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Sequential applications of diquat to control flowering rush (*Butomus umbellatus* L.) in mesocosms

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ABSTRACT

Flowering rush (*Butomus umbellatus* L.) is an aggressive, invasive, aquatic plant spreading throughout water bodies in the northern United States and southern Canada, displacing many native aquatic/wetland plants. This can disrupt ecosystem processes and affect human uses of water bodies. Operational management in Detroit Lakes, MN, reduced flowering rush biomass and propagules by > 80% using two sequential, submersed applications of diquat (0.37 mg L^{-1}) per growing season (4 wk apart). However, in dense colonies, long-term control has taken years to achieve, suggesting a more aggressive treatment regime may be necessary. A mesocosm study was initiated in 2015 and repeated in 2016 to further investigate diquat (0.37 mg L^{-1}; 12 h exposure time) efficacy using one to four biweekly (every other week) sequential herbicide applications to improve flowering rush control. All treatments reduced flowering rush aboveground and belowground biomass and propagule (rhizome buds) density compared with non-treated reference plants ($P < 0.001$) at 8 and 52 wk after initial treatment (WAIT). There were no differences among diquat treatments, regardless of the number of applications. Diquat treatments reduced aboveground biomass 57 to 99% and 62 to 100% at 8 and 52 WAIT, respectively. Diquat treatments reduced belowground biomass 73 to 92% and 71 to 100% at 8 and 52 WAIT, respectively. Propagules were reduced 65 to 97% and 67 to 100% by treatments at 8 and 52 WAIT, respectively. This research suggests a more aggressive treatment protocol will not benefit resource managers; however, these results need to be field verified before existing treatment protocols are altered.

Key words: chemical control, herbicide timing, invasive species, nuisance species, submersed application.

INTRODUCTION

Flowering rush (*Butomus umbellatus* L.), a perennial aquatic/wetland plant, native to Eurasia, is becoming a widespread pest across the northern United States and Canada (Core 1941, Countryman 1970, Anderson et al. 1974, Kliber and Eckert 2005). Flowering rush can thrive on the wetland margins of water bodies as an emergent plant in shallow, littoral areas (depth < 1.4 m) and/or as a fully submersed plant in deeper waters (depth > 1.4 m; Hroudova et al. 1996, Marko et al. 2015, Madsen et al. 2016c). In addition, flowering rush can rapidly outcompete native plants and decrease biodiversity of native flora and fauna (Core 1941, Countryman 1970, Bellaud 2009). Flowering rush primarily reproduces and colonizes new sites via vegetative means, most notably rhizome fragments and rhizome bud production and dispersal (Hroudova et al. 1996). Control of flowering rush propagules should be a key focus of management efforts as the primary propagules (rhizome buds) easily separate from other plant structures and sprout within the parent colony, which can increase plant density within the parent colony, or propagules may float away with potential to colonize new sites. Flowering rush densities can exceed hundreds of ramets per square meter and can negatively affect water use for humans (Marko et al. 2015, Madsen et al. 2016c).

Currently, there are limited submersed chemical-control options available to resource managers that provide adequate control of flowering rush biomass and propagules (Madsen et al. 2012, Madsen et al. 2013, Madsen et al. 2014, Madsen et al. 2016a, Madsen et al. 2016b, Poovey et al. 2012, Poovey et al. 2013, Turnage and Madsen 2015, Wersal et al. 2014). To date, most research on chemical control of flowering rush documented in peer-reviewed journals has been conducted as small-scale field trials in the Detroit Lakes, MN (Madsen et al. 2016a) or as growth chamber or mesocosm studies at research facilities in Mississippi (Poovey et al. 2012, Poovey et al. 2013, Madsen et al. 2016b, Wersal et al. 2014). Most of these studies investigated the efficacy of systemic and contact herbicides for control of flowering rush; however, only contact herbicides were tested in field sites (Madsen et al. 2016a). Contact herbicides typically have short concentration exposure time (CET) requirements to control nuisance vegetation (Netherland 2009). Little information exists regarding calculated exposure times in aquatic systems known to contain flowering rush (Skogerboe 2010, Wersal and Madsen 2011, Getsinger et al. 2013). Skogerboe (2010) reported half-lives of 4 to 78 h

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in the Detroit Lakes. Wersal and Madsen (2011) reported herbicide half-lives of 8 to 22 h in Noxon Rapids Reservoir, MT. In addition, in Noxon Rapids Reservoir, Getsinger et al. (2013) reported whole-plot half-lives of 2 to 33 h. Most field, mesocosm, and growth-chamber research regarding flowering rush control with short CET herbicides has focused on exposure times (ETs) between 6 and 72 h (Madsen et al. 2016a, Madsen et al. 2016b, Poovey et al. 2012, Poovey et al. 2013); however, results of these trials have varied regarding control of flowering rush biomass and propagule density.

Poovey et al. (2012) conducted growth-chamber experiments on populations of flowering rush from Idaho (Lake Pend O'Reille) and Minnesota (Detroit Lakes) using the contact herbicides diquat, flumioxazin, and endothall at multiple CETs. Poovey et al. (2012) found that Minnesota plants treated once with submersed applications of diquat (0.37 mg L^{-1}) reduced flowering rush shoot, but not root (including rhizomes), biomass 4 wk after treatment (WAT) at 6 and 12 h ETs. Similarly, one endothall treatment (1.5 and 3.0 mg L^{-1}) at 12 and 24 h ETs, respectively, also reduced flowering rush shoot, but not root, biomass. One submersed flumioxazin treatment (0.2 mg L^{-1}) did not reduce biomass of Minnesota plants at 12 or 24 h ETs (Poovey et al. 2012). Flowering rush shoots from Idaho were reduced 6 WAT by flumioxazin (0.4 mg L^{-1}) and endothall (3.0 mg L^{-1}) at a 24-h ET, whereas root biomass was reduced only by the endothall treatment (Poovey et al. 2012). Lesser CETs of flumioxazin had no effect on flowering rush shoot or root biomass from Idaho (Poovey et al. 2012).

Poovey et al. (2013), in growth chamber experiments on Minnesota and Idaho populations of flowering rush, found that endothall (1.5 mg L^{-1}) and flumioxazin (0.4 mg L^{-1}), at a 24-h ET, controlled roots and shoots of Minnesota populations. Endothall also controlled shoots and roots of Idaho populations, whereas flumioxazin only controlled shoots (Poovey et al. 2013). Additionally, Poovey et al. (2013) showed that, by 8 WAT, flowering rush shoots produced by plants from Idaho recovered and were equal to reference plant levels from those herbicide treatments, whereas plants from Minnesota did not. Neither herbicide controlled rhizomes, which is essential to long-term flowering rush control because they are the main carbohydrate-storage structure used for overwintering and propagule production of flowering rush (Marko et al. 2015) of either population (Poovey et al. 2013).

Madsen et al. (2016b) conducted a mesocosm trial in which diquat (0.19 mg L^{-1}) was applied once as a subsurface injection with an ET of 72 h. This resulted in control of aboveground and belowground biomass as well as propagule density at 8 WAT (Madsen et al. 2016b). Addition of fluridone (0.03 mg L^{-1}) as a static treatment did not enhance the efficacy of the flowering rush control (Madsen et al. 2016b).

Field trials in the Detroit Lakes showed that two submersed applications of diquat (0.37 mg L^{-1}) herbicide (4 wk apart) per growing season provided $> 80\%$ control of rhizome buds and plant biomass in flowering rush beds (Madsen et al. 2016a). Furthermore, that protocol did not appear to affect native plant biodiversity at treatment sites (Madsen et al. 2016a). Madsen et al. (2016a) is the only documented field study, to our knowledge, pertaining to flowering rush control, which shows control of flowering rush biomass and propagule density using an herbicide with short CET requirements.

Currently, a low abundance of flowering rush propagules (< 30 per m^2) still remain in the sediments of previously treated plots in the Detroit Lakes system compared with reference plots (100s m^{-2}; Turnage et al. 2018). This persistence is likely attributed to a number of factors (i.e., shallow water depth, dock placement and shape/design, flowering rush intermixed with desirable plant species) that make it difficult to treat the entire system uniformly, thus creating a spatial refugia and allowing flowering rush to remain long after herbicide treatment (Turnage, pers. obs.). In addition, all rhizome buds do not sprout at the same time; therefore, some rhizome buds may start to grow after the herbicide has been applied, which creates a temporal refugia that facilitates flowering rush persistence. Spatial and temporal refugia permits flowering rush plants time to grow and produce more rhizome buds before the next herbicide treatment, thus complicating control of this species.

Increasing the number of diquat treatments per growing season and shortening the time interval between treatments to 2 wk is a more aggressive treatment protocol than what is currently recommended/deployed. Thus, we speculate this modified protocol may increase the level of plant control by reducing the availability of temporal refugia. Therefore, a mesocosm trial was initiated to determine whether shortened intervals from 4 to 2 wk between sequential diquat treatments combined with an increased number of treatments would improve flowering rush control, as measured by decreased aboveground and belowground biomass and rhizome bud density.

MATERIALS AND METHODS

This study was conducted at the Aquatic Plant Research Facility at the Mississippi State University R. R. Foil Plant Science Research Center. The study was initiated in early June 2015 and repeated in 2016. Flowering rush was grown in 1,140-L (300 gal.) outdoor mesocosms filled with pond water to a volume of 216 L (41 cm or 16 inch depth). Flowering rush was established by placing two 7.6-cm (3-in.) rhizome fragments, with at least one attached bud, in 3.78-L pots filled with sand and amended with a slow release fertilizer[1] to stimulate growth. Nine pots of flowering rush were placed in each of the 20 mesocosms, and plants were allowed to acclimate for 1 mo before herbicide application.

Before the first herbicide application, one pot per mesocosm was harvested to establish a pretreatment baseline of plant growth. Harvesting consisted of separating plant tissue into aboveground and belowground biomass and recording rhizome bud numbers per pot. Harvested biomass was placed in labeled paper bags and dried in a forced air oven for 5 d at 70 C (158 F). After drying was complete, plant biomass was weighed, and weights were recorded.

After the pretreatment harvest was completed, diquat[2] (0.37 mg L^{-1}) was applied via submersed injection to 16

Table 1. Treatment timing of sequential submersed diquat applications to flowering rush in 2015 and 2016 in a mesocosm setting under various treatment times.

Treatment	Treatment Timing			
	0 WAIT[1]	2 WAIT	4 WAIT	6 WAIT
Reference	NA	NA	NA	NA
Single[2]	X			
Double	X	X		
Triple	X	X	X	
Quadruple	X	X	X	X

[1]Abbreviations: WAIT, weeks after initial treatment; X, herbicide was administered at this time; NA, not applicable.
[2]Treatments designated as single received one diquat application (0.37 mg L^{-1}), double received two applications, triple received three applications, and quadruple are those mesocosms that received four applications. Herbicide treated water remained in mesocosms for 12 h.

mesocosms that contained flowering rush; most (> 50%) of the plant biomass was subsurface in all mesocosms. A 12-h ET was used because it falls within ET ranges found in field settings of water bodies containing flowering rush (Skogerboe 2010, Wersal and Madsen 2011, Getsinger et al. 2013) and matches ETs used in other small-scale chemical control studies (Poovey et al. 2012, Poovey et al. 2013, Madsen et al. 2016b). Diquat was applied (early July) to all 16 herbicide-treated mesocosms. After the 12-h ET was completed, mesocosms were drained and refilled with herbicide-free water (Table 1). At 2 wk after initial treatment (WAIT), a second submersed injection was applied to those 12 mesocosms designated to receive two, three, or four diquat applications (Table 1) for a 12-h ET. At 4 WAIT, a third diquat treatment was applied to eight mesocosms designated to receive three and four sequential applications (Table 1) for a 12-h ET. At 6 WAIT, a fourth diquat treatment was administered to the four mesocosms that received a fourth herbicide application (Table 1) for a 12-h ET. In addition, a nontreated reference was included (Table 1). Each treatment was replicated four times for a total of 20 mesocosms. At 8 WAIT (late August), four pots of flowering rush were randomly selected and harvested from each mesocosm to assess short-term effects of sequential diquat applications on treated plants. At 52 WAIT, the remaining four pots in each mesocosm were harvested to assess long-term effects of sequential diquat applications on flowering rush. Plants were harvested and processed in the same manner as pretreatment specimens.

Response variables were analyzed statistically via an ANOVA. Because of a year effect, data were not pooled. Differences detected in treatment means by ANOVA were further separated by a Fisher's Protected LSD test at the 0.05 significance level (Analytical Software 2009).

RESULTS AND DISCUSSION

All diquat applications significantly reduced flowering rush biomass and rhizome bud density over short-term and long-term periods when compared with the nontreated reference (Figures 1 and 2). Furthermore, all diquat treatments had the same level of control within a given year (Figures 1 and 2), which suggests one application of diquat was equally efficacious as multiple applications for controlling flowering rush in mesocosms. In the 2015 study, no flowering rush tissues were detected 52 WAIT for any treatment, whereas in 2016, flowering rush recovered from all herbicide treatments by 52 WAIT.

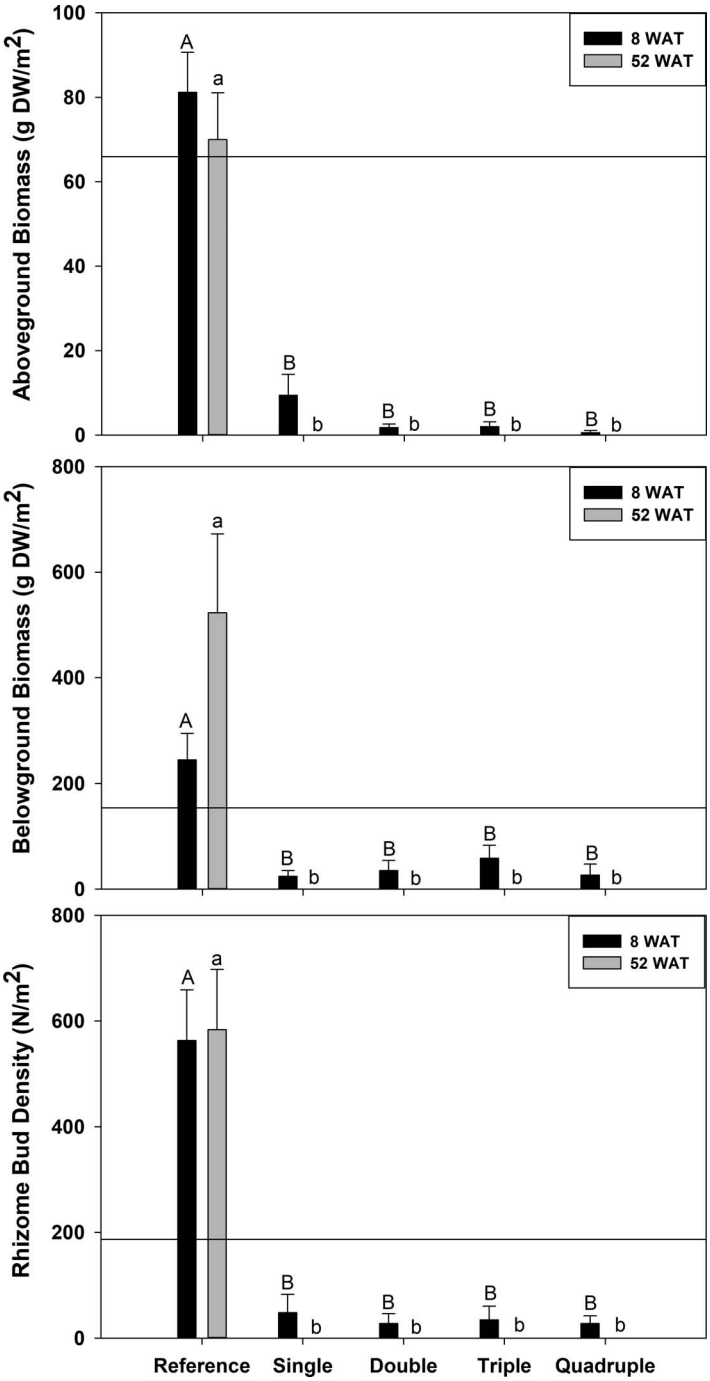

Figure 1. Year 1 (2015) flowering rush aboveground biomass (top), belowground biomass (middle), and rhizome bud density (bottom) response to a single or sequential (double, triple, or quadruple application) subsurface application of diquat every 2 wk. The horizontal lines represent pretreatment biomass. Error bars are 1 standard error of the mean. Bars sharing the same letter within a particular harvest date at 8 and 52 wk after initial treatment are not significantly different according to Fisher's Protected LSD test ($P = 0.05$); $n = 4$.

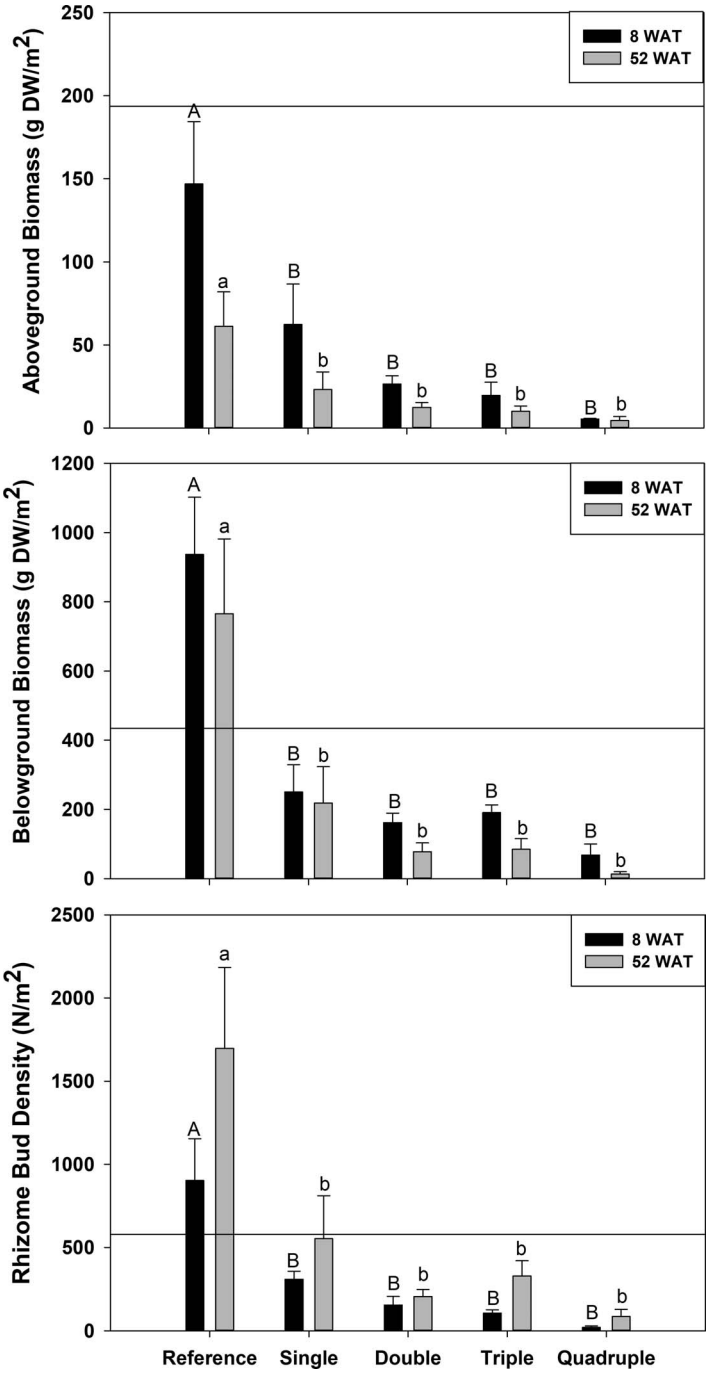

Figure 2. Year 2 (2016) flowering rush aboveground biomass (top), belowground biomass (middle), and rhizome bud density (bottom) response to a single or sequential (double, triple, or quadruple application) subsurface application of diquat every 2 weeks. The horizontal lines represent pretreatment biomass. Error bars are 1 standard error of the mean. Bars sharing the same letter within a particular harvest date at 8 and 52 wk after initial treatment are not significantly different according to Fisher's Protected LSD test ($P = 0.05$); $n = 4$.

In 2015, flowering rush aboveground biomass was reduced 88 to 99% by diquat treatments at 8 WAIT and 100% at 52 WAIT (Figure 1). Also in 2015, flowering rush belowground biomass was reduced 76 to 90% by diquat treatments at 8 WAIT and 100% by 52 WAIT (Figure 1). Flowering rush rhizome bud density was reduced 91 to 95% and 100% by diquat treatments at 8 and 52 WAIT, respectively, in 2015 (Figure 1). In 2016, diquat treatments reduced flowering rush aboveground biomass 57 to 96% at 8 WAIT and 62 to 92% at 52 WAIT (Figure 2). Belowground biomass was reduced 73 to 92% at 8 WAIT and 71 to 98% at 52 WAIT by subsurface diquat treatments in the 2016 trial (Figure 2). In 2016, flowering rush rhizome bud density was reduced 65 to 97% at 8 WAIT and 67 to 94% at 52 WAIT (Figure 2).

Madsen et al. (2016a) conducted field trials for the management of flowering rush in the Detroit Lakes, MN, using two diquat treatments (0.38 mg L^{-1}) applied 1 mo apart (June and July) and found that flowering rush aboveground biomass, belowground biomass, and rhizome bud densities were reduced 99%, 82%, and 83%, respectively, during the growing season, which was similar to our findings. Additionally, flowering rush biomass and rhizome buds were reduced after one application of diquat when compared with the nontreated reference plants and remained suppressed after the second diquat application (Madsen et al. 2016a). Data from the current study suggest that subsequent (second, third, and fourth) diquat applications every 2 wk may be unnecessary because they did not provide further biomass reductions of flowering rush after diquat was applied at 0.37 mg L^{-1} and plants were exposed for 12 h (Figures 1 and 2). Similar to our findings, Poovey et al. (2012) showed that one diquat (0.37 mg L^{-1}) application with ETs of 6 and 12 h reduced aboveground flowering rush biomass. In contrast to our findings, Poovey et al. (2012) showed a single diquat application did not reduce belowground flowering rush biomass.

Herbicide application timing can be a critical factor in successful reduction of nuisance vegetation. The early part of the growth cycle of some perennial plants is typically considered a weak point because carbohydrate reserves in belowground structures have been depleted to produce emergent plant growth, and energy production in foliage has not yet reached a point where reserves have been replenished by photosynthesis (Aldous 1935, Madsen 1997, Madsen and Owens 1998). Flowering rush usually reaches its peak height $<$ 1 mo after sprouting, but peak rhizome bud density occurs a few months later (Marko et al. 2015). This would suggest flowering rush energy reserves in rhizomes are depleted to initiate emergent growth during early summer until photosynthesis within the leaves is able to support both growth and rhizome bud production for overwintering. Sequential diquat treatments in the current and previous research (Madsen et al. 2016a) applied diquat early in the growth cycle (1 mo after planting and in June, respectively) of flowering rush, which likely coincided with a weak point in the plant's life cycle. Surprisingly, these data suggest diquat applied during a weak point in the growth cycle of flowering rush induces stress from which plants are unable to recover. Furthermore, the present work shows that a single diquat application at maximum labeled rate under 12-h exposure periods early in the flowering rush life cycle is sufficient for short-term and long-term control. However, concentration exposure time (CET) in field sites may differ from those in mesocosms because of herbicide

dissipation and/or water movement, which may necessitate the need for follow-up diquat applications to control flowering rush in field settings.

Because diquat reduced flowering rush biomass over both the short and long term (Figures 1 and 2), it is beneficial to resource managers. Marko et al. (2015) showed that flowering rush belowground tissues had a higher starch content than aboveground tissues throughout the growing season, suggesting that control of flowering rush should focus on reduction of belowground tissues. Diquat is not typically used for control of belowground plant tissues because it is a contact herbicide with limited translocation (Shaner 2014). It lacks root absorption in the sediment and is active on aboveground tissues that are capable of photosynthesis (Shaner 2014). However, use of diquat to reduce emergent flowering rush may force plants to deplete energy reserves in belowground tissues to survive herbicide-induced stress by depleting carbohydrates to regrow emergent tissues (i.e., leaves). This, in turn, could reduce belowground plant structures without the herbicide actually contacting those structures, which is similar to repeated mechanical control events (Armellina et al. 1996, Seiger and Merchant 1997, Zaller 2004). If stored plant carbohydrates in flowering rush rhizomes are allocated to survival of individual plants after an herbicide treatment, they are unlikely to be available for rhizome bud production, which, in turn, can decrease the number of rhizome buds available to sprout at a later date. Consequently, this could reduce the overall density of individual flowering rush colonies.

Because diquat typically reduces emergent nuisance vegetation within days after application, public perception of management activities is generally positive. Additionally, because diquat can reduce flowering rush propagules below pretreatment levels with just one application, resource managers may be able to reallocate resources to other issues in their management areas. Resource managers should also periodically rotate herbicide modes of action (i.e., protoporphyrinogen oxidase inhibitors, inhibitor of lipid and protein biosynthesis) to reduce the potential for development of herbicide resistance to diquat (Koschnick et al. 2006). Resource managers also need alternative herbicide-treatment options, tank mixtures, or application methods (i.e., foliar applications) in areas in which diquat applications are restricted or in areas in which sediment resuspension (i.e., shorelines) occurs via wave and/or wind activity. Suspended sediments and organic matter negatively affect diquat by irreversibly binding diquat molecules (Shaner 2014). Flumioxazin and endothall would be excellent candidates to rotate with diquat because of their relatively short ET requirements. Careful consideration of waterbody characteristics (i.e., pH, water exchange) is necessary when selecting these (or any) herbicides for controlling flowering rush because both will likely need a longer ET than diquat to control flowering rush (Poovey et al. 2012), and flumioxazin use in water bodies with lower pH is recommended because it rapidly breaks down in high pH (pH $>$ 9) waters (Mudge et al. 2010, Shaner 2014).

Future studies should focus on timing of single diquat application (late vs. early season relative to plant phenology) because limited evidence suggests late-season herbicide applications can effectively control flowering rush (Wersal et al. 2014). Future studies should also focus on control with alternative nonchemical and integrated control techniques for flowering rush as well as multiple diquat CET-use protocols in flowing aquatic systems in which a 12-h ET may not be feasible.

SOURCES OF MATERIALS

[1]Osmocote 19–6–12 (N–P–K) fertilizer, Scotts-Sierra Horticultural Products Company, 14111 Scottslawn Rd. Marysville, OH 43041.

[2]Harvester® Aquatic herbicide (Diquat dibromide), Applied Biochemists, a Lonza Business, W175N11163 Stonewood Dr., Ste. 234, Germantown, WI 53022.

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