Influence of winter on herbicide efficacy for control of giant salvinia (*Salvinia molesta*)

CHRISTOPHER R. MUDGE AND BRADLEY T. SARTAIN*

INTRODUCTION

The invasive aquatic fern, giant salvinia (Salvinia molesta Mitchell), continues to spread throughout the United States, disrupting transportation, hindering water uses, affecting desirable native plant communities, and increasing mosquito breeding habitat in ponds, lakes, rivers, and bayous (Jacono 1999, Jacono and Pitman 2001, Nelson et al. 2001). The Louisiana Department of Wildlife and Fisheries estimated that 52,496 acres of giant salvinia were present in public water bodies throughout the state in 2014 and 19,440 acres were chemically managed (A. Perret, pers. comm.). In 2015, the Texas Parks and Wildlife Department determined that more than 20 public water bodies were infested with giant salvinia in Texas (Texas Parks and Wildlife Department 2015).

Since the introduction of giant salvinia, mesocosm trials have been conducted to screen herbicides (Nelson et al. 2001, Glomski and Getsinger 2006, Mudge et al. 2012), evaluate spray volume (Nelson et al. 2007), and determine the influence of seasonality/time of year for herbicide efficacy against giant salvinia (Mudge et al. 2016). During the past few years, giant salvinia in Louisiana has been managed primarily with foliar applications of a combination of glyphosate, diquat, and two adjuvants (a nonionic surfactant with buffering agents and a nonionic organosilicone surfactant) (Mudge et al. 2014, Mudge et al. 2016). This spray mixture is used during the growing season of April through October (Mudge et al. 2016), whereas winter management (when applicable) utilizes foliar applications of diquat and one surfactant (Mudge et al. 2014). Conversely, giant salvinia is controlled with glyphosate and diquat during the growing season and winter, respectively, by state and federal agencies in Texas (T. Corbett, pers. comm.).

Although recent mesocosm research investigated combinations of glyphosate, diquat, and surfactants, as well as other combinations during the spring, summer, and fall (Mudge et al. 2016), small-scale winter treatments have not been evaluated. During the winter, the most widely used treatment to manage plant populations in Louisiana is diquat (1.7 kg ai ha⁻¹) plus one surfactant (A. Perret, pers. comm.). However, replicated research has not been conducted to determine if this treatment is more efficacious than other stand-alone and combination treatments. In addition, many natural resource agencies do not manage aquatic plants during the winter because of speculation that herbicides are not effective when plants are slow growing or dormant in response to shorter photoperiods and decreased temperatures.

Foliar herbicide applications during the winter (November to March) may offer an opportunity for natural resource agencies to achieve better control than treatments administered during the peak growing season. Decreased temperatures and shortened photoperiods slow plant growth and may cause significant injury (i.e., tissue necrosis and desiccation) to existing plant stands, thus reducing plant biomass. Also, mat thickness is often reduced to one or two layers during winter. Consequently, minimal biomass and the absence of a thick plant mat may allow herbicides to come in contact with the entire stand or mat, although uptake of herbicide into unhealthy or dormant plants may be limited. In addition, the number of applications throughout the growing season could be reduced if plants are controlled before peak growing season, thus decreasing the probability of developing large lake-wide infestations. Therefore, mesocosm research was conducted to 1) determine if control can be achieved during the winter when plant growth is minimal, and 2) determine the influence of winter/cold temperatures on herbicide efficacy.

MATERIALS AND METHODS

Three outdoor mesocosm trials were conducted at the Louisiana State University (LSU) AgCenter Aquaculture Research Facility in Baton Rouge, LA to evaluate the efficacy of herbicides against mature giant salvinia during the winters of 2014 to 2015 and 2015 to 2016. Trials were initiated and treated in December 2014 (Trial 1) and 2015 (Trials 2 and 3). Plants used in this research were collected from cultures maintained at LSU Aquaculture. Equal amounts of fresh plant material, enough to cover approximately 85% of the water surface, were placed in 76-L plastic containers (49.5-cm diam by 58.4-cm height). Containers for Trial 1 were filled with a 2 : 1 mixture of pond (pH 7) and well (pH 8.0) water to achieve a final pH of

^{*}First author: Research Biologist, U.S. Army Engineer Research and Development Center, Louisiana State University School of Plant Environmental and Soil Sciences, Baton Rouge, LA 70803. Second author: Graduate Research Assistant, Louisiana State University School of Plant Environmental and Soil Sciences, Baton Rouge, LA 70803. Corresponding author's E-mail: Christopher.R.Mudge@usace.army.mil. and in revised form October 5, 2017.Month 2017

Table 1. Effect of foliar aquatic herbicide treatments on giant salvinia dry weight (G \pm 1 standard error) during the winters of 2014 to 2015 and 2015 to 2016.

Herbicide Treatment	Rate (g ai ha ⁻¹) ¹	2014 to 2015 Uncovered Trial 1 ^{2,3}	2015 to 2016 Uncovered Trial 2	2015 to 2016 Covered Trial 3
$\begin{array}{l} \text{Control} \\ \text{G}^4 + \text{D} + \text{NISBA} + \text{NIOS} \end{array}$	0	3.66 ± 3.95 a	36.43 ± 1.08 a	58.53 ± 2.07 a
	3,364.1 + 560.1 + 0.25% v/v + 0.094% v/v	0.00 ± 0.00 b	9.97 ± 1.85 b	16.40 ± 4.07 c
G + F + MVO	3,364.1 + 71.5 + 0.25% v/v	$0.00 \pm 0.00 \text{ b}$	$0.56 \pm 0.52 \text{ d}$	22.69 ± 2.57 bc
G + C + MVO	3,364.1 + 66.6 + 0.25% v/v	$0.01 \pm 0.02 \text{ b}$	$1.74 \pm 1.38 \text{ c}$	17.82 ± 3.18 c
E + F + MVO	592.9 + 143.0 + 0.25% v/v	$0.00 \pm 0.00 \text{ b}$	$4.12 \pm 1.28 \text{ c}$	$21.78 \pm 2.45 \text{ bc}$
D + MVO	1,682.2 + 0.25% v/v	$0.00 \pm 0.00 \text{ b}$	$0.28 \pm 0.23 \text{ d}$	$2.18 \pm 1.76 \text{ d}$
D + SBA	$\begin{array}{l} 1,682.2 + 0.25\% \mathrm{v/v} \\ 4,205.2 + 0.25\% \mathrm{v/v} \end{array}$	$0.00 \pm 0.00 \text{ b}$	$0.96 \pm 0.72 \text{ d}$	$3.96 \pm 2.51 \text{ d}$
G + MVO		$0.01 \pm 0.02 \text{ b}$	$5.72 \pm 1.40 \text{ c}$	$30.67 \pm 3.35 \text{ b}$

¹Glyphosate and endothall applied as g ae ha⁻¹.

 $^{2}2014$ to 2015 trial harvested 8 wk after treatment; 2015 to 2016 trials harvested 15 wk after treatment.

³Means within a column followed by the same letter are not significantly different according to the Student-Newman-Keuls method at $P \le 0.05$; n = 4.

⁴Abbreviations: G, glyphosate; D, diquat; NISBA, nonionic surfactant and buffering agent; NIOS, nonionic organosilicone surfactant; F, flumioxazin; MVO, modified vegetable oil; C, carfentrazone; E, endothall (dipotasium salt); SBA, spray binder adjuvant.

ca. 7.2, whereas Trials 2 and 3 containers were filled with well water and amended with the sphagnum moss (30 g of dry material per tank) to achieve a final pH of ca. 6.8. Despite the initial differences in water pH, the pH was 6.5 (\pm 0.1 standard error) at the time of herbicide application. All tanks were amended with 2.1 g of Miracle-Gro^{®1} (24–18–16 N–P–K) fertilizer initially and again immediately before herbicide treatment. Water volume was maintained at ca. 60 L throughout the experiments. The plastic containers were placed inside larger plastic tanks (1,136 L) partially filled with water to help maintain a consistent water temperature. Culture techniques were adapted from previous giant salvinia research (Mudge et al. 2012, 2016).

Plants were allowed to acclimate to container conditions for 2 wk before herbicide application. At the time of herbicide treatment, coverage was ca. 100%, with mean dry weights of 17.35 ± 1.04 , 27.78 ± 2.09 , and 48.17 ± 3.60 g container⁻¹ for Trials 1, 2, and 3, respectively. Containers in Trials 1 and 2 were maintained under natural conditions. Trial 1 simulated open water (i.e., middle of lake or canal) and colder winter conditions where freeze damage is likely to occur, whereas Trial 2 mimicked a milder winter or conditions where frost damage and no freeze are likely to occur. In contrast, Trial 3 containers were temporarily covered with tarps when air temperature decreased below 0° C. Tarps were placed over the containers before sunset on the evening before freezing temperatures were anticipated and were removed once air temperature increased above 0° C. The purpose of covering was to prevent water and plant freezing, with the goal of simulating a mild winter or conditions where vegetation is protected by tree canopy. Hourly air temperature data were obtained from a local weather station (LSU AgCenter 2016) and hourly water temperature data were recorded using loggers² during Trial 1. Loggers were deployed to collect air and water temperature data during Trials 2 and 3; however, data collected were unreliable and are not included in this research.

Herbicides evaluated included carfentrazone³, diquat⁴, endothall⁵, flumioxazin⁶, and glyphosate⁷ alone and in combination as well as with various adjuvants⁸, ⁹, ¹⁰, ¹¹ (Table 1). A nontreated control was also used to compare plant growth in the absence of herbicide. Treatments were randomly assigned and replicated four times. Herbicide treatments were applied to the foliage of giant salvinia using a forced-air CO₂-powered sprayer at an equivalent of 935 L ha⁻¹ diluent delivered through a single TeeJet^{®12} 80-0067 nozzle at 20 psi. All viable giant salvinia biomass was harvested 8 (Trial 1) and 15 (Trials 2 and 3) wk after treatment (WAT), dried to a constant weight (65° C), and recorded as dry weight biomass. Trial 1 data failed assumptions of normality and equal variance, so means were separated using 95% confidence intervals. Data from Trials 2 and 3 were subjected to ANOVA, with means separated using the Student–Newman–Keuls method ($P \leq 0.05$).

RESULTS AND DISCUSSION

All herbicide treatments resulted in plant injury 1 to 5 d after treatment (DAT), regardless of winter trial. In particular, plants treated with the contact herbicides carfentrazone, diquat, endothall, and flumioxazin alone or in combination with another herbicide produced rapid injury symptoms < 2 DAT. During Trial 1 (uncovered), new frond production was documented 7 to 10 DAT in plants treated with diquat and either surfactant, whereas all other herbicide-treated plants continued to decline in health through the first 2 wk after herbicide application. Air temperature at 14 and 16 DAT was at or below 0° C for 5 and 15 h, respectively (LSU AgCenter 2016). Consequently, all herbicide-treated plants, including those recovering from the diquat treatment, became necrotic and lost buoyancy within 3 d after the initial freeze event. Plants in Trial lwere subjected to a total of 40 h of freezing temperatures, with a range of -6.67 to 23.89° C throughout the duration of the experiment (Table 2). Although water temperature remained at or above 0.22° C throughout the 8wk trial, air temperature decreased to -5° C or less for 5 h. In contrast, plants treated in Trial 2 (uncovered) were exposed to fewer freeze events (27 h below freezing) and temperatures did not fall below -2.22° C. The warmer temperatures $(13.81^{\circ} \text{ C} \pm 6.41^{\circ} \text{ C})$ experienced during the 2015 to 2016 winter likely prevented complete plant control and ultimately allowed recovery before the final harvest 15 WAT. Although loggers failed to collect usable water

Table 2. Temperature data (C \pm 1 standard error) collected during the 2014 to 2015 and 2015 to 2016 giant salvinia winter herbicide efficacy trials.

Trial	1	1	2 and 3
Study duration	23 December 2014 to 16 February 2015	23 December 2014 to 16 February 2015	18 December 2015 to 29 March 2016
Temperature Source ¹	Air	Water	Air
Average temp	10.00 ± 5.86	10.42 ± 4.09	13.81 \pm 6.41
Range	-6.67 to 23.89	0.22 to 21.92	-2.22 to 27.22
Freezing hours ²	40	0	27

¹Air temperature data were collected from a weather station operated by the Louisiana State University AgCenter, whereas the water temperature data were collected from temperature loggers placed ca. 10 cm below the water surface.

²Number of hours air temperature was at or below 0 C throughout the duration of the trials.

temperature data, plants treated in Trial 3 (covered) did not experience freezing temperatures because tarps protected the tanks during freeze events (pers. obs.).

All herbicide treatments in Trial 1 reduced plant dry weight by 99 to 100% compared with nontreated controls 8 WAT (Table 1). In Trials 2 and 3, herbicide treatments reduced plant biomass 73 to 99% and 48 to 96%, respectively, thus indicating better control when plants are exposed to colder conditions. In general, diquat (regardless of surfactant) provided the best control of all herbicides in Trials 2 and 3. These results support current use of diquat at 3,364.1 g ai ha^{-1} (96 oz A^{-1}) for winter giant salvinia management in Louisiana and Texas. There were no general control trends with the other treatments in Trials 2 and 3. Mudge et al. (2016) evaluated similar herbicide treatments and found that most of the treatments were highly efficacious against giant salvinia in the spring and summer; however, treatments involving glyphosate + diquat or endothall + flumioxazin provided substantially less control during the fall, when slow plant growth likely caused decreased herbicide efficacy.

In addition to evaluating herbicide efficacy during the winter, the objective of these experiments was also to determine the influence of colder temperatures on plant survival. Plants in Trial 1 were subjected to the coldest temperatures and herbicide treatments resulted in 99 to 100% control. Trials 2 and 3 experienced less severe conditions (i.e., fewer freezing events and number of hours below 0° C), most notably in Trial 3, where plants were covered and sheltered from extreme temperatures. Therefore, as the severity of the winter increased (i.e., colder and freezing temperatures), efficacy increased. In addition to temperature data, biomass was an indicator of plant health throughout these trials. Plants used in Trials 2 and 3 actively grew throughout the winter and control plant dry weight increased by 8.65 and 10.36 g during Trials 2 and 3, respectively, from pretreatment to harvest. Conversely, dry weight of control plants in Trial 1 significantly decreased (from 17.35 to 3.66 g) as a result of exposure to a colder winter with an average temperature of $10.00^{\circ} \text{ C} \pm 5.86^{\circ} \text{ C}$ and 40 h of temperatures at or below freezing.

Giant salvinia can tolerate infrequent frosts or freezes, since buds can be sheltered from freezing temperatures by larger fronds. Whiteman and Room (1991) suggested that giant salvinia will persist in areas that experience frost but not ice formation, whereas Harley and Mitchell (1981) stated that continuous temperatures below 0° C can be lethal. Owens et al. (2004) conducted an acute-exposure study to determine the survivability of giant salvinia to low temperatures (4, -3, and -16° C) at various exposure times (1, 4, 8, 15, 24, and 48 h) and found that plant survival decreased as time below freezing and ice formation increased. Plants exposed to -16° C for 48 h were completely killed, whereas all other temperature/exposure treatments failed to provide control, which provides evidence of giant salvinia's hardiness when exposed to short periods of cold weather. Plants used in the current research were in the tertiary growth stage, but were only a single layer thick and were unprotected from colder air temperatures. This research demonstrates that the use of herbicides during colder temperatures can be a useful tool for control of giant salvinia.

Previous research reported that glyphosate efficacy on several terrestrial species increased as temperature increased (Adkins et. al. 1998, Waltz et al. 2004), but these findings are inconsistent with other research. Zhou et al. (2007) found that exposing glyphosate-treated velvetleaf (Abutilon theophrasti Medik.) to posttreatment temperatures of 5 and 12° C for 48 h enhanced control of the species, but cold stress before treatment adversely affected glyphosate efficacy. Also, Reddy (2000) reported that absorption and translocation of glyphosate in redvine (Brunnichia ovata [Walt.] Shinners) was higher in plants maintained at 15/10° C than at 25/20° C (day/night). Variable results of temperature effects on the translocation and absorption of glyphosate are most likely due to different bioassay species and temperature regimes (Zhou et al. 2007). In our research, four of the eight treatments utilized glyphosate alone or in combination with another herbicide. Plants in Trial 1 experienced 13 h of subzero temperatures 14 DAT and all herbicide treatments resulted in 100% control. Our research provides the first published data that describe temperature/winter influence on efficacy of carfentrazone, diquat, flumioxazin, or endothall.

When herbicides were applied in these trials, plants were not completely brown but instead retained enough green coloration to be considered relatively healthy for the winter. Future research should investigate herbicide application after plants have been subjected to temperatures near or below 0° C to determine whether herbicide uptake and efficacy are decreased when plants are unhealthy or stressed (as they would likely be in February and March) because plant conditions including slow growth, brown in coloration, loss in buoyancy, etc. may influence herbicide uptake, translocation, and efficacy.

These data provide evidence that herbicide treatments are useful when applied in December, especially when applied before cold conditions. Therefore, efforts to chemically manage giant salvinia should not cease at the conclusion of the growing season, but should instead continue through early to midwinter. We did not detect differences in herbicide efficacy under severe winter conditions; thus any of the treatments evaluated in these studies should be effective. Although all herbicide treatments were efficacious in Trial 3 (covered), plants exhibited less injury and more recovery compared with uncovered plants in Trials 1 and 2. These experiments should be conducted under milder winter conditions (natural or artificial) to determine if level of control is subject to change.

SOURCES OF MATERIALS

¹Miracle-Gro[®] all purpose plant food, The Scotts Company, P.O. Box 606, Marysville, OH 43040.

 $^2\mathrm{HOBO}^{\circledast}$ Water Temperature Pro v2 data logger, Onset Computer Corporation, 470 MacArthur Blvd., Bourne, MA 02532.

 $^3\mathrm{Stingray^{\circledast}},$ SePRO Corporation, 11550 N. Meridian St., Suite 600, Carmel, IN 46032.

⁴Tribune™, Syngenta Crop Protection, P.O. Box 18300, Greensboro, NC 24719.

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

⁶Clipper™, Valent USA Corporation, P.O. Box 8025, Walnut Creek, CA 94596.

⁷Roundup Custom[™], Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167.

 8 Turbulence $^{\rm TM},$ Winfield Solutions, LLC, P.O. Box 64589, St. Paul, MN 55164.

 $^{9}\mathrm{Aqua}\textsc{-King}$ Plus®, Winfield Solutions, LLC, P.O. Box 64589, St. Paul, MN 55164.

 $^{10}\mathrm{AirCover^{\tau s}},$ Winfield Solutions, LLC, P.O. Box 64589, St. Paul, MN 55164.

 $^{11} \mathrm{Surf}\text{-}\mathrm{AC}^{\circledast}$ 910, Drexel Chemical Company, P.O. Box 13327, Memphis, TN 38113.

¹²TeeJet[®], Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60187.

ACKNOWLEDGEMENTS

This research was supported by the U.S. Army Engineer Research and Development Center Aquatic Plant Control Research Program and the LSU Vice President for Agriculture Assistantship. The authors are appreciative of technical assistance provided by Alex Perret and Jonathan Winslow of the Louisiana Department of Wildlife and Fisheries. The authors thank Terry Corbett and Jill Day for providing reviews of this manuscript. Permission was granted by the Chief of Engineers to publish this information. Citation of trade names does not constitute endorsement or approval of the use of such commercial products.

LITERATURE CITED

- Adkins SW, Tanpipat S, Swarbrick JT, Boersma M. 1998. Influence of environmental factors on glyphosate when applied to *Avena fatua* or *Urochlua panicoides*. Weed Res. 38:129–138.
- Glomski LM, Getsinger KD. 2006. Carfentrazone-ethyl for control of giant salvinia. J. Aquat. Plant Manage. 44:136–138.
- Harley KLS, Mitchell DS. 1981. The biology of Australian weeds 6. Salvinia molesta D.S. Mitchell. J. Aust. Inst. Agr. Sci. 67–76.
- Jacono C. 1999. Salvinia molesta (D. S. Mitchell) invades the United States. Aquatics. 21(1):4-9.
- Jacono C, Pitman B. 2001. Salvinia molesta: Around the world in 70 years. ANS Digest (4)2:13-16.
- LSU AgCenter. 2016. http://weather.lsuagcenter.com/reports.aspx?r=4. Accessed October 3, 2016.
- Mudge CR, Heilman MA, Theel HJ, Getsinger KD. 2012. Impact of subsurface and foliar penoxsulam and fluridone applications on giant salvinia. J. Aquat. Plant Manage. 50:116–124.
- Mudge CR, Perret AJ, Winslow JR. 2014. Evaluation of new herbicide combinations for managing giant salvinia in Louisiana. Proc. Aquat. Plant Manage. Soc. 54:34.
- Mudge CR, Perret AJ, Winslow JR. 2016. Evaluation of foliar herbicide and surfactant combinations for control of giant salvinia at three application timings. J. Aquat. Plant Manage 54:32–36.
- Nelson LS, Glomski LM, Gladwin DN. 2007. Effect of glyphosate rate and spray volume on control of giant salvinia. J. Aquat. Plant Manage. 45:58– 61.
- Nelson LS, Skogerboe JG, Getsinger KD. 2001. Herbicide evaluation against giant salvinia. J. Aquat. Plant Manage. 39:48–53.
- Owens CS, Smart RM, Stewart RM. 2004. Low temperature limits of giant salvinia. J. Aquat. Plant Manage. 42:91–94.
- Reddy KN. 2000. Factors affecting the toxicity, absorption and translocation of glyphosate in redvine (*Brunnichia ovata*). Weed Technol. 14:457– 462.
- Texas Parks and Wildlife. 2015. Texas ramping up fight against aquatic invasive species. http://tpwd.texas.gov/newsmedia/releases/? req=20151218a. Accessed February 25, 2016.
- Waltz AL, Martin AR, Roeth FW, Lindquist JL. 2004. Glyphosate efficacy on velvetleaf varies with application time of day. Weed Technol. 18:931– 939.
- Whiteman JB, Room PM. 1991. Temperatures lethal to Salvinia molesta Mitchell. Aquat. Bot. 40:27–35.
- Zhou J, Tao BT, Messersmith CG, Nalewaja JD. 2007. Glyphosate efficacy on velvetleaf (Abutilon theophrasti) is affected by stress. Weed Sci. 55:240–244.