Evaluations of Systemic Aquatic Herbicides for Controlling Two Populations of Submersed Flowering Rush

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1 Introduction

Background

Flowering rush (*Butomus umbellatus* L.), a monocot introduced from Eurasia, has become invasive in the northern US. It grows as an emergent plant along shorelines and as a submersed plant in deeper water of lakes and rivers (>3 m). Flowering rush can form monospecific stands, interfering with intended water uses and crowding out native plants. The reproductive biology of flowering rush is quite complex and varies among populations (Kliber and Eckert 2005). The triploid or sterile phenotype spreads clonally by rhizomes and lateral rhizome buds. The diploid or fertile phenotype spreads through seed dispersal and reproductive structures (bulbils) that form on the root and inflorescence and break off to generate new plants. Once established, however, both phenotypes of flowering rush are difficult to control.

Aquatic herbicides may be an effective option to control flowering rush, yet systematic research of herbicide efficacy is lacking. Because both emergent and submersed forms create nuisance conditions, both morphologies require independent control strategies. Investigators are evaluating the use of herbicides on the emergent form of the plant (Rice et al. 2009), and on the submersed form following dewatering (T. Woolf, Idaho State Department of Agriculture, pers. comm.). Another strategy would involve aqueous application of herbicides to plant populations in the submersed stages of growth. Success or failure of a submersed herbicide treatment depends upon the aqueous herbicide concentration that comes in contact with the target plant concomitant with the length of time the target plant is exposed to the dissipating herbicide concentration. Understanding this concentration/exposure time (CET) relationship is critical in achieving desirable control of nuisance submersed plants (Getsinger and Netherland 1997).

Since submersed flowering rush grows in flowing waterways, such as the Detroit Lakes, MN, and Lake Pend Oreille, ID, management of this plant with herbicides has been inconsistent and unpredictable. Herbicide applications in these systems are typically subject to more extreme environmental variables than applications made to lakes with limited water flow. Most notably, run of the river reservoirs have variable water exchange patterns that will impact aqueous distribution of herbicides resulting in reduced chemical exposure times against target plants, and likely reduced efficacy.

Potential herbicide exposure time of Detroit Lakes was investigated by collecting chemical residues from operational treatments conducted in 2010 (Skogerboe 2010). Treatment areas were 0.40 ha (1 acre) with target concentrations of 3 mg active ingredient (ai) L^{-1} (ppm) of endothall (dipotassium salt) [7-oxabicyclo (2.2.1) heptane-2,3-dicarboxylic acid]. Results of residue analyses indicated that herbicide exposure times were short in all plots, with residue half-lives ranging from 4 to 78 h and initial concentrations 20 to 40% of target concentrations. Based on this field information, a series of small-scale evaluations was undertaken. The first set of experiments focused on using different concentrations of contact herbicides under relatively short aqueous exposure time scenarios for controlling the submersed growth stage of flowering rush (Poovey et al. 2011). The second set of experiments was undertaken based on results from the contact herbicide experiments with a focus on using operational concentrations of systemic herbicides, and combinations of systemic herbicides with contact herbicides, for controlling submersed flowering rush.

Systemic herbicides can be classed by their mode-of-action; they include synthetic auxins and specific plant enzyme inhibitors. Some systemic herbicides may be broad spectrum in activity, and can be used to control many submersed plant species; other systemic herbicides are highly specific, and are used to selectively control certain aquatic plants. Systemic products differ from contact ones in that they translocate throughout the plant, and under ideal conditions, can provide complete control of the target weed. Enhanced efficacy is achieved when herbicides are applied to young, actively growing plants rather than mature, slowly growing plants. Exposure time depends on herbicide mode-of-action. For example, exposure times for the synthetic auxin herbicides range from 1 to 4 days, while herbicides that target a specific plant enzyme (eg. acetolactate synthase herbicides) may require 45 to 100 days for plant uptake, translocation, and eventual necrosis. To maintain adequate herbicide concentrations for many weeks, these herbicides are applied to large coves, the entire littoral zone, or the whole lake. Repeat or booster applications also may be necessary to maintain concentrations. When CET requirements are met, systemic herbicides typically provide up to one to two years of control, including the year of treatment. In addition to weed control, aquatic plant management with systemic herbicides seeks to minimize damage to native vegetation through the choice of herbicide, use rate, and timing of application (Poovey and Getsinger 2005).

Synthetic auxin herbicides are chemicals that act similarly to the plant hormone indole-3-acetic acid (IAA). Uptake of these herbicides leads to uncontrolled cell division and growth, which results in

vascular tissue destruction. Once they are absorbed into plant tissues, stem epinasty and browning occurs 1 to 2 days after application. After 7 to 14 days, shoots start to decay and plant death occurs in another 14 days. Triclopyr (3,5,6-trichloro-2-pyridinyloxyactetic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) are synthetic auxin herbicides used for aquatic weed management. The maximum label rate for submersed applications of triclopyr is 2.5 mg acid equivalent (ae) L^{-1} and 4.0 mg ae L^{-1} for 2,4-D. In addition, there are two formulations of 2,4-D available for aquatic weed control: the amine formulation, which is a liquid, and the low-volatile butoxyethyl ester formulation, which is a granular. Triclopyr and 2,4-D are routinely used for management of Eurasian watermilfoil (*Myriophyllum spicatum* L.) in the Midwest and Pacific Northwest. Exposure times for both products require 1 to 3 days for effective control of susceptible submersed species (Green and Westerdahl 1990, Netherland and Getsinger 1992), and regrowth can occur in 4 to 8 weeks following initial application. Broad-leaf plants (dicots) are more susceptible to synthetic auxins than narrow-leaf plants (monocots), such as grasses (ie. flowering rush); however, herbicide activity on monocot aquatic plants has been reported (Belgers et al. 2007).

Other systemic herbicides interrupt biosynthetic pathways by blocking the production of specific plant enzymes. Two examples are herbicides with modes-of-actions that inhibit production of phytoene desaturase (PDS) and acetolactate synthase (ALS). PDS is a necessary enzyme in carotene biosynthesis and carotene pigments are essential for plants to photosynthesize (Bartels and Watson 1978). ALS is a necessary enzyme in the biosynthesis of branched-chain amino acids (isoleucine, valine, and leucine), which are protein building blocks and integral to plant growth (Tranel and Wright 2002).
In these experiments, we used one PDS-inhibitor and two ALS-inhibitors:

- PDS-fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone)
- ALS-imazamox (2-[4,5-dihydro-4 methyl-4-(1-methylethyl)-5oxo-1*H*-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid)
- ALS-bispyribac-sodium (sodium 2,6-bis[(4,6-dimethoxy-2-pyrimidinyl)oxy]benzoate).

Both PDS and ALS chemistries require long exposure times to be efficacious on susceptible plants. Fluridone is effective with exposures of 45 to 90 days (Netherland and Getsinger 1993, 1995), while imazamox and bispyribac are effective with exposure times ranging from 30 to 100 days. Growth cessation of susceptible plants is immediate; however, existing biomass can remain healthy and functional for extended periods of time. If the treatment is effective, target plant regrowth usually does

not occur for more than 12 months. Fluridone is a broad-spectrum herbicide, but can be used to selectively control target weeds with low application rates (Netherland et al. 1997, Getsinger et al. 2002). Conversely, imazamox and bispyribac are highly specific for certain emergent and submersed aquatic macrophyte species (Getsinger et al. 1994, Chiconela et al. 2004, Koschnick et al. 2007, Glomski and Netherland 2008).

Objectives

The overall objective was to evaluate systemic aquatic herbicides for the control of submersed growth form of flowering rush from two populations, Minnesota and Idaho. A series of small-scale evaluations were undertaken as an initial effort to determine herbicide efficacy against flowering rush. The first set of experiments focused on using different concentrations of contact herbicides under relatively short aqueous exposure time scenarios for controlling the submersed growth stage of flowering rush from Minnesota and Idaho (Poovey et al. 2011). A second set of experiments was undertaken with a focus on using systemic herbicides for controlling submersed flowering rush. These experiments also used flowering rush from Minnesota and Idaho. Results of these evaluations will be used to provide guidance for management of submersed flowering rush in the Detroit Lakes, Pelican River Watershed, Minnesota and for diploid flowering rush in Lake Pend Oreille, Idaho.

Specific objectives were:

1) Determine ploidy and identify genetic differences between Minnesota and Idaho flowering rush;
2) Evaluate synthetic auxin herbicides, 2,4-D amine, 2,4-D ester, triclopyr, and combinations of these herbicides with contact herbicides endothall and flumioxazin for control of both Minnesota and Idaho flowering rush;
3) Determine susceptibility of Minnesota and Idaho flowering rush to systemic herbicides with enzyme-specific modes of action, fluridone, bispyribac, and imazamox, in a static exposure time.

2 Ploidy and Genetic Analyses of Minnesota and Idaho Flowering Rush

For ploidy and genetic analyses, flowering rush rhizomes were field-collected from Detroit Lakes, Pelican River Watershed, MN, and Lake Pend Oreille, ID and shipped overnight to ERDC. Rhizomes were surrounded by sediment and subjected to a cold (4°C) dark treatment for at least 3 wk before sprouting. Rhizomes (4 to 5 cm in length) were washed, placed in culture solution (Smart and Barko 1985) that was aerated, and allowed to sprout in an environmental growth chamber for 5 wk.

Sprouted rhizomes were propagated in potting soil that was fertilized with 150 mg L^{-1} ammonium chloride. Planted beakers were placed in aquaria (volume=48 L) filled with culture solution (Smart and Barko 1985) amended with chelated iron (0.1 mg L^{-1}). Plants grew for 5 wk prior to harvesting the shoots and roots. Shoot and roots were harvested, washed, and shipped overnight to Grand Valley State University, Muskegon, MI.

Amplified Fragment Length Polymorphism (AFLP) was the genetic technique employed to detect potential genetic differences between plant populations. According to Vos et al. (1995):

> The AFLP technique is based on the selective Polymerase Chain Reaction (PCR) amplification of restriction fragments from a total digest of genomic DNA. The technique involves three steps: (i) restriction of the DNA and ligation of oligonucleotide adapters, (ii) selective amplification of sets of restriction fragments, and (iii) gel analysis of the amplified fragments. PCR amplification of restriction fragments is achieved by using the adapter and restriction site sequence as target sites for primer annealing. The selective amplification is achieved by the use of primers that extend into the restriction fragments, amplifying only those fragments in which the primer extensions match the nucleotides flanking the restriction sites. Using this method, sets of restriction fragments may be visualized by PCR without knowledge of nucleotide sequence. The method allows the specific co-amplification of high numbers of restriction fragments. The number of fragments that can be analyzed simultaneously, however, is dependent on the resolution of the detection system. Typically 50-100 restriction fragments are amplified and detected on denaturing polyacrylamide gels. The AFLP technique

provides a novel and very powerful DNA fingerprinting technique for DNAs of any origin or complexity.

There were 136 AFLP bands analyzed, including:

- Eco-AGG, in which there were 61 alleles shared by all individuals, with no polymorphic alleles.
- Eco-ACA, in which there were 75 alleles shared by all individuals, with no polymorphic alleles.

Other markers included:

- Chloroplast DNA
    - trnL-trnF sequences, with no differences between plant populations.
    - trnK (matK) sequences, with no differences between plant populations.
- Nuclear DNA
    - ITS sequences, with no differences between plant populations.

There were no genetic differences between the Minnesota and Idaho plant populations.

Ploidy was determined cytologically using guard cell length following the methods of Kliber and Eckert (2005). They found that plant populations with unknown ploidy could be classified because triploid populations have had significantly larger mean (±1 SE) guard cell length than diploids; 47.20 ±0.64 µm for triploids versus 40.39 ±0.93 µm for diploids. Since the highest mean guard cell size for diploid populations (42.63 µm) was below the lowest triploid population (42.65 µm), plant populations could be classified as triploid if mean guard cell length >42.63 µm. Using this criterion, both Minnesota and Idaho flowering rush populations were determined to be triploid.

3 Synthetic Auxin Herbicides Combined with Contact Herbicides for Controlling Submersed Flowering Rush

Materials and Methods

This experiment was conducted in a walk-in controlled environment growth chamber (48 m^2) at the US Army Engineer Research and Development Center (ERDC) in Vicksburg, MS. Ambient conditions were set to provide optimum conditions for submersed plant growth: air temperature of 21 ±2°C, light intensity of 700 µmol m^{-2} sec^{-1}, and photoperiod of 14 h:10 h light:dark cycle.

Flowering rush rhizomes were field-collected from Detroit Lakes, Pelican River Watershed, MN, and Lake Pend Oreille, ID and shipped overnight to ERDC. Rhizomes were surrounded by sediment and subjected to a cold (4°C) dark treatment for at least 3 wk before sprouting. Rhizomes (4 to 5 cm in length, 4 to 5 g FW) were then washed to remove sediment, placed in culture solution (Smart and Barko 1985) that was aerated, and allowed to sprout in the environmental growth chamber for 3 wk.

Two different sediments were used for propagating sprouted rhizomes from each plant population based on observations from the field. Minnesota plants were planted in silty topsoil (Black Kow® Topsoil, Black Gold Compost Co., Oxford, FL). The pH of the potting soil (5.3) to was raised to 6.8 by adding 150 mg L^{-1} of calcium carbonate and 1.5 g L^{-1} of sodium bicarbonate to make it more alkaline. The Idaho plants were planted in topsoil that had remnants of bark, which was more acidic (pH=5.2; Scotts® Premium Topsoil, the Scotts Company, Marysville, OH). Both sediments were fertilized with 150 mg L^{-1} ammonium chloride.

One sprouted Minnesota rhizome containing one shoot (7.7 ±1.0 g FW, shoot length=33 ±2.4 cm, root length=20 ±1.3 cm; n=20) was planted to a depth of 4 cm in 1 L high-density polyethylene (HDPE) beakers filled with Black Kow Topsoil. One sprouted Idaho rhizome containing one shoot (11 ±1.0 g FW, shoot length=34 ±2.9 cm, root length=11 ±0.9 cm; n=20) was planted to a depth of 4 cm in 1 L HDPE beakers filled with Scotts Premium Topsoil. A 2-cm layer of masonry sand was added to the sediment surface in each beaker to prevent dispersion of nutrients and sediment into the water column. Two beakers of each plant population were placed in each aquarium (volume=48 L) filled with culture

solution (Smart and Barko 1985) amended with chelated iron (0.1 mg L^{-1}). Plants grew for three weeks prior to herbicide application.

Herbicide concentrations evaluated were selected based on several factors. Since aqueous exposure times in the Detroit Lakes and Lake Pend Oreille can be short (<24 hr), high concentrations of each product, except endothall, were used. A low rate of endothall was chosen based on a previous experiment in which 1.5 mg ai L^{-1} for a 24 hr exposure provided >75% control (Poovey et al. 2011). An exposure period of 24 hr was used for all treatments. In addition, an exposure period of 48 hr was used for triclopyr (Table 1). Using high concentrations with these exposure periods provide a strong indication of efficacy; if a product is not efficacious using this CET, then it will probably not be effective in a flowing system.

For herbicide application, stock solutions of endothall (Aquathol® K, United Phosphorus Inc), flumioxazin (Clipper®, Valent USA Corp.), 2,4-D amine (DMA 4 IVM, Dow AgroSciences LLC), and triclopyr (Renovate®, SePRO Corp.) were prepared by diluting formulation concentrates in distilled water. A special liquid 2,4-D ester formulation of Navigate® was provided by NuFarm Americas Inc. for this experiment; it also was diluted in distilled water for stock preparation. From the stock, each herbicide was applied subsurface using a pipette to provide nominal concentrations in the treatment aquaria for the appropriate exposure time (Table 1). Untreated reference aquaria were included to assess plant growth in the absence of herbicide exposure. Immediately, following herbicide exposure times, all aquaria, including references, were drained and filled with fresh culture solution two times to remove all aqueous herbicide residues. The experiment was concluded 6 wk after treatment (WAT) to allow enough time for plants to potentially recover.

Water samples were collected 25 cm below the water surface from all treatments after herbicide exposure (24 or 48 hr) to ensure nominal herbicide concentrations were achieved. In addition, water samples of flumioxazin were taken 1 hr after application because of its potential to rapidly hydrolyze at high pH levels (Katagi 2003). Samples were stored at 4°C until shipped for analysis. Endothall, 2,4-D, and triclopyr residues were analyzed in house at ERDC using the enzyme-linked immunosorbent assay (ELISA) technique. Flumioxazin residues were analyzed by Valent USA Corporation using high-performance liquid chromatography (HPLC).

Table 1. Exposure times and concentrations of contact and systemic herbicides evaluated against field-collected Minnesota and Idaho flowering rush.

Herbicide Treatment	Mode-of-Action	Concentration	Exposure Time (h)
Endothall	Contact	1.5 mg ai L^{-1}	24
Flumioxazin	Contact	0.4 mg ai L^{-1}	24
2,4-D amine	Systemic	4.0 mg ae L^{-1}	24
2,4-D ester	Systemic	4.0 mg ae L^{-1}	24
Triclopyr	Systemic	1.25, 2.5 mg ae L^{-1}	24, 48
Triclopyr + Endothall	Systemic + Contact	1.25 mg ae L^{-1} Triclopyr 1.5 mg ai L^{-1} Endothall	24
Triclopyr + Flumioxazin	Systemic + Contact	1.25 mg ae L^{-1} Triclopyr 0.4 mg ai L^{-1} Flumioxazin	24
Triclopyr + 2,4-D amine	Systemic + Systemic	1.25 mg ae L^{-1} Triclopy 4.0 mg ae L^{-1} 2,4-D amine	24
Reference		0	24

Water temperature and pH were measured with a handheld multi-parameter probe (model 556, YSI, Yellow Springs, OH) before herbicide application. Water temperatures in aquaria were 21 ±0.02 °C during the experiment, while pH was 8.6 at time of treatment.

Herbicide efficacy was assessed by measuring shoot, root, and rhizome biomass. At 6 WAT, biomass from one beaker in each aquarium was harvested, dried, and weighed for a dry weight (DW) measurement (g DW). A growth recovery assessment was also included where shoots were clipped at the sediment surface from one beaker, and it was placed back in the aquarium for an additional 2 wk to monitor re-growth. Afterwards, biomass was harvested, dried, and weighed.

Treatments were randomly assigned to individual aquaria and replicated four times, including the reference (n=4). All shoot, root, and rhizome data were analyzed using one-way analysis of variance (ANOVA) to determine herbicide effects. If effects were significant ($p \leq 0.05$), means were compared using Fisher's Least Significant Difference test (LSD).

Results and Discussion

Aqueous herbicide residues (mean ±1 SE) after the 24 hr exposure time are shown in Table 2. Residues for both 2,4-D formulations and triclopyr were within 3% of nominal concentrations. Endothall residues were 1.8 ±0.06 mg ai L^{-1}, 21% higher than the nominal concentration of 1.5 mg ai L^{-1}. Flumioxazin residues were 0.2 ±0.01 mg ai L^{-1} at 1 hr after treatment. Given that sample recovery was 85% for fortified samples, these flumioxazin concentrations are within range of concentrations and half-lives reported by Mudge et al. (2010) in a greenhouse experiment. In mesocosms containing hydrilla (*Hydrilla verticillata* (Lf.) Royle) with a water column pH >8.5, the half-life of 0.4 mg ai L^{-1} flumioxazin was 1.7 h. Flumioxazin is rapidly degraded by hydrolysis with an average half-life of 4.1 d, 16.1 h, and 17.5 min at pH 5.0, 7.0, and 9.0, respectively (Katagi 2003). Surprisingly, flumioxazin was still detected 24 hr after treatment with residues of 0.06 mg ai L^{-1}. Hydrolysis of flumioxazin may have been slowed by diurnal temperature fluctuations in the aquaria since water temperatures slightly decrease during the dark cycle, after the growth chamber lights turn off.

Table 2. Herbicide residues of contact and systemic herbicides in aquaria 24 hours after treatment.

Herbicide Treatment	Nominal Concentration	Actual Concentration
Endothall n=8	1.5 mg ai L^{-1}	1.8 ± 0.06 mg ai L^{-1}
Flumioxazin[1] n=4	0.4 mg ai L^{-1}	[1]0.2 ± 0.01 mg ai L^{-1} [2]0.06 ± 0.07 mg ai L^{-1}
2,4-D amine n=8	4.0 mg ae L^{-1}	3.9 ± 0.03 mg ae L^{-1}
2,4-D ester n=4	4.0 mg ae L^{-1}	3.9 ± 0.27 mg ae L^{-1}
Triclopyr n=20	1.25 mg ae L^{-1}	1.28 ± 0.03 mg ae L^{-1}
Triclopyr n=8	2.5 mg ae L^{-1}	2.5 ± 0.11 mg ae L^{-1}

[1]Herbicide residues collected 1 hour after treatment

[2]Herbicide residues collected 24 hours after treatment

Reduction of Minnesota shoot biomass occurred with endothall (76%) and endothall combined with triclopyr (85%), flumioxazin (63%) and flumioxazin combined with triclopyr (82%). These treatments were statistically similar indicating that the addition of triclopyr did not substantially improve control. Plants exposed to these herbicides showed symptoms of herbicide injury by 1 WAT. Plants in endothall treatments had brown stems while plant stems in flumioxazin treatments were purple. By 4 WAT, plants in all endothall and flumioxazin treatments were necrotic. There was little shoot recovery from endothall treatments compared to the reference (Figure 2A) due to significant herbicide effects on root biomass (Figure 1B).

Compared to the reference, Minnesota root biomass reduction occurred in all plants (Figure 1B); however, only the endothall treatment reduced root biomass below pretreatment levels, signifying that root biomass continued to accumulate despite herbicide application. Although triclopyr reduced root biomass compared to the untreated reference, it was not effective in reducing shoot biomass (Figure 1A) or eliminating shoot re-growth (Figure 2A) compared to the reference with the CETs evaluated in this experiment. Similarly, both 2,4-D amine and ester were effective in reducing root biomass (Figure 1B), but did not reduce shoot biomass compared to the reference; however, shoot biomass for these treatments were reduced below pretreatment levels (Figure 1A). Flowering rush treated with triclopyr and 2,4-D exhibited initial herbicide symptoms of chlorosis and epinasty along the stems rather than the apices (Figure 3). By 3 WAT, plants were growing vigorously with mostly green healthy shoots, although some browning was evident on a few decayed stems.

Reduction of Idaho shoot biomass ranged from 73 to 82% with endothall and endothall combined with triclopyr, respectively (Figure 4A). Likewise, flumioxazin (49%) and flumioxazin combined with triclopyr (65%) reduced Idaho shoot biomass compare to the reference. Two other treatments that reduced shoot biomass >50% included 2,4-D combined with triclopyr and 2.5 mg ae L^{-1} triclopyr for the 48 hr exposure time. Treatments that reduced biomass by 50% or more (or below pretreatment levels) were statistically similar (LSD=0.488), including the flumioxazin treatment.

Herbicide effects on root biomass followed the same general trend as shoot biomass (Figure 4B). Treatments that significantly reduced root biomass compared to the reference were endothall combined with triclopyr, 2.5 mg ae L^{-1} triclopyr for 48 hr exposure period, endothall, and 2,4-D amine combined with triclopyr. Like the Minnesota plants, no treatments reduced root biomass below

pretreatment levels. Shoot recovery occurred in all Idaho plants (Figure 2B) as substantial root and rhizome biomass remained beneath the sediment to sustain plant recovery. Most shoots sprouted within 1 wk after shoot removal.

Rhizome biomass was not impacted by herbicide treatments in either the Minnesota or Idaho populations. Nonetheless, rhizome biomass was below pretreatment levels in plants exposed to endothall, including combinations with triclopyr, for both plant populations (Figures 1C and 4C). In addition, rhizome biomass was below pretreatment levels for all combinations of triclopyr with flumioxazin and 2,4-D as well as the maximum label rate of triclopyr for 48 hr exposure time (Figure 4C).

Results from this experiment compare favorably to the results of the small-scale contact herbicide experiment, where 1.5 mg ai L^{-1} endothall for a 24 hr exposure time provided good control of shoot biomass (>80% at 4 WAT), and a treatment of 3 mg ai L^{-1} significantly reduced both shoot and root biomass in the Idaho population (Poovey et al. 2011). These data further confirm endothall efficacy where root biomass was reduced in both the Minnesota and Idaho plant populations in this experiment. Emergent and floating-leaf plants that may be growing in mixed communities with flowering rush would probably not be negatively affected by the endothall CET used in this small-scale experiment. In an outdoor mesocosm study, spatterdock (*Nuphar lutea* spp. *advena*), pickerelweed (*Pontederia cordata*), or cattail (*Typha latifolia* L.) were not injured by endothall concentrations of 1.5 mg ai L^{-1} with a 24 hr exposure time (Skogerboe and Getsinger 2002). In another study, water lily (*Nymphaea odorata* Aiton.) and arrowhead (*Sagittaria latifoliaI* Willd.) were significantly impacted by 2.0 mg ai L^{-1} endothall in a static exposure of 120 h; however, these species are not likely to be impacted at lower concentrations and exposure times (Skogerboe and Getsinger 2001). It is unknown how the addition of triclopyr will impact selectivity of endothall when used in combination, and this combined treatment needs further investigation.

Flumioxazin has the potential to control submersed flowering rush at the maximum label rate (0.4 mg ai L^{-1}) even when the water column pH≥9. In this experiment, flumioxazin was effective against Minnesota and Idaho flowering rush, verifying its efficacy against Idaho flowering rush in the previous experiment (Poovey et al. 2011). Preliminary experimental data indicate that 0.4 mg ai L^{-1} may negatively impact water lily and spatterdock; however, re-growth may occur within the growing season of herbicide application (authors' unpublished data). In a greenhouse study by Mudge (2007), subsurface treatments

of 0.4 mg ai L^{-1} flumioxazin injured sagittaria (*Sagittaria lancifolia* L.) and maidencane (*Panicum hemitomonI* Schult.); however, these plants were tolerant of foliar flumioxazin applications using the maximum label rate (841 g ha^{-1} or 12 oz ac^{-1}). Selectivity of flumioxazin and flumioxazin combinations against floating-leaf and emergent native plants requires further investigation.

Use of synthetic auxin herbicides for control of triploid flowering rush had mixed results, with reduction of shoot biomass ranging from 27 to 48% for triclopyr (all CETs) and ~50% for 2,4-D (both formulations) in Minnesota plants. Root biomass reduction occurred in all but the 1.25 mg ae L^{-1} triclopyr treatments. In Idaho plants, there was little shoot or root reduction compared to the references. The combination of 2,4-D and triclopyr did not substantially enhance efficacy over each product alone. Selectivity of synthetic auxins differs between 2,4-D and triclopyr, and may differ between 2,4-D formulations (authors' personal observations). American bulrush (*Schoenoplectus americanus* Pers.) has been reported as tolerant to high concentrations of triclopyr (2 mg ae L^{-1}) and moderate concentrations of 2,4-D ester (2.5 mg ae L^{-1}) using a 24 hr exposure time; however, soft-stem bulrush (*S. taberbaemontanil* (C.C. Gmel.) Palla) biomass was significantly reduced with these CETs in the same experiment (Glomski et al. 2009). In another experiment, (Glomski and Nelson 2008) found that water lily was initially injured by triclopyr (2 mg ae L^{-1}) and 2,4-D ester (2.5 mg ae L^{-1}) using a 24 hr exposure time. Plants recovered from the triclopyr treatment, but not the 2,4-D ester treatment. These same herbicide treatments were used against spatterdock, where shoot biomass was reduced by 48% following herbicide application; however, root biomass was not affected by herbicide treatment, and re-growth from roots and rhizomes was observed. Since submersed applications of the synthetic auxins provided only fair control (50%) of submersed flowering rush in this experiment at CETs that compromise selectivity, additional research with these products should focus on foliar applications on the emergent form of flowering rush.

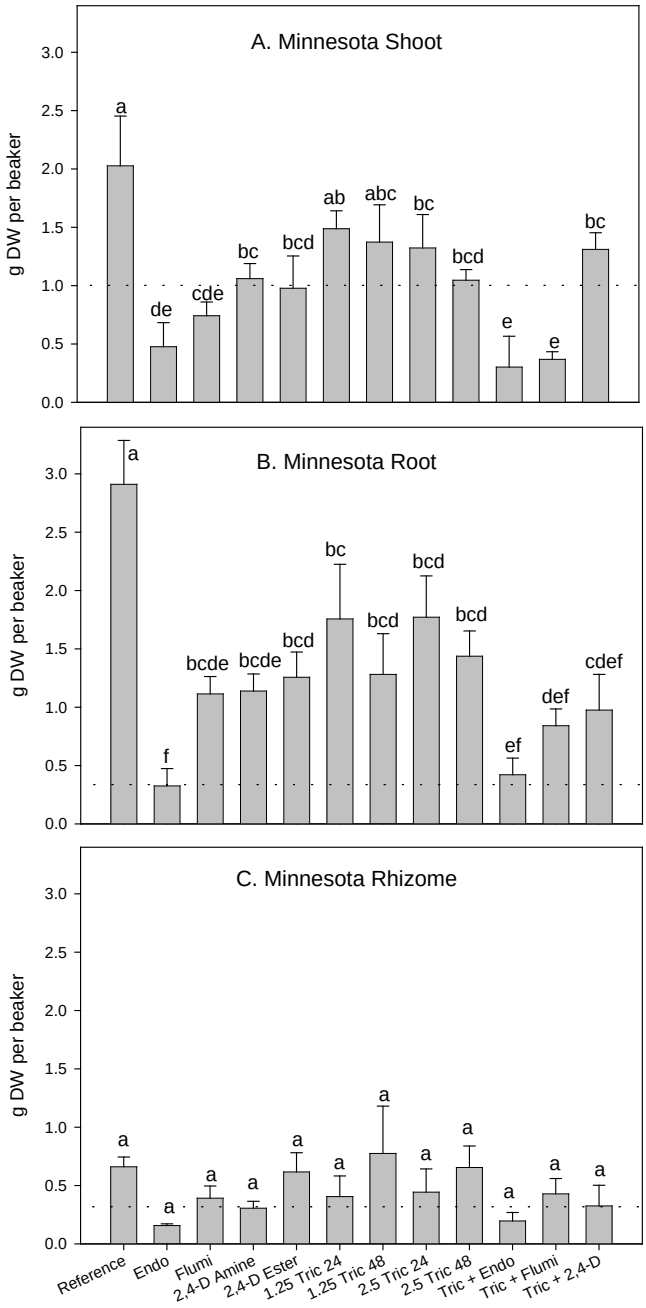

Figure 1. Minnesota flowering rush (mean ±1 SE, n=4) A) shoot, B) root, and C) rhizome biomass (g DW) 6 weeks after treatment with aquatic herbicides 1.5 mg ai L^{-1} endothall (Endo), 0.4 mg ai L^{-1} flumioxazin (Flumi), 4.0 mg ae L^{-1} 2,4-D amine, 4.0 mg ae L^{-1} 2,4-D ester, 1.25 or 2.5 mg ae L^{-1} triclopyr (Tric) and combinations of triclopyr (1.25 mg ae L^{-1}) with endothall(1.5 mg ai L^{-1}), flumioxazin (0.4 mg ai L^{-1}), and 2,4-D amine (4.0 mg ae L^{-1}). Numbers in front of herbicide abbreviations represent herbicide concentrations (mg ae L^{-1}) followed by exposure time (h). Treatments with the same letter are not significantly different (ANOVA $p \leq 0.05$ shoot LSD=0.663; root ANOVA $p \leq 0.05$, LSD=0.786; rhizome ANOVA p=0.32).

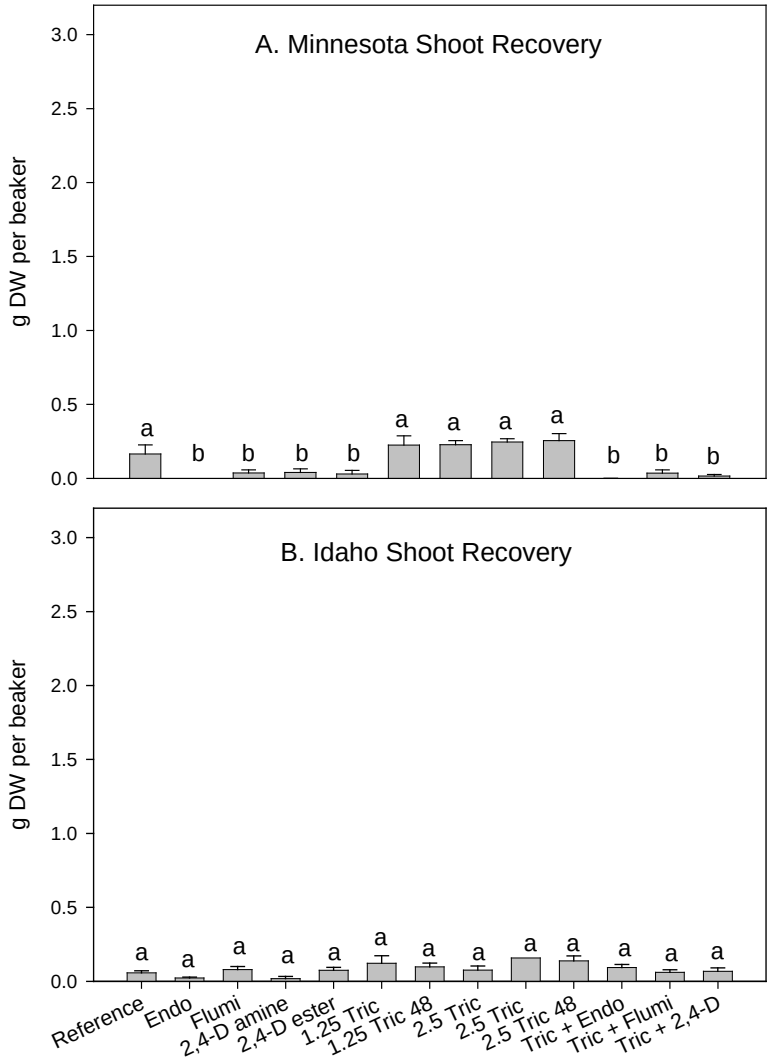

Figure 2. A) Minnesota and B) Idaho flowering rush shoot biomass (g DW, mean ±1 SE, n=4) collected as an assessment of recovery 8 weeks after treatment with aquatic herbicides 1.5 mg ai L^{-1} endothall (Endo), 0.4 mg ai L^{-1} flumioxazin (Flumi), 4.0 mg ae L^{-1} 2,4-D amine, 4.0 mg ae L^{-1} 2,4-D ester, 1.25 or 2.5 mg ae L^{-1} triclopyr (Tric) and combinations of triclopyr (1.25 mg ae L^{-1}) with endothall(1.5 mg ai L^{-1}), flumioxazin (0.4 mg ai L^{-1}), and 2,4-D amine (4.0 mg ae L^{-1}). Numbers in front of herbicide abbreviations represent herbicide concentrations (mg ae L^{-1}) followed by exposure time (h). Treatments with the same letter are not significantly different (Minnesota ANOVA $p \leq 0.05$, LSD=0.095; Idaho ANOVA $p=0.058$).

Figure 3. Chlorosis and epinasty along the stem of Minnesota and Idaho flowering rush 1 week after treatment with 1.25 mg ae L^{-1} triclopyr for a 24-hour exposure time.

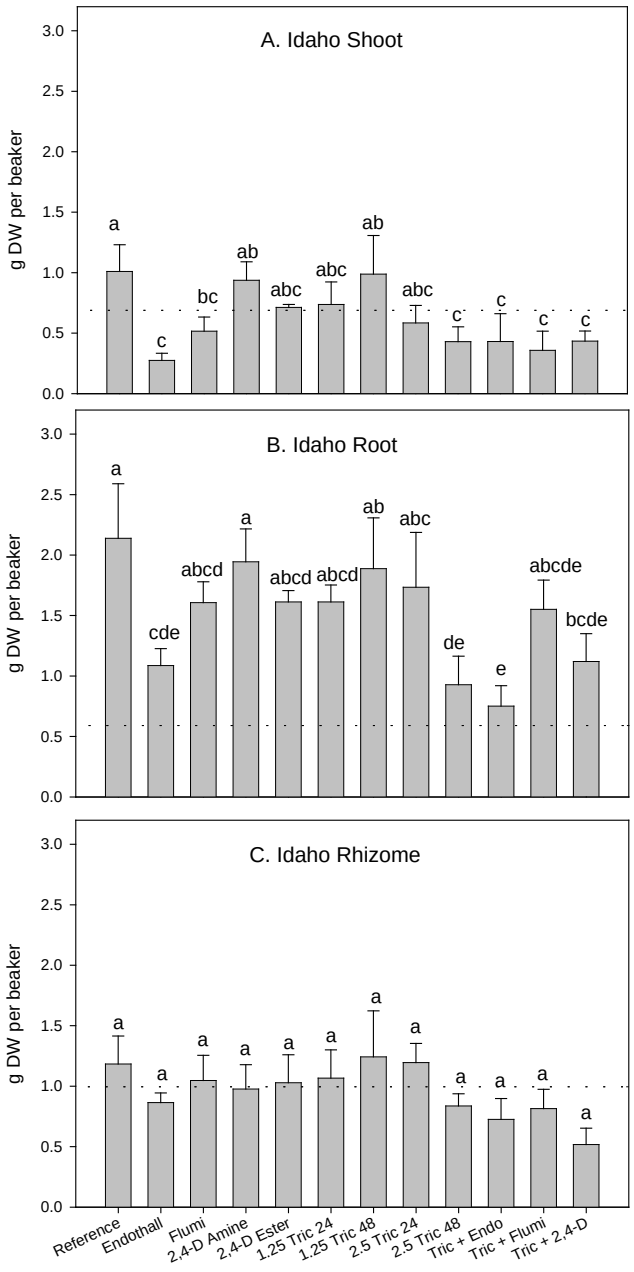

Figure 4. Idaho flowering rush (mean ±1 SE, n=4) A) shoot, B) root, and C) rhizome biomass (g DW) 6 weeks after treatment with 1.5 mg ai L^{-1} endothall (Endo), 0.4 mg ai L^{-1} flumioxazin (Flumi), 4.0 mg ae L^{-1} 2,4-D amine, 4.0 mg ae L^{-1} 2,4-D ester, 1.25 or 2.5 mg ae L^{-1} triclopyr (Tric) and combinations of triclopyr (1.25 mg ae L^{-1}) with endothall(1.5 mg ai L^{-1}), flumioxazin (0.4 mg ai L^{-1}), and 2,4-D amine (4.0 mg ae L^{-1}). Numbers in front of herbicide abbreviations represent herbicide concentrations (mg ae L^{-1}) followed by exposure time (h). Treatments with the same letter are not significantly different (shoot ANOVA $p \leq 0.05$, LSD=0.448; root ANOVA $p \leq 0.05$, LSD=0.800; rhizome ANOVA $p=0.39$).

4 Herbicides with Enzyme-Specific Modes of Action against Submersed Flowering Rush

Materials and Methods

This experiment was conducted in a walk-in controlled environment growth chamber (48 m^2) at the US Army Engineer Research and Development Center (ERDC) in Vicksburg, MS. Ambient conditions were set to provide optimum conditions for submersed plant growth: air temperature of 21 ±2°C, light intensity of 594 µmol m^{-2} sec^{-1}, and photoperiod of 14 h:10 h light:dark cycle.

Flowering rush rhizomes were field-collected from Detroit Lakes, Pelican River Watershed, MN, and Lake Pend Oreille, ID and shipped overnight to ERDC. Rhizomes were surrounded by sediment and subjected to a cold (4°C) dark treatment for at least 3 wk before sprouting. Rhizomes (4 to 5 cm in length, 4 to 5 g FW) were then washed to remove sediment, placed in culture solution (Smart and Barko 1985) that was aerated, and allowed to sprout in the environmental growth chamber for 5 wk.

Two different sediments were used for propagating rhizomes from each plant population based on observations from the field. Minnesota plants were planted in silty topsoil (Black Kow® Topsoil, Black Gold Compost Co., Oxford, FL). The pH of the potting soil (5.3) was raised to 7.1 by adding 150 mg L^{-1} of calcium carbonate and 1.5 g L^{-1} of sodium bicarbonate to make it more alkaline. The Idaho plants were planted in topsoil that had remnants of bark, which was more acidic (pH=5.2; Scotts® Premium Topsoil, The Scotts Company, Marysville, OH). Both sediments were fertilized with 150 mg L^{-1} ammonium chloride.

One sprouted Minnesota rhizome containing one shoot (5.1 ±0.8 g FW, shoot length=24 ±2 cm, root length=5.3 ±1.5 cm; n=15) was planted to a depth of 4 cm in 1 L high-density polyethylene (HDPE) beakers filled with Black Kow Topsoil. One sprouted Idaho rhizome containing one shoot (5.9 ±0.6 g FW, shoot length=23 ±1.5 cm, root length=9.3 ±0.6 cm; n=15) was planted to a depth of 4 cm in 1 L HDPE beakers filled with Scotts Premium Topsoil. A 2-cm layer of masonry sand was added to the sediment surface in each beaker to prevent dispersion of nutrients and sediment into the water column. Two beakers of each plant population were placed in each aquarium (volume=48 L) filled with culture solution (Smart and Barko 1985) amended with chelated iron (0.1 mg L^{-1}). Plants grew for 5 wk prior to herbicide application.

Herbicide concentrations were selected based on current operational use patterns for ALS and PDS herbicides against submersed plants: low use rates (<100 µg ai L^{-1}) with long-term exposures of 4 to 12 wk. For herbicide application, stock solutions of fluridone (Sonar® AS, SePRO Corp.), bispyribac-sodium (Tradewind®, Valent USA Corp.), and imazamox (Clearcast®, SePRO Corp.) were prepared by diluting formulation concentrates in distilled water. From the stock, each herbicide was applied subsurface using a pipette to provide nominal concentrations in the treatment aquaria for a static exposure of 5 wk (Table 3). Untreated reference aquaria were included to assess plant growth in the absence of herbicide exposure. After 5 wk, the experiment was concluded.

Table 3. Concentrations of plant-enzyme specific herbicides evaluated against field-collected Minnesota and Idaho flowering rush. Plants were exposed to herbicide treatments for 5 weeks.

Herbicide	Concentration (µg ai L^{-1})
Fluridone	10, 20
Bispyribac	20, 40
Imazamox	50, 100
Reference	0

Results and Discussion

Compared to the reference, herbicide treatments in this experiment were not effective in significantly reducing shoot and rhizome biomass of either the Minnesota or Idaho flowering rush (Figures 5 and 6). The 100 µg ai L^{-1} imazamox and 40 µg ai L^{-1} bispyribac treatments reduced Idaho root biomass by 63and 53%, respectively (Figure 6B). Root biomass of treated and reference plants for both populations decreased during the experiment compared to pretreatment biomass taken 5 weeks earlier.

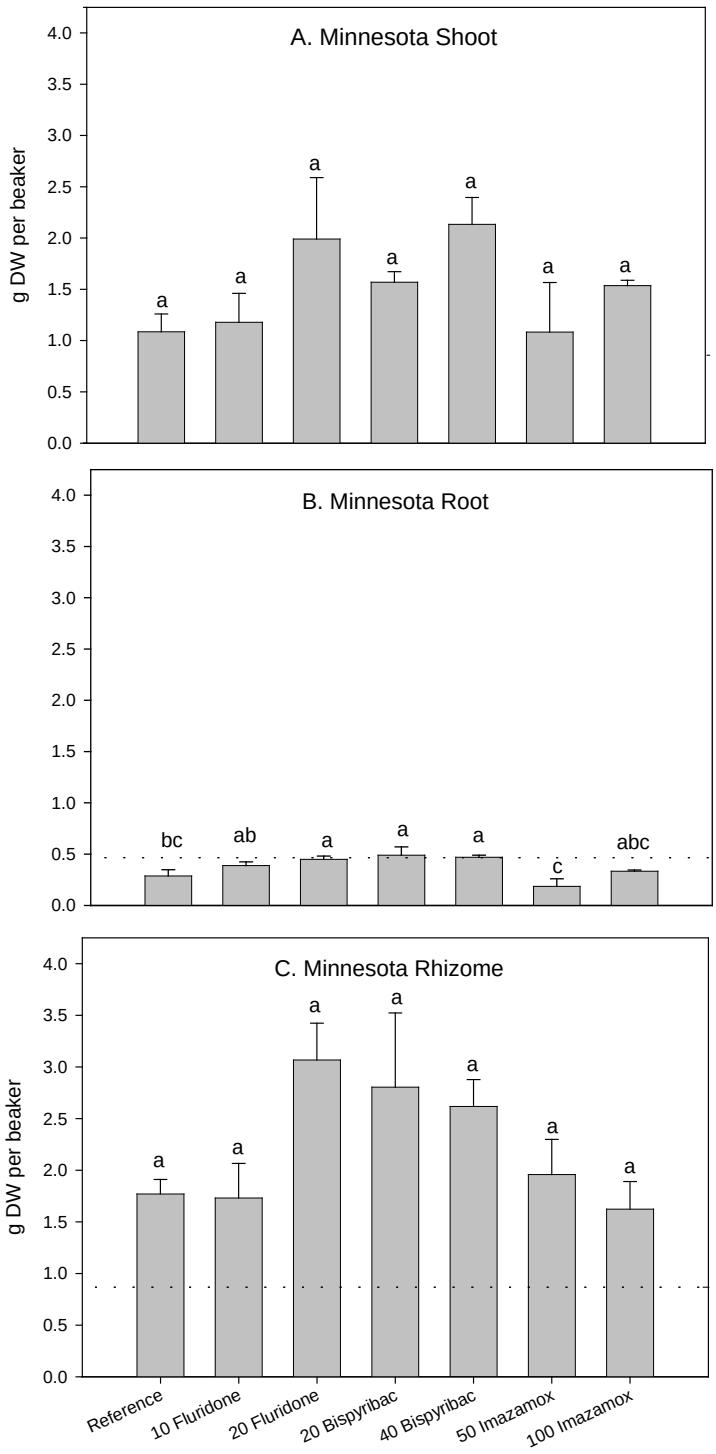

Figure 5. Minnesota flowering rush (mean ±1 SE, n=3) A) shoot, B) root, and C) rhizome biomass (g DW) 5 weeks after treatment with aquatic herbicides fluridone, bispyribac, and imazamox. Numbers in front of herbicide active ingredient represent herbicide concentrations (µg ai L^{-1}). Treatments with the same letter are not significantly different (shoot ANOVA p≤0.05, LSD=0.448; root ANOVA p≤0.05, LSD=0.800; rhizome ANOVA p=0.39).

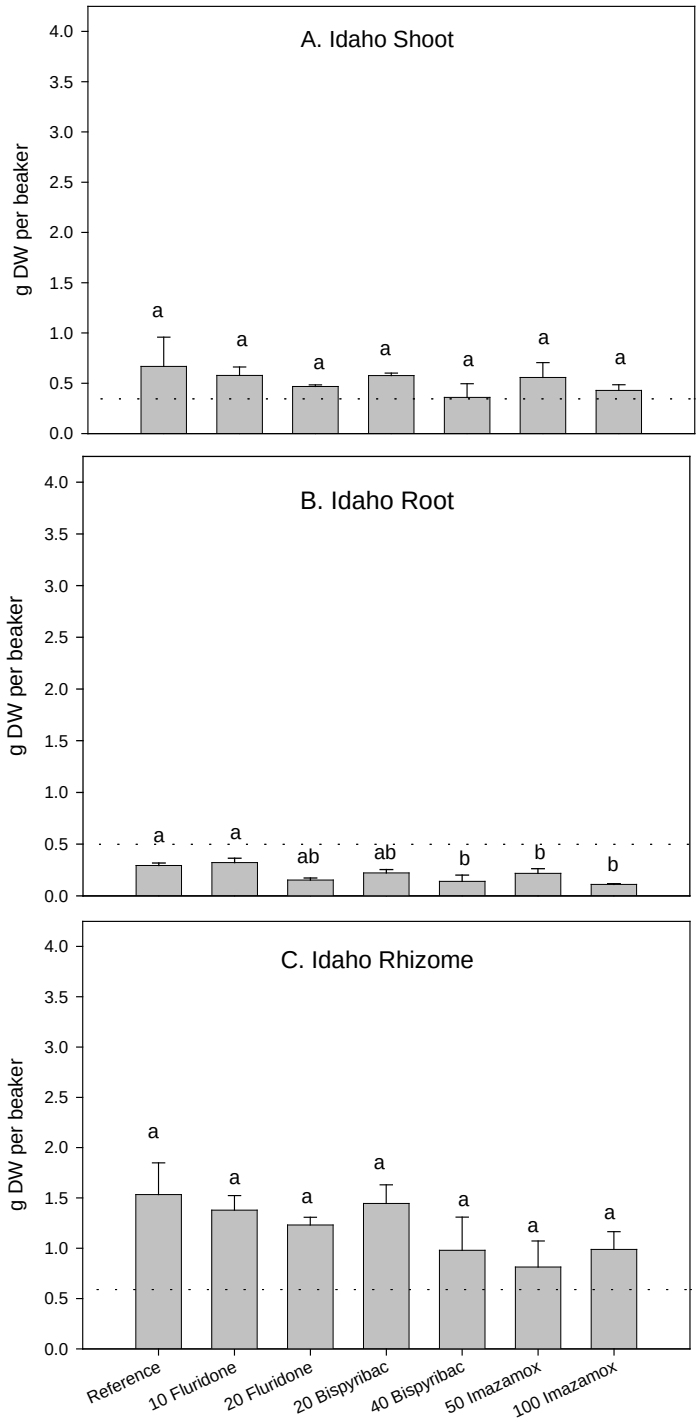

Figure 6. Idaho flowering rush (mean ±1 SE, n=3) A) shoot, B) root, and C) rhizome biomass (g DW) 5 weeks after treatment with aquatic herbicides fluridone, bispyribac, and imazamox. Numbers in front of herbicide active ingredient represent herbicide concentrations (µg ai L^{-1}). Treatments with the same letter are not significantly different (shoot ANOVA p≤0.05, LSD=0.448; root ANOVA p≤0.05, LSD=0.800; rhizome ANOVA p=0.39).

Visually, all herbicide treatments produced slight bleaching and some browning of shoots through 2 WAT. For example, in the 20 and 40 µg ai L^{-1} bipyribac treatments, plant shoots were light green and yellow due to herbicide effects; however, plants were still vigorous. Shoots remained stressed until the end of the experiment, yet biomass was not affected by herbicide treatment (Figure 7).

Lack of efficacy may be attributed to inherent tolerance of submersed flowering rush to subsurface applications of these plant enzyme-specific herbicides. Differential response of emergent and submersed aquatic plants to ALS-inhibiting herbicides has been reported (Getsinger et al. 1994, Chiconela et al. 2004, Koschnick et al. 2007, Glomski and Netherland 2008). Slight changes in the molecular structure of these herbicides greatly affect the potency and spectrum of susceptibility in plants (Ladner 1991). For example, soft-stem bulrush, was more susceptible to concentrations of bispyribac than imazamox, but maidencane, pickerelweed (*Pontederia cordata* L.), and sagittaria were more susceptible to imazamox than bispyribac in an outdoor mesocosm experiment (Koschnick et al. 2007).

Higher doses and/or longer exposure times of the herbicides tested might result in better control of flowering rush. Herbicide doses were chosen based on use patterns developed for ALS and PDS chemistries for control of submersed aquatic plants such as hydrilla and Eurasian watermilfoil, which currently focus on low use rates (<100 µg ai L^{-1}) with long-term exposures of 4 to 12 wk (Netherland et al. 1993). Given that fluridone, bispyribac, and imazamox require long exposure times for submersed treatments, it is not likely that these herbicides would be appropriate tools to control flowering rush, especially since they are best used in large contiguous areas where there is limited water flow.

Combination of herbicide applications with another management practice, such as dewatering or bareground applications, may provide better control of flowering rush biomass. Application of fluridone to newly sprouted vegetative propagules (tubers) after dewatering was successful in controlling monoecious hydrilla in Lake Gaston, a run of the river reservoir on the border of North Carolina and Virginia (Nawrocki 2011). One advantage of this integrated approach is that submersed application techniques would only require delivery of product to shallow water if application is immediately after dewatering (reducing the amount of herbicide required); however, herbicide treatments in operational applications would be difficult if sprouting of flowering rush rhizomes is non-seasonal or random.

Figure 7. Flowering rush from Idaho (on left) and Minnesota (M, on right) that was treated with 20 µg ai L^{-1} bispyribac 2 wk after herbicide application.

5 Conclusions and Recommendations

Conclusions:

Based on the results of these small-scale experiments, the following conclusions can be drawn about the effect of aquatic herbicides on the submersed growth stage of flowering rush:

a. Idaho and Minnesota flowering rush populations were both triploid and genetically identical.

b. Responses of both plant populations to the herbicides, concentrations, and exposure times followed similar patterns.

c. Endothall alone and endothall + triclopyr: Low concentrations of endothall (1.5 mg ai L^{-1}) for exposure times of 24 h reduced shoot and root biomass of Minnesota and Idaho submersed flowering rush by >75%. When shoots were harvested from these treatments, there was no new shoot growth from rhizomes observed in these treatments. A combination of endothall (1.5 mg ai L^{-1}) with triclopyr (1.25 mg ae L^{-1}) did not enhance efficacy for either plant population.

d. Flumioxazin alone and flumioxazin + triclopyr: Flumioxazin alone reduced shoot biomass by 63% and 49% in Minnesota and Idaho plants, respectively. The addition of 1.25 mg ae L^{-1} triclopyr in combination with 0.4 mg ai L^{-1} flumioxazin increased control of both flowering rush populations by almost 20%; however, this combination was statistically similar to flumioxazin alone.

e. Triclopyr and 2, 4-D: Shoot and root biomass reductions ranged from 5 to 50% in both Minnesota and Idaho flowering rush using maximum concentrations of 2,4-D (4 mg ae L^{-1}) and triclopyr (2.5 mg ae L^{-1}) for 24-hr exposure times. Triclopyr concentrations of 2.5 mg ae L^{-1} for a 48-hr exposure time significantly reduced shoot and root biomass of Idaho flowering rush.

f. ALS and PDS herbicides: A 5-wk static exposure of low doses of ALS (bispyribac and imazamox) and PDS (fluridone) herbicides in submersed applications did not significantly reduce shoot or root biomass of Minnesota flowering rush. Idaho root biomass was reduced by >50 % with 40

µg ai L^{-1} bispyribac and 100 µg ai L^{-1} imazamox; however, shoot biomass was not affected by these herbicide treatments.

g. Compared to the reference, systemic herbicide treatments in either experiment did not significantly reduce rhizome biomass of Minnesota and Idaho flowering rush. Colonization from rhizomes is the primary means for spread of triploid flowering rush infestations.

Recommendations:

Based on the results of this investigation, the following recommendations are provided:

a. Foliar spray applications of systemic herbicides against the emergent stage of flowering rush in an outdoor mesocosm experiment or field plots located in shallow water should be investigated. Although the CETs of some systemic herbicides in subsurface applications were not efficacious against submersed flowering rush in this experiment (2,4-D, triclopyr, bispyribac, imazamox), foliar spray applications of these products may be effective against emergent flowering rush. For example, 50 and 100 µg ai L^{-1} imazamox for a 5-wk static exposure did not reduce plant biomass in this experiment; however, a foliar application of imazamox (4.7 L per ha or 2 qt per ac) was effective in suppressing flowering rush for more than one growing season in a field demonstration in Flathead Lake, MT (Rice et al. 2009). Rice et al. (2009) have conducted some work on the emergent form with triclopyr and imazamox; however, additional quantitative research on these and other herbicides, as well as herbicide combinations, are warranted.

b. Validate results of these experiments in an outdoor mesocosm study with the objective of expanding CET relationships to include lower herbicide concentrations and shorter exposure times for contact herbicides, or contact and systemic herbicide combinations. This study would use an early spring application strategy against submersed flowering rush and incorporate three ecologically important native aquatic plants that occupy the same habitat, such as hardstem bulrush (*Scirpus acutus*), spadderdock (*Nuphar advena*), and water lily (*Nymphaea odorata*). Aadvantages of early spring treatments are threefold: young flowering rush plants with low biomass would be more susceptible to herbicides, limit reproduction potential of flowering rush,

and mitigate impacts on collateral non-target native vegetation that is still in winter quiescence (Netherland et al. 2000, Poovey et al. 2002, Skogerboe et al. 2008).

c. Small-scale evaluation of additional combinations of flumioxazin with other herbicides (eg. diquat) may further enhance its effectiveness against submersed plants. Combinations should include lower flumioxazin concentrations and shorter exposure times to determine a range of CETs that may be efficacious and selective. Although water pH may be a factor for flumioxazin efficacy, current experiments suggest that this herbicide may have potential to control flowering rush despite high water column pH (≥8). Efficacy should be enhanced in water bodies with lower pH; however, experimental work in neutral water column pH would verify this.

d. Compare triploid and diploid flowering rush to determine if there are substantial differences in herbicide response using laboratory bench assays on sprouted rhizomes. This approach has proven useful in determining response of aquatic plants to enzyme specific herbicides (Glomski and Netherland 2011).

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