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Does the aquatic herbicide 2,4-D and a nonionic surfactant affect survival of salvinia weevil?

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ABSTRACT

Chemical and biological control methods are often integrated to manage unwanted plants. However, the application of 2,4-D near giant salvinia (*Salvinia molesta* Mitchell) may hinder biological control efforts by the salvinia weevil (*Cyrtobagous salviniae* Calder and Sands). Limited efforts have been made to examine the susceptibility of salvinia weevil to direct and indirect applications of 2,4-D. The objectives of these studies were to 1) determine the direct and indirect impact of 2,4-D amine (0.62 and 1.85 g ai ha^{-1}) and nonionic surfactant (0.25% v/v) on adult salvinia weevil survivorship, and 2) determine how different application rates of herbicide affect salvinia weevil survivorship. In a growth chamber experiment, the application of 2,4-D directly to salvinia weevil resulted in 9% mortality, whereas surfactant alone or in combination with the herbicide resulted in mean insect mortality of 21%. In a mesocosm experiment, the application of 2,4-D alone and in combination with surfactant to giant salvinia infested with the salvinia weevil resulted in 9 to 23% mortality, and the application of surfactant alone or in combination with herbicide resulted in 9 to 19% indirect mortality. Giant salvinia treated with 2,4-D resulted in a significant reduction in biomass relative to the nontreated plants. The mesocosm experiment provided evidence that 2,4-D alone and in combination with a surfactant has limited negative impacts on salvinia weevil mortality and future biological control programs should continue when using these methods together.

Key words: biological control, chemical control, *Cyrtobagous salviniae*, integrated pest management, *Salvinia molesta*, surfactants.

INTRODUCTION

Giant salvinia (*Salvinia molesta* Mitchell) is a free-floating aquatic fern that has become problematic throughout the southeastern United States, particularly in Louisiana and Texas (Tipping et al. 2008). Native to southern Brazil, giant salvinia is considered an invasive species in several tropical and subtropical countries (McFarland et al. 2004). Giant salvinia was first reported in the United States in the late 1990s and quickly spread across the southeastern United States (Johnson 1995), and has been found in 13 states and 2 U.S. territories (Thayer et al. 2017). Giant salvinia has negatively affected Louisiana from an economic and ecological perspective because of its ability to form extensive mats in lakes, ponds, reservoirs, and bayous (Thayer et al. 2017). The proliferation of giant salvinia in water bodies displaces native plant species, alters water quality (Flores and Carlson 2006), and inhibits recreational activities (Smith and Gary 2014). Under favorable environmental conditions, giant salvinia can double its coverage in as few as 36 h and form thick mats in approximately 10 d (Johnson et al. 2010). The dominant or tertiary growth stage of giant salvinia is a densely packed mat of individual plants that can be easily spread through fragmentation (Room and Kerr 1983). Dense mats of giant salvinia not only block access to water bodies, but also block the sunlight, which results in the loss of submersed aquatic plants (McFarland et al. 2004).

Giant salvinia removal or eradication is difficult and cost prohibitive. In the United States, management of giant salvinia includes mechanical, physical, chemical, and biological control. When managing isolated or small plant infestations, chemical control is the most rapid control method (Thomas and Room 1986); however, as mat increases in coverage and thickness, chemical control becomes costly and often requires multiple applications throughout a growing season. The salvinia weevil (*Cyrtobagous salviniae* Calder and Sands; Coleoptera: Curculionidae) is a South American beetle that is a specialist feeder on giant salvinia (Tipping et al. 2008) and has been extensively used in biological control programs in the United States for the past decade (Tipping 2004, Sullivan et al. 2011, Sullivan and Postle 2012, Wahl et al. 2016). The salvinia weevil was first introduced from Brazil as a biocontrol agent into Australia during the 1980s, and it has successfully controlled giant salvinia infestations (Room et al. 1981, Room et al. 1985). Weevils collected from this Australian population were first released in Texas and Louisiana in 2001 and since have resulted in successful establishment throughout the southeastern United States (Tipping 2004, Flores and Carlson 2006, Tipping et al. 2008, Mukherjee et al. 2017).

To control unwanted aquatic broadleaf plants, natural-resource managers typically spray the herbicide 2,4-D since it is highly efficacious against terrestrial and aquatic broadleaf species, but has limited activity on grasses (Vencil 2002). 2,4-D is a systemic herbicide that is taken up by roots and leaves, and interferes with a plant's ability to maintain hormone balance (Jervais et al. 2008), leading to uncontrolled cell growth that results in rapid injury and gradual death (Vencil 2002). Controlling unwanted aquatic vegetation with 2,4-D can be a successful endeavor; however, use of

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this herbicide may affect biological control efforts. Target and nontarget aquatic plant species often occur in mixed populations; for example, water hyacinth [*Eichhornia crassipes* (Mart.) Solms] commonly occupies the same habitat as giant salvinia. 2,4-D is highly efficacious when applied to the foliage of water hyacinth (Egglar 1953) and ineffective against giant salvinia (Wersal and Madsen 2007). However, the impact of 2,4-D applications on the salvinia weevil in locations where management of water hyacinth or other broadleaf weeds occurs in proximity to giant salvinia is unknown. In the northern distribution of giant salvinia, strictly relying on biological control is not feasible because of weevil mortality during severe winters (Obeysekara et al. 2015). In these regions, integrated management utilizing chemical and biological control is necessary. Unfortunately, the impacts of herbicides and surfactants on biological control agents is limited.

Previous research has been conducted to examine the impact of various herbicides and nonionic surfactants on giant salvinia (Nelson et al. 2001, Fairchild et al. 2002; Mudge et al. 2016). However, there is a limited amount of data on the impacts of herbicides and nonionic surfactants (NISs) on salvinia weevil mortality (Mudge et al. 2013). Therefore, the objectives of our studies were to determine the direct and indirect impact of the commonly used aquatic herbicide 2,4-D amine and a NIS on salvinia weevil survivorship and giant salvinia quality.

MATERIALS AND METHODS

Direct impact of 2,4-D and surfactant on weevil survival

Two laboratory bioassays were conducted in June and July 2017 at the Department of Entomology, Louisiana State University (LSU) in Baton Rouge, LA to determine the impacts of 2,4-D on adult weevil survival. Giant salvinia was reared at LSU in 568-L outdoor tanks that contained reverse-osmosis (RO) water and rainwater (pH 6.5) and was fertilized with Miracle-Gro®[1] (24–8–16, N–P–K) to maintain water concentration of 10 mg L^{-1} N. A systemic insecticide (2.94% imidacloprid[2]) was applied to foliage of plants initially to control aphids and caterpillars. To ensure the insecticide had dissipated, a 3-mo waiting period was implemented before the plants were used for experiments. Adult weevils were collected and transferred to the plants on campus. Weevils were collected from an outdoor weevil-rearing pond in St. Martinville, LA, live extracted via Berlese funnels (Knutson and Mukherjee 2012), and into bags that contained 2 g of fresh giant salvinia and fertilized tank water. Live extracting weevils involves placing giant salvinia infested with salvinia weevils in a Berlese funnel and drying the plants with a lightbulb suspended above the funnel (Wahl et al. 2016). As the giant salvinia dries, weevils exit the plants and are collected in bags at the bottom of the funnel. Upon extraction, weevils were placed into 11-L dishpans (25 by 31 by 14 cm) containing fresh giant salvinia and RO water. The dishpans were covered with a fine mesh screen, placed in an environmental growth chamber,[3] and allowed to acclimate for 7 d at 24 C (± 1 C), 80% relative humidity (± 10%) and a 14-h : 10-h (light : dark) photoperiod.

TABLE 1. AQUATIC HERBICIDES AND SURFACTANTS APPLIED DIRECTLY TO ADULT SALVINIA WEEVILS IN LABORATORY AND MESOCOSM TRIALS.

Treatment	Rate
Direct application experiment	
Nontreated	—
Reverse osmosis water	—
Low 2,4-D	0.62g ai ha^{-1}
High 2,4-D	1.85g ai ha^{-1}
Low 2,4-D + surfactant	0.62g ai ha^{-1} + 0.25% v/v
High 2,4-D + surfactant	1.85g ai ha^{-1} + 0.25% v/v
Surfactant	0.25% v/v
Indirect application experiment	
Nontreated	—
Low 2,4-D	0.62g ai ha^{-1}
High 2,4-D	1.85g ai ha^{-1}
Low 2,4-D + surfactant	0.62g ai ha^{-1} + 0.25% v/v
High 2,4-D + surfactant	1.85g ai ha^{-1} + 0.25% v/v
Surfactant	0.25% v/v

Treatments included 2,4-D amine[4] (0.62 and 1.85 g ai ha^{-1}), a NIS[5] at 0.25% v/v, and combinations of 2,4-D and the NIS (Table 1). Solutions of 2,4-D and the surfactant were prepared by diluting products into separate containers of RO water at the equivalent to a diluent of 935 L ha^{-1}. The herbicide and surfactant used in this research are commonly applied throughout Louisiana to manage water hyacinth and other invasive aquatic plants (Shanks 2014a, Shanks 2014b, Salyers 2016). A NIS was selected since most 2,4-D labels specify this classification/chemistry of surfactants (Thompson et al. 1996). Nontreated and RO water controls were added to account for insect mortality in the absence of herbicide or surfactant exposure.

After acclimation, five adult weevils per replicate ($n = 10$; 50 weevils per treatment) were removed from giant salvinia using soft forceps and placed into a Petri dish for exposure. Each treatment was replicated 10 times. This study was designed to replicate an operational application of 2,4-D near giant salvinia in which weevils come in direct contact with the herbicide/surfactant spray. From the stock solutions, each treatment was applied directly to weevils in Petri dishes using a micropipette set to deliver 2 µl of solution per weevil (Mudge et al. 2013). After exposure, weevils remained in the Petri dish for 10 min before being transferred to mesh-covered 250-ml beakers containing 2.5 g (± 0.2 g) of fresh-weight giant salvinia and 150 ml of nutrient-amended water.

At 7 d after treatment (DAT), salvinia weevils were removed from giant salvinia, placed on Petri dishes, and individually examined for mortality. Since weevils become immobile and rigid when moved, an acclimation period of 10 min was utilized before determining mortality (Mudge et al. 2013). All data were analyzed using analysis of variance (ANOVA) and means separated using Student–Newman–Keuls (SNK) method ($P \leq 0.05$).

Indirect impact of 2,4-D and surfactant on salvinia weevil and giant salvinia

To determine the indirect impacts of 2,4-D sprayed directly on giant salvinia and the weevil survival, a field experiment was conducted in June 2017 in an earthen pond at the LSU AgCenter Reproductive Biology Center in St.

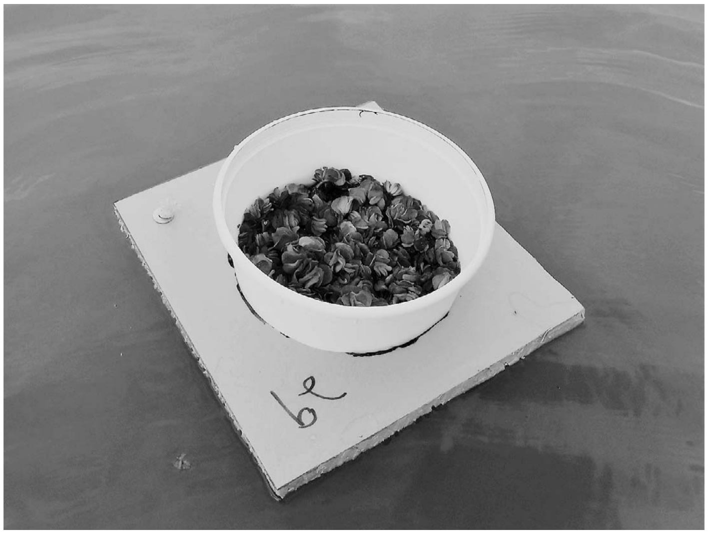

Figure 1. Giant salvinia inside floating mesocosm constructed from polyethylene container and foam block. Mesocosm confined salvinia and salvinia weevils for the indirect application experiment.

Gabriel, LA. Weevil-free giant salvinia was collected from a rearing pond located at the LSU AgCenter Iberia Research Station in Jeanerette, LA. Similarly to the laboratory studies, giant salvinia was transferred to the 568-L outdoor tanks located at LSU and cultured under similar conditions. Giant salvinia was treated with a systemic insecticide (2.94% imidacloprid) to control *Samea multiplicalis* (Guenée) (Lepidoptera). As previously mentioned, a 3-mo waiting period was implemented to allow the insecticide to dissipate before introducing weevils. Adult weevils were collected from the same outdoor weevil-rearing pond in St. Martinville, LA, and live extracted from giant salvinia in June 2017. Methods for live weevil extraction, growth-chamber rearing, and culture techniques were the same as the direct-impact trials. After the 7-d growth chamber acclimation period, mesocosms and weevils were then deployed to the pond (pH 6.8).

Treatments included 2,4-D amine (0.62 and 1.85 g ai ha^{-1}), a NIS (0.25% v/v), and combinations of 2,4-D and the surfactant. Weevil only, herbicide–surfactant free, and weevil-free, herbicide–-surfactant-free controls were also included to account for insect mortality in the absence of herbicide and surfactant exposure. Floating mesocosms were constructed with a 5.62-L polyethylene container (22 by 17 cm) to contain giant salvinia and weevils. The bottom of the polyethylene container was removed, and the container was placed inside a 30-cm^2 square sheet of 1.3-cm-thick foam (Obeysekara et al. 2015). Additionally, the top and bottom of the mesocosms were covered with fine mesh to prevent weevil escape. Mesocosms were held in place by attaching the foam to bricks with a nylon rope (Figure 1).

In each mesocosm, approximately 0.1 kg of giant salvinia was utilized to cover the water surface. Immediately after giant salvinia establishment, 15 adult weevils per replicate (n = 5; 75 weevils per treatment) were added to each container. Weevils and plants were allowed 5 d to acclimate to mesocosm conditions. Pond water was fertilized with Magic Carpet Professional Fertilizer[6] (33–0–0, N–P–K) to a concentration of

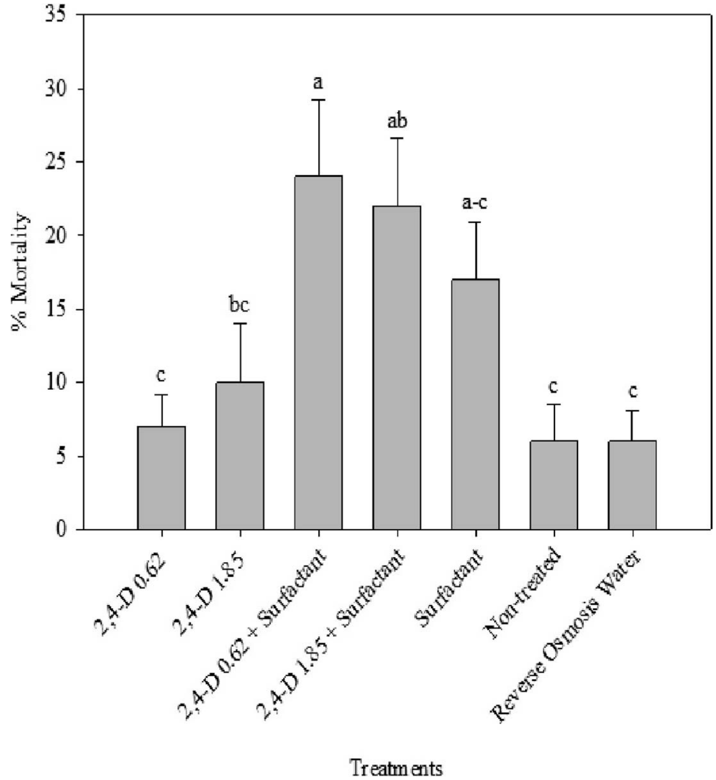

Figure 2. Percent mortality of adult salvinia weevils 7 d after exposure to the aquatic herbicide 2,4-D (amine) and a nonionic surfactant (0.25% v/v) in a laboratory setting. Numbers following 2,4-D on x-axis represent herbicide rates in g ai ha^{-1}. Treatments with the same letter are not significant according to Student–Newman–Keuls (SNK) method at $P \leq 0.05$; $n = 10$ and each replicate contained five weevils rated as live or dead.

10 mg L^{-1} N by dissolving fertilizer in water and then distributing the nutrient water throughout the pond. After the acclimation period, treatments were applied to the foliage of salvinia using a forced-air CO_2-powered sprayer at an equivalent of 935 L ha^{-1} of diluent delivered through a single TeeJet[7] 80-0067 nozzle at 20 psi. In addition, pretreatment plant biomass was harvested on the same day as herbicide application. At 7 DAT, all viable giant salvinia biomass was harvested from each mesocosm. Before weevil extraction via Berlese funnels, plant fresh wet weight was collected by suspending giant salvinia from each mesocosm in a fine mesh net for 5 min to remove excess water weight. Giant salvinia was then placed in Berlese funnels for 24 h and extracted weevils were preserved in 80% ethanol and counted. Once extraction was completed, giant salvinia was placed in a drying over for 24 h at 65 C and dry-weight biomass was recorded. The effect of treatments on the number of weevils extracted and plant weight was analyzed in Sigma Plot using ANOVA and means separated by the SNK method ($P \leq 0.05$).

RESULTS AND DISCUSSION

Direct impact of 2,4-D and surfactant on salvinia weevil

There were no significant differences in adult salvinia weevil mortality between the first and second trials ($F = 0.68$;

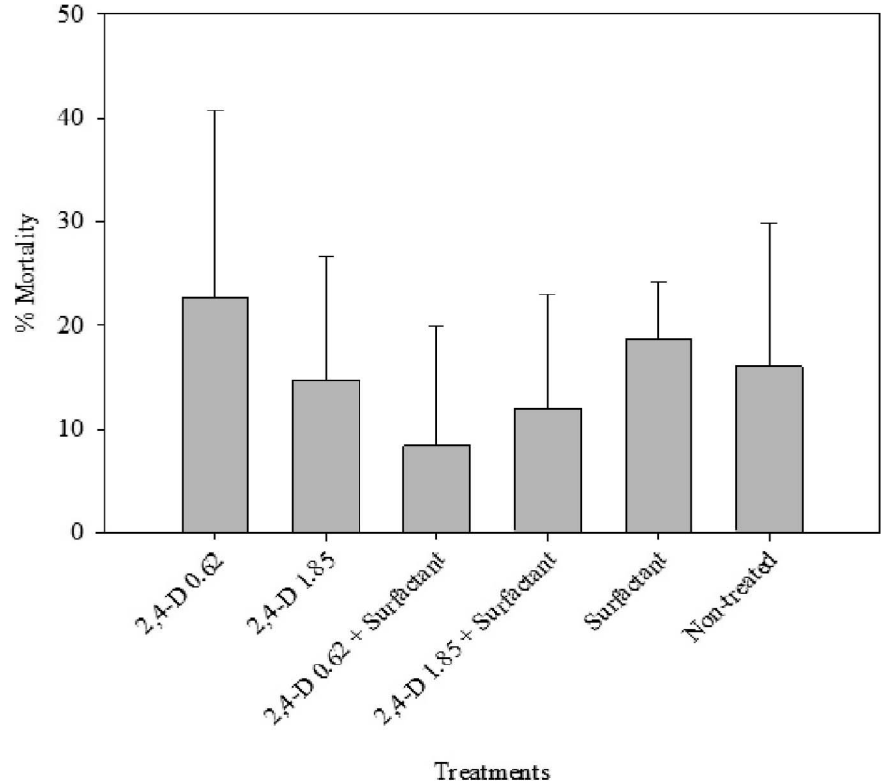

Figure 3. Percent mortality of adult salvinia weevils 7 d after exposure to the aquatic herbicide 2,4-D (amine) and a nonionic surfactant (0.25% v/v) in a mesocosm setting. Numbers following 2,4-D on *x*-axis represent herbicide rates in g ai ha^{-1}. Treatments with the same letter are not significant according to Student–Newman–Keuls (SNK) method at $P \leq 0.05$; $n = 5$ and each replicate contained 15 weevils rated as live or dead.

df = 1,126; $P = 0.41$); therefore, data from the two laboratory bioassays were pooled. The nontreated and RO water treatments resulted in 6% mortality at 7 DAT (Figure 2). Similarly, the direct application of the low rate of 2,4-D (0.62g ai ha^{-1}) to the insects resulted in 7% weevil mortality, which was not different from the nontreated and water treatments. The surfactant alone and in combination with the herbicide resulted in 17 to 24% mortality (Figure 2). The addition of the NIS was more toxic to salvinia weevils than the two herbicide rates applied alone ($F = 4.47$; df = 6,133; $P < 0.001$). Previous research investigated the direct impact of the aquatic herbicides flumioxazin and penoxsulam on survival of adult weevils and found $\leq$ 5% mortality when applied directly to the insects (Mudge et al. 2013). Furthermore, directly applying a surfactant (nonionic and buffering agent blend) at 0.25% v/v alone and in combination with the aforementioned herbicides to salvinia weevils resulted in 20 to 47% mortality (Mudge et al. 2013). Additionally, when examining four surfactants on weevil mortality, Mudge et al. (2013) found that mortality ranged from 22 to 41%, and all surfactants increased mortality relative to the control. 2,4-D is not believed to be toxic to insects, including honeybees (*Apis mellifera* L.) (Jervais et al 2008). However, some surfactants can be toxic to ecosystems and organisms, and can increase the diffusion of other environmental contaminants (Krogh et al. 2003, Lechuga et al. 2016). A study examining honeybee survival found that 4 of 11 commercially available surfactants were toxic and 2 of the surfactants resulted in 100% mortality (Goodwin and McBrydie 2000). Risch (1980) found that spraying squash beetle (*Acalymma thiemei* Baly; Coleoptera) with a surfactant alone or in combination with a fungicide increased mortality by 7 to 13% relative to applying water alone. Previous research also indicated that inert ingredients, such as nonylphenol polyethoxylates and the solvent *N*-methyl-2-pyrrolidone (NMP), may be more toxic than the active ingredient in surfactants (Mullin et al. 2015). Lan et al. (2004) examined acute toxicity of NMP to *Daphnia magna* and found that NMP was highly toxic, with a 50% lethal concentration of 1.2 mg L^{-1}. In addition, organosilicone surfactants are a blend of silicone with NISs (Miller and Westra 1998) and have been found to be toxic to numerous insects and mites (Imai et al. 1995, Purcell and Schroeder 1996, Cowles et al. 2000, Tipping et al. 2003). Although it is not completely understood how surfactants affect insects, research exploring the various modes of action has been conducted. One theory is that the oily texture of the surfactant may block respiratory openings and lead to asphyxiation (Davidson et al. 1991). Another common hypothesis is that surfactants disrupt cell membranes and remove waxy layers around cuticles, leading to water loss and desiccation of insects (Ebeling 1945, Ebeling 1975, Hadley 1994). This same phenomenon can cause harm to plant tissues and cause burning of leaves (Miller and Westra 1998). Surfactants also lower water surface tension and assist the penetration of water molecules into plant tissue (Miller and Westra 1998), which may lead to increased penetration of water and chemicals into the weevils.

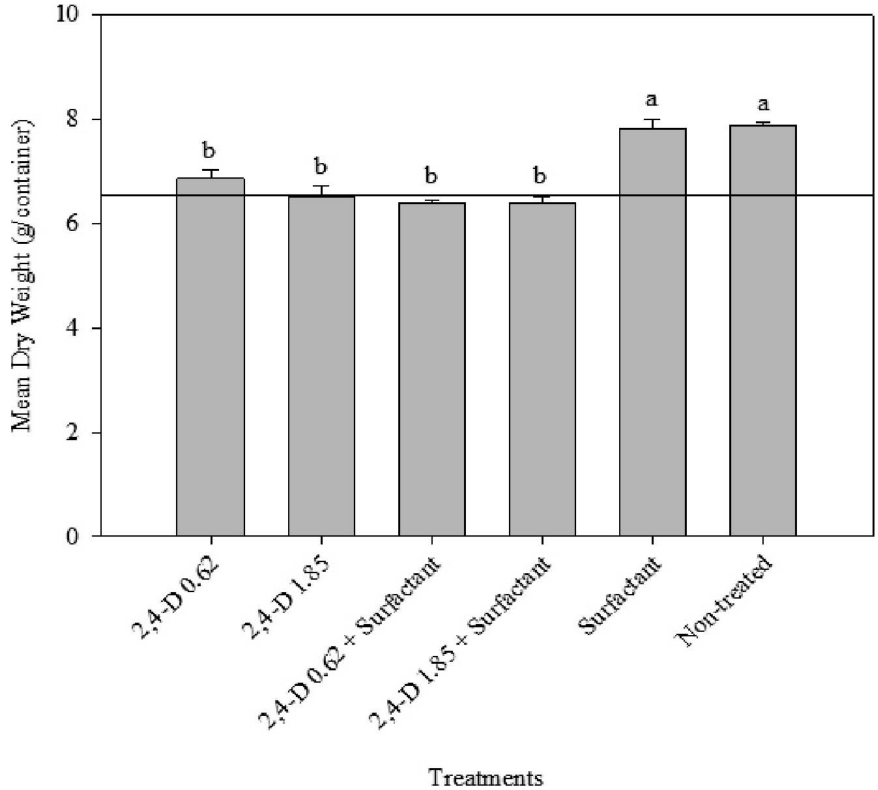

Figure 4. Impact of 2,4-D and a nonionic surfactant on giant salvinia dry weight (± SE) 7 d after exposure in a mesocosm trial. Numbers behind 2,4-D represent herbicide rates in g ai ha^{-1}. Horizontal line represents pretreatment weight. Means with the same letter are not significantly different according to Student–Newman–Keuls (SNK) method at $P \leq 0.05$; $n = 5$.

Additional research examining the mode in which surfactants cause harm to salvinia weevils is needed.

On the basis of these results, the addition of a surfactant has the potential for increased adult salvinia weevil mortality under worst-case scenarios. Under these scenarios, herbicide and surfactant would be applied to other problematic vegetation that grow among thin mats of giant salvinia that contain salvinia weevils. The chances of a weevil encountering the spray would be increased relative to the thickness of the mat. As a result, mortality would likely increase as the number of plant layers decreases. However, under real-world scenarios, weevils would likely be less affected since 2,4-D and surfactant are applied to the foliage of giant salvinia and only the top layer of the mat receives spray. Although weevils can be found walking, feeding, or mating on top of the mat, they are more common within the fronds and near buds (Sands et al. 1983). Herbicide and surfactant sprayed on giant salvinia may persist for a few hours before being absorbed by plants or evaporating (Munro et al. 1992), which results in a limited exposure period for weevils to come in contact with the spray solution.

Indirect impact of 2,4-D and surfactant on salvinia weevil

In the mesocosm experiment, the application of 2,4-D and surfactant to weevil-infested giant salvinia did not result in increased weevil mortality 7 DAT ($F = 0.62$; df = 5,23; $P = 0.69$; Figure 3). Weevil mortality was observed in the nontreated reference containers and was statistically similar to containers that received herbicide and surfactant. All treatments, regardless of product applied, resulted in 9 to 23% weevil mortality at the conclusion of the experiment.

Under real-world scenarios, the application of herbicide and surfactant had minimal impact on salvinia weevil mortality. Percent mortality between herbicide and surfactant treatments did not differ; 15% and was not different from the control, 16%. Adult salvinia weevils can be found in the floating leaves or subaquatic portion of giant salvinia; thus the portion of adult weevil population on the frond surface versus concealed during the day is unknown. However, since weevil mortality was lower in the indirect mesocosm trial than in the direct application bioassay, we believe insect behavior may limit exposure and reduce an opportunity for increased mortality.

In the mesocosm experiment, the application of 2,4-D to the foliage of giant salvinia directly affected plant biomass ($F = 23.12$; df = 5,24; $P \leq 0.001$; Figure 4). Pretreatment biomass was 6.6 g dry weight, thus indicating an increase in plant growth during the 7-d acclimation period. At 7 DAT, the nontreated control and surfactant-only treatments resulted in 1.2× greater biomass (mean 7.9 g) compared with herbicide + surfactant combination treatment (mean 6.6 g). There were no differences in plant weight among the two rates of 2,4-D alone and in combination with the surfactant.

Application of 2,4-D to control other unwanted plants will decrease giant salvinia biomass and could possibly reduce ongoing biological control efforts. Although the herbicide will not directly affect weevil survivorship, it may indirectly influence weevil behavior by reducing host plant quality. Lower plant quality may influence adult weevils to fly (Micinski et al. 2016) to seek a healthier food source. This dispersal behavior may extend the time required to successfully manage giant salvinia using biological control. Additionally, adult weevils typically seek out and lay eggs near the nitrogen-rich developing buds of giant salvinia (Sands et al. 1983). If plant quality is compromised, weevils may search for higher-quality plants or cease reproduction until plant quality increases, thus slowing biological control. In addition, further research is needed to determine whether diminished plant quality affects salvinia weevil behavior, feeding preferences, and survival of larvae.

The application of 2,4-D and surfactant, in conjunction with biological control, should have minimal negative impacts on salvinia weevil mortality for future biological control efforts in the United States, particularly Louisiana. Because of weevil behavior, mortality due to direct contact with 2,4-D and surfactant should be minimal. Indirect effect by reduction of plant quality may alter weevil movement and reproduction, but this short-term effect should not affect biological control of giant salvinia. On the basis of these findings, natural resource managers may continue using 2,4-D and a NIS to control other unwanted aquatic plants alongside giant salvinia, and not expect a decline in biological control of giant salvinia.

SOURCES OF MATERIALS

[1]Miracle-Gro All Purpose Plant Food, The Scotts Miracle-Gro Company, 14111 Scottslawn Rd., Marysville, OH 43041.

[2]Bayer Advanced® Tree & Shrub Insect Control, SBM Life Science Corp., 101 Winstead Dr., Suite 500, Cary, NC 27513.

[3]Model I-36VL, Percival Scientific Inc., 505 Research Dr., Perry, IA 50220.

[4]Weedar® 64, Nufarms Americas, 11901 South Austin Ave., Alsip, IL 60803.

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

[6]Magic Carpet™ Professional Fertilizer, Agri-AFC, LLC, PO Box 2207, Decatur, AL 35609.

[7]TeeJett, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60187.

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