SOURCES OF MATERIAL

[1]Miracle-Gro® Water Soluable All Purpose Plant Food, The Scotts Company, PO Box 606 Marysville, OH 43040

[2]Reward® Landscape and Aquatic Herbicide, Syngenta Professional Products, PO Box 18300 Greensboro, NC 27419

[3]Stingray®, FMC Corporation, 1735 Market Street, Philadelphia, PA 19103.

[4]CygnetPlus®, Brewer International, PO Box 690037, Vero Beach, FL 32969.

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Evaluations of contact aquatic herbicides for controlling two populations of submersed flowering rush

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ABSTRACT

Flowering rush (Butomus umbellatus L.) is a rapidly spreading invasive aquatic plant in the northern United States. Introduced from Eurasia, it grows as an emergent plant along shorelines and as a submersed plant in deeper water of lakes and rivers. Because submersed flowering rush grows in fluctuating water levels, management of this plant has been inconsistent and unpredictable. Two small-scale experiments were conducted to evaluate contact herbicide efficacy on the submersed form of flowering rush from two triploid populations, one from Minnesota and one from Idaho. In the first experiment, various concentrations and exposure times of diquat, endothall, and flumioxazin were applied to Minnesota flowering rush. In the second experiment, concentration–exposure time relationships were investigated for flumioxazin against Idaho flowering rush.

One treatment of endothall was used to compare flumioxazin, a newly registered compound, with an older chemistry. Results of both experiments showed that contact herbicides are effective against flowering rush. Although flumioxazin (200 µg ai L^{-1}) did not significantly reduce shoot biomass for exposure periods of 12 or 24 h, concentrations of diquat (370 µg ai L^{-1}) for exposure times of 6 and 12 h, and endothall (1500 and 3000 µg ai L^{-1}) for exposure times of 12 and 24 h reduced shoot biomass of Minnesota submersed flowering rush by >70%; however, these treatments did not significantly impact root biomass. Lateral rhizome buds, which serve as a source of annual reinfestation, were found in all treatments. Concentrations of flumioxazin (400 µg ai L^{-1}) and endothall (3000 µg ai L^{-1}) for exposure times of 24 h controlled Idaho submersed flowering rush by successfully reducing shoot and root biomass by >70%. Application strategies for complete control of triploid flowering rush shoots and roots with contact herbicides may require repeat applications and/or combinations with each other and systemic herbicides. Further evaluation of herbicides for controlling triploid as well as diploid flowering rush is warranted.

Key Words: Butomus umbellatus, diquat, endothall, flumioxazin, triploid.

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INTRODUCTION

Flowering rush (*Butomus umbellatus* L.), a monocot introduced from Eurasia, has become invasive in the northern United States. 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 (Boutwell 1990, Les and Mehrhoff 1999). 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, each morphology requires an independent control strategy. Focusing on the submersed plants, success or failure of herbicide treatment depends on 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 to achieve desirable control of nuisance submersed plants (Getsinger and Netherland 1997).

Because submersed flowering rush grows in waterbodies with fluctuating water levels, management of this plant 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, lakes or a chain of lakes connected by a river have variable water exchange patterns that will impact aqueous distribution of herbicides resulting in reduced chemical exposure times against target plants and reduced efficacy. One option for control of flowering rush in these environments is the use of contact herbicides.

Contact herbicides are products that are fast-acting, usually requiring short exposure times that range from 6 to 24 h, depending on the active ingredient. They have a broad spectrum of activity and can be used to control most submersed plant species. The disadvantage of these products is that they rarely kill the entire plant, with the exception of annual plants and very young perennial plants (with poorly developed rootstock or root crown tissue). Robust perennial species treated with contact herbicides usually have the ability to recover from the herbicide exposure, and typically regrow from rootstock or root crown tissue located at or below the surface of the sediment.

In two separate experiments, we evaluated endothall (dipotassium salt) [7-oxabicyclo (2.2.1) heptane-2,3-dicarboxylic acid], diquat [6,7-dihydro-dipyrido (1,2-a:2',1'-c) pyrazinediium dibromide], and flumioxazin (2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione) for the control of submersed flowering rush under relatively short aqueous exposure time scenarios. In one experiment, all three herbicides were used against the flowering rush field-collected from Minnesota. In another experiment, we used flowering rush that was field-collected from Idaho for a CET study of flumioxazin, which was recently registered by the US Environmental Protection Agency (EPA) for aquatic use. We incorporated one endothall treatment as a comparison using an older chemistry. Results of these evaluations will be used to provide guidance for management of submersed flowering rush in water bodies with short contact times.

MATERIALS AND METHODS

Minnesota flowering rush experiment

Flowering rush rhizomes were field-collected on 19 July 2010 from Big Detroit Lake, Pelican River Watershed, Minnesota. The Pelican River Watershed is composed of five natural lakes and two reservoirs connected by short segments of the Pelican River. These lakes are considered "ice-block" lakes, and are located in an outwash plain. Big Detroit Lake is one of the natural lakes in the watershed. It has an 840 ha surface area with 12.4 km of shoreline. The average depth is 5.6 m, and the maximum depth is 25 m. The littoral zone encompasses 40% of the lake. The shore of Big Detroit Lake is heavily populated and lined with residential development. Flowering rush occupies 25 ha of the lake.

After collection, rhizomes were shipped overnight to the US Army Engineer Research and Development Center (ERDC) in Vicksburg, Mississippi. Rhizomes surrounded by sediment were subjected to a cold treatment (4 C) for at least 3 weeks before sprouting. Rhizomes (4 to 5 cm in length) were then washed to remove sediment, placed in culture solution (Smart and Barko 1985) that was aerated, and allowed to sprout in an environmental growth chamber (48 m²) for 3 weeks.

Ambient conditions were set to provide optimum conditions for submersed plant growth: air temperature of 21 ± 2 C, light intensity ranging from 353 to 517 μmol m^{-2} sec^{-1}, and photoperiod of 14 h:10 h light:dark cycle. On 31 August 2010, one sprouted rhizome (1 to 3 shoots; shoot length = 27 ± 1.6 cm) was planted to a depth of 4 cm in 1 L high-density polyethylene (HDPE) beakers filled with fertilized (150 mg L^{-1} ammonium chloride) potting soil[1] and kitty litter[2]. A thin layer of masonry sand (2 cm) was added to the sediment surface to prevent dispersion of nutrients and sediment into the water column. Three beakers 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 3 weeks to achieve the initial formation of a surface canopy (~60 cm) prior to herbicide application.

Herbicide application rates (Table 1) were selected based on several factors. Medium to high concentrations of each product were used because aqueous exposure times can be short where flowering rush grows. For example, exposure times in Detroit Lakes ranged from 4 to 78 h in field trials using 3000 μg active ingredient (ai) L^{-1} endothall (Skogerboe 2010).

For herbicide application, stock solutions of endothall (Aquathol® K)[3], diquat (Reward®)[4] and flumioxazin (Clipper®)[5] 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 the appropriate exposure

Herbicide Treatment	Concentration (μg ai L^{-1})	Exposure (h)
Endothall	1500, 3000	12, 24
Diquat	370	6, 12
Flumioxazin	200	12, 24
Reference	0	24

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 three times to remove all aqueous herbicide residues. The experiment was concluded on 19 October 2010, 4 weeks after treatment (WAT).

Water samples were collected 25 cm below the water surface from the diquat and endothall treatments at the end of each exposure time to ensure nominal herbicide concentrations were achieved. Samples were stored at 4 C until shipped for analysis. Endothall residues were analyzed in-house at ERDC using the enzyme-linked immunosorbent assay (ELISA) technique (Toth 1999), which can detect endothall concentrations as low as 7 μg ai L^{-1}. Diquat residues were analyzed using HPLC-UV method following EPA protocol 549.1 by Pacific Agricultural Labs, Portland, Oregon. Water samples were not collected or analyzed for flumioxazin due to its rapid degradation at pH levels observed in this experiment (9.4 ± 0.1). Investigations have shown that flumioxazin degrades rapidly in pH >8.5 under laboratory and mesocosm conditions, with an aqueous half-life ranging from 17.5 to 102 min (Katagi 2003, Mudge 2007, Mudge et al. 2010).

Water temperature was measured continuously with an Optic Stowaway® Temperature Probe[6] in reference aquaria. Water temperatures in aquaria were 21 ± 0.02 C during the experiment. The pH was measured with a handheld multi-parameter probe[7].

Treatments were randomly assigned to individual aquaria and replicated three times, including the reference (n = 3). Herbicide efficacy was assessed by harvesting shoot and root biomass at 4 WAT. Biomass from all beakers in each aquarium were harvested, dried, and weighed for a dry weight measurement (g DW). Shoot and root biomass were analyzed using one-way analysis of variance (ANOVA) to determine herbicide effects. If effects were significant (p ≤ 0.05), means were compared using the Tukey test.

Idaho flowering rush experiment

Flowering rush shoots, roots, and associated rhizomes were field-collected on 10 August 2010 from Lake Pend Oreille, a large natural lake in northern Idaho that stretches for 105 km beyond Albeni Falls Dam, which sits on the Pend Oreille River. The lake is fed by the Clark Fork and Pack Rivers and has a surface area of 380 km². The lake basin is deep and steep-sided with a maximum depth of 377 m and average depth of 164 m. The lake is located in a valley surrounded by mountains, national forests, and few small towns. Flowering rush coverage ranges from 20 to 81 ha.

After collection, plants were shipped overnight to ERDC. The next day, plants were washed and then floated in culture solution (Smart and Barko 1985) for one day before planting. The Idaho flowering rush experiment was also conducted in a walk-in controlled environment growth chamber (52 m²) at ERDC with ambient conditions similar to the Minnesota flowering rush experiment described above.

On 13 August, whole plants (shoots, roots, and rhizomes) were planted to a depth of 4 cm in 1 L HDPE beakers filled with fertilized (300 mg L^{-1} ammonium chloride) potting soil[1]. A thin layer of silica sand (2 cm) was added to the sediment surface to prevent dispersion of nutrients and sediment into the water column. Two beakers were placed in each aquarium (volume = 48 L) filled with culture solution amended with chelated iron (0.1 mg L^{-1}). After 10 days, new shoots were emerging from the sediment surface, while older shoots were senescing. Plants grew for another week before herbicide application to allow the newly emerged shoots to reach the water surface (50 cm); senesced shoots were removed from the water column. Plants were young and actively growing at the time of herbicide application on 31 August. A handheld multi-parameter probe[7] was used to measure water temperature and pH before herbicide application. Average water temperature was 22 C and pH was 9.0.

Flumioxazin application rates (Table 2) were selected to further refine CETs for this product against submersed flowering rush, and the endothall rate was used as a comparison with a product that was used in field trials (Skogerboe 2010). For herbicide application, stock solutions of each product were made and applied as in the previous experiment. The experiment continued for 6 WAT and was completed on 12 October 2010.

Treatments were randomly assigned to individual aquaria and replicated four times, including the reference (n = 4). Herbicide efficacy was assessed by harvesting shoot and root biomass at 6 WAT. Biomass from all beakers in each aquarium were harvested, dried, and weighed for a dry weight measurement (g DW). Shoot biomass and root biomass were analyzed using one-way ANOVA to determine herbicide effects. If effects were significant (p ≤ 0.05), means were compared using the Tukey test.

Ploidy and genetic variation of flowering rush populations

Ploidy of the Minnesota flowering rush population has been documented to be triploid by Lui et al. (2005). Ploidy of Idaho flowering rush was determined using guard cell length following the methods of Kliber and Eckert (2005).

Herbicide Treatment	Concentration (μg ai L^{-1})	Exposure Time (h)
Flumioxazin	50	24
	100	24
	200	12, 24
	400	12, 24
Endothall	3000	24
Reference	0	24

Plant populations with unknown ploidy can be classified using this method because triploid populations have had significantly larger mean guard cell length than diploids. Plant populations could be classified as triploid if mean guard cell length was >42.63 µm. We prepared slides for guard cell measurement by preserving the plant material in 3:1 ethanol and glacial acetic acid. This solution showed no cellular swelling compared to deionized water or saline. The plant material was mounted on slides in 3:1 ethanol and glacial acetic acid and sealed with acrylic. Measurements of a minimum of five guard cells per plant were obtained from four different plants within the Idaho population. The mean guard cell length was then used to infer ploidy.

We also examined genetic variation in the Minnesota (n = 6 individuals) and Idaho (n = 10 individuals) populations using amplified fragment length polymorphisms (AFLPs). Genomic DNA was extracted from fresh emergent vegetation using DNeasy Plant Mini Kits.[8] AFLPs were prepared as described in Thum et al. (2011). We examined two selective primer pairs (EcoR1-ACA/ MseI-CAT and EcoR1-AGG/ MseI-CAT). Selective amplification products were run on an automated DNA sequencer[9] using the internal size standard MapMarker1000 ROX[10]. We scored the AFLP data with GeneMapper v4.0,[11] limiting our analysis to fragments between 50 and 500 bp in length. Allele bins were determined using a peak height threshold (PHT) of 300 relative fluorescence units (RFU) and a bin width of 0.75 bp to ensure that only strong bands were included in the binset. We then automatically scored the data with the bin set using a PHT of 30 RFU, but visually checked and edited all allele calls.

RESULTS AND DISCUSSION

Minnesota flowering rush experiment

Aqueous diquat residues (mean ±1 SE) were measured at 390 ± 7 µg ai L^{-1} (n = 6) compared to the nominal concentration of 370 µg ai L^{-1}. Aqueous endothall residues (mean ± 1 SE) were measured at 1822 ± 61 µg ai L^{-1} (n = 6) and 3363 ± 53 µg ai L^{-1} (n = 6) compared to the nominal concentrations of 1500 and 3000 µg ai L^{-1}, respectively. Because the recovery range was 100 to 110% of endothall in the ELISA analyses, the increase of 10% in actual concentrations compared to nominal concentrations is typical for aqueous residues of aquatic herbicides.

Significant reduction of shoot biomass ranged from 74 to 95% for the endothall and diquat CETs evaluated (Figure 1A). Plants in the diquat treatments showed symptoms of herbicide injury (chlorotic and disintegrating stems) at 4 days after treatment. By 1 WAT, endothall treatments were exhibiting chlorosis and browning. By 4 WAT, plants dosed with diquat and endothall were necrotic; however, new healthy shoots were emerging from the sediment. Endothall treatments also had healthy green tissue at the lower levels of older shoots at the conclusion of the experiment. Neither the concentration nor the exposure time made a difference in the level of control achieved with endothall because all treatments were statistically similar; therefore, a concentration of 1500 µg ai L^{-1} for a 12 h exposure was as effective as a concentration of 3000 µg ai L^{-1} for a 24 h exposure. Flumioxazin applied at 200 µg

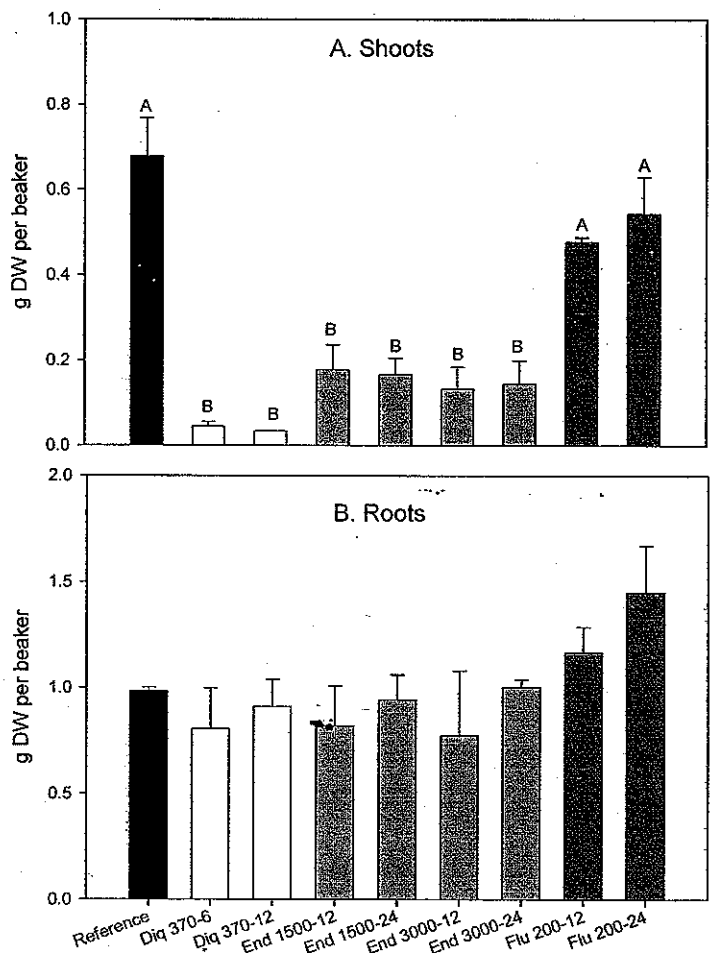

Figure 1. Shoot (A) and root (B) biomass (g DW) of Minnesota flowering rush (mean ± 1 SE, n = 4) at 4 weeks after treatment with diquat (Diq), endothall (End), and flumioxazin (Flu). Numbers behind herbicide abbreviations represent herbicide concentrations (µg ai L^{-1}) with exposure time (h). Treatments with the same letter are not significantly different (Tukey test, p ≤ 0.05).

ai L^{-1} (50% maximum label rate) for 12 or 24 h exposure was not effective because shoot biomass was not significantly different from the untreated reference. By 1 WAT, plant stems in the flumioxazin treatments were chlorotic and red, but these early injury symptoms did not lead to major loss of mass at 4 WAT, with some shoots remaining green and viable. The lack of efficacy may be related to the high aqueous pH at time of treatment (9.4), thereby increasing herbicide concentration and/or exposure time required for plant death.

None of the contact herbicide treatments evaluated significantly reduced root biomass compared to the untreated reference (Figure 1B). All treatments had lateral rhizome buds present at the end of the experiment. Moreover, root biomass increased by almost 100% compared to pretreatment levels (0.44 g DW per beaker).

It is uncertain if the treatment of newly sprouted rhizomes would provide better control of flowering rush root biomass. Although application of contact herbicides to newly sprouted vegetative propagules (tubers) was successful in controlling hydrilla in small-scale studies (Van and Conant 1988), her-

bicide treatments in operational applications would be difficult if sprouting of flowering rush rhizomes is nonseasonal or random.

Idaho flowering rush experiment

Flumioxazin concentrations of 400 µg ai L^{-1} for a 24 h exposure time reduced shoot biomass by 82% and root biomass by 72% (Figure 2A). Visually, these plants had a collapsed canopy with dead shoots floating in the water column. Healthy green shoots were present growing from the sediment with a few shoots at the water surface.

When reducing the concentration by 50% (200 µg ai L^{-1} for 24 h) or exposure time by 50% (400 µg ai L^{-1} for 12 h), the efficacy of flumioxazin was reduced by 30 to 40%. These plants had intact canopies with healthy green shoots as well as brown dead ones. Although some reddening of stems and necrosis occurred in plants dosed with 50 and 100 µg ai L^{-1} for 24 h and 200 µg ai L^{-1} for 12 h, shoot biomass in these treatments was comparable to the reference. Shoot biomass in plants dosed with 3000 µg ai L^{-1} endothall for 24 h was reduced by >90%.
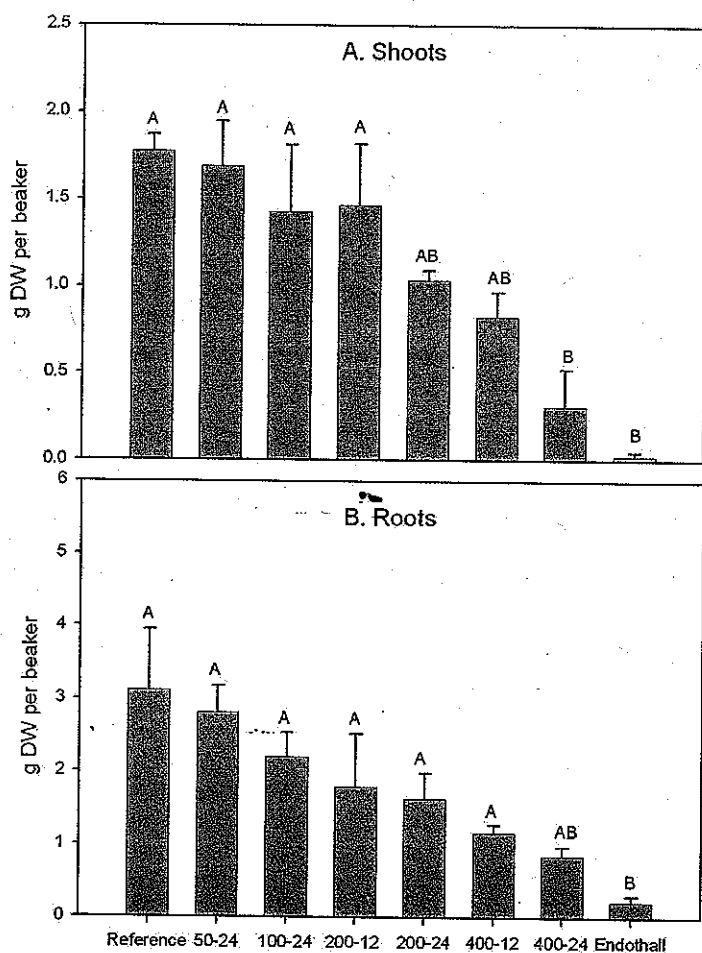

Figure 2. Shoot (A) and root (B) biomass (g DW) of Idaho flowering rush (mean ± 1 SE, n = 4) at 6 weeks after treatment with flumioxazin and endothall (3000 µg ai L^{-1}; 24 h exposure time). Flumioxazin concentrations (µg ai L^{-1}) are followed by exposure time (h). Treatments with the same letter are not significantly different (Tukey test, p ≤ 0.05).

Despite a trend of decreased root biomass with increasing herbicide concentration, root biomass for all flumioxazin treatments was statistically similar to the untreated reference due to treatment variation between replicates (Figure 2B). Plants dosed with 400 µg ai L^{-1} for a 24 h exposure time were similar in root biomass to both the untreated reference and the endothall treatment. Reference plants and those that were dosed with 50 and 100 µg ai L^{-1} had new rhizome growth. Few rhizomes were harvested in the 200 and 400 µg ai L^{-1} flumioxazin treatments, and no rhizomes were recovered in the endothall treatments.

Flumioxazin has the potential to control submersed flowering rush at the maximum label rate (400 µg ai L^{-1}), even with a water column pH >9. Because flumioxazin potentially hydrolyzes in minutes at the high pH levels evaluated in this study (Katagi 2003), flowering rush and other aquatic plants are able to rapidly uptake flumioxazin. In a mesocosm study, the half-life of flumioxazin was 1.7 h at pH >8.5 in the water column, yet hydrilla was able to rapidly uptake flumioxazin with significant phytotoxic effects at 400 µg ai L^{-1} (Mudge et al. 2010); however, for successful control of hydrilla (>70% biomass reduction) either higher concentrations or lower pH levels are required (Mudge and Haller 2010). Increased efficacy of flowering rush would likely be achieved in waterways with neutral pH ranges (pH = 7) because hydrolysis of flumioxazin is 16 to 18 h at lower pH levels (Katagi 2003, Mudge et al. 2010). Further testing of flumioxazin efficacy in a neutral pH would be beneficial in determining whether flowering rush is inherently tolerant or susceptible to this product.

Ploidy and genetic variation of flowering rush populations

Like the Minnesota population (Lui et al. 2005), the Idaho flowering rush population was determined to be triploid (mean ±1 SE guard cell length = 58.9 ± 1.6 µm). Triploid plants produce more shoot, root, and rhizome biomass, as well as more numerous lateral rhizome buds than diploid plants; therefore, limited seed production in triploids is compensated for by more intensive vegetative reproduction (Hroudová and Zákravský 1993). In these experiments, the root:shoot of reference Minnesota plants was 1.4 and the root:shoot for Idaho reference plants was 1.8. These ratios are similar to findings from a greenhouse experiment using emergent flowering rush that was predominately field-collected from locations in Midwestern North America (Lui et al. 2005).

The two selective primer pairs resulted in 136 bands in our AFLP analysis. Surprisingly, all of the individuals from both of our lakes were genetically identical for their AFLP genotypes. In addition, we analyzed several plants from each of four other populations (Silver and Long Lakes in WA and Noxon and Thompson Reservoirs in MT; data not shown), and these individuals also had identical AFLP profiles to the Minnesota and Idaho populations examined here. This suggests that these individuals are either all truly genetically identical (i.e., are different ramets of the same genet), or that genetic diversity for AFLPs is extremely low for flowering rush. Our results stand in contrast to earlier studies examining genetic variation in flowering rush that employed random amplified polymorphic DNA (RAPD) markers (Eckert

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et al. 2003, Kliber and Eckert 2005). Guard cell analysis of the four populations not evaluated with herbicides in these experiments indicated that they were also triploid (mean guard cell length range = 52.8 ± 2.0 to 60.1 ± 2.7 μm). Thus, one explanation for the identical genetic profiles is that all six of these flowering rush populations are closely related or identical triploid genotypes, which has implications for their potential expansion and management; however, it is also possible that genetic variation may be tied to the molecular techniques employed, in which variation is lower for AFLPs than for RAPDs. In either case, further study of diploid and triploid flowering rush genotypes is warranted.

As expected for genetically similar plants, flowering rush shoot and root biomass from both populations responded similarly to flumioxazin concentrations and exposure times that were used in both experiments. Conversely, endothall concentrations of 3000 μg ai L^{-1} for a 24 h exposure period greatly reduced root biomass of Idaho plants, but not Minnesota plants. One explanation may be the difference in treatment timing for each experiment. In the Minnesota experiment, herbicide treatments were applied to plants that grew for 3 weeks from sprouted rhizomes, while in the Idaho experiment herbicides were applied to field-collected mature plants that had undergone senescence and regrowth.

The results of these small-scale experiments show that contact herbicides are effective against flowering rush. Concentrations of diquat (370 μg ai L^{-1}) for exposure times of 6 and 12 h and endothall (1500 and 3000 μg ai L^{-1}) for exposure times of 12 and 24 h reduced shoot biomass of Minnesota submersed flowering rush by >70%; however, these treatments did not significantly impact root biomass. Concentrations of flumioxazin (400 μg ai L^{-1}) and endothall (3000 μg ai L^{-1}) for exposure times of 24 h controlled Idaho submersed flowering rush by successfully reducing shoot and root biomass by >70%.

Application strategies for complete control of triploid flowering rush with contact herbicides may require repeat applications or higher concentrations (as in the case of endothall and flumioxazin). Combinations of contact herbicides may also improve efficacy, such as the combination of flumioxazin and diquat, or the combination of endothall and diquat, which enhanced hydrilla control in another experiment (Pennington et al. 2001). Combination of contact herbicides with systemic herbicides, perhaps the synthetic auxins, would augment control of both flowering rush populations, with the potential to reduce roots and rhizomes as well as lateral root buds. For example, endothall has been combined with the auxin-type compounds, 2,4-D and triclopyr, for increased control of Eurasian watermilfoil (Madsen et al. 2010). Although synthetic auxin herbicides are typically selective for dicot species, activity against monocots has been reported (Belgers et al. 2007).

Validation of these experimental results in a larger experimental system should be conducted. Using a mixed plant community of nontarget vegetation would help determine species selectivity. Early spring applications would be advantageous when using contact herbicides against submersed flowering rush because impacts on collateral nontarget native vegetation that is still in winter quiescence would be mitigated (Netherland et al. 2000, Poovey et al. 2002, Skogerboe et al. 2008). Moreover, young triploid flowering rush plants with less biomass than mature plants would be more susceptible to herbicides, and vegetative reproduction potential would be reduced. Early spring applications also would be beneficial for selectively controlling diploid flowering rush to minimize reproduction through seeds and bulbils; however, herbicide response of diploid plants still needs to be determined.

ACKNOWLEDGMENTS

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[2]Special Kitty, Cat Litter, WalMart, Vicksburg, MS
[3]Aquathol® K, United Phosphorus Inc., King of Prussia, PA
[4]Reward® Syngenta Corp., Wilmington, DE
[5]Clipper® Valent Corp., Walnut Creek, CA
[6]Optic Stowaway® Temperature Probe, Onset Computer Corp., Bourne, MA
[7]YSI Model 556, Handheld Multi-Parameter Probe, YSI, Yellow Springs, OH
[8]DNeasy® Plant Mini Kits, Qiagen, Valencia, CA
[9]ABI 3130xl DNA sequencer, Applied Biosystems, Carlsbad, CA
[10]MapMarker®, 1000 ROX, BioVentures, Inc., Murfreesboro, TN
[11]GeneMapper®, v4.0, Applied Biosystems, Carlsbad, CA

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Does hydrilla grow an inch per day? Measuring short-term changes in shoot length to describe invasive potential

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INTRODUCTION

The ability of invasive plants such as hydrilla (*Hydrilla verticillata* [L.f.] Royle) and Eurasian watermilfoil (*Myriophyllum spicatum* L.) to grow and rapidly expand has been well described in both popular and scientific literature (Steenis 1967, Adams and McKracken 1974, Johnson and Manning 1974, Haller 1976, Sutton et al. 1992). Infestations are often reported in terms of percent cover or percent frequency at the lake scale, while dry weight biomass per square meter is generally reported for scientific studies at laboratory and field scales. Studies have also focused on growth from vegetative propagules and subsequent propagule production as endpoints (Van and Steward 1990, Sutton et al. 1992, Spencer et al. 2000). Nonetheless, when viewed in terms of overall productivity, submersed aquatic plants produce much less biomass when compared to their terrestrial counterparts (Westlake 1963, Grace and Wetzel 1978). Moreover,

prior studies have demonstrated that Eurasian watermilfoil is not particularly productive when biomass production is compared to other submersed native species (Grace and Wetzel 1978, Smith and Barko 1990). While the vast majority of scientific trials have focused on changes in biomass or propagule production as an endpoint for evaluating growth or response of hydrilla or Eurasian watermilfoil to control methods, measuring the change in total stem length may be a useful technique for explaining rapid rates of lateral plant spread in a water body. Emphasis has often been placed on hydrilla and Eurasian watermilfoil concentrating biomass in a surface canopy (Haller and Sutton 1975, Grace and Wetzel 1978, Madsen 1997), yet production of numerous lateral stems and stolons and the subsequent rates of extensions may better explain rapid radial expansion. Hydrilla stolons, for example, have been documented to expand a colony radially at a rate of 4 cm d^{-1} (Madsen and Smith 1999). While the statement that "hydrilla can grow an inch a day" (Langeland 1996) initially sounds impressive, it is unlikely this rate of extension would explain the ability of hydrilla to form large contiguous surface canopies on hundreds of thousands of acres in lakes and reservoirs.

To evaluate the rate of growth of hydrilla and Eurasian watermilfoil, a series of mesocosm trials were conducted to mea-

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