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Effect of Winter Herbicide Applications on Bald Cypress (*Taxodium distichum*) and Giant Salvinia (*Salvinia molesta*)

Bradley T. Sartain[1] and Christopher R. Mudge[2]

[1]Former Graduate Research Assistant, Louisiana State University School of Plant Environmental and Soil Sciences, Baton Rouge, LA, USA and [2]Research Biologist, U.S. Army Engineer Research and Development Center, Louisiana State University School of Plant Environmental and Soil Sciences, Baton Rouge, LA, USA

Research Article

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Author for correspondence:
Christopher R. Mudge, Research Biologist, U.S. Army Engineer Research and Development Center, Louisiana State University School of Plant Environmental and Soil Sciences, Baton Rouge, LA 70803.
(Email: Christopher.R.Mudge@usace.army.mil)

Abstract

Immense stands of bald cypress [*Taxodium distichum* (L.) Rich.] make it difficult for herbicide applicators to access the free-floating fern giant salvinia (*Salvinia molesta* Mitchell), which can be found growing under the canopy of the trees. The difficulty of accessing these areas, as well as avoiding direct contact of herbicides with tree foliage, provides a substantial amount of nonmanaged plant material capable of rapidly reinfesting treated sites, thus making management efforts null and void. Herbicide application during the winter, when bald cypress sheds its leaves, may be an ideal time to manage *S. molesta* and minimize negative impacts on the nontarget tree. Therefore, the herbicides diquat, glyphosate, flumioxazin, and glyphosate + diquat were evaluated at one of the three application timings (December, January, or February) against *S. molesta* and immature bald cypress during the winter. All herbicide treatments, except diquat applied in February of year 1, reduced *S. molesta* biomass 40% to 100%. In addition, flumioxazin applications during December, January, and February provided ≥70% *S. molesta* control with little or no negative impacts to bald cypress health. A treatment by timing interaction revealed that trees exposed to flumioxazin did not result in a significant decrease in average leaf length when compared with reference trees 20 wk after bud break at any of the three application timings. In addition, bald cypress exposed to flumioxazin produced the highest probability of a refoliation pattern equivalent to the nontreated reference trees. Although complete tree mortality was not documented in either study, delayed and abnormal leaf formation, reduced leaf length, irregular canopy formation, or no negative effects were observed among herbicide-treated bald cypress. This research suggests that winter herbicide applications over the top of dormant bald cypress may be a practical management technique for controlling severe infestations of *S. molesta*.

Introduction

The invasive aquatic fern giant salvinia (*Salvinia molesta* Mitchell) has continued to spread across the United States since it was first documented in 1995 in South Carolina (Johnson 1995). It exhibits very rapid growth and biomass can double in as little as 36 h under optimal conditions (Johnson et al. 2010). It has the ability to form extensive mats, sometimes up to a meter thick (McFarland et al. 2004; Thomas and Room 1986), causing water resources to be negatively affected immediately after infestation. Dense *S. molesta* growth impedes navigation, irrigation, and recreational use of infested water bodies (Pimentel et al. 1999), leading not only to environmental impacts, but also to economic impacts and public health concerns (McFarland et al. 2004). These negative impacts can lead to situations in which *S. molesta* needs to be intensively managed to limit its growth and spread to surrounding water bodies.

A significant portion of the *S. molesta* infestations throughout the southeastern United States occur in lakes, bayous, and backwaters that are filled with bald cypress [*Taxodium distichum* (L.) Rich.]. Bald cypress is a slow-growing, long-lived tree species, and due to its ability to withstand prolonged flooding, it can be found in lakes, ponds, swamps, and other bottomland or waterlogged areas (Samuelson and Hogan 2003). Cypress swamps are highly valued because of their ecological and economic importance, particularly their support of habitat for waterfowl, wading birds, small mammals, and other wildlife species (Samuelson and Hogan 2003). In addition, cypress swamps slow floodwaters, act as sediment and pollution traps, and provide storm surge protection in areas susceptible to hurricanes and other severe weather events (Liu et al. 2009; Wharton 1977). Unfortunately, bald cypress swamps are not readily regenerating and are converting to marsh and open water (Myers et al. 1995). Poor

Management Implications

Giant salvinia (*Salvinia molesta* Mitchell) is a highly invasive floating aquatic fern that exhibits very rapid growth, with biomass capable of doubling in as little as 36 h under optimal conditions. It has the ability to form extensive mats, up to a meter thick, causing water resources to be negatively affected immediately after infestation. A significant portion of the *S. molesta* infestations throughout the southeastern United States occur in lakes, bayous, and backwaters that are filled with bald cypress [*Taxodium distichum* (L.) Rich.]. Cypress swamps are highly valued because of their ecological and economic importance, particularly their support of wildlife habitat, protection from storm surge, slowing of floodwaters, and ability to filter sediment and pollution. Unfortunately, bald cypress stands make it difficult for herbicide applicators to access large populations of *S. molesta*. The inability of applicators to access these areas provides a substantial amount of nonmanaged plant material capable of rapidly reproducing and reinfesting previously treated sites, thus making management efforts null and void. The shedding of bald cypress leaves during the winter may provide an opportunity for aquatic herbicides to be applied uniformly to *S. molesta*, either by boat or aircraft, which would otherwise be shaded during the growing season by dense bald cypress canopies. Because the tree is such a valued asset, it is too risky to attempt a large-scale winter herbicide application in a field scenario that may have irreversible negative impacts on the health of bald cypress. Therefore, mesocosm trials were conducted to investigate the effects of herbicide applications over-the-top of immature bald cypress trees during winter to determine whether adequate *S. molesta* control can be attained using this application technique. If successful, wintertime aerial herbicide applications could be a tool for managing large areas of *S. molesta* in dense bald cypress stands.

regeneration and a loss of cypress swamps, particularly in Louisiana, are the result of altered hydrologic regimes, increased flooding, increased salinity, and other physical/biological factors (Myers et al. 1995).

Salvinia molesta is frequently found growing underneath the canopy of extensive bald cypress stands, thus making it difficult for herbicide applicators to access entire plant populations. Vegetation control programs that negatively impact bald cypress are discouraged and often avoided at all costs. The difficulty facing herbicide applicator crews in accessing these isolated areas and in avoiding direct contact of herbicides with tree foliage results in considerable amounts of *S. molesta* that are capable of rapidly reinfesting previously treated sites.

Unlike most trees in the Cupressaceae family, bald cypress is deciduous and sheds its leaves during the winter. The annual shedding of cypress leaves may allow herbicides to be applied uniformly to *S. molesta* that would otherwise be shaded during the growing season by dense bald cypress canopies. Fixed-wing airplanes and helicopters are potential management tools that could be used in these situations to provide adequate herbicide coverage to areas inaccessible to watercraft. It is unknown whether aquatic herbicide applications over the top of dormant bald cypress would provide successful *S. molesta* control with minimal or no injury to bald cypress. Because the tree is a valued commodity throughout the southeastern United States, it is too risky to attempt a large-scale wintertime herbicide application in a field scenario that may negatively, and irreversibly, affect the health of bald cypress trees. Therefore, mesocosm trials were conducted in a controlled setting to investigate the effects of herbicide applications over the top of immature bald cypress trees when foliage is minimal or completely shed to determine whether adequate *S. molesta* control can be attained using this application technique.

Materials and Methods

On October 5, 2015, and October 25, 2016, 75 bald cypress trees (1.5 to 3 m in height) were purchased from a local nursery and maintained in 58-L planting pots at the Louisiana State University (LSU) AgCenter Aquaculture Research Facility in Baton Rouge, LA. The potted trees were placed into 64-L secondary containers to prevent soil water loss through the bottom of the planting pots. *Salvinia molesta* was collected from a stock population that was maintained at LSU Aquaculture and cultured in 76-L (49.5-cm diameter by 58.4-cm height) plastic containers filled with approximately 60 L of pond water (pH 8.5). Pond water was amended with sphagnum moss (14 g) to lower the pH to < 7.0. In addition, 2.1 g of Miracle-Gro® (24-8-16, All Purpose Plant Food, Scotts, P.O. Box 606 Marysville, OH 43040) was applied to each container at planting and every 2 wk until mid-December. Fertilization resumed in mid-March and continued every 2 wk until final harvest.

Each container of *S. molesta* and potted cypress tree was randomly assigned to a respective treatment and grouped into rows containing 5 alternating trees and 5 containers of *S. molesta*. Five herbicide treatments included: diquat (Tribune™, Syngenta Crop Protection, P.O. Box 18300, Greensboro, NC 24719) (1.6 kg ai ha^{-1}), glyphosate (Roundup Custom™, Monsanto Company, 800 N. Lindbergh Boulevard, St Louis, MO 63167) (3.3 kg ae ha^{-1}), flumioxazin (Clipper™, Valent USA, P.O. Box 8025, Walnut Creek, CA 94596) (0.2 kg ai ha^{-1}), and a tank mix of glyphosate (3.3 kg ae ha^{-1}) + diquat (0.5 kg ai ha^{-1}). Herbicide treatments were applied at three application timings (December, January, or February) to represent early-, mid-, or late-winter herbicide applications. Each herbicide treatment was replicated five times at each of the three application timings. All herbicide treatments contained a combination of a nonionic organosilicone surfactant (AirCover™, Winfield Solutions, P.O. Box 64589 St Paul, MN 55164) (0.1% v/v) and nonionic surfactant (Aqua-King Plus®, Winfield Solutions) (0.25% v/v). A nontreated reference was also included for bald cypress and *S. molesta* to evaluate plant health throughout the duration of the study.

Applications were made over the top of bald cypress trees and *S. molesta* on December 15, 2015, January 13, 2016, and February 12, 2016, during the first trial; and on December 14, 2016, January 12, 2017, and February 14, 2017 during the second trial. Pretreatment *S. molesta* biomass collected 1 h before the December treatments in 2015 to 2016 and 2016 to 2017 was 42.0 and 34.9 g dry weight, respectively. Plants were in the tertiary growth stage and arranged as a single plant layer across the water surface. A CO_2-powered sprayer and custom-built spray boom equipped with two 8002VS (TeeJet®, Spraying Systems, P.O. Box 7900, Wheaton, IL 60187) nozzles at 275 kPa was used. Treatments were applied at an equivalent of 94 L ha^{-1} to simulate an aerial aquatic herbicide application at approximately 0.25 and 2.25 m above the tree and *S. molesta* canopies, respectively. An artificial buffer constructed from tarp and PVC pipe was used while administering treatments to minimize herbicide drift to adjacent treatments. To quantify *S. molesta* health at treatment, an

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additional pretreatment tank was included for each treatment and harvested before herbicide application.

To evaluate bald cypress injury, leaf length was assessed weekly at the first sign of bud break (March) until leaf growth slowed considerably (July). Nondestructive leaf measurements included five leaf-length measurements per branch on three predetermined and marked branches that were at least 30 cm in length for a total of 15 leaf measurements per tree. Selected branches represented the fifth branch from the base of the tree, the fifth branch from top of the tree, and a randomly selected branch between the upper and lower selected branches. In addition, bald cypress refoliation (i.e., the production of leaves following herbicide applications) was assessed each year at 5-wk intervals over a 20-wk period. Ratings were recorded on a 1 to 5 scale, where 1 = equivalent to the reference, 2 = slight malformation, 3 = moderate malformation, 4 = moderate to severe malformation, and 5 = severe malformation. Refoliation assessment compared leaf spacing, leaf-length variability, leaf orientation, and overall visual aesthetics of treated trees compared with reference trees.

Harvest of all viable *S. molesta* plant material for each application timing was made 12-wk after the February application. This harvest date was chosen to measure plant response well into the growing season to evaluate long-term effects. Harvested *S. molesta* plant material was dried to a constant weight (65 C) and recorded as grams dry weight biomass (g DW^{-1}). A three-way ANOVA was conducted using PROC GLIMMIX in SAS® v. 9.4 (2017) statistical software at $P \leq 0.05$ significance level. The analysis indicated years were significant ($P < 0.0001$), and thus data were analyzed separately by year. Treatments were subjected to a one-way ANOVA and Fisher's protected LSD ($P \leq 0.05$) mean separation procedure and indicated that reference treatment means were significant in comparison to herbicide treatment means in 2015 to 2016 (hereafter referred to as year 1) and 2016 to 2017 (hereafter referred to as year 2); thus dry weight biomass data were analyzed further without reference biomass data using a two-way ANOVA at $P \leq 0.05$ significance level to test for herbicide treatment, application timing, and interaction effects. Significant means were further separated using the Fisher's protected LSD method ($P \leq 0.05$).

Leaf measurement data, collected at 20 wk after bud break (WABB), were subjected to a three-way ANOVA using SAS® v. 9.4 (SAS Institute 2017) statistical software at $P \leq 0.05$ significance level. Bald cypress data were not significant between years; thus data were combined across years and analyzed with year designated as a random variable. Fixed effects included: treatment, application timing, and branch location. If significant, means were further separated using the Fisher's protected LSD method ($P \leq 0.05$). Tree refoliation uniformity ratings were analyzed with a generalized linear mixed model using SAS® (SAS Institute2017) statistical software. The generalized linear mixed model was used to develop a proportional odds model that focused on the probabilities of the various response categories for each treatment, rather than calculating the average rating under the assumption that the data were normally distributed (Gbur et al. 2012). To provide a visual representation of leaf growth throughout the duration of the study, mean leaf lengths of bald cypress recorded during each data-collection period were averaged across treatment, plotted, and subjected to an exponential growth to maximum nonlinear regression

$$\left[y = a\left(1 - e^{-bx}\right)\right]$$

where y = response variable, a = initial or maximum response variable, x = explanatory variable (time: wabb), b = rate constant.

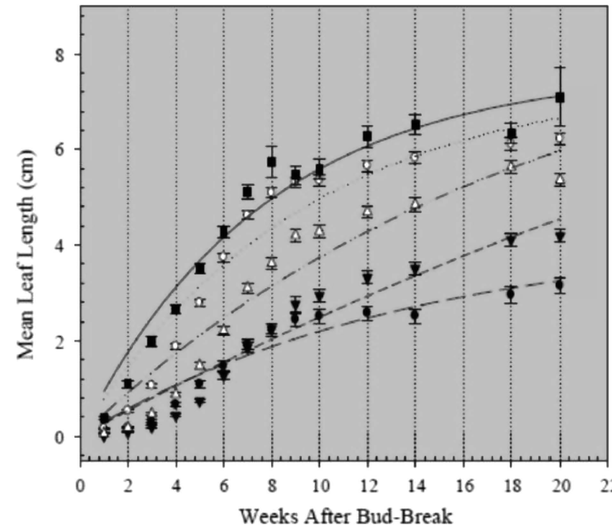

Figure 1. Mean leaf length of bald cypress, averaged across timing, in response to the aquatic herbicides diquat (1.6 kg ai ha^{-1}) (filled circle), flumioxazin (0.2 kg ai ha^{-1}) (open circle), glyphosate (3.3 kg ae ha^{-1}) (filled triangle), glyphosate (3.3 kg ae ha^{-1}) + diquat (0.5 kg ai ha^{-1}) (open triangle), and reference treatments (filled square). All measurements are reported as mean leaf length (± SE). Leaf growth was estimated using nonlinear regression [Exponential growth rise to a maximum ($y = a(1\text{-}e^{-bx})$)] for the diquat ($y = 4.236{*}1\text{-}e^{-0.0731x}$; $r^2 = 0.917$), flumioxazin ($y = 7.530{*}1\text{-}e^{-0.1077x}$; $r^2 = 0.921$), glyphosate ($y = 1.424{*}1\text{-}e^{-0.0192x}$; $r^2 = 0.9270$), glyphosate + diquat ($y = 9.236{*}1\text{-}e^{-0.0519x}$; $r^2 = 0.9171$), and reference ($y = 7.692{*}1\text{-}e^{-0.1298x}$; $r^2 = 0.9583$) treatments.

Results and Discussion

Taxodium distichum (Bald Cypress)

ANOVA of bald cypress average leaf length revealed a significant effect for herbicide, timing, branch location, and treatment by timing interaction at 20 WABB (Table 1). Leaf length, averaged across all timings, for nontreated and flumioxazin-treated bald cypress did not differ at 20 WABB and produced the highest average leaf lengths of 7.07 cm and 6.18 cm, respectively (Figure 1). In response to glyphosate + diquat treatments, mean leaf length (5.37 cm) was significantly less in comparison to nontreated trees, but not significantly different from flumioxazin-treated trees. Bald cypress subjected to glyphosate alone and diquat alone produced the smallest average leaf lengths of 4.18 cm and 3.13 cm, respectively. Tree mortality was not documented in any treatment throughout the study period.

Analysis of the treatment by timing interaction revealed that trees exposed to flumioxazin at any of the three application timings did not result in a significant decrease in average leaf length when compared with nontreated reference trees at 20 WABB (Figure 2). The leaves of nontreated and flumioxazin-treated trees grew an average of 1 cm wk^{-1} up to 7 WABB. After 7 WABB, leaf length increased slowly throughout the remainder of the observation period. In addition, no visual injury symptoms were recorded for flumioxazin-treated trees. These results are consistent with those of Osiecka and Minogue (2012), who reported no defoliation or malformed foliation following flumioxazin applications of 290 and 490 g ai ha^{-1} to dormant bald cypress seedlings. Flumioxazin applications also resulted in the highest percentage of healthy seedlings when compared with over-the-top applications of sulfometuron, imazapyr, hexazinone, and aminopyralid to dormant bald cypress seedlings (Osieka and Minogue 2012). Although there are limited data on bald cypress response to flumioxazin, other conifer species have been

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Table 1. Grams (g) dry weight response of *Salvinia molesta* (mean ± SE) to the aquatic herbicides diquat, flumioxazin, glyphosate, and glyphosate + diquat, applied at three timings during the winter of year 1 (2015 to 2016) and year 2 (2016 to 2017).

Treatment[a]	Rate kg ai ha^{-1}[b]	Treatment application month[c] December	January	February
		Year 1		
Nontreated reference[c,d]	0.0	————————— 47.5 ± 3.4 ————————		
Diquat	1.6	0.6 ± 0.6e	0.0 ± 0.0e	45.6 ± 4.3a
Flumioxazin	0.2	0.0 ± 0.0e	2.0 ± 2.0e	14.1 ± 3.6cd
Glyphosate	3.4	9.7 ± 6.3cde	19.3 ± 3.8bc	9.6 ± 4.1cde
Glyphosate + diquat	3.4 + 0.5	28.6 ± 5.9b	3.6 ± 2.2e	7.1 ± 4.0de
		Year 2		
Nontreated reference[d]	0.0	————————— 21.4 ± 4.6 ————————		
Diquat	1.6	0.0 ± 0.0b	0.6 ± 0.4b	0.0 ± 0.0b
Flumioxazin	0.2	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b
Glyphosate	3.4	3.7 ± 2.3a	0.0 ± 0.0b	0.0 ± 0.0b
Glyphosate + diquat	3.4 + 0.5	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b

[a]Treatments included a nonionic organosilicone surfactant + nonionic surfactant added at 0.1% and 0.25% v/v, respectively.
[b]Glyphosate applied as kg ae ha^{-1}.
[c]Means within each year followed by the same letter are not significantly different according to Fisher's protected LSD test ($P \leq 0.05$); $n = 5$.
[d]Nontreated reference biomass was not included in the statistical analysis.

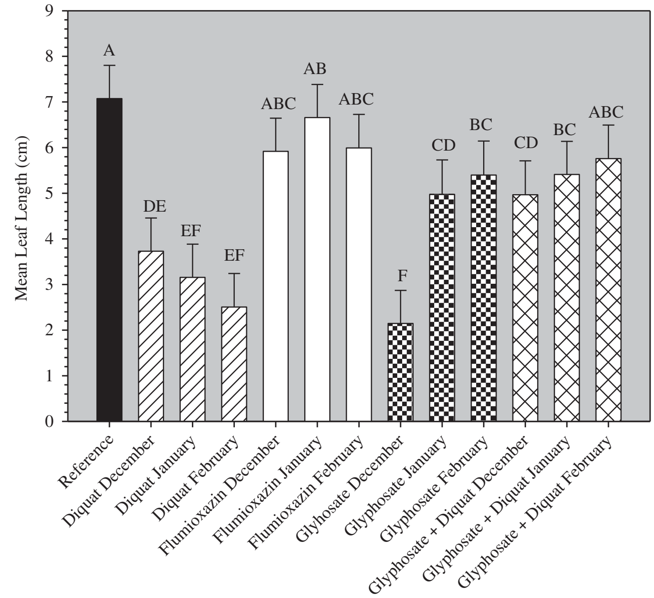

Figure 2. Mean leaf length (± SE) of bald cypress 20 wk after bud break (WABB) in response to winter-applied herbicide treatments averaged across treatment and timing. The aquatic herbicides diquat (1.6 kg ai ha^{-1}), flumioxazin (0.2 kg ai ha^{-1}), glyphosate (3.3 kg ae ha^{-1}), and diquat (0.5 kg ai ha^{-1}) + glyphosate (3.3 kg ae ha^{-1}) were applied over the top of dormant bald cypress trees at the equivalent of 94 L ha^{-1} in December, January, or February of year 1 (2015 to 2016) and year 2 (2016 to 2017). Bars with different letters indicate a significant mean leaf length difference at 20 WABB according to Fisher's protected LSD test ($P \leq 0.05$); $n = 150$.

documented as flumioxazin tolerant. Richardson and Zandstra (2009) documented significantly longer leader lengths following over-the-top fall applications of flumioxazin to dormant Fraser fir [*Abies fraseri* (Pursh) Poir.] when compared with nontreated trees at 10 mo after treatment (MAT). In addition, flumioxazin applications over the top of Colorado blue spruce (*Picea pungens* Englem.) resulted in 0% visual injury at 6 and 9 MAT (Richardson and Zandstra 2009). Kuhns and Harpster (2002) reported Fraser fir and Douglas fir [*Pseudosuga menziesii* (Mirb.) Franco] seedlings were not affected by over-the-top spring applications of flumioxazin at 850 g ai ha^{-1}, which is approximately four times greater than the rate tested in the current study. Flumioxazin tolerance in cotton (*Gossypium hirsutum* L.) has been associated with plant age and restricted herbicide uptake in mature barked stems compared with immature chlorophyllus stems (Ferrell and Vencill 2003; Price et al. 2004). The trees used in the current study would be classified as young (2 to 3 yr old) considering the longevity of bald cypress; however, all had fully developed bark. These characteristics may have led to an increased tolerance of flumioxazin applications in the present study.

Leaf length in response to the glyphosate + diquat treatment was not affected by application timing. Visual injury and shorter average leaf length was documented for glyphosate + diquat treatments compared with the nontreated control; however, leaf length was not significant in comparison to flumioxazin treatments at 20 WABB. Visual leaf injury symptoms observed in glyphosate + diquat–treated trees included abnormal leaf proliferation (i.e., witches'-broom), reduced leaf size, chlorosis, and leaf strapping.

Leaf length in response to glyphosate alone was equivalent and greater when applied in January and February compared with December. Glyphosate applications in January and February resulted in average leaf lengths equivalent to the glyphosate + diquat treatments at 20 WABB (Figure 2). Glyphosate treatments during December severely impacted leaf growth and average length failed to exceed 2.5 cm throughout the study. To date, no literature on the phytotoxicity of glyphosate to dormant bald cypress is available, but it has been documented in other conifer species. Radosevich et al. (1980) reported fall applications of glyphosate did cause minor injury to Douglas fir, white fir [*Abies concolor* (Gord. & Glend.) Lindl], and red fir (*Abies magnifica* A. Murray bis.), but growth was not inhibited. Glyphosate injury in conifers has also been shown to vary considerably due to application timing. Radosevich et al. (1980) documented that herbicide applications to conifers that were dormant (early spring) or had ceased seasonal growth (fall) were more tolerant to glyphosate applications than conifers exposed during active growth periods (July). King and Radosevich (1985) documented decreased injury when glyphosate was applied to sugar pine (*Pinus lambertiana* Douglas), red fir, white fir, and Douglas fir in September, but injury increased significantly when glyphosate was applied in October. Willoughby (1996) reported no impact on the growth of Douglas fir, Corsican pine (*Pinus nigra* Arnold), lodgepole pine (*Pinus contorta* Douglas), Norway spruce [*Picea abies* (L.) Karst.], and Scots pine (*Pinus sylvestris* L.) from high rates of glyphosate applied in March; however, the same herbicide rate applied in January significantly impacted tree growth and survival.

Increased glyphosate injury in December was possibly due to trees not being completely dormant, and some still maintaining leaves, thus allowing the herbicide to contact portions of green leaves, tissue in and around bud scars, or buds that had not completely closed. In cotton, glyphosate is readily absorbed by green stems, allowing it to accumulate in fruiting structures leading to floral abnormalities, pollen sterility, and boll abortion (Pline et al. 2002). Leaves of glyphosate-treated trees documented severe witches'-broom characteristics and stunting, and in some instances, leaves failed to fully emerge before the conclusion of the trials.

Although diquat is a broad-spectrum foliar herbicide, rapid cell death following absorption and acropodial xylem flow limits translocation from treated leaves (Senseman 2007); thus, it was anticipated that diquat would produce minimal or no injury in dormant bald cypress trees. Unfortunately, diquat, regardless of timing, severely impacted not only leaf length, but caused severe nonuniform flushing. Branches exposed to diquat failed to produce more than a couple of leaves per branch, and apical shoot meristem dieback was common in the upper auxiliary branches. As a result of the shoot dieback, excessive branching from the trunk occurred, possibly to offset reduced leaf production at the auxiliary branches.

Diquat efficacy has been reported in nondeciduous conifer species and is commonly used to control lodgepole pine, mountain pine (*Pinus mugo* Turra), and Douglas fir during winter in New Zealand (Gous et al. 2010). In addition, the closely related compound paraquat has been reported to cause tip dieback and delayed flushing in European beech (*Fagus sylvatica* L.) seedlings (Harmer et al. 2000). Based on the results of the current study and previous research, diquat tolerance in woody plants is species specific and influenced by additional factors such as herbicide rate, timing, presence/absence of green plant material, and environmental conditions at application.

Average leaf length of bald cypress was also influenced by the branch location (top, middle, or bottom) on the tree ($P = 0.0005$) and varied in response to the application. As expected, the upper branches of the tree canopy came in contact with the herbicide solution more so than the middle and bottom branches, resulting in the top branches exhibiting a mean leaf length (4.24 cm) 14% shorter compared with middle (4.91 cm) and bottom (4.98 cm) branches (Figure 3).

Refoliation ratings of flumioxazin-exposed trees resulted in the highest probability of producing a refoliation pattern equivalent to nontreated reference trees and the lowest probability of producing an extremely malformed refoliation pattern, with values of 0.9124 and 0.0025, respectively (Table 2). Both glyphosate and the glyphosate + diquat tank-mixture treatments were most likely to produce a slightly malformed refoliation pattern, with estimated probabilities of 0.3734 and 0.3817, respectively. Diquat-treated trees resulted in excessive trunk branching, failure to produce new leaves, high length variability in leaves that were produced (e.g., alternating abnormally long and stunted leaves on the same branch), and the highest probability of developing a severe malformation refoliation rating (0.5788).

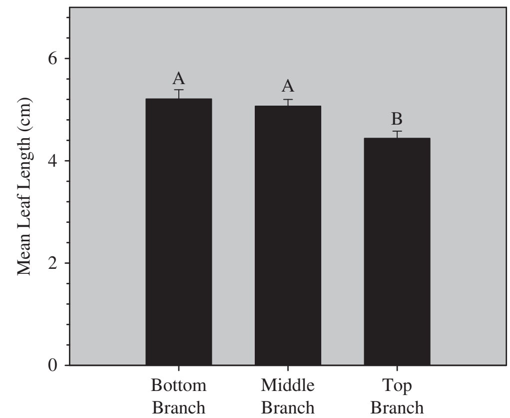

Figure 3. Mean (± SE) leaf length of bottom, middle, and top branches of bald cypress averaged across all treatments and timings. The aquatic herbicides diquat (1.6 kg ai ha^{-1}), flumioxazin (0.2 kg ai ha^{-1}), glyphosate (3.3 kg ae ha^{-1}), and diquat (0.5 kg ai ha^{-1}) + glyphosate (3.3 kg ae ha^{-1}) were applied over the top of dormant bald cypress trees at the equivalent of 94 L ha^{-1} in December, January, or February of year 1 (2015 to 2016) and year 2 (2016 to 2017). Bars with different letters indicate a significant mean leaf length difference at 20 wk after bud break according to Fisher's protected LSD test ($P \leq 0.05$); $n = 625$.

Table 2. Estimated probability of bald cypress developing malformed refoliation following over-the-top applications of the aquatic herbicides diquat (1.6 kg ai ha^{-1}), flumioxazin (0.2 kg ai ha^{-1}), glyphosate (3.3 kg ae ha^{-1}), and glyphosate (3.3 kg ae ha^{-1}) + diquat (0.5 kg ai ha^{-1}) at the equivalent of 94 L ha^{-1} during the winter.

		Estimated probability[a]			
Rating[b]	Rating equivalent	Diquat	Flumioxazin	Glyphosate	Glyphosate + diquat
1	Equal to reference	0.0200	0.9194	0.2121	0.2336
2	Slightly malformed	0.0790	0.0642	0.3734	0.3817
3	Moderately malformed	0.1067	0.0093	0.1829	0.1744
4	Highly malformed	0.2155	0.0046	0.1346	0.1236
5	Severely malformed	0.5788	0.0025	0.0970	0.0867

[a] A generalized linear mixed model was used to develop the estimated probability of receiving each refoliation rating for each treatment.
[b] Ratings were collected at 5-wk intervals over 20 wk of each year ($n = 8$). Ratings are based on a scale of 1 to 5.

Salvinia molesta

The ANOVA for *S. molesta* biomass revealed a significant year effect ($P < 0.0001$); thus years were analyzed separately. The ANOVA tests for treatment effects in year 1 and year 2 indicated that reference treatments were significant in comparison to herbicide treatments for both years; thus reference data were excluded and a two-way ANOVA was used to test for significant treatment, timing, and treatment by timing interaction effects. Statistical analysis of year 1 data showed a significant treatment effect ($P = 0.0144$), timing effect ($P < 0.0001$), and treatment by timing interaction ($P < 0.0001$). Analysis of year 2 data showed a significant treatment by timing interaction ($P = 0.0268$); however, there was not a significant herbicide treatment or application timing effect ($P = 0.0930$ and $P = 0.1293$, respectively).

In year 1, glyphosate + diquat provided increased control compared with glyphosate alone in January (93% vs. 60%, respectively), but not December (Table 1). February applications of glyphosate and glyphosate + diquat produced similar results with 80% and 85% control, respectively. *Salvinia molesta* control

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with diquat and flumioxazin alone was greater than glyphosate and glyphosate + diquat applied in December (99% and 100% vs. 80% and 40%, respectively) and equal to glyphosate + diquat in January of year 1 (100% and 96% vs. 93%, respectively). The results of glyphosate + diquat applications in December of year 1 contrast with those reported by Mudge et al. (2016), who reported that fall (October) applications of glyphosate + diquat in combination with various adjuvants yielded 83%, 72%, and 77% control. In addition, Mudge and Sartain (2018) documented a 73% reduction of *S. molesta* biomass following December applications of glyphosate + diquat. Efficacy differences are most likely due to varying weather patterns and harvest dates.

Salvinia molesta control was ≥99% when diquat or flumioxazin was applied in December, and December glyphosate applications provided ≥80% control during both years (Table 1). The glyphosate + diquat tank mix resulted in 100% control at all timings in year 2, in contrast to glyphosate + diquat applications in year 1 that provided 40%, 93%, and 85% control in December, January, and February, respectively. The reduced efficacy of glyphosate + diquat applications in December of year 1 may have been due to plants not receiving complete exposure to the herbicide solution. Although extensive efforts were made to prevent herbicide drift to adjacent treatments, the height of the boom above the *S. molesta* canopy may have allowed for reduced herbicide-to-plant contact, resulting in less control. All herbicide treatments in February of year 2 resulted in 100% *S. molesta* control, which is contrary to year 1, when diquat, flumioxazin, glyphosate, and glyphosate + diquat control was documented to be 4%, 70%, 80%, and 85%, respectively.

Reduced efficacy of diquat applications in February is likely due to the limited amount of healthy plant material present on the upper plant canopy at the time of treatment. These results are in agreement with data from Sartain and Mudge (2018). *Salvinia molesta* exhibited noticeable visible freeze damage to the majority of the exposed fronds by early February, which made it difficult for the herbicide solution to contact healthy, actively growing plant tissue.

These results indicate that herbicide applications in winter over the top of bald cypress are a practical management technique for controlling *S. molesta*. Flumioxazin applied during December and January controlled *S. molesta* at least 96% and ≥70% when applied in February with little to no negative effects on leaf growth or refoliation of bald cypress. Although, *S. molesta* control was ≥80% when the aquatic herbicides diquat, glyphosate, and glyphosate + diquat were applied at three timings, these treatments negatively impacted bald cypress leaf length and refoliation. None of the treatments evaluated led to complete tree mortality and could be potential tools for *S. molesta* management; however, the probability of bald cypress injury is significantly increased with glyphosate, diquat, and glyphosate + diquat applications.

In order to maximize *S. molesta* control, herbicides should be applied during December and January, when the majority of the target plant material has minimal freeze damage. If infestations are more severe (i.e., multiple plant layers), multiple applications will be necessary. Late winter applications (February) may be beneficial in the event of a colder winter that severely reduces the amount of plant material present; however, diquat should be avoided unless a considerable amount of healthy green plant material is present. *Salvinia molesta* control is likely to vary depending on the environmental conditions present at application and may vary from one year to the next. Even though bald cypress foliage will be minimal and/or absent during winter, it can be expected that portions of spray solution will be intercepted by the stems of bald cypress. Although herbicide contact to *S. molesta* foliage will likely be impacted if treatments are administered over the top of dense bald cypress stands, the reduced risk of bald cypress injury and the ability to manage large areas (>100 hectares per day) is a distinct advantage compared with applications via watercraft that may only manage ≤ 10 hectares per day. Future research should examine the products tested in the current research on more mature bald cypress. The trees used in the current study were relatively young considering the longevity of a bald cypress, whereas older trees have greater bark development and metabolic capacity and may be more tolerant to the herbicides evaluated in this research. In addition, other registered aquatic herbicides and application techniques (i.e., submersed applications) should be investigated to determine their effects on bald cypress and their utility for *S. molesta* control during the winter.

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