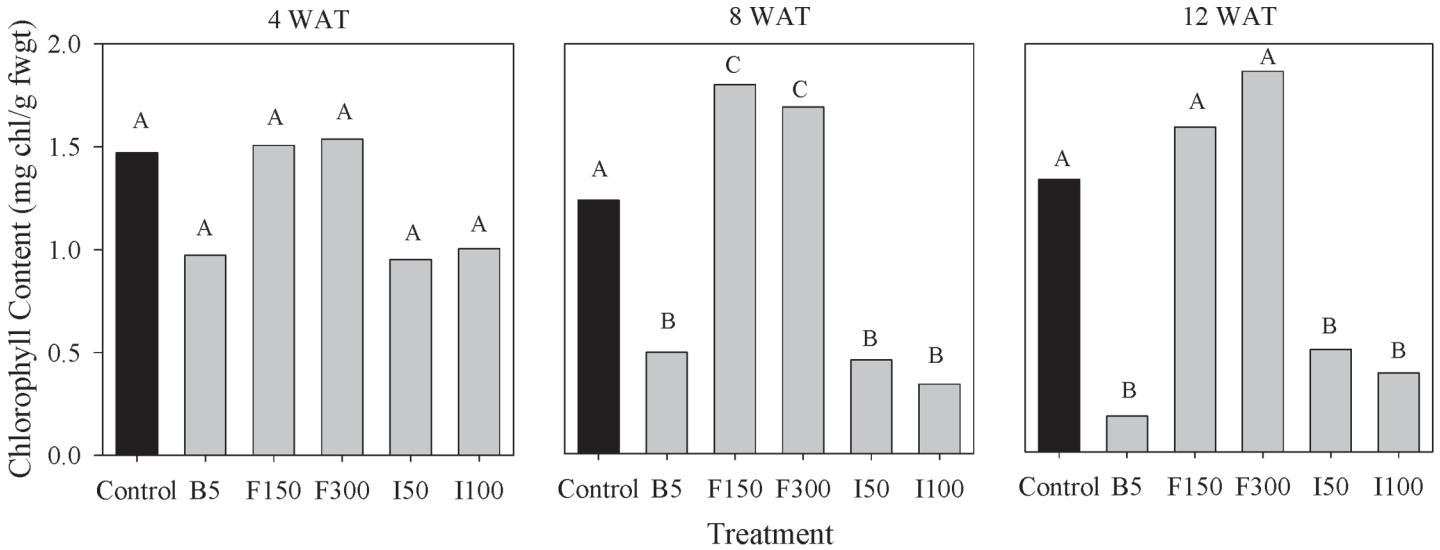

Figure 4. Effects of bensulfuron-methyl at 5 µg ai L^{-1} (B5), flurprimidol 150 and 300 µg ai L^{-1} (F150 and F300), and imazamox at 50 and 100 µg ai L^{-1} (I50 and I100) on chlorophyll content (mg chl/g fwgt) of hydrilla 4, 8, and 12 weeks after treatment (WAT). Different letters within a graph differ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

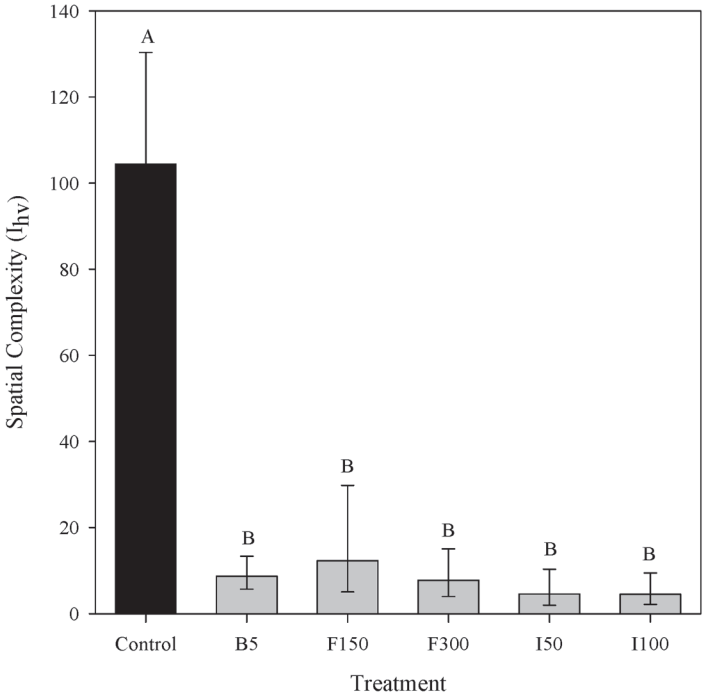

Figure 5. The effects of bensulfuron-methyl at 5 µg ai L^{-1} (B5), flurprimidol 150 and 300 µg ai L^{-1} (F150 and F300), and imazamox at 50 and 100 µg ai L^{-1} (I50 and I100) on mean hydrilla spatial complexity (I_{hv}) from 4, 6, and 8 weeks after treatment (WAT) using a repeated measures analysis.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

LITERATURE CITED

Akaike H. 1973. Information theory and an extension of the maximum likelihood principle. In: B. N. Petrov and F. Csaki (*eds.*). Second international symposium on information theory. Academiai Kiado, Budapest.

Anderson L. 1988. Growth regulator activity of bensulfuron methyl in aquatic plants, pp. 127-145. In: J. E. Kaufman and H. E. Westerdahl (*eds.*). Chemical vegetation management. Plant Growth Regulator Society of America, San Antonio, Texas.

Arnon DI. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidases in *Beta vulgaris*. Plant Physiol. 24:1-15.

BASF. 2008. Clearcast herbicide label. Last accessed 1 June 2009. http://www.cdms.net/LDat/ld7J8005.pdf.

Crowder LB, Cooper WE. 1979. Structural complexity and fish-prey interactions in ponds: a point of view, pp. 2-10. In: D. L. Johnson and R. A. Stein (*eds.*). Response of fish to habitat structure in standing water. American Fisheries Society, North Central Division, Special Publication 6, Bethesda, MD.

Crowder LB, Cooper WE. 1982. Habitat structural complexity and the interaction between bluegills and their prey. Ecology 63:1802-1813.

Dibble ED, Harrel SL. 1997. Largemouth bass diets in two aquatic plant communities. J. Aquat. Plant Manage. 35:74-78.

Dibble ED, Killgore KJ, Dick GO. 1996a. Measurement of plant architecture in seven aquatic plants. J. Freshw. Ecol. 11(3):311-318.

Dibble ED, Killgore KJ, Harrel SL. 1996b. Assessment of fish-plant interactions. Am. Fish. Soc. Symp. 16:357-372.

Dibble ED, Thomaz SM, Padial AA. 2006. Spatial complexity measured at a multi-scale in three aquatic plant species. J. Freshw. Ecol. 21(2):239-247.

Dibble ED, Thomaz SM. 2009. Use of fractal dimension to assess habitat complexity and its influence on dominant invertebrates inhabiting tropical and temperate macrophytes. J. Freshw. Ecol. 24:93-102.

Dieh, S. 1988. Foraging efficiency of three freshwater fishes: effects of structural complexity and light. Oikos 53:207-214.

[FDEP] Florida Department of Environmental Protection. 2007. Status of the aquatic plant maintenance program in Florida public water: annual report for fiscal year 2006-2007. Accessed 15 May 2009. http://www.dep.state.fl.us/lands/invaspec/2ndlevpgs/pdfs/Aquatic_06-07.pdf

Gotceitas V, Colgan P. 1989. Predator foraging success and habitat complexity: quantitative test of the threshold hypothesis. Oecologia 80:158-166.

Haller WT. 1978. Hydrilla, a new and rapidly spreading aquatic weed problem. Circular S-245. Agricultural Experiment Station, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida.

Haller WT, Sutton DL. 1975. Community structure and competition between hydrilla and vallisneria. Hyacinth Contr. J. 13:48-50.

Hiscox JD, Israelstam GF. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. 57:1332–1334.

Jones RL. 1973. Gibberellins: their physiological role. Annu. Rev. Plant Physiol. 24:571-598.

Lembi CA, Chand T. 1992. Response of hydrilla and Eurasian watermilfoil to flurprimidol concentrations and exposure times. J. Aquat. Plant Manage. 30:6-9.
MacDonald GE, Netherland MD, Haller WT. 2001. Discussion of fluridone "tolerant" hydrilla. Aquatics 23(3):4-8.
Michel A, Arias RS, Scheffler BE, Duke SO, Netherland M, Dayan FE. 2004. Somatic mutation-mediated evolution of herbicide resistance in the nonindigenous invasive plant hydrilla (*Hydrilla verticillata*). Mol. Ecol. 13:3229-3237.
Nelson LS. 1996. Growth regulation of Eurasian watermilfoil with flurprimidol. J. Plant Growth Regul. 15:33-38.
Nelson LS. 1997. Response of hydrilla and American pondweed to flurprimidol. J. Aquat. Plant Manage. 35:50-54.
Netherland MD, Lembi CA. 1992. Gibberellin synthesis inhibitor effects on submersed aquatic weed species. Weed Sci. 40:29-36.
Perret AJ. 2007. A multi-scale approach to evaluate the effect of the invasive aquatic plant hydrilla (*Hydrilla verticillata*) on littoral zone habitat of juvenile largemouth bass (*Micropterus salmoides*). M.S. thesis. Mississippi State University, Mississippi State, MS.
Richardson RJ. 2008. Aquatic plant management and the impact of emerging herbicide resistance issues. Weed Tech. 22:8-15.
SAS. 2003. SAS 9.1 Qualification tools user's guide. SAS Institute, Inc., Cary, NC.
Savino JF, Stein RA. 1982. Predator-prey interaction between largemouth bass and bluegills as influenced by simulated, submersed vegetation. T. Am. Fish. Soc. 111:255-265.
Smart RM, Barko JW. 1985. Laboratory culture of submersed freshwater macrophytes on natural sediments. Aquat. Bot. 21:251-263.
Theel HJ, Dibble ED. 2008. An experimental simulation of an exotic aquatic macrophyte invasion and its influence on foraging behavior of bluegill. J. Freshw. Ecol. 23:79-89.
Theel HJ, Dibble ED, Madsen JD. 2008. Differential influence of a monotypic and diverse native aquatic plant bed on a macroinvertebrate assemblage; an experimental implication of exotic plant induced habitat. Hydrobiologia. 600:77-87.
Valley RD, Bremigan MT. 2002. Effects of macrophyte bed architecture on largemouth bass foraging: implications of exotic macrophyte invasions. T. Am. Fish. Soc. 131:234-244.
[WSSA] Weed Science Society of America. 2007. Herbicide handbook. 9th ed. S. A. Senseman (*ed.*). Lawrence, KS. 458 pp.
Wersal RM, Madsen JD. 2007. Comparison of imazapyr and imazamox for control of parrotfeather (*Myriophyllum aquaticum* (Vell.) Verdc.). J. Aquat. Plant Manage. 45:132-136.

J. Aquat. Plant Manage. 50: 135-144

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(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

*First and third authors: Graduate Student, Professor, Agronomy Department, University of Florida, Gainesville, FL 32611; Second author: US Army ERDC Research Biologist, Gainesville, FL 32653. Corresponding author's Email: sberger@ufl.edu. Received for publication December 18, 2011 and in revised form April 19, 2012.

in many waterbodies throughout the northern tier of the United States. Invasive submersed 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. 2009). 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).

Fluridone[1] (1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl]-4(1*H*)-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-pryidinyl]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) fluorometery 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 fluorometery has been used to study irradiance stress (Ralph et al. 1998), salinity stress (Kamermans 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 fluorometery 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, Annis 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 1g 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 fluorometer[2] 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 ($\alpha = 0.05$) 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.

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*).

Population	Species	Location
Study 1		
Townline	hybrid	Michigan
Frog	hybrid	Wisconsin
Auburn	EWM	Minnesota
Texas	EWM	Texas
Study 2		
Townline	hybrid	Michigan
Indian	hybrid	Michigan
Auburn	EWM	Minnesota
Texas	EWM	Texas
North Carolina	EWM	North Carolina

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, Annis 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 immunosorbant 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

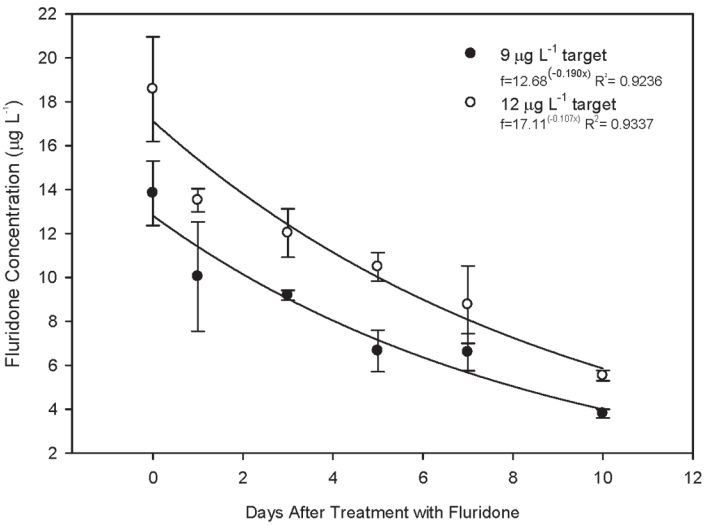

Figure 1. Decay curve of fluridone concentration in 9 and 12 µg L^{-1} target mesocosm tanks sampled 0, 1, 3, 5, 7, and 10 d after treatment (DAT). Data are presented as means ± standard error (n =4). Curves indicate exponential decay regression ($f = a^{(-bx)}$).

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}).

	7 WAT		11 WAT	
fluridone (µg L^{-1})	Susceptible[a] %	Townline %	Susceptible %	Townline %
5	44.9 ± 10.0*[bc]	98.1 ± 2.2	15.4 ± 5.8 *	124.6 ± 9.1
10	13.9 ± 6.9 *	98.4 ± 4.0	2.7 ± 2.7 *	119.2 ± 13.7
20	5.3 ± 3.9	23.5 ± 14.9	0	0

[a]Susceptible populations indicate three combined populations of invasive watermilfoils originating from Minnesota, Texas, and Wisconsin.
[b]Values indicate means with standard error (n = 6).
[c]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}).

	7 WAT		11 WAT	
fluridone (µg L^{-1})	Susceptible[a] %	Townline %	Susceptible %	Townline %
5	9.3 ± 3.3 *[bc]	111.2 ± 11.3	2.3 ± 0.9*	91.1 ± 4.4
10	2.0 ± 1.6 *	92.3 ± 16.1	0.08 ± 0.07*	81.4 ± 5.9
20	2.7 ±2.6	10.3 ± 7.3	0	0

[a]Susceptible populations indicate 3 combined populations of invasive watermilfoils originating from Minnesota, Texas, and Wisconsin.
[b]Values indicate means with standard error (n = 6).
[c]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 at 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 4. Above-ground biomass of each watermilfoil population used in Study 2 sampled from control mesocosms. Biomass was sampled 6 and 8 weeks after treatment (WAT).

	Biomass (g)	
Population	6 WAT[ab]	8 WAT
Townline	1.025[c]	0.565[d]
Indian	1.923[b]	1.518[c]
Auburn	2.011[b]	1.015[c]
Texas	1.838[b]	2.388[b]
North Carolina	2.679[a]	3.333[a]

[a]Values represent mean of four replications.
[b]Letters within each column represent differences between populations at each sampling date found using Fisher's Protected LSD ($\alpha = 0.05$).

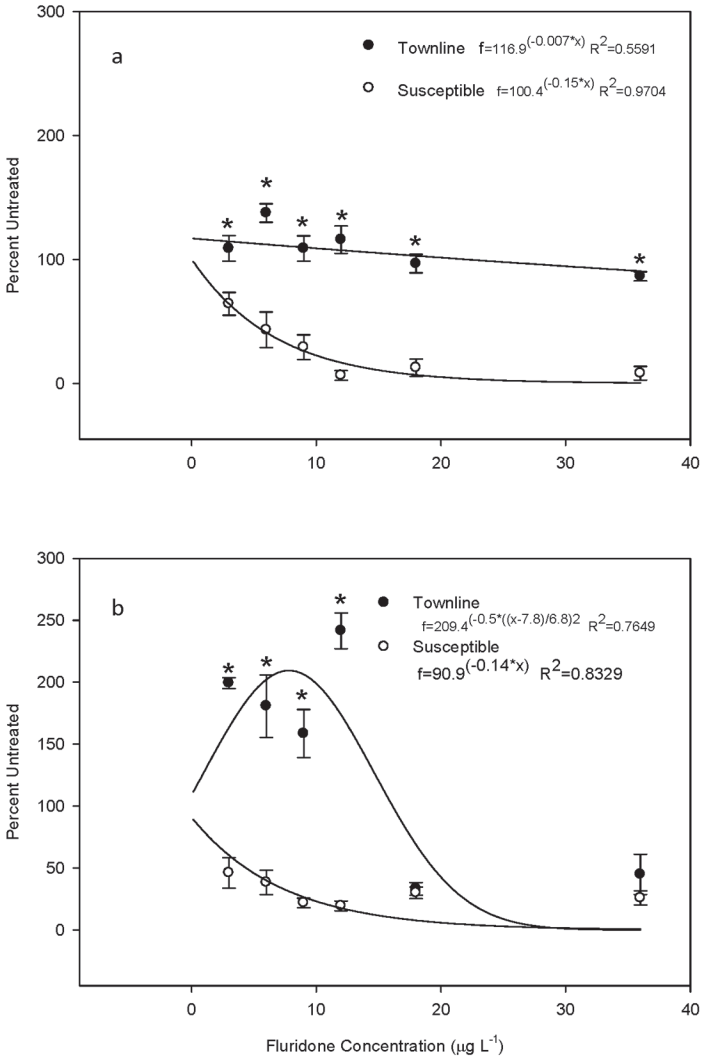

Figure 2. Fluorescence yield (a) and dry biomass (b) of suspected fluridone tolerant Townline hybrid watermilfoil and four combined susceptible populations of invasive watermilfoils 6 weeks after treatment with fluridone. Data are presented as percent of the untreated control as means ± standard error (n = 4). Curves indicate nonlinear regression. Significant differences within each concentration between Townline and susceptible populations, found using Fisher's Protected LSD ($\alpha = 0.05$), are marked with an asterisk.

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.

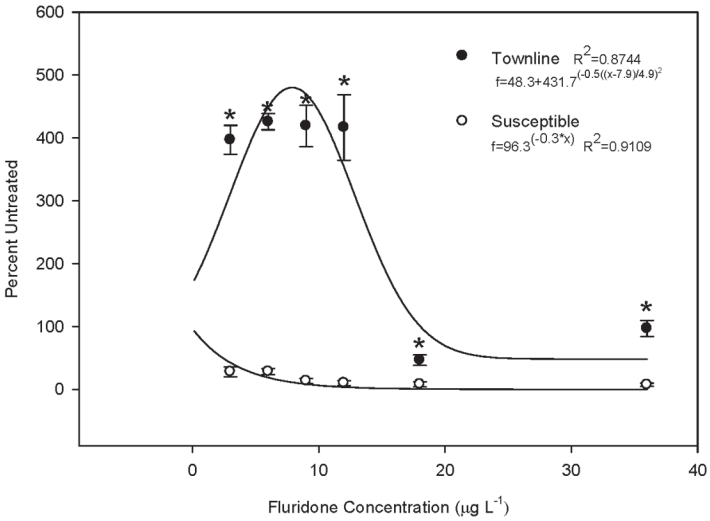

Figure 3. Dry biomass of suspected fluridone tolerant Townline hybrid watermilfoil and four combined populations of susceptible invasive watermilfoils 8 weeks after treatment with fluridone. Data are presented as percent of the untreated control as means ± standard error (n = 4). Curves represent nonlinear regression. Significant differences within each concentration between Townline and the combined susceptible populations, found using Fisher's Protected LSD ($\alpha = 0.05$), 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 2012, Thum et al. 2012) 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

[1]Fluridone (SONAR)- 480 g L^{-1} suspension concentrate liquid formulation Sonar A.S. ™ (SePRO Corporation, Carmel, IN).
[2]PAM 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

Berger ST. 2012. Characterization of a suspected herbicide tolerant hybrid watermilfoil (*Myriophyllum spicatum* x *M. sibiricum*). MS thesis. University of Florida.

Bolhar-Nordenkampf HR, Long SP, Baker NR, Oquist G, Schreibers U, Lechner EG. 1989. Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. Funct. Ecol. 3:497-514.

Bowes G, Holaday AS, Haller WT. 1979. Seasonal variation in the biomass, tuber density, and photosynthetic metabolism of hydrilla in three Florida lakes. J. Aquat. Plant Manage. 17:61-65.

Crowell WJ, Proulx NA, Welling CH. 2006. Effects of repeated fluridone treatments over nine years to control Eurasian watermilfoil in a mesotrophic lake. J. Aquat. Plant Manage. 44:133-136.

Durako MJ, Kunzelman JI. 2002. Photosynthetic characteristics of Thalassia testudinum measured in situ by pulse-amplitude modulated (PAM) fluorometery: methodological and scale-based considerations. J. Aquat. Bot. 73:173-185.

Elmore MT, Brosnan JT, Kopsell DA, Breeden GK. 2011. Methods of assessing bermudagrass [*Cynodon dactylon*] responses to HPPD-inhibiting herbicides. Crop Sci. 51(6):2840-2845.

Ferrell JA, Earl HJ, Vencil WK. 2003. The effect of selected herbicides on CO_2 assimilation, chlorophyll fluorescence, and stomatal conductance in johnsongrass (*Sorghum halepense* L.). Weed Sci. 51(1):28-31.

Getsinger KD, Madsen JD, Koschnick TJ, Netherland MD, Stewart RM, Honnel DR, Staddon AG, Owens CS. 2001. Whole lake applications of Sonar for selective control of Eurasian watermilfoil. Technical Report TR-07-1, US Army Engineer Waterways Experiment Station, Vicksburg, MS. 37 pp.

Getsinger KD, Madsen JD, Koschnick TJ, Netherland MD. 2002a. Whole lake fluridone treatments for selective control of Eurasian watermilfoil: I. Application strategy and herbicide residues. Lake Reserv. Manage. 18:181-190.

Getsinger KD, Stewart RM, Madsen JD, Way AS, Owens CS, Crosson HA, Burns AJ. 2002b. Use of whole-lake fluridone treatments to selectively control Eurasian watermilfoil in Burr Pond and Lake Hortonia, Vermont. Technical Report TR-02-39, US Army Engineer Waterways Experiment Station, Vicksburg, MS. 79 pp.

Glomski LM, Netherland MD. 2010. Response of Eurasian and hybrid watermilfoil to low use rates and extended exposures of 2,4-D and triclopyr. J. Aquat. Plant Manage. 48:12-14.

Gressel J, Segel LA. 1990. Modeling the effectiveness of herbicide rotations and mixtures as strategies to delay of preclude resistance. Weed Technol. 4:186-198.

Haynes D, Ralph P, Prange J, Dennison B. 2000. The impact of the herbicide diuron on photosynthesis in three species of tropical seagrass. Mar. Pollut. Bull. 41:288-293.

Ireland CR, Percival MP, Baker NR. 1986. Modification of the induction of photosynthesis in wheat by glyphosate, an inhibitor of amino acid metabolism. J. Exp. Bot. 37:299-308.

Jasieniuk M, Brûlé-Babel AL, Morrison IN. 1996. The evolution and genetics of herbicide resistance in weeds. Weed Sci. 44:176-193.

Kamermans P, Hemminga MA, deJong DJ. 1999. Significance of salinity and silicon levels for growth of a formerly estuarine eelgrass (*Zostera marina*) population. Mar. Biol. 133:527-539.
Madsen JD, Sutherland JW, Bloomfield JA, Eichler LW, Boylen CW. 1991. The decline of native vegetation under dense Eurasian watermilfoil canopies. J. Aquat. Plant Manage. 29:94-99.
Michel A, Arias RS, Scheffler BE, Duke SO, Netherland MD and Dayan FE. 2004. Somatic mutation-mediated evolution of herbicide resistance in the nonindigenous invasive plant hydrilla (*Hydrilla verticillata*). Mol. Ecol. 13:3229-3237.
Moody ML, Les DH. 2002. Evidence of hybridity in invasive watermilfoil (*Myriophyllum*) populations. P. Nat. Acad. Sci. USA. 99:14867-14871.
Netherland, MD, Getsinger KD. 1995. Laboratory evaluation of threshold fluridone concentrations for controlling hydrilla and Eurasian watermilfoil. J. Aquat. PlantManage. 33:33-36.
Netherland, MD, Getsinger KD, Skogerboe JD. 1997. Mesocosm evaluation of the species selective potential of fluridone. J. Aquat. Plant Manage. 35:41-50.
Poovey AG, Slade JG, Netherland MD. 2007. Susceptiblity of Eurasian watermilfoil (*Myriophyllum spicatum*) and a milfoil hybrid (*M. spicatum* x *M. sibiricum*) to triclopyr and 2, 4-D amine. J. Aquat. Plant Manage. 45:111-115.
Puri A, MacDonald GE, Haller WT, Singh M. 2006. Phytoene and βcarotene response of fluridone-susceptible and –resistant hydrilla (Hydrilla verticillata) biotypes to fluridone. Weed Sci. 54: 995-999.
Ralph PJ, Gademann T, Dennison WC. 1998. In situ seagrass photosynthesis measured using a submersible, pulse-amplitude modulated fluorometer. Mar. Biol. 132:367-373.
Roley SS, Newman RM. 2008. Predicting Eurasian watermilfoil invasions in Minnesota. Lake Reserv. Manage. 24:361-369.
Slade JG, Poovey AG, Netherland MD. 2007. Efficacy of fluridone on Eurasian and hybrid watermilfoil. J. Aquat. Plant Manage. 45:116:118.
Sturtevant AP, Hatley N, Pullman GD, Sheick R, Shorez D, Bordine A, Mausolf R, Lewis A, Sutter R, Mortimer A. 2009. Molecular characterization of Eurasian watermilfoil, northern watermilfoil, and the invasive interspecific hybrid in Michigan lakes. J. Aquat. Plant Manage. 47:128-135.
Thum RA, Heilman MA, Hausler PJ, Huberty LE, Tyning P, Wcisel DJ, Zuellig MP, Berger S, Netherland MD. Forthcoming 2012. Field and laboratory documentation of reduced fluridone sensitivity by a hybrid watermilfoil biotype (*Myriophyllum spicatum* x *Myriophyllum sibiricum*). J. Aquat. Plant Manage. 50.
[WSSA] Weed Science Society of America. 1998. "Herbicide resistance" and "herbicide tolerance" defined. Weed Technol. 12:789.

J. Aquat. Plant Manage. 50: 141-146

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 fluri-

*First, sixth, and seventh authors: Assistant Professor, Research Associate, Graduate Student, Annis 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; Nintha 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-41H-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 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 hydrilla (*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 PlanT-EST™, 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 grid vertices. Sampling locations were programmed into a handheld Global Positioning System (GPS).

We quantified watermilfoil abundance at each sampling point within the lake using a rake-toss index. While such an index has some obvious limits to its precision, it yields a sufficient qualitative picture of watermilfoil abundance at a given location, especially for pre- and post-treatment comparisons. At each sampling location, we averaged the index from two rake tosses thrown in distinctly different directions off the bow of the boat. Watermilfoil that was clearly dead was not counted. Our index values for each throw were as follows:

(0) Rake contained no living watermilfoil.

(1) Live watermilfoil comprised <5% of the rake tine space.

(2) Live watermilfoil comprised between 5 and 25% of the tine space.

(3) Live watermilfoil was common on the rake, but occupied <50% of the rake tine space.

(4) Rake was densely covered with live watermilfoil: >50% of the rake tine space.

We also conducted a χ^2 analysis to statistically test for deviation from the expected response to fluridone. We expected at least 80% of the sites with watermilfoil in the pretreatment survey to be devoid of watermilfoil in the post-treatment survey (i.e., 80% die-back); thus, we calculated our expected number of sites with watermilfoil for the post-treatment survey to be 20% of the sites with watermilfoil in the pretreatment survey. Because fluridone may take several weeks to impact the plant population, we performed these calculations by comparing data from the initial survey (28 Apr) and last survey of the summer (13 Aug).

Table 1. Fluridone concentrations in surface water of Townline Lake during Spring 2010 Sonar A.S. Treatment. Repeat (bump) application of 3.3 µg L^{-1} was applied 18 May. Surface samples were collected from four sites on lake (three littoral, one open water).

Date (Days after initial treatment)	30 April 2010 (2 DAT)	11 May 2010 (13 DAT)	27 May 2010 (29 DAT)	24 June 2010 (57 DAT)
µg L^{-1} Fluridone	**4.2 ± 1.1**	**2.7 ± 1.3**	**5.3 ± 0.2**	**3.7 ± 0.2**

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

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

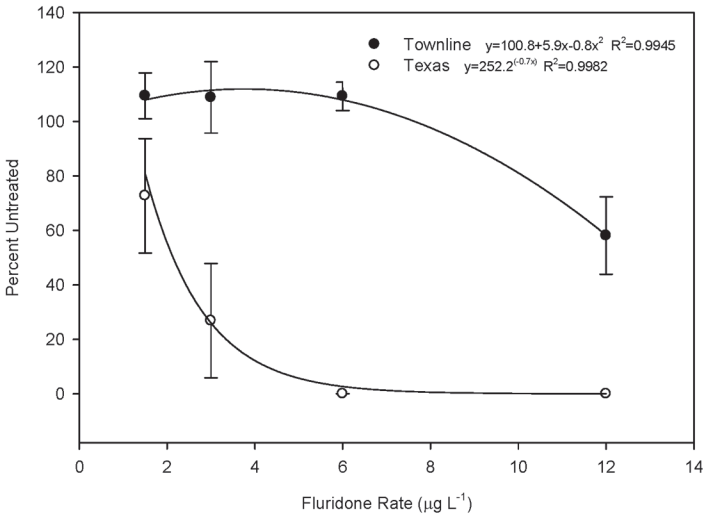

Figure 1. Hybrid watermilfoil collected from Townline Lake, MI, and a strain of Eurasian watermilfoil collected from the Lewisville Aquatic Ecosystem Research Facility, TX (Texas), were subjected to static exposures of fluridone in April 2010; shoot biomass was harvested at 60 days. Data are presented as percent biomass of the untreated control. Symbols represent means +95% confidence intervals (n = 4) and curves represent nonlinear regression.

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 65 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 (χ^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.

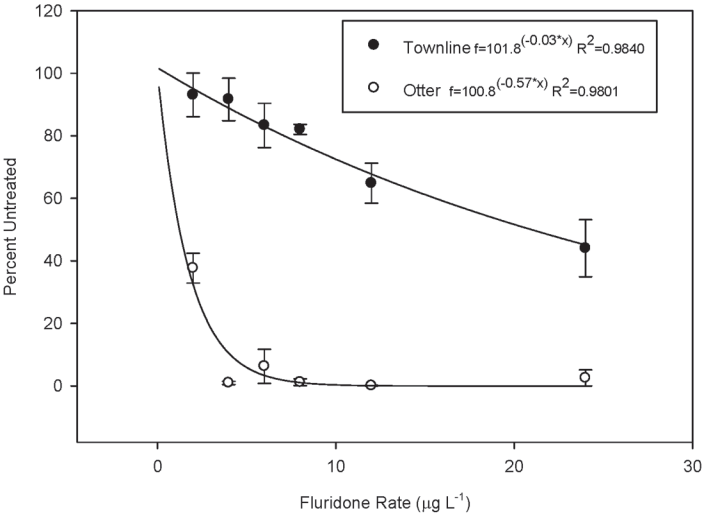

Figure 2. Hybrid watermilfoil collected from Townline Lake, MI, and a separate strain of hybrid watermilfoil collected from Otter Lake, MN (Otter) were subjected to static exposures of fluridone in May 2010 and shoot biomass was harvested at 60 days. Data are presented as percent biomass of the untreated control. Symbols represent means +95% confidence intervals (n = 5) and curves represent nonlinear regression.

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 Stebbins1954, Barton 2001, Rieseberg et al. 2003, Kim et al.

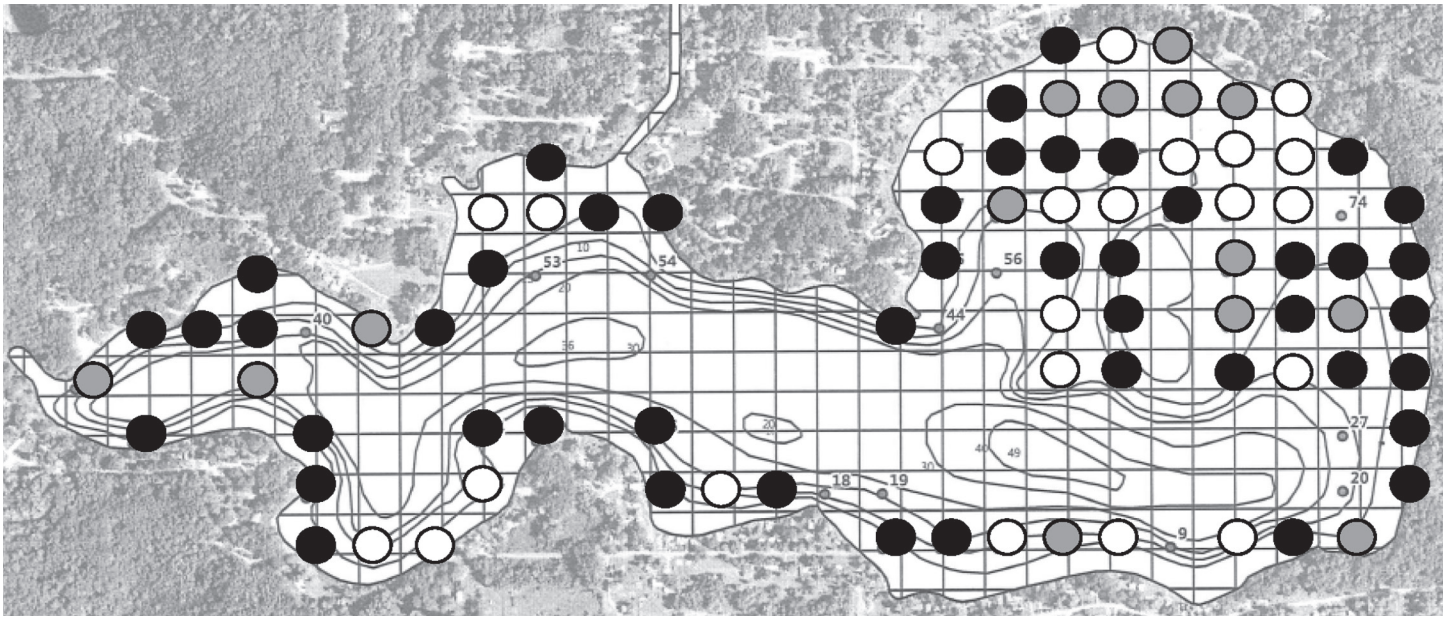

Figure 3. Change in abundance of hybrid watermilfoil in Townline Lake, MI, as determined by a rake-toss index (average of two rake tosses), before (28 Apr) vs. 107 days after (13 Aug) initial fluridone treatment at a target rate of 6 µg L^{-1}. Black circles: increase in abundance; gray circles: no change in abundance; white circles: decrease in abundance. Small gray dots with numbers are survey sites where no watermilfoil was found before or after treatment.

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 sensitivities of watermilfoil strains should be conducted and that further research on factors that may contribute to increased tolerance is warranted.

SOURCES OF MATERIALS

Fluridone (SONAR): 480 g L^{-1} suspension concentrate liquid formulation Sonar A.S. ™ (SePRO Corporation, Carmel, IN)

FasTEST: Internal High Performance Liquid Chromatographic (HPLC) internal methods for fluridone and other aquatic herbicide analysis

ACKNOWLEDGMENTS

Thanks to Caleb James, Heather Hayward, Patricia Phillips, and Amanda Mercer for help with the molecular identifications of watermilfoils. In addition, comments and suggestions from three anonymous reviewers improved the initial draft of this manuscript. This study was funded in part by a Michigan Department of Environmental Quality grant awarded to RAT. Parts of this research were funded under the US Army Aquatic Plant Control Research Program and permission to publish this information was granted by the Chief of Engineers.

LITERATURE CITED

Anderson E, Stebbins G. 1954. Hybridization as an evolutionary stimulus. Evolution. 8:378-388.

Arias RS, Netherland MD, Scheffler BE, Puri A, Dayan FE. 2005. Molecular evolution of herbicide resistance to phytoene desaturase inhibitors in *Hydrilla verticallata* and its potential use to generate herbicide-resistant crops. Pest Manage. Sci. 61:258-268.

Arnold ML, Martin NH. 2010. Hybrid fitness across time and habitats. Trends Ecol. Evol. 25:530-536.

Barton NH. 2001. The role of hybridization in evolution. Mol. Ecol. 10:551-568.

EDI, Inc. 1978. Feasibility study for the improvement of Townline Lake, Montcalm Co., MI.

Ellstrand NC, Schierenbeck KA. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? Proc. Natl. Acad. Sci. USA. 97:7043-7050.

Getsinger KD, Madsen JD, Koschnick TJ, Netherland MD, Stewart RM, Honnell DR, Staddon AG, Owens CS. 2001. Whole-lake applications of SonarTM for selective control of Eurasian watermilfoil. Technical Report ERDC/EL TR-01-7; US Army Engineer Research and Development Center; Vicksburg, MS.

Getsinger K, Madsen JD, Koschnick T, Netherland MD. 2002. Whole lake fluridone treatments for selective control of Eurasian watermilfoil: I. Application strategy and herbicide residues. Lake Resev. Manage. 18:181-190.

Hamel KS, Madsen JD, Getsinger KD. 2001. The use of 2,4-D for selective control of an early infestation of Eurasian watermilfoil in Loon Lake, Washington. J. Aquat. Plant Manage. 39:117-125.

Hauxwell J, Knight S, Wagner K, Mikulyuk A, Nault M, Porzky M, Chase S. 2010. Recommended baseline monitoring of aquatic plants in Wisconsin: sampling design, field and laboratory procedures, data entry and analysis, and applications. Wisconsin Department of Natural Resources Bureau of Science Services; PUB-SS-1068 2010. Madison, WI, USA.

Kim M, Cui M, Cubas P, Gillies A, Lee K, Chapman MA, Abbott RJ, Coen E. 2008. Regulatory genes control a key morphological and ecological trait transferred between species. Science. 14:1116-1119.

Madsen J, Getsinger KD, Stewart RM, Owens CS. 2002. Whole lake fluridone treatments for selective control of Eurasian watermilfoil: II. Impacts on submersed plant communities. Lake Reserv. Manage. 18:191-200.

Michel A, Arias RS, Scheffler BE, Duke SO, Netherland MD, Dayan FE. 2004. Somatic mutation-mediated evolution of herbicide resistance in the nonindigenous invasive plant hydrilla (*Hydrilla verticillata*). Mol. Ecol. 13:3229-3237.

MESB Sonar Investigation Panel. 1999. Evaluation of the Use of Sonar in Michigan. Michigan Environmental Science Board, Lansing, MI.

Moody ML, Les DH. 2002. Evidence of hybridity in invasive watermilfoil (*Myriophyllum*) populations. Proc. Natl. Acad. Sci. USA. 99:14867-14871.

Moody ML, Les DH. 2007. Geographic distribution and genotypic composition of invasive hybrid watermilfoil (*Myriophyllum spicatum x M. sibiricum*) populations in North America. Biol. Invasions. 9:559-570.

Newman R. 1996. Effects of a potential biocontrol agent, *Euhrychiopsis lecontei*, on Eurasian watermilfoil in experimental tanks. Aquat. Bot. 53:131-150.

Poovey AG, Slade JG, Netherland MD. 2007. Susceptibility of Eurasian watermilfoil (*Myriophyllum spicatum*) and a milfoil hybrid (*M. spicatum x M. sibiricum*) to triclopyr and 2,4-D amine. J. Aquat. Plant Manage. 45:111-115.

Rieseberg LH, Raymond O, Rosenthal DM, Lai Z, Livingstone K, Nakazato T, Durphy JL, Schwarzbach AE, Donovan LA, Lexer C. 2003. Major ecological transitions in wild sunflowers facilitated by hybridization. Science. 301:1211-1216.

Sprecher SL, Netherland MD, Stewart AB. 1998. Phytoene and carotene response of aquatic plants to fluridone under laboratory conditions. J. Aquat. Plant Manage. 36:111-120.

Sturtevant AP, Hatley N, Pullman GD, Sheick R, Shorez S, Bordine A, Mausolf R, Lewis A, Sutter R, Mortimer A. 2009. Molecular characterization of Eurasian watermilfoil, northern watermilfoil, and the invasive interspecific hybrid in Michigan lakes. J. Aquat. Plant Manage. 47:128-135.

Thum RA, Lennon JT, Connor J, Smagula AP. 2006. A DNA fingerprinting approach for distinguishing native and non-native milfoils. Lake Reserv. Manage. 22:1-6.

Thum RA, Zuellig MP, Johnson RL, Moody ML, Vossbrinck C. 2011. Molecular markers reconstruct the invasion history of variable leaf watermilfoil (*Myriophyllum heterophyllum*) and distinguish it from closely related species. Biol. Invasions. 13:1687-1709.

Unmuth JM, Sloey DJ, Lillie RA. 1998. An evaluation of close-cut mechanical harvesting of Eurasian watermilfoil. J. Aquat. Plant Manage. 36: 93-100