

Interactions of herbicides, surfactants, and the giant salvinia weevil (*Cyrtobagous salviniae*) for control of giant salvinia (*Salvinia molesta*)

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ABSTRACT

Herbicides and the biological control agent *Cyrtobagous salviniae* Calder and Sands (giant salvinia weevil, Coleoptera: Curculionidae) are the most effective means to manage the floating aquatic invasive giant salvinia (*Salvinia molesta* Mitchell) in North America. Limited efforts have been made to integrate these technologies and no information is available on the direct and indirect impacts of herbicides on giant salvinia weevils. Flumioxazin 2-[7-Fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-indole-1,3(2H)-dione and (penoxsulam) 2-(2,2-difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl)benzenesulfonamide, applied directly to the weevils resulted in less than 5% weevil mortality, whereas the addition of an aquatic surfactant (nonionic and buffering agent [surfactant A]) alone and in combination with the herbicides resulted in 20 to 47% giant salvinia weevil mortality. Alternate surfactants, including a methylated vegetable oil and organo-silicone blend (surfactant B), a silicone-polyether copolymer (surfactant C), and a nonionic organo-silicone blend (surfactant D) resulted in 22 to 23% mortality. In a mesocosm experiment, all weevil and herbicide treatments (alone or combination) resulted in 52 to 97% reductions in giant salvinia biomass by 4 wk after treatment (WAT). By the conclusion of the experiment (6 WAT), flumioxazin, flumioxazin plus giant salvinia weevil, and penoxsulam plus giant salvinia weevil resulted in 98 to 100% plant control. Treatment with the giant salvinia weevil alone caused a significant reduction in biomass (68%) and continued to damage plant tissue at 6 WAT. The mesocosm experiment also provided evidence of the minimal indirect impacts herbicides and surfactants will have on the giant salvinia weevil. The experiment also demonstrated that giant salvinia weevils were capable of surviving at least 4 wk on plant material treated with foliar applications of flumioxazin and penoxsulam.

Key words: biological control, chemical control, flumioxazin, foliar-applied herbicides, integrated pest management, penoxsulam.

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INTRODUCTION

Giant salvinia is a free-floating, mat-forming aquatic fern native to southeastern Brazil (Forno and Harley 1979) that has become problematic in water bodies throughout the southeastern United States, Puerto Rico, and Hawaii. This species dominates water bodies where dense infestations disrupt transportation, hinder water uses, impact desirable native plant communities, and increase mosquito breeding habitat (Jacono 1999, Jacono and Pitman 2001, Nelson et al. 2001). It is estimated that under optimal growth conditions, plants can double in coverage every 36 to 53 h (Cary and Weerts 1983, Johnson et al. 2010). This plant has become especially problematic in Texas and Louisiana and, by 2004, had been reported in 4 reservoirs, 5 rivers (or streams) and 20 ponds in Texas alone (Owens et al. 2004). Although an estimate of current total acreage in Texas is not available, 17 major water bodies are confirmed to be infested by giant salvinia (H. Elder, pers. comm.). In 1999, an initial infestation in Louisiana estimated to be 400 A expanded to > 28,340 ha in 20 lakes, 7 bayous or rivers, the Atchafalaya River basin, the Red River, and the coastal fresh water marsh from Lafitte to Morgan City (Johnson et al. 2010).

Management of giant salvinia has been attempted via chemical, biological, mechanical, and physical control methods (Madsen and Wersal 2009), with chemical and biological methods being more widely used in the United States. When applied to smaller or less dense populations of giant salvinia, herbicide treatments can selectively and precisely provide rapid control. Although herbicide control programs have increased over recent years, giant salvinia infestations continue to expand (Sanders et al. 2010). In an attempt at management, rearing, harvesting, and release of the giant salvinia weevil (*Cyrtobagous salviniae* Calder and Sands), a biological control agent, has increased in recent years (Harms et al. 2009, Sanders et al. 2010). The giant salvinia weevil, originally occurring in southeastern Brazil, Bolivia, Paraguay, and northern Argentina (Wibmer and O'Brien 1986, Calder and Sands 1985), was first released in the United States in 2001 at sites in Louisiana and Texas, with a subsequent reduction in plant populations observed at release sites (Tipping 2004, Tipping et al. 2008). Successful control of giant salvinia below problematic levels has also been achieved in Zimbabwe, South Africa, Senegal, Mauritania, and India, often within 2 yr after initial stocking (Jayanth 1987, Cilliers 1991, Chikwenhere and Keswani 1997, Pieterse et al. 2003, Diop and Hill 2009). Despite the

reported success of this biocontrol agent in other parts of the world, limited distribution of the giant salvinia weevil and minimal large-scale releases in the United States have likely hindered potential effectiveness (Mudge and Harms 2012). In addition, severe winters can limit the increase and spread of giant salvinia weevil populations in the spring, and maintenance of populations may be necessary (Tipping et al. 2008).

In contrast to a herbicide- or biocontrol-focused management program, a more prudent approach may be to combine technologies to achieve rapid biomass reduction and long-term control of giant salvinia. Minimal research has been conducted to determine the potential of combining herbicides and biological agents (Center et al. 1999) or mycoherbicides (Nelson et al. 1998) for the management of aquatic plants. Possible candidates for evaluations should include the recently registered, reduced-risk aquatic herbicides. These newer chemistries are highly plant-specific (minimal toxic impacts on animals), applied at very low use rates (g ai ha^{-1}) and concentrations ($\mu\text{g ai L}^{-1}$), and possess a high degree of selectivity against target plants, thereby minimizing damage to desirable vegetation (Koschnick et al. 2007, Mudge 2007, Glomski and Mudge 2009).

In exploring the compatibility of these 2 technologies, the direct and indirect impacts of herbicides on the giant salvinia weevil should be considered, as impacts to giant salvinia weevil fitness may alter their long-term effectiveness. In addition to herbicides, surfactants typically used in combination with foliar-applied herbicides should be examined for their impact on the giant salvinia weevil. Surfactants, which are a type of adjuvant, improve the emulsifying, dispersing, spreading, and wetting, as well as increasing the spray coverage on the foliage to aid in herbicide uptake by the plant (Ferrell et al. 2008). Surfactants enhance the herbicide application or efficacy and do not necessarily provide control as stand-alone treatments. The inclusion of certain ingredients in adjuvant formulations is regulated by the U.S. Environmental Protection Agency, but the manufacture and use of these products is not stringently tested and regulated (Tu et al. 2001). Only a few U.S. states regulate adjuvants and require the disclosure of ingredients, results from efficacy trials, and data from environmental and toxicological studies (Tu et al. 2001, Washington State Department of Agriculture 2012, Witt 2012).

The impact of various surfactants and herbicides has been evaluated on several invertebrates, including beetles, spider mites, midges, and the water hyacinth weevil (Wolfenbarger and Holscher 1967, Haag 1986, Pellissier 1988, Buhl and Faerber 1989, Cowless et al. 2000). Unfortunately, no data exist on the impact of herbicides and surfactants on the giant salvinia weevil. Therefore, the objectives of this research were to (1) determine the direct impact of recently registered aquatic herbicides and surfactants on the giant salvinia weevil, (2) determine the indirect impact of herbicides on giant salvinia weevil survival in a controlled setting, and (3) determine if the combined technologies are more efficacious against giant salvinia than each method used alone.

MATERIALS AND METHODS

Direct impacts of herbicides and surfactants on giant salvinia weevils

Experiment 1. This experiment was conducted at the U.S. Army Engineer Research and Development Center (ERDC) in Vicksburg, MS. Adult giant salvinia weevils were collected from cultures in aboveground rearing boxes ($1.5 \times 3.0 \times 0.6$ m deep [$4.9 \times 9.8 \times 2.0$ ft]) at the U.S. Army Engineer Research and Development Center, Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX. Weevils were reared on giant salvinia supplemented with nutrients approximately every month to maintain 10ppm mg L^{-1} nitrogen (Miracle-Gro^{®1}) and 3 mg L^{-1} iron.² Weevils were harvested for experimentation on 3 separate occasions from May to June 2011. Collection occurred through Berlese funnel extraction (Harms et al. 2009) into glass jars containing moist paper towels, which were shipped overnight to ERDC. Weevils were immediately transferred onto fresh giant salvinia contained in 1.056 gallon plastic containers with nutrient (Miracle-Gro)-amended water. Weevils were allowed to acclimate in a walk-in growth chamber for 6 d at a temperature of 80.6° and a 14-h : 10-h (light : dark) photoperiod.

Prior to herbicide exposure, 15 adult weevils per replication ($n = 3$; 45 weevils per treatment) were removed from the giant salvinia plants with forceps and placed into petri dishes for exposure. Treatments were replicated 3 times and randomly assigned. Solutions of flumioxazin, (quick-acting contact herbicide) and penoxsulam, (a slow-acting systemic herbicide) were prepared by diluting formulation concentrates in distilled water equivalent to a diluent of 200 gallons. The aquatic herbicides flumioxazin³ (12 oz A^{-1}) and penoxsulam⁴ (5.6 oz A^{-1}) were used for the experiment (Table 1). In addition, a surfactant (nonionic and buffering agent blend) at 0.25% v/v was evaluated alone and in combination with the herbicides to determine direct impacts on the giant salvinia weevil. This study was designed to simulate conditions typical of a commercial herbicide application to control giant salvinia in which weevils would come in direct contact with the herbicide/surfactant spray. From the stock solutions, each treatment was applied directly to the weevils in the petri dishes using a micropipette set to deliver $2\ \mu\text{L}$ of solution per weevil. Nontreated and water controls were also included to assess insect mortality in the absence of herbicide/surfactant exposure. After treatment, weevils were kept in the petri dishes for 10 min before being transferred to mesh-topped 5 oz beakers containing 0.140 oz fresh giant salvinia in nutrient-amended water. Beakers were then returned to the growth chamber. Herbicide/surfactant rates and application technique chosen for this study were designed to provide a worst-case exposure scenario. Since the giant salvinia weevil is nocturnal (Schotz and Sands 1988), the likelihood of direct exposure to the herbicide mixture during a daytime field application is low.

At 7 d after treatment (DAT), all giant salvinia weevils were transferred to petri dishes and individually examined for mortality. Since weevils became immobile and rigid

TABLE 1. AQUATIC HERBICIDES AND SURFACTANTS APPLIED DIRECTLY TO ADULT GIANT SALVINIA WEEVILS.

Treatment	Rate ^{1,2}	Type/class
Experiment 1		
Control	—	—
Deionized water	—	—
Flumioxazin	429 g ai ha ⁻¹	PPO ³ inhibitor
Flumioxazin + surfactant A ⁴	429 g ai ha ⁻¹ + 0.25% v/v	PPO inhibitor + nonionic and buffering agent blend
Penoxsulam	98 g ai ha ⁻¹	ALS inhibitor
Penoxsulam + surfactant A	98 g ai ha ⁻¹ + 0.25% v/v	ALS inhibitor + nonionic and buffering agent blend
Surfactant A	0.25% v/v	Nonionic and buffering agent blend
Experiment 2		
Deionized water	—	—
Surfactant A	0.25% v/v	Nonionic and buffering agent blend
Surfactant B	0.25% v/v	Methylated vegetable oil and organo-silicone blend
Surfactant C	0.25% v/v	Silicone-polyether copolymer
Surfactant D	0.25% v/v	Nonionic organo-silicone blend

¹Two microliters of treatment solution applied to each weevil.

²The herbicides and surfactants were mixed in deionized water at an equivalent of 1,870 L ha⁻¹ diluent to provide a solution similar to a commercial herbicide application to control giant salvinia.

³Abbreviations: PPO, protoporphyrinogen oxidase; ALS, acetolactate synthase.

⁴Aquatic surfactants: A, nonionic and buffering agent blend; B, methylated vegetable oil and organo-silicone blend, C, silicone-polyether copolymer; and D, a nonionic organo-silicone blend.

when disturbed, an acclimation period of 10 min was allowed before observation. All data were analyzed using analysis of variance and means separated using the Student-Newman-Keuls (SNK) method ($P \leq 0.05$).

Experiment 2. An additional experiment was conducted to evaluate the direct effects of 4 surfactants on adult giant salvinia weevils. Solutions of 4 commonly used aquatic surfactants (Table 1) were prepared and applied to the weevils in August 2011, following methods developed in Experiment 1. All surfactants were prepared to provide a mixture of 0.25% v/v. A water control was also included to assess mortality in the absence of surfactant exposure. After treatment, weevils remained in the petri dishes for 10 min, were transferred to fresh giant salvinia (4 g) in 150-ml mesh-topped beakers, and then placed in the growth chamber. The treatments were replicated 4 times and randomly assigned. At 7 DAT, mortality was assessed and statistically analyzed in the same manner as the herbicide experiment.

Indirect impact of herbicides with surfactant on giant salvinia and the giant salvinia weevil

A mesocosm trial was conducted at ERDC from June to July 2011 to assess the ability of the giant salvinia weevil to survive on herbicide-damaged plant material, and to evaluate the compatibility of 2 herbicides and the weevil for control of giant salvinia. The study was designed to treat weevil-infested plants instead of introducing the insect onto fresh material prior to herbicide application. A high-density weevil culture box from LAERF was chosen as the weevil source for this experiment. Initially, samples (240 g fresh weight [F.W.]) of weevil-infested giant salvinia were collected and divided into 2 equal subsamples (each 120 g F.W.). One subsample was processed using Berlese funnel extraction to obtain adult weevil population estimates prior to commencement of the experiment, and the other subsample was shipped overnight to ERDC for experimentation. Giant salvinia weevil counts ranged from 41 to 53 weevils (10 to 14 weevils per experimental unit) per treatment. In

addition, noninfested giant salvinia was collected from a nearby weevil-free culture at the LAERF. This material was utilized for all weevil-free treatments and was cultured under identical conditions (light, nutrient amendments, etc.). Prior to plant/weevil shipment to ERDC, whole plant nitrogen was measured at 2.5% (data not shown), which is in the optimal range for weevil development and reproduction (Forno and Bourne 1985).

The weevil-infested and weevil-free plants were placed in 18.9-L buckets containing 12-L of water amended with Miracle-Gro (36-6-6, 41.7 mg L⁻¹) and allowed to acclimate 5 d. Foliar herbicide treatments included flumioxazin (214 g ai ha⁻¹) and penoxsulam (49 g ai ha⁻¹) applied alone, and in combination with a surfactant (nonionic and buffering agent blend) at 0.25% v/v. Herbicide treatments were applied to the foliage of weevil-infested and weevil-free giant salvinia using a forced air CO₂-powered sprayer at an equivalent of 935 L ha⁻¹ diluent delivered through a single TeeJet® 80-0067 nozzle⁵ at 20 psi. The treatments were replicated 10 times and randomly assigned.

At 2, 4, and 6 WAT, plant biomass was destructively harvested from 3, 3, and 4 of the replicates, respectively. Counts of surviving weevils were obtained by placing all harvested plant material into Berlese funnels regardless of treatment (i.e., whether or not weevils were expected to be present) and collecting weevils into 70% EtOH for subsequent enumeration. Plant fresh weights were obtained prior to Berlese extraction. After extraction, plants were transferred to a drying oven (55 C) until constant weight was achieved, and then weighed to obtain dry weights. Weevil population estimates were made on a per-kilogram basis. All giant salvinia dry weight and weevil count data were analyzed using analysis of variance and means were separated using the SNK method at $P \leq 0.05$ when differences were detected. Also, dry weight data were analyzed using nonlinear regression (exponential decay, $y = b_0 e^{-bx}$). Regression models were used to determine the estimated time required to reduce plant dry weight biomass by 50% (ET₅₀). The ET₅₀ values were calculated using the slope of the line.

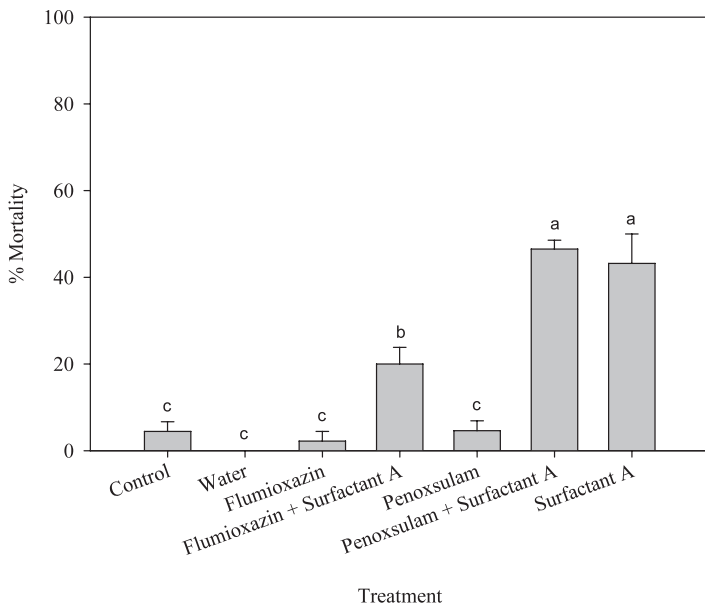


Figure 1. Percentage of mortality of adult giant salvinia weevils exposed to the aquatic herbicides flumioxazin (429 g ai ha⁻¹) and penoxsulam (98 g ai ha⁻¹) and surfactant A (nonionic and buffering agent blend; 0.25% v/v) 7 d after treatment. The herbicides and surfactant were mixed in distilled water at an equivalent of 1,870 L ha⁻¹. Treatments with the same letter are not significant according to Student-Newman-Keuls method at $P \leq 0.05$; $n = 3$ and each replication contained 15 weevils rated as live or dead.

RESULTS AND DISCUSSION

Direct impacts of herbicides and surfactants on the giant salvinia weevil

Experiment 1. In the direct impact herbicide experiment, control and water treatments resulted in 4 and 0% giant salvinia weevil mortality, respectively, 7 DAT (Figure 1). The aquatic herbicides flumioxazin and penoxsulam resulted in less than 5% mortality, which was not different from the control or water treatments. Conversely, the aquatic surfactant alone and in combination with the herbicides resulted in 20 to 47% weevil mortality. The surfactant alone and penoxsulam plus surfactant treatments resulted in significantly greater mortality than all other treatments.

Experiment 2. All surfactants, regardless of chemistry or classification, resulted in mortality greater than the control treatment in the surfactant experiment (Figure 2). Surfactants B, C, and D resulted in 22 to 23% giant salvinia weevil mortality, while surfactant A resulted in 41% mortality. These data (Figure 2) were similar to the herbicide experiment (Figure 1) with regard to the level of mortality compared to the control and water treatments. In general, these particular classes of surfactants were more injurious to the giant salvinia weevil than the 2 herbicides evaluated in this research. In addition, surfactant A resulted in a similar percentage of mortality in both experiments. Previous research demonstrated the aquatic herbicides 2,4-D, diquat, and glyphosate resulted in minimal water hyacinth weevil mortality when applied up to 6 times maximum recommended rate (Haag 1986, Pellessier 1988, Grodowitz and Pellessier 1989). Only 1 of the 78 herbicide/

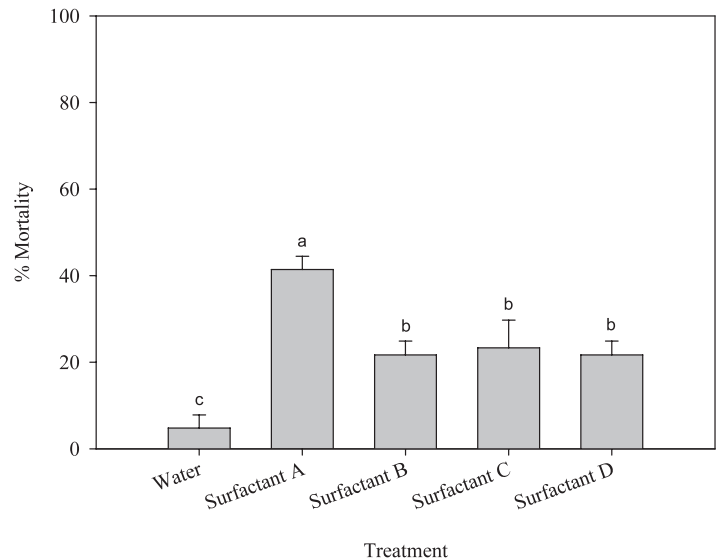


Figure 2. Percentage of mortality of adult giant salvinia weevils exposed to 4 aquatic surfactants: (A) nonionic and buffering agent blend, (B) methylated vegetable oil and organo-silicone blend, (C) silicone-polyether copolymer, and (D) a nonionic organo-silicone blend 7 d after treatment. All surfactants (0.25% v/v) were mixed in distilled water at an equivalent of 1,870 L ha⁻¹. Treatments with the same letter are not significant according to Student-Newman-Keuls method at $P \leq 0.05$; $n = 4$ and each replication contained 15 weevils rated as live or dead.

surfactant treatments (polyvinyl polymer/sinking agent) evaluated against the water hyacinth weevil resulted in significant mortality (13.3%) (Pellessier 1988). In addition, a nonionic invert oil and a limonene-based surfactant caused 73 to 94% water hyacinth weevil mortality (Haag 1986). In our research, giant salvinia weevil mortality was $\leq 47\%$ for all herbicide, surfactant, or combination treatments.

Based on these results, surfactant A (nonionic and buffering agent) has the potential to cause increased mortality levels in giant salvinia weevils treated under worst-case scenarios. However, under operational conditions, these aquatic herbicides and surfactants are applied to the foliage of the plants and, due to the nocturnal nature of this insect, are not likely to come in direct contact with the weevils during field applications. In addition, adult weevils are subaquatic in nature and can be found on or under fronds, within buds, or among the root-like modified leaves of giant salvinia plants (Johnson et al. 2010). Consequently, direct contact with the herbicide and surfactant mixture in the field will likely be at a minimum and mortality should be less likely to occur.

Indirect impacts of herbicides with surfactant on giant salvinia and the giant salvinia weevil

At the first harvest (2 WAT), flumioxazin and penoxsulam applied alone were more efficacious against giant salvinia than the giant salvinia weevil alone and herbicide plus weevil combination treatments (Table 2). These treatments resulted in 63 and 36% reductions of dry weight biomass, respectively. Giant salvinia displayed injury symptoms, including necrosis and chlorosis of the fronds and some

TABLE 2. IMPACT OF THE GIANT SALVINIA WEEVIL AND HERBICIDE TREATMENTS ON GIANT SALVINIA AND INDIRECT IMPACT OF HERBICIDES ON THE WEEVIL.

Treatment	2 WAT ¹		4 WAT		6 WAT	
	Dry Weight (g) ²	% W Change ³	Dry Weight (g)	% W Change	Dry Weight (g)	% W Change
C	10.3 ± 0.5 a	—	10.9 ± 0.9 a	—	13.8 ± 0.5 a	—
W	9.7 ± 0.5 a	-18	5.0 ± 0.7 b	+49	4.4 ± 1.8 b	+323
F ⁴	3.8 ± 0.7 c	—	0.3 ± 0.1 c	—	0.3 ± 0.3 c	—
P	6.6 ± 0.3 b	—	5.3 ± 1.0 b	—	0.0 ± 0.0 c	—
F + W	9.0 ± 0.9 a	-18	2.3 ± 1.2 bc	+4	0.0 ± 0.0 c	NRB
P + W	8.8 ± 0.6 a	-48	5.3 ± 1.3 b	-90	0.0 ± 0.0 c	NRB

¹Abbreviations: WAT, weeks after treatment; C, control; W, weevil; F, flumioxazin; P, penoxsulam; NRB, no remaining biomass.

²Treatments with the same letter are not significant according to Student-Newman-Keuls method at $P \leq 0.05$; $n = 3, 3,$ and 4 for $2, 4,$ and 6 WAT harvests, respectively.

³Percentage of change (+ or -) in adult weevils per kilogram fresh weight giant salvinia from the inception of the study to that particular harvest.

⁴Flumioxazin and penoxsulam herbicide treatments applied at 214 and 49 g ai ha⁻¹, respectively, with a surfactant (nonionic and buffering agent blend, 0.25% v/v).

detachment of the rootlike modified leaves by 1 WAT when treated with either herbicide (data not shown).

All weevil-alone, herbicide-alone, or weevil plus herbicide treatments resulted in 52 to 97% reductions in giant salvinia biomass by 4 WAT (Table 2). Visually, all treatments decreased plant biomass, injured or damaged plants, and resulted in open water in the buckets. By the conclusion of the experiment (6 WAT), flumioxazin, flumioxazin plus giant salvinia weevil, penoxsulam, and penoxsulam plus giant salvinia weevil treatments resulted in 98 to 100% plant control. The weevil-alone treatment caused a significant reduction in biomass (68%) and damage was still occurring at the conclusion of the experiment 6 WAT.

The ET₅₀ values based on the nonlinear equation were 5.5, 1.3, 2.7, 2.4, and 3.0 wk for the weevil, flumioxazin, penoxsulam, flumioxazin plus weevil, and penoxsulam plus weevil treatments, respectively (Figure 3). Giant salvinia exposed to any of the treatments showed similar trends in reduction of biomass over time (Figure 3). The ET₅₀ values demonstrate the amount of time it would require to have a substantial impact on giant salvinia with these control options.

The number of weevils per kilogram found on giant salvinia decreased 18, 18, and 48% for the weevil, flumioxazin plus weevil, and penoxsulam plus weevil treatments, respectively, at 2 WAT compared to the initial counts (Table 2). The 18% weevil decrease in the weevil and flumioxazin plus weevil treatments could be attributed to natural mortality or stress incurred during transport. Conversely, it is unknown if the significant decline in the number of weevils in the penoxsulam plus weevil treatment 2 WAT was due to direct toxicity or if it was caused indirectly through loss of key nutrients and extractable compounds necessary for development or reproduction of the weevils. Penoxsulam is an acetolactate synthase (ALS)-inhibiting herbicide that prevents the production of the branched-chain amino acids isoleucine, leucine, and valine (LaRossa and Schloss 1984, Senseman 2007). Such ALS herbicides inhibit plant growth 7 to 14 d after application and typically result in plant death by 4 WAT (Senseman 2007). The mode of action of the slow-acting penoxsulam may have contributed to the unfavorable plant conditions as weevils ultimately starved or were unsuccessful in seeking healthy or palatable plant material. The percentage of change in weevils per kilogram continued to decrease (90%)

4 WAT with the penoxsulam treated plants, whereas weevil counts increased with the other treatments.

At the conclusion of the experiment, there were no giant salvinia weevils remaining on herbicide-treated plants due to the lack of remaining plant biomass. However, in the weevil-only treatment, there was a 323% increase in the number of weevils per kilogram by the conclusion of the experiment. The increase in weevils per kilogram is likely attributed to the reduction in plant biomass (i.e., the ratio of weevils to biomass increased as weevil number increased or remained steady and plant biomass decreased), oviposition by adults (present at study initiation), and production of a new generation of weevils, which reached adulthood by the study's end. Previous research demonstrated that the giant salvinia weevil life cycle could be completed in 6 wk (Forno et al. 1983).

The mesocosm experiment also provided evidence of the minimal indirect impacts that surfactants and flumioxazin have on giant salvinia weevil survival when these products

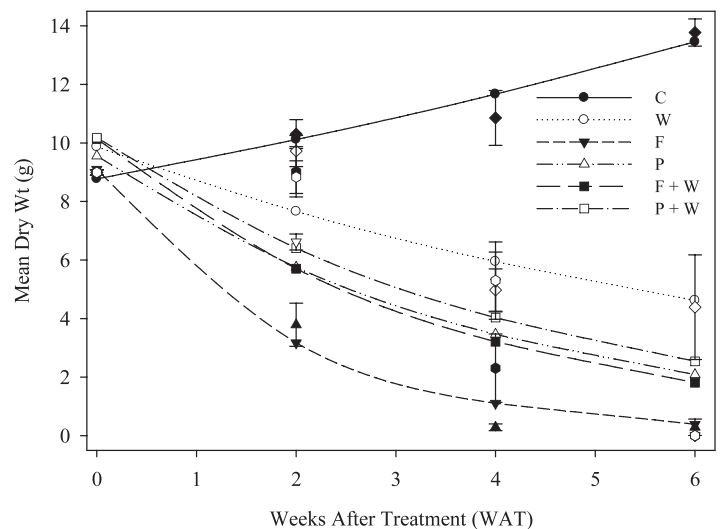


Figure 3. The effect of no treatment (control, C), the giant salvinia weevil (W), flumioxazin (F), penoxsulam (P), flumioxazin + weevil (F + W), and penoxsulam + weevil (P + W) on giant salvinia dry weight (\pm SE) over a 6-wk period. Lines represent predicted values for the weevil ($y = 9.8634e-0.1265x$, $r^2 = 0.91$), flumioxazin ($y = 9.1000e\text{minus}; 0.5272x$, $r^2 = 0.97$), penoxsulam ($y = 9.5538e-0.2538x$, $r^2 = 0.92$), flumioxazin + weevil ($y = 10.1362e-0.2874x$, $r^2 = 0.87$), and penoxsulam + weevil ($y = 10.1761e-0.2315x$, $r^2 = 0.89$) treatments; $n = 3, 3,$ and 4 for harvests at $2, 4,$ and 6 wk after treatment, respectively.

are applied directly to weevil-infested plant material. Although the nonionic and buffering agent surfactant (Surfactant A) treatment resulted in substantial mortality in the worst-case laboratory trials when applied directly to the insect (Figures 1 and 2), weevils were still present on giant salvinia 4 WAT in the mesocosm trial (Table 2). However, penoxsulam plus surfactant decreased the number of weevils per kilogram throughout the course of the study. It is uncertain if the decline in weevil population can be attributed to direct mortality or starvation due to unpalatable plant material. Regardless of the herbicide used, these results support the notion that weevils are unlikely to come in contact with the herbicide/surfactant spray solution. More research should be conducted to determine indirect impacts of herbicides used operationally (i.e., diquat and glyphosate) on the giant salvinia weevil.

Under these conditions, the combination of the giant salvinia weevil and penoxsulam or flumioxazin did not shorten the time needed for giant salvinia control compared to the herbicides used alone. However, these data demonstrated the capability of the giant salvinia weevil to survive for at least 4 wk on plant material treated with foliar-applied herbicides. Flumioxazin is a fast-acting contact herbicide that will injure plants relatively quickly with possible plant regrowth in a few days to weeks after treatment (Senseman 2007, Mudge et al. 2010). Conversely, penoxsulam requires several days or weeks to injure plants, followed by minimal regrowth (Senseman 2007). Regardless of the speed of herbicide efficacy, these results indicate the potential for a contact or systemic herbicide to be utilized in a field setting where giant salvinia weevil has been released. Further research should be conducted to integrate these technologies in a field setting and verify the results of these small-scale studies. In addition, future research should investigate lower herbicide rates in combination with weevils to decrease costs and possibly increase weevil survival since more plant material will be available for weevil longevity.

Our mesocosm study also provided evidence that flumioxazin plus surfactant A will have minimal indirect impacts on the giant salvinia weevil with regard to mortality. The giant salvinia weevil has the potential to survive on flumioxazin-treated plants for at least 4 wk or as long as plant material is available, whereas the number of weevils per kilogram decreased 90% by 4 WAT when weevil-infested plants were treated with penoxsulam at half of the maximum label rate. Further research is needed to determine if this combination will inhibit weevil establishment if plants are treated with herbicide/surfactant prior to weevil introduction. Although the direct impact research examined a worst-case scenario, there are a number of surfactants and herbicides available for aquatic use that have not resulted in significant mortality of other weevil species (Pellissier 1988) and should be considered. In conclusion, the research conducted in these laboratory and mesocosm studies did not provide enough evidence to conclude that the giant salvinia weevil and the aquatic herbicides flumioxazin and penoxsulam are advantageous when used in combination; there was no increased efficacy obtained by combining weevils with herbicide treatments.

However, this research represents the first step in combining the technologies for control of giant salvinia and additional experimentation should be conducted under controlled and field conditions to further examine the suitability of an integrated management strategy.

SOURCES OF MATERIALS

¹Miracle-Gro® Lawn Fertilizer, The Scott's Company, PO Box 606, Marysville, OH 43040.

²Green Light® Iron & Soil Acidifier, The Green Light Company, PO Box 17985, San Antonio, TX 78217.

³Clipper™ Herbicide, Valent USA Corporation, PO Box 8025, Walnut Creek, CA 94596.

⁴Galleon®, SePRO Corporation, 11550 North Meridian St., Suite 600, Carmel, IN 46032.

⁵TeeJet®, Spraying Systems Co., PO Box 7900, Wheaton, IL 60187.

ACKNOWLEDGMENTS

This research was supported by the U.S. Army Aquatic Plant Control Research Program. Appreciation is extended to Katharine DeRossette, Ford Biedenharn, Emily Yeoman, and Daniel Markbreiter for assistance throughout the research. Kurt Getsinger and Michael Grodowitz assisted in study protocol development and provided reviews of this manuscript. Permission was granted by the Chief of Engineers to publish this information. Citation of trade names does not constitute endorsement or approval of the use of such commercial products.

LITERATURE

- Buhl KJ, Faerber NL. 1989. Acute toxicity of selected herbicides and surfactants to larvae of the midge *Chironomus riparius*. Environ. Contain. Toxicol. 18:530–536.
- Calder AA, Sands DPA. 1985. A new Brazilian *Cyrtobagous* Hustache (Coleoptera: Curculionidae) introduced into Australia to control salvinia. J. Aust. Entomol. Soc. 24:57–64.
- Cary PR, Weerts PGJ. 1983. Growth of *Salvinia molesta* as affected by water temperature and nutrition. I. Effects of nitrogen level and nitrogen compounds. Aquat. Bot. 16:163–172.
- Center TD, Dray FA, Jubinsky GP, Grodowitz MJ. 1999. Biological control of water hyacinth under conditions of maintenance management: Can herbicides and insects be integrated? Environ. Manag. 23:241–256.
- Chikwenhere GP, Keswani CL. 1997. Economics of biological control of Kariba weed (*Salvinia molesta* Mitchell) at Tengwe in north-western Zimbabwe—A case study. Int. J. Pest Manag. 43:109–112.
- Cilliers CJ. 1991. Biological control of water fern, *Salvinia molesta* (Salviniaceae), in South Africa. Agric. Ecosyst. Environ. 37:219–224.
- Cowless RS, Cowless EA, McDermott AM, Ramoutar D. 2000. “Inert” formulation ingredients with activity: Toxicity of trisiloxane surfactant solutions to twospotted spider mites (Acari: Tetranychidae). J. Econ. Entomol. 93:180–188.
- Diop O, Hill MP. 2009. Quantitative post-release evaluation of biological control of floating fern, *Salvinia molesta* D.S. Mitchell (Salviniaceae), with *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae) on the Senegal River and Senegal River delta. Afr. Entomol. 17:64–70.
- Ferrell JA, MacDonald GE, Sellers B. 2008. Adjuvants. University of Florida, Institute of Food and Agricultural Sciences. SS-AGR-109. <http://edis.ifas.ufl.edu/pdf/files/WG/WG05000.pdf>. Accessed September 21, 2010.
- Forno IW, Bourne AS. 1985. Feeding by adult *Cyrtobagous salviniae* on *Salvinia molesta* under different regimes of temperature and nitrogen content and the effects on plant growth. Entomophaga 30:279–286.
- Forno IW, Harley KLS. 1979. The occurrence of *Salvinia molesta* in Brazil. Aquat. Bot. 6:185–187.

- Forno IW, Sands DPA, Sexton W. 1983. Distribution, biology and host specificity of *Cyrtobagous singularis* Hustache [Coleoptera: Curculionidae] for the biological control of *Salvinia molesta*. *Bull. Entomol. Res.* 73:85–95.
- Glomski LM, Mudge CR. 2009. Effect of Submersed Applications of Bispyribac-Sodium on Non-Target Emergent Vegetation. APCRP Technical Notes Collection (ERDC/TN APCRP-CC-12). U.S. Army Engineer Research and Development Center, Vicksburg, MS. <http://led.erdcd.usace.army.mil/qaqual>.
- Grodowitz MJ, Pellessier D. 1989. Effects of chemical applications on the biological control agents of waterhyacinth. pp. 71–84. In: Proceedings, 23rd Annual Meeting, Aquatic Plant Control Research Program, U.S. Army Engineer Waterways Experiment Station, Miscellaneous Paper A-89-1, Vicksburg, MS.
- Haag KH. 1986. Effects of herbicide application on mortality and dispersive behavior of waterhyacinth weevils, *Neochetina eichhorniae* and *Neochetina bruchi* (Coleoptera: Curculionidae). *Environ. Entomol.* 15:1192–1198.
- Harms N, Grodowitz MJ, Nachtrieb J. 2009. Mass-rearing *Cyrtobagous salviniae* Calder and Sands for the management of *Salvinia molesta* Mitchell. U.S. Army Engineer Research and Development Center ERDC/TN APCRP-BC-16, Vicksburg, MS. 10 pp.
- Jacono C. 1999. *Salvinia molesta* (D. S. Mitchell) invades the United States. *Aquatics* 21(1):4–9.
- Jacono C, Pitman B. 2001. *Salvinia molesta*: Around the world in 70 years. *Aquat. Nuisance Sp. Dig.* 4:13–16.
- Jayanth KP. 1987. Biological control of the water fern *Salvinia molesta* infesting a lily pond in Bangalore (India) by *Cyrtobagous salviniae*. *Entomophaga* 32:163–165.
- Johnson S, Sanders D, Eisenberg L, Whitehead K. 2010. Fighting the blob: Efforts to control giant salvinia. *La. Agric.* 2010(winter):6–9.
- Koschnick TJ, Netherland MD, Haller WT. 2007. Effects of three ALS-inhibitors on five emergent native plant species in Florida. *J. Aquat. Plant Manag.* 45:47–51.
- LaRossa RA, Schloss JV. 1984. The sulfonylurea herbicide sulfometuron methyl is an extremely potent and selective inhibitor of acetolactate synthase in *Salmonella typhimurium*. *J. Biol. Chem.* 259:8753–8757.
- Madsen JD, Wersal RM. 2009. Giant Salvinia. Invasive Plant Atlas of the MidSouth. <http://www.gri.msstate.edu/ipams/species.php?CName=Giant%20salvinia>. Accessed September 9, 2011.
- Mudge CR. 2007. Characterization of Flumioxazin as an Aquatic Herbicide. Ph.D dissertation. University of Florida, Gainesville, FL. 120 pp.
- Mudge CR, Haller WT, Netherland MD, Kowalsky JK. 2010. Evaluating the influence of pH-dependent hydrolysis on the efficacy of flumioxazin for hydrilla control. *J. Aquat. Plant Manag.* 48:25–30.
- Mudge CR, Harms NE. 2012. Development of an integrated pest management approach for controlling giant salvinia using herbicides and insects. U.S. Army Engineer Research and Development Center, APCRP Bulletin. ERDC APCRP-A-12-1, Vicksburg, MS. 9 pp.
- Nelson LS, Shearer JF, Netherland MD. 1998. Mesocosm evaluation of integrated fluridone-fungal pathogen treatments on four submersed plants. *J. Aquat. Plant Manag.* 36:73–77.
- Nelson LS, Skogerboe JG, Getsinger KD. 2001. Herbicide evaluation against giant salvinia. *J. Aquat. Plant Manag.* 39:48–53.
- Owens CS, Smart RM, Stewart RM. 2004. Low temperature limits of giant salvinia. *J. Aquat. Plant Manag.* 42:91–94.
- Pellessier DC. 1988. Effects of Selected Herbicides and Associated Adjuvants on *Neochetina eichhorniae*, the Mottled Waterhyacinth Weevil. M.S. thesis. University of Southwestern Louisiana, Lafayette, LA. 74 p.
- Pieterse AH, Kettunen M, Diouf S, Ndao I, Sarr K, Tarvainen A, Kloff S, Hellsten S. 2003. Effective biological control of *Salvinia molesta* in the Senegal River by means of the weevil *Cyrtobagous salviniae*. *Ambio* 32:458–461.
- Sanders D, Lorio W, Perret A. 2010. Progress in Controlling Giant Salvinia in Louisiana. <http://www.apms.org/programs/2010program.pdf>. Accessed November 6, 2012.
- Schotz M, Sands DPA. 1988. Diel pattern of feeding and oviposition by *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae). *Aust. Entomol. Mag.* 15:31–32.
- Senseman SA. (ed.). 2007. *Herbicide Handbook*. 9th ed. Weed Science Society of America, Lawrence, KS. 458 pp.
- Tipping PW. 2004. Giant Salvinia. pp. 174–177. In: E. M. Coombs, J. K. Clark, G. L. Piper, and A. F. Cofrancesco (eds.). *Biological Control of Invasive Plants in the United States*. Oregon State University Press, Corvallis, OR.
- Tipping PW, Martin MR, Center TD, Davern TM. 2008. Suppression of *Salvinia molesta* Mitchell in Texas and Louisiana by *Cyrtobagous salviniae* Calder and Sands. *Aquat. Bot.* 88:196–202.
- Tu M, Hurd C, Randall JM. 2001. *Weed Control Methods Handbook: Tools & Techniques for Use in Natural Areas*. <http://digitalcommons.usu.edu/cgi/viewcontent.cgi?article=1532&context=govdocs>. Accessed Aug 19, 2013.
- Washington State Department of Agriculture. 2012. Pesticide Registration. <http://agr.wa.gov/pestfert/pesticides/productregistration.aspx#Adjuvants>. Accessed November 2, 2012.
- Wibmer CJ, O'Brien CW. 1986. Checklist of the weevils of South America. *Mem. Amer. Entomol. Inst.* 39:1–563.
- Witt JM. 2012. Agricultural Spray Adjuvants. <http://psep.cce.cornell.edu/facts-slideself/facts/gen-peapp-adjuvants.aspx>. Accessed November 2, 2012.
- Wolfenbarger DA, Holscher CE. 1967. Contact and fumigant toxicity of oils, surfactants, and insecticides to two aphid and three beetle species. *Fla. Entomol.* 50:27–36.