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Response of four *Vallisneria* taxa to aquatic herbicides

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Vallisneria taxa response **(short title)**

Abstract

Native aquatic macrophytes such as *Vallisneria americana* Michx. are often desirable in aquatic ecosystems due to the ecological benefits they provide but are threatened by competition from invasive taxa including nonnative *Vallisneria* taxa and hydrilla (*Hydrilla verticillata* L.f. Royle). Identifying potential selective herbicide management options can provide options to minimize impacts to native taxa in restoration and aquatic invasive plant management programs. Greenhouse mesocosm experiments were conducted in 2023 to investigate herbicide efficacy on two native eelgrass species (*V. americana* and *V. neotropicalis* Vict.), two nonnative taxa (*V. australis* S.W.L. Jacobs & Les and *V. spiralis* × *V. denseserrulata* Makino) and *H. verticillata*. Herbicide applications included endothall, diquat, florpyrauxifen-benzyl, fluridone, and flumioxazin and select combinations of these herbicides used in *H. verticillata* management. Endothall alone provided 90 to 100% aboveground biomass reduction at 3000 µg L^{-1} with at least 24 hours of continuous or intermittent exposure to all native and invasive species at six weeks after exposure, whereas florpyrauxifen-benzyl applied alone resulted in minimal aboveground biomass reduction. A 45 day of exposure of fluridone (10 µg L^{-1}) resulted in 95% biomass reduction on *V. americana* and 7 to 48% other tested taxa. The combination of flumioxazin and florpyrauxifen-benzyl resulted in 90 to 100% aboveground biomass reduction and endothall combined with florpyrauxifen-benzyl resulted in 93 to 100% aboveground biomass reduction across taxa. Reductions in belowground biomass mirrored trends observed in aboveground biomass. No treatments selectively controlled invasive *Vallisneria* without injury to native *Vallisneria*, although efficacy was observed on hydrilla. These insights provide an understanding for differences between these *Vallisneria* for researchers moving forward with selectively targeting *H. verticillata* in the presence of native *Vallisneria* species and two new aquatic invasive plants. Future research should expand treatment scenarios, increase the study period, and identify potential integrated plant management strategies for field scenarios.

Key words: aquatic; native species; invasive species; native species; submersed vegetation; herbicide

Management Implications

Diverse communities of submersed aquatic vegetation are ecologically beneficial to aquatic ecosystems; however, nonnative plants commonly disrupt these communities and their associated benefits. *Vallisneria* species are recognized as desirable plants for aquatic resource managers. However, recent documentation of multiple non-native *Vallisneria* taxa in the United States has prompted concerns in management of aquatic systems. The primary objective was to evaluate the response of two native eelgrass taxa (*Vallisneria americana* and *V. neotropicalis*) and two nonnative taxa (*V. australis* and hybrid *V. spiralis* × *V. denseserrulata*) to common aquatic herbicide treatments in a small-scale mesocosm study. This research also provided efficacy data for new aquatic invasive taxa. Managers may control native and invasive *Vallisneria* taxa with endothall or combinations of endothall and diquat, endothall and florpyrauxifen-benzyl, and florpyrauxifen-benzyl and flumioxazin. Florpyrauxifen-benzyl applied alone does not provide nominal above or belowground biomass control compared to some of the other treatments tested. Fluridone reduced native *V. americana* above and belowground biomass but is less likely to suppress *V. australis, V. neotropicalis,* or *V. spiralis* × *V. denseserrulata*. Control of invasive hydrilla with minimal damage to native *Vallisneria* was also observed with florpyrauxifen-benzyl treatments. These observations may be of particular interest to managers looking to control hydrilla growing intermingled with native *Vallisneria* taxa, especially in restoration sites.

Introduction

Diverse, native submersed aquatic vegetation (SAV) assemblages are essential for aquatic ecosystems as they provide fish and wildlife habitat, sediment stabilization, reduce wave action, and improve water quality (Dodd et al. 2021; Gettys and Haller 2013; Korschgen et al. 1987; Moore et al. 2010; Owens et al. 2008). However, native aquatic plant communities are sensitive to invasion by nonnative plants which reduce biodiversity and habitat quality as well as negatively impact human uses of aquatic systems such as hydropower, flood control, irrigation, and recreation (Langeland 1996; Madsen and Sand-Jensen 1991; Smart et al. 1994; Zhang and Boyle 2010). Aquatic ecosystem restoration efforts often include revegetation plantings of native plants (Canfield and Hoyer 1992; Gettys and Haller 2013). In the United States, American eelgrass (*Vallisneria americana* Michx.) is arguably the most common and sought after

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submersed plant species used in restoration projects due to ease of propagation, resistance to invasive species pressure, tolerance of wave action, growth in a variety of soil and water characteristics and ecosystem services provided including stabilizing water and soil quality, and serving as a food source or host to food sources for invertebrate and vertebrate species (Gettys and Haller 2013; Henry 2017; Korschgen and Green 1988; Moore et al 2010).

Vallisneria is a genus with global distribution; however, *V. americana* is native to North America, primarily found in eastern North America, with some distribution in Europe and Asia as well (Korschgen and Green 1988; Les et al. 2008; Martin and Mort 2023; Mesterházy et al 2021). *Vallisneria* taxa are herbaceous, monocotyledonous, and form rosettes at the hydrosoil surface (Godfrey and Wooten 1979). *Vallisneria* taxa are dioecious with female flowers in long sessile stalks at the water surface and male flowers on short stalks near the basal rosette that detach and float free to the water surface (Les et al. 2008; Martin and Mort 2023). *Vallisneria* also reproduces asexually via stolons, which produce clonal daughter plants and some species, such as *V. americana*, also reproduce via subterranean turions. Unlike most aquatic macrophytes which are canopy forming, *Vallisneria* is a meadow-forming plant with biomass more evenly distributed in the water column (Best et al. 2004; Haller and Sutton 1975; Hauxwell et al. 2007).

Recently, genetic divergence between United States native populations of *V. americana* was recognized which suggested the resurrection of *V. neotropicalis* Vict., previously consolidated with *V. americana* (Gorham et al. 2021; Les et al. 2008; Martin and Mort 2023). Previously, these taxa were considered separate ecotypes of *V. americana*, despite considerable phenological differences including annual vs perennial habits of overwintering and the lack of turion production in southern ecotypes, thought to be a function of geography or environmental factors (Godfrey and Wooten 1979; McFarland and Schafer 2008; Smart et al. 2005). Anecdotal evidence indicates there is a geographic delineation separating *V. americana* and *V. neotropicalis,* but the exact distribution is not currently understood and requires further investigation.

The presence of non-native *Vallisneria* species, cultivars, and hybrids has also been recently documented in the United States, primarily *V. spiralis* and *V. spiralis* L. × *V. denseserrulata* Makino in the Southeastern United States and *V. australis* S.W.L. Jacobs & Les in California (CDFA 2021; Gorham et al. 2021; Martin and Mort 2023). Given the prevalence of different *Vallisneria* species in the aquarium and water garden communities, it is possible some

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of these new infestations have arisen from accidental releases. *Vallisneria australis* and *V. neotropicalis* have been identified as new invasions in Europe and the hybrid *V. spiralis* L. × *V. denseserrulata* has been documented in Japan (Mesterházy et al. 2021; Wasekura et al. 2016). Hybrids between *V. australis* and one of the United States native species (*V. americana* or *V. neotropicalis*) are commercially available in the aquarium trade mistakenly labeled as *V. americana* (Martin and Mort 2023). Unfortunately, some restoration efforts have unknowingly planted non-native *Vallisneria* taxa as plants collected from Crystal River, FL were identified as *V. spiralis* L. × *V. denseserrulata* following a restoration project (Martin and Mort 2023). However, we cannot yet determine the contribution of spread that restoration efforts may have played compared to aquarium-based releases.

Differentiation between cryptic species and hybrids via plant morphology alone is often difficult with closely related taxa, especially *Vallisneria*. Hybridization between macrophytes is likely more common than currently recognized and has been observed in *Myriophyllum* and *Nymphoides* (Harms et al. 2021; Parks et al. 2016; Tavalire et al. 2012). Multiple *Vallisneria* taxa have been documented to coexist in waterbodies, so selective management actions would be desirable to suppress invasive populations while mitigating adverse effects to native populations (Gorham et al. 2021).

To combat invasions by other species and restore SAV communities, aquatic resource managers have been motivated to identify and cultivate *Vallisneria* populations with growth characteristics favorable for restoration efforts. This includes establishing or re-establishing self-sustaining populations that are fast growing, tolerant to chemical and non-chemical management activity, resistant to invasion, competition, and environmental disturbance. Unfortunately, these characteristics are also common in many weedy species. *Vallisneria* may also slow or prevent invasions by introduced plant species, especially in low nutrient sediments, where it has been shown to outcompete *Hydrilla verticillata* (L.f.) Royle in mesocosm trials (Owens et al. 2008; Van et al. 1999). Chadwell et al. (2008) observed reduced *H. verticillata* establishment via fragmentation alone in field trays with *V. americana* but in plots where *H. verticillata* subterranean turions were not removed, *V. americana* did not prevent colonization. The competitive pressure posed by exotic macrophytes such as *H. verticillata* and Eurasian watermilfoil (*Myriophyllum spicatum* L.) has bolstered the need for selective management options of ecologically important species such as *Vallisneria* (Beets et al. 2019; Mudge 2013 Netherland et al. 1997).

Effective and selective control of aquatic invasive plants is commonly achieved using registered herbicides and is dependent on a concentration and exposure time (CET) that is plant and herbicide specific. Concentration and exposure time requirements have been widely studied primarily on *H. verticillata* and *M. spicatum*. The required exposure times are not always feasible in water bodies where increased water exchange occurs. In these systems increasing application rate is not always desired as this can reduce selectivity and increase costs due to required herbicide volumes (Nault et al. 2014; Netherland 2015; Netherland and Getsinger 1995; Netherland et al. 1991, 1997). Netherland (2015) observed equivalent efficacy on monoecious *H. verticillata* between intermittent and continuous exposure to fluridone in mesocosm experiments. These intermittent exposures represent the CET requirements separated by non-treated periods and these separation periods may need to be adjusted based on mode of action (Darnell 2022). The concept of intermittent exposures builds on the practice of injecting fluridone over long periods of time. Results have indicated that pausing application during a dilution event would not negatively impact efficacy and subsequent applications may build on the initial exposure period (Darnell 2022; Netherland 2015).

Vallisneria, presumed to be *V. americana* has shown sensitivity to fluridone at concentrations 1.5 to 20 µg L^{-1} in mesocosms; however, field studies indicate recovery from initial phytotoxicity often occurs and increase in *Vallisneria* frequency can occur (Mudge 2013; Netherland et al. 1997; Smith and Pullman 1997; Valley et al. 2006). Fluridone concentrations below 12 µg L^{-1} with 35 to 60 days of exposure are generally employed to control *H. verticillata* and *M. spicatum* while providing selectivity (Gettys and Leon 2021; Netherland 1995; Netherland et al. 1993). Endothall applications of 0.5 to 3.4 mg L^{-1} with 24 hours to three weeks of exposure have provided control of various *Vallisneria* taxa in mesocosm and field studies (Dugdale et al. 2022; Mudge 2013; Skogerboe and Getsinger 2002). Mudge (2013) reported suppression of suspected *V. americana* with diquat at 100 µg L^{-1} and excellent control with combinations of endothall and flumioxazin at (500 µg L^{-1} + 50 µg L^{-1}). Combinations of endothall and diquat have proven effective on *H. verticillata*, with indication of additive effects when the two herbicides are combined (Chiconela and Haller 2013; Pennington et al. 2001; Skogerboe et al. 2004). *Vallisneria* has shown limited sensitivity to florpyrauxifen-benzyl at 3 to

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48 µg L^{-1} for 6 hours to 15 days, although this may be dependent on plant age and size (Beets et al. 2019; Dodd et al. 2022; Sperry et al. 2021). There is a lack of knowledge concerning efficacy of combinations of florpyauxifen-benzyl and other aquatic registered herbicides.

Previous studies have largely focused on selectivity for what were thought to be one or two native ecotypes of *V. americana*, but there is a lack information concerning differential herbicide sensitivity in *Vallisneria* taxa (Beets et al. 2019; Mudge 2013; Netherland and Glomski 2014; Skogerboe and Getsinger 2002). Additionally, these studies only differentiated northern and southern V*allisneria*, or narrowleaf and broadleaf, assuming these plants to be *V. americana*. This is likely no longer a valid assumption in future studies without confirmation through genetic testing. *Hydrilla verticillata* has historically been identified as a major threat to native *Vallisneria* populations and has a large body of research for comparison of efficacious management options. As such, hydrilla was included for comparison of *Vallisneria* control to a well-researched species. The objective of this research was to evaluate the response of *Vallisneria* taxa to a variety of aquatic herbicide concentration and exposure times used in *H. verticillata* management and determine their utility in management of invasive *Vallisneria* taxa.

Methods

Mesocosm experiments were conducted and repeated under greenhouse conditions in the summer of 2023 (trial 1 in June and trial 2 in July) at North Carolina State University in Raleigh, NC (35.810278, -78.721714) to evaluate the response of *Vallisneria* taxa to common aquatic herbicide treatments. Plant material for the four *Vallisneria* taxa were sourced from the following sites: *V. americana* from the Connecticut River (41.48333, -72.50656), *V. neotropicalis* from Lake Gaston, NC (36.49792, -77.84371), *V. australis* from Lake Mattamuskeet, NC (35.52013, -76.1088), and *V. spiralis* × *V. denseserrulata* from Wheeler Lake, AL (34.62456, -86.98359). Monoecious hydrilla was originally sourced from an impoundment in Granville County, NC (36.136993, -78.794979). Genetic confirmation of *Vallisneria* was performed using ITS sequencing (Les et al. 2008) at Montana State University. Single rosettes of each *Vallisneria* population were planted in 0.52-L pots containing topsoil (Timberline Soil Top Soil, Oldcastle® Lawn & Garden, Inc) with slow-release fertilizer (Osmocote Smart Release 15-9-12®, Scotts) at a rate of 3 g L^{-1} soil and covered with a sand cap to reduce nutrient leaching into the water column. Water used in this study was conditioned tap water (API Tap Water Conditioner®, Mars

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Fishcare North America) with a pH of 7.8 – 8.2. A single sprouted apical stem of hydrilla was planted in a 0.09 L cup with the same amended topsoil to serve as a bioindicator for treatment efficacy. Plants were established for four weeks prior to study initiation in 16-L mesocosms.

Following the establishment period, one pot of each taxon was placed in a 16-L mesocosm containing 12-L of conditioned tap water to minimize cross contamination among taxa. Each mesocosm was considered an experimental unit and was treated with one of eleven treatments containing diquat, endothall, florpyrauxifen-benzyl, flumioxazin, fluridone, or a combination of these herbicides (Table 1). Nontreated controls and a pretreatment biomass harvest control were included in the experiment as references. Following predetermined herbicide exposure periods, plants were placed in new mesocosms containing non-treated water for a 6-week recovery period. Treatments were applied via syringe using herbicide stock solutions. The experiment was set up as a randomized complete block design with four replications. Mesocosms were maintained under 50% shade to reduce light stress, minimize algal growth, and regulate water temperature. Mean water temperature was 26.7° C and pH was 8.1 at time of treatment. Mesocosms were maintained at a consistent water level via addition of conditioned tap water.

Six weeks after treatment (WAT), above and belowground biomass was harvested, placed in a forced-air dryer for 72 hours at 65° C, and weighed. Fluridone treatments were harvested six weeks after the end of their exposure period due to long exposure requirements of fluridone. Likewise, an additional set of nontreated controls was included for each fluridone harvest time. Biomass data were normalized using their respective nontreated controls to calculate percent biomass reduction using the following equation:

$$Biomass\ Reduction\ (\%) = \frac{Mean\ Control\ Biomass - Sample\ Biomass}{Mean\ Control\ Biomass} \times 100 \qquad [1]$$

Biomass reduction was subjected to mixed model analysis of variance (ANOVA) for each herbicide treatment ANOVA with trial and block as random effects taxa as a fixed effect. Where appropriate, means were separated using a Tukey honest significant difference (HSD) in JMP Pro 17 (JMP Pro v.17, SAS Institute).

Results and Discussion

Trial and block factors were not significant when included in separate mixed model ANOVAs for each herbicide, so results between trials were pooled. *Vallisneria americana* was highly sensitive to most herbicide treatments, resulting in increased control compared to the other *Vallisneria* taxa tested. Diquat alone for 12 hours at 370 µg L^{-1} resulted in 84% biomass reduction of *V. americana* 6 WAT (Table 2). Biomass reduction from this treatment was similar in *V. australis* and *V. spiralis* × *V. denseserrulata* (59 and 63% respectively) but was significantly lower in *V. neotropicalis* (11%). Visual injury was observed 1 to 2 WAT in sensitive taxa. Reductions in belowground biomass did not significantly differ between the four tested taxa when exposed to diquat alone despite a wide range of biomass reduction (Table 3).

Endothall alone for 12 hours at 2000 µg L^{-1} resulted in 99% aboveground biomass reduction of *Vallisneria americana* and 97% reduction of *V. spiralis* × *V. denseserrulata* biomass 6 WAT (Table 2). Aboveground biomass reduction was significantly lower for *V. australis* (70%) and *V. neotropicalis* (82%) than the other two taxa. Visual injury was observed in all taxa within 1 WAT. Belowground biomass reduction was significantly lower in *V. australis* (25%) than the other tested taxa (Table 3). One *V. americana* replicate in this treatment produced a winter bud/turion despite a substantial amount of injury to this contact herbicide. Endothall alone (3000 µg L^{-1}) under a 24-hour continuous exposure resulted in 99 to 100% aboveground biomass reduction of *V. americana*, V. *spiralis* × *V. denseserrulata*, and *V. neotropicalis*, but biomass reduction was slightly lower in *V. australis* (94%). A similar trend was observed in belowground biomass but there was no significant difference in the reduction among the tested taxa. When the 24-hour exposure of endothall was split into three 8-hour exposures and a 40-hour rest between applications, there was no significant difference in above or belowground biomass reduction between the *Vallisneria* taxa and biomass reduction was comparable to continuous endothall exposure at the same concentration and exposure time.

Florpyrauxifen-benzyl alone for 72 hours at 30 µg L^{-1} resulted in 20% and 23% aboveground biomass reduction for *V. australis* and V. *spiralis* × *V. denseserrulata,* respectively, while growth was observed in *V. americana* (-44%) and no impact to *V. neotropicalis* was observed 6 WAT (Table 2). This concentration and exposure time resulted in minimal (-11 to 33%) impact to belowground biomass and no significant differences between taxa were observed (Table 3). Similar trends were observed when the 72-hour exposure period was broken up into three 24-hour exposure periods. Aboveground biomass of *V. australis* and *V. spiralis* × *V. denseserrulata* were reduced 20% and 38%, respectively, with growth (-40%) of *V. americana* and no effect on *V. neotropicalis*. Minimal impacts on belowground biomass (-14 to 24%) were observed in the intermittent applications of florpyrauxifen-benzyl. No injury to *Vallisneria* was observed when florpyrauxifen-benzyl was applied alone. The observed growth in aboveground biomass may be a result of hormesis, or the augmented growth due to sublethal auxin-mimic exposure, which has been observed in *Elodea canadensis* (Michx.) and *Egeria densa* (Planch.) when exposed to florpyrauxifen-benzyl (Cedergren et al. 2007; Howell et al. 2022; Mudge et al. 2021).

Fluridone applied at 10 µg L^{-1} with a 45-day exposure reduced aboveground biomass of *Vallisneria americana* by 95%, *V. australis* by 7%, and *V. neotropicalis* 6% (Table 2). Growth and a lack of visual injury was observed in V. *spiralis* × *V. denseserrulata* with this treatment (-48% biomass reduction). This trend was also observed for belowground biomass of all taxa (Table 3). Interestingly, when fluridone was split into three 15-day applications with a 6-day rest period, aboveground biomass reduction on V. *spiralis* × *V. denseserrulata* was 60%, which was similar to the aboveground biomass reduction on *V. americana* and significantly higher than the efficacy on *V. australis*. Intermittent applications of fluridone resulted in a 10% reduction in *V. australis* belowground biomass and 35% for *V. neotropicalis* belowground biomass. Chlorosis, a common symptom of fluridone applications (Netherland and Getsinger 1995; Netherland et al. 1997; Sprecher et al. 1998) was observed in *V. australis* leaves treated with fluridone, however this symptom did not persist, which is reflected in the lack of biomass reduction six weeks after the end of the exposure period.

A 12-hour exposure of endothall (2000 µg L^{-1}) plus diquat (370 µg L^{-1}) reduced *V. americana* aboveground biomass by 98% and V. *spiralis* × *V. denseserrulata* by 100% 6 WAT (Table 4). Aboveground biomass reductions from this herbicide combination were lower in *V. australis* (11%) and *V. neotropicalis* (62%). A similar trend was observed in belowground biomass, with reductions in belowground biomass between 41 and 100% for *V. australis* and *V. americana* (Table 5). The combination of flumioxazin at 300 µg L^{-1} and florpyrauxifen-benzyl at 30 µg L^{-1} for a 48-hour exposure resulted in 97, 98, and 99% aboveground biomass reduction of V. *spiralis* × *V. denseserrulata*, *V. australis*, and *V. americana*, respectively. Efficacy of this combination treatment against aboveground biomass remained high for *V. neotropicalis* (91%).

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There were no significant differences in belowground biomass reduction between taxa when treated with this herbicide combination (79 to 99%). Combinations of 2000 µg L^{-1} endothall and 30 µg L^{-1} florpyrauxifen-benzyl were highly efficacious against all *Vallisneria*, with 100% aboveground biomass reduction of *V. americana* and V. *spiralis* × *V. denseserrulata* and 99.9% in *V. neotropicalis*. Aboveground biomass reduction was significantly lower in *V. australis* (94%), but still considered efficacious. Similar to the previous herbicide combination, there were no differences in belowground biomass reduction across plant species (77 to 100%).

Hydrilla verticillata was included as a bioindicator in each treatment since these treatments were selected from mesocosm trials targeting monoecious and dioecious *H. verticillata* (Beets, unpublished data; Darnell 2021). Rapid symptomology was observed in *H. verticillata* treated with all herbicides except fluridone and the 12-hour exposure of endothall. Six weeks after treatment, *H. verticillata* aboveground biomass was reduced 80 to 100% except for the 12-hour exposure of endothall (Table 6). This treatment only resulted in a 7% reduction in aboveground biomass. While an 83% reduction in belowground biomass was observed, this was likely due to several replicates in this treatment detaching from the sediment and persisting as fragments. Reductions in belowground biomass did not significantly differ between treatments, with several treatments resulting in 100% belowground biomass reduction.

These results are promising for long-term *H. verticillata* control as reductions in belowground biomass can be indicative of low regrowth potential via root crown or subterranean turions (Haller et al. 1976; Nawrocki et al. 2016; Netherland et al. 1997; Steward 1980). The high level of efficacy demonstrated by the intermittent applications of endothall, florpyrauxifen-benzyl, and fluridone also further validates preliminary studies for intermittent applications. Monoecious *H. verticillata* overwinters as subterranean turions, so early season management is often desirable before biomass has peaked and has proven highly efficacious and small plants such as those utilized in this study can be representative of plants at this growth stage in the field (Langeland and Pesacreta 1986; Nawrocki et al. 2016). While not the main objective of the study, we find it important to highlight that hydrilla bioindicator plants were well managed utilizing florpyrauxifen-benzyl treatments with biomass reductions of 96% while native *V. americana* remained relatively unharmed (Table 6). Fluridone applications provided similar selectivity between hydrilla and *V. neotropicalis* (Tables 2 and 3). *Hydrilla verticillata* can outcompete native species such as *V. americana* and *V. neotropicalis*. Therefore, identification of

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selective management options is important, even if they are not effective against the exotic *Vallisneria* taxa (Beets et al. 2019; Chadwell et al. 2008; Haller and Sutton 1975).

There is a current lack of knowledge about management strategies for the newly documented invasive hybrid species *V. spiralis* × *V. denseserrulata*, and the populations of *Vallisneria australis* that have been identified in the United States. The results of this research can provide an initial understanding of potentially efficacious management actions. Additionally, comparisons to historical datasets are complicated and may not be accurate because previous research may not have accurately identified the *Vallisneria* species being studied without genetic confirmation of the tested species (Gorham et al. 2021; Martin and Mort 2023).

Native *Vallisneria* of various previously classified ecotypes has shown sensitivity to endothall at concentrations of 0.5 mg L^{-1} and higher, thus validating the findings of this research (Mudge 2013; Skogerboe and Getsinger 2001, 2002). These previous studies have also indicated recovery from viable root crowns and turions, so treatment timing may play an important role in recovery, and further investigation into direct effects on *Vallisneria* turions is warranted to improve strategies for effective selective management of these native species. Similarly, Turnage and Madsen (2015) observed expansion of *Vallisneria* in Minnesota lakes following diquat applications, and Mudge (2013) observed minimal biomass reduction in *Vallisneria* exposed to diquat.

The observation of minimal impact on native *Vallisneria* with florpyrauxifen-benzyl are corroborated with previous mesocosm and field studies, indicating that florpyrauxifen-benzyl alone is not an effective control method of *Vallisneria* but does provide a selective management option of hydrilla when *Vallisneria* is a non-target species (Beets et al. 2019; Dodd et al. 2022; Sperry et al. 2021). *Vallisneria* sensitivity to fluridone has been variable in previous mesocosm and field studies, which may be partially explained by differentiation between *V. americana* and *V. neotropicalis*, or other *Vallisneria* taxa, as seen in this study (Getsinger et al. 2001, 2002; Nelson et al. 1998; Netherland et al. 1997; Poovey et al. 2004). Several divergences in efficacy compared to previous studies were observed. Dugdale et al. (2012) observed a 90% reduction in *V. australis* treated with diquat, while diquat efficacy was minimal in this study (Tables 2&3). Previous studies have indicated low efficacy of flumioxazin when applied alone and in combination with endothall or diquat (Mudge 2013; Mudge et al. 2010). However, combinations

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with flumioxazin have not been fully investigated at higher doses, especially in combination with florpyrauxifen-benzyl.

With the resurrection of the distinction between *V. americana* and *V. neotropicalis*, future studies must confirm which species are being utilized, and new evaluations are warranted to properly correlate selectivity with the species present. This study presents new evidence that previous differences in efficacy between *V. americana* populations may in fact have been differences in response between these two species, especially when comparing Northern to Southern populations in the United States. While the observed differences in herbicide response are indicative of variation between species, it is possible that the observed differences are limited to the tested populations. Further investigation and replication are needed to see if there is a noticeable within-taxa variation to herbicide response in *Vallisneria*.

Previous field and mesocosm studies have indicated that *Vallisneria australis* is sensitive to, but not completely controlled, by similar concentrations and exposure times that were effective in reducing above and belowground biomass in this study (Clements et al. 2018; Dugdale et al. 2019, 2022). Dugdale et al. (2019) also observed improved endothall efficacy on *Vallisneria australis* in flowing mesocosms compared to quiescent mesocosms. There is a general lack of information concerning the efficacy of other herbicides on *V. australis* to further corroborate our findings. However, the findings of this research with *V. australis*, especially florpyrauxifen-benzyl, flumioxazin, and fluridone are similar to the results observed with other species. Further investigation into the effects of water flow on herbicide efficacy can greatly improve management strategies, especially in lotic systems such as the San Joaquin River Delta in CA where invasions of *Vallisneria australis* have been identified (CDFA 2021).

Female flowers were observed in all *Vallisneria* taxa included in this study. However, no male flowers were observed in the hybrid *V. spiralis* × *V. denseserrulata* or *V. australis*. Male flowers have not been observed in the hybrid *Vallisneria* in other studies, indicating propagation may primarily be occurring via asexual reproduction (Gorham et al. 2021; Wasekura et al. 2016). Considerable genetic plasticity has been observed in *V. australis* and its documented hybridization with native *Vallisneria* indicates these female flowers are not sterile, or male plants are also present in the United States (Martin and Mort 2023). The possibility of hybridization between these invasive and native taxa should be investigated further, and further field monitoring is needed to confirm the lack of male plants in the United States, considering the issues that have arisen with hybridization between native and invasive species such as *M. spicatum* L. × *M. sibiricum* Kom. and *Nymphoides cristata* × *N. aquatica* (J.F.Gmel.) Kuntze (Harms et al. 2021; Parks et al. 2016).

In conclusion, these small-scale mesocosm experiments provide an initial understanding of herbicide response in two new invasive aquatic species, as well as two native species that were previously understood to be the same species. No herbicide treatments tested definitively provided selectivity between invasive and native *Vallisneria* taxa. However, hydrilla was selectively managed while minimal damage to native *Vallisneria* was observed for several well-established treatments, and these results further document the efficacy of intermittent exposures. Future research should scale up the size and study length in experimental design (i.e., larger mesocosms or small field plots and longer than 6-week evaluation periods) to provide a more operational understanding of selective management practices. Given the observed resilience of *V. australis* and the hybrid *V. spiralis* × *V. denseserrulata*, rapid endeavors should be made to improve understanding of efficacious management options as well as phenological and growth patterns to improve timing of management efforts. Turion production was not consistent throughout the trial for statistical analysis and was only observed in *V. americana*, thus future studies are needed to determine what factors lead to turion formation, including temperature, season, and herbicide application.

There are several key concepts future research endeavors should seek to investigate to improve management of a spreading problematic invasive plant. 1) Potential hybridization of non-native *Vallisneria* taxa with native *Vallisneria* taxa, as well as identifying other *Vallisneria* taxa that may be present in the United States, given the genus' prevalence in the aquarium and water garden trade. 2) Due to the similar morphology of plants in this genus, improvements in genetic confirmation of *Vallisneria* populations should be implemented prior to treatment to inform herbicide treatment decisions and improve potential selectivity. 3) Improve the understanding of the ecological and anthropological impacts these non-native *Vallisneria* taxa have on aquatic ecosystems. 4) Identify phenological and growth differences between *Vallisneria* taxa to better inform management actions and the potential spread of these plants. 5) Identify new herbicide use patterns, potential new herbicide chemistries, and use integrated pest management strategies to provide selective management options for *Vallisneria* taxa.

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Competing Interests

The authors declare none.

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Table 1. Herbicides evaluated in a greenhouse trial examining control of four *Vallisneria* taxa and hydrilla in Raleigh, NC in 2023 .

Common Name	Trade Name	Manufacturer
Diquat	Reward®	Syngenta, Greensboro, NC, USA
Endothall dipotassium salt	Aquathol® K	UPL, King of Prussia, PA, USA
Florpyrauxifen-benzyl	Procellacor® SC	SePRO, Carmel, IN, USA
Flumioxazin	Clipper® SC	NuFarm, Alsip, IL, USA
Fluridone	Sonar Genesis®	SePRO, Carmel, IN, USA

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Table 2. Aboveground biomass reduction of four *Vallisneria* taxa 6 weeks after in-water herbicide application (6 weeks after end of exposure period for fluridone).

	Herbicide							
	Diquat	Endothall	Endothall	Endothall[a]	FB[b]	FB[c]	Fluridone	Fluridone[d]
Concentration ($\mu g\ L^{-1}$)	370	2000	3000	3000	30	30	10	10
Exposure time (hours)	12	12	24	24	72	72	45 days	45 days
	----------------% Biomass Reduction[e,f]----------------							
Taxa								
V. americana	84 a	99 a	100 a	99 a	-44 b	-40 b	95 a	64 a
V. australis	59 a	70 bc	94 b	98 a	20 a	20 a	7 b	21 b
V. neotropicalis	11 b	82 c	99 a	100 a	-1 ab	6 ab	6 b	36 ab
V. spiralis × *V. denseserrulata*	63 a	97 ab	100 a	100 a	23 a	38 a	-48 c	60 a

[a]Intermittent exposure (8-hour + 40-hour rest [3x])

[b]Florpyrauxifen-benzyl

[c]Intermittent exposure (24-hour + 6-day rest [3x])

[d]Intermittent exposure (15-day + 6-day rest [3x])

[e]Mean response within a column followed by the same letter do not differ according to Fisher's Protected LSD ($P \leq 0.05$)

[f]Negative values indicate plant growth

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Table 3. Belowground biomass reduction of four *Vallisneria* taxa 6 weeks after in-water herbicide application (6 weeks after end of exposure period for fluridone).

	Herbicide							
	Diquat	Endothall	Endothall	Endothall[a]	FB[b]	FB[c]	Fluridone	Fluridone[d]
Concentration (μg L^{-1})	370	2000	3000	3000	30	30	10	10
Exposure time (hours)	12	12	24	24[b]	72	72	45 days	45 days
	--% Biomass Reduction[e,f]--							
Taxa								
V. americana	64 a	91 a	95 a	96 a	24 a	17 a	83 a	76 a
V. australis	-1 a	25 b	86 a	95 a	-11 a	-14 a	3 b	10 c
V. neotropicalis	6 a	86 a	99 a	99 a	-16 a	-1 a	15 b	35 bc
V. spiralis × *V. denseserrulata*	43 a	72 a	91 a	91 a	33 a	24 a	-74 c	41 b

[a] Intermittent exposure (8-hour + 40-hour rest [3x])

[b]Florpyrauxifen-benzyl

[c]Intermittent exposure (24-hour + 6-day rest [3x])

[d]Intermittent exposure (15-day + 6-day rest [3x])

[e]Mean response within a column followed by the same letter do not differ according to Fisher's Protected LSD ($P \leq 0.05$)

[f]Negative values indicate plant growth

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Table 4. Aboveground biomass reduction of four *Vallisneria* taxa 6 weeks after in-water herbicide applications. Herbicides were applied simultaneously at indicated rates.

	Herbicide		
	Endothall + Diquat	Flumioxazin + FB[a]	Endothall + FB
Concentration ($\mu g\ L^{-1}$)	2000 + 370	300 + 30	2000 + 30
Exposure time (hours)	12	48	48
	---------------------------% Biomass Reduction[b]-----------------------		
Taxa			
V. americana	98 a	99 a	100 a
V. australis	11 b	98 a	94 b
V. neotropicalis	62 b	91 b	99 a
V. spiralis × *V. denseserrulata*	100 a	97 a	100 a

[a] Florpyrauxifen-benzyl

[b]Mean response within a column followed by the same letter do not differ according to Fisher's Protected LSD ($P \leq 0.05$)

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Table 5. Belowground biomass reduction of four *Vallisneria* taxa 6 weeks after in-water herbicide applications. Herbicides were applied simultaneously at indicated rates.

	Herbicide		
	Endothall + Diquat	Flumioxazin + FB[a]	Endothall + FB
Concentration (µg L^{-1})	2000 + 370	300 + 30	2000 + 30
Exposure time (hours)	12	48	48
	---------------------------% Biomass Reduction[c]-----------------------		
Taxa			
V. americana	100 a	99 a	100 a
V. australis	41 c	95 a	83 a
V. neotropicalis	58 bc	80 a	77 a
V. spiralis × *V. denseserrulata*	85 ab	79 a	90 a

[a] Florpyrauxifen-benzyl

[b]Mean response within a column followed by the same letter do not differ according to Fisher's Protected LSD ($P \leq 0.05$)

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Table 6. Biomass reduction of hydrilla 6 weeks after in-water herbicide application (6 weeks after end of exposure period for fluridone).

Herbicide	Rate	Exposure Time	Aboveground	Belowground
	$\mu g\ L^{-1}$		-----------------% Biomass Reduction[a]---------------	
Diquat	370	12 hours	96 a	100 a
Endothall	2000	12 hours	7 b	83 a
Endothall	3000	24 hours	91 a	86 a
Endothall	3000	24 hours	99 a	100 a
Florpyrauxifen-benzyl	30	72 hours	96 a	100 a
Florpyrauxifen-benzyl	30	72 hours	97 a	100 a
Fluridone	10	45 days	80 a	75 a
Fluridone	10	45 days	99 a	100 a
Endothall + Diquat	2000 + 370	48 hours	98 a	100 a
Flumioxazin + Florpyrauxifen-benzyl	300 + 30	48 hours	99 a	100 a
Endothall + Florpyrauxifen-benzyl	3000 + 30	48 hours	100 a	100 a

[a]Mean response within a column followed by the same letter do not differ according to Fisher's Protected LSD ($P \leq 0.05$)

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