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Note

Mesocosm evaluation of three herbicides on Eurasian watermilfoil (*Myriophyllum spicatum*) and hybrid watermilfoil (*Myriophyllum spicatum* × *Myriophyllum sibiricum*): Developing a predictive assay

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INTRODUCTION

Reported difficulties in managing hybrid watermilfoils [Eurasian watermilfoil (*Myriophyllum spicatum* L.) × northern watermilfoil (*Myriophyllum sibiricum* Komarov] have generated significant interest and concern from aquatic plant managers across the northern tier of the United States. Hybridity between various watermilfoils was first suggested by Patten (1954), yet it was not until the mid-2000s that improved techniques for genetic identification documented several new finds of novel hybrid watermilfoil genotypes (Moody and Les 2002, 2006; Thum et al. 2006; Sturtevant et al. 2009; Thum et al. 2012). These findings have since been associated with multiple, anecdotal claims of management failures, presumably because of the increased vigor or herbicide tolerance of hybrid watermilfoils. Initial laboratory studies evaluating two hybrid watermilfoil genotypes did not confirm increased tolerance to higher use rates of several aquatic herbicides (Poovey et al. 2007, Slade et al. 2007). Nonetheless, as continued claims of reduced herbicide control were conveyed by resource managers, research documented greater tolerance of specific hybrid watermilfoil genotypes to 2,4-D and fluridone (Glomski and Netherland 2010; Berger et al. 2012, 2015; Thum et al. 2012; LaRue et al. 2013). These studies suggest a more-complex picture, whereby specific hybrid watermilfoil genotypes may express varying levels of tolerance to lower concentrations of specific herbicides. Hybridity may be linked to increased invasive potential of watermilfoils (Thum and Lenon 2006), increased herbicide tolerance, or both. A hybrid watermilfoil that is more competitive and tolerant to herbicides would represent a novel genotype with potential for spread (and presumably greater impact than either parent) to nearby water bodies (Roley and Newman 2008).

Although published research has been limited to evaluating a few hybrid watermilfoil populations against single herbicides, there are anecdotal claims that hybrids show increased tolerance to registered aquatic herbicides in general. To address the question of potential for differential herbicide tolerance by hybrid watermilfoils, we obtained a hybrid with confirmed tolerance to the herbicide fluridone (Townline Lake, MI), two hybrids from Wisconsin lakes with reported tolerance to 2,4-D (Frog and English lakes), and a Eurasian watermilfoil population from Lake Minnetonka, MN. Each watermilfoil population was exposed to the herbicides 2,4-D, endothall, and diquat at selected concentration and exposure time scenarios in outdoor mesocosm facilities. The study objective was to determine the feasibility of using short-term, efficacy assessments on rooted plants to compare the response of four watermilfoil accessions to three different herbicides widely used for watermilfoil control.

MATERIALS AND METHODS

Hybrid watermilfoil accessions maintained at the University of Florida, Center for Aquatic and Invasive Plants (Gainesville, FL) were originally collected from Townline Lake in western Michigan, and English and Frog lakes in northern Wisconsin in 2012. A Eurasian watermilfoil accession was collected from Lake Minnetonka, MN. Before establishing these cultures, plants had been confirmed as either hybrid or Eurasian genotypes by Grand Valley State University (Allendale, MI). In May 2013, 4-cm tips were cut from stems of mature plants, established in 100-ml containers with potting soil, and fertilized with Osmocote[1] (14–3–14, N–P–K) at a rate of 1 g kg^{-1}. Containers with plants from each watermilfoil accession were then placed in 3.78-L pots, and the pots were transferred into 1,000-L concrete tanks (0.6 m depth). In late May 2013, after 21 d of

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Table 1. Percentage of biomass compared with untreated reference plants for four watermilfoil accessions at 1, 2, and 4 wk after treatment (WAT) following application of the herbicides diquat, endothall, and 2,4-D at varying concentrations and exposure times ($n = 6$). Letters represent differences within an herbicide/exposure treatment between the four watermilfoil accessions.

		Diquat 0.37 mg/L		Endothall 1.5 mg/L		2,4-D 0.5 mg/L				
Taxon	Source	8 h	24 h	8 h	24 h	8 h	24 h	48 h	96 h	144 h
1 WAT										
Eurasian	Minnetonka	7 b	0 b	92	71 b	23 c	41 c	0 c	0 b	0
Hybrid	Townline	137 a	39 a	96	64 b	64 b	57 b	0 c	0 b	0
Hybrid	Frog	4 b	0 b	90	59 b	54 b	33 c	20 b	26 a	0
Hybrid	English	3 b	0 b	86	82 a	71 a	77 a	75 a	28 a	0
2 WAT										
Eurasian	Minnetonka	3 b	0 b	113 a	73 a	23 c	43bc	0 c	0 b	0
Hybrid	Townline	69 a	31 a	55 c	41 c	64 a	54 b	0 c	0 b	0
Hybrid	Frog	1 b	0 b	76 b	68 b	54 b	31 c	29 b	26 a	0
Hybrid	English	6 b	0 b	84 b	75 b	77 a	72 a	65 a	38 a	0
4 WAT										
Eurasian	Minnetonka	11 b	3 b	92 b	121 a	28 c	35 b	0 c	0 c	0 b
Hybrid	Townline	115 a	42 a	66 c	44 c	43 c	51 b	2 c	0 c	0 b
Hybrid	Frog	0 c	0 b	107 a	83 b	77 b	43 b	50 b	16 b	0 b
Hybrid	English	0 c	0 b	117 a	121 a	108a	98 a	97 a	42 a	18 a

growth, the 3.78-L containers with plants were removed and placed into 95-L tanks for herbicide exposure. Plants were treated with diquat at 0.37 mg ai L^{-1}, endothall at 1.5 mg ae L^{-1}, and 2,4-D at 0.5 mg ae L^{-1}. These treatments represent the maximum allowable concentration for diquat, half the recommended rate for endothall, and one-eighth the maximum allowable concentration for 2,4-D. Diquat- and endothall-treated plants were exposed to herbicide concentrations for 8 and 24 h before being thoroughly rinsed and moved back into six 1,000-L grow-out tanks. The 2,4-D–treated plants were exposed for 8, 24, 48, 96, and 168 h and were then moved into the grow-out tanks. These treatment concentrations and exposures were chosen to discern potential differences in response between watermilfoil accessions. Each treatment was replicated six times (i.e., each replicate had one pot of each watermilfoil accession). Plants were harvested at 1, 2, and 4 wk after treatment (WAT), and aboveground shoot biomass was dried to a constant weight at 70 C for 48 h. Plant data are presented as the percentage of biomass of the untreated reference for each watermilfoil accession. These data were subjected to ANOVA, and means comparing the response of each watermilfoil accession within each herbicide/exposure treatment were separated via a Duncan's multiple range test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

The initial 4-cm shoots (0.05 ± 0.1 g dry wt.) exhibited healthy growth and all watermilfoil accessions had formed extensive root systems during the pretreatment growth period. Initial pretreatment watermilfoil biomass range was 1.5 g (English), 1.2 g (Minnetonka), 1.0 g (Frog), and 0.8 g (Townline). Final biomass of untreated control plants at 4 wk ranged from 1.5 to 2.1 g dry weight, suggesting that additional growth was limited in these small containers.

By 1 WAT, diquat had reduced the biomass of the Minnetonka, English, and Frog watermilfoil accessions by $> 90\%$ (Table 1). These treatments led to a rapid and near-complete collapse of plant tissue, and the trends persisted through the 2 and 4 WAT evaluations. In contrast, the Townline population showed greater tolerance to diquat throughout the evaluation (Table 1). Increasing the diquat exposure period from 8 to 24 h increased activity on the Townline plants. These data suggest the feasibility of a rapid comparative evaluation of diquat on different watermilfoil accessions.

Results for endothall were variable, and watermilfoil control was generally poor after exposure periods of 8 and 24 h (Table 1). Biomass of the Townline population showed a greater reduction when compared with the other three populations. Overall, these results suggest the additional development work to determine optimal concentration and exposures for comparing endothall activity on watermilfoils should be considered.

Treatments with 2,4-D confirmed field reports of increased tolerance of both the Frog and English lake hybrid watermilfoil populations (Table 1). The ability to detect this trend at 1 WAT was somewhat unexpected. However, sensitive plants showed severe injury after a 48-h exposure, and these results remained consistent through the 4-wk evaluation. The response of the English and Frog plants suggest different levels of sensitivity to 2,4-D for these two genotypes. Although increasing exposure times to 144 hrs resulted in greater overall control of all four watermilfoil accessions, plants from Frog and English Lakes were consistently more tolerant to 2,4-D after the 48- and 96-h exposures.

These results confirmed differences in response of watermilfoil populations to widely used herbicides, and they further suggested that these differences might be detected as early as 1 WAT for herbicides such as diquat and 2,4-D. Prior work by Berger et al. (2015) suggested that fluridone impacts on different watermilfoil populations could be detected within 1 WAT using pigment response. Therefore, it was encouraging that the current studies provided biomass data that allowed for determination of differences between watermilfoil populations by 1 WAT. The reduced response of the Townline Lake population to diquat is the first report, to our knowledge, of the potential for increased tolerance of a hybrid watermilfoil to diquat. Interestingly, the Townline population has also been documented as more tolerant to the herbicide fluridone (Berger et al. 2012). In contrast, the hybrids from Wisconsin were highly susceptible to diquat but demonstrated greater tolerance to 2,4-D. Frog and English lakes have been targeted with whole-lake 2,4-D at concentrations of about 0.3 mg L^{-1}, and rapid, late-season watermilfoil recovery has been noted in both of these lakes (J. Skogerboe, pers. comm.). The potential for this type of treatment to result in rapid selection pressure resulting in dominance of hybrid watermilfoils has been discussed by LaRue et al. (2013). Although laboratory and mesocosm studies are not necessarily predictive of the response in the field, these assays allow for rapid documentation of increased tolerance to these herbicides. This information can be important to managers when they choose the product, use rate, and timing for a given management action. As these studies are further refined, the ultimate goal will be to develop an assay or genetic test that can predict how a given hybrid is likely to respond to a given herbicide and use strategy (e.g., timing, concentration, formulation).

The ability to predict how a specific hybrid may respond to a selected herbicide or combination of herbicides would be of value to resource managers. Our study results suggest that standardization of methods to evaluate comparative sensitivity of various watermilfoils is warranted. Recent studies utilizing an Organization for Economic Cooperation and Development (OECD) protocol to determine Eurasian watermilfoil sensitivity to a new arylpicolinate herbicide suggest this method may merit further evaluation (Netherland and Richardson 2016). This growth chamber assay utilizes rooted plants and standard culture and growth conditions. It can easily be modified to accommodate various concentrations and exposure scenarios of interest and the small-scale nature of this test allows for testing numerous treatments across a broad range of concentrations and exposures. Given the large number of hybrid populations and potential for multiple unique patterns of herbicide susceptibility (as well as potential herbicide and combination recommendations), the ability to develop a rapid, standard screening method is important. These small-scale and rapid screening methods will ultimately require validation to confirm predictions of field outcomes. The initial objective would be to develop a method that allows us to determine whether a hybrid watermilfoil is likely to respond to a given herbicide in a manner different from Eurasian watermilfoil. To date there are no data to suggest that Eurasian watermilfoil collected from different sites shows variable tolerances to herbicides; however, that requires further validation. Future research should focus on evaluating, validating, and standardizing screening strategies to ensure method optimization. Although some types of genetic test—e.g., GenTEST[2] for fluridone sensitivity of hydrilla [*Hydrilla verticillata* (L. f.) Royle]—would ultimately be preferable to a multistep bioassay, the complexity of hybrid watermilfoil tolerance to various herbicides may preclude that type of testing. At a minimum, development of a genetic approach would still require careful laboratory validation to determine herbicide sensitivity of different hybrid watermilfoil populations.

SOURCES OF MATERIALS

[1]Osmocote (14-3-14), Scotts Miracle-Gro Company, 14111 Scottslawn Road, Marysville, OH 43041.

[2]GenTEST, SePRO Corporation, 11550 North Meridian Street, Suite 600, Carmel, IN 46032.

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