

## Research Article

**Cite this article:** Hoff HK and Thum RA (2022) Hybridization and invasiveness in Eurasian watermilfoil (*Myriophyllum spicatum*): is prioritizing hybrids in management justified? *Invasive Plant Sci. Manag* **15**: 3–8. doi: [10.1017/inp.2022.4](https://doi.org/10.1017/inp.2022.4)

Received: 22 July 2021

Revised: 2 November 2021

Accepted: 11 January 2022

First published online: 2 February 2022

### Associate Editor:

Rob J. Richardson, North Carolina State University

### Keywords:

2,4-D; genetic variation; herbicide response; *Myriophyllum sibiricum*; vegetative growth

### Author for correspondence:

Hannah K. Hoff, Department of Plant Sciences and Plant Pathology, Montana State University, P.O. Box 173150, Bozeman, MT 59717. (Email: [hannah.hoff@montana.edu](mailto:hannah.hoff@montana.edu))

# Hybridization and invasiveness in Eurasian watermilfoil (*Myriophyllum spicatum*): is prioritizing hybrids in management justified?

Hannah K. Hoff<sup>1</sup>  and Ryan A. Thum<sup>2</sup> 

<sup>1</sup>Research Associate, Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT, USA and <sup>2</sup>Associate Professor, Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT, USA

## Abstract

Hybridization can play an important role in the evolution of invasiveness. Eurasian watermilfoil (*Myriophyllum spicatum* L.) is a widespread aquatic invasive plant species that hybridizes with native northern watermilfoil (*Myriophyllum sibiricum* Kom.). Previous studies have found mixed evidence for whether hybrid watermilfoil (*Myriophyllum spicatum* × *sibiricum*) and pure *M. spicatum* differ in vegetative growth rate and herbicide response. While several studies have emphasized variation in these traits among *M. spicatum* × *sibiricum* genotypes, variation within *M. spicatum* has not been considered. Therefore, it is unclear how much genetic variation influences invasive traits and management outcomes within *M. spicatum* versus between *M. spicatum* and *M. spicatum* × *sibiricum*. If *M. spicatum* × *sibiricum* genotypes are always more invasive than *M. spicatum* genotypes, simply distinguishing taxa may be sufficient for identifying lake management priorities; however, if significant phenotypic overlap is observed between taxa, distinguishing individual genotypes may be important for tailoring management strategies. We performed replicated trials of a vegetative growth and 2,4-D assay to measure clonal variation in growth rate and herbicide response of *M. spicatum* and *M. spicatum* × *sibiricum*. Our results indicate that *M. spicatum* × *sibiricum* exhibits higher average vegetative growth than *M. spicatum*, regardless of whether it was treated with subsurface applications of 2,4-D. We did not observe interactions between taxon and treatment or between genotype and treatment. Despite differences between *M. spicatum* and *M. spicatum* × *sibiricum* in average vegetative growth, there was substantial overlap between taxa. For example, we found that the fastest-growing genotype of pure *M. spicatum* did not differ significantly in average growth from the fastest-growing *M. spicatum* × *sibiricum* genotype. The potential for overlap between these invasive *Myriophyllum* taxa suggests that distinguishing and characterizing genotypes may be more informative for management than simply distinguishing between *M. spicatum* and *M. spicatum* × *sibiricum*.

Evolutionary biologists have long hypothesized that genetic variation plays an important role in invasion success by facilitating adaptation to the environmental pressures of the novel habitat (Baker 1965; Lee 2002; Mooney and Cleland 2001). High levels of genetic variation have been documented in invasive species (Ward et al. 2008), and previous studies have demonstrated that phenotypic variation within species can be as extreme as phenotypic variation across species (Albert et al. 2010). Intraspecific trait variation may therefore influence factors associated with invasion success as much as interspecific variation (Des Roches et al. 2018; Wellband et al. 2017). However, because invasions are often studied at the species level, the role of genetic variation in invasive species management is rarely considered.

In clonal species, genetic variation is described between clonal lineages (genotypes), and trait variation between these genotypes may impact invasion success. For example, with sufficient trait variation, different genotypes may successfully persist in the presence of different environmental conditions, thus extending the ecological range of the broader taxon (terHorst and Lau 2015). In clonal invasive plants, trait variation between genotypes may also be relevant to the ability of the broader taxon to adapt to management tools such as herbicides (Michel et al. 2004).

The range of genetic diversity present in an invasive plant species may also be impacted by hybridization events (Ellstrand and Schierenbeck 2000; Pierce et al. 2017; Zalapa et al. 2010). Plant invaders capable of sexual reproduction may hybridize with related taxa in the novel habitat, and a hybrid taxon may exhibit increased vigor compared with its parental taxa due to high levels of heterozygosity (Stebbins 1985). Furthermore, the generation of novel hybrid genotypes increases the genetic variation of an invasive population, and one of these hybrid genotypes may represent better adaptations to certain environmental conditions than any

© The Author(s), 2022. Published by Cambridge University Press on behalf of the Weed Science Society of America. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.



### Management Implications

*Myriophyllum spicatum* × *sibiricum* (hybrid watermilfoil) has been associated with faster growth rates and increased tolerance to herbicides compared with pure invasive *Myriophyllum spicatum* (Eurasian watermilfoil) and has thus become prioritized for management. Because *M. spicatum* and *M. spicatum* × *sibiricum* are morphologically similar, taxon-level genetic identification has commonly been used to identify priority populations of *M. spicatum* × *sibiricum*.

In our assay, *M. spicatum* × *sibiricum* exhibited higher average growth than *M. spicatum*. However, we observed substantial overlap in vegetative growth between taxa. Thus, not all *M. spicatum* × *sibiricum* genotypes are fast growers, and some pure *M. spicatum* genotypes can be as invasive as some *M. spicatum* × *sibiricum* genotypes. While we did not see variation in 2,4-D response between taxa or between genotypes, relatively fast-growing genotypes in control tanks tended to be relatively fast growers in treatment tanks, with the same pattern occurring for slow growers. This genotype-level variation in vegetative growth, combined with the relationship between growth in control and growth in treatment, suggests that relatively fast-growing genotypes may have higher remaining biomass after a treatment with 2,4-D than relatively slow-growing genotypes. Identifying lake management priorities may therefore depend on identifying particularly fast-growing genotypes, rather than simply looking for hybrid genotypes.

Due to clonal reproduction, genotypes of invasive *Myriophyllum* can be tracked across the landscape, and characterizing a single genotype for invasive trait values (e.g., vegetative growth, response to commonly used herbicides) may be applicable to multiple lakes. If a particular genotype is identified to be a relatively fast grower, managers considering herbicide applications to treat populations of that genotype may consider alternatives to 2,4-D that result in lower posttreatment biomass values.

parental genotypes (Ellstrand and Schierenbeck 2000). In species with clonal ability, better-adapted genotypes may be propagated indefinitely (Balloux et al. 2003). Due to the addition of genetic variation and potential for increased invasiveness, invasive hybrids have become a focus of invasive species research and management (Blair and Hufbauer 2010; Ellstrand and Schierenbeck 2000; Rieseberg and Willis 2007).

Eurasian watermilfoil (*Myriophyllum spicatum* L.) is an invasive aquatic plant species, and one of the most widespread and heavily managed aquatic weeds in the United States (Bartodziej and Ludlow 1998; Cofrancesco 1993). Due to concerns about impacts on native plant communities and human recreation, *M. spicatum* is heavily managed using several systemic and contact herbicides (e.g., 2,4-D, fluridone, triclopyr, floryprauxifen-benzyl, endothall).

*Myriophyllum spicatum* hybridizes with northern watermilfoil (*Myriophyllum sibiricum* Kom.), which is native to North America. Hybrid watermilfoil (*Myriophyllum spicatum* × *sibiricum*) was identified as a cryptic invader long after *M. spicatum* invasion was documented in North America due to morphological similarity with the parental taxa (Moody and Les 2002, 2007). Since its recognition, concerns about increased vigor in *M. spicatum* × *sibiricum* compared with pure *M. spicatum* have made *M. spicatum* × *sibiricum* a focus of genetic study (LaRue et al. 2013; Moody and

Les 2007; Taylor et al. 2017). As *M. spicatum* and *M. spicatum* × *sibiricum* are visually similar, taxon-level genetic identification (Grafe et al. 2014) is commonly used to identify *M. spicatum* × *sibiricum* populations for prioritization. In addition, *Myriophyllum* taxa reproduce clonally, and molecular genetic studies have identified distinct genotypes within both *M. spicatum* and *M. spicatum* × *sibiricum* that may exhibit distinct sets of traits (Pashnick and Thum 2020; Zuellig and Thum 2012).

General concerns about invasiveness of hybrid taxa have led to concerns about increased invasiveness in *M. spicatum* × *sibiricum* compared with pure *M. spicatum*. Laboratory studies comparing average growth and herbicide response in *M. spicatum* and *M. spicatum* × *sibiricum* have documented faster growth and increased tolerance to herbicide in *M. spicatum* × *sibiricum* (LaRue et al. 2013; Taylor et al. 2017). Similarly, a field study of 2,4-D treatment impacts in 23 Wisconsin lakes documented relatively better control in lakes with pure *M. spicatum* compared with most lakes with *M. spicatum* × *sibiricum* populations (Nault et al. 2017). However, two studies comparing a single genotype of *M. spicatum* with a single genotype of *M. spicatum* × *sibiricum* concluded that the two taxa did not differ in vegetative growth rate or in response to the systemic herbicides 2,4-D, triclopyr, or fluridone (Poovey et al. 2007; Slade et al. 2007). A disparity thus exists between studies examining differences in invasive traits between *M. spicatum* and *M. spicatum* × *sibiricum*. This disparity suggests that variation within *M. spicatum* and *M. spicatum* × *sibiricum* may impact comparisons of invasive traits at the taxon level.

Numerous studies have emphasized variation between *M. spicatum* × *sibiricum* genotypes in vegetative growth and herbicide response (e.g., LaRue et al. 2013; Taylor et al. 2017). In contrast, although numerous genotypes of pure *M. spicatum* have been identified (Thum et al. 2020; Zuellig and Thum 2012), comparatively little attention has been paid to whether there is statistically significant and biologically meaningful variation in invasive traits (e.g., vegetative growth) and herbicide response in *M. spicatum* (but see Chorak and Thum 2020; Thum and McNair 2018). Therefore, it is unclear how important genetic variation within *M. spicatum* versus between pure *M. spicatum* and *M. spicatum* × *sibiricum* is in influencing invasive traits and management outcomes. In this study, we compared clonal variation within and between *M. spicatum* and *M. spicatum* × *sibiricum* for two traits associated with their invasiveness—vegetative growth rate and response to the systemic herbicide 2,4-D.

### Materials and Methods

To make comparisons between and within taxa, our assay included four different genotypes each of pure *M. spicatum* and *M. spicatum* × *sibiricum* hybrids (Table 1). The microsatellite multilocus genotypes for each taxon were determined in a previous study (Thum et al. 2020), and a culture of each genotype was started from a single meristem collected in the field and vegetatively propagated in the Montana State University Plant Growth Center (Bozeman, MT).

While different lakes often contain different genotypes of invasive *Myriophyllum*, some genotypes may be shared among lakes (Thum et al. 2020). For example, the pure *M. spicatum* genotypes used in our assay (E-1–E-4; Table 1) have each been found in more than one lake (Thum et al. 2020; Thum, unpublished data), and characterizing their vegetative growth rates and herbicide responses may therefore inform management in lakes outside the specific lakes that they were collected from for our assay.

**Table 1.** Names and locations of lakes where genotypes used in the vegetative growth assay were collected.

Genotype <sup>a</sup>	Taxon	Lake <sup>b</sup>	County	State
E-1	Eurasian	Langford	Gogebic	Michigan
E-2	Eurasian	Bair	Cass	Michigan
E-3	Eurasian	Coeur d'Alene	Kootenai	Idaho
E-4	Eurasian	Spring	Ottawa	Michigan
H-1	Hybrid	Coeur d'Alene	Kootenai	Idaho
H-2	Hybrid	White	Oakland	Michigan
H-3	Hybrid	Spring	Ottawa	Michigan
H-4	Hybrid	White	Oakland	Michigan

<sup>a</sup>E, Eurasian watermilfoil (*Myriophyllum spicatum*); H, hybrid watermilfoil (*Myriophyllum spicatum* × *sibiricum*). Number codes were assigned for figure clarity based on mean growth rate rank. All four *M. spicatum* genotypes have been found in multiple waterbodies in the United States (Thum et al. 2020; RAT, unpublished data).

<sup>b</sup>Lake location information corresponds to the lake from which stems were collected for this assay.

Additionally, if multiple genotypes are present within a lake, genotype-level differences in invasive trait values may lead to changes in population composition over time. For example, a faster-growing genotype may displace a slower-growing genotype, or a herbicide-resistant genotype may replace a more sensitive genotype following a herbicide application. Of particular interest to managers is whether *M. spicatum* × *sibiricum* genotypes will displace *M. spicatum* genotypes. Therefore, we included two pairs of *M. spicatum* and *M. spicatum* × *sibiricum* genotypes that were found in the same lake (E3+H1 from Coeur d'Alene Lake, and E-4+H3 from Spring Lake; Table 1) to assess whether heightened concerns about managing *M. spicatum* × *sibiricum* genotypes in those lakes would be warranted. In addition, multiple genotypes of the same taxon can co-occur in a lake, and we included a pair of co-occurring *M. spicatum* × *sibiricum* genotypes (H-2+H-4 from White Lake; Table 1).

To measure both vegetative growth and 2,4-D response in *M. spicatum* and *M. spicatum* × *sibiricum*, we conducted a greenhouse treatment versus control assay. We replicated the assay twice on all eight genotypes (hereafter referred to as Trials 1 and 2), with planting dates staggered by 6 wk between trials. For each trial, we vegetatively propagated cultures of each genotype to generate enough meristems for the assay. For each of the two trials, six 378.5-L steel stock tanks (60 by 60 by 120 cm) were filled with approximately 300 L of Smart and Barko (1985) buffered water. On June 25, 2020 (Trial 1), and August 6, 2020 (Trial 2), a 10-cm stem of each genotype was planted into a 0.53-L plastic cup filled with soil (1:1:1 topsoil/sand/peat) and capped with quartz sand. To account for tank variance, three cup plantings of each genotype were randomly placed in each tank. After planting, plants acclimated for 6 wk. Both trials were conducted under greenhouse conditions with ambient and supplemental incandescent lighting to maintain a 16-h light:8-h dark period. Air bubblers were placed in each tank and remained throughout each trial to maintain aeration of the water. Water temperatures in tanks ranged from 18 to 22 C in both trials, and water filtered by reverse osmosis was replaced weekly as it evaporated.

After the 6-wk establishment period, we treated three random tanks in each trial with 2,4-D (Alfa Aesar, Thermo Fisher Scientific, Lancashire, LA, UK), for a target concentration exposure time (CET) of 500 µg L<sup>-1</sup> for 72 h. We recognize that 2,4-D efficacy is a function of both herbicide concentration and exposure time. Further, we recognize that target concentrations for 2,4-D in operational applications are often higher than 500 µg L<sup>-1</sup>. However, patterns of dilution and dissipation commonly result in doses that are lower than the target CET. For example, Nault

et al.'s (2012) field study found that the mean 2,4-D concentration between 0 and 3 d after treatment for a lake with a target CET of 2,500 µg L<sup>-1</sup> was 613 µg L<sup>-1</sup>. Given that we could only do one treatment level because of inherent space constraints, we chose 500 µg L<sup>-1</sup> as our treatment concentration, because we believe that is a more realistic representation of doses that are achieved in field applications compared with higher doses. We chose 72 h as our exposure time, because that CET combination is predicted to control *M. spicatum* (Green and Westerdahl 1990). Herbicide concentrations in Trial 1 ranged from 493 to 571 µg L<sup>-1</sup>. Herbicide concentrations in tanks in Trial 2 at 1 h posttreatment were 364 to 371 µg L<sup>-1</sup>, and thus did not reach the target dose required. Following the 72-h treatment exposure period, all tanks were drained, flushed with 125 L of Smart and Barko (1985) buffered water, and refilled with 300 L of Smart and Barko (1985) buffered water. We harvested all viable material above the sediment at 6 wk after treatment, dried it to a constant weight at 44 C, and measured for dry weight biomass.

We tested for differences in growth between taxa and among genotypes using a linear mixed-effects model in R package LME4 (Bates et al. 2015). Taxon, genotype, and 2,4-D treatment (control vs. treated), as well as the interaction terms between taxon and 2,4-D treatment and between genotype and 2,4-D treatment, were treated as fixed effects. To ensure that variation in treatment concentration between Trial 1 and Trial 2 did not impact overall results, we tested main effects and interactions using a linear mixed-effects model on each trial individually. As the main effect of treatment was significant in all cases and there were no significant interactions detected, data were pooled across trials, and we included trial in the linear model as a random block effect. Treatment, taxon, and genotype (nested within taxon), as well as interactions between taxon and treatment and between genotype and treatment, were included as fixed effects in the linear model. In the growth assay, each genotype was grown in three separate cup plantings (replicates) within each tank. However, neither tank nor replicate were included as random effects in the model, because the model fit (using the Akaike information criterion) was improved without them. Genotype was nested within taxon to test for differences in growth (dry weight) between and within taxa. Overall data were log<sub>10</sub> transformed to meet the normality assumption of ANOVA.

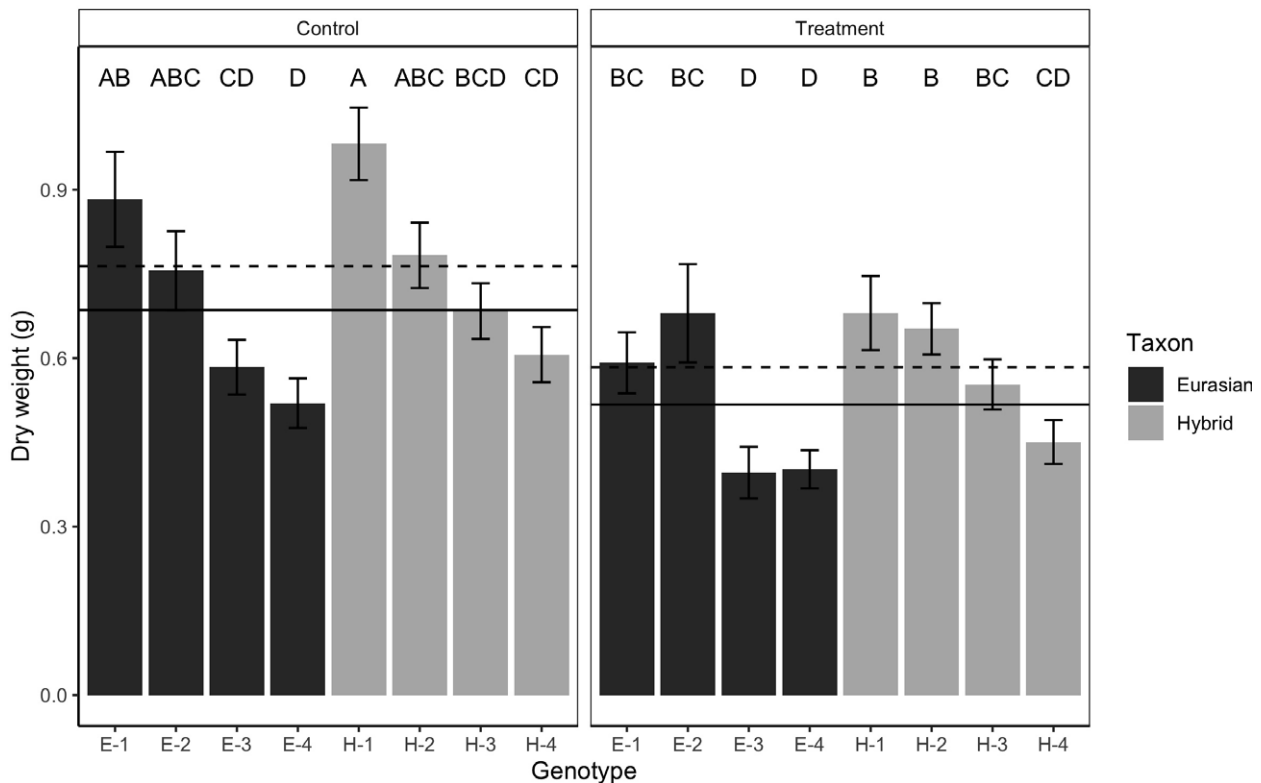
We measured treatment response for each taxon and genotype as the difference in mean dry weight between control and treatment. As differences in growth and 2,4-D response between taxa were a priori contrasts, we used the EMMEANS package in R to calculate estimated marginal means and performed contrasts between taxa for mean dry weight in both control and treatment (Lenth 2020; Supplementary Appendix S1). Finally, to assess variation among genotypes, we used a Tukey's honestly significant difference test to conduct post hoc pairwise comparisons for each genotype and treatment combination.

## Results and Discussion

Even when auxinic herbicide treatments result in undetectable levels of invasive *Myriophyllum*, regrowth commonly occurs. Thus, control efforts rarely eradicate populations completely (Netherland 2014). While it is possible that regrowth occurs due to seedbank recruitment or fragment recolonization from untreated areas of a lake, regrowth may also occur in treated areas from plants that are not completely killed during auxinic herbicide treatment (Thum et al. 2017). Vegetative growth rate may thus

**Table 2.** ANOVA (type II Satterthwaite's method) table for the linear mixed regression model [dry weight ~ taxon\*treatment\*genotype(taxon) + (1|trial)] determining the effects of *Myriophyllum* taxon and genotype as well as effect of the 500  $\mu\text{g L}^{-1}$  2,4-D treatment on dry weight.

	Sum of squares	Mean squares	df	F-value	P-value
Taxon	0.066	0.066	1	3.217	0.074
Treatment	1.229	1.229	1	59.640	<0.001
Genotype (taxon)	1.824	0.304	6	14.750	<0.001
Taxon $\times$ treatment	0.002	0.002	1	0.090	0.764
Genotype (taxon) $\times$ treatment	0.142	0.024	6	1.145	0.337



**Figure 1.** Average dry weight ( $g \pm SE$ ) values for eight genotypes of invasive *Myriophyllum*: four *Myriophyllum spicatum* (black bars) and four *Myriophyllum spicatum*  $\times$  *sibiricum* (gray bars). Each of the genotypes is represented by a letter+number combination for figure clarity. The letter indicates the taxon associated with each genotype: E, Eurasian watermilfoil (*Myriophyllum spicatum*); H, hybrid watermilfoil (*Myriophyllum spicatum*  $\times$  *sibiricum*). The number indicates its within-taxon ranking in mean growth rate. The dashed line denotes mean dry weight for *M. spicatum*  $\times$  *sibiricum* hybrids in each treatment type, and the solid line denotes mean dry weight for pure *M. spicatum* in each treatment type. Different letters represent significant differences between genotypes across both control and treatment.

impact regrowth after treatment as well as biomass accumulation before treatment. In our assay, all plants in treatment tanks showed signs of auxin injury (i.e., epinasty, stem curling) following subsurface application of 2,4-D. However, despite a period of initial injury following exposure to 2,4-D, regrowth via axillary meristems was common from incomplete kill of treated plants.

To test whether 2,4-D had a negative impact on invasive *Myriophyllum* biomass, we included treatment as a main effect. Despite underdosing in Trial 2, untreated plants grew significantly better than plants that were treated with a subsurface application of 2,4-D ( $F = 59.64$ ,  $P < 0.001$ ; Table 2). We thus expect that 2,4-D treatment will lead to reduced overall biomass of invasive *Myriophyllum* when used in lake management.

We tested for differences in vegetative growth between pure *M. spicatum* and *M. spicatum*  $\times$  *sibiricum* hybrids by including taxon as a main effect. The main effect of taxon was marginally detectable using the full model ( $F = 3.217$ ,  $P = 0.0740$ ; Table 2). Contrasts supported that *M. spicatum*  $\times$  *sibiricum* had significantly higher average vegetative growth than *M. spicatum* in both control

( $t = -2.456$ ,  $P = 0.0147$ ) and 2,4-D-treated plants ( $t = -3.105$ ,  $P = 0.0021$ ; Figure 1). These findings suggest that hybridization may play a role in increased growth rate and that *M. spicatum*  $\times$  *sibiricum* may be more invasive on average than pure *M. spicatum*.

Genotypes E-4 and H-3, as well as E-3 and H-1, are two *M. spicatum*–*M. spicatum*  $\times$  *sibiricum* pairs of genotypes that co-occur in the same lake (Table 1). In the E-4+H-3 pair, the *M. spicatum*  $\times$  *sibiricum* genotype had higher average vegetative growth compared with the *M. spicatum* genotype under treatment (Figure 1). In the E-3+H-1 pair, the *M. spicatum*  $\times$  *sibiricum* genotype had higher average vegetative growth compared with the *M. spicatum* genotype in both treatment and control (Figure 1). This is consistent with the broader pattern of higher average vegetative growth in *M. spicatum*  $\times$  *sibiricum* compared with *M. spicatum*. Thus, in the absence of genotype-specific data, prioritization of *M. spicatum*  $\times$  *sibiricum* genotypes in lake management may be justified. Further, the observed differences in growth rates between co-occurring genotypes may lead to

changes in the composition of these lakes over time if the faster-growing genotype is able to displace the slower-growing genotype.

Herbicide tolerance is defined as differences between species in their ability to survive and reproduce following a herbicide treatment, and some managers have speculated that *M. spicatum* × *sibiricum* hybrids are generally more tolerant to 2,4-D than pure *M. spicatum*. If *M. spicatum* × *sibiricum* was more tolerant to 2,4-D treatment than *M. spicatum*, we would expect to see an interaction between taxon and treatment in our analysis. However, there was no difference between taxa in the degree to which 2,4-D decreased dry weight ( $F = 0.090$ ,  $P = 0.764$ ; Table 2), suggesting that hybrids are not generally more tolerant to 2,4-D, per se. However, we did observe higher average overall biomass in *M. spicatum* × *sibiricum* compared with *M. spicatum* when treated with 2,4-D (Figure 1), which may influence the perception that *M. spicatum* × *sibiricum* is more difficult to control because there is more overall biomass of hybrids after 2,4-D treatment.

We observed variation in vegetative growth rate between genotypes ( $F = 14.75$ ,  $P < 0.001$ ; Table 2). For example, a pair of *M. spicatum* × *sibiricum* genotypes (H-2 and H-4) that co-occur in the same lake (Table 1) differed in average vegetative growth when treated with 2,4-D (Figure 1). Thus, genotypes of the same taxon may differ in management-relevant traits. Additionally, while genetic study has focused on variation in *M. spicatum* × *sibiricum* (LaRue et al. 2013; Moody and Les 2007; Taylor et al. 2017), we documented substantial variation in pure *M. spicatum* (Figure 1).

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. In this study system, resistance would be represented by an unusually flat slope in the line between its average biomass in control and in treatment relative to other genotypes. If some genotypes were more resistant to 2,4-D treatment than others, we would thus expect to see an interaction between genotype and treatment. However, we observed no differences between genotypes in the degree to which 2,4-D treatment decreased their dry weight ( $F = 1.145$ ,  $P = 0.337$ ; Table 2). Rather, relatively fast growers in control tanks tended to be relatively fast growers in treatment tanks, with the same pattern occurring for slow growers (Figure 1). These results are consistent with a previous study testing the effects of 500 and 1,000  $\mu\text{g L}^{-1}$  2,4-D on different genotypes of *M. spicatum* × *sibiricum* (Taylor et al. 2017) and indicate that vegetative growth in the absence of treatment may be a strong predictor of growth in the presence of 2,4-D. Therefore, while we did not detect 2,4-D resistance in the *M. spicatum* or *M. spicatum* × *sibiricum* genotypes screened, we expect that fast-growing genotypes will have relatively higher remaining biomass following 2,4-D treatment, which may lead to greater difficulty in control. Further, although we did not detect evidence for resistance in the genotypes we studied, it is possible that other genotypes may be resistant to 2,4-D, and continued genetic monitoring and genotype-level characterization is warranted.

The fastest-growing genotype of *M. spicatum* (E-1) present in this experiment is geographically widespread across its novel range and has been documented in multiple states across the United States (Thum et al. 2020). The E-1 genotype also occurs in high abundance in more localized areas; for example, a survey of 62 lakes in Minnesota from 2017 to 2018 found that the E-1 genotype accounted for 93% of *M. spicatum* infestations in Minnesota (Thum et al. 2020). As growth rate has been tied to colonization success in clonal species (Herben et al. 2014), fast growth rates

may have played a role in the ability of the E-1 genotype to colonize and establish in new water bodies upon introduction. Further, as fast-growing genotypes in control tanks tended to also exhibit fast growth in treatment (Figure 1), the E-1 genotype may be able to maintain relatively high biomass in lakes treated with 2,4-D and may pose management concerns similar to those surrounding genotypes of *M. spicatum* × *sibiricum*.

While we observed significant differences in vegetative growth between *M. spicatum* and *M. spicatum* × *sibiricum* in control and treatment, there was significant overlap between taxa. For example, the fastest-growing *M. spicatum* genotype (E-1) did not differ significantly in average growth from the fastest-growing *M. spicatum* × *sibiricum* genotype (H-1) in control or in treatment (Figure 1). This suggests that *M. spicatum* genotypes may be comparatively invasive compared with some *M. spicatum* × *sibiricum* genotypes. Further, the slowest-growing *M. spicatum* genotype (E-4) did not differ significantly from the slowest-growing *M. spicatum* × *sibiricum* genotype (H-4) in control or in treatment (Figure 1). Thus, not all *M. spicatum* × *sibiricum* genotypes are fast growers. As *M. spicatum* and *M. spicatum* × *sibiricum* are visually similar, genetic identification is commonly used to distinguish these taxa (Grafe et al. 2014). However, this overlap suggests that management decisions purely based on the *Myriophyllum* taxa that are present in a lake may fail to account for overlap between *M. spicatum* and *M. spicatum* × *sibiricum* and that monitoring at the genotype level may be important for tailoring lake management strategies.

Genotype-level variation in vegetative growth, as well as the relationship between growth in control and growth in treatment, suggests that relatively fast-growing genotypes may have higher remaining biomass than relatively slow-growing genotypes following subsurface application of 2,4-D. Identifying lake management priorities may therefore depend on identifying particularly fast-growing genotypes, rather than simply identifying populations of *M. spicatum* × *sibiricum*. If a genotype is identified to be a particularly fast grower, managers considering herbicide applications to treat populations of that genotype may consider alternatives to 2,4-D that result in greater biomass declines posttreatment.

Overall, we observed significant differences between *M. spicatum* and *M. spicatum* × *sibiricum* in vegetative growth in control and treatment and no difference in how the two taxa responded to 2,4-D. Thus, prioritization of *M. spicatum* × *sibiricum* in lake management may often be warranted if monitoring at the genotype level is not possible. However, transitioning from taxon-level identification to genotype-level identification is feasible due to the development of fingerprinting methods that distinguish genotypes (Thum et al. 2020; Wu et al. 2013). The potential for overlap between taxa in management-relevant traits suggests that continued genetic monitoring at the genotype level is the most reliable way to predict how a certain genotype or set of co-occurring genotypes will grow and respond to management.

**Supplementary material.** To view supplementary material for this article, please visit <https://doi.org/10.1017/inp.2022.4>

**Acknowledgments.** This project was supported by the Michigan Invasive Species Grant Program ([www.michigan.gov/invasives](http://www.michigan.gov/invasives)), with additional support from the Montana Agricultural Experiment Station (Project MONB00249). We thank Greg Chorak and Ashley Wolfe for assisting in experimental setup and takedown. We also thank Matt Lavin, Jane Mangold, Jennifer Lachowicz, Jeff Pashnick, Greg Chorak, Katie Gannon, Zach Kuzniar, and Ashley Wolfe for providing courtesy reviews of this paper. No conflicts of interest have been declared.

## References

- Albert CH, Thuiller W, Yoccoz NG, Soudant A, Boucher F, Saccone P, Lavorel S (2010) Intraspecific functional variability: extent, structure and sources of variation. *J Ecol* 98:604–613
- Baker HG (1965) Characteristics and modes of origin of weeds. Pages 147–169 in Baker HG, Stebbins GL, eds. *The Genetics of Colonizing Species: Proceedings of the First International Union of Biological Sciences Symposia on General Biology*. New York: Academic
- Balloux F, Lehmann L, de Meeüs T (2003) The population genetics of clonal and partially clonal diploids. *Genetics* 164:1635–1644
- Bartodziej JW, Ludlow J (1998) Aquatic vegetation monitoring by natural resource agencies in the United States. *Aquatics* 20:15–18
- Bates, D, Mächler, M, Bolker, B, Walker, S (2015) Fitting linear mixed-effects models using {lme4}. *J Stat Softw* 67:1–48
- Blair AC, Hufbauer RA (2010) Hybridization and invasion: one of North America's most devastating invasive plants shows evidence for a history of interspecific hybridization. *Evol Appl* 3:40–51
- Chorak GM, Thum RA (2020) Identification of resistant clones of Eurasian (*Myriophyllum spicatum*) and hybrid (*Myriophyllum spicatum* × *sibiricum*) watermilfoil to an operational rate of fluridone. *Invasive Plant Sci Manag* 13:247–251
- Cofrancesco AF Jr (1993) Biological control. Pages 71–78 in *Proceedings of the 27th Annual Meeting, Aquatic Plant Control Research Program*. Miscellaneous Paper A-93-2. Vicksburg, MS: U.S. Army Engineer Waterways Experiment Station
- Des Roches S, Post DM, Turley NE, Bailey JK, Hendry AP, Kinnison MT, Schweitzer JA, Palkovacs EP (2018) The ecological importance of intraspecific variation. *Nat Ecol Evol* 2:57–64
- Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? *Proc Natl Acad Sci USA* 97:7043–7050
- Grafe SF, Boutin C, Pick FR, Bull RD (2014) A PCR-RFLP method to detect hybridization between the invasive Eurasian watermilfoil (*Myriophyllum spicatum*) and the native northern watermilfoil (*Myriophyllum sibiricum*), and its application in Ontario lakes. *Botany* 93:117–121
- Green WR, Westerdahl HE (1990) Response of Eurasian watermilfoil to 2,4-D concentrations and exposure times. *J Aquat Plant Manage* 28:27–32
- Herben T, Nováková Z, Klimešová J (2014) Clonal growth and plant species abundance. *Ann Bot* 114:377–388
- 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). *Evol App* 6: 462–471
- Lee CE (2002) Evolutionary genetics of invasive species. *Trends Ecol Evol* 17:386–391
- Lenth, R (2020) emmeans: Estimated Marginal Means, aka Least-Squares Means. R Package v. 1.4.6. <https://CRAN.R-project.org/package=emmeans>. Accessed: January 5, 2021
- 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
- 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* × *M. sibiricum*) populations in North America. *Biol Invasions* 9:559–570
- Mooney HA, Cleland EE (2001) The evolutionary impact of invasive species. *Proc Natl Acad Sci USA* 98:5446–5451
- Nault M, Barton M, Hauxwell J, Heath E, Hoyman T, Mikulyuk A, Netherland M, Provost S, Skogerboe J, Van Egeren S (2017) Evaluation of large-scale low-concentration 2,4-D treatments for Eurasian and hybrid watermilfoil control across multiple Wisconsin lakes. *Lake Reservoir Manage* 34:115–129
- Nault M, Mikulyuk A, Hauxwell J, Skogerboe J, Asplund T, Barton M, Wagner K, Hoyman T, Heath E (2012) Herbicide treatments in Wisconsin lakes: building a framework for scientific evaluation of large-scale herbicide treatments in Wisconsin lakes. *NALMS Lake Line* 32:19–24
- Netherland MD (2014) A manager's definition of aquatic plant control. Pages 215–222 in Gettys LA, Haller WT, Petty DG, eds. *Biology and Control of Aquatic Plants: A Best Management Practices Handbook*. 3rd ed. Marietta, GA: Aquatic Ecosystem Restoration Foundation
- Pashnick J, Thum RA (2020) Comparison of molecular markers to distinguish genotypes of Eurasian watermilfoil, northern watermilfoil, and their hybrids. *J Aquat Plant Manage* 58:61–66
- Pierce AA, Gutierrez R, Rice AM, Pfennig KS (2017) Genetic variation during range expansion: effects of habitat novelty and hybridization. *Proc R Soc Lond B Biol Sci* 284:20170007
- Poovey AG, Slade JG, Netherland MD (2007) Susceptibility of Eurasian watermilfoil (*Myriophyllum spicatum*) and a milfoil hybrid (*M. spicatum* × *M. sibiricum*) to triclopyr and 2,4-D amine. *J Aquat Plant Manage* 45: 111–115
- Rieseberg LH, Willis JH (2007) Plant speciation. *Science* 317:910–914
- Slade JG, Poovey AG, Netherland MD (2007) Efficacy of fluridone on Eurasian and hybrid watermilfoil. *J Aquat Plant Manage* 45:116–118
- Smart RM, Barko JW (1985) Laboratory culture of submersed freshwater macrophytes on natural sediments. *Aquat Bot* 21:251–263
- Stebbins GL (1985) Polyploidy, hybridization, and the invasion of new habitats. *Ann MO Bot Gard* 72:824–832
- Taylor LL, McNair JN, Guastello P, Pashnick J, Thum RA (2017) Heritable variation for vegetative growth rate in ten distinct strains of hybrid watermilfoil. *J Aquat Plant Manage* 55:51–57
- terHorst CP, Lau JA (2015) Genetic variation in invasive species response to direct and indirect species interactions. *Biol Invasions* 17:651–659
- Thum RA, Chorak GM, Newman RM, Eltawley JA, Latimore J, Elgin E, Parks S (2020) Genetic diversity and differentiation in populations of invasive Eurasian (*Myriophyllum spicatum*) and hybrid (*Myriophyllum spicatum* × *sibiricum*) watermilfoil. *Invasive Plant Sci Manag* 13:59–67
- Thum RA, McNair JN (2018) Inter- and intraspecific hybridization affects vegetative growth and invasiveness in Eurasian watermilfoil. *J Aquat Plant Manage* 56:24–30
- Thum RA, Parks S, McNair JM, Tynning P, Hausler P, Chadderton L, Tucker A, Monfils A (2017) Survival and vegetative regrowth of Eurasian and hybrid watermilfoil following operational treatment with auxinic herbicides in Gun Lake, Michigan. *J Aquat Plant Manage* 55:103–107
- Ward SM, Gaskin JF, Wilson LM (2008) Ecological genetics of plant invasion: what do we know? *Invasive Plant Sci Manage* 1:98–109
- Wellband KW, Pettitt-Wade H, Fisk AT, Heath DD (2017) Differential invasion success in aquatic invasive species: the role of within- and among-population genetic diversity. *Biol Invasions* 19:2609–2621
- Wu ZG, Yu D, Xu XW (2013) Development of microsatellite markers in the hexaploid aquatic macrophyte, *Myriophyllum spicatum* (Haloragaceae). *Appl Plant Sci* 1:2
- Zalapa JE, Brunet J, Guries RP (2010) The extent of hybridization and its impact on the genetic diversity and population structure of an invasive tree, *Ulmus pumila* (Ulmaceae). *Evol Appl* 3:157–168
- Zuellig MP, Thum RA (2012) Multiple introductions of invasive Eurasian watermilfoil and recurrent hybridization with native northern watermilfoil in North America. *J Aquat Plant Manage* 50:1–19