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The response of giant salvinia to foliar herbicide applications at three winter timings

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ABSTRACT

The growth habits of giant salvinia (*Salvinia molesta* D.S. Mitchell) make it difficult to get adequate coverage and control with foliar-applied herbicides during the growing season. Winter may be an opportune time for natural resource managers to use foliar-applied herbicides to achieve favorable control without making multiple applications throughout the growing season. Therefore, outdoor mesocosm trials were conducted to investigate the efficacy of the aquatic herbicides glyphosate, diquat, flumioxazin, and glyphosate + diquat against mature giant salvinia during the winter of 2015 to 2016 (yr 1) and 2016 to 2017 (yr 2). Dry wt data 12 wk after the February application showed no significant herbicide treatment by application timing interaction. However, significant differences were noted for herbicide treatment and application timing in yr 1, but not yr 2. Plant response in yr 1 and 2 varied significantly because of dissimilar environmental conditions (i.e., yr 2 plants being exposed to colder temperatures for longer periods). Diquat, flumioxazin, glyphosate, and glyphosate + diquat treatments reduced giant salvinia biomass by 27, 45, 55, and 55%, respectively. Applications during January and February resulted in greater control than those applied in December (47 and 50% vs. 33%, respectively). Year 2 giant salvinia control was $\geq$ 99% for all herbicide treatments and application timings. Herbicide applications, in combination with extended periods of subfreezing temperatures, can increase giant salvinia control during the winter. In the event of a milder winter, in which temperatures do not remain at or below freezing for a consecutive $\geq$ 9 h, herbicide applications just before a minor freeze event can increase herbicide efficacy. However, applications of contact herbicides to freeze-damaged fronds later in the winter can lead to decreased control because of minimal healthy plant material being contacted with herbicide solution.

Key words: biomass, diquat, flumioxazin, freeze, glyphosate, mesocosm, timing.

INTRODUCTION

The first documentation of giant salvinia (*Salvinia molesta* D.S. Mitchell) in the United States occurred in 1995 in South Carolina (Johnson 1995). Since its introduction, it has spread across the southern United States and as far west as California and Hawaii (Thayer et al. 2018). Giant salvinia was first documented in Louisiana in 1998 on the Toledo Bend Reservoir, which lies on the Louisiana–Texas border (Horst and Mapes 2000) and has since dispersed across Louisiana and East Texas. These infestations have caused ecological and economic impacts, including reduction of desirable native plant species, disruption of transportation and irrigation, decreased recreational use, and increased mosquito breeding habitat (Jacono 1999, Jacono and Pittman 2001, Nelson et al. 2001), among others. These negative impacts have led to active management operations for controlling giant salvinia in Louisiana and Texas.

Small-scale research and large-scale field operations have shown that herbicides registered for aquatic use in the United States are capable of managing giant salvinia infestations (McFarland et al. 2004), but its growth habits make it difficult to get adequate control with foliar herbicide applications. Rapid growth and the ability to quickly form multiple plant layers often prevents foliar-applied herbicides from reaching fronds and plants that are found under the upper mat layers (Horst and Mapes 2000). Chemical management in Louisiana is primarily administered with a combination of the aquatic herbicides glyphosate + diquat and two adjuvants during the growing season from April through October (Mudge et al. 2014, Mudge et al. 2016). In 2016, the Louisiana Department of Wildlife and Fisheries chemically managed > 8,000 ha of giant salvinia in public water bodies throughout the state (A. Perret, pers. comm.).

Well-established giant salvinia infestations are typically multiple plant-layers thick and require multiple herbicide applications throughout the year to achieve adequate control (Mudge et al. 2016). The winter months may be an ideal time for natural resource managers to use foliar-applied herbicides to achieve favorable control. During periods of cold weather, many plant layers are often not present, plant biomass is substantially reduced, and as a result, fewer herbicide applications might be required when plant growth is much slower compared with the spring, summer, and fall. Winter applications may also allow herbicides to contact a larger portion of the target plant population because one layer of plant material is likely present during December, January, and February.

Typically, higher temperatures increase herbicide absorption and/or translocation resulting in improved herbicide efficacy (Pline et al. 1999). Many natural resource agencies speculate that herbicides are not effective for managing aquatic plants during the winter because plants are slow growing or dormant because of the lower

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temperatures and shorter photoperiods (Mudge and Sartain 2018). Although the metabolic activity of most plant species is suppressed during the winter, compared with during the growing season, the effectiveness of a particular herbicide can vary across temperatures and species. For example, glyphosate efficacy for control of bermudagrass [*Cynodon dactylon* (L.) Pers.] improved at 32 C, compared with 22 C (Jordan 1977), and johnsongrass [*Sorghum halepense* (L.) Pers] control was greater at 35 C, compared with 24 C (McWhorter et al. 1980). In contrast, control of large crabgrass [*Digitaria sanguinalis* (L.) Scop.] and common waterhemp (*Amaranthus rudis* J. D. Sauer) with mesotrione was greater at 18 C, compared with 32 C (Johnson and Young 2002). The improved efficacy at a lower temperature may be due to the herbicide being metabolized more slowly (Godar et al. 2015).

In Louisiana, winter applications of diquat (1.7 kg ai ha^{-1}] + one surfactant are sometimes used to manage severe giant salvinia infestations (Mudge et al. 2014). In addition, federal and state agencies in Texas have managed giant salvinia with glyphosate + diquat treatments during winter (T. Corbett, pers. comm.). However, a few replicated studies have been conducted to evaluate herbicide efficacy on giant salvinia during the winter months (Mudge and Sartain 2018) and replicated research investigating stand-alone and/or combination treatments at multiple winter timings have not been conducted. To investigate herbicide efficacy on giant salvinia at various winter application timings, the following mesocosm research was conducted.

MATERIALS AND METHODS

Outdoor mesocosm trials were conducted at the Louisiana State University (LSU) AgCenter Aquaculture Research Facility in Baton Rouge, LA, to investigate herbicide efficacy against mature giant salvinia during the winter of 2015 to 2016 (yr1) and 2016 to 2017 (yr2). Tertiary growth–stage plants, collected from stock tanks at LSU Aquaculture, were cultured in 90 76-L plastic containers (49.5 cm diam by 58.4 cm ht) filled with approximately 60 L of pond water (pH 8.5) amended with sphagnum moss (14 g) to lower the pH to < 7.0. Tertiary growth–stage plants possess fronds that are larger and more robust than plants in the primary and secondary growth stage, and it is commonly referred to as the mat-forming growth stage in which plants begin to mat on top of one another (Mitchell and Tur 1975). On 18 September 2015 and 29 September 2016, equal amounts of fresh plant material, enough to cover approximately 85% of the water surface, were placed in each 76-L tank. In addition, 2.1 g of Miracle-Gro[1] Water Soluble Lawn Food (24–8–16) was applied to each container at planting and every 2 wk until mid-December. Fertilization resumed in mid-March and continued every 2 wk in both trials until final harvest. Culture and planting techniques were adapted from previous giant salvinia research (Nelson et al. 2007, Mudge et al. 2012, Mudge et al. 2016).

Plants received foliar herbicide applications at one of the three application timings. Treatments during the initial trial, hereafter referred to as yr 1, were applied 12 December 2015, 12 January 2016, and 11 February 2016. Treatments during the repeat trial, hereafter referred to as yr 2, were applied 6 December 2016, 4 January 2017, and 6 February 2017. These timings were chosen to compare early, mid-, and late-winter applications to determine which provided the best giant salvinia control. Each treatment was replicated five times at the three application timings. Nontreated reference plants were used to compare plant growth in the absence of herbicide and five pretreatment samples were collected before each application timing to quantify plant biomass. Hourly air-temperature data during both trials were obtained from a local weather station (LSU AgCenter 2017).

Before the December treatment period in both trials, plants were green, healthy, and matted several layers thick ($>$ 5 cm). Herbicide treatments were administered with a CO_2-powered sprayer at an equivalent of 935 L ha^{-1} diluent delivered through a single TeeJet 80-0067 nozzle[2] at 20 psi. Herbicide treatments included diquat[3] (1.1 kg ai ha^{-1}), glyphosate[4] (3.3 kg ae ha^{-1}), flumioxazin[5] (0.2 kg ai ha^{-1}), glyphosate (3.3 kg ae ha^{-1}) + diquat (0.5 kg ai ha^{-1}), and a nontreated control. All herbicide treatments contained a combination of a nonionic organosilicone surfactant[6] (0.1% v/v) and a nonionic surfactant[7] (0.25% v/v). Final plant harvest was conducted 12 wk after the February treatment. That harvest date was chosen to measure plant response well into the growing season as opposed to 12 wk after the treatment for each application timing. On 9 May 2016 and 1 May 2017, viable plant material were collected, dried at 65 C, and recorded as grams dry wt (g DW^{-1}) biomass.

Because of varying winter conditions between yr 1 and 2 (i.e., plants were exposed to colder conditions in yr 2), dry wt data in each year were analyzed separately. Data were subjected to a two-way ANOVA using the Proc Glimmix procedure in SAS[8] statistical software at $P \leq 0.05$ significance level, with herbicide treatment and application timing as fixed effects. Type III tests were used to test for significant fixed effects. The ANOVA indicated all herbicide treatments were significant in comparison to the nontreated control; thus, the same statistical procedure was conducted to make comparisons among herbicide treatments and timings. Least-square means were used for herbicide treatment and timing comparisons with means separated using a Fisher's Protected LSD method.

RESULTS AND DISCUSSION

Regarding biomass reduction 12 wk after the February application, there was no significant herbicide treatment by application timing interaction in yr 1 ($P \leq 0.0840$) or 2 ($P \leq 0.4757$). However, significant differences were noted for herbicide treatment and application timing in yr 1, but not yr 2 (Table 1). Diquat, flumioxazin, glyphosate, and glyphosate + diquat treatments, averaged across application timings, reduced giant salvinia biomass by 27, 45, 55, and 55%, respectively. Applications during January and February resulted in greater control compared with those applied in December (47 and 50% vs. 33%, respectively). In yr 2, giant salvinia control was $\geq$ 99% and herbicide treatment ($P = 0.3818$) and application timing ($P = 0.4007$) were not significant.

Table 1. The response of giant salvinia [g dry wt (DW) biomass ± standard error (SE)] to the aquatic herbicides diquat, flumioxazin, glyphosate, and glyphosate + diquat, applied during the winter of 2015 to 2016 and 2016 to 2017.

Treatment Factor[1]		Giant Salvinia Dry Weight (g DW^{-1}) 2015–2016	2016–2017
Herbicide[2,3]	Rate (kg ai ha^{-1})[4]	Year 1	Year 2
Reference[5]	0.0	64 ± 3	18 ± 9
Diquat	1.1	47 ± 3 a	0 ± 0 ns
Flumioxazin	0.2	35 ± 3 b	0 ± 0 ns
Glyphosate	3.3	28 ± 3 b	0 ± 0 ns
Glyphosate + diquat	3.3 + 0.5	32 ± 3 b	0 ± 0 ns
Application timing			
December		41 ± 2 a	0 ± 0 ns
January		34 ± 2 b	0 ± 0 ns
February		32 ± 2 b	0 ± 0 ns

[1] Data results for each treatment factor are pooled over all levels of the other factor. Means within a column for each treatment factor followed by the same letter are not significantly different according to Fisher's Protected LSD test at $P \leq 0.05$. ns, not significant.

[2] Diquat, flumioxazin, glyphosate, and glyphosate + diquat applied at 1.1, 0.2, 3.3, and 3.3 + 0.5 kg ai ha^{-1}, respectively.

[3] A nonionic organosilicone surfactant + a nonionic surfactant were included with each treatment at 0.1% and 0.25% (v/v), respectively.

[4] Glyphosate was applied in g ae ha^{-1}.

[5] Reference biomass was not included in the statistical analysis.

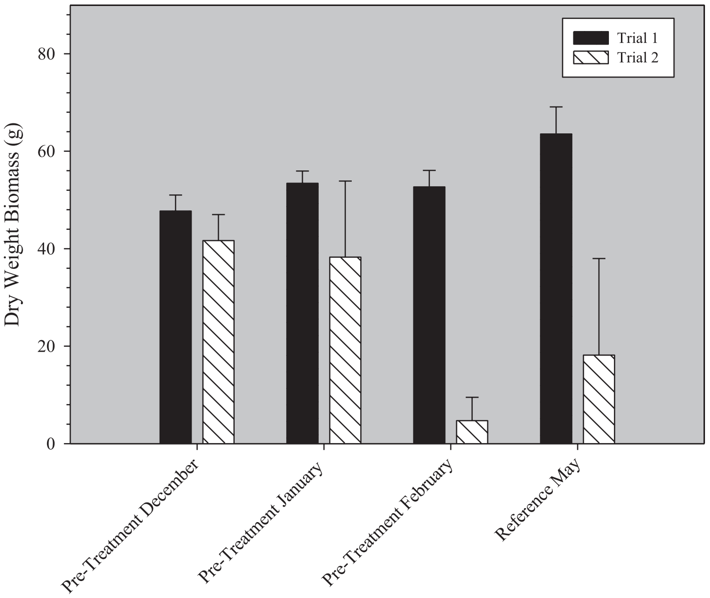

Figure 1. Dry weight biomass (mean ± SE) of giant salvinia harvested pretreatment and from nontreated reference during the winter for yr 1 (2015 to 2016) and yr 2 (2016 to 2017). Bars represent pretreatment biomass at each application timing ($n = 5$) and biomass of the nontreated reference ($n = 15$).

Year 1

In yr 1, December applications of diquat, flumioxazin, and glyphosate + diquat resulted in plant injury < 1 wk after treatment (WAT). As expected, glyphosate injury was slower to develop, and injury symptoms did not become visible until 3 WAT. Although diquat and flumioxazin treatments resulted in injury < 5 d after treatment, plant recovery was observed by 2 WAT. Visually, the upper layers of plant material appeared necrotic and nonviable; however, the necrotic plant tissue on the surface sheltered healthy, actively growing fronds underneath the upper plant layer. This sheltering effect was also observed by Mudge and Sartain (2018), who reported winter herbicide treatments were less efficacious to giant salvinia protected from cold air temperatures when compared with exposed plants. In addition, Mudge et al. (2016) documented substantially less giant salvinia control with glyphosate + diquat and endothall + flumioxazin during the fall when plant growth was slower compared with these same treatments applied during the spring and summer months.

Plant material was subjected to freezing temperatures before herbicide applications in January and February, and treatments applied in January and February of yr 1 were more effective than applications in December. Giant salvinia buds are capable of tolerating infrequent frosts and freezes (Whiteman and Room 1991). Larger fronds can provide protection from cold air temperatures, thus allowing viable buds to persist (Harley and Mitchell 1981). Individual fronds or whole plants exposed to heavy frosts or subfreezing temperatures could die off or be substantially reduced in biomass, but water and upper portions of the plant mat can insulate stems and lateral buds that protect the plant during unfavorable conditions and ultimately lead to recovery in the spring (Owens et al. 2004). Cold-exposure studies have documented that the average water temperature is likely to be 1.4, 1.6, and 1.8 times greater in low, medium, and high density giant salvinia biomass treatments, respectively, compared with open-water treatments when plants were exposed to 0 C for 14 h (Moshman 2017). Minor freeze- and frost-related injury symptoms from cold exposure were noted before the January treatment in yr 1. Weather-related injury symptoms became more severe in February, as observed on control plants, and pretreatment biomass samples indicated that dry wt biomass remained relatively constant at [mean ± standard error (SE)] 53.4 ± 2.5 g in January to 52.7 ± 3.4 g in February (Figure 1). Year 1 reference plants displayed freeze damage in most of the emergent fronds during the February treatment. The excessive amount of freeze-damaged fronds may have led to decreased diquat efficacy at the February application timing. Diquat applications in January provided 45% control compared with only 20% in February applications (data not shown). Although the plant biomass was slightly less in February, visual observations documented only a small percentage of the upper emergent fronds were healthy with green tissue present. Because diquat is not actively translocated throughout the plant, it is less effective when it is not able to contact healthy plant material or when it is applied to injured/nonviable tissue, which was the case in February 2016 for yr 1. These data are not supportive of previous research by Mudge and Sartain (2018) that documented diquat provided ≥ 93% control of giant salvinia when applied during the winter; however, treatments were administered earlier in the winter before the beginning of cold weather. Most likely, differences in harvest date and plant architecture (single layer vs. multiple

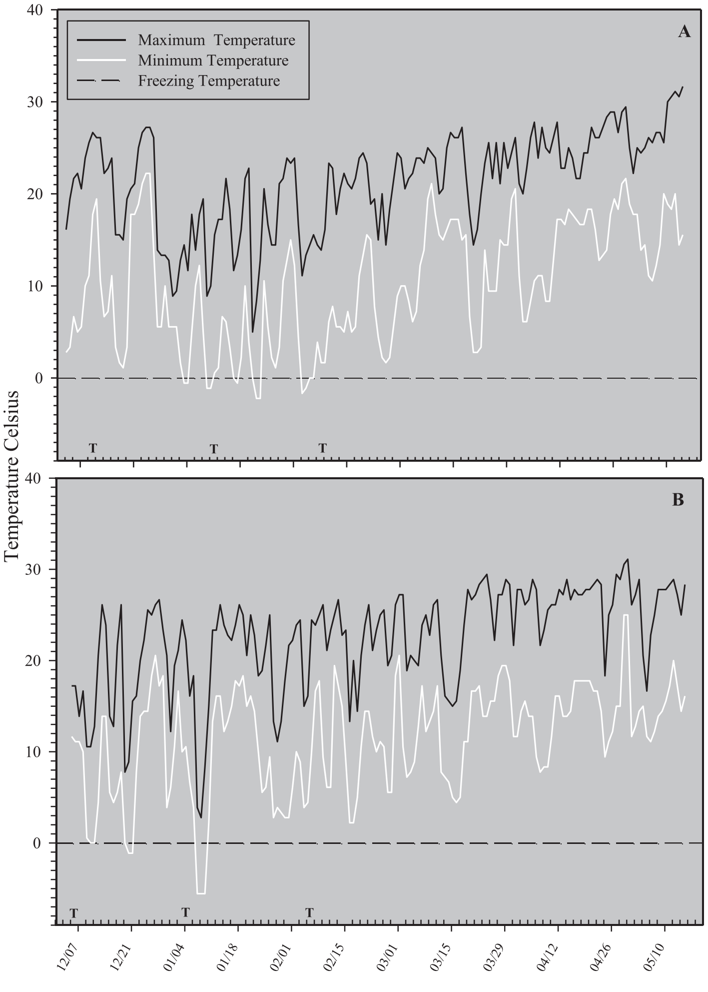

Figure 2. Maximum and minimum daily air temperatures (C) Temperatures 3 December 2015 to 20 May 2016 (A) and 3 December 2016 to 20 May 2017 (B). The symbol "T" along the x axis represents the date of the herbicide application.

layers of plant material) contributed to those differences between the current and previous research (Mudge and Sartain 2018).

Table 2. Duration giant salvinia was exposed to air temperatures that remained continuously at or below 0 C during yr 1 (2015 to 2016) and yr 2 (2016 to 2017), and the minimum temperature reached during each freeze event.

Timing	Duration (h) at/below 0 C	Minimum Temperature[1,2] (C)
Year 1		
5 January 2016	4	−0.6
11 January 2016	3	−1.1
18 January 2016	1	0.0
23 January 2016	2	0.0
24 January 2016	9	−2.2
5 February 2016	5	−1.7
6 February 2016	2	0.0
7 February 2016	1	0.0
Year 2		
11 November 2016	2	0.0
10 December 2016	3	0.0
19 December 2016	2	0.0
20 December 2016	6	−1.1
6–7 January 2017[3]	16	−5.6
7–8 January 2017	17	−5.6
8 January 2017	1	0.0

[1] Air temperature data were collected from a local weather station operated by the Louisiana State University Agricultural Center in Baton Rouge, LA.

[2] Minimum temperature recorded over the duration of each freeze event.

[3] Multiple dates indicate that temperatures remained at or below 0 C from one date to the next.

Year 2

All December treatments in yr 2 resulted in $\geq$ 10% visual injury to giant salvinia, when compared with the reference, at 1 WAT. The rapid visual-injury symptoms observed on plants treated with glyphosate was unexpected because this treatment produced substantially slower injury when applied to giant salvinia in the previous year. Plants in yr 2 were exposed to freezing temperatures 5 d after the December application (Figure 2), which may have contributed to the faster visual injury. It has been documented in several terrestrial plant species that glyphosate efficacy increases as temperature increases (Adkins et al. 1998; Waltz et al. 2004); however, these data have been inconsistent with other findings. Zhou et al. (2007) documented that glyphosate treated velvetleaf (*Abutilon theophrasti* Medik.) exposed to posttreatment temperatures of 5 and 12 C for 48 h enhanced glyphosate control of velvetleaf, but cold stress before treatment decreased glyphosate efficacy. Reddy (2000) reported that absorption and translocation of glyphosate in redvine [*Brunnichia ovata* (Walter) Shinners] was greater in plants maintained at 15/10 C (day/night) temperature than at 25/20 C. The 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). December-treated plants had been exposed too multiple minor freeze events at 2 WAT (Figure 2) and, by 4 WAT, were reduced to a single layer of plant material. Although all plants were exposed to minor freezes, only the herbicide-treated plants were reduced to a single plant layer, whereas the reference plants and those that were yet to be treated (i.e., January and February treatments) still maintained multiple layers of plant material.

Four days after the January treatment in yr 2, a prolonged freezing period occurred. During that time, air temperatures remained at or below freezing (as low as –5.6 C) for 33 h during a 38-h period (Table 2). Those temperatures resulted in ice formation up to 8 cm thick in the experimental containers. The effects of the prolonged freeze period were immediately evident. Pretreatment biomass decreased 87% from January 2017 to February 2017, and plant material that had not been treated was reduced to a single layer. The response of giant salvinia to the prolonged freeze is in agreement with data generated by Whiteman and Room (1991) that documented temperatures below –3 C can be lethal to giant salvinia buds if exposure exceeds 2 h. Although freezing temperatures can have lethal effects on giant salvinia and/or decrease the number of plants to a remnant population, regrowth will often occur from underlying, insulated plants or from

TABLE 3. AVERAGE AIR TEMPERATURE [C ± STANDARD DEVIATION (SD)] THAT GIANT SALVINIA WAS EXPOSED TO DURING EACH MONTH OF YR 1 (2015 TO 2016) AND YR 2 (2016 TO 2017).

Timing	Year 1	Year 2
Month	3 December 2015 to 20 May 2016	3 December 2015 to 20 May 2016
	Average Temperature[1]	
December	15.7 ± 6.3	13.7 ± 6.4
January	9.7 ± 5.4	14.2 ± 6.9
February	12.9 ± 5.8	16.7 ± 5.3
March	18.1 ± 4.6	18.3 ± 5.3
April	20.1 ± 4.3	20.9 ± 4.8
Range	−2.2 to 29.4 C	−5.6 to 30.6° C
Freezing hours[2]	27	45

[1] Air temperature data were collected from a local weather station operated by the Louisiana State University Agricultural Center in Baton Rouge, LA.

[2] Number of hours air temperature was at or below 0 C throughout the duration of each trial.

plants not subjected to prolonged periods of subfreezing temperatures (Horst and Mapes 2000, McFarland et al. 2004). An acute-exposure study (Owens et al. 2004), in which giant salvinia was exposed to low temperatures (4, −3, and −16 C) for various exposure times (1, 4, 8, 15, 24, and 48 h), documented that exposure to −16 C for 48 h led to complete mortality, whereas the other treatments failed to provide complete control. In the current study, the prolonged freeze period did not provide complete control of the nontreated plant material. However, biomass was reduced to a single plant layer, which allowed for excellent herbicide coverage to the minimal remaining biomass during February treatments.

Plant response in yr 1 and 2 varied significantly because of dissimilar environmental conditions. Air temperatures from 5 December to 15 May were relatively mild during both winters. The number of days with air temperatures at or below freezing was 13 d in yr 1 and 8 d in yr 2. Although yr 1 had more days at or below freezing, in comparison to yr 2, plants were only exposed to at total of 27 h of temperatures at or below freezing compared with 45 h in yr 2 (Table 3).

These data provide evidence that successful control of giant salvinia in the winter is possible; however, control will be more dependent on environmental conditions and will be influenced by herbicide application timing relative to freeze/frost events. Herbicide applications in combination with extended periods of subfreezing temperatures can increase giant salvinia control during the winter. In the event of a milder winter, in which temperatures do not remain at or below freezing for a consecutive $\geq$ 9 h, herbicide applications just before minor freeze events can increase herbicide efficacy. However, applications of contact herbicides to freeze-damaged fronds later in the winter can lead to decreased control because of minimal healthy plant material being contacted with herbicide solution. Overall, both trials provided some level of control when compared with nontreated plant material. Therefore, efforts to chemically manage giant salvinia should continue beyond the growing season into mid to early winter and/or begin in late winter as opposed to early or mid spring. Future research should evaluate other herbicide chemistries, application rates, and temperature regimes when managing giant salvinia to more accurately predict the level of control.

SOURCES OF MATERIALS

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

[2] TeeJet™, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60187.

[3] Tribune™, Syngenta Crop Protection, P.O. Box 18300, Greensboro, NC 24719.

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

[5] Clipper™, Valent USA Corporation, P.O. Box 8025, Walnut Creek, CA 94596.

[6] AirCover™, Winfield Solutions LLC, P.O. Box 64589, St. Paul, MN 55164.

[7] Aqua-King Plus®, Winfield Solutions LLC, P.O. Box 64589, St. Paul, MN 55164.

[8] SAS® version 9.4 statistical software, SAS Institute Inc., Cary, NC, USA.

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