

## Case Study

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# Low carrier volume herbicide trials and UAAS support management efforts of giant salvinia (*Salvinia molesta*): a case study

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## Abstract

Expanding the current aquatic herbicide portfolio, reducing total spray volumes, or remotely delivering herbicide using novel spray technologies could improve management opportunities targeting invasive aquatic plants, where options are more limited. However, research on giant salvinia (*Salvinia molesta* Mitchell) response to foliar herbicide applications at carrier volumes  $\leq 140$  L ha<sup>-1</sup> is incomplete. Likewise, no data exist documenting *S. molesta* control with unoccupied aerial application systems (UAAS). Following the recent >100-ha incursion of *S. molesta* in Gapway Swamp, NC, a case study was developed to provide guidance for ongoing management efforts. In total, three field trials evaluated registered aquatic and experimental herbicides using a 140 L ha<sup>-1</sup> carrier volume. Select foliar applications from UAAS were also evaluated. Results at 8 wk after treatment (WAT) indicated the experimental protoporphyrinogen oxidase inhibitor, PPO-699-01 (424 g ai ha<sup>-1</sup>), in combination with endothall dipotassium salt (2,370 g ae ha<sup>-1</sup>) provided 78% visual control, whereas control when PPO-699-01 (212 g ai ha<sup>-1</sup>) was applied alone was lower at 35%. Evaluations also showed diquat (3,136 g ai ha<sup>-1</sup>) alone, glyphosate (4,539 g ae ha<sup>-1</sup>) alone, and metsulfuron-methyl (42 g ai ha<sup>-1</sup>) alone achieved 86% to 94% visual plant control at 8 WAT. Sequential foliar applications of diquat, flumioxazin (210 g ai ha<sup>-1</sup>), and carfentrazone (67 g ai ha<sup>-1</sup>) at 6 wk following exposure to in-water fluridone treatments were no longer efficacious by 6 WAT due to plant regrowth. Carfentrazone applications made from a backpack sprayer displayed greater control than applications made with UAAS deploying identical carrier volumes at 2 WAT; however, neither application method provided effective control at 8 WAT. Additional field validation is needed to further guide management direction of *S. molesta* control using low carrier volume foliar applications.

## Introduction

Giant salvinia (*Salvinia molesta* Mitchell) is an invasive, free-floating aquatic fern that has progressively colonized wetland waterways throughout the southern United States over the past three decades (EDDMapS 2022; Johnson 1995). Native to Brazil (Forno and Harley 1979), the federally noxious weed rapidly forms dense vegetation mats up to 1-m thick (Thomas and Room 1986), which can disrupt irrigation schedules, clog drainages, limit recreational opportunities, and result in human health concerns (McFarland et al. 2004). Ecologically, *S. molesta* poses threat to habitat diversity by altering water chemistry (e.g., dissolved oxygen and pH) and restricting light availability to other aquatic biotas (McFarland et al. 2004; Owens et al. 2005). Once established, the floating fern is difficult to control due to the complexities of the waterways it occupies, its prolific growth rates (Kaufman and Kaufman 2007), and effective vegetative dispersal mechanisms (Glomski and Mudge 2013; Nelson et al. 2001).

In North Carolina, *S. molesta* was discovered in nine southeastern counties by the early 2000s (NCWRC 2015), but was considered eradicated in 2008 following rapid response measures with herbicides (W Batten, personal communication). The recent discovery (2020) of a new infestation in a cypress [*Taxodium distichum* (L.) Rich.] swamp system has again prompted rapid response techniques from local, state, and federal agencies (ERDC 2021; Rashash 2020). Initial evaluations from July 2020 revealed *S. molesta* had invaded approx. 100 ha within Gapway Swamp, NC (R Emens, personal communication), which prompted immediate plant control efforts using foliar-applied herbicides to prevent further plant spread to surrounding waterways. Nonchemical aquatic plant management techniques such as mechanical harvesting and biological control agents (e.g., salvinia weevil [*Cyrtobagous salviniae* (Calder and Sands)])

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### Management Implications

Since escaping from the aquaria trade in the mid-1990s, *Salvinia molesta* (giant salvinia) continues to invade waterways throughout the southern United States. Because *S. molesta* is a free-floating fern having inherent management challenges, plant eradication largely relies on herbicides. While some existing foliar techniques do provide consistent control of *S. molesta*, application volumes with these herbicides are generally high (>468 L ha<sup>-1</sup>). These traditional carrier volumes can ultimately limit the scale of plant treatment, especially when using backpack sprayers or unoccupied aerial application systems (UAAS) having limited tank capacities (≤10 L). The recent discovery of *S. molesta* in a North Carolina cypress swamp prompted research to broaden ongoing plant management tactics deploying carrier volumes 3-fold lower than conventional application volumes (140 L ha<sup>-1</sup>). Field experiments indicated diquat (3,136 g ai ha<sup>-1</sup>) alone, glyphosate (4,539 g ae ha<sup>-1</sup>) alone, and metsulfuron-methyl (42 g ai ha<sup>-1</sup>) alone provided the longest-lasting *S. molesta* control. The experimental protoporphyrinogen oxidase inhibitor, PPO-699-01 (424 g ai ha<sup>-1</sup>), also showed potential for *S. molesta* management. This herbicide was most efficacious when tank mixed with either endothall dipotassium salt (2,370 g ae ha<sup>-1</sup>) or 2,4-D (2,129 g ae ha<sup>-1</sup>). Additional UAAS evaluations are required to examine other foliar herbicides and carrier volumes, which may provide adequate control of *S. molesta*. Managers attempting *S. molesta* control at reduced carrier volumes may wish to conduct an additional foliar spray operation (~6 wk) to minimize plant recolonization.

can reduce *S. molesta* density (Cilliers 1991; Cozad et al. 2019; Westbrook 2010). However, herbicides or integrated management techniques including herbicides are largely utilized for eradication and rapid response efforts due to high efficacy and selectivity (McFarland et al. 2004; Nelson et al. 2001).

Chemical control of *S. molesta* in cypress swamps has been successful in Louisiana and Texas (Cozad et al. 2019), yet management using herbicide applications is often challenging due to limited watercraft accessibility and environmental constraints (e.g., trees provide refuge) (Sartain and Mudge 2018a). Previous research has demonstrated subsurface (i.e., in-water) applications of the aquatic herbicides chelated copper, endothall dipotassium salt, fluridone, penoxsulam, and bispyribac-sodium have the potential to reduce floating plant biomass under greenhouse and field settings (Glomski and Mudge 2013; Glomski et al. 2003; Mudge and Netherland 2020; Mudge et al. 2012). However, these types of herbicide treatments can be cost prohibitive in broad invasion scenarios, typically require long exposures (10 to 12 wk), or are restricted due to localized constraints (e.g., irrigation restrictions), which can limit operational implementation for resource managers. Therefore, foliar-applied herbicides have largely remained the most common treatment technique for *S. molesta* management.

The systemic herbicide glyphosate has persisted as one of the most effective foliar treatment options for managing *S. molesta* (Mudge et al. 2016; Nelson et al. 2007). Still, the contact herbicides diquat, flumioxazin, and carfentrazone-ethyl (hereinafter, carfentrazone) have shown favorable results used as stand-alone treatments or as tank-mix partners (Glomski and Getsinger 2006; Nelson et al. 2001; Richardson et al. 2008). Efficacy evaluations for 2,4-D at 0.75 to 3.0 kg ae ha<sup>-1</sup> have also demonstrated 92% to 95%

*S. molesta* control in greenhouse settings (Diatloff et al. 1979). Similarly, 2,4-D (0.62 and 1.85 g ai ha<sup>-1</sup>) and nonionic surfactant (NIS) (0.25% v/v) suppressed *S. molesta* growth in a mesocosm study (Wahl et al. 2018); however, low herbicide efficacy was reported in Brazil when 2,4-D was applied at 1.34 kg ae ha<sup>-1</sup> to *S. molesta* in a reservoir (Martins et al. 2002). In the United States, 2,4-D has not been evaluated in an operational setting and is not readily utilized for *S. molesta* control. The herbicide does, however, provide excellent control of the free-floating plant water hyacinth [*Eichhornia crassipes* (Mart.) Solms] (Enloe et al. 2022), which commonly associates with *S. molesta* in mixed stands. In recent studies, the herbicide metsulfuron-methyl at rates ≤42 g ai ha<sup>-1</sup> provided ≥98% control when applied to *S. molesta* foliage (Prevost et al. 2021; Sartain and Mudge 2018b). However, this herbicide can only be applied under the Federal Insecticide, Fungicide, and Rodenticide Act Special Local Need (SLN) 24(c) label in Texas, Louisiana, Mississippi, Alabama, and South Carolina (Mudge 2020). While several foliar-applied herbicides have been trialed and utilized to successfully combat *S. molesta* (Glomski and Mudge 2013; Mudge et al. 2016; Nelson et al. 2007; Prevost et al. 2021; Richardson et al. 2008; Sartain and Mudge 2019), there remains an interest in expanding the currently available aquatic herbicide portfolio while using lower carrier volumes (e.g., 140 L ha<sup>-1</sup>) to increase operational efficiency and performance.

Typically, aquatic herbicides are delivered at total spray volumes between 468 and 1,870 L ha<sup>-1</sup> (Haller 2020; Nelson et al. 2007). While this foliar application strategy has been successful with numerous herbicides and plant targets (Sperry and Ferrell 2021), there are associated hindrances that make “high” spray-volume applications undesirable for current management tactics. Research has established carrier volume directly affects spray deposition, herbicide activity, and ultimately aquatic plant control (Moreira et al. 1999; Nelson et al. 2007; Sperry and Ferrell 2021; Sperry et al. 2022; Van et al. 1986; Willard et al. 1998). Limitations of high carrier volumes (e.g., 935 L ha<sup>-1</sup>) include reduced spray retention at low floating plant densities, thus resulting in spray loss to the water column (Mudge et al. 2021), and reduced herbicide concentration per spray droplet (Knoche 1994). In general, herbicide applications made with lower carrier volumes are preferred because of time savings when filling spray tanks (Nelson et al. 2007), public perception (Sperry and Ferrell 2021), increased spray retention on plant targets (Sperry et al. 2022), and the increased performance of some herbicides on floating plant species (Nelson et al. 2007; Sperry and Ferrell 2021; Van et al. 1986). In a mesocosm study, Sartain and Mudge (2018a) evaluated winter herbicide applications on *S. molesta* and *T. distichum* using a carrier volume representative of an aerial application (94 L ha<sup>-1</sup>). While treatment efficacy varied by application timing (month and year), Sartain and Mudge (2018a) found low spray-volume applications of diquat, glyphosate, flumioxazin, and glyphosate + diquat provided 40% to 100% control of *S. molesta*.

Traditional spray application techniques made with hand wands often requires applicators to navigate hazardous environmental conditions (e.g., trees, submersed objects and stumps) to achieve adequate spray coverage for plant control. Inaccessible regions within a waterbody can also completely restrict foliar applications due to obstruction (e.g., trees and tussocks) and limited water depth (e.g., too shallow for watercraft), making spray attempts futile. Over the past decade, unoccupied aerial application systems (UAAS) have gained popularity in terrestrial settings to remotely deliver herbicides in complex environments that limit access for ground-based operations (Göktoğan et al. 2010; Lan

et al. 2017; Wang et al. 2019; Xue et al. 2016). Aquatic herbicide applicators have expressed the desire to integrate UAAS in spray operations to overcome inherent environmental complexities in aquatics and potentially decrease exposure to herbicides during treatment. However, commercially available UAAS typically have tank volumes of  $\leq 10$  L to conform to Federal Aviation Administration regulations (i.e., FAA 14 CFR Part 107). Existing application strategies limit UAAS operational use and further signify the importance of reevaluating carrier volumes for foliar spray tasks. To date, literature regarding UAAS practices among aquatic site applications remains limited; however, positive results have been documented when implementing UAAS for site-specific weed management over conventional broadcast herbicide applications in terrestrial sites (Hunter et al. 2020). While studies do exist comparing the efficacy and efficiency of UAAS and standard backpack applications in terrestrial settings (Gertsis and Karampekos 2021; Hunter et al. 2020; Martin et al. 2020), none have directly correlated herbicide efficacy holding carrier volume constant. There remains a clear need to investigate the performance of herbicides delivered from UAAS to traditional ground-based applications made at low carrier volumes for aquatic weed management.

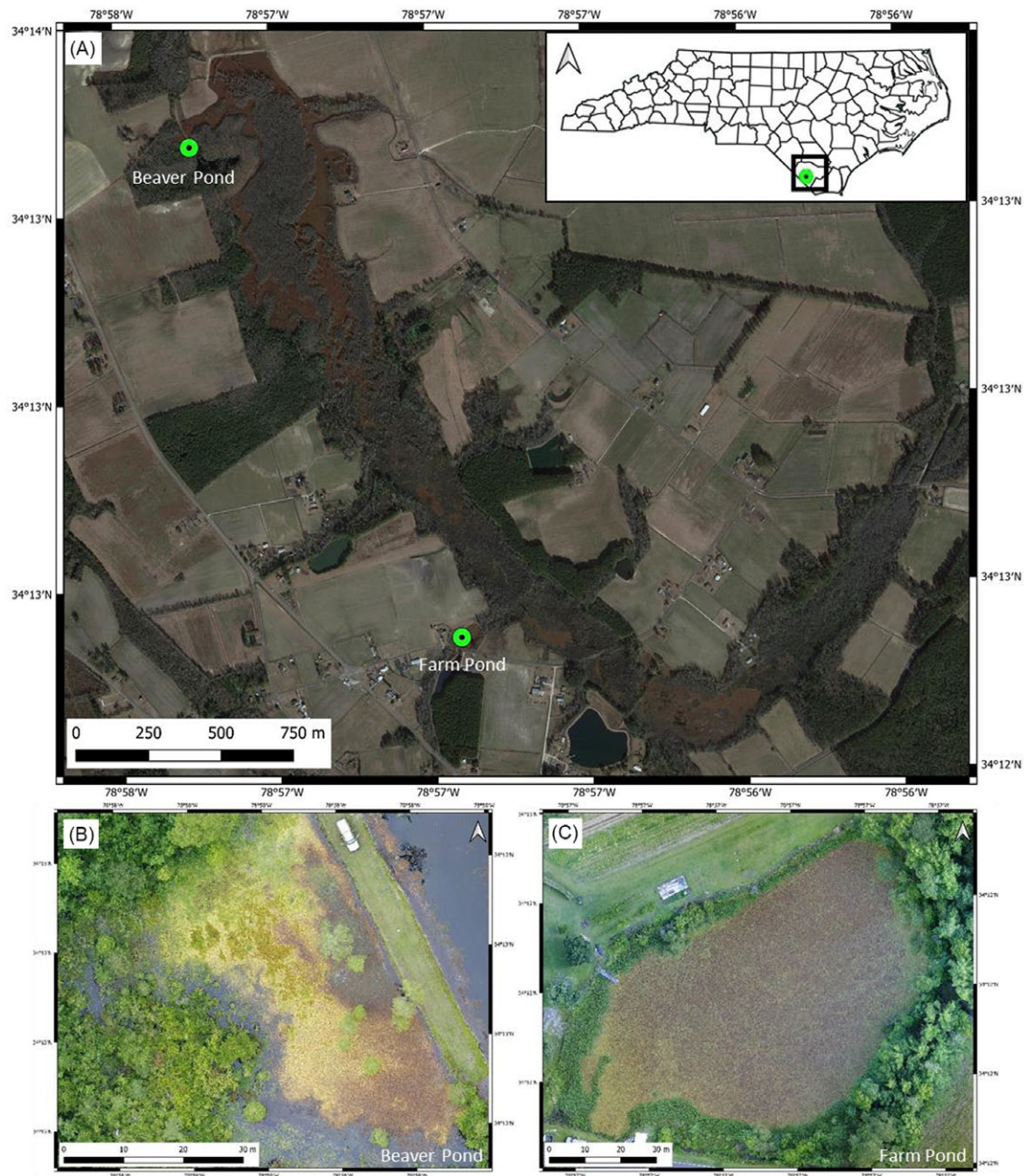
Although spray application techniques in terrestrial settings generally outpace weed management strategies in aquatics, there is an opportunity to adopt similar spray technology and techniques to enhance current floating plant management strategies. As weed science practices evolve with time, it is important to evaluate novel foliar spray methods to increase herbicide efficacy while increasing spray application efficiency. We hypothesize the reduction of carrier volume will achieve comparable results to current management tactics and thereby provide an additional application strategy for management. The objectives of this research are to: (1) evaluate the efficacy of aquatic and experimental herbicides at reduced carrier volumes for *S. molesta* control efforts; (2) determine the effectiveness of diquat, flumioxazin, and carfentrazone applied as sequential treatments following in-water fluridone applications; and (3) document the performance of carfentrazone when applied with an UAAS versus foliar backpack applications. Findings are reported as a case study to inform current and future *S. molesta* management efforts.

## Materials and Methods

Three field trials were conducted within select sites of Gapway Swamp (Columbus County, NC; 34.21°N, 78.94°W) to evaluate the efficacy of registered aquatic and experimental herbicides against tertiary-growth *S. molesta* during the summer of 2021 (Figure 1). Two trials occurred within a local farm pond (0.61 ha) directly adjacent to the main swamp system, and one trial occurred at a nearby beaver pond (0.24 ha) connected via culvert to the swamp. Before herbicide applications, floating quadrats (0.79 m<sup>2</sup>) constructed of polyvinyl chloride pipe (2.54-cm diameter) and floatation foam (7.62-cm diameter) were deployed to serve as plots for the selected herbicide treatments. Floating quadrats were spaced 0.9 m apart using parachute cordage and secured in place using metal conduit anchors. Trials were initiated July 13, 20, and 28 (Trials 1, 2, and 3, respectively) and were monitored weekly over the 8-wk study period. Ambient environmental conditions at the time of the herbicide applications consisted of partly sunny skies with air temperatures of 28 C ( $\pm 0.7$  SD) and wind speeds not exceeding 9.7 km h<sup>-1</sup>. At the time of herbicide treatment, plots were 100% covered with 7- to 10-cm-thick *S. molesta*. Nontreated

plots (0.79 m<sup>2</sup>) had pretreatment biomass values of  $328.0 \pm 58.5$  g dry weight.

Trial 1 (farm pond) included varying rates of two experimental products, PPO-699-01 (in development; protoporphyrinogen oxidase inhibitor) and PPO-393-01 (in development; protoporphyrinogen oxidase inhibitor), and the registered aquatic herbicides 2,4-D amine (WSSA Group 4; synthetic auxin) and endothall dipotassium salt (WSSA Group 31; protein phosphatase inhibitor). Trial 2 (farm pond) evaluated the herbicides carfentrazone (WSSA Group 14; protoporphyrinogen oxidase inhibitor), diquat (WSSA Group 22; photosystem I diversion), flumioxazin (WSSA Group 14; protoporphyrinogen oxidase inhibitor), glyphosate (WSSA Group 9; 5-enolpyruvylshikimate-3-phosphate synthase inhibitor), metsulfuron-methyl (WSSA Group 2; acetolactate synthase inhibitor), and penoxsulam (WSSA Group 2; acetolactate synthase inhibitor). Previous research suggests a sequential “bump” application of fluridone (WSSA Group 12; carotenoid biosynthesis inhibitor) is often required 6 to 8 wk after initial treatment to maintain suitable in-water herbicide concentrations to control established *S. molesta* populations (Mudge et al. 2012). While single subsurface fluridone treatments of 10 to 20  $\mu\text{g L}^{-1}$  often severely injure *S. molesta* (Mudge et al. 2012), regrowth occurs with frequency in well-established populations (AW Howell, personal observation), and managers have suggested the desire to spot treat areas using foliar-applied herbicides to control remaining plants. Likewise, the application of foliar herbicide treatments is generally more economical for control of targeted areas of regrowth than subsurface fluridone applications. The contact herbicides carfentrazone, diquat, and flumioxazin, are commonly deployed for *S. molesta* management in South Carolina (C Moorer, personal communication). Therefore, Trial 3 (beaver pond) evaluated these three herbicides made as a sequential application following an in-water treatment of fluridone that had occurred 6 wk before trial initiation. A complete list of the herbicides used in these trials is included in Table 1. All herbicide treatments included a nonionic surfactant (Induce<sup>®</sup>, Helena Agri-Enterprises, Collierville, TN, USA) at 0.25% v/v. For each trial, a nontreated control was included to compare plant vigor without herbicide. All treatments followed a randomized complete block design and included four replicates. Foliar applications were done from a boat using a CO<sub>2</sub>-pressurized backpack sprayer with a handheld boom fit with two XR110015-VP nozzles (TeeJet<sup>®</sup> nozzles, Spraying Systems, Wheaton, IL, USA) calibrated to deliver an application volume of 140 L ha<sup>-1</sup>. Applications were made approximately 0.4 m above the plant canopy and when wind speeds were  $< 3.2$  km h<sup>-1</sup>, to avoid spray drift onto adjacent plots. At both of the trial locations, a UAAS was deployed to compare the efficacy of carfentrazone backpack foliar applications and low-altitude (3 m above ground level [m AGL]) aerial carfentrazone foliar applications. Carfentrazone was chosen for ground and aerial application comparisons because the herbicide was the most widely utilized foliar treatment deployed during initial *S. molesta* eradication efforts at Gapway Swamp. Aerial applications were done with a DJI Agras MG-1 octocopter (Da-Jiang Innovations, Shenzhen, China) consisting of four AIXR11002-VP (TeeJet<sup>®</sup> nozzles, Spraying Systems) nozzles calibrated to deliver 140 L ha<sup>-1</sup>. Ground-based visual assessments of efficacy were not feasible due to inaccessibility and concern of plot disturbance. For all trials, aerial imagery was collected with a DJI Phantom 4 Advanced (Da-Jiang Innovations) small unoccupied aerial system (sUAS) at 9 and 61 m AGL to evaluate pretreatment *S. molesta* vigor and plant injury or regrowth following herbicide applications. Treatments were



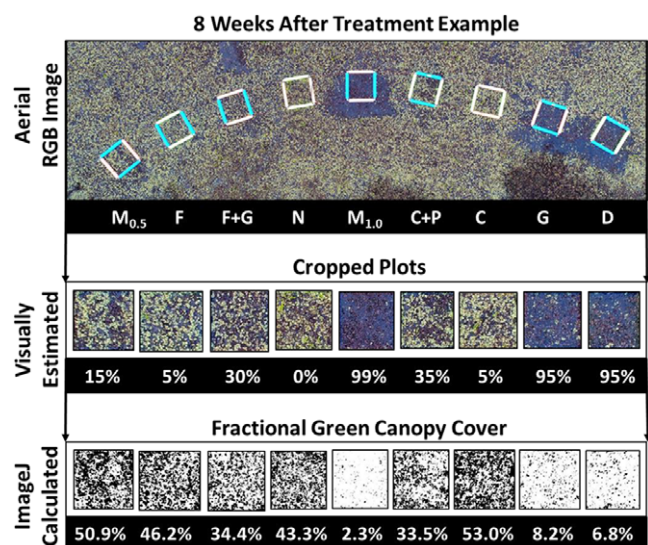
**Figure 1.** Map of the *Salvinia molesta* infestation site at Gapway Swamp (Columbus County, NC; 34.21°N, 78.94°W) and study locations used for the three field trials conducted during the summer of 2021 (A). *Salvinia molesta* (dark brown foliage) completely occupied all open water within the *Taxodium distichum* (gray foliage) swamp as depicted in the January 23, 2021, satellite image. Field Trial 3 was conducted at the beaver pond (B; pretreatment aerial image captured July 20, 2021), while field Trials 1 and 3 were conducted at the farm pond (C; pretreatment aerial image captured June 30, 2021).

compared using visual estimations from aerial imagery to determine plant control (0% = no plant control to 100% = complete necrosis) among treatments at each evaluation. Additionally, fractional green canopy cover (FGCC) was calculated from collected aerial imagery in ImageJ software (National Institutes of Health, Bethesda, MD, USA), following methods similar to those described by Ali et al. (2013), to provide a quantitative measure of the remaining viable plant material at 8 WAT. This binary image-processing procedure utilizes hue, saturation, and brightness color thresholding parameters, calibrated to the nontreated reference plots to separate green plant material (pixels) from the non-green background (i.e., water

and necrotic plant tissues) to calculate the percentage of green pixels (Figure 2). Following image collection, plots were harvested of viable floating plant material at 8 WAT, and plants were force air-dried for 72 h at 60 C, then recorded as dry biomass. All data were subjected to ANOVA, and means were separated using Fisher's protected LSD ( $P \leq 0.05$ ) in RStudio (v. 4.0.3; R Core Team 2020) using the AGRICOLAE (de Mendiburu 2020) and DPLYR packages (Wickham et al. 2021). Statistical comparisons of plant control by application technique between backpack and foliar treatments used Student's *t*-test ( $\alpha = 0.05$ ) in RStudio. A Pearson correlation analysis was performed comparing calculated FGCC percentages

**Table 1.** Herbicides evaluated in the three field trials (Gapway Swamp, NC) examining the control of *Salvinia molesta* under low carrier volume applications in 2021.

Common name	Trade name	Manufacturer	Trial
2,4-D amine	Weedar® 64	NuFarm, Alsip, IL, USA	1 (farm pond)
Carfentrazone	Stingray®	SePRO, Carmel, IN, USA	2, 3 (farm and beaver ponds)
Diquat	Reward®	Syngenta, Greensboro, NC, USA	2, 3 (farm and beaver ponds)
Endothall dipotassium salt	Aquathol® K	UPL, King of Prussia, PA, USA	1 (farm pond)
Flumioxazin	Clipper® SC	NuFarm, Alsip, IL, USA	2, 3 (farm and beaver ponds)
Glyphosate	Rodeo®	Corteva, Indianapolis, IN, USA	2 (farm pond)
PPO-393-01	Experimental	UPL, King of Prussia, PA, USA	1 (farm pond)
PPO-699-01	Experimental	UPL, King of Prussia, PA, USA	1 (farm pond)
Metsulfuron-methyl	MSM 60®	Alligare, Opelika, AL, USA	2 (farm pond)
Penoxsulam	Galleon® SC	SePRO, Carmel, IN, USA	2 (farm pond)



**Figure 2.** Example workflow developed to evaluate *Salvinia molesta* response to various foliar herbicide applications in Trial 2 captured from aerial imagery at 8 wk after treatment (WAT). Abbreviations of herbicides listed under the original RGB image indicate M<sub>0.5</sub>, metsulfuron-methyl (21 g ai ha<sup>-1</sup>); F, flumioxazin (210 g ai ha<sup>-1</sup>); F+G, flumioxazin (210 g ai ha<sup>-1</sup>) + glyphosate (4,539 g ae ha<sup>-1</sup>); N, nontreated; M<sub>1.0</sub>, metsulfuron-methyl (42 g ai ha<sup>-1</sup>); C+P, carfentrazone (67 g ai ha<sup>-1</sup>) + penoxsulam (70 g ai ha<sup>-1</sup>); C, carfentrazone (67 g ai ha<sup>-1</sup>); G, glyphosate (4,539 g ae ha<sup>-1</sup>); and D, diquat (3,136 g ai ha<sup>-1</sup>). Percentages listed under the cropped plot images represent visually estimated control values of each respective experimental unit. Percentages listed under the binary images (black and white) illustrate fractional green canopy cover (FGCC) calculated from the cropped images in ImageJ software.

and visual control estimates to measure the strength of the relationship between the metrics. Where appropriate, herbicide tank mixtures were examined further to calculate whether control was synergistic, antagonistic, or additive at 8 WAT using Colby's method:

$$E = (X + Y) - \left( \frac{XY}{100} \right) \quad [1]$$

where  $E$  is the expected control (%) of two herbicides applied as a tank mixture,  $X$  is control (%) of herbicide A applied alone, and  $Y$  is the control (%) of herbicide B when applied alone. When the observed control is greater than expected, the tank mixture is synergistic; when observed control is less than expected, the tank mixture is antagonistic (Colby 1967). If the observed and expected control levels are equal, the tank mixture is then considered additive (Colby 1967).

## Results and Discussion

### Trial 1. Evaluation of Experimental Herbicides Alone and in Combination

Within 1 WAT, all treatment plots showed varying levels of herbicide injury, which included chlorotic and necrotic fronds (data not shown). Visual control estimates at 2 WAT indicated PPO-699-01 in combination with endothall or 2,4-D provided the greatest injury to treated *S. molesta* with 96% and 94% control, respectively (Table 2). Previous studies have shown endothall alone or as a tank-mix partner in foliar spray solutions provides rapid injury symptoms to *S. molesta* within days to 2 WAT (Mudge and Netherland 2020; Mudge et al. 2016; Nelson et al. 2001) and support the additive injury benefits shown in the present trial when endothall was included with PPO-699-01 as a tank-mix partner. Across all treatments, visual control levels peaked at 4 WAT with no difference between PPO-699-01 single or combination treatments (85% to 94% visual control). Plant injury from combinations of PPO-699-01 with PPO-393-01 progressed more slowly than from the combinations of PPO-699-01 with either endothall or 2,4-D; however, by the 6 WAT evaluation, there were no differences between the highest rate of PPO-699-01 alone and PPO-699-01 with either rate of PPO-393-01, endothall, or 2,4-D. PPO-699-01 alone at 212 g ai ha<sup>-1</sup> provided the least control during these later evaluations. At the trial conclusion (8 WAT), all treatments showed varying levels of regrowth. However, due to a high degree of variability in regrowth by 8 WAT, only PPO-699-01 in combination with endothall provided significant improvement in visual control (78%) over PPO-699-01 applied alone at the lowest rate (35%). Computer-aided estimates of FGCC conveyed results similar to visual control estimates at 8 WAT, with PPO-699-01 in combination with endothall providing the least green canopy cover (31% less FGCC than the nontreated reference plots) and therefore the greatest plant control. Dry weight data showed a response similar to the visual and calculated image analysis metrics. Reduction in biomass was greatest with PPO-699-01 in combination with endothall; however, all other treatments did not differ from one another (Table 2).

### Trial 2. Evaluation of Commonly Applied Foliar Herbicides Alone and in Combination

At 1 WAT, all foliar herbicide applications resulted in chlorotic and necrotic fronds (data not shown), and by 2 WAT, all treatments provided 84% to 97% visual control (Table 3). Apart from plots treated with carfentrazone alone or flumioxazin + glyphosate, control continued to increase at 4 WAT. Carfentrazone alone showed the highest recovery at this 4 WAT evaluation. However, varying levels of plant recovery were observed among all treatment plots

**Table 2.** Visually estimated control, fractional green canopy cover (FGCC), and dry weights ( $\pm$ SE) of *Salvinia molesta* following foliar herbicide applications in Trial 1 at the farm pond (Gapway Swamp, NC).

Herbicide <sup>c</sup>	Rate <sup>d</sup>	Weeks after treatment <sup>a,b</sup>				FGCC <sup>e</sup>	Dry weight
		2	4	6	8		
	g ai ha <sup>-1</sup>	%				%	g
PPO-699-01 + endothall dipotassium salt	424 + 2,370	96 a	94 a	94 a	78 a	20 a	178.7 $\pm$ 31.1 a
PPO-699-01 + 2,4-D amine	424 + 2,129	94 a	94 a	92 a	73 ab	31 abc	208.1 $\pm$ 24.1 ab
PPO-699-01 + PPO-393-01	424 + 208	69 bc	93 a	91 a	66 ab	29 ab	285.4 $\pm$ 29.4 bc
PPO-699-01 + PPO-393-01	424 + 104	79 b	90 a	83 ab	46 ab	34 abcd	251.5 $\pm$ 28.2 b
PPO-699-01	424	69 bc	89 a	85 ab	53 ab	47 cd	230.1 $\pm$ 23.7 ab
PPO-699-01	212	64 c	85 a	71 b	35 bc	42 bcd	245.6 $\pm$ 18.7 ab
Nontreated	0	0 d	0 b	0 c	0 c	51 d	328.0 $\pm$ 39.2 c

<sup>a</sup>Visual control rated on 0% to 100% scale: 0% = no plant response to 100% = complete necrosis.

<sup>b</sup>Mean responses within a column followed by the same letter do not differ according to Fisher's protected LSD ( $P \leq 0.05$ ).

<sup>c</sup>Nonionic surfactant at 0.25% v/v included with all herbicide applications.

<sup>d</sup>Endothall and 2,4-D are reported in g ae ha<sup>-1</sup>.

<sup>e</sup>FGCC at harvest: 0% = no green canopy cover to 100% = complete green canopy cover.

**Table 3.** Visually estimated control, fractional green canopy cover (FGCC), and dry weights ( $\pm$ SE) of *Salvinia molesta* following foliar herbicide applications via backpack and unoccupied aerial application systems in Trial 2 at the farm pond (Gapway Swamp, NC).

Herbicide <sup>c</sup>	Rate <sup>d</sup>	Weeks after treatment <sup>a,b</sup>				FGCC <sup>e</sup>	Dry weight
		2	4	7	8		
	g ai ha <sup>-1</sup>	%					g
Backpack							
Metsulfuron-methyl	21	84 b	97 a	10 c	6 d	53 a	336.8 $\pm$ 27.0 a
Metsulfuron-methyl	42	95 a	100 a	88 a	86 ab	19 c	226.2 $\pm$ 37.5 a
Flumioxazin	210	95 a	98 a	73 ab	50 c	34 bc	283.8 $\pm$ 15.1 a
Glyphosate	4,539	97 a	100 a	96 a	94 a	17 c	256.8 $\pm$ 25.1 a
Flumioxazin + Glyphosate	210 + 4,539	90 ab	65 ab	5 c	3 d	51 ab	255.3 $\pm$ 15.8 a
Carfentrazone	67	89 ab	53 b	2.5 c	1 d	51 ab	279.8 $\pm$ 31.9 a
Carfentrazone + Penoxsulam	67 + 70	94 ab	97 a	64 b	58 bc	29 c	314.7 $\pm$ 40.7 a
Diquat	3,136	90 ab	99 a	91 a	87 ab	15 c	214.2 $\pm$ 20.8 a
Nontreated	0	0 c	0 c	0 c	0 d	60 a	334.8 $\pm$ 46.4 a
Unoccupied aerial application system <sup>f</sup>							
Carfentrazone	67	74*	55	0	0	45	252.7 $\pm$ 20.1

<sup>a</sup>Visual control rated on 0% to 100% scale: 0% = no plant response to 100% = complete necrosis.

<sup>b</sup>Mean responses within a column followed by the same letter do not differ according to Fisher's protected LSD ( $P \leq 0.05$ ).

<sup>c</sup>Nonionic surfactant at 0.25% v/v included with all herbicide applications.

<sup>d</sup>Glyphosate is reported in g ae ha<sup>-1</sup>.

<sup>e</sup>FGCC at harvest: 0% = no green canopy cover to 100% = complete green canopy cover.

<sup>f</sup>An asterisk (\*) following the metric indicates the mean response by application method differs for carfentrazone using Student's *t*-test ( $\alpha = 0.05$ ).

beyond 4 WAT. At 7 WAT, no difference was detected between diquat, flumioxazin, glyphosate, and metsulfuron-methyl at 42 g ai ha<sup>-1</sup>, which provided 73% to 96% control (Table 3). A significant rate response ( $P > 0.05$ ) was observed at 7 and 8 WAT for metsulfuron-methyl, as plots treated at 42 g ai ha<sup>-1</sup> improved visual control 78 to 80 percentage points greater than plots treated with metsulfuron-methyl at 21 g ai ha<sup>-1</sup> (7 and 8 WAT, respectively). Sartain and Mudge (2018b) indicated metsulfuron-methyl at 21 and 42 g ai ha<sup>-1</sup> provided >98% control in a 12-wk mesocosm study, which contradicts the difference in metsulfuron rate response presented in the current trial. However, previous research has shown that mesocosm results do not necessarily align with field assessments due to environmental variability (Netherland and Getsinger 2018). This could explain the significant rate response difference for metsulfuron-methyl in the present trial not being observed in previous mesocosm trials. By 8 WAT, foliar treatments of carfentrazone, flumioxazin + glyphosate, and metsulfuron-methyl at 21 g ai ha<sup>-1</sup> provided the least control ( $\leq 6\%$ ) (Table 3). Antagonism was detected when flumioxazin and glyphosate were tank mixed, as flumioxazin alone and glyphosate alone

provided 47 and 91 percentage points greater *S. molesta* control than the tank-mixed treatment, respectively. While flumioxazin is a broad-spectrum contact herbicide (Schardt and Netherland 2020), it is possible that rapid cell death ultimately limited the systemic properties of glyphosate on *S. molesta*, thus reducing the effectiveness of the tank mix. However, previous mesocosm research contradicts the calculated flumioxazin + glyphosate antagonism in the present trial, because these herbicides in combination provided >98% *S. molesta* control at 7 to 8 WAT (Mudge and Sartain 2018; Mudge et al. 2016). While all treatments displayed *S. molesta* recovery 8 WAT, plots treated with diquat alone, glyphosate alone, and metsulfuron-methyl alone at 42 g ai ha<sup>-1</sup> did achieve 87%, 94%, and 86% visual plant control, respectively. Further, there was no difference at 8 WAT in visual control estimates for diquat, glyphosate, and metsulfuron-methyl at 42 g ai ha<sup>-1</sup> (Table 3).

Imagery analysis of FGCC closely supported the visual estimates of control at 8 WAT, with diquat, glyphosate, and metsulfuron-methyl at 42 g ai ha<sup>-1</sup> providing the least green canopy cover (45%, 43%, and 41% less FGCC than the nontreated

**Table 4.** Visually estimated control, fractional green canopy cover (FGCC), and dry weights ( $\pm$ SE) of *Salvinia molesta* following select sequential foliar herbicide applications overtop plants treated 6 wk prior with fluridone at the beaver pond (Gapway Swamp, NC).

Herbicide <sup>c</sup>	Rate	Weeks after treatment <sup>a,b</sup>				FGCC <sup>d</sup>	Dry weight
		2	4	6	8		
Backpack	g ai ha <sup>-1</sup>	%				%	g
Flumioxazin	210	99 a	91 a	38 a	0 a	68 a	217.0 $\pm$ 13.2 a
Carfentrazone	67	98 ab	83 a	35 a	0 a	70 a	178.0 $\pm$ 59.0 a
Diquat	3,136	97 b	83 a	43 a	0 a	65 a	144.7 $\pm$ 31.9 a
Nontreated	0	0 c	0 b	0 b	0 a	75 a	357.6 $\pm$ 44.4 b
Unoccupied aerial application system <sup>e</sup>							
Carfentrazone	67	88*	76	—	0	63	293.5 $\pm$ 49.1

<sup>a</sup>Visual control rated on 0% to 100% scale: 0% = no plant response to 100% = complete necrosis.

<sup>b</sup>Mean responses within a column followed by the same letter do not differ according to Fisher's protected LSD ( $P \leq 0.05$ ).

<sup>c</sup>Nonionic surfactant at 0.25% v/v included with all herbicide applications. Applications were made via backpack and unoccupied aerial application systems in Trial 3.

<sup>d</sup>FGCC at harvest: 0% = no green canopy cover to 100% = complete green canopy cover.

<sup>e</sup>An asterisk (\*) following the metric indicates the mean response by application method differs for carfentrazone using Student's *t*-test ( $\alpha = 0.05$ ).

reference plots, respectively) and therefore the greatest control (Table 3). It should be noted the FGCC calculations for carfentrazone + penoxsulam, diquat, glyphosate, and metsulfuron-methyl at 42 g ai ha<sup>-1</sup> were not different according to ANOVA ( $P = 0.05$ ), which does differ from visual control evaluations at the trial conclusion at 8 WAT (Table 3). Dry weight data did not provide separation between treatments ( $P = 0.103$ ); further, no treatments differed from the nontreated reference biomass (Table 3). This response is likely due in part to the tertiary growth present at the time of herbicide application. Previous studies have documented limited plant control following foliar-applied herbicides to multiple plant layers due to difficulty in herbicide penetration through the entire plant canopy (Mudge et al. 2016; Sartain and Mudge 2018b). We hypothesize *S. molesta* recolonization experienced among treatment plots at approximately 4 WAT was from second- and third-layer plant material that remained shielded by the top-most plant layer during the foliar applications. While contact herbicides like flumioxazin initially provide rapid injury symptoms, greenhouse and field studies have indicated *S. molesta* recovery when contact herbicides are applied alone (Richardson et al. 2008; Sartain and Mudge 2019). Because living *S. molesta* can remain trapped under dead biomass, results from visual and calculated plant control best describe the initial plant responses to treatment (Figure 2). Results from Pearson correlation analysis showed a strong inverse association when FGCC image calculations are used as a surrogate to visual control observations for gauging plant response to treatment ( $r = -0.90$ ). Therefore, similar image analysis techniques are encouraged to monitor *S. molesta* response to herbicide in future studies, particularly in plant management scenarios having limited site access.

*Salvinia molesta* treated with the UAAS provided 80% visual control at 1 WAT (data not shown). However, UAAS treatments began recovering by 2 WAT, with carfentrazone backpack applications showing greater plant control over UAAS treatments (Table 3). Nevertheless, there was no difference between carfentrazone application methods beyond the 4 WAT evaluation ( $\alpha = 0.05$ ), and plants had fully recovered at 8 WAT regardless of the treatment method (Table 3). The lack of control at trial conclusion is not surprising based on previous evaluations of carfentrazone that indicated retreatment might be necessary beyond 4 WAT to control remaining viable plant tissues leading to regrowth (Glomski and Getsinger 2006). Ramsdale and Messersmith (2001) evaluated the effects of adjuvant type on carfentrazone efficacy in terrestrial settings and showed 9% to 12% improved control of flax

(*Linum usitatissimum* L.) and oilseed sunflower (*Helianthus annuus* L.) when a methylated vegetable oil (MVO) was included in the spray solution in place of NIS. Because *S. molesta* is guarded by trichomes on the surface of plant fronds, which reduces spray penetration and thus limits herbicide effectiveness (McFarland et al. 2004), the inclusion of a more appropriate spray adjuvant or higher adjuvant rate (i.e., MVO or NIS at 1% v/v) may have improved plant control over the inclusion of NIS at 0.25% v/v in the present trial. Future evaluations should investigate the variability between ground and aerial applications of additional contact herbicides with varying spray adjuvants and the influence on *S. molesta* control.

### Trial 3. Evaluation of Select Contact Herbicides as a Sequential Application to In-Water Fluridone Exposures

*Salvinia molesta* was partially bleached and chlorotic (data not shown) following an in-water application of fluridone 6 wk before the foliar treatment trial initiation (Figure 1B). Sequential foliar applications of carfentrazone, diquat, and flumioxazin to the fluridone-treated plants resulted in rapid plant injury, with necrotic fronds appearing within 1 WAT, whereas nontreated reference plots displayed signs of recovery (healthy green fronds) following the previous fluridone treatment (i.e., 7 wk following initial fluridone exposure; data not shown). Although visual control for all herbicides peaked at 97% to 99% at 2 WAT, observations of *S. molesta* recovery did occur across all treatment plots by 4 WAT (Table 4). At the 2 WAT evaluation, carfentrazone applications made with the backpack technique displayed greater control than applications made via the UAAS, which mimics Trial 2 results using these spray techniques (Tables 3 and 4). However, no difference was observed between carfentrazone application methods at any other evaluation time point ( $\alpha = 0.05$ ). Due to the fact that carrier volume was held constant in these trials, we speculate that other factors such differences in spray droplet size and distribution (droplets per unit area) between the backpack and UAAS treatments influenced the performance of the contact herbicide, carfentrazone (data not shown). *Salvinia molesta* control was not considered efficacious for management by 6 WAT (35% to 43% visual control) due to rapid recolonization, and complete plant recovery was observed for all foliar treatments by the trial's conclusion at 8 WAT (Table 4). Results from the quantitative measurements of FGCC further support visual control estimates, with no difference detected between treated and nontreated plants at the 8-wk harvest (Table 4). While dry weight data at 8 WAT indicated all herbicide treatments significantly reduced plant

biomass 39% to 60% compared with nontreated plots ( $P = 0.027$ ), no difference was detected between treatments, and suitable plant control was not achieved.

Floating plant control frequently relies on foliar herbicide application strategies during early invasion scenarios and when targeting persistent or escape plants following active management. However, results from the present trial generally do not support the use of a single foliar application of the contact herbicides carfentrazone, diquat, or flumioxazin as a surrogate to maintaining, or increasing, *S. molesta* control when fluridone concentrations are in a lag phase (below lethal concentrations). While all foliar treatment and application methods provided excellent visual control (88% to 99%) within 2 WAT (Table 4), initial treatment efficacy was not synonymous with long-term effectiveness. Further, plant recovery following treatment occurred more rapidly than previously documented (Glomski and Getsinger 2006; Mudge and Sartain 2018; Mudge et al. 2012; Nelson et al. 2001; Richardson et al. 2008; Sartain and Mudge 2019). The rapidity of plant regrowth observed within plots among all foliar treatments suggests mat thickness likely contributed to decreased herbicide performance, as *S. molesta* mats were 7- to 10-cm thick at trial initiation. Another likely explanation for reduced herbicide performance was the decomposed and injured plant material present from the fluridone application, which could have shielded newly formed *S. molesta* fronds from the foliar spray; thus, new growth likely originated from tissue not exposed to herbicide. Previous research supports this hypothesis, as Sartain and Mudge (2019) attributed the presence of necrotic plant tissue to reducing the efficacy of diquat and flumioxazin when foliar applied to *S. molesta*. Wersal and Madsen (2010) also observed a similar shielding response when common salvinia (*Salvinia minima* Baker) was sprayed with diquat at  $130.8 \text{ g ai ha}^{-1}$  to surface plant material, which allowed plants underneath the mat surface to recolonize. Because contact herbicides are not actively translocated within the plant phloem (Shaner 2014), the fast-acting nature of carfentrazone, diquat, and flumioxazin treatments evaluated in the present trial also may have limited the longevity of plant control (Sartain and Mudge 2019). There remains the need to evaluate the performance of additional slower-acting foliar herbicides such as glyphosate or penoxsulam as sequential applications to in-water fluridone exposure and the influence of *S. molesta* growth stage at time of the sequential application (i.e., primary, secondary, or tertiary stage).

In conclusion, data from the Gapway Swamp, NC, field trials revealed some low carrier volume foliar applications ( $\leq 140 \text{ L ha}^{-1}$ ) may provide an effective alternative to the more commonly delivered spray volumes of 468 to  $1,870 \text{ L ha}^{-1}$  for floating plant control (Haller 2020; Nelson et al. 2007). While select experimental and registered aquatic foliar herbicides controlled 73% to 94% of *S. molesta* in the tertiary growth stage, sequential foliar applications would be necessary to maintain plant control beyond 6 WAT. Further, we recognize that some of the herbicides evaluated in this case study are not currently labeled for aquatic site applications in North Carolina (i.e., experimental PPO and metsulfuron-methyl), which may restrict their use if desired for management. Still, these data provide value for future *S. molesta* control efforts in North Carolina and management of additional invasion sites throughout the United States. The inclusion of aerial image capture at each evaluation further enhanced separation of plant response to treatment over time. Image analytics previously described provide rapid quantitative assessment of plant phytotoxicity, and future use of sUAS true-color imagery as an alternative evaluation technique to gauge *S. molesta* treatment success is encouraged. Future

research should continue identifying low-volume application strategies with other herbicides and tank-mix partners and evaluate UAAS-directed in-water application techniques with appropriate herbicides for *S. molesta* management.

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