Small-Scale Evaluation of Diquat, Endothall, and 2,4-D on Eurasian Watermilfoil and Three Hybrid Watermilfoils

by

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Introduction

This report provides data on trials that were initiated in the spring of 2013 to determine if differences in herbicide response between Eurasian watermilfoil and three hybrid watermilfoils could be ascertained under outdoor mesocosm conditions. While initial studies evaluating higher rates of 2,4-D did not support significant differences in response between a Eurasian and hybrid population (Poovey et al. 2007). Subsequently, several papers have been published suggesting differences in response between Eurasian and hybrid watermilfoils to the herbicides 2,4-D and fluridone, especially at lower concentrations (Glomski and Netherland 2010, LaRue et al. 2013, Thum et al. 2013, Berger et al. 2013, Berger et al. 2014). While the focus of these papers has been limited to just a few hybrid populations, the results have led to some anecdotal claims suggesting that all hybrids show increased tolerance to herbicides.

To address the question of potential for differential herbicide tolerance by hybrid watermilfoils we obtained a hybrid with confirmed tolerance to the herbicide fluridone (Lake Townline, Michigan), two hybrids from Wisconsin with suspected tolerance to 2,4-D (Frog and English lakes) and Eurasian watermilfoil from Lake Minnetonka, MN. Each milfoil population was exposed to 2,4-D, endothall, and diquat under a range of concentration and exposure time scenarios in outdoor mesocosms.

Materials and Methods

Eurasian milfoil collected from lakes English, Frog, Townline and Minnetonka were grown in culture at the Center for Aquatic and Invasive Plants. In May 2013, 4cm tips were cut from stems of mature plants and planted in 100 ml containers filled with potting soil and fertilized with Osmocote pellets (14:3:14) at a rate of 1 g kg -1. The containers with plants were placed in 1 L pots that were placed in 1,000 L concrete tanks (Figure 1). In June 2013 after 4 weeks of growth, plants were removed and placed into 95 L tanks for treatment. 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, ½ the recommended maximum for endothall, and 1/8 the maximum allowable concentration for 2,4-D. Diquat and endothall treated plants were exposed to herbicide concentrations for 8 and 24 hours before being thoroughly rinsed and moved back into the 1000 L grow-out tank. The 2,4-D treated plants were exposed for 8, 24, 48, 96 and 168 hours and were then moved into the grow-out tank. Plants were visually rated and harvested at 1, 2 and 4 WAT. Each treatment was replicated six times. Plant data are presented as fresh weight per container (dry weight data are available, but have not been analyzed). Data were subjected to ANOVA and means separated via a Duncan's multiple range test (α=0.05)

Figure 1. Eurasian and hybrid milfoil established for mesocosm evaluations of three herbicides.

Results and discussion

At 1 WAT diquat and 2,4-D treatments were highly effective against the Minnetonka milfoil (Figure 2). The plants were actively growing at the time of treatment, and the herbicide exposure to diquat and 2,4-D generally led to rapid and near complete collapse of the plant tissue. In contrast, the Townline milfoil treated with diquat for 8 hours resulted in greater biomass than the untreated reference. Increasing the exposure period to diquat from 8 to 24 hrs resulted in greater efficacy. For both the Frog and English Lake plants, the exposures to diquat resulted in similar results to that observed for Lake Minnetonka (complete collapse of tissue); however, response to 2,4-D was reduced compared to that observed for EWM from Minnetonka (Figure 2). Endothall generally showed limited activity at 1 WAT. While a 1 week assessment is relatively close to the application date, it represents an opportunity to determine if certain populations are either highly sensitive (e.g Minnetonka EWM to diquat and 2,4-D) or more tolerant. In this case, the speed of diquat and 2,4-D activity on Lake Minnetonka suggests a highly sensitive population, while reduced activity of diquat was noted for Townline Lake and reduced activity of 2,4-D was noted fo Frog and English lakes.

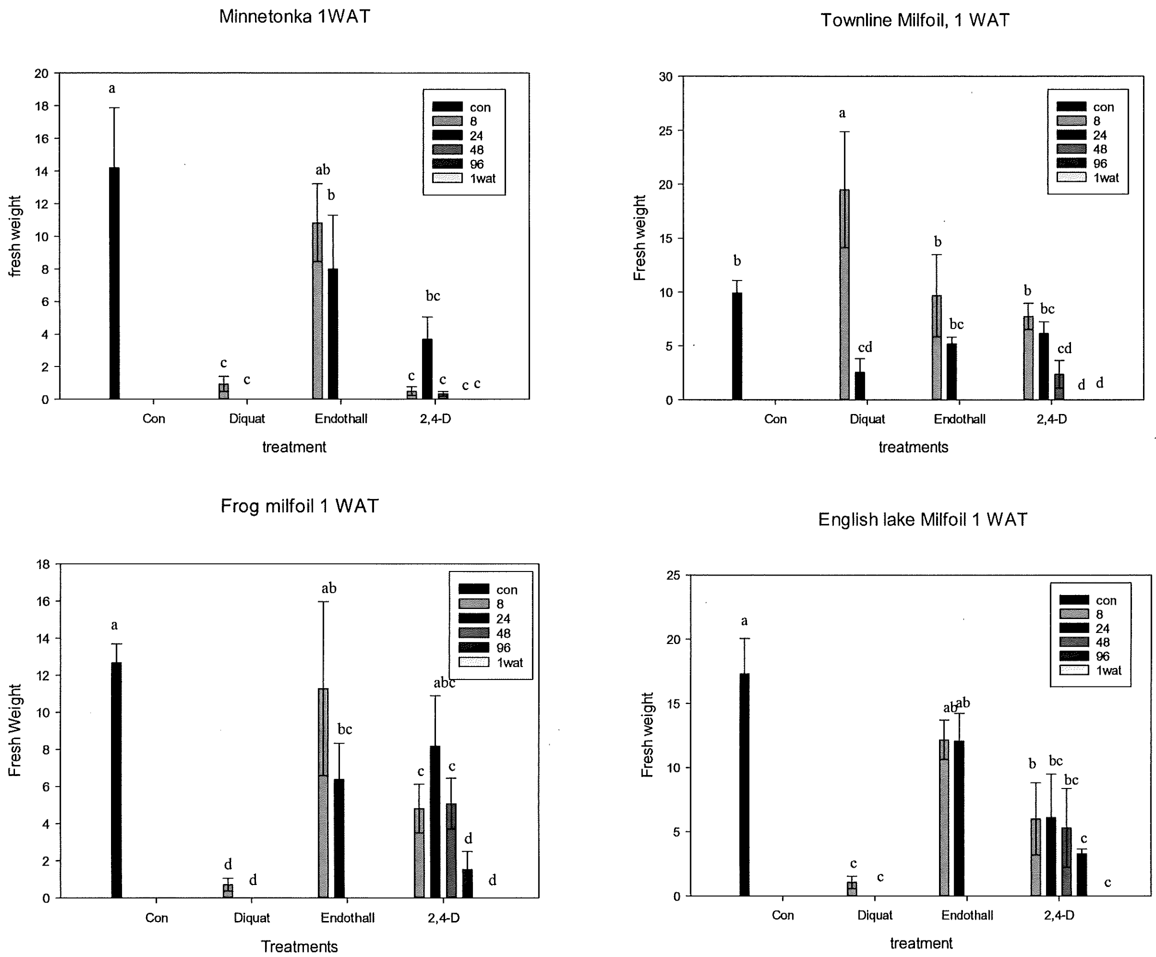

Figure 2. Response of EWM and three hybrid watermilfoils at 1 week following exposure to diquat, endothall, and 2,4-D at varying concentrations and exposure times.

By 2 WAT EMW from Lake Minnetonka remained highly susceptible to both diquat and 2,4-D treatments (Figure 3). While biomass for the Townline milfoil was reduced compared to the untreated reference following exposure to diquat, these plants were producing new healthy tissue at 2 WAT. In contrast, the English, Frog, and Minnetonka milfoils were severely injured by the diquat and there was very limited viable tissue remaining by 2 WAT (Figure 3). The English Lake plants were recovering from the all 2,4-D exposures, while the Townline and Frog plants were showing evidence of recovery following the shorter exposures to the 2,4-D applications. As noted in the 1 WAT assessment, the endothall treatments were not very effective with the exception of 50 to 70% biomass reduction of the Townline plants.

Diquat, Endothall, and 2,4-D on watermilfoils

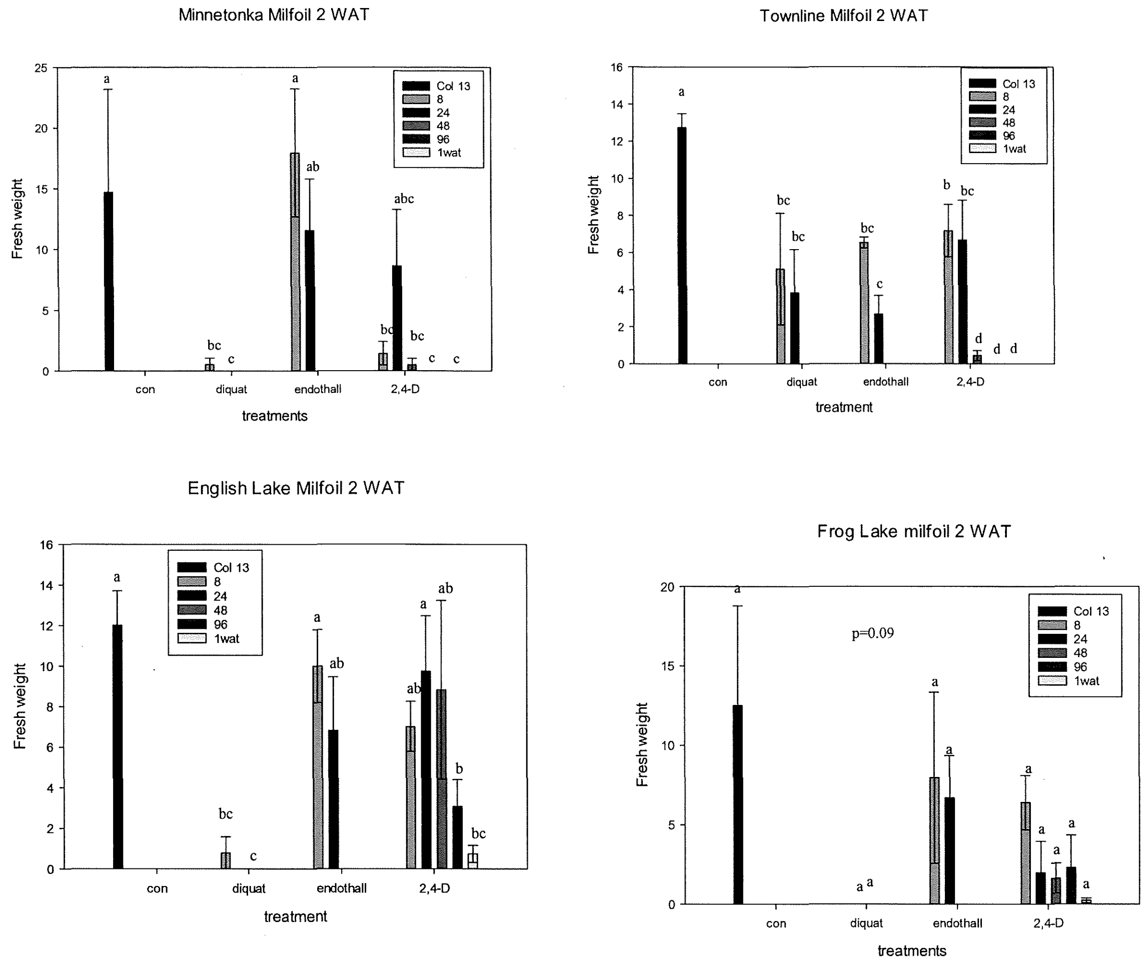

Figure 3. Response of EWM and three hybrid watermilfoils at 2 weeks following exposure to diquat, endothall, and 2,4-D at varying concentrations and exposure times.

At 4 WAT, plants from Minnetonka, Frog and English remained severely impacted following both diquat exposures (Figure 4). In contrast, the Townline plants exposed for 8 hours were not different from the untreated reference. Plants exposed to diquat for 24 hours showed much greater biomass reduction; however, these plants remained green with evidence of new growth following the initial injury. As noted at 1 and 2 weeks, the EWM from Minnetonka showed the greatest susceptibility to 2,4-D while the hybrid plants from English Lake showed potential for recovery across a range of 2,4-D exposures. The Townline plants showed a clear trend with decreasing biomass following longer 2,4-D exposure periods while the Frog plants tolerated exposures through 48 hrs. New shoot growth was apparent on these plants.

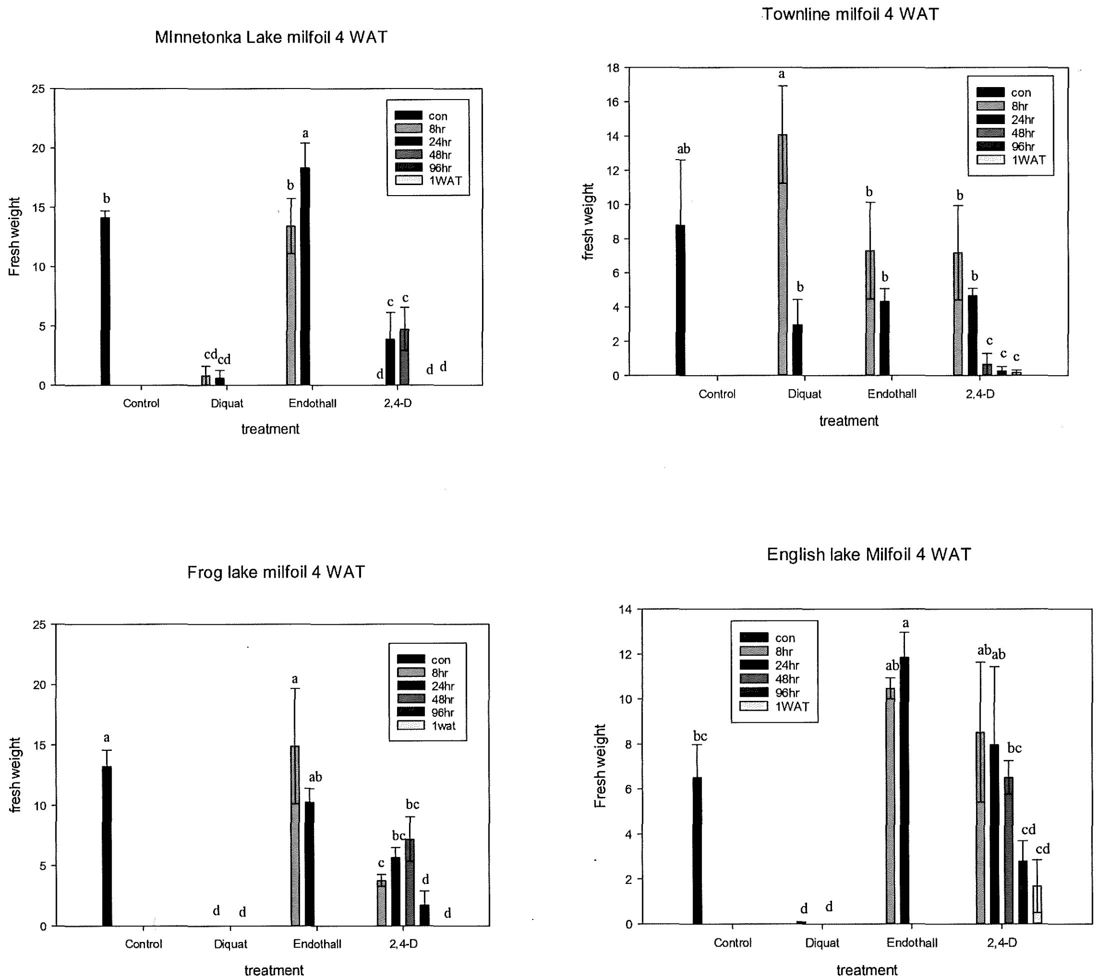

Figure 4. Response of EWM and three hybrid watermilfoils at 4 weeks following exposure to diquat, endothall, and 2,4-D at varying concentrations and exposure times.

Table 1. Response in grams fresh weight of EWM and three hybrid watermilfoils at 4 weeks following exposure to diquat, endothall, and 2,4-D at varying concentrations and exposure times

			Diquat		Endothall		2,4-D			
Taxon	**Source**	**Control**	***8 h***	***24 h***	***8 h***	***24 h***	***8 h***	***24 h***	***48 h***	***96 h***
Eurasian	Minnetonka	14	2	1	13	17	4	5	0	0
hybrid	Townline	9	14	3	6	4	7	5	1	0.5
Hybrid	Frog	14	0	0	15	11	3	6	7	2
hybrid	English	7	0.2	0	11	12	9	8	7	3

Discussion:

Overall these results suggest differences between hybrids and Eurasian watermilfoil in response to selected herbicides; nevertheless, there were also differences among the hybrids. In particular, the increased tolerance of the Townline Lake population to maximum concentrations of diquat is the first report of potential differential tolerance to diquat. Interestingly the hybrids from Wisconsin were highly susceptible to diquat while demonstrating increased tolerance to 2,4-D when compared to the plants from Lake Minnetonka (especially the plants from Lake English). In the case of Lake's Frog and English, these Wisconsin lakes were targeted with whole-lake 2,4-D at concentrations of ~ 0.3 mg/L. Rapid late-season milfoil recovery was noted in both of these lakes and the current mesocosm results would be supportive of these findings. We have stressed in numerous publications and presentations that increased tolerance to the lower herbicide concentrations is important in understanding the ability of these plants to recover following operational applications. While both hybrid and Eurasian watermilfoil may remain sensitive to the higher concentrations of herbicides, the rapid dispersion of herbicide concentrations following operational treatments can result in low level exposure to sub-lethal concentrations. The potential for these treatments to result in selection pressure resulting in dominance by hybrid milfoils is currently under investigation in select Minnesota lakes.

This study suggests that further evaluation of herbicide response by hybrid and Eurasian watermilfoil populations is warranted. Our approach was to evaluate the short-term response by different accessions of watermilfoils to selected herbicides. This approach allows for rapid assessment of different populations, yet extrapolating these results to the field requires further research. Given the large number of hybrid populations and potential for each to show unique patterns of herbicide susceptibility, the ability to develop a rapid screening method is important. Improved validation of various small-scale and rapid screening methods is necessary to increase confidence that results obtained at the small-scale predict field outcomes. At this point, researchers have been able to demonstrate that less than expected reductions (e.g. Fluridone on Townline Lake and 2,4-D on Lakes English and Frog) can yield corresponding lab and mesocosm data; nevertheless, this is very different than lab or mesocosm results being able to predict operational results. Future research should focus on evaluating and validating these screening methods to insure method optimization.

CITATIONS

Berger ST, Netherland MD, MacDonald GE (2012) Evaluating fluridone sensitivity of multiple hybrid and Eurasian watermilfoil accessions under mesocosm conditions. J Aquat Plant Manage 50:135-144.

Berger, ST, GE MacDonald, and MD Netherland. 2015. Laboratory documentation of multiple herbicide tolerance to fluridone, norflurazon, and topramazone in a hybrid watermilfoil (*Myriophyllum spicatum* X *M. sibiricum*) population. Weed Sci. 63:235-241.

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.

LaRue EA, Zuellig MP, Netherland MD, Heilman MA, Thum RA. 2013. Hybrid watermilfoil lineages are more invasive and less sensitive to a commonly used herbicide than their exotic parent (Eurasian watermilfoil). Evolutionary Applic. 6(3):462-471.

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. Journal of Aquatic Plant Management 45:111-115.

Thum RA, Heilman MA, Hausler PJ, Huberty LE, Tyning P, Wcisel DJ, Zuellig MP, Berger ST, Netherland MD. 2012. Field and laboratory documentation of reduced fluridone sensitivity of a hybrid watermilfoil biotype (*Myriophyllum spicatum* x. *Myriophyllum sibiricum*). Journal of Aquatic Plant Management 50:141-146