The Effects Diquat on Mixed Stands of Flowering Rush and Hardstem Bulrush in Lake Sallie, Minnesota 2016 – Interim Report



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Introduction

The invasive aquatic plant flowering rush (*Butomus umbellatus*) is capable of utilizing habitat occupied by the native hardstem bulrush (*Shoenoplectus acutus*) in the Detroit Lakes system. Currently, the standing protocol for controlling flowering rush in infested areas is to inject two diquat herbicide treatments into the water column four weeks apart during the growing season.

Because bulrush is a desirable native species herbicide treatments haven't historically been administered in areas with bulrush due to the unknown effects of diquat on the species within these lakes. However, in 2015 the MN DNR permitted a pilot study utilizing two sites in Lake Sallie (Turnage et al. 2016a) that had flowering rush and hardstem bulrush. One site served as an untreated reference while the other received diquat treatments according to the abovementioned protocol.

Turnage et al. (2016a) showed that diquat treatments were effective at controlling flowering rush while allowing hardstem bulrush to increase in prevalence within a growing season.

The work presented in this document is a follow up study to the Turnage et al. (2016a) that is intended to determine if diquat treatments over multiple years will continue to control flowering rush without decreasing hardstem bulrush prevalence.

Materials and Methods

Study protocol in 2016 followed that utilized in 2015 (Turnage et al. 2016) so as to make comparisons across and within years.

Two five acre sites were established on Lake Sallie, MN: a treatment site and an untreated reference in 2015 (Figure 1). The untreated reference falls completely within a larger reference site (LS-REF-1) that is being used for another larger study of diquat usage on flowering rush in the entire Detroit Lakes system (Turnage and Madsen 2015; Turnage et al. 2016b). The presence of hardstem bulrush and flowering rush has been documented at this reference site for the last five years (Madsen et al. 2014; Turnage and Madsen 2015). Lake Sallie is immediately upstream of Lake Melissa on the Pelican River. Both the treatment and the reference site on Lake Sallie had bulrush and flowering rush present. Diquat was applied to the proposed treatment site using the 2015 treatment protocol: two applications of diquat herbicide at 0.37 ppm spaced four weeks apart during the growing season.

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A grid of data collection points were placed in both the reference and the treatment plots (28 and 27 points respectively). This is an increase of one sampling point at the treatment site. At each point data was collected within a $0.1m^2$ (13 inch on a side) area that was demarcated by a PVC frame (Figure 2). A Garmin 78 SC handheld GPS unit was used for navigation to survey points during each data collection effort. Data was collected in a non-destructive manner by Minnesota Department of Natural Resources and Pelican River Watershed District personnel and analyzed by the Geosystems Research Institute of Mississippi State University.

Stem (or leaf) count and maximum stem height from the sediment were recorded for hardstem bulrush that fell within PVC frames at each survey point. Heights were measured to the nearest cm. Presence or absence of flowering rush and percent cover by flowering rush was also recorded within PVC frames at each point. Data was recorded prior to each herbicide treatment and again four weeks after the last treatment (three data collection efforts).

A paired T-test was used to compare means within each plot within a year and among the first data collection efforts of each year. Statistical tests were carried out in the software package Statistix 9.0 (Analytical Software 2009). Data were only analyzed within plots as there were no replicates of each treatment with which to do a more rigorous statistical analysis (i.e. Analysis of Variance).

Results and Discussion

Reference Site

Paired t-tests of 2016 reference site data utilized all data from all sampling points while paired t-tests analyzing changes from 2015 to 2016 only utilized sample points common to both years (19 points). This was done due to the lack of pretreatment sampling at points 20–28 in the 2015 study (Turnage et al. 2016a).

Neither bulrush stem count or stem height significantly changed after the first or second herbicide application (p>0.05; Table 1; Figure 3 & 4) nor had they declined from 2015 pretreatment levels (p>0.05; Table 2; Figure 5).

Neither, the presence (frequency) nor percent cover of flowering rush statistically changed (p>0.05) in the reference site during the 2016 growing season (Table 1; Figure 6 & 7). However, the pretreatment prevalence (frequency) of flowering rush in the reference site statistically decreased by 24% from 2015 to 2016 in the reference site (p=0.0419; Table 2; Figure 5). Similarly, flowering rush percent cover statistically declined by 23% (p=0.0457) from 2015 to 2016 (Table 2; Figure 5). This could be due to the lack of data points in the 2015 pretreatment dataset or differences in time of sprouting from year to year as timing of environmental factors (i.e. water temperature or ice cover) can vary from year to year. Comparison of final sampling efforts within the reference site each year showed no change in flowering rush prevalence (p=0.7691, paired t-test) or percent cover (p=0.8959, paired t-test) from 2015 to 2016 suggesting that flowering rush may not have actually declined within the reference site.

Treatment Site

Bulrush stem count and stem height did not significantly change (p>0.05; Table 1; Figure 3 & 4) in the treatment site during 2016 or from 2015 to 2016 (p>0.05; Table 2; Figure 5). This would suggest that submersed diquat treatments have no effect on hardstem bulrush.

Flowering rush presence and percent cover did not decline (p>0.05) after the first diquat application but both significantly declined by 64% and 56% respectively after the second application (Table 1; Figure 6 & 7). However, statistical analysis of flowering rush pretreatment frequency and percent cover showed no difference from 2015 to 2016 (p>0.05; Table 2; Figure 8). This is most likely due to an abundance of propagules within the sediments of the treatment site that are able to quickly replenish above ground biomass after an herbicide treatment or re-infestation of the site from external propagules that drift in from other sites. However, without destructive sampling and analysis of belowground tissues (i.e. propagule mass and number) this can't be determined at this time.

Conclusions

Subsurface applications of diquat in mixed stands of bulrush and flowering rush appear to give selective control over flowering rush while simultaneously benefiting bulrush. This is most likely done by releasing bulrush from competition pressures associated with the growth rate and characteristics (density) of flowering rush.

It should be noted that diquat applications should not be made with an airboat as this has the potential to create a mist from treated water that can drift onto aerial portions of bulrush leaves thus damaging them as seen in the 2015 study (Turnage et al. 2016a).

Future efforts should include more reference and treatment sites so as to analyze data with more robust statistical tests (i.e. Analysis of Variance).

Future studies should incorporate destructive sampling techniques to further assess changes in belowground structures (i.e. rhizome biomass, flowering rush rhizome bud and ramet number) of both hardstem bulrush and flowering rush. These tissues represent nutrient reserves and propagules needed for each species to overwinter and persist from year to year thus analysis of each would be appropriate to determine potential long term changes in each species due to herbicide applications.

References

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Tables and Figures

Characteristic	Plot	Treatment One	Treatment Two	P-values (Treatment One)	P-values (Treatment Two)
Bulrush Stem	Reference	24%	-10%	0.5093	0.7070
Count	Treatment	51%	1%	0.2404	0.9637
Bulrush Height	Reference	5%	7%	0.8066	0.7462
	Treatment	39%	8%	0.3175	0.6048
Flowering Rush	Reference	-10%	0%	0.4242	0.7457
Frequency	Treatment	-27%	*-64%	0.2646	0.0165
Flowering Rush	Reference	-9%	-8%	0.5244	0.9458
% Cover	Treatment	-25%	*-56%	0.3479	0.0397

Table 1. Changes in plant characteristics at the reference and diquat treatment sites on Lake Sallie over the 2016 growing season. Percentages with an asterisk beside them represent statistically significant changes. P-values in bold type represent statistically significant changes.

Table 2. Changes in mean plant characteristics at the reference and diquat treatment sites on Lake Sallie from 2015 to 2016. Percentages with an asterisk beside them represent statistically significant changes. P-values in bold type represent statistically significant changes.

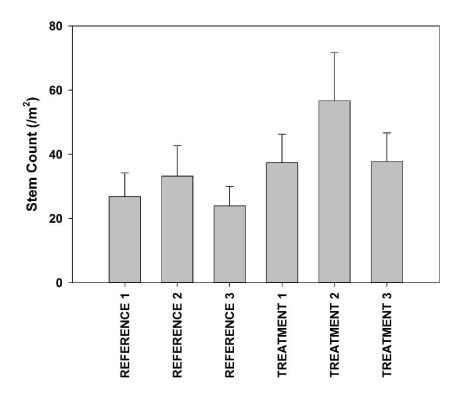
Characteristic	Plot	2015	2016	% Change	P-value
Bulrush	Reference	29	36	24%	0.5099
Stem Count (/m ²)	Treatment	40	37%	8%	0.8035
Bulrush	Reference	38	54	42%	0.1902
Height (cm)	Treatment	52	42	-19%	0.4542
Flowering	Reference	17	13	*-24%	0.0419
Rush Frequency	Treatment	12	11	-8%	0.6632
Flowering	Reference	0.75	0.58	*-23%	0.0457
Rush % Cover	Treatment	0.38	0.33	-13%	0.4514



Figure 1. Map of Lake Sallie, MN showing the treatment site and reference site locations for a diquat treatments within bulrush stands.

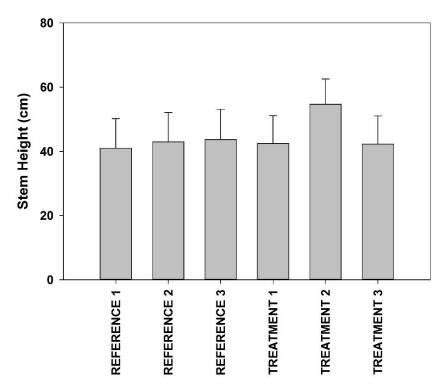


Figure 2. Image of $0.1m^2$ sampling device and flowering rush.



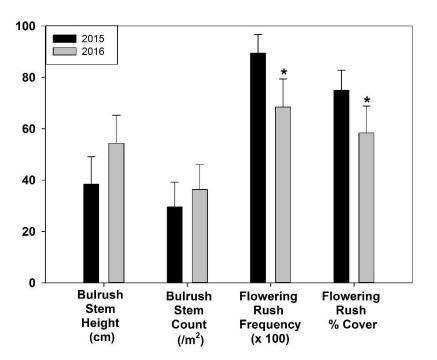
Hardstem Bulrush Stem Count

Figure 3. Hardstem bulrush stem (leaf) count in the reference and treatment sites on Lake Sallie. Numbers after site names represent data collection efforts (1=June, 2=August, & 3=September). Reference and treatment site data were analyzed separately using paired t-tests. Bars with an asterisk above them are statistically different from the first data collection effort of a given site.



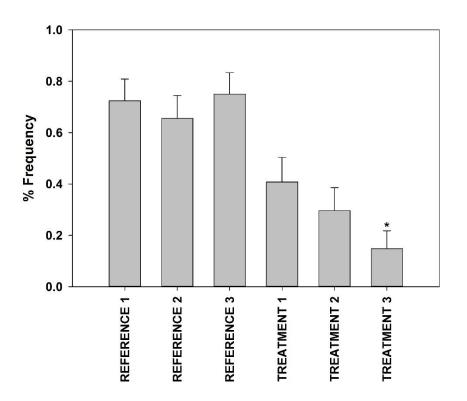
Hardstem Bulrush Maximum Stem Height Above Sediment

Figure 4. Hardstem bulrush stem height (cm) above sediment in the reference and treatment sites on Lake Sallie. Numbers after site names represent data collection efforts. Numbers after site names represent data collection efforts (1=June, 2=August, & 3=September). Reference and treatment site data were analyzed separately using paired t-tests. Bars with an asterisk above them are statistically different from the first data collection effort of a given site.



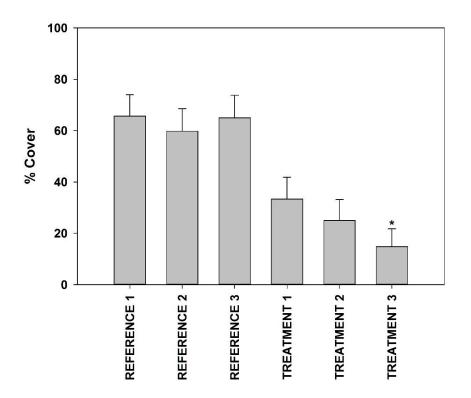
2015 vs. 2016 Reference Site Data

Figure 5. Changes in hardstem bulrush (stem/leaf count and stem height) and flowering rush (frequency and %-cover) in the Lake Sallie reference site from 2015 to 2016. Each variable was analyzed separately using paired t-tests. 2016 bars with an asterisk above them are statistically different from 2015 data.



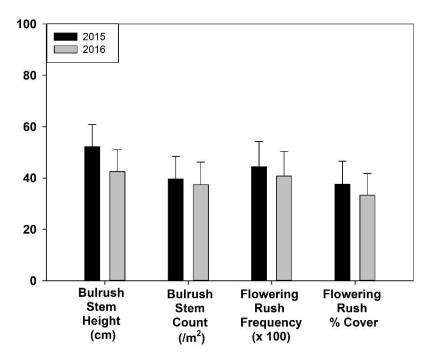
% Frequency of Flowering Rush

Figure 6. Percent flowering rush frequency in the reference and treatment sites on Lake Sallie. Numbers after site names represent data collection efforts (1=June, 2=August, & 3=September). Reference and treatment site data were analyzed separately using paired t-tests. Bars with an asterisk above them are statistically different from the first data collection effort of a given site.



% Cover of Flowering Rush

Figure 7. Percent flowering rush cover in the reference and treatment sites on Lake Sallie. Numbers after site names represent data collection efforts (1=June, 2=August, & 3=September). Reference and treatment site data were analyzed separately using paired t-tests. Bars with an asterisk above them are statistically different from the first data collection effort of a given site.



2015 vs. 2016 Treatment Site Data

Figure 8. Changes in hardstem bulrush (stem/leaf count and stem height) and flowering rush (frequency and %-cover) in the Lake Sallie treatment site from 2015 to 2016. Each variable was analyzed separately using paired t-tests. 2016 bars with an asterisk above them are statistically different from 2015 data.