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Note

Mesocosm and field evaluation of Eurasian and hybrid watermilfoil response to endothall in Jefferson Slough, Montana

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INTRODUCTION

Aquatic plant management decisions and outcomes are influenced by a wide range of variables, such as site-specific hydrology and water quality. In addition, genetic variation in the plant species targeted for control can play a role in management outcomes. Individuals and populations of a species may not all be genetically identical, and different genotypes may exhibit variation in management-relevant traits, such as growth and herbicide sensitivity. For example, mutations in the phytoene desaturase gene of hydrilla (*Hydrilla verticillata* (L. f.) Royle) can confer resistance to fluridone (Michel et al. 2004). Likewise, individual biotypes of fanwort (*Cabomba caroliniana* A. Gray) exhibit different responses to some herbicides (Bultemeier et al. 2009). Explicit consideration of genetic variation may therefore facilitate predictions regarding efficacy of proposed control tactics in specific water bodies. Yet, aquatic plant management projects rarely consider genetic variation when designing and evaluating treatment plans.

Eurasian watermilfoil (*Myriophyllum spicatum* L.; EWM) is one of the most widespread and frequently managed invasive aquatic plant species across the northern tier of the United States. Management is primarily through herbicides, and several local factors are considered when making herbicide decisions. For example, herbicide formulations and use patterns will be influenced by local hydrological, chemical, and biological factors. All of these can influence the impacts on target and nontarget species. However, recent research has demonstrated that EWM is more genetically diverse than originally recognized. There are numerous genotypes of at least two genetically distinct biotypes of EWM, as well as many genotypes from frequent hybridization between both biotypes of EWM and native northern watermilfoil (*Myriophyllum sibiricum* Komarov) (Moody and Les 2002, Moody and Les 2007, Sturtevant et al. 2009, Zuellig and Thum 2012, LaRue et al. 2013a,b). Individual populations may be composed of a single or multiple genotypes, and different populations of EWM and hybrid watermilfoils (HYB) are often composed of different sets of genotypes (R. A. Thum, unpub. data). Nevertheless, genetic variation is not commonly considered during operational management programs for EWM.

One emerging concern among water resource managers is whether, and how often, hybrid genotypes pose unique management challenges compared with wild-type ("pure") EWM. Several studies have demonstrated that EWM and HYB can have different responses to some herbicides (Glomski and Netherland 2010, Thum et al. 2012, Berger et al. 2012, LaRue et al. 2013a, Berger et al. 2015, Netherland and Willey 2017), whereas other studies have found no difference in the response of EWM and HYB from the populations studied (Poovey et al. 2007, Slade et al. 2008). Given the evident variability in growth and response to herbicide among genotypes, managers would benefit from studies that can help predict whether the specific genotypes present in their water bodies will respond differently to proposed control tactics. This information would be especially important for water bodies where EWM and HYB co-occur.

Jefferson Slough, near Cardwell, MT, is one location where EWM and HYB co-occur. EWM was first discovered in the slough in 2011, and a genetic survey of the slough in 2014 found that the watermilfoil population was composed of both EWM and HYB. In the upstream reaches of the slough, only EWM was found, whereas HYB dominated the downstream reaches. A small segment near the middle of the slough contained a mixture of both (Figure 1).

Initial watermilfoil control efforts in Jefferson Slough focused on hand pulling, but this method was deemed ineffective after 3 yr. Because of factors such as flowing and turbid water, a 3 mg L^{-1} endothall treatment was proposed on the basis of laboratory concentration and exposure time studies (Netherland et al. 1991, Skogerboe and Getsinger 2002) and demonstrated control in the field (Parsons et al. 2004). However, in light of other studies demonstrating the potential for differential growth and herbicide response by EWM and HYB, Jefferson Slough managers were interested in determining whether there was any evidence for differences in endothall response by the two different biotypes; in particular, whether hybrids would be tolerant to the proposed endothall treatment.

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In this study, our goal was to determine whether the proposed endothall treatment in Jefferson Slough would have similar short-term efficacy (within the growing season) on EWM and HYB. To do so, we conducted a greenhouse assay to compare vegetative growth and response to 3 mg L^{-1} endothall by EWM and HYB collected from Jefferson Slough in 2015. Then, we performed pre- and posttreatment sampling to evaluate the efficacy of an operational 3 mg L^{-1} endothall treatment in Jefferson Slough in 2016.

MATERIALS AND METHODS

Growth and endothall response study

In August 2015, we collected plants throughout Jefferson Slough to establish cultures in the greenhouse for the growth and endothall response experiment. In 2014, we established 100 permanent sampling points spaced at approximately 70-m intervals throughout a 9.6-km portion of the slough that was known to have watermilfoil. On the basis of a 2014 genetic survey of these points, we expected to find EWM in upstream reaches (points 1 to 39) and HYB in downstream reaches (points 56 to 100), and indeed, visual identifications suggested that this was the case for the 2015 samples. Nevertheless, we randomly sampled 23 plants from our 2015 collections at these points to confirm their identifications using restriction enzyme banding patterns for the internal transcribed spacer (ITS; Thum et al. 2006, Grafé et al. 2015). Furthermore, microsatellite genotype data on these samples (data not shown, but see Taylor et al. 2017 for details on the method) indicated that only one genotype of EWM and one genotype of HYB were present in 2015, suggesting that the slough was dominated by a single genotype each that had extensively spread via clonal reproduction.

After confirming identities, we established cultures in the greenhouse. We mixed all of the plants from sampling points with EWM together. Similarly, we mixed all of the plants from sampling points with HYB together. We then randomly selected plants from each group and planted approximately 25 plants into each of 16 7.6-L pots per taxon. Pots were filled with potting soil supplemented with 2.2 mg kg^{-1} of a controlled-release fertilizer.[1] Afterward, we randomly assigned four pots of each taxon to each of four 568-L tanks. Tanks were filled with dechlorinated tap water from Montana State University, supplemented with a continuous supply of CO_2 and a liquid medium on the basis of Smart and Barko (1985). Natural light in the greenhouse was supplemented with a full-spectrum sodium lamp[2] to create a 14 : 10 h light : dark cycle. These plants were allowed to grow in the greenhouse for approximately 2 mo to remove or minimize any maternal or environmental effects originating from the field collections.

Two months after planting, we harvested plants from these cultures to use for our experiment. We randomly assigned three 12-cm apical segments to each of 48 2.4-L pots for each taxon. Pots were filled with the same soil and Osmocote formulation as described above. Three pots of each taxon then were randomly allocated to each of 16 208-L barrels that were filled with water and nutrients as described above, and light as described above. Within each barrel, we used a mesh netting to ensure that EWM and HYB did not intermingle in the water column. Plants were allowed to grow for 3 wk, at which point most had grown to the water surface.

Four of the 208-L barrels were randomly assigned to the endothall treatment; the other four remained as untreated controls. Treated plants were exposed to 3 mg L^{-1} endothall[3] for 12 h, after which the water was completely flushed via continuous flow for 1 hour. We collected water samples from each tank at the time of treatment to confirm that the target concentrations were reached, and immediately after flushing to confirm that the endothall was removed. Three weeks after exposure, we harvested all living plant material (roots and shoots) for biomass measurement. We oven-dried plant tissue at 43 C for 1 wk, and measured for total biomass (roots and shoots) to the nearest 0.01 g. We analyzed biomass data using a split-plot ANOVA, with tank as the main plot and taxon as the split plot. We also performed two *a priori* contrasts: one comparing EWM and HYB in the untreated controls and one comparing EWM and HYB in the 3 mg L^{-1} endothall treatment.

Operational endothall treatment and evaluation

The operational herbicide treatment was performed by a commercial applicator on 13 July 2016. A 9.65-km stretch of the slough that covered the most upstream site known to have EWM and HYB down to the confluence with the Boulder River was treated with endothall[4] at a target concentration of 3 mg L^{-1} (3 ppm) for 12 h. It was not a requirement of the herbicide application permit to determine endothall concentrations, so water samples were not collected. However, endothall was applied using a drip system that was calibrated to achieve the target exposure based on a rhodamine WT (RWT) fluorescent dye[5] study conducted by the applicator 2 d before the endothall application. A high correlation between endothall and RWT has been previously established.

We conducted pre- and posttreatment sampling (8 and 9 July 2016 and 26 August 2016, respectively) at the 100 predetermined sampling points described above. At each point, we sampled watermilfoil biomass by tossing a rake approximately 1 m from one side of the boat. Any debris or nonmilfoil plant species were removed on site. We collected approximately 3- to 5-cm sections of one to three apical meristems of representative plants from each rake toss to confirm identifications using ITS as previously described. The watermilfoil samples were then oven-dried at 43 C for 1 wk, and biomass was measured to the nearest 0.01 g. We tested for differences in average posttreatment biomass using an unpaired t test and used a Zelen's test to find changes in frequency of occurrence of each taxon. Because of a mixture of EWM and HYB in points 39, 40, 41, 50, and 55, as well as the difficulty and unreliability in separating the biomass of these taxa at these locations, these points were excluded from the trial.

RESULTS AND DISCUSSION

An ANOVA from our greenhouse study the year before treatment (2015) detected significant main effects of

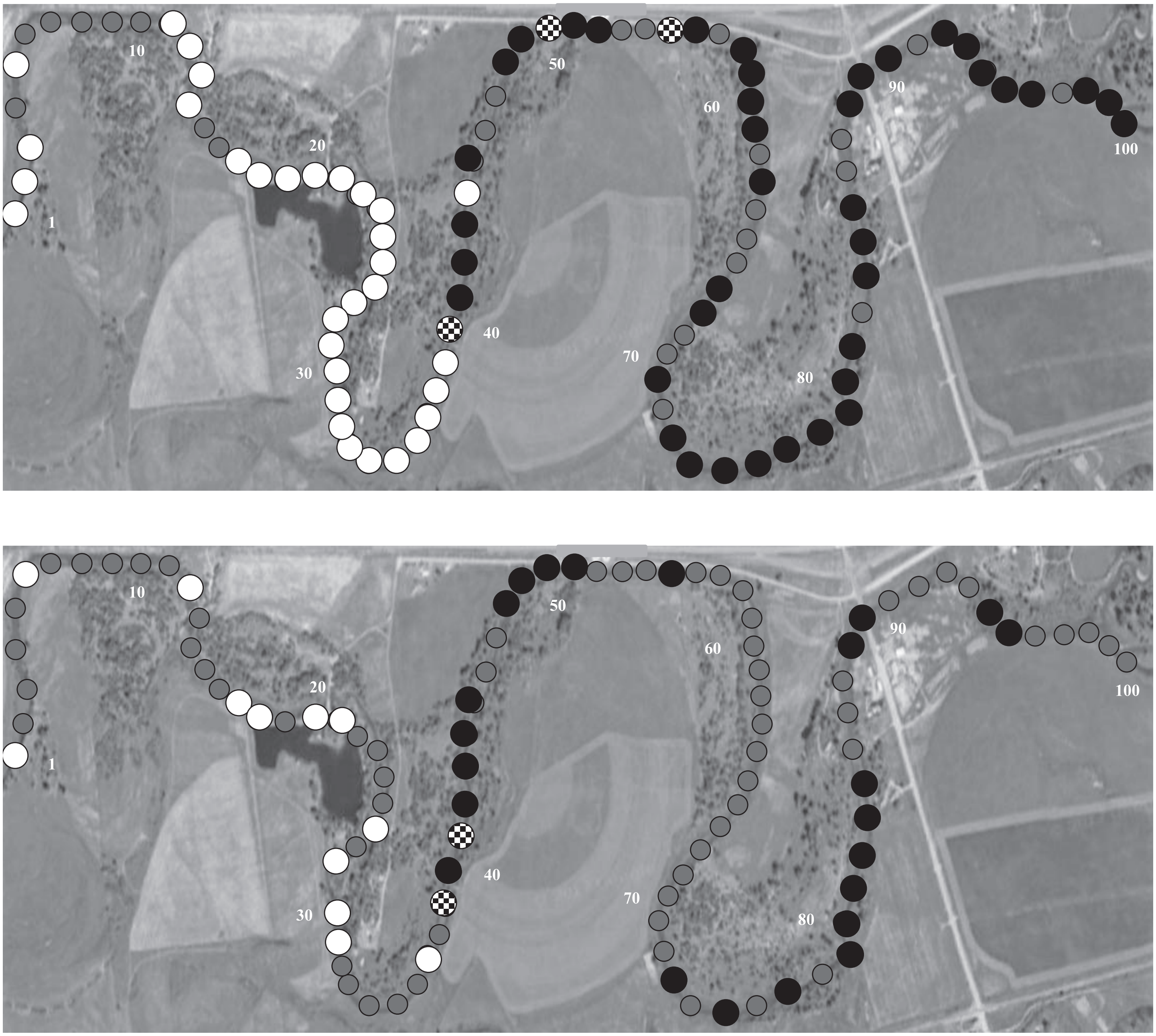

Figure 1. Map of the study area (Jefferson Slough, near Cardwell, MT) indicating the occurrence of Eurasian watermilfoil (EWM) (white circles) and hybrid watermilfoil (HYB) (black circles) during the pre- (top) and posttreatment sampling (bottom) (8 and 9 July 2016 and 26 August 2016, respectively). Points where the taxa co-occurred are indicated with black-and-white checkered circles. Smaller gray circles are points where no watermilfoil was found. Numeric labels are provided every 10 points for orientation.

endothall treatment (0 vs. 3 mg L^{-1}) and taxon (EWM vs. HYB) (Figure 2; Table 1). The interaction between taxon and endothall treatment was not significant ($P = 0.10$). *A priori* contrasts revealed a significant difference between EWM and HYB biomass in untreated (control) tanks ($P = 0.011$; Figure 2), but no significant difference between EWM and HYB biomass in tanks treated with 3 mg L^{-1} endothall for 12 h ($P = 0.41$ Figure 2). Similar to our greenhouse results, EWM and HYB were reduced to similar average biomass in Jefferson Slough after operational treatment with endothall (unpaired $t_{32} = 0.95$, $P = 0.35$; Figure 3). Furthermore, a Zelen's test for changes in frequency of occurrence indicated that the change in the proportion of points occupied by EWM and HYB did not significantly differ ($P = 1$).

On the basis of the greenhouse and field results, we conclude that EWM and HYB genotypes in Jefferson Slough at the time of treatment in 2016 were similarly susceptible to endothall under the prescribed treatment conditions (3 mg L^{-1} for approximately 12 h). The similar response to endothall by EWM and HYB present in Jefferson Slough at the time of this study reinforces that tolerance to herbicides

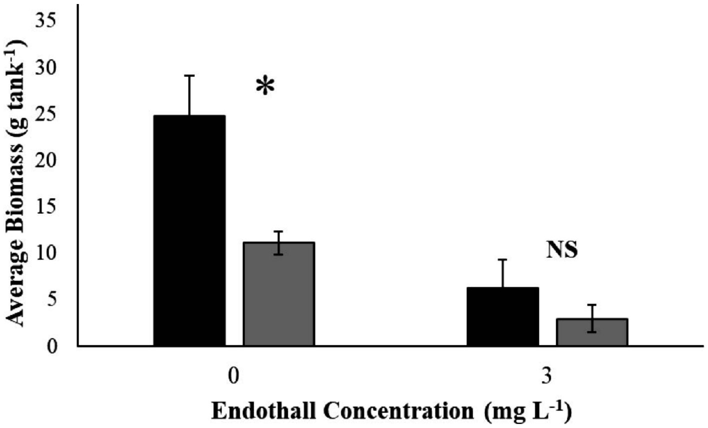

Figure 2. Total biomass (root and shoot, g tank^{-1}) of hybrid watermilfoil (HYB) (black bars) and Eurasian watermilfoil (EWM) (light gray bars) 3 wk after treatment with 0 and 3 mg L^{-1} endothall treatment for 12 h in the mesocosm study. Error bars are 1 standard error of the mean; $n = 4$. We used *a priori* contrasts to test for differences between EWM and HYB under 0 and 3 mg L^{-1}. A significant difference is indicated by an asterisk (*). NS indicates a nonsignificant difference.

is not a general property of all hybrid watermilfoils, but depends on the specific genotype (Netherland and Willey 2017).

Although there was no evidence for higher tolerance to endothall by Jefferson Slough HYB, we did observe vegetative growth differences that may have important management implications in the slough over time. In the greenhouse, Jefferson Slough HYB grew significantly more than EWM under controlled conditions (contrast $P = 0.01$; Figure 2). This result is consistent with previous studies that have identified faster vegetative growth rates of hybrid watermilfoils compared with EWM (LaRue et al. 2013a, Taylor et al. 2017, Thum and McNair 2018). In contrast, there was significantly greater biomass of EWM compared with HYB in Jefferson Slough before endothall treatment (unpaired $t_{66} = 2.72$, $P = 0.008$). However, it is important to note that EWM and HYB were located in different areas of the slough (upstream and downstream, respectively), whereas the greenhouse comparison is more appropriate because they were grown in a common environment. Where EWM and HYB were found growing intermixed in Jefferson Slough (points 39 to 55), we did observe a qualitative shift toward a higher relative frequency of HYB (Figure 1). In this section, there were 11 HYB points and 5 EWM points pretreatment. Post-treatment, there were 12 HYB points and 2 EWM points. The number of sample points in this portion of the slough was too small to determine whether this shift was statistically significant. However, this result, along with the greenhouse results, suggests a relatively higher potential for regrowth or re-establishment of HYB compared with EWM after treatment. This could mean that Jefferson Slough HYB may require more frequent treatment to achieve sufficient long-term control compared with EWM. Therefore, continued monitoring and further study of regrowth potential is warranted to determine whether changes in frequency of occurrence differ between EWM and HYB over the long term (see also Parks et al. 2016).

Table 1. ANOVA of biomass for hybrid versus Eurasian watermilfoil (Taxon) for two levels of endothall treatment (0 and 3 mg L^{-1}) in the mesocosm study.

Factor	df	Sum of Squares	Mean Square	*F*-Value	*P*-Value
Treatment	1	713.9	713.9	20.2	0.004
Residuals	6	8.5	2.8		
Taxon	1	289.0	289.0	10.2	0.018
Treatment × taxon	1	107.5	107.5	3.8	0.099
Residuals	6	169.7	28.2		

Although the specific EWM and HYB genotypes found in Jefferson Slough at the time of this study did not show significant differences in their response to endothall, it is important to note that, to the best of our knowledge, there was only one genotype of each taxon present, on the basis of microsatellite genotyping. In our case, the comparative data from the mesocosm trials on EWM and HYB collected from the Jefferson Slough helped provide confidence that the proposed operational endothall treatment would be similarly effective on the two specific genotypes present. It is important to keep in mind, however, that watermilfoil genotypes can differ in their responses to herbicides, including endothall (Netherland and Willey 2017), 2,4-D (Glomski and Netherland 2010, LaRue et al. 2013a, Netherland and Willey 2017), triclopyr (Glomski and Netherland 2010), diquat (Netherland and Willey 2017), and fluridone (Berger et al. 2012, Thum et al. 2012, Berger et al. 2015). Thus, different responses may be found among water bodies that are composed of different genotypes, and further studies of different populations are warranted. In addition, it is possible for the genetic composition to change over time within the same water body such that a watermilfoil population that is currently dominated by susceptible genotypes could become dominated by tolerant genotypes in the future. Genetic monitoring of populations could

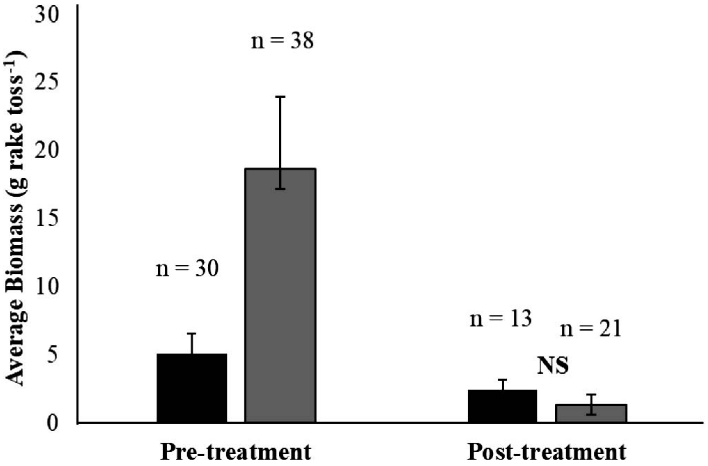

Figure 3. Total biomass (root and shoot, g toss^{-1}) of hybrid watermilfoil (HYB) (black bars) and Eurasian watermilfoil (EWM) (light gray bars) before and 6 wk after operational endothall treatment in Jefferson Slough. Each bar represents the mean biomass measurement (± standard error) of all points characterized by the taxon. We used an unpaired *t* test to test for differences between EWM and HYB average biomass posttreatment; NS indicates a nonsignificant difference.

therefore potentially be used to determine if genetic shifts have occurred over the course of a management program, which could be used to trigger additional growth and herbicide response comparisons of any newly identified genotypes of concern within a given water body. Where feasible, we recommend that aquatic plant managers quantify and monitor genetic diversity in their system, and use that information to design small-scale evaluations to predict plant response to proposed treatment tactics before operational treatment.

SOURCES OF MATERIALS

[1]Osmocote (19–6–12), Scotts Miracle-Gro Company, 14111 Scottslawn Road, Marysville, OH 43041.

[2]Full-spectrum sodium lamp, General Electric Multi-Vapor MVR1000/C/U, East Cleveland, OH 44110.

[3]Aquathol K, United Phosphorus Incorporated, 630 Freedom Business Center, Suite 402, King of Prussia, PA 19406.

[4]Cascade, United Phosphorus Incorporated, 630 Freedom Business Center, Suite 402, King of Prussia, PA 19406.

Keyacid Rhodamine WT Liquid, Keystone Aniline Corporation, 3002 West Weldon Avenue, Phoenix, AZ 85017.

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