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Selective control of flowering rush in mesocosms and field sites

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ABSTRACT

Flowering rush is an invasive aquatic plant species that is spreading across the northern United States and southern Canada. Flowering rush can displace many native aquatic plant species such as hardstem bulrush, an emergent aquatic plant that is used as spawning habitat by many native fish species. Previous studies show that repeated applications of contact herbicides can control flowering rush; however, it is unknown if these herbicides can be used to selectively control flowering rush co-occuring with hardstem bulrush. The purpose of this study was to determine if selective control of flowering rush was possible with repeat contact herbicide applications in field and mesocosms trials. In field trials, flowering rush leaf density was reduced 99% and 92% at 8 wk after initial treatment (WAIT) in years one and two, respectively, whereas hardstem bulrush leaf density was not affected. In mesocosms, flowering rush and hardstem bulrush were exposed to repeat submersed injections of the contact herbicides diquat, endothall, copper, carfentrazone-ethyl, and flumioxazin. Endothall reduced aboveground biomass of flowering rush by 69% compared to reference plants at 8 WAIT; no other herbicides affected aboveground biomass of flowering rush. Diquat reduced belowground biomass by 77% compared to reference plants at 8 WAIT, but the other herbicides had no effect. None of the herbicides tested in mesocosms affected above- or belowground biomass of hardstem bulrush when compared to nontreated reference plants at 8 WAIT. Future studies should investigate concentration exposure time requirements of endothall and diquat for flowering rush control.

Key words: Butomus umbellatus, chemical control, diquat, endothall, invasive species.

INTRODUCTION

Flowering rush (*Butomus umbellatus* L.) is an invasive aquatic plant that is spreading across the northern United States and southern Canada (Core 1941, Countryman 1970, Anderson et al. 1974, Kliber and Eckert 2005). Flowering rush is native to Eurasia, but was introduced to North America in the late 1800s, probably in shipping ballast (Bellaud 2009). In the native and introduced ranges, two biotypes of flowering rush (diploid and triploid) exist suggesting multiple introductions to North America (Kliber and Eckert 2005). Both biotypes are capable of aggressive growth and rely primarily on vegetative reproduction to expand within a site and to colonize new areas (Hroudova et al. 1996). Flowering rush can grow as submersed or emergent plants and thrives as either a marginal species on the edge of waterbodies, as an emergent in shallow water, or fully submersed in deeper aquatic sites (Hroudova et al. 1996, Marko et al. 2015, Madsen et al. 2016b).

Flowering rush leaves arise from underground rhizomes and grow vertically through the water column at high densities (Crow and Hellquist 2000); this can negatively impact access to aquatic resources for human recreational (i.e., skiing, boating, fishing), agricultural (irrigation canals), and drainage purposes (Marko et al. 2015, Madsen et al. 2016b). Additionally, flowering rush displaces native plants, thereby disrupting ecosystem processes in infested sites (Marko et al. 2015, Madsen et al. 2016b).

Hardstem bulrush [*Schoenoplectus acutus* (Muhl. ex Bigelow) A. Love & D. Love] is an emergent plant species that has been displaced by flowering rush in the Detroit Lakes chain of lakes (major basins are Big and Little Detroit Lakes, Lake Melissa, and Lake Sallie) on the Pelican River near the city of Detroit Lakes, MN (46° 48′ 44.388″ N, 95° 50′ 35.7828″ W; degrees, minutes, and seconds). Similar to flowering rush, hardstem bulrush leaves arise from rhizomes under the sediment surface and extend up into the water column (Crow and Hellquist 2000). Hardstem bulrush is highly valued by the Minnesota Department of Natural Resources to reduce shoreline erosion and as habitat for spawning and young-of-the-year fish (Radomski and Goeman 2001, Reed and Pereira 2009). A permit from the Minnesota Department of Natural Resources is required for any management of emergent vegetation in public waters, including hardstem bulrush (Minnesota Department of Natural Resources 2019). Flowering rush has been in the Pelican River system for decades (Marko et al. 2015). Resource managers have attempted to control flowering rush in the Detroit Lakes via mechanical and chemical control options. Turnage et al. (2019b) showed that mechanical control of flowering rush is possible if frequently repeated. However, harvesting of nuisance aquatic vegetation has also been shown to have negative aspects, namely the release and spread of vegetative propagules (Culpepper and Decell 1978, Haller 2009b).

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The extent of flowering rush infestation was such in the Detroit Lakes system that mechanical control options were abandoned for chemical control measures to maximize financial resources for management activities and to reduce the spread of flowering rush propagules within the system. Since 2011, resource managers in the Detroit Lakes have been managing flowering rush with submersed herbicide applications (Madsen et al. 2016a). However, permits for herbicide applications were not issued in areas that contain hardstem bulrush due to the plants' value as spawning habitat for native fish (Reed and Pereira 2009). Mixed stands of hardstem bulrush and flowering rush that are not treated can act as source populations for flowering rush propagules that can be dislodged and colonize new areas or recolonize managed sites in the Detroit Lakes system. Selective control of flowering rush in mixed stands with bulrush is desirable because it would lessen the potential sources of flowering rush for reinfestation in this system.

Currently, resource managers in the Detroit Lakes are using two applications of diquat (0.37 mg L^{-1}) administered 1 mo apart for operational control of monotypic flowering rush stands (Madsen et al. 2016a, Turnage et al. 2018). Due to the prohibition of treating mixed stands of flowering rush and hardstem bulrush with diquat, it is unknown if selective control with submersed diquat applications is possible; however, Madsen et al. (2016a) documented that native plant and macroalgae species in the Detroit Lakes persisted or recolonized diquat-treated sites within weeks of diquat applications. Similarly, Parsons et al. (2019) showed that both macroalgae and some pondweeds (*Potamogeton* spp.) in Silver Lake, WA were capable of persisting in sites treated with diquat.

There are currently five contact herbicides registered for general use in aquatic sites in the U.S.: diquat, endothall, copper, flumioxazin, and carfentrazone-ethyl (Anonymous 2016a, Anonymous 2016b, Anonymous, 2016c, Shaner 2014), although endothall has been documented as having some systemic properties (Ortiz et al. 2019). Dye studies conducted in the Detroit Lakes, MN determined that water exchange rates would be more favorable to the use of contact herbicides than systemic for controlling flowering rush (Madsen et al. 2012). Diquat (0.37 mg L^{-1}) at 6- and 12-h exposure times (ET) controlled aboveground biomass of flowering rush from Minnesota populations but not belowground biomass (Poovey et al. 2012). Similarly, endothall (1.5 mg L^{-1} and 3.0 mg L^{-1} a.i.; dipotassium salt) for 12 and 24 ET controlled aboveground but not belowground biomass of flowering rush from Minnesota, whereas endothall (3.0 mg L^{-1}) with a 24 h ET controlled above- and belowground biomass from Idaho flowering rush populations (Poovey et al. 2012). Flumioxazin (0.4 mg L^{-1}) with a 24-hr ET controlled aboveground but not belowground biomass of flowering rush from Idaho, but flumioxazin (0.2 mg L^{-1}) at 12 and 24 h ET did not affect plants from Idaho or Minnesota (Poovey et al. 2012). However, these earlier studies did not consider how selective these applications could be on nontarget vegetation.

Therefore, the objectives of these studies were to 1) determine if diquat could selectively control flowering rush in field trials using the current operational control pattern for the Detroit Lakes system and 2) determine if other contact herbicides could selectively control flowering rush when grown with hardstem bulrush in mesocosms.

MATERIALS AND METHODS

Field trials

Permitting by the Minnesota Department of Natural Resources limited field test sites; therefore, two 2.02 ha (5-ac) sites with flowering rush and hardstem bulrush in Lake Sallie, MN were selected to test for selective control of flowering rush using repeated diquat applications in 2015 and 2016. One site was a reference site and the other was a treatment site. The reference site was located immediately north (upstream) of where the Pelican River entered Lake Sallie. The treatment site was located 0.8 km (0.5 mi) south (downstream) of the reference site and the Pelican River entrance in order to prevent cross contamination of herbicide into the reference site. Diquat[1] (0.37 mg L^{-1}) was applied as a submersed injection twice (June 30 and August 10 in 2015 and 29 June and 2 August in 2016) each year to the treatment site; average depth was approximately 0.9 m (3 ft) in each site. The permit did not allow destructive sampling within hardstem bulrush sites; therefore, presence/absence was recorded and leaf number of each species was counted at multiple points across the sites (26 points for the treatment site and 19 for the reference site) to measure diquat control. Presence/absence data allowed for the calculation of percent occurrence of each species in each plot. Leaves were counted in June prior to treatment and again 8 wk after initial treatment (WAIT). A polyvinyl chloride (PVC) frame (0.1 m^2) was placed on the water surface at each point and leaves within the frame were counted for each species.

Paired *t*-tests were used to analyze leaf densities within sites for each species pre- and posttreatment each year. Two-by-two contingency table analyses were used to detect differences in the percent occurrence of infested points within a site for each year. All statistics were conducted at the $P = 0.05$ significance level (Analytical Software 2009, R Core Team 2018).

Mesocosm trials

This study was conducted in 2016 and again in 2017 at the Aquatic Plant Research Facility (APRF) at Mississippi State University (MSU). Flowering rush and hardstem bulrush were grown in 20 outdoor 378 L (100 gal) mesocosms. Six 3.78 L (1 gal) pots of sand amended with a slow-release fertilizer[2] were placed in each mesocosm. Three pots per mesocosm were planted with flowering rush rhizomes (8 cm long) and three were planted with hardstem bulrush rhizomes (8 cm long). Mesocosms were filled to a volume of 216 L (40.6 cm [16]-in depth). Plants were allowed to establish for 2 mo prior to exposure to herbicides. Prior to herbicide treatments, plants in two mesocosms were harvested to establish a plant growth baseline. Harvesting consisted of removing plants from pots and separating plant structures into above- and belowground biomass. Biomass was placed in labeled paper bags then placed in a forced air oven for 5 d at 70 C to remove moisture from plant tissues. After drying, biomass was weighed, and weights recorded.

After the pretreatment harvest, the remaining mesocosms were buffered to a pH of 6.5 to 7.0 prior to herbicide application to prevent rapid breakdown of some herbicides (protoporphyrinogen oxidase [PPO] inhibitors). In total, there was a nontreated reference and five herbicide application rates: diquat[3] (0.19 mg L^{-1}), endothall[4] (3.0 mg L^{-1}), copper-ethylenediamine[5] (1.0 mg L^{-1}), carfentrazone-ethyl[6] (0.2 mg L^{-1}), and flumioxazin[7] (0.4 mg L^{-1}). Four WAIT, herbicides were applied again. Twelve hours after each application, mesocosms were drained and refilled with nontreated water. At 8 WAIT, plants were harvested in the same manner as pretreatment specimens.

Normality was confirmed prior to running a mixed ANOVA procedure using year as a random effect and biomass as a fixed effect (R Core Team 2018). Any differences detected in treatment means were further separated using a Tukey's post-hoc test at the $P = 0.05$ significance level (R Core Team 2018).

RESULTS AND DISCUSSION

Field trials

Diquat applications reduced flowering rush mean leaf density 8 WAIT by 99% in 2015 ($P = 0.0068$) and 93% in 2016 ($P = 0.0149$) when compared to pretreatment leaf densities (Figure 1A). Mean leaf density prior to diquat applications was 189 and 27 leaves m^{-2} in 2015 and 2016, respectively. Posttreatment density was 1.5 and 1.9 leaves m^{-2} in 2015 and 2016, respectively. Diquat applications did not affect hardstem bulrush leaf densities 8 WAIT either year when compared to pretreatment leaf densities (Figure 1A). Leaf densities within the reference plot did not change for either species (Figure 1B). Hardstem bulrush leaf density ranged from 19 to 61 leaves m^{-2} across both plots and years.

The percent occurrence (presence or absence) of flowering rush in the treatment site declined from 42 to 12% (71% decrease; $P = 0.0124$) in 2015 and from 39 to 12% (69% decrease; $P = 0.0250$) in 2016 at 8 WAIT. Hardstem bulrush percent occurrence in the treatment site did not change in either year, and ranged from 46 to 62% over both years ($P < 0.05$). In the reference site, neither flowering rush nor hardstem bulrush percent occurrence changed either year ($P < 0.05$). Flowering rush ranged from 46 to 79% occurrence, whereas hardstem bulrush ranged from 39 to 47% occurrence in the reference site over both years.

This work shows similar results to other studies: repeated diquat applications reduced aboveground flowering rush and percent occurrence in field sites (Madsen et al. 2016a, Parsons et al. 2019). This is the first work to show that diquat selectively controls flowering rush growing in mixed stands with hardstem bulrush in field sites (Figure 1A). Madsen et al. (2016a) reported a 99% reduction in aboveground flowering rush biomass and 60% reduction in infested survey points in a single growing season following two submersed applications of diquat with minimal impacts to native species. Parsons et al. (2019) showed a 96% reduction of flowering rush aboveground biomass in one growing season using the same protocol as Madsen et al. (2016a); however, the percent occurrence of flowering rush in their treatment site did not decline until the third year of treatments. Parsons et al. (2019) also did not detect any negative impacts to the percent occurrence of native plant species growing in their treatment site.

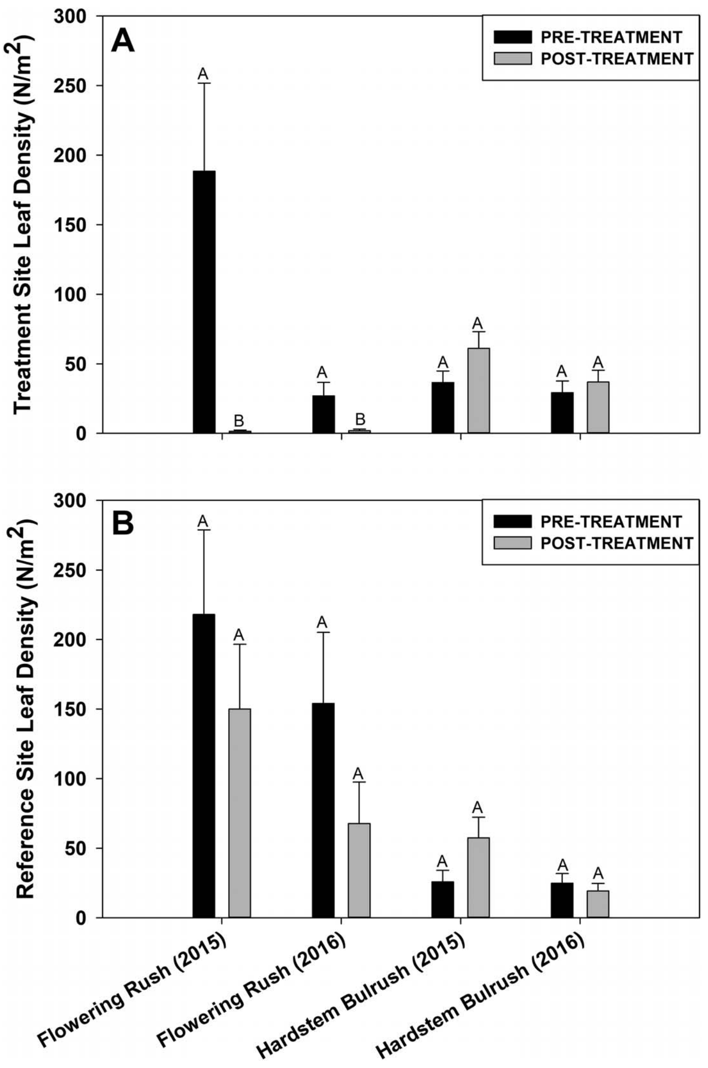

Figure 1. Leaf density of flowering rush and hardstem bulrush in treatment (A) and reference plots (B) pre- and post-treatment with diquat (0.37 mg L^{-1}) in June and July of 2015 and 2016 at a 2.02 ha (5 ac) field site in Lake Sallie, MN. Species were analyzed separately within sites and years. Error bars are one standard error of the mean. Shaded bars that share a letter within a species and year are not different from one another using a paired *t*-test at the *P* .05 significance level.

Mesocosm trials

There were no statistical differences among herbicide treatments (diquat, endothall, copper, carfentrazone-ethyl, and flumioxazin) for flowering rush aboveground biomass; however, endothall reduced aboveground biomass 68.7% when compared to nontreated reference plants 8 WAIT while the other herbicides did not (Table 1). Similarly, there

Table 1. Percent reduction of flowering rush and hardstem bulrush aboveground (AG) and belowground (BG) biomass by contact herbicides when compared to a non-treated reference. Superscript letters denote level of significance; within a column, treatments with the same letters are not significantly different from one another at the $P = 0.05$ significance level using a mixed model ANOVA followed by a Tukey's post hoc test.

Treatment	Flowering Rush AG	Flowering Rush BG	Hardstem Bulrush AG	Hardstem Bulrush BG
Reference	NA[1] a	NA[1] a	NA[1] a	NA[1] a
Diquat 0.19 mg L^{-1}	−58.5 ab	−77.2 b	−23.0 a	−19.5 a
Endothall 3.0 mg L^{-1}	−68.7 b	−68.5 ab	−39.2 a	−43.4 a
Copper 1.0 mg L^{-1}	−33.6 ab	−57.6 ab	−20.6 a	−4.15 a
Carfentrazone-ethyl 0.2 mg L^{-1}	−30.4 ab	−32.8 ab	−26.5 a	−15.5 a
Flumioxazin 0.4 mg L^{-1}	−61.3 ab	−53.9 ab	−62.4 a	−41.6 a

[1] Biomass units are grams dry weight per square meter (g DW m^{-2}). The mean reference value for flowering rush AG was 207.2 g DW m^{-2} with a standard error of 56.15; for flowering rush BG was 1,295.1 g DW m^{-2} with a standard error of 490.01; for hardstem bulrush AG was 1,179.36 g DW m^{-2} with a standard error of 177.62; and for hardstem bulrush BG was 3,584.22 g DW m^{-2} with a standard error of 348.79.

were no statistical differences between herbicide treatments (diquat, endothall, copper, carfentrazone-ethyl, and flumioxazin) for flowering rush belowground biomass (Table 1), although diquat was the only herbicide to significantly reduce belowground biomass (77.2%) compared to non-treated reference plants at 8 WAIT. None of the herbicide treatments (diquat, endothall, copper, carfentrazone-ethyl, or flumioxazin) affected hardstem bulrush above- or belowground biomass at 8 WAIT when compared to reference plants (Table 1).

Poovey et al. (2012) found that one application of endothall (1.5 and 3.0 mg L^{-1}) at 12 and 24 h ET controlled aboveground, but not belowground flowering rush biomass from Minnesota 4 wk after treatment (WAT); however, they found that endothall (3.0 mg L^{-1}) with a 24 h ET controlled both above- and belowground biomass of flowering rush from Idaho 6 WAT. Both flowering rush populations were found to be triploid, suggesting that even within biotypes some populations can respond differently than others to chemical control measures. Flowering rush stock cultures at the MSU APRF were originally collected in the Detroit Lakes, MN, which are the same populations as the Minnesota plants used by Poovey et al. (2012). Interestingly, we found that two sequential endothall treatments controlled aboveground but not belowground flowering rush biomass 8 WAIT, which, when taken with the findings of Poovey et al. (2012), suggest the second endothall application might not enhance control of the Minnesota flowering rush. In contrast to Poovey et al. (2012), we did not observe flowering rush belowground biomass control by endothall (Table 1). Poovey et al. (2012) found that diquat (0.37 mg L^{-1}) at 6 and 12 h ET controlled aboveground flowering rush biomass, whereas we found that a reduced diquat rate (0.19 mg L^{-1}) with a 12 h ET only controlled belowground biomass (Table 1). Our findings matched those of Poovey et al. (2012) with no flowering rush biomass reduction using flumioxazin (0.4 mg L^{-1}) with a 12 h ET.

Diquat (0.37 mg L^{-1}) with a 12 h ET controlled both above- and belowground flowering rush biomass 8 WAIT (Turnage et al. 2019a), but the present research found that only belowground biomass was controlled with the reduced rate (Table 1). Higher diquat rates (0.37 mg L^{-1}) sustained control to 52 WAIT (Turnage et al. 2019a). Interestingly, one maximum rate diquat application (0.37 mg L^{-1}) provided the same level of flowering rush biomass control as four maximum rate applications (Turnage et al. 2019a), whereas this work found that two applications at a reduced rate did not control aboveground biomass (Table 1), suggesting that research investigating the concentration/exposure time (CET) relationship of diquat and flowering rush control should be conducted.

In another study, two applications of diquat (0.19 mg L^{-1}) with a 12 h ET controlled both above- and belowground biomass 16 WAIT; however, by 52 WAIT biomass was no different from reference plants (Turnage et al. 2019b). The present research found that only belowground flowering rush biomass was controlled with this protocol 8 WAIT (Table 1). The findings of Turnage et al. (2019b) and this work suggest that although in-season belowground flowering rush biomass control is possible with reduced diquat rates, another study suggests higher diquat rates and/or longer ETs are needed to attain long-term control (Turnage et al. 2019a).

Care should be taken to assess environmental variables in aquatic environments (water pH, water temperature, turbidity, depth, etc.) prior to using any herbicide labeled for use in these environments because these variables can impact herbicide efficacy on target plants. For example, hydrolysis of flumioxazin and carfentrazone-ethyl (PPO inhibitors) increased as water pH increased (Ngim and Crosby 2001, Koschnick et al. 2004, Mudge et al. 2010). Mudge et al. (2010) showed that flumioxazin efficacy against hydrilla [*Hydrilla verticillata* (L.f.) Royle] decreased as water pH increased. In another example, endothall was readily metabolized by aquatic microbes as a source of carbon (Sikka and Saxena 1973). This metabolism likely increases as water temperatures rise and microbial metabolism increases. Diquat rapidly binds to sediment particles and organic matter (Shaner 2014) and was shown to decrease in efficacy against Brazilian elodea (*Egeria densa* Planch.) as turbidity increased (Poovey and Getsinger 2002). Lastly, depth can affect herbicide efficacy because liquid herbicides typically do not move through thermoclines that develop in lakes that stratify; thus, herbicide applicators might need to inject herbicide at multiple depths or use granular herbicides in order to control target vegetation growing through a thermocline (Haller 2009a).

No negative impacts of submersed herbicide applications to hardstem bulrush biomass were detected in field sites (Figure 1A) or mesocosms (Table 1), suggesting that the contact herbicides utilized in this study could be beneficial for selective control of other susceptible nuisance vegetation that might grow in mixed stands with this species. The results of our field and mesocosm trials suggest that diquat can be used operationally for selective flowering rush control when intermixed with hardstem bulrush. Future studies should determine if the selective control of flowering rush by endothall holds in field settings prior to recommendation for operational use in intermixed stands with hardstem bulrush. Future studies should also investigate concentration exposure time relationships of diquat and endothall for flowering rush control because these two contact herbicides have shown the most activity on flowering rush in this and other work (Poovey et al. 2012, Madsen et al. 2016a, Parsons et al. 2019, Turnage et al. 2019a,b); such a study would also be beneficial to resource managers with flowering rush infestations in flowing waters.

SOURCES OF MATERIALS

[1] Reward® Landscape and Aquatic Herbicide, Syngenta Crop Protection, LLC, P.O. Box 18300, Greensboro, NC 27419.

[2] Osmocote 19–6–12 fertilizer, Scotts-Sierra Horticultural Products Company, 14111 Scottslawn Rd., Marysville, OH 43041.

[3] Harvester® Aquatic Herbicide (Diquat dibromide), Applied Biochemists, W175N11163 Stonewood Dr. Ste. 234, Germantown, WI 53022.

[4] Aquathol K® Aquatic Herbicide, UPL NA., 630 Freedom Business Center Drive, King of Prussia, PA 19406.

[5] Harpoon® Aquatic Herbicide (Copper-ethylenediamine), Applied Biochemists, W175N11163 Stonewood Dr. Ste. 234, Germantown, WI 53022.

[6] Stingray® Aquatic Herbicide, SePRO Corporation, 11550 North Meridian Street, Suite 600, Carmel, IN 46032.

[7] Clipper SC® Aquatic Herbicide, Nufarm Americas Inc., 11901 South Austin Avenue, Alsip, IL 60803.

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