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Heritable variation for vegetative growth rate in ten distinct genotypes of hybrid watermilfoil

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ABSTRACT

Previous studies of hybrid watermilfoil (*Myriophyllum spicatum* × *sibiricum*) demonstrate that they can exhibit unique traits such as fast vegetative growth and reduced response to herbicide. However, molecular genetic studies also demonstrate that hybrid watermilfoils are genetically diverse, and we have few data about how different hybrid watermilfoil genotypes grow and respond to commonly used herbicides, such as 2,4-D. Here, we asked whether vegetative growth rate, including in the presence of 2,4-D, is a heritable trait that differs between hybrid genotypes. We compared the vegetative growth rate of 10 different hybrid genotypes collected from across the northern tier of the United States and covering a range of the overall genetic diversity exhibited by hybrid watermilfoils. Our results demonstrate that variation in vegetative growth rate, including when exposed to 2,4-D, is due in part to genetic differences (i.e., is heritable). Although vegetative growth differed between genotypes, we observed a clear trend for higher vegetative growth rates in hybrid watermilfoil genotypes compared to two Eurasian watermilfoil (*Myriophyllum spicatum* L.) reference genotypes. Two hybrid genotypes exhibited unusually fast vegetative growth rates relative to the other hybrid and Eurasian watermilfoil genotypes. A comparison of microsatellite markers to Eurasian, northern (*Myriophyllum sibiricum* Komarov), and hybrid watermilfoils collected across North America demonstrated that the two fast-growing genotypes are not closely related, but have arisen from independent hybridization events involving two different biotypes of Eurasian watermilfoil. Based on these data, we suggest that relatively faster vegetative growth may be a common, but not universal, phenomenon in hybrid watermilfoils.

Key words: 2,4-D, Eurasian watermilfoil, herbicide tolerance, *Myriophyllum sibiricum*, *Myriophyllum spicatum*, northern watermilfoil.

INTRODUCTION

Nonnative Eurasian watermilfoil (*Myriophyllum spicatum* L.; hereafter EWM) is a widespread aquatic invasive weed in the United States. EWM hybridizes with its native North American sister species, northern watermilfoil (*Myriophyllum sibiricum* Komarov; hereafter NWM) (Moody and Les 2002, 2007; Zuellig and Thum 2012), resulting in hybrid watermilfoil.

There is increasing concern among some water resource managers that hybrid watermilfoils may be more invasive and difficult to control than wild-type ("pure") EWM. For example, laboratory and field studies identified a population of hybrid watermilfoils in Townline Lake, Michigan, that was highly tolerant to the systemic herbicide fluridone (Berger et al. 2012, 2015; Thum et al. 2012). Similarly, LaRue et al. (2013b) provided laboratory evidence for increased vegetative growth rate and tolerance to the auxinic herbicide 2,4-D for hybrid watermilfoil populations compared to EWM populations collected throughout Michigan. However, other studies have concluded that hybrid and EWM watermilfoils exhibit similar growth and herbicide response. For example, Poovey et al. (2007) compared one hybrid population (Otter Lake, Minnesota) to one EWM population (Pierson Lake, Minnesota) and concluded that the two responded similarly to the synthetic auxins 2,4-D and triclopyr. Similarly, Slade et al. (2007) compared the same hybrid population as above (Otter Lake) to one EWM population (Medicine Lake, Minnesota) and concluded that the two responded similarly to fluridone. Other studies have demonstrated that different hybrid populations can grow and respond to herbicides differently, including the following: 2,4-D and triclopyr response in hybrids collected from White Bear Lake versus Otter Lake (both in Minnesota) (Glomski and Netherland 2010); fluridone response in hybrids collected from Townline Lake and Indian Lake (both Michigan), Frog Lake (Wisconsin), and EWM collected from Auburn Lake (Minnesota), and established cultures from two research facilities in North Carolina and Texas (Berger et al. 2012); and diquat, endothall, and 2,4-D response in hybrids collected from Townline Lake (Michigan), Frog Lake (Wisconsin), and English Lake (Wisconsin), and EWM collected from Lake Minnetonka, Minnesota (Netherland and Willey 2017).

Although there are several studies comparing growth and herbicide response among populations of EWM and hybrid watermilfoil, the total number of hybrid populations that have been studied is small (but see LaRue et al. 2013b). It is therefore presently unclear how much variation exists in growth patterns among different hybrid genotypes, and how commonly hybrid watermilfoil will exhibit faster vegetative growth compared to EWM, including when exposed to herbicide. Are cases of faster-growing hybrid watermilfoil the rule or the exception? Molecular genetic studies of hybrid watermilfoils demonstrate that they are genetically diverse (Zuellig and Thum 2012, LaRue et al. 2013a,b), so it

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Table 1. Names and locations of lakes where genotypes used in the growth study were collected. H = hybrid, E = Eurasian watermilfoil.

Lake	Genotype	County	State	Latitude	Longitude
Antoine	H-1	Dickinson	Michigan	45°50′12.75″N	88°01′53.99″W
Fawn	H-2	Adams	Wisconsin	43°43′25.63″N	89°48′55.10″W
Forest	H-3	Oakland	Michigan	42°35′42.47″N	83°18′01.30″W
Halls	H-4	Isabella	Michigan	43°34′52.36″N	85°03′58.48″W
Hayden	H-5	Kootenai	Idaho	47°45′42.23″N	116°42′51.17″W
Hayward	H-6	Sawyer	Wisconsin	46°00′29.52″N	91°28′18.33″W
Houghton	H-7	Roscommon	Michigan	44°20′26.74″N	84°44′13.90″W
Ice	H-8	Iron	Michigan	46°06′03.66″N	88°36′54.94″W
Mattoon	H-9	Kittitas	Washington	46°58′38.60″N	120°33′02.50″W
Portage	H-10	Houghton	Michigan	47°03′33.59″N	88°29′48.55″W
Norway	E-1	Dickinson	Michigan	46°04′11.03″N	87°50′10.20″W
Sawyer	E-2	Dickinson	Michigan	46°10′55.60″N	88°03′40.86″W

is possible that there is considerable variation among genotypes in how they grow and respond to herbicides. On the other hand, it is possible that hybrids exhibit similar patterns of growth despite genetic diversity. For example, if hybrid vigor is common across hybridization events with different parental Eurasian and northern watermilfoil, then hybrids may commonly (not necessarily universally) display higher vegetative growth compared to parental EWM. Thus, further characterization of growth and herbicide response patterns exhibited by different hybrid watermilfoil genotypes that span the range of genetic diversity is needed.

In this study, we specifically ask whether vegetative growth rate is heritable—including when treated with the auxinic herbicide 2,4-D—by studying 10 distinct genotypes of hybrid watermilfoils. In addition, we compare the vegetative growth rates of these 10 hybrid genotypes to two representative genotypes of EWM in order to explore how commonly hybrid watermilfoils may exhibit faster vegetative growth compared to EWM.

MATERIALS AND METHODS

Plant material

We isolated one hybrid plant stem (i.e., a single genotype) from each of 10 lakes across the northern tier of the United States (Table 1). Plants were confirmed as EWM or hybrid using an internal transcribed spacer restriction analysis (Grafé et al. 2015). We vegetatively propagated each individually chosen plant in order to ensure that we would measure vegetative growth rates on individual genotypes. We chose to sample a single plant from each lake, as opposed to multiple plants from one lake, to ensure that the hybrid genotypes used in our study were all genetically different. Different lakes tend to harbor distinct hybrid watermilfoil genotypes (Zuellig and Thum 2012, LaRue et al. 2013b), but we commonly find a dominant genotype within individual lakes (R. A. Thum, unpub. data). Therefore, sampling multiple individuals from the same lake would likely have resulted in sampling the same genotype (clone), whereas sampling one individual from multiple lakes ensured that we sampled different genotypes across a range of genetic diversity exhibited by hybrid watermilfoils.

In addition to our 10 hybrid genotypes, we isolated two EWM genotypes to use as reference genotypes to compare with hybrid growth (Table 1). These two genotypes were chosen from populations studied by LaRue et al. (2013b) and represent the range of high and low EWM vegetative growth exhibited in that study.

To obtain enough plant material from each genotype for our vegetative growth assays, individual stems were planted in separate 18.9-L buckets containing potting soil supplemented with 1 g kg^{-1} Osmocote[1] (19 : 6 : 12, nitrogen : phosphorus : potassium) and capped with sand to prevent soil dispersing into the water column. We vegetatively propagated each genotype by removing and replanting lateral branches. Members of each genotype were planted in the same bucket, and all buckets were randomly placed in a 1,136-L mesocosm. Cultures were checked daily to ensure there was no cross-contamination between genotypes within the same tank. All tanks were filled with filtered water from Muskegon Lake, Michigan, and lit with a full-spectrum sodium lamp[2] on a 14 : 10-h light : dark cycle with water temperature ranging from 21 to 24 C throughout all studies. Cultures were maintained in this manner until each genotype had a sufficient number of stems for the vegetative growth assays.

Genetic analysis

To confirm that we were working with different genotypes, we genotyped the clones in this study using eight microsatellite markers (Myrsp1, Myrsp5, Myrsp9, Myrsp12, Myrsp13, Myrsp14, Myrsp15, and Myrsp16 from Wu et al. 2013). We also collected microsatellite genotype data from these same markers for EWM, NWM, and hybrids sampled from across the northern tier of the United States (Table 2) in order to illustrate the range of hybrid genetic diversity that our genotypes represented. To do this, we conducted a principal coordinates analysis, as implemented in the R package POLYSAT (Clark and Jasieniuk 2011).

Vegetative growth assays

Growth assays were designed to assess the effects of two experimental factors: genotype and 2,4-D exposure. The genotype factor had 12 levels (10 hybrid watermilfoil and two EWM genotypes) while the 2,4-D exposure factor had three levels: aqueous concentrations of 0 (control), 500, and 1,000 μg L^{-1} acid equivalent of analytical grade 2,4-D.[3] These 2,4-D concentrations were chosen to represent

Table 2. Locations of samples included in the genetic analysis to illustrate the range of genetic diversity covered by the hybrid genotypes in the vegetative growth assays. The number of unique genotypes of each taxon from each population is provided. EWM = Eurasian watermilfoil, HWM = hybrid watermilfoil, NWM = northern watermilfoil.

			Number of Unique Genotypes		
Lake	County	State	EWM	HWM	NWM
Red Tail	Weld	Colorado	—[1]	—	1
Cocolalla	Bonner	Idaho	1	1	2
Couer D'Alene	Kootenai/Benewah	Idaho	—	2	—
Hayden	Kootenai	Idaho	—	2	
Pend Oreille	Bonner/Kootenai	Idaho	2	1	3
Priest	Bonner	Idaho	1	—	1
Wilson Pond	Kennebec	Maine	—	—	1
Houghton	Roscommon	Michigan	—	2	—
Lake Louise	Dickinson	Michigan	—	—	1
Long Lake	Barry	Michigan	1	1	—
Christmas Lake	Hennepin/ Carver	Minnesota	1	—	2
Lake Minnetonka - Gray's Bay	Hennepin/ Carver	Minnesota	—	3	—
Lake Minnetonka - North Arm	Hennepin/ Carver	Minnesota	—	2	—
Lake Minnetonka - Smiths Bay	Hennepin/ Carver	Minnesota	—	2	2
Lake Minnetonka - St Albans	Hennepin/ Carver	Minnesota	1	1	—
Lake Minnetonka - Veteran's Bay	Hennepin/ Carver	Minnesota	—	2	—
Jefferson Slough	Jefferson	Montana	2	2	—
Noxon Reservoir	Sanders	Montana		3	—
Selmac	Josephine	Oregon	1	—	—
Berry	Menominee	Wisconsin	2	1	—
Hancock	Oneida	Wisconsin	—	2	1
Moshawquit	Menominee	Wisconsin	—		1
Silver	Kenosha	Wisconsin		1	

[1]Dash indicates not present.

realistic exposure concentrations in the field (Green and Westerdahl 1990, Bugbee et al. 2003). This yielded 36 treatments. Each genotype was represented by nine replicate ramets in each treatment for a total of 324 plants tested. Due to logistical constraints, it was necessary to divide the experiment into three temporal (complete) blocks, with three replicates of each genotype per treatment, yielding 108 total plants per block.

At the start of the vegetative growth assay, three replicates of each genotype from the cultures described above were planted individually in a randomly assigned grid location in a 1,136-L mesocosm, and this was repeated in each of three tanks (i.e., three replicates of each genotype in each of three tanks). These plants were allowed to grow for a 6-wk period before starting the growth assay. During this time, we vegetatively propagated each plant by cutting 11.8-cm apical meristems and replanting them at least once in order to minimize potential maternal effects during the experiment. After this initial grow-out, an 11.8-cm apical cutting was harvested from each plant for use in the assay. We blotted each plant with a paper towel and recorded its initial wet weight, wrapped it individually in a permeable netting, and randomly exposed it to one of the three 2,4-D exposure levels for 48 h. Each cutting was then individually planted in a 115-ml Cone-tainer pot[4] containing soil supplemented with 1 g kg^{-1} Osmocote, capped with sand, and placed in a mesocosm (also filled with soil supplemented with the fertilizer and capped with sand) to grow for 3 wk. At the end of the growth period, we measured length gained (final length minus 11.8 cm) and wet weight gained (final wet weight minus initial wet weight). Measurements were made on a total of 27 replicates of each genotype (three replicates per exposure level × three exposure levels × three experimental blocks).

Statistical analysis

We tested for differences in growth between genotypes using a mixed model ANOVA. Genotype, 2,4-D exposure, and the genotype × 2,4-D exposure interaction term were treated as fixed effects. In addition, we included block as a fixed effect in our model. Finally, we nested genotypes within taxon (10 hybrid, 2 EWM) to test for overall differences between EWM and hybrids. Our dependent variables were length gained and wet weight gained, but for brevity we only present results for length gained because the two growth measures were strongly correlated (Spearman's rho = 0.86, $P < 0.001$) and qualitatively similar. Data for independent variables were transformed using the Box-Cox method (Box and Cox 1964, Sakia 1992) to satisfy the ANOVA assumptions of approximately Gaussian residuals with homogenous variance. We also ran separate ANOVA models for each herbicide treatment level to test whether there was an overall difference between hybrid and EWM genotypes at each level of 2,4-D exposure. For the models within each treatment, we $\log_{10}$-transformed data to meet assumptions for ANOVA. We performed post-hoc pairwise comparisons between clones within each herbicide treatment using Tukey's honest significant difference test.

We used Kendall's nonparametric test for rank correlation to determine whether mean vegetative growth in controls and 2,4-D treatments were positively correlated across hybrid genotypes. For these correlation calculations, we removed the block effects by subtracting the corresponding within-block mean across genotypes and 2,4-D

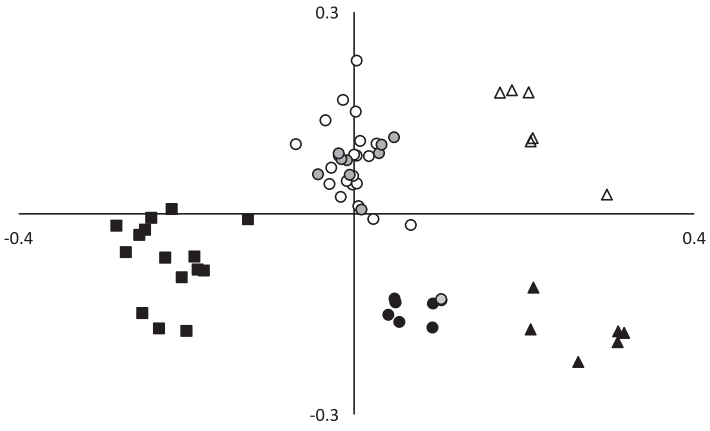

Figure 1. Principal coordinates analysis of all unique genotypes using eight microsatellite loci. Black squares are northern watermilfoil. White and black triangles are Eurasian watermilfoil biotypes 1 and 2, respectively (see Zuellig and Thum 2012). White and black circles are hybrid watermilfoil with Eurasian watermilfoil biotypes 1 versus 2 as the Eurasian parent, respectively. Gray circles represent the microsatellite genotypes of the hybrid watermilfoil genotypes used in the vegetative growth study.

exposure levels. For each genotype, we averaged over blocks to obtain a single adjusted mean growth for each combination of genotype and 2,4-D exposure.

We estimated broad-sense heritability (the proportion of variation in vegetative growth rate that can be attributed to variation in genotype) as the ratio of among-genotype variance to total variance across blocks, within each 2,4-D exposure level and the control (Falconer and Mackay 1996, Lynch and Walsh 1998). A significant effect of genotype in the model indicates that the trait is heritable, but note that the heritability estimate is not a universal value, but is a function of the genetic diversity present in the study population, and the experimental conditions (see the "Results and Discussion" section for further details).

All statistical analyses were performed with R version 3.1.2 (R Development Core Team 2014) using a statistical significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Genetic variation

All 10 hybrid genotypes used in this study were genetically different from one another and covered a range of the overall genetic diversity found in hybrid watermilfoils using our eight microsatellite loci. Comparison with microsatellite data from other EWM, NWM, and hybrids collected from several populations across the northern tier of the United States distinguished the two previously identified EWM biotypes (EWM1 and EWM2; Zuellig and Thum 2012). Concordantly, we identified two distinct groups of hybrid watermilfoils that correspond to hybridization between northern watermilfoil and each of the two genetically distinct biotypes of EWM, as each hybrid group showed a clear affinity to one of two genetically distinct clusters of EWM (Figure 1). Nine of our study genotypes were in a group of hybrid watermilfoils that were common in the Midwest but distributed broadly throughout the Midwest and western United States. Our remaining study genotype (H-5) aligns more closely with a group of hybrid watermilfoils that were found in the western United States. Our two EWM genotypes were in the EWM1 group.

Variation in vegetative growth

In our overall model, we found significant main effects of taxon (EWM vs hybrid), genotype, 2,4-D exposure, and block on length gained, but we detected no significant interaction between genotype and 2,4-D exposure (Table 3, Figure 2). Thus, while genotypes clearly differ in their vegetative growth, we found no significant differences between genotypes in the degree to which length decreased with increasing 2,4-D concentration. This suggests that while some genotypes grow faster than others, their responses to 2,4-D are similar. Interestingly, we found a significant effect of taxon at each 2,4-D exposure level, demonstrating that our hybrid genotypes had greater vegetative growth, on average, compared to the two EWM references (Table 4).

We found clear evidence for heritable variation in vegetative growth rate among distinct hybrid watermilfoil genotypes. Estimates of broad-sense heritability for length gained (the proportion of variation in length gained that can be attributed to genetic differences among the study genotypes) were 0.34, 0.22, and 0.27 for the 0-, 500-, and 1,000-μg L^{-1} 2,4-D exposure levels, respectively (e.g., 34% of the phenotypic variation in the controls can be attributed to genetic differences among the genotypes; the remaining phenotypic variation in the control is attributable to random environmental factors, such as differences in microsites or individual development, etc.). Broad-sense heritability is of general interest because the evolutionary potential of populations is determined in part by heritability. Broad-sense heritability of vegetative growth rate should be of specific interest to aquatic plant managers because it will determine in part the evolutionary potential of populations to evolve increased growth rates over time, which presumably would lead to populations that exhibit

Table 3. ANOVA of length gained for hybrid and Eurasian watermilfoil genotypes. Data were transformed using the Box-Cox method. df = degrees of freedom, SS = sum of squares, MS = mean square, EWM = Eurasian watermilfoil.

Factor	df	SS	MS	*F* Value	*P* Value
Taxon (EWM vs. hybrid)	1	18.69	18.69	56.80	< 0.001
Genotype (taxon)	10	32.89	3.29	10.00	< 0.001
Exposure (0, 500, 1000 μg L^{-1} 2,4-D)	2	55.79	27.89	84.77	< 0.001
Genotype (taxon) × exposure	22	8.20	0.37	1.13	0.31
Block	2	8.76	4.38	13.31	< 0.001
Residuals	286	94.11	0.33		

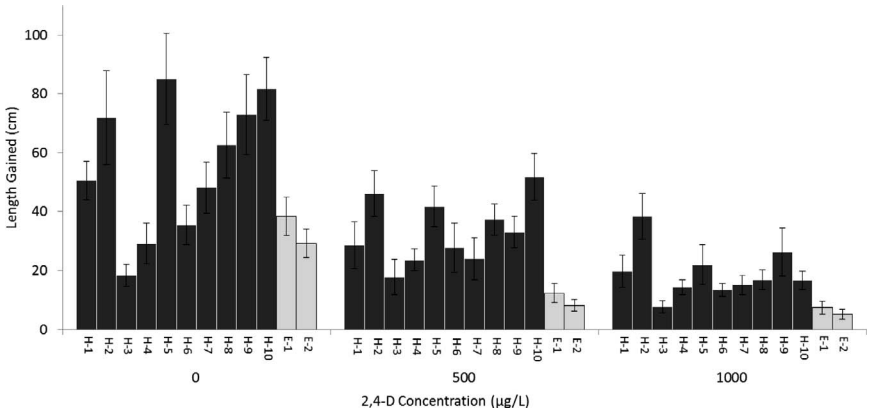

Figure 2. Growth of hybrid watermilfoil genotypes (dark gray bars) and Eurasian watermilfoil reference genotypes (light gray bars) in two concentrations of 2,4-D and a control after 3 wk of growth posttreatment. Each bar represents the mean of the nine replicates across blocks. Error bars represent one standard error of the mean. Along the x-axis, "H" refers to hybrid genotypes from different lakes and "E" refers to Eurasian genotypes from different lakes. The same genotype from each lake is present in both treatments and the control.

greater levels of nuisance growth, lower reductions in biomass following treatment with 2,4-D, or both.

It is important to note that the heritability of a trait is specific to the population in which it is measured, and that there is no "universal heritability" of a trait (Conner and Hartl 2004). Our sampling design was intended to broadly sample different hybrid genotypes to test the general hypothesis that different genotypes can exhibit different growth properties. Therefore, the "population" that we studied is composed of hybrid watermilfoils collected from different lakes, and our study genotypes were not intended to represent entire local populations. Our design illustrates the concept that faster-growing hybrid watermilfoil genotypes could displace slower-growing genotypes where two or more genotypes differing in growth rates occur together. However, determining the potential for evolution within an individual lake would require estimating heritability specifically for that lake, which would require conducting a similar study as the one here using different genotypes collected from that specific lake. If a lake were composed entirely of a single genotype, then the heritability for that lake would be zero unless or until one or more different genotypes that differed in growth rate invaded the lake. Therefore, future studies aiming to quantify the evolutionary potential of growth rate in individual lakes should initially focus on lakes where molecular marker data clearly demonstrate the co-occurrence of different genotypes.

We did not find a significant interaction between genotype and 2,4-D exposure, so there is no evidence that genotypes differ in sensitivity to 2,4-D. Furthermore, vegetative growth rates in the controls and each of the two levels of 2,4-D exposure were correlated across genotypes. Adjusted mean vegetative growth of cuttings exposed to 500 µg L^{-1} of 2,4-D was significantly and positively correlated with adjusted mean vegetative growth in the control (Kendall's tau = 0.78 and Holm-adjusted $P < 0.001$). Similarly, adjusted mean vegetative growth in 1,000 µg L^{-1} 2,4-D was significantly and positively correlated with adjusted mean vegetative growth in the control (Kendall's tau = 0.60, Holm-adjusted $P = 0.008$). Thus, relatively fast growers tended to be relatively fast growers whether they were treated with 2,4-D or not, and similarly for relatively slow growers. However, some genotypes did not significantly differ from our EWM references in the controls, whereas

Table 4. ANOVA of length gained for hybrid and Eurasian watermilfoil genotypes for each 2,4-D treatment level. Data were $\log_{10}$-transformed. df = degrees of freedom, SS = sum of squares, MS = mean square.

Factor	df	SS	MS	*F* Value	*P* Value
0 µg L^{-1}					
Taxon	1	0.36	0.36	5.95	< 0.001
Genotype (taxon)	10	4.14	0.41	6.82	< 0.001
Block	2	0.77	0.38	6.32	0.003
Residuals	94	5.71	0.06		
500 µg L^{-1}					
Taxon	1	3.99	3.99	35.16	< 0.001
Genotype (taxon)	10	3.79	0.38	3.34	< 0.001
Block	2	4.31	2.15	18.99	< 0.001
Residuals	94	10.67	0.11		
1000 µg L^{-1}					
Taxon	1	3.63	3.63	29.58	< 0.001
Genotype (taxon)	10	7.99	4	32.54	< 0.001
Block	2	3.79	0.38	3.09	0.002
Residuals	94	11.54	0.12		

they did in positive 2,4-D exposures (Figure 2), suggesting potential variation in dose-response curves between genotypes that our study did not have sufficient power to detect. Therefore, further investigation into dose-response curves using greater numbers of exposure levels and larger sample sizes may reveal differences in 2,4-D sensitivity among genotypes.

Given the possibility for different hybrid genotypes to exhibit significant differences in vegetative growth properties, and observations from molecular genetic analyses that different populations are composed of different genotypes (Zuellig and Thum 2012; LaRue et al. 2013a,b; R. A. Thum, unpub. data), populations should not be expected to necessarily exhibit the same level of nuisance growth or control from herbicide(s) (see also Glomski and Netherland 2010, Berger et al. 2012, Thum et al. 2012). However, we recognize that our study design used a single genotype from each lake, and that a single genotype may not be representative of how an entire population will grow and respond. Nevertheless, our results illustrate the potential for genetically distinct populations to exhibit different growth properties, which is consistent with anecdotal observations by managers who have had different experiences with hybrids in different lakes.

Interestingly, vegetative growth of hybrid genotypes as a group was higher than that of the EWM reference genotypes in all exposure levels (Figure 2; Table 4), as indicated by the significant taxon term in our ANOVA model. In fact, only one hybrid genotype (H-3) consistently showed vegetative growth rates lower than the two EWM reference genotypes across exposure levels. In contrast, two hybrid genotypes (H-5 and H-10) consistently exhibited significantly higher vegetative growth than the EWM reference genotypes across exposure levels, and three other hybrid genotypes (H-2, H-8, and H-9) exhibited significantly higher vegetative growth than the EWM reference genotypes in one or more exposure levels (tables of pairwise comparisons between genotypes within and across treatments available from the corresponding author upon request). Importantly, two hybrid genotypes that consistently exhibited higher vegetative growth rates compared to the EWM reference genotypes (H-5 and H-10) clearly result from independent hybridization events with distinct EWM biotypes (Figure 1; see also Zuellig and Thum 2012), indicating that their relatively fast growth rates have independently arisen from two hybridization events with different parental backgrounds. In addition, the remainder of our study genotypes represent a broad range of genetic variation among hybrids with EWM1 as the EWM parent, which we posit results from multiple independent hybridization events (see also Zuellig and Thum 2012). Thus, relatively faster vegetative growth in hybrids has most likely occurred in multiple independent hybridization events (see also LaRue et al. 2013b) and may be common, but not universal, across distinct hybrid genotypes. However, we note that determining how commonly hybrids exhibit faster growth rates compared to EWM will require comparison to a larger number of EWM genotypes, as EWM is genetically diverse, including distinct biotypes and distinct genotypes within biotypes (Figure 1; see also Zuellig and Thum 2012). Future studies should also include comparisons to NWM.

We recognize two additional and important limitations to our study that future studies should address. First, our data are based on a vegetative growth assay that does not use rooted, intact plants (see LaRue et al. 2013b). We observed considerable variation even within genotypes, some of which is possibly attributable to our assay conditions. Future studies should make similar comparisons using rooted plants, and promising new protocols are now available (e.g., Netherland and Richardson 2016). Second, more work could be done to untangle the potential effects of genetic variation attributable to different hybrid classes and/or different parental backgrounds. For example, first-generation hybrids may exhibit hybrid vigor whereas later-generation hybrids may exhibit hybrid breakdown (Burke and Arnold 2001). Similarly, different hybridization events with different parental backgrounds may lead to differences in vegetative growth. Future studies employing controlled crosses and/or more powerful molecular marker datasets that can estimate hybrid class (e.g., F_1 vs. later generations and backcrosses) or hybridization events with different parents may provide important insight into the underlying causes of different vegetative growth rates among hybrid genotypes.

Predicting when and where Eurasian and hybrid watermilfoil will exhibit nuisance growth and/or tolerance to control measures, such as treatment with 2,4-D, would improve the long-term and broad-scale management outcomes for watermilfoil. Ultimately, more studies that combine genetic information with growth and response data on individual genotypes are needed to have a more complete understanding of genetic variation and its impacts on management outcomes. We encourage managers to explicitly recognize the potential for genetic variation within and among the lakes they manage, and to consider participating in research collaborations by conducting genetic monitoring where possible (e.g., Parks et al. 2016), and by assisting researchers with the identification of specific genotypes that warrant further scientific characterization of their growth and herbicide response properties. Characterization of a large number of genotypes, combined with research on the genetic mechanisms underlying growth and herbicide response, may eventually lead to genetic assays that can be used to predict the spread, impact, and control outcomes for specific genotypes. We suggest that our approach, complemented by genetic monitoring of populations over time, will lead to a greater understanding of when and where hybrid populations will respond favorably to established control techniques versus requiring alternative ones.

SOURCES OF MATERIALS

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

[2]Full-spectrum sodium lamp, Sylvania M1000/U M47/S Metalarc, Ledvance, Wilmington, MA 01887.

[3]Analytical Grade 2,4-D, Thermo Fisher Scientific, 168 Third Avenue, Waltham, MA 02451.

[4]Cone-tainer, Ray Leach Cone-tainers™ through Stuewe & Sons, Inc., 31933 Rolland Drive, Tangent, OR 97389.

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