J. Aquat. Plant Manage. 59: 14–19

Evaluation of foliar and subsurface applications of metsulfuron-methyl for control of giant salvinia

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ABSTRACT

Giant salvinia (*Salvinia molesta* D.S. Mitchell) is an invasive floating aquatic fern that has rapidly spread throughout Louisiana and Texas since the late 1990s. Management is difficult due to the limited number of efficacious herbicides. Recently a Section 24(c) registration [Special Local Need (SLN) label] was granted in Louisiana and Texas for the use of metsulfuron-methyl (MSM), but studies are lacking to determine the optimum use patterns for selective control of giant salvinia in public waterbodies. Metsulfuron applied to the foliage of giant salvinia at 2.6, 5.3, 10.5, 21.1, 42.1, 84.1, and 168.2 g ha^{-1} resulted in growth cessation and necrosis 1 wk after treatment (WAT) with gradual stunting and decline throughout the 8-wk trial. Based on dry weight biomass, the calculated foliar lethal dose required to control 90% of the test population (LD_{90}) of MSM when applied to the foliage of giant salvinia was 3.83 g ha^{-1}, which is less than one-tenth of the max use rate (42.1 g ha^{-1}). Additional trials evaluating foliar applications of MSM (42 g ha^{-1}) in combination with currently registered aquatic herbicides found that $\geq$99% control was achieved when MSM was mixed with carfentrazone, diquat, flumioxazin, or glyphosate. Combination treatments provided faster visual injury compared to MSM alone but resulted in no differences among treatments at the conclusion of the trial. Subsurface static applications of MSM at 10 to 80 μg L^{-1} provided >98% control (10 WAT), and the calculated EC_{90} (effective concentration required to control 90% of the test population) based on dry weight was 1.87 μg L^{-1}. The results of these experiments demonstrate that metsulfuron is highly efficacious on giant salvinia when applied to the foliage and subsurface.

Key words: ALS inhibitor, carfentrazone, chemical control, diquat, flumioxazin, glyphosate, tank mix.

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INTRODUCTION

Since its introduction into the southern United States in 1995, the free-floating aquatic fern giant salvinia has become a nuisance in lakes, rivers, and reservoirs (Johnson 1995, Jacono 1999). This invasive plant is capable of doubling its biomass in as little as 36 h under optimal growing conditions (Johnson et al. 2010). Due to its rapid growth rate, giant salvinia has the capability to outcompete other vegetation for resources such as nutrients, light, and surface area (Mitchell and Tur 1975) and can quickly form a monoculture. Further implications of giant salvinia infestations include, but are not limited to, hindering recreational activities, restricting irrigation capacity, clogging water intakes and impairing flood control, decreasing waterfront property values, and impacting rice, crawfish, and catfish farming operations (Horst and Mapes 2000; Nelson 2014). Although listed as a Federal Noxious Weed by the U.S. Department of Agriculture, prohibiting importation to the United States and transportation across state lines (McFarland et al. 2004), giant salvinia has spread to 13 states: Alabama, Arizona, Arkansas, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, North Carolina, South Carolina, Texas, and Virginia (Thayer et al. 2018).

Management of giant salvinia has proven difficult. Although there are viable mechanical and biological control strategies (Chilton et al. 2002, Madsen and Wersal 2009), chemical control is the most widely used (Netherland 2014), and 10 of the 15 Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Section 3 registered aquatic herbicides have demonstrated some level of activity, but the majority of these herbicides fail to provide long-term control (Nelson et al. 2001, Glomski et al. 2003, Glomski and Getsinger 2006, Mudge and Harms 2012, Mudge et al. 2012, 2013, Glomski and Mudge 2013, Mudge 2016). However, diquat, glyphosate, and their combination are the most widely used and cost-effective treatments and have been used almost exclusively during the growing season and winter by the Louisiana Department of Wildlife and Fisheries (LDWF) for over 20 yr (Madsen and Wersal 2009, Mudge et al. 2016, Sartain and Mudge 2018). Due to the reliance on two herbicide mechanisms of action, there is a need to find additional active ingredients to manage giant salvinia and prevent the development of herbicide resistance.

The previously registered acetolactate synthase (ALS) - inhibiting herbicides bispyribac-sodium and penoxsulam have also been evaluated for activity on giant salvinia in mesocosm settings. Bispyribac provided up to 69 and 73% control when applied subsurface under static conditions and to the foliage of plants, respectively, at 12 wk after treatment (WAT) (Glomski and Mudge 2013). However, all plants, even at the highest rate/concentration evaluated, recovered within 2 to 6 WAT regardless of application method; therefore, bispyribac is not currently operationally utilized due the lack of long-term control. Conversely, giant salvinia exposed to penoxsulam at 10 and 20 μg L^{-1} for 12 wk were reduced in biomass by >85%; however, plants recovered once the product was removed from the water column (Mudge et al. 2012).

Metsulfuron-methyl (MSM) is an ALS-inhibiting herbicide that is labeled for use in wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), pastures, turf, right-of-way, and industrial sites to control broadleaf weeds, brush, and deciduous trees (Shaner 2014, Bayer 2019a). In addition, MSM has a SLN registration (FL-170005) in Florida for the control of Old World climbing fern [OWCF, *Lygodium microphyllum* (Cav.) R. Br.] (Bayer 2019b, Bayer 2019c). This label allows use of MSM in freshwater marshes and hydric forests, as long as the water is not used for irrigation or drinking.

The effectiveness of MSM for controlling OWCF, coupled with a low toxicity profile (Shaner 2014), subsequently led to evaluation for giant salvinia control. Preliminary herbicide trials by Sartain and Mudge (2018) demonstrated that foliar applications of MSM were capable of providing 98 to 100% control of giant salvinia at rates ≤42 g ha^{-1}. From these data, a SLN label was approved by Louisiana and Texas to use MSM to control giant salvinia in public waterways (Alligare 2019, Alligare 2020, Bayer 2020).

The SLN registrations in Louisiana and Texas warrant additional research to refine foliar rates and develop use patterns for managing giant salvinia with MSM. Although MSM application is restricted to a foliar use pattern, subsurface applications (i.e., in-water treatments) should be investigated to determine plant sensitivity to this herbicide in the event that a Section 3 aquatic label is granted in the future. Therefore, the objectives of this research were to 1) evaluate foliar application rates to determine the most effective rate of MSM to control giant salvinia, 2) evaluate MSM in combination with currently registered aquatic herbicides for compatibility and increased efficacy, and 3) determine efficacy of subsurface MSM applications.

MATERIALS AND METHODS

General plant establishment

Three outdoor mesocosm trials were conducted and repeated at the Louisiana State University (LSU) Aquaculture Research Facility in Baton Rouge, LA, in 2018 (May and July) and 2019 (May). Giant salvinia was sourced from populations maintained at the LSU Aquaculture Research Facility. Plant propagation techniques were adapted from previous giant salvinia research (Nelson et al. 2007, Mudge et al. 2012, Mudge et al. 2016, Sartain and Mudge 2018). For all trials, plants in the tertiary, or mat-forming, growth stage (Mitchell and Tur 1975) were cultured under full sunlight in 76-L high-density polyethylene (HDPE) containers (49.5 cm diameter by 58.4 cm height) filled with approximately 60 L of pond water (pH 8.5). Sphagnum moss (14 g dry weight) was added to each water-filled container to lower the pH (6.5 to 7.0) and mimic water pH conditions where giant salvinia thrives (Cary and Weerts 1984, Owens et al. 2005). Equal amounts of plant material, enough to cover approximately 70% of the water surface, were placed in each container. Fertilizer[1] (2.1 g) was added to each container at planting and every other week throughout the duration of the trials to maintain plant growth. Plants were allowed to acclimate for 2 wk before herbicide treatments were administered. At the time of treatment, plants had reached 100% coverage and were thinned to a single layer to ensure complete herbicide contact. In the subsurface titration trial, plants were approximately two layers thick, but the thicker plant stand did not limit herbicide contact to the plants since MSM was applied into the water column.

Treatments were arranged in a completely randomized design with five replicates per treatment. Within each trial, biomass from five extra containers was collected pretreatment to measure plant biomass (dry weight) prior to herbicide application. In addition, a nontreated control (NTC) was included in each trial to monitor plant growth in the absence of the herbicide treatments. The NTC plants demonstrated healthy growth and increased 145 to 277% in dry weight when compared to pretreatment plants in all trials (data not shown).

Foliar rate titration trial

Metsulfuron[2] was applied to the foliage of giant salvinia at 2.6, 5.3, 10.5, 21.1, 42.1, 84.1, and 168.2 g ha^{-1}. All treatments included a nonionic surfactant[3] at 0.25% (v/v) and were applied using a CO_2-powered sprayer calibrated to deliver 935 L ha^{-1} through a single TeeJet® brass flat fan 800067 nozzle[4] at 20 psi. As the herbicide was applied, a shielding device was used to minimize herbicide drift to adjacent containers/plants. At 8 WAT, all viable plant material was harvested, dried (65 C), and weighed. Dry weight data were analyzed using nonlinear regression (exponential decay, $y = b_0e^{-bx}$) with the PROC NLIN procedure in SAS®[5] (version 9.4) statistical software. Regression models were used to determine the LD_{90} value for MSM applied to the foliage of giant salvinia. Data were pooled across trials because slopes of regression lines were not significantly different at the 95% confidence interval level.

Foliar combination trial

Herbicides evaluated in the foliar combination trial included MSM alone and in combination with glyphosate[6], diquat[7], flumioxazin[8], and carfentrazone[9] (Table 1). Glyphosate + diquat was also included as an operational standard (Mudge et al. 2016). All herbicide treatments included the same nonionic surfactant (0.25% v v^{-1}) and application procedures as described above. Following herbicide application, phytotoxicity (%) was assessed visually at 1, 3, 7, 14,

Table 1. Giant salvinia injury and control resulting from foliar applied herbicides.[1]

Treatment	Rate[2] (g ha^{-1})	% Injury 3 DAT[3,4]	7 DAT	14 DAT	28 DAT	56 DAT	Dry Biomass (g ± SE) 56 DAT
Non-treated control	0	0 a	0 a	0 a	0 a	0 a	65.2 ± 5.6 a
Metsulfuron	42	10 a	50 b	70 b	90 b	98 b	0.6 ± 0.3 a
Metsulfuron + Glyphosate	42 + 1121	30 b	60 bc	70 b	95 b	100 b	0.0 ± 0.0 a
Metsulfuron + diquat	42 + 280	30 b	50 b	70 b	90 b	98 b	0.4 ± 0.2 a
Metsulfuron + Flumioxazin	42 + 72	40 bc	70 c	80 bc	90 b	100 b	0.0 ± 0.0 a
Metsulfuron + Carfentrazone	42 + 67	40 bc	60 bc	80 bc	90 b	98 b	0.5 ± 0.3 a
Glyphosate + diquat	3364 + 561	50 c	70 c	90 c	90 b	96 b	2.2 ± 2.5 a

[1]Data obtained by visually estimating giant salvinia injury on a scale of 0 to 100%, where 0 = no injury and 100 = complete plant death.
[2]Herbicides rates listed as g active ingredient (a.i.) ha^{-1}, except glyphosate, which was applied as g acid equivalent (a.e.) ha^{-1}.
[3]Abbreviation: DAT, days after treatment.
[4]Means within a column followed by the same letter are not significantly different based on Fisher's protected LSD test ($P < 0.05$; $n = 10$).

28, and 56 d after treatment (DAT). Visual estimates of giant salvinia injury were determined on a scale of 0 to 100, where 0 = no plant injury (i.e., no chlorotic/necrotic tissue) and 100 = complete plant death. At 56 DAT all viable biomass was harvested, dried, and weighed. Dry weight data were subjected to an analysis of variance (ANOVA), and a post-hoc test (Fisher's protected LSD) was conducted to determine significant differences among treatments ($P < 0.05$). Due to no significant differences between the initial and repeat trial, data were pooled across experiments.

Subsurface concentration titration trial

Subsurface applications of MSM were administered directly to the water column at concentrations of 1, 2.5, 5, 10, 20, 40, and 80 μg L^{-1} (parts per billion, ppb). The herbicide was mixed into a 1 g L^{-1} solution with water, applied at the appropriate concentration with a pipette directly into the water column, and stirred thoroughly. Since subsurface applications of MSM have not been previously documented for controlling giant salvinia, plants were subjected to a static exposure for the duration of the experiment (10 wk). At 10 WAT, all viable plant material was harvested, dried, and weighed. Dry weight data were analyzed using the PROC NLIN procedure, and regression models were used to determine the EC_{90}, the effective concentration of MSM required to cause a 90% reduction in dry weight compared to NTC plants. Data were pooled across trials because slopes of regression lines were not significantly different at the 95% confidence interval level.

RESULTS AND DISCUSSION

Foliar rate titration trial

Giant salvinia exposed to foliar applications of MSM resulted in necrosis and malformation of new fronds as early as 7 DAT for all rates tested (data not shown). Plant recovery, through the development of new/healthy fronds under and at the water surface, was documented as early as 14 DAT for plants exposed to 2.6 and 5.3 g ha^{-1}; however, no regrowth was documented for plants exposed to rates ≥10.5 g ha^{-1}. The type and progression of giant salvinia injury symptoms noted in the current research were similar to those observed by Sartain and Mudge (2018) when MSM was applied at 21 to 84 g ha^{-1}. The calculated LD_{90} of MSM when applied to giant salvinia foliage was 3.83 g ha^{-1} (Figure 1). These results confirm previous research illustrating that MSM is highly effective against giant salvinia when applied to the foliage at very low use rates (Sartain and Mudge 2018). Similarly, MSM demonstrated 100% efficacy against the highly invasive plant OWCF at 40 to 80 g ha^{-1} (Langeland and Link 2006) and is currently used to control OWCF under the Florida SLN label at 21.1 to 84.1 g ha^{-1} (Bayer 2019b). Under the current SLN labels, MSM can be applied to the foliage of giant salvinia at 21.1 to 42.1 g ha^{-1} (Alligare 2019, 2020). Future field research is required to evaluate MSM at these rates against plants in an operational setting to verify the small-scale results.

Foliar combination trial

Based on the results in the foliar rate titration trial, MSM applied alone at 21.1 to 42.1 g ha^{-1} would require several days to observe injury symptoms on giant salvinia. Tank mix

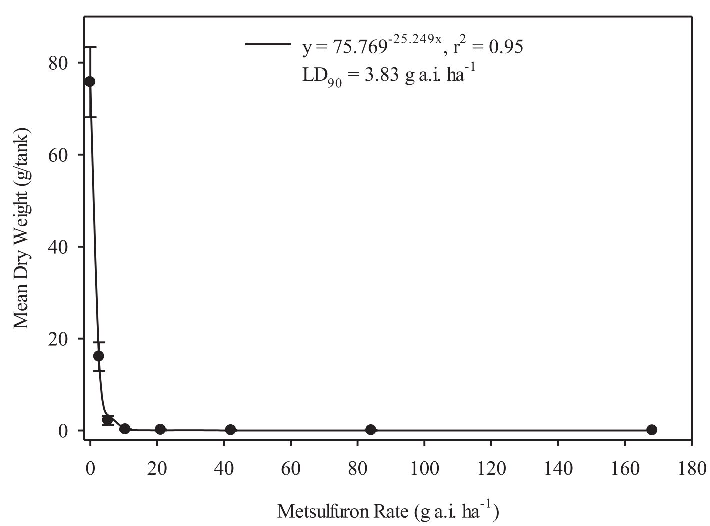

Figure 1. The effect of foliar applications of metsulfuron on giant salvinia dry weight 8 wk after treatment. Data are shown as dry weight means ± standard error ($n = 10$). A nonionic surfactant (0.25% v/v) was added to all treatments. LD_{90} = lethal dose 90, rate of metsulfuron required to control 90% of the giant salvinia test population. Mean pretreatment dry weight = 20.5 g.

partners such as contact or faster acting herbicides would be beneficial since aquatic applicators often reply on next-day rapid injury (i.e., visual markers) to determine where to continue spraying on consecutive days (Mudge and Netherland 2014, 2015). Metsulfuron is compatible with several herbicides, and tank mixes are recommended on MSM herbicide labels (Bayer 2019a, USEPA 2019) and by weed control suggestion guides (Stephenson et al. 2020). Therefore, as a follow-up trial, several herbicides were investigated in combination with MSM to determine if speed of injury would be enhanced while evaluating compatibility. All combination treatments with MSM plus carfentrazone, diquat, flumioxazin, or glyphosate, as well as the comparison treatment of glyphosate + diquat, resulted in ≥30% plant injury 1 to 3 DAT (Table 1). Metsulfuron + flumioxazin and MSM + carfentrazone treatments were not different than glyphosate + diquat at 3 DAT and provided more rapid visual injury compared to MSM + glyphosate and MSM + diquat. Although MSM + glyphosate and MSM + diquat did not provide injury as quickly as other treatments, visual injury was more prominent than MSM alone (10%) at 3 DAT. However, by 14 DAT, visual injury differences among MSM treatments were not detectible. Foliar applications of glyphosate + diquat resulted in >90% giant salvinia injury by 14 DAT with substantial necrosis and desiccation (Table 1). All plants treated with tank mixes involving MSM were necrotic and began to desiccate by 28 DAT, and the majority of the plants fell to the bottom of the container prior to the conclusion of the trial. At 56 DAT, all MSM treatments resulted in ≥99% control (Table 1). Despite injury being slower to develop for MSM applied alone, there were no differences among treatments at the conclusion of the trial. Similarly, glyphosate + diquat reduced plant biomass by 97% when compared to the NTC at 56 DAT.

These data provide evidence that the 42.1 g ha^{-1} rate of MSM approved by the Louisiana Department of Agriculture & Forestry (LDAF), Texas Department of Agriculture (TDA), and U.S. Environmental Protection Agency (USEPA) when tank mixed with carfentrazone, diquat, flumioxazin, or glyphosate was highly efficacious and demonstrated no negative effects when applied to the foliage of giant salvinia. Injury symptoms developed more rapidly from the combination treatments compared to metsulfuron alone, but the overall level of plant control was not negatively impacted and the same by the conclusion of the trials. These results will assist natural resource agencies with managing large populations of giant salvinia, particularly when aquatic sites are continuously sprayed and applicators require site markers to denote areas sprayed the previous day. Since MSM requires several days to provide injury symptoms, the addition of the nonselective contact herbicides flumioxazin, carfentrazone, and diquat offer rapid visual markers (hours to 1 d), which can aid in distinguishing treated versus untreated sites. Previous research (Mudge and Netherland 2014, 2015) demonstrated a similar concept when the slow-acting ALS herbicides penoxsulam, imazamox, and bispyribac were combined with several contact herbicides to provide rapid injury and control to water hyacinth [*Eichhornia crassipes* (Mart.) Solms] and water lettuce (*Pistia stratiotes* L.) compared to the ALS herbicides applied alone.

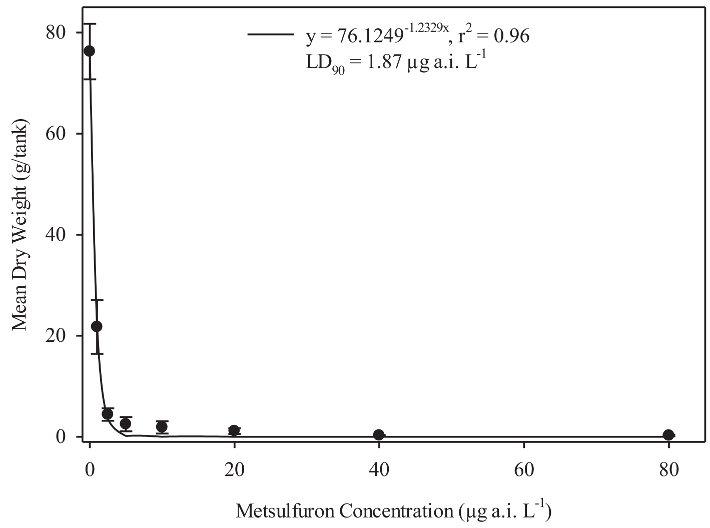

Figure 2. The effect of subsurface applications of metsulfuron on giant salvinia dry weight 10 wk after treatment. Metsulfuron was applied as a single application and plants were exposed to the herbicide under static conditions. Data are shown as dry weight means ± standard error ($n = 10$). EC_{90} = effective concentration 90, concentration of metsulfuron required to control 90% of the giant salvinia test population. Mean pretreatment dry weight = 40.2 g.

In addition, tank mixing MSM with another herbicide could provide resistance management instead of applying one herbicide and be a suitable rotation partner for the glyphosate + diquat mixture (Mudge et al. 2016).

Subsurface concentration titration trial

Visual estimation of injury was 10 to 20% at 7 DAT and >50% at 14 DAT for plants exposed to MSM concentrations ≥10 µg L^{-1}. Plants treated with the lower concentrations (1, 2.5, and 5 µg L^{-1}) did not show any injury symptoms until 14 DAT, and plants became increasingly necrotic from 14 to 21 DAT; however, at 28 DAT, healthy buds began to emerge below the water surface. Unlike the lower concentration treatments, no plant recovery occurred at doses ≥10 µg L^{-1}. The calculated EC_{90} for MSM applied subsurface under static conditions was 1.87 µg L^{-1} (Figure 2). It can be concluded that MSM is highly active as an in-water treatment against giant salvinia in the tertiary growth stage. Metsulfuron has also shown activity when applied subsurface to common duckweed (*Lemna minor* L.) and Eurasian watermilfoil (*Myriophyllum spicatum* L.), with EC_{50} values of 0.36 and 0.816 µg L^{-1}, respectively (USEPA 2020a, 2020b). The high level of efficacy at extremely low concentrations of MSM on these target plants is a favorable characteristic for aquatic use. The current data as well as previous findings (USEPA 2020a, 2020b) could be used as supporting material if an herbicide registrant were considering applying for a Section 3 aquatic label for MSM use in the United States. However, additional research would be required for this method of application to be utilized in an operational setting. Since these experiments were conducted under static conditions, future research should investigate the impact of concentration exposure times to determine the required concentration and exposure time to control target plant species. The effect of water pH on in-water activity of MSM should also be investigated since sulfonylurea herbicides are degraded at faster rates by acid hydrolysis, which increases at lower pH levels (Grey and McCullough 2012). Metsulfuron half-life ranges from 4 to 9.6 days at pH 5.2 and 116 days at pH 7.1 (National Center for Biotechnology Information 2019). Since giant salvinia thrives at a pH < 7.5 (Cary and Weerts 1984) and has been shown to decrease water pH over time, MSM might degrade more rapidly in waterbodies where this plant thrives. A short herbicide half-life is environmentally favorable but could result in decreased efficacy at lower pH levels due to faster degradation.

The results of these three experiments demonstrate that MSM is highly active against giant salvinia when applied to the foliage or as a subsurface treatment. When applied to the foliage at rates of 10.5 to 168.2 g ha^{-1}, MSM treated plants were slow to develop injury symptoms, but $>99\%$ control was achieved by 8 WAT and one-tenth of the max label use rate (3.83 g ha^{-1}) would reduce dry biomass by 90% based on the calculated LD_{90}. Metsulfuron was also compatible in tank mix combinations with glyphosate, flumioxazin, diquat, and carfentrazone, which did not enhance control from metsulfuron used alone but did increase speed of injury and would provide visual markers for aquatic applicators. Although subsurface applications of MSM are prohibited on the current SLN label, these data provide evidence that this compound is highly effective ($>99\%$ control at ≥ 10 μg L^{-1}) and support a new use pattern on future labels (SLN or Section 3). The high level of MSM activity on giant salvinia as a foliar and subsurface treatment further supports previous research (Sartain and Mudge 2018), indicating that this herbicide is a viable control option for this highly invasive floating fern.

SOURCES OF MATERIALS

[1]Miracle-Gro® Lawn Fertilizer (24-8-16), The Scotts Company, P.O. Box 606, Marysville, OH 43040.

[2]MSM 60®, Alligare LLC, 13 N. 8th Street, Opelika, AL 36801.

[3]Surf-AC® 910, Drexel Chemical Company, P.O. Box 13327, Memphis, TN 38113.

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

[5]SAS® software version 9.4, SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513.

[6]Roundup Custom™, Bayer CropScience LP, P.O. Box 12014, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709.

[7]Reward®, Syngenta Crop Protection LLC, P.O. Box 18300, Greensboro, NC 27419.

[8]Clipper®, Valent U.S.A. Corporation, P.O. Box 8025. Walnut Creek CA 94596.

[9]Stingray®, SePRO Corporation, 11550 N. Meridian St., Suite 600, Carmel, IN. 46032.

ACKNOWLEDGEMENTS

Support for this work was provided by the Louisiana State University Agricultural Center and the Louisiana Department of Wildlife and Fisheries Aquatic Plant Control Program. Appreciation is extended to Trista Galivan, Daniel Humphreys, Shelby Sirgo, Bennett Judice, Ashley Weaver, and Patrick Saucier for technical assistance throughout this research. This document was reviewed in accordance with U.S. Army Engineer Research and Development Center policy and approved for publication. Citation of trade names does not constitute endorsement or approval of the use of such commercial products. The authors declare no conflicts of interest.

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