Note

Evaluation of diquat, endothall, and diquat plus endothall under short exposure times for the management of flowering rush (*Butomus umbellatus*)

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INTRODUCTION

Flowering rush (Butomus umbellatus L.) possesses a dynamic ability to establish and thrive in the littoral zones of quiescent and flowing water systems, either as an emergent plant along shorelines (up to 1.3 m) or as a submersed plant in deeper water (up to 6 m) (Countryman 1970, Madsen et al. 2016c). Once established, flowering rush can form monotypic stands that crowd out desirable native vegetation, limit recreational water use, reduce water flow, and impact native fish species (Boutwell 1990, Parkinson et al. 2010). Subsequently, its ability to grow in a variety of habitats and conditions has led to its spread and establishment in water bodies where a high rate of water exchange can occur over a relatively short period of time. This presents a unique challenge for the management of this species using submersed herbicide applications because water exchange can be too rapid to maintain adequate herbicide concentrations in potential treatment areas (Getsinger et al. 1996).

One such waterbody is the McNary Reservoir (Wallula Lake; 15,378 ha) on the Columbia River in the tricities area of Washington State. McNary is a run-of-the-river reservoir, and as such acts as a hydrodynamic system—with constantly flowing water. Flowering rush was first reported in the upper portion of the reservoir at the mouth of the Yakima River in 2008. As of 2019, the plant has been documented at numerous locations within the reservoir, in small, isolated patches and stands < 1 ha in size. The majority of the flowering rush in these locations never breaks the water surface and remains in the submersed growth habit. In the shallow littoral zones (1 to 2 m) adjacent to the reservoir shoreline, flowering rush grows in mixed stands of other submersed species (e.g., elodea, milfoils, pondweeds); however, in deeper areas (2 to 6 m) there is limited competitive

pressure from other submersed plants. This dynamic reservoir system presents a complex matrix to determine which treatment options will be best to control flowering rush—particularly using herbicides in short concentration exposure time (CET) settings. Water exchange evaluations at multiple flowering rush sites during 2018 and 2019 demonstrated that rhodamine WT (RWT) dye dissipated quickly, and dye half-lives ranged between 0.5 and 8.0 h.

Currently, there are limited strategies for providing longterm selective control of flowering rush, particularly in hydrodynamic systems. Attempts to mechanically harvest flowering rush in Detroit Lakes, MN, during the 1990s and 2000s were ineffective (Marko et al. 2015) and likely resulted in its spread throughout the watershed due to the displacement of rhizomes and rhizome buds, which are an important factor for flowering rush dispersal (Hroudova et al. 1996). Turnage et al. (2019b) documented mechanical clipping of flowering rush shoots to be an effective management technique; however, it did not provide any additional level of control in comparison to sequential herbicide applications. Inefficiencies associated with timely removal and disposal of clipped/harvested vegetation and high operating costs (Bryant 1970, Bryant 1974, Culpepper and Decell 1978, Haller 2009) further limit the use of mechanical control for large-scale flowering rush management operations. At present, there are no biological control agents available for flowering rush.

Small-scale research and field demonstrations have documented some success with submersed treatments of contact herbicides, but multiple treatments are often necessary to provide acceptable levels of control (Poovey et al. 2012, Poovey et al. 2013, Madsen et al. 2016a,b, Parsons et al. 2019, Turnage et al. 2019a,b). Water exchange evaluations with RWT dye, in conjunction with endothall (dipotassium salt, 3 mg L^{-1}) or diquat (0.37 mg L^{-1}) treatments targeting flowering rush at Detroit Lakes, MN, revealed that dye concentrations dissipated quickly (half-life: 2 to 12 h) out of treatment plots (Skogerboe 2010). Subsequently, endothall treatments were not effective at reducing above- or belowground biomass following a single treatment; however,

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two sequential diquat treatments (0.37 mg L^{-1}) were deemed effective at reducing aboveground biomass (Madsen et al. 2012). Additional larger scale field demonstrations at Detroit Lakes, MN, ultimately showed that multiple diquat treatments annually were effective in reducing above- and belowground biomass as well as rhizome bud density with minimal adverse effects on native plant communities (Madsen et al. 2013, Madsen et al. 2016b, Turnage et al. 2016). These findings indicate that sequential contact herbicide treatments on an annual basis can contribute to long-term flowering rush control by reducing vegetative growth and exhausting energy reserves (i.e., roots, rhizomes).

To date, small-scale research evaluating contact herbicides under short (e.g., less than 6 h) CETs for the management of flowering rush is lacking, because much of the available literature has primarily focused on submersed species such as hydrilla (Hydrilla verticillata L.f. Royle) and Eurasian watermilfoil (Myriophyllum spicatum L.) (Netherland et al. 1991, Poovey and Skogerboe 2003, Glomski et al. 2005, Skogerboe et al. 2006, Mudge and Theel 2011). In addition, a commercially available premix formulation of diquat plus endothall (hereafter referred to as diquat plus endothall) has not been previously evaluated for flowering rush control. Therefore, the objective of this study was to evaluate maximum concentrations of the herbicides diquat, endothall, and diquat plus endothall, across a range of short exposure times, for managing flowering rush. It is hypothesized that the diquat and diquat plus endothall treatment will perform similarly, because the diquat concentration is essentially equivalent when each is applied at the maximum labeled rate. However, diquat plus endothall labeling allows for drip or metered applications in nonirrigation, flowing water sites (United Phosphorous Inc. 2017); whereas other available aquatic use diquat products do not have the necessary labeling language for these applications. Thus, providing these data is beneficial for resources managers because it offers supporting documentation to pursue proper permitting and the development of appropriate management plans linked to water exchange processes specific to the targeted treatment area, particularly in sites where a drip or metered application might be warranted.

MATERIALS AND METHODS

Studies were conducted at the U.S. Army Engineer Research and Development Center (USAERDC) in Vicksburg, MS to evaluate diquat, endothall, and diquat plus endothall under short CETs for managing flowering rush. The studies were conducted in temperature $(25 \pm 1 \text{ C})$ and light (14 : 10 light vs. dark) controlled environmental growth chambers equipped with 55-L aquaria from April to August 2019 (run one) and September 2019 to January 2020 (run two). Plants were cultured from rhizome collected from the Columbia River at Kennewick, WA (46°12'49.75"N, 119°04'35.70"W) utilizing a hand-operated Ponar^{®1} grab sampler. Healthy rhizome segments (5 to 8 cm in length) were utilized for planting during both experimental runs. Planting methods consisted of placing one rhizome segment with at least one attached bud into 946-ml plastic pots filled

with topsoil² and amended with 1.04 ± 0.05 g of slowrelease fertilizer³ to stimulate plant growth. Each segment was placed onto the topsoil and capped with a 1-cm layer of masonry sand to prevent suspension of soil and loss of nutrients in the water column. Three pots were placed into each of the 55-L aquaria and filled with approximately 15 cm of nutrient-amended water (Smart and Barko 1985). In order to facilitate successful sprouting and growth of shoots, the water level in each aquarium was maintained at 15 cm for a period of 2 wk. At 2 wk after planting (WAP) all potted rhizomes had produced healthy green shoots (20 to 40 cm in length) and aquaria water level was increased from 15 to 45 cm. At 3 WAP, the water level was increased to 60 cm where it was maintained throughout the duration of the study. By 4 WAP, flowering rush shoots had begun to spread across the top and/or emerge approximately 10 to 15 cm above the water surface.

The experimental design consisted of a 3 by 3 factorial plus a nontreated reference, also referred to as an "augmented factorial" (Lentner and Bishop 1993, Marini 2003), with herbicide and exposure time as main effect factors. Each aquarium, containing three pots of flowering rush, was randomly assigned to one of three herbicide treatments: diquat⁴ (0.37 mg L^{-1}) , endothall⁵ (5 mg L^{-1}) , and diquat plus endothall⁶ (0.36 mg L^{-1} + 1.8 mg L^{-1}). At treatment, flowering rush plants appeared vibrant and healthy, possessing rigid, upright, shoots either emerging and/or spreading across the water surface. Herbicide treatments were administered at the maximum labeled rate as an in-water injection and maintained for 3-, 6-, and 12-h exposure times. At the termination of assigned exposure periods, aquaria were drained and refilled twice with reverse osmosis (RO) water and a third time with fresh growth culture solution (Smart and Barko 1985) to remove aqueous herbicide residues.

Each herbicide and exposure time combination was replicated during run one and (n = 4) and run two (n = 3). Due to a limited amount of rhizome material, replicates for run two were scaled down from four to three replicates. Harvest of viable flowering rush shoots and rhizome biomass were conducted prior to treatment and at 4, 8, and 12 wk after treatment (WAT). Harvest consisted of removing one pot from each aquarium at each of the assigned harvest periods. All viable shoot and rhizome biomass were collected, sorted, dried at 65 C, and weighed to the nearest 0.01 g. In order to make comparisons more easily to previously published small-scale and field-scale flowering rush research, dry weight biomass per pot was used to estimate g dry-weight (DW) biomass m⁻². Pretreatment biomass for shoot and rhizome material were 89.1 \pm 12.7 and 75.6 \pm 7.4 g DW m⁻² respectively.

Shoot and rhizome biomass data were subject to two-way ANOVA in SAS[®] version 9.4⁷ using a generalized linear mixed model (Proc Glimmix) to test for significant herbicide, exposure time, and interaction effects at each harvest period. The experimental design and the analysis was chosen to evaluate the relative contribution of "herbicide" and "exposure time" to the reduction of flowering rush shoot and rhizome biomass. Experimental run was included in the model as a random effect.

		Dry Weight Bio	mass g m ⁻² (Percent Bioma	ss Reduction Compared to	the Reference)	
	4 W.	AT	8 W.	AT	12 W	AT
	Rhizome	Shoot	Rhizome	Shoot	Rhizome	Shoot
Reference ¹	165.8 ± 39.6	78.6 ± 21.8	283.8 ± 85.0	58.9 ± 24.3	231.5 ± 102.3	42.4 ± 16.1
Main effect factor Herbicide ²						
Diquat	$59.9 \pm 8.5 \text{ NS} (64 \pm 5\%)$	3.4 ± 0.8 NS (96 $\pm 1\%$)	50.0 ± 9.5 NS (82 $\pm 4\%$)	2.2 ± 0.5 NS (96 $\pm 1\%$)	34.1 ± 9.2 NS $(85 \pm 4\%)$	$1.5 \pm 0.6 \text{ a} (96 \pm 2\%)$
Endothall	58.6 ± 10.3 NS $(65 \pm 6\%)$	6.0 ± 1.9 NS $(92 \pm 3\%)$	53.6 ± 10.8 NS (81 $\pm 4\%$)	8.5 ± 3.3 NS $(85 \pm 6\%)$	42.5 ± 9.8 NS $(82 \pm 4\%)$	$8.8 \pm 3.2 \text{ b} (79 \pm 8\%)$
Diquat + Endothall	57.3 ± 12.1 NS $(65 \pm 7\%)$	2.3 ± 0.8 NS $(97 \pm 1\%)$	47.0 ± 10.2 NS $(83 \pm 4\%)$	3.1 ± 1.4 NS $(95 \pm 2\%)$	30.7 ± 7.9 NS $(87 \pm 3\%)$	$1.5 \pm 0.5 a (97 \pm 1\%)$
Exposure time						
$\hat{3}$ h	56.7 ± 10.6 NS ($66 \pm 6\%$)	3.9 ± 1.2 NS $(95 \pm 2\%)$	54.7 ± 9.6 NS (81 $\pm 6\%$)	3.9 ± 1.3 NS $(93 \pm 2\%)$	48.3 ± 11.6 NS $(79 \pm 5\%)$	5.7 ± 2.4 NS $(87 \pm 6\%)$
6 h	65.8 ± 10.6 NS $(60 \pm 6\%)$	5.3 ± 1.8 NS $(93 \pm 2\%)$	37.9 ± 8.2 NS $(87 \pm 3\%)$	$1.5 \pm 0.7 \text{ NS} (97 \pm 1\%)$	33.0 ± 6.6 NS ($86 \pm 3\%$)	2.6 ± 1.2 NS $(94 \pm 3\%)$
12 h	53.3 ± 9.7 NS ($68 \pm 6\%$)	2.6 ± 0.8 NS $(97 \pm 1\%)$	58.0 ± 11.9 NS $(80 \pm 4\%)$	8.5 ± 3.3 NS $(85 \pm 6\%)$	26.0 ± 7.4 NS (89 $\pm 3\%$)	3.5 ± 2.4 NS $(92 \pm 6\%)$
¹ Reference data were not ir ² Data for each main effect fi separation test ($\alpha = 0.01$); n	ncluded in the statistical analysis actor are pooled over all levels $\alpha = 21$.	f the other factor. Means withi	in a column for each main effect	factor followed by the same le	etter are not significantly differe	nt based on LSMEANS mean

Treatment (WAT) with submersed applications of diquat (0.37 mg L^{-1}), endothall (5 mg L^{-1}),

AFTER '

WK .

SE) 4, 8, AND 12

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TABLE 1. THE RESPONSE OF FLOWERING RUSH RHIZOME AND SHOOT BIOMASS (MEAN

Nontreated reference data are presented but were not included in the statistical analysis. No significant interaction effects were detected at any of the three harvest periods; thus, if significant main effects were detected, means were separated by least square means (LSMeans) tests at 99% significance level ($\alpha = 0.01$).

RESULTS AND DISCUSSION

Plant injury in response to all herbicide treatments occurred very rapidly. Flowering rush shoots became chlorotic and began to lose integrity in as little as 2 d after treatment (DAT) and shoots were necrotic by 1 WAT. At 4 WAT, all herbicides performed similarly and reduced flowering rush rhizome and shoot biomass 64 to 65% and 92 to 97% of the nontreated reference, respectively (Table 1). In addition, all exposure times evaluated performed similarly, reducing rhizome and shoot biomass 60 to 68% and 93 to 97% of the nontreated, respectively (Table 1). Harvest data 8 WAT resulted in no differences among herbicides or exposure times; however, at 12 WAT diquat and diquat plus endothall provided a greater reduction of shoot biomass, 96 and 97%, of the nontreated reference respectively, compared to endothall (79%) (Table 1).

These results agree with previously conducted small-scale studies investigating submersed applications of diquat and/or endothall for the management flowering rush. Poovey et al. 2012 reported diquat (0.37 mg L^{-1} at 6- and 12-h exposures) and endothall (1.5 mg L^{-1} for 12- and 24-h exposure) effectively reduced shoot biomass 4 WAT. In addition, mesocosm trials by Madsen et al. (2016c) and Turnage et al. (2019a) documented a single application of diquat to be effective at reducing above- and belowground biomass at 8 and 52 WAT, respectively. In the current study, significantly greater control of flowering rush shoot biomass 12 WAT with diquat and diquat plus endothall compared to endothall alone indicates that diquat was the primary driver of flowering rush control and the addition of endothall did not provide any increased efficacy. Further, these data indicate that diquat or products containing diquat can be effective at controlling flowering rush at CETs greater than or equal to 3 h.

It should be noted that under operational treatment scenarios, in areas of high bulk water exchange, it is unlikely that the herbicide concentrations evaluated in the current studies would be maintainable utilizing traditional submersed herbicide application techniques due to dilution and dissipation out of the treatment site. Alternative treatment strategies such as drip or metered applications are commonly used to manage nuisance vegetation in flowing water systems and might be a potential option for the management of flowering rush in areas with high bulk water exchange. Currently, all available aquatic-use, diquat-only products lack the necessary labeling for these application methods. However, the diquat- plus- endothall formulation is labeled for flowing water drip or metered applications (United Phosphorous Inc. 2017) and provides managers with an additional management tool for treatment areas where drip or metering applications of diquat plus endothall might be practical. Further, diquat use rates vary depending upon specific labels, and maximum efficacy might not be achievable because some labels limit use, and application rates are calculated per surface acre only up to a specified water depth. This could increase difficulties when managing flowering rush in areas where the average water depth is greater than that specified on those product labels.

Due to no significant exposure time effect at any of the harvest periods, it can be concluded that all exposures resulted in equal control of flowering rush shoot and rhizome biomass under controlled experimental conditions. In addition, no significant herbicide effect was detected for rhizome biomass at any of the three harvest periods. However, the significant herbicide effect for control of flowering rush shoots 12 WAT indicates that diquat or products containing diquat are the best option for optimal reductions of flowering rush shoot biomass. Nonetheless, this research indicates that the contact herbicides evaluated, if applied in areas where CETs are greater than or equal to 3 h, can be effective tools for managing vegetative (e.g., shoots/leaves) and reproductive (e.g., rhizomes) structures of flowering rush. Future research should investigate the utility of drip or metered applications and herbicide efficacy under shorter CETs (< 3 h) against mature, well-established plants.

SOURCES OF MATERIALS

¹Petite Ponar[®] Grab Sampler, Wildco, 86475 Gene Lasserre Blvd., Yulee, FL 32097.

²Gardenese Topsoil[®], Phillips Bark, 428 County Farm Lane, Brookhaven, MS 39601.

³Osmocote[®], The Scotts Company, P.O. Box 606, Marysville, OH 43040.

⁴Reward[®], Syngenta Crop Protection LLC, P.O. Box 18300, Greensboro, NC 24719-8300.

 5 Aquathol K®, United Phosphorous Limited, 630 Freedom Business Center, Suite 402, King of Prussia, PA 19406.

⁶Aquastrike, United Phosphorous Limited, 630 Freedom Business Center, Suite 402, King of Prussia, PA 19406.

 $^7\mathrm{SAS}$ version 9.4 statistical software. SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513-2414.

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