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Research Article

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Abstract

Invasive *M. spicatum sensu lato* strains can differ in their growth, spread, impacts, and herbicide response. For example, strains of Eurasian (*Myriophyllum spicatum* L.) and hybrid (*Myriophyllum spicatum* × *Myriophyllum sibiricum* Kom.) watermilfoil have been characterized as resistant or susceptible to specific herbicides (e.g., fluridone and 2,4-D). Identifying resistant and susceptible strains can inform managers as to whether a specific herbicide should be used to treat a water body. However, to date, no centralized location existed to house and share *M. spicatum* and *M. spicatum* × *M. sibiricum* strain and herbicide response information. To address this need, we built MilfoilMapper, a publicly available, user-friendly R Shiny application that houses invasive *M. spicatum sensu lato* strain distribution and herbicide response information. To date, we have identified 290 strains from more than 300 lakes across the United States sent by state agencies, aquatic plant managers, and citizen scientists. Although some strains are found only in a single lake, some strains have been found in multiple lakes. Therefore, strain information obtained from either the field or the lab can be applied to additional lakes where these strains are found. We encourage people to incorporate genetic surveying and monitoring into their *M. spicatum* management plans to help identify strains that should be prioritized for herbicide characterization. We believe MilfoilMapper will facilitate and encourage these actions by providing a centralized, interactive platform for tracking *M. spicatum* and *M. spicatum* × *M. sibiricum* strain data, enabling lake managers, stakeholders, and state agencies to share experiences and resources to improve the efficacy and efficiency of invasive *M. spicatum sensu lato* management.

Introduction

Genetic information and tools have been applied across taxonomic groups to assist management where resistance to a control method has been found, including: antimicrobial resistance (Bengtsson-Palme et al. 2023; Hadfield et al. 2018), insecticide resistance (Faria et al. 2017; Knox et al. 2014; Rodbell et al. 2022), herbicide resistance (Chorak and Thum 2020; Comont and Neve 2020; Gaines et al. 2010; Heap 2024), and fungicide resistance (Fontaine et al. 2019; Lucas et al. 2015; Massi et al. 2021). Pretreatment identification of resistant populations using genetic markers can inform management and control.

Recent studies demonstrate that genetic variation can be relevant to aquatic weed management as well. For example, herbicide resistance has been documented in hydrilla [*Hydrilla verticillata* (L.f.) Royle] (Michel et al. 2004), landoltia [*Landoltia punctata* (G. Mey.) D.H. Les & D.J. Crawford] (Koschnick et al. 2017), and Eurasian watermilfoil (*Myriophyllum spicatum* L. *sensu lato*) (Berger et al. 2012, 2015a, 2015b; Chorak and Thum 2020; Thum et al. 2012). However, genetic studies of resistance are relatively rare for aquatic weeds compared with agricultural weeds, and thus molecular assays for resistance are relatively rare (but see Benoit and Les 2013; Michel et al. 2004). Therefore, the development of molecular tools to predict herbicide response could improve aquatic plant management outcomes.

For clonal organisms, such as many aquatic plants, characterizing herbicide response of clones (i.e., genets) can inform management decisions in the short term, while longer-term studies to identify herbicide response genes are being conducted. For example, clones that are widespread, rapidly increasing, and/or have been noted by managers to exhibit lower than desired management response could be prioritized for herbicide response characterization, which could inform management efforts in all locations where they are found. Therefore, DNA fingerprinting approaches (e.g., microsatellites and single-nucleotide polymorphism [SNP] assays) that can identify ramets of the same genet and distinguish unique genets can be a

Management Implications

MilfoilMapper provides data on watermilfoil strain occurrence, distribution, diversity, and herbicide response to support effective management of *Myriophyllum spicatum* (Eurasian watermilfoil). Although herbicide treatment efficacy is influenced by hydrological and technical factors (e.g., dilution, dissipation, degradation, delivery), it is also clear that genetic composition influences herbicide efficacy. Therefore, strain-specific herbicide response data can guide treatment decisions. Treatment of strains with a herbicide they are resistant to should be avoided, while treating susceptible strains is more likely to result in effective control. Out of 290 identified watermilfoil strains, 22 have been characterized for their response to fluridone and/or 2,4-D. Seven of the 22 characterized strains are resistant to either fluridone or 2,4-D, and we suspect there are additional strains that are resistant in the field to one or both herbicides but have not yet been identified.

If herbicide response data are unavailable on MilfoilMapper, users can identify lakes with the strain of interest and collaborate with local managers to share insights or conduct herbicide response studies. We identified that *M. spicatum* and *Myriophyllum spicatum* × *Myriophyllum sibiricum* (hybrid watermilfoil) strains show distinct distribution patterns across the United States. *Myriophyllum spicatum* strains are more widespread, with three strains accounting for 80% of occurrences in MilfoilMapper, making their herbicide response data highly impactful for management. In contrast, *M. spicatum* × *M. sibiricum* strains are more genetically diverse and geographically restricted, leading to greater variability in herbicide response. It is therefore especially important to carefully observe *M. spicatum* × *M. sibiricum* response to treatments.

valuable, short-term management tool for the management of clonal aquatic plants (e.g., Chorak and Thum 2020; Gannon et al., 2022; Hoff and Thum 2022; Thum et al. 2020).

Myriophyllum spicatum and its hybrids with native northern watermilfoil (*Myriophyllum sibiricum* Kom.) are among the most widespread and heavily managed aquatic weeds in the United States (Bartodziej and Ludlow 1997; Confrancesco 1993). Within *M. spicatum*, its native sister species, *M. sibiricum*, and their hybrid *M. spicatum* × *M. sibiricum*, there are distinct genotypes, or strains, that are produced through sexual reproduction (Hartleb et al. 1993; LaRue et al. 2013; Thum and McNair 2018; Thum et al. 2020). Therefore, unique *M. spicatum* and *M. spicatum* × *M. sibiricum* clonal lineages (hereafter "strains") can spread indefinitely through clonal propagation both within and between lakes (Smith and Barko 1990).

Strains of invasive *Myriophyllum* differ in their response to herbicide treatment (Berger et al. 2012, 2015a, 2015b; Chorak and Thum 2020; Hoff and Thum 2022; Netherland and Willey 2017). As such, strain-level information about invasive *Myriophyllum* populations is relevant to management. Because some strains are found in multiple lakes (Thum et al. 2020), herbicide treatment options can be better evaluated when the particular strain(s) in a lake are characterized for their herbicide response. For example, a fluridone-resistant strain of *M. spicatum* × *M. sibiricum* is commonly found in Michigan lakes, whereas fluridone-susceptible strains are also commonly found in Michigan lakes (Chorak and Thum 2020; Thum et al. 2020). Therefore, genetic screening for these strains can help determine whether fluridone is an appropriate herbicide to use in a given Michigan lake.

Even in the absence of herbicide response data, strain composition information can help inform management (Gannon et al., 2022). For example, utilizing pre- and posttreatment strain surveys on a lake can help identify whether the relative frequencies of different *M. spicatum* and *M. spicatum* × *M. sibiricum* strains change after a herbicide treatment. If so, this observation could help to identify strains for further study in laboratory herbicide response assays. Additionally, aquatic plant managers and stakeholders whose lakes share strains could share their management experiences, or even judiciously design field studies to assess management alternatives.

Although genetic variation is clearly important for *M. spicatum* management outcomes, a centralized location that provides access to *M. spicatum* and *M. spicatum* × *M. sibiricum* strain information is currently lacking. Here, we present an R Shiny application designed to address this need—MilfoilMapper.

Materials and Methods

Strain Identification and Distribution

To date, 4,911 *Myriophyllum* tissue samples have been collected and identified to the strain level from across 15 different U.S. states through the participation of state agencies, aquatic plant management consultants, and citizen scientists. Additionally, some data contained in the MilfoilMapper database have been previously published (Chorak and Thum 2020; Eltawely et al. 2020; Gannon et al., 2022; Hoff and Thum 2022; Thum et al. 2020). New strain data are added to MilfoilMapper every season, and more than 200 strains have been added to the database since the most recent publications cited above.

To characterize strain composition for a lake, we recommend contributors target a representative sample of 20 to 50 plants from around the lake. However, there was no standardized sampling protocol used for the samples included in MilfoilMapper due to the differing sampling methods, objectives, and constraints among collectors. For example, some samples were collected as a part of quantitative vegetative mapping (e.g., point-intercept surveys), whereas others were collected in a stratified way across locations where *Myriophyllum* was present in the water body (e.g., meandering shoreline surveys). In some cases, plant samples were submitted with little to no information on the sampling protocol used. When more than 20 plants were sampled, we typically processed a subsample of 20 plants. If fewer than 20 plants were provided for a water body, all plants were processed.

In some cases, the exact latitude and longitude values were provided for each individual sample for a given water body. However, in most cases, we do not have those data. Therefore, we decided to standardize all strain occurrences to a centroid latitude–longitude point within a water body. These values were taken from state Department of Natrual Resources (DNR) websites when available. If these data were unavailable, a centroid latitude–longitude was selected using Google Maps.

Plant samples were generally dried with silica gel before being shipped to the laboratory for processing. In some cases, plant samples were shipped live, and fresh material was either immediately dried with silica gel or flash-frozen with liquid nitrogen and stored in a −80 C freezer until DNA extraction. DNA was extracted from all samples using the Qiagen DNeasy Plant Mini Kit (Valencia, CA) following the manufacturer's protocol. DNA extractions were duplicated for ~10% of all samples to assist with scoring of microsatellites.

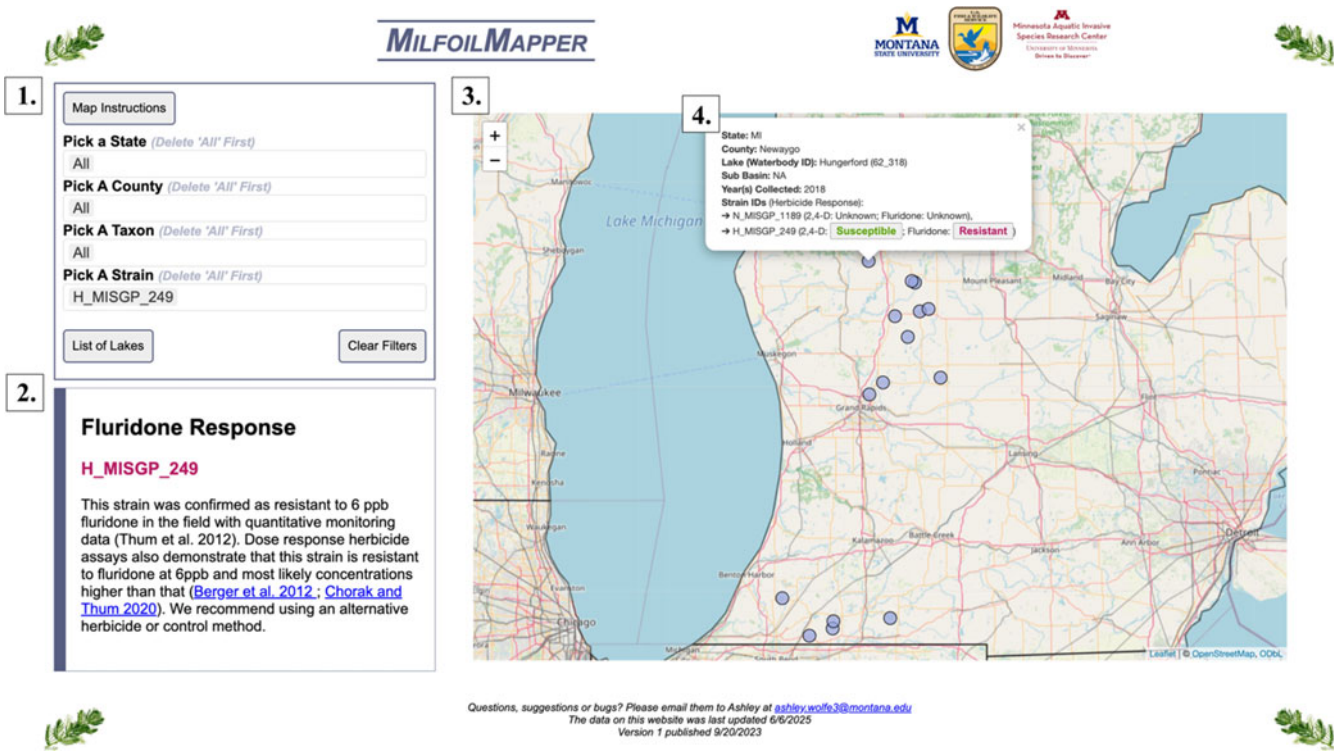

Figure 1. Illustration of MilfoilMapper components. (1) The database can be queried by geography (state, county, lake) or genetic factors (taxon or strain). This example filters data for the strain H_MISGP_294. The "List of Lakes" button will return a list based on the query (not shown). (2) An information box, where additional details about herbicide response information and strain nomenclature are displayed. (3) Interactive map showing the distribution of strains based on the results of a given query. In this example, H_MISGP_249 occurs in two discrete clusters in the central and southwestern part of Michigan. (4) Selecting an individual water body produces a list of all strains that have been identified there, including buttons displaying herbicide response information. At present, herbicide response information is limited to two common herbicides for *Myriophyllum spicatum sensu lato* control: 2,4-D and fluridone. If a herbicide response button is selected, additional information populates box 2. In this example, the selected lake, Hungerford Lake, MI, displays the two strains identified in this lake: H_MISGP_249 is indicated as Susceptible to 2,4-D and Resistant to fluridone, whereas H_MISGP_1189 is indicated as having unknown herbicide responses.

Plants were genotyped using the same methods described in Thum et al. (2020). Briefly, we genotyped plants using eight microsatellite loci developed by Wu et al. (2013; Myrsp 1, Myrsp 5, Myrsp 9, Myrsp 12, Myrsp 13, Myrsp 14, Myrsp 15, and Myrsp 16) under the PCR conditions described therein. Fluorescently labeled microsatellite PCR products were sent to the University of Illinois–Urbana-Champaign's Core Sequencing Facility for fragment analysis on an Applied Biosystems 3730xl sequencer (Applied Biosystems, Foster City, CA).

Microsatellites were scored using GeneMapper v. 5.0 (Applied Biosystems). Because *M. spicatum*, *M. sibiricum*, and *M. spicatum* × *M. sibiricum* are hexaploid, exact strain identifications cannot be determined, as the numbers of allele copies are ambiguous. Therefore, we treated microsatellites as dominant, binary data (i.e., presence or absence of each possible allele at each locus) using R (R Core Team 2022) and the R package POLYSAT (Clark and Jasieniuk 2011). We delineated distinct multi-locus strains (hereafter, simply "strains") using Lynch distances and a threshold of 0 in POLYSAT (Clark and Jasieniuk 2011). Each unique microsatellite strain identification was given a unique identification code. Individuals with the same microsatellite strain identification were assumed to be ramets of the same genetic clone and were therefore given the same identification code in MilfoilMapper.

Each strain present in MilfoilMapper is assigned one of four different herbicide response labels (Susceptible, Resistant, Of Concern, or Unknown) based on herbicide characterization data (Supplementary Table 1). "Susceptible" and "Resistant" labels are given to strains that have both field and laboratory observations of herbicide efficacy that comport with one another. Strains labeled as "Of Concern" have been brought to our attention by lake managers as possibly resistant based on qualitative observations but have not yet been included in a quantitative and controlled laboratory study of herbicide response. Finally, strains without any herbicide response observations are labeled as "Unknown."

MilfoilMapper Creation

We created an R Shiny application—MilfoilMapper—to house and share compiled *M. spicatum* and *M. spicatum* × *M. sibiricum* strain information (Figure 1). We chose to create our website using the R package SHINY (Chang et al., 2024) because it is a user-friendly way (creators are not required to be familiar with HTML, CSS, or JavaScript) to build web applications that can house and visualize voluminous data for free for both users and developers. MilfoilMapper can provide two pieces of information for identified strains to guide management decisions: (1) herbicide response for one or more herbicides commonly used for control and (2) occurrence and geographic distribution information. The application is available at: https://thumlab-msu-watermilfoilapp.shinyapps.io/milfoil_app/. A copy of all source code is contained in a GitHub repository: https://github.com/AshleyW406/MilfoilMapper.git.

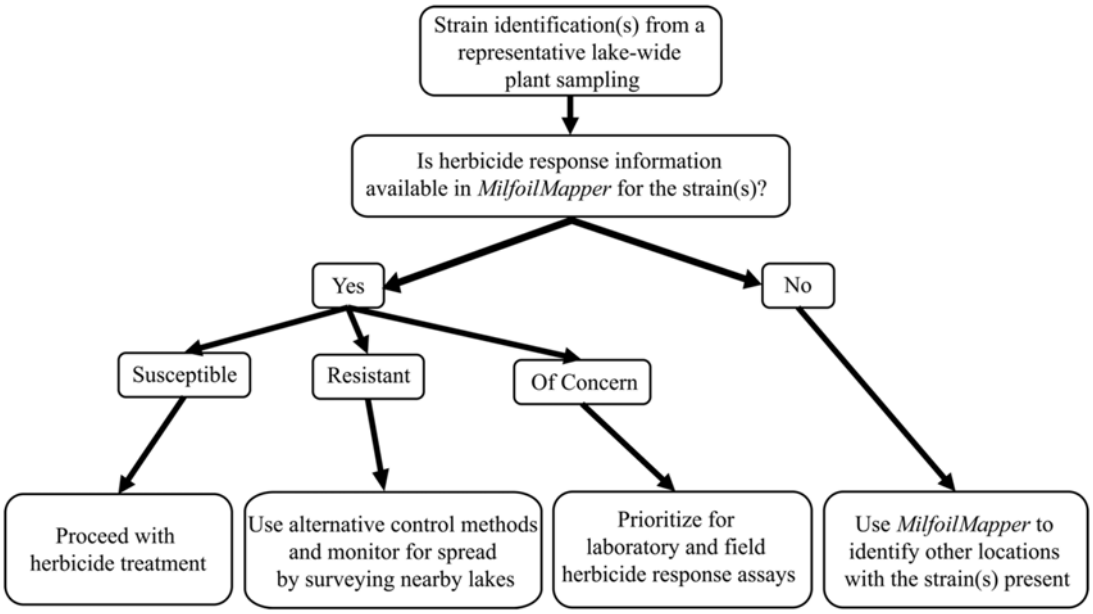

Figure 2. Workflow for *Myriophyllum spicatum sensu lato* strain identification(s) to help inform management decisions. The most direct impact on decision making will occur when herbicide response data are available. However, in the absence of herbicide response data, information on strain distribution and occurrence can still inform management decisions. We always recommend quantitative monitoring of a strain's herbicide response in the field.

Use of a Large-Language Model

Authors acknowledge the limited use of a large-language model (LLM), ChatGPT 4.0, in the preparation of this article. Specifically, it was used for troubleshooting RStudio code for figure creation and for rewording select sentences to improve clarity in the original drafts of the manuscript. The tool was not used to generate scientific content, develop ideas, analyze data, or interpret results. All scientific work and manuscript writing are entirely the original work of the authors.

Results and Discussion

MilfoilMapper serves as a centralized, living repository that integrates strain and herbicide response data to help inform management strategies based on the strains present in a lake and whether those strains are common or regionally unique (Figure 2). When available, herbicide response data can be used to directly inform herbicide prescriptions. When herbicide response data are not available for a given strain, MilfoilMapper allows users to identify other lakes where the strain occurs, facilitating communication among managers and potentially pooling resources needed to conduct field or laboratory studies. Collectively, these components of MilfoilMapper provide valuable guidance for developing and modifying comprehensive management plans for *M. spicatum* and *M. spicatum* × *M. sibiricum*.

In the following sections, we discuss the current status and future prospects of the major components of MilfoilMapper: (1) strain identification; (2) diversity, occurrence, and distribution of strains; and (3) herbicide response.

Strain Identification

The strains currently in MilfoilMapper are distinguished with eight multi-locus microsatellite genotypes. We recognize the small number of microsatellite markers can potentially result in erroneous strain identifications. For example, two individuals may share the same multi-locus genotype by chance instead of through shared ancestry by clonal reproduction. The hexaploid nature of our microsatellite markers requires that we score microsatellite data as dominant and precludes us from calculating probabilities of identity for individuals with the same genotype. However, given the extensive clonal propagation of *M. spicatum sensu lato*, we believe assigning the same strain identification to individuals with the same multi-locus genotype is reasonable. In addition, somatic mutations can accumulate during clonal propagation, and individuals that share ancestry through clonal reproduction could exhibit different herbicide responses. For example, invasive *H. verticillata* in the United States has evolved fluridone resistance through somatic mutations (Michel et al. 2004). Finally, scoring errors could potentially identify two individuals of the same clone as different strains. However, we have used extensive duplicate samples to identify microsatellite loci and alleles that are robust, and we manually inspect every microsatellite chromatogram for accurate scoring.

Given the limitations of microsatellite markers, *M. spicatum* and *M. spicatum* × *M. sibiricum* strain identification could potentially be improved with a modernized approach based on SNP markers. JJ Pashnick and RA Thum (unpublished) piloted a panel of targeted SNPs for genotyping-by-sequencing in *M. spicatum sensu lato* (see Baetscher et al. [2018] and Campbell et al. [2015] for similar methodologies). Implementation of this approach was initially hampered by difficulty in assigning reads to homeologous subgenomes, as well as unequal amplification of target SNPs and inconsistent amplification among individuals. The recent completion of a high-quality genome (Hannay et al., unpublished data) and further refinement of the original SNPs identified for genotyping-by-sequencing could facilitate the development and implementation of an SNP-based assay to replace the microsatellite approach currently used in MilfoilMapper.

Although it is possible for independent laboratories to collect the same genetic data to identify and distinguish *M. spicatum sensu*

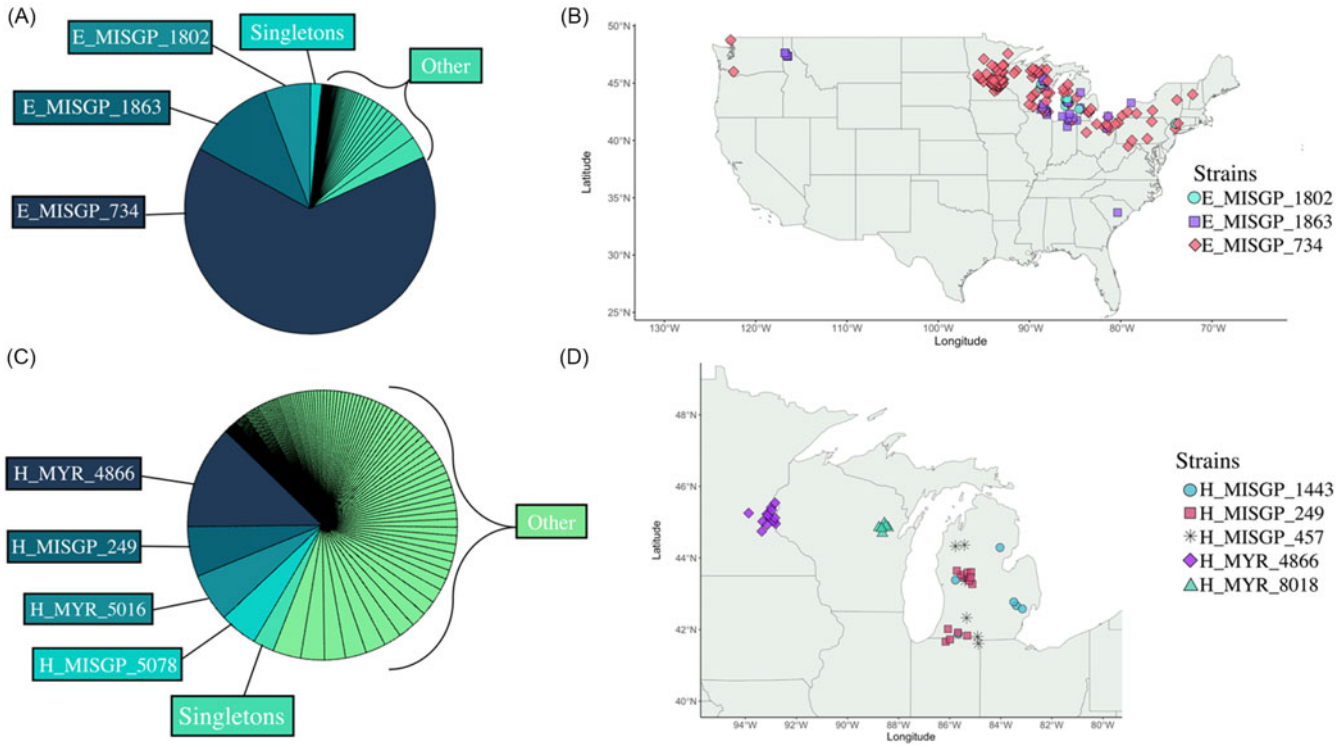

Figure 3. The geographic distribution and occurrence of *Myriophyllum spicatum* (A and B) and *Myriophyllum spicatum* × *Myriophyllum sibiricum* strains (C and D). (A) Relative frequencies of *M. spicatum* strain occurrences ($n = 2,420$). (B) Geographic distribution of the three most common *M. spicatum* strains. (C) Relative frequencies of *M. spicatum* × *M. sibiricum* strain occurrences ($n = 2,490$). (D) Geographic distribution of widespread *M. spicatum* × *M. sibiricum* strains. Strains on this map are considered widespread because they were found in five or more lakes. "Singletons" are strains that have been found once in a single lake. "Other" includes all other strain occurrences.

lato strains, combining data from different labs should be done with careful curation. Microsatellite allele sizes will differ depending on whether the primers are labeled with fluorescent tags directly versus using a universal fluorescent labeling system (e.g., Shimizu et al. 2002), where the former will yield smaller PCR products than the latter for the same alleles. Similarly, scoring decisions can differ from person to person. We have carefully chosen allele bins based on a large number of duplicate samples, and therefore standardization of the methods used to identify and distinguish *M. spicatum sensu lato* strains is possible. Similarly, even though the development of an SNP-based identification method may facilitate automated scoring of genotyping-by-sequencing data, collated data from different laboratories would require careful curation to account for sequencing errors and genotype likelihoods. Therefore, we recommend a central curator be identified if multiple laboratories collaborate to collate microsatellite or SNP data on strain identification and occurrence.

Diversity, Occurrence, and Distribution of Strains

The current data contained in MilfoilMapper are a much-expanded version of the dataset presented in Thum et al. (2020), who reported on 103 water bodies in Minnesota and Michigan. We have now identified strains from an additional 220 water bodies in 15 states. Diversity, occurrence, and distribution patterns in this expanded dataset are similar to those identified in Thum et al. (2020).

Although we recommend sampling approximately 20 to 50 plants from across a lake to characterize strain composition, not all of the lakes in MilfoilMapper were sampled that way (see "Materials and Methods"). Currently, the strain data in MilfoilMapper are presence–absence. Therefore, any given lake may contain more strains than are reported, and this is obviously more likely in lakes with small sample sizes compared with lakes with large sample sizes. Furthermore, strain composition can vary over time (see Gannon et al. 2022) and thus strains present at a previous time may no longer be present, and vice versa. Future versions of MilfoilMapper may be improved by allowing users to see data for individual sampling events (e.g., sample sizes and dates).

Even though most lakes are dominated by a single *M. spicatum sensu lato* strain ($n = 180$), some lakes are more diverse and contain multiple strains ($n = 143$). Because different strains can respond to herbicides differently, it is important to consider strain composition when planning and assessing herbicide treatments. If multiple strains are present, managers should consider tracking relative strain frequencies over time to detect any shifts in strain frequency (see Gannon et al. 2022; Parks et al. 2016). Strains that increased in their relative frequency could then be prioritized for laboratory herbicide assays. However, MilfoilMapper does not currently break down sampling events and is therefore not recommended as a data analysis tool for strain monitoring, per se.

The proportion of strains that have been found in multiple locations, and the geographic breadth of their distributions, differ between *M. spicatum* and *M. spicatum* × *M. sibiricum* strains (Figure 3). Out of 85 total *M. spicatum* strains identified, 35 were singletons found only one time in a single lake, making up 1.4% of all *M. spicatum* strain occurrences. Out of the remaining 50 strains, 7 *M. spicatum* strains have been identified in multiple water bodies. Of these, three strains were particularly common and geographically widespread (E_MISGP_734, E_MISGP_1863, E_MISGP_1802; see

also Thum et al. 2020). Furthermore, these three strains make up 82% of all *M. spicatum* water body occurrences in MilfoilMapper.

In contrast, out of the 204 *M. spicatum* × *M. sibiricum* strains identified to date, 56 are singletons, making up 2% of all *M. spicatum* × *M. sibiricum* strain occurrences (Figure 3). Of the remaining 148 strains, 29 have been found in multiple waterbodies, making up only 48% of all *M. spicatum* × *M. sibiricum* occurrences in MilfoilMapper. However, unlike the three *M. spicatum* strains discussed earlier, none of the *M. spicatum* × *M. sibiricum* strains found in multiple locations have a broad geographic range. Instead, *M. spicatum* × *M. sibiricum* strains found in multiple locations tend to be geographically restricted. Some of these widespread *M. spicatum* × *M. sibiricum* strains have a more clumped distribution (H_MISGP_249, H_MYR_4866, H_MYR_8018) and others are more dispersed across a state (H_MISGP_1443, H_MISGP_457) (see Figure 3D). These patterns may be due to strains spreading from lake to lake via dispersal vectors (e.g., boats and birds) with specific movement patterns. Alternatively, some strains may be more likely to spread and establish due to their growth or management response. For example, invasive *Myriophyllum* populations in Michigan are commonly managed with fluridone, and the fluridone-resistant hybrid strain H_MISGP_249 is relatively common in the state, potentially because it can outcompete other strains in lakes managed with fluridone.

The occurrence and geographic distribution patterns described have important management implications. Lakes containing *M. spicatum* are most likely to have one of a small number of common and widespread lineages, especially E_MISGP_734 (Figure 3). Therefore, herbicide response characterization of these common *M. spicatum* strains would have a relatively large impact on informing herbicide decisions for a large number of lakes across the United States. In fact, these three widespread *M. spicatum* strains have all been prioritized for fluridone and 2,4-D characterizations and are listed as "Susceptible" to both herbicides in MilfoilMapper. These *M. spicatum* strains should be prioritized for studies to characterize their response to other commonly used and newly developed herbicides.

In contrast to *M. spicatum*, we do not see any *M. spicatum* × *M. sibiricum* strains that are as common or geographically widespread as the three *M. spicatum* strains discussed earlier (Figure 3). Instead, *M. spicatum* × *M. sibiricum* strains are genetically more diverse, and comparatively more geographically restricted than pure *M. spicatum* strains. This pattern suggests that *M. spicatum* × *M. sibiricum* strains have arisen many times independently from different *M. spicatum* and *M. sibiricum* parents in different locations (Thum et al. 2020; Zuellig and Thum 2012). Further, the greater genetic diversity of *M. spicatum* × *M. sibiricum* suggests that we can expect to observe more variation in herbicide response among populations of *M. spicatum* × *M. sibiricum* compared with pure *M. spicatum*.

Admittedly, the more restricted distribution of *M. spicatum* × *M. sibiricum* strains limits the ability to apply *M. spicatum* × *M. sibiricum* strain–specific information across multiple locations. It is therefore especially important to carefully observe *M. spicatum* × *M. sibiricum* response to herbicide treatments and to prioritize those strains for laboratory herbicide response characterization based on field and observations of control efficacy and how common and geographically widespread they are. Because of their generally restricted distribution patterns, once *M. spicatum* × *M. sibiricum* strains have been characterized for herbicide response, we recommend that nearby lakes be surveyed for the presence of the same strains. For example, the *M. spicatum* × *M. sibiricum* strain H_MISGP_249 is resistant to operational fluridone treatment levels in Michigan (Berger et al. 2012, 2015a, 2015b; Chorak and Thum 2020; Thum et al. 2012), Since its original discovery and characterization, it has been identified in 12 additional lakes. Further, these lakes occur in two discrete clusters located in central and southwestern Michigan (see Figures 1 and 3). Surveys of nearby lakes where this hybrid strain is identified can thus preclude costly fluridone treatment failures. Similarly, lakes containing hybrid strains that are known to be susceptible to a particular herbicide can reasonably predict similar responses to control methods used in nearby lakes with the same strains. Therefore, although there is considerable genetic diversity in *M. spicatum* × *M. sibiricum* strains, and their distributions are generally restricted, genetic and herbicide response information provided by MilfoilMapper has the potential to inform management decisions and practices for *M. spicatum* × *M. sibiricum* strains.

Herbicide Response

The ultimate goal of MilfoilMapper is to provide a living catalog of strain distribution and herbicide response information. Currently, the number of strains characterized is small (22 out of 290 strains identified from genetic surveys) and limited to only two commonly used herbicides (fluridone and 2,4-D) (see Supplementary Table 1). While we believe MilfoilMapper will be a valuable tool to provide an important platform to serve as a repository for additional information, as well as increasing *M. spicatum sensu lato* control efficiency and efficacy, there are admittedly several important limitations in populating MilfoilMapper with strain information and in interpreting and utilizing that information.

The most important limitation to populating MilfoilMapper with herbicide response information is logistical difficulty associated with conducting herbicide dose–response studies. Dose–response studies for aquatic plants require considerable time and space, and it is impossible to exhaustively characterize the large number of strains for response to the number of aquatic herbicides used for invasive *Myriophyllum* control. Therefore, it is necessary to prioritize the strains and herbicides targeted for characterization.

Quantitative and qualitative field observations are critical for prioritizing herbicide response characterization. In fact, we initially identified all the resistant strains in MilfoilMapper through field observations. The four fluridone-resistant strains (H_MISGP_249, E_MISGP_380, H_MISGP_1443, H_MISGP_1861) and two 2,4-D strains (H_MYR_5016, H_MYR_15816) were initially brought to our attention via manager observations of low efficacy in the field and subsequently confirmed with controlled laboratory study (Berger et al. 2012; Chorak and Thum 2020; Thum et al. 2012; GM Chorak and RA Thum, unpublished data; HK Hoff and RA Thum, unpublished data; RM Newman and J Gerritsen, unpublished data; AL Wolfe and RA Thum, unpublished data) (see Supplementary Table 1). Similarly, a 2,4-D–resistant strain (N_MYR_15319) was initially identified through quantitative field monitoring of strain composition before and after herbicide treatments (see Gannon et al., 2022) and was then subsequently confirmed with controlled laboratory study. We suspect there are additional strains in the field that are resistant to one or both herbicides but have not yet been identified and/or brought to our attention for characterization.

Further, field observations and data to identify susceptible strains are equally important. Therefore, we strongly encourage managers to conduct genetic surveys of lakes to determine how many and which strains are present for interpretation of their control results and to consider integrating strain monitoring data into quantitative evaluations of herbicide efficacy whenever feasible.

Although MilfoilMapper lists strains as "Resistant" versus "Susceptible," it is important to recognize that herbicide response is not binary (Thum et al. 2023). Because there are different herbicide treatment use patterns in different states, and because the achieved efficacy of a treatment will depend on strains' specific dose–response curves to the herbicide used, a particular strain may be satisfactorily controlled in one place with one use pattern and not in another with a different use pattern. For example, the strains currently marked as "Resistant" versus "Susceptible" to fluridone in MilfoilMapper are classified as such based on reference to the operational rate of 6 μg L^{-1} fluridone treatment as it is applied in Michigan (see Premo et al. 1999), where fluridone is used more commonly than in other midwestern states. However, fluridone use for *M. spicatum sensu lato* control is increasing in nearby states (e.g., Minnesota and Wisconsin) but is often used at lower concentrations. Therefore, a strain labeled as "Susceptible" in reference to 6 μg L^{-1} fluridone may not be controlled sufficiently at a lower concentration.

Given the limitations of a binary "Resistant" versus "Susceptible" scheme for herbicide response, MilfoilMapper could be improved by reporting standardized, quantitative herbicide response values (e.g., EC_{50}) or including more detailed dose–response information for each strain and herbicide. Currently, the available resources for *M. spicatum sensu lato* herbicide response characterization do not provide a centralized laboratory to conduct herbicide studies, which in turn precludes a standard protocol for herbicide characterization. The development of collaborations among multiple laboratories and the development and implementation of standardized screening procedures (including standardized reference clones) would therefore improve the utility of MilfoilMapper. Nevertheless, the current approach and information available in MilfoilMapper should provide helpful guidance for managers.

It is important to acknowledge that management decisions and efficacy are influenced by a number of site-specific characteristics besides genetic composition and that poor efficacy can be observed even on strains that are genetically susceptible to an herbicide. For example, improper dose calculation or delivery could result in failure to achieve sufficient concentration and exposure time for effective control (Nault et al. 2018; Schardt and Netherland 2021; West et al. 1983). Similarly, dilution and dissipation of a herbicide can prevent sufficient concentration and exposure times, especially for treatments applied to small areas of a water body. Finally, the extent of herbicide degradation (e.g., temperature, light, water chemistry, and microbial composition depending on the specific herbicide) could limit the achieved concentration and exposure time.

Conclusions

Although we have identified areas for improvement, we believe MilfoilMapper will facilitate improved management efforts by providing a centralized, interactive platform for tracking *M. spicatum* and *M. spicatum* × *M. sibiricum* strains. We advocate for the integration of more widespread genetic surveying and monitoring into *M. spicatum* and *M. spicatum* × *M. sibiricum* management to help identify and prioritize strains with documented herbicide response as well as identifying strains to characterize for herbicide response.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/inp.2025.10032

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