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Efficacy of subsurface and foliar penoxsulam and fluridone applications on giant salvinia

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ABSTRACT

Giant salvinia (*Salvinia molesta* Mitchell) continues to be problematic and spread throughout the southern portion of the United States. Traditional management of this invasive weed has been application of the foliar herbicides diquat, glyphosate, and combinations of the two. Unfortunately, thick surface mats that limit contact with foliar sprays and fast recovery potential have resulted in mixed efficacy. Three experiments were conducted to determine the efficacy of

subsurface and foliar penoxsulam and subsurface fluridone applications on giant salvinia. These studies were conducted to determine concentration exposure time (CET) relationships, determine if repeat applications can be as effective as single static applications of each respective herbicide, and if subsurface or foliar applications will control mature giant salvinia compared to standard foliar treatments used operationally. In the CET experiment, both herbicides were more effective at growth regulating or controlling giant salvinia when exposed ≥ 8 wk, regardless of concentration. All penoxsulam concentrations evaluated (5 to 40 $\mu\text{g a.i. L}^{-1}$) resulted in initial growth regulation of giant salvinia as early as 1 week after treatment, followed by either new healthy growth (1 to 4 wk exposure) or tissue destruction (>4 wk exposure). Static penoxsulam treatments (10 and 20 $\mu\text{g L}^{-1}$) decreased plant

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dry weight 88 to 100% compared to the nontreated control. Penoxsulam foliar (24 h exposure) plus fluridone subsurface, penoxsulam foliar (24 h exposure), and penoxsulam foliar (static) reduced plant biomass to below pretreatment level in the third experiment. All herbicide treatments, except fluridone subsurface (20 µg a.i. L⁻¹ with 8 wk exposure), were as effective or provided greater giant salvinia control than the standard operational mix of glyphosate plus diquat plus two surfactants. These data confirm that penoxsulam and fluridone can be used operationally to control giant salvinia.

Key words: ALS inhibitor, aquatic fern, chemical control, diquat, exotic weed, foliar herbicide application, glyphosate, PDS inhibitor, *Salvinia molesta*, subsurface herbicide application

INTRODUCTION

Giant salvinia is a free floating, mat-forming aquatic fern native to southeastern Brazil (Forno and Harley 1979) that has become invasive in many parts of the world. Giant salvinia has become problematic in water bodies throughout the southeastern United States, as well as Puerto Rico and Hawaii, dominating coves and quiescent bays where dense infestations disrupt transportation, hinder water uptake, 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 Louisiana and Texas. In 1999, an initial infestation in Louisiana estimated to be <400 A expanded to >70,000 A over 20 lakes, 7 bayous or rivers, the Atchafalaya Basin, the Red River, and the coastal fresh water marsh from Lafitte to Morgan City by 2010 (Johnson et al. 2010). By 2004, giant salvinia had been reported in four reservoirs, five rivers (or streams), and 20 ponds in Texas (Owens et al. 2004). In 2011, the Louisiana Department of Wildlife and Fisheries treated more than 17,000 A of giant salvinia with herbicides (A.J. Perret, 2012, pers. comm.).

Management of giant salvinia has been attempted via chemical, biological, mechanical, and physical control methods, with chemical being more widely used in the United States (Madsen and Wersal 2009). Herbicides such as diquat (6,7-dihydrodipyrido[1,2- α :2',1'-c] pyrazinediium ion) and glyphosate (N-(phosphonomethyl glycine) are currently recommended for control of this floating plant species (Nelson et al. 2007, Madsen and Wersal 2009). Since the inception of chemical control of giant salvinia, herbicides have traditionally been applied as foliar applications with moderate to good success, but chemical contact with all frond surfaces is difficult to achieve. Thus dense infestations of giant salvinia often require multiple herbicide applications to insure that underlying plants receive treatment (Nelson et al. 2007). The limited leaf surface of giant salvinia makes treatment with foliar applied herbicides difficult because plants form dense vegetative mats up to 1 m thick (Thomas and Room 1986), thus sheltering plants from surface-sprayed herbicides. Based on high growth rates with nutrient additions in controlled studies (Owens and Smart 2010), plants that escape effective foliar exposure have an ability for rapid recovery, utilizing released nutrients from partially controlled vegetation.

The acetolactate synthase (ALS) inhibiting herbicide penoxsulam (2-(2,2-difluoroethoxy)-6-(trifluoromethyl)-N-(5,8-dimethoxy[1,2,4] triazolo-[1,5c]pyrimidin-2-yl)-benzenesulfonamide) recently received federal registration for control of aquatic plants (Wersal and Madsen 2010). Previous research demonstrated penoxsulam was efficacious against giant salvinia at low use rates as subsurface and foliar applications (Richardson and Gardner 2007). In addition, the phytoene desaturase (PDS) inhibitor fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone) (Senseman 2007) has shown varying levels of control with rates ranging from 45 to 90 µg L⁻¹ in Florida (McFarland et al. 2004). Although penoxsulam and fluridone activity on giant salvinia is known, limited research has been conducted to determine optimal use patterns of each herbicide. Therefore, a series of studies were conducted to (1) determine concentration exposure time (CET) relationships for penoxsulam and fluridone efficacy for giant salvinia, (2) compare the efficacy between single static and repeat applications of penoxsulam and fluridone, and (3) determine if subsurface or foliar applications of penoxsulam and fluridone will control mature giant salvinia compared to the field standard foliar treatment (i.e., glyphosate plus diquat) typically used in Louisiana and Texas. In addition, this research presents the first published accounts of injury symptoms exhibited by giant salvinia treated with penoxsulam and fluridone.

MATERIALS AND METHODS

Experimental design. All studies were conducted at the US Army Engineer Research and Development Center (US-AERDC) in Vicksburg, Mississippi. Giant salvinia used in this research was collected from cultures maintained at US-AERDC. Equal amounts of fresh plant material, enough to cover approximately 75% of the water surface, were placed inside 76 L plastic containers (49.5 cm diameter by 58.4 cm height). The amount of plant material (g dry weight) added to the containers for the CET, single static versus multiple applications, and subsurface versus foliar experiments was 12.7 ± 0.3, 11.8 ± 0.4, and 10.2 ± 0.4 g, respectively. The containers were filled with tap water amended with high nitrogen lawn fertilizer¹ at a rate 41.6 mg L⁻¹. The fertilizer was added to the experimental units every 4 wk throughout the course of the experiments. Additionally, 5 mL of aquatic dye² was added to the water column to reduce light penetration and algal growth, particularly when containers did not have a full salvinia canopy. Water level was maintained weekly at 60 L (44.5 cm in height). The plastic containers were placed inside larger plastic tanks (946 or 1136 L) partially filled with water to help maintain a consistent water temperature. Culture techniques were adapted from previous giant salvinia research (Nelson et al. 2001, 2007).

CET experiment. The CET study was conducted from June to October 2008 to determine the concentration exposure requirements of penoxsulam and fluridone to control giant salvinia. Plants acclimated to container conditions for 7 days prior to herbicide treatment and thus developed a dense single layer of mature salvinia covering 100% of the water's surface (24.8 ± 0.1 g dry weight). Subsurface penoxsulam³ and fluridone⁴ treatments were applied to giant salvinia at various CET scenarios (Table 1). A nontreated reference was

TABLE 1. SUBSURFACE PENOXSUMAM AND FLURIDONE TREATMENTS APPLIED TO GIANT SALVINIA 7 DAYS AFTER ESTABLISHMENT IN THE CET EXPERIMENT.

Treatment	Concentration ($\mu\text{g a.i. L}^{-1}$)	Exposure (wk)
Penoxsulam	5	4
Penoxsulam	5	8
Penoxsulam	5	12
Penoxsulam	10	2
Penoxsulam	10	4
Penoxsulam	10	8
Penoxsulam	10	12
Penoxsulam	20	1
Penoxsulam	20	2
Penoxsulam	20	4
Penoxsulam	20	8
Penoxsulam	20	12
Penoxsulam	40	1
Penoxsulam	40	2
Penoxsulam	40	4
Fluridone	10	4
Fluridone	10	8
Fluridone	20	4
Fluridone	20	8
Control	0	—

also used to compare plant growth in the absence of herbicide. Both herbicides were dispensed from a stock solution to the water surface in each plastic container, followed by thorough mixing. After each designated exposure time, the plants were transferred to clean plastic containers filled with fresh untreated water amended with fertilizer and dye. The study was concluded 16 wk after treatment (WAT), 4 wk after the last plants were removed from herbicide treatment. Treatments were randomly assigned and replicated four times.

Water samples were collected 1 day after treatment (DAT) and 1, 2, 4, and 8 WAT for penoxsulam treatments and 1 DAT and 1, 2, and 4 WAT for fluridone treatments to verify initial herbicide concentrations and subsequent degradation. All water samples were frozen and shipped to the SePRO Corporation laboratory (Whitakers, NC) for penoxsulam and fluridone analysis using a combination of immunoassay and high performance liquid chromatographic methods (FasTEST™). At 16 WAT, all viable giant salvinia biomass was harvested, dried to a constant weight (70 C for 144 h), and recorded as dry weight biomass. Data were subjected to analysis of variance (ANOVA) and means separated using Fisher's Protected LSD ($p = 0.05$).

Single static vs. multiple applications experiment. This study was conducted at USAERDC from May to September 2009 to compare the effectiveness of repeat, multiple applications of penoxsulam and fluridone versus single static applications of each respective herbicide. Various application scenarios were primarily designed to simulate field use patterns where plants are treated with a higher initial dose followed by a lower dose for an extended period of time. The procedures for this experiment were similar to the CET study. Plants were allowed to acclimate to container conditions for 7 days. At herbicide treatment, a dense single layer of mature salvinia (23.1 ± 1.0 g dry weight) covered 100% of the water surface. All herbicide treatments were dispensed from a stock solution to the water surface in each container, followed by

thorough mixing to achieve nominal concentrations. Herbicides evaluated in the study included penoxsulam or fluridone at various initial concentrations (5 to $160 \mu\text{g L}^{-1}$), initial exposure times (1 to 16 wk), and successive treatments (5 to $20 \mu\text{g L}^{-1}$) (Table 2). Regardless of herbicide type, number of treatments, or exposure period, herbicide exposure totaled 16 wks. An example of one herbicide treatment scheme was as follows: penoxsulam applied at $5 \mu\text{g L}^{-1}$ exposed (Ex) for 4 wk followed by (Fb) a second application of penoxsulam at $20 \mu\text{g L}^{-1}$ (P5Ex4Fb20). Herbicide treatments designated as Fb treatments were accomplished by transferring the treated plants to clean containers filled with fresh untreated water amended with fertilizer and dye. The water was then treated with the Fb treatment immediately after transfer into new containers. Treatments were randomly assigned and replicated four times.

Water samples were collected 1 DAT and 1, 2, 4, 8, 10, 12, and 16 WAT for penoxsulam 5 and $20 \mu\text{g L}^{-1}$ static treatments (P5Ex16 and P20Ex16) and 8 WAT for fluridone $20 \mu\text{g L}^{-1}$ exposed for 4 wk Fb $5 \mu\text{g L}^{-1}$ treatment (F20Ex4Fb5) to verify initial herbicide concentrations and subsequent degradation. The final harvest procedure was similar to the CET experiment at 16 WAT. Data were subjected to ANOVA and means were separated using Fisher's Protected LSD ($p = 0.05$).

Subsurface vs. foliar experiment. This study was conducted at the USAERDC in Vicksburg, Mississippi, from June to October 2010 to determine the effect of subsurface or foliar applications of penoxsulam alone and in combination with subsurface applications of fluridone on mature giant salvinia biomass compared to the standard foliar treatment (glyphosate plus diquat plus two surfactants) used in Louisiana (D.E. Sanders and A.J. Perret, 2010, pers. comm.). Plants were allowed to acclimate to container conditions for 33 days. At the time of herbicide treatment, a dense layer of mature salvinia (39.5 ± 1.2 g dry weight) about 7.6 to 10.2 cm thick, (3 to 4 plant layers) had formed in the containers. Herbicide treatments included: penoxsulam at $20 \mu\text{g L}^{-1}$ (static), fluridone at $20 \mu\text{g L}^{-1}$ (static), penoxsulam at $10 \mu\text{g L}^{-1}$ (static) plus fluridone at $20 \mu\text{g L}^{-1}$ (static), penoxsulam at 59.57 g

TABLE 2. SUBSURFACE HERBICIDE TREATMENT SCENARIOS APPLIED TO GIANT SALVINIA 7 DAYS AFTER ESTABLISHMENT IN THE SINGLE STATIC VS. MULTIPLE APPLICATIONS EXPERIMENT.

Treatment	Initial Concentration ^b	Exposure (wk)	Follow up Concentration	Abbreviation
Penoxsulam	5	4	20	P5Ex4Fb20 ^c
Penoxsulam	5	16	—	P5Ex16
Penoxsulam	10	2	5	P10Ex2Fb5
Penoxsulam	10	4	5	P10Ex4Fb5
Penoxsulam	10	16	—	P10Ex16
Penoxsulam	20	2	5	P20Ex2Fb5
Penoxsulam	20	4	5	P20Ex4Fb5
Penoxsulam	20	4	10	P20Ex4Fb10
Penoxsulam	20	16	—	P20Ex16
Penoxsulam	40	2	5	P40Ex2Fb5
Penoxsulam	80	2	5	P80Ex2Fb5
Penoxsulam	160	1	5	P160Ex1Fb5
Fluridone	20	4	5	F20Ex4Fb5
Fluridone	20	8	5	F20Ex8Fb5

^b $\mu\text{g a.i. L}^{-1}$.

^cAbbreviations: P, penoxsulam; F, fluridone; Ex, exposed; Fb, followed by.

a.i. ha⁻¹ (24 h exposure) plus fluridone at 20 µg L⁻¹ (static), penoxsulam at 59.57 g ha⁻¹ (24 h exposure), penoxsulam at 59.6 g ha⁻¹ (static), and glyphosate⁵ at 3.36 kg acid equivalent (a.e.) ha⁻¹ (24 h exposure) plus diquat⁶ at 280.35 g a.i. ha⁻¹ (24 h exposure; Table 3). A nonionic + buffering agent surfactant at 0.25% v/v⁷ was added to the penoxsulam foliar treatments and nonionic + buffering agent (Aqua-King Max) plus nonionic organosilicone⁸ surfactants were added to the glyphosate plus diquat treatments. All subsurface herbicide treatments were dispensed from a stock solution to the water surface in each container, followed by thorough mixing to achieve nominal concentrations. Foliar herbicide treatments were applied to the foliage of giant salvinia using a forced air CO₂-powered sprayer at an equivalent of 935 L ha⁻¹ diluent delivered through a single TeeJet®⁹ 80-0067 nozzle at 20 psi.

Subsurface or foliar herbicide treatments designated as “static” treatments were accomplished by treating the water column with the appropriate herbicide once and allowing the herbicide to degrade naturally without re-treatment for the duration of the experiment. Treatments designated as a “24 h exposure” were accomplished by transferring the treated plants to clean containers filled with fresh untreated water amended with fertilizer and dye 24 h after treatment. The subsurface fluridone (static) plus foliar penoxsulam (24 h) treatment was accomplished by re-treating the water with fluridone immediately after transfer into new experimental units. Because penoxsulam is efficacious against giant salvinia as a subsurface treatment, removal of plants 24 h after the foliar treatment eliminated the possibility of herbicide uptake from spray solution that failed to reach plants and reached the water column. Water samples were collected 1 DAT and 1, 2, 4, 8, and 11 WAT for penoxsulam 20 µg L⁻¹ (static), fluridone 20 µg L⁻¹ (static), and penoxsulam 59.57 g ha⁻¹ (static) treatments to verify initial herbicide concentrations and subsequent degradation.

Final harvest procedure was similar to the CET experiment 11 WAT. Treatments were randomly assigned and replicated four times. Data were subjected to ANOVA and means separated using Fisher’s Protected LSD (p = 0.05).

RESULTS AND DISCUSSION

CET experiment. Giant salvinia treated with penoxsulam exhibited injury symptoms including necrosis of older leaves as well as chlorosis and growth regulation of newer leaves. Within 2 WAT, numerous small young leaves emerged from

meristematic tissue and had a tightly rolled appearance. These symptoms were similar to the injury symptom “witches broom,” a common symptom of plants treated with ALS inhibiting herbicides. Witches broom is characterized by the release of apical dominance and subsequent outgrowth of lateral buds, symptoms that can be duplicated by treating seeds and seedlings with cytokinins (Murai et al. 1980). Witches broom has been observed on the aquatic weed parrotfeather when imazapyr and imazamox were applied as foliar treatments (Wersal and Madsen 2007) and on the submersed plant hydrilla (*Hydrilla verticillata* [L. f.] Royle) when treated with imazamox (Netherland 2011). In this study, new leaves and existing plant material were growth regulated as early as 1 WAT and typically lasted through 8 WAT. ALS herbicides inhibit the production of the amino acids valine, leucine, and isoleucine in plants by binding to the ALS enzyme, consequently resulting in decreased protein and enzyme synthesis and a rapid cessation of growth (Anderson 1996, Tranel and Wright 2002). For aquatic weed control, growth regulation can be defined as a partial or complete cessation of target weed growth for a sustained period that assists management objectives without notable reductions in weed biomass present prior to treatment.

Those plants exposed to penoxsulam for at least 8 wk began to lose buoyancy and entire plants lost integrity 8 to 10 WAT. Herbicide injury symptoms developed over time; however, once the plants were removed from penoxsulam-treated water, healthy new leaves developed without ALS symptoms. This occurred regardless of penoxsulam concentration or length of exposure. Symptoms were almost nonexistent within 2 to 4 wk after plants were removed from penoxsulam-treated water.

All fluridone-treated plants exhibited initial chlorosis followed by tissue desiccation. Those plants exposed to 10 or 20 µg L⁻¹ fluridone for 4 wk recovered and produced a large amount of new growth by 8 WAT. Only those plants receiving fluridone at 20 µg L⁻¹ for an 8 wk exposure continued to show injury symptoms through the midpoint of the experiment. However, once plants were removed from herbicide-treated water, recovery occurred.

Thirteen of the 19 penoxsulam and fluridone treatments significantly reduced giant salvinia biomass 20 to 99% of the nontreated control 16 WAT (Figure 1). Although many of these treatments statistically reduced plant biomass compared with nontreated plants, only seven treatments provided >45% control. The only treatments to provide >85% control

TABLE 3. SUBSURFACE AND FOLIAR HERBICIDE TREATMENTS APPLIED TO MATURE GIANT SALVINIA 33 DAYS AFTER ESTABLISHMENT IN SUBSURFACE VS. FOLIAR EXPERIMENT.

Herbicide Treatment	Application	Concentration/Rate
Penoxsulam	Subsurface	20 µg a.i. L ⁻¹
Fluridone	Subsurface	20 µg a.i. L ⁻¹
Penoxsulam + Fluridone	Subsurface	10 µg a.i. L ⁻¹ + 20 µg a.i. L ⁻¹
Penoxsulam ^a	Foliar (24 h)	59.57 g a.i. ha ⁻¹
Penoxsulam ^a	Foliar (Static)	59.57 g a.i. ha ⁻¹
Penoxsulam ^a + Fluridone	Foliar (24 h) + Subsurface	59.57 g a.i. ha ⁻¹ + 20 µg a.i. L ⁻¹
Glyphosate + Diquat ^b	Foliar	3.36 kg a.e. ha ⁻¹ + 280.35 g a.i. ha ⁻¹
Control	—	—

^aNonionic + buffering agent surfactant (0.25% v/v) added.

^bNonionic + buffering agent (0.25% v/v) and nonionic organo-silicone (0.125% v/v) surfactants added.

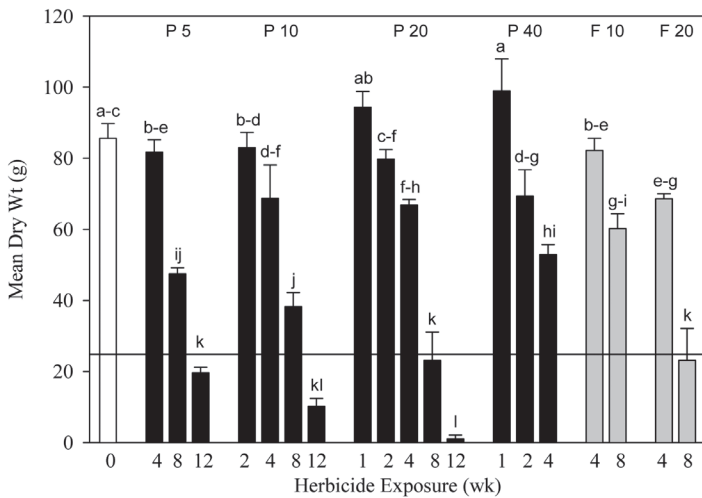


Figure 1. Effect of exposure time and concentration of penoxsulam (P) and fluridone (F) on giant salvinia dry weight (mean \pm standard error) 16 weeks after treatment (WAT). Numbers behind herbicide abbreviations represent herbicide concentrations in $\mu\text{g a.i. L}^{-1}$. Horizontal line represents mean pretreatment biomass for giant salvinia. Means with the same letter are not significant according to Fisher's Protected LSD test at $p = 0.05$; $n = 4$.

were penoxsulam at 10 and 20 $\mu\text{g L}^{-1}$ exposed for 12 wk. Fluridone at 20 $\mu\text{g L}^{-1}$ (8 wk exposure) was equally effective as penoxsulam at 5 (12 wk exposure), 10 (12 wk exposure), or 20 $\mu\text{g L}^{-1}$ (8 wk exposure). Overall, results indicated that penoxsulam and fluridone were not effective when applied at low or elevated concentrations for short exposure periods. All short exposure treatments resulted in initial injury, but plants quickly recovered once the herbicide was removed. This indicates the importance of long term exposure for achieving acceptable giant salvinia control with penoxsulam or fluridone using only subsurface application.

Initially, herbicide concentrations at the monitored 20 $\mu\text{g L}^{-1}$ rate of each herbicide were 26.0 and 10.8 $\mu\text{g L}^{-1}$ for penoxsulam and fluridone, respectively, 1 DAT (Table 2). These concentrations were 23% higher (penoxsulam) and 46% lower (fluridone) than the targeted concentration of 20 $\mu\text{g L}^{-1}$. At 1 WAT, levels were 24.6 and 17.0 ppb for penoxsulam and fluridone, respectively, suggesting possible incomplete mixing or other temporary artifact in measured doses of fluridone at 1 DAT. Herbicide concentrations slowly decreased over time. The gradual decline of both herbicides may be attributed to the large amount of plant biomass blocking light from reaching the water column. Over time, more light was able to penetrate into the water as plants were controlled, especially those treatments where plants were exposed to herbicides for ≥ 8 wk. In aqueous systems, fluridone is degraded primarily via photolysis, while penoxsulam degradation is via photolysis and microbial activity (Senseman 2007). Initially, fluridone was more injurious and efficacious on giant salvinia than penoxsulam for concentrations evaluated in this study, as indicated by injury symptoms through 8 WAT. However, once giant salvinia was removed from fluridone-treated water, plants recovered more quickly than plants exposed and removed from penoxsulam-treated water.

Single static vs. multiple applications experiment. Throughout the course of the study, giant salvinia exhibited injury symptoms including growth regulation, witches

broom, necrosis, and plant desiccation when treated with penoxsulam or exhibited chlorosis, necrosis, and plant desiccation when treated with fluridone. The injury symptoms in this study were visually similar to the CET study. All treatments in this experiment exposed giant salvinia plants to penoxsulam or fluridone for 16 wk. In comparison, plants in the CET study were able to recover to some degree after removal (1 to 12 WAT) from the herbicide-treated water by 16 WAT. Giant salvinia plants treated in the CET experiment were exposed to herbicide-free water for 4 to 15 wk by the conclusion of the experiment.

All penoxsulam and fluridone treatments resulted in a decrease in giant salvinia dry weight 16 WAT with all but one of the treatments decreasing mean dry weight $\geq 75\%$ and to less than pretreatment level (Figure 2). Static penoxsulam treatments (5, 10, and 20 $\mu\text{g L}^{-1}$) decreased plant dry weight 76 to 100%, but an increase in control resulted as the penoxsulam concentration increased. In general, a longer initial penoxsulam (4 to 16 wk) or fluridone (8 wk) exposure followed by a low dose of the same herbicide provided greater giant salvinia efficacy compared to a high dose, short exposure treatment. The multiple application treatment techniques evaluated in this study were more effective than treating giant salvinia with 5 to 40 $\mu\text{g L}^{-1}$ penoxsulam or fluridone for ≤ 8 wk as done in the CET study. Based on these results, the additional exposure period is necessary to control or suppress this resilient weed.

Two of the treatments included penoxsulam applied at 5 or 20 $\mu\text{g L}^{-1}$ for a 4 wk exposure followed by 20 and 5 $\mu\text{g L}^{-1}$, respectively (Table 2). Both herbicide treatments resulted in a decrease in giant salvinia biomass by the conclusion of

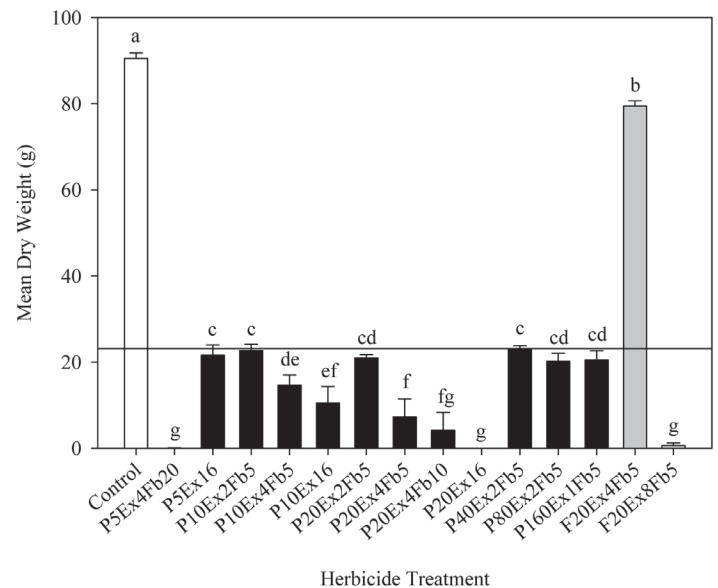


Figure 2. Effect of single static and multiple penoxsulam (P) and fluridone (F) subsurface applications ($\mu\text{g a.i. L}^{-1}$) coupled with exposure time on the growth of giant salvinia dry weight (mean) 16 weeks after treatment (WAT). Horizontal line represents mean pretreatment biomass for giant salvinia. Herbicide treatment abbreviation example as follows: penoxsulam applied at 5 $\mu\text{g L}^{-1}$ exposed (Ex) for 4 wk followed (Fb) by 20 $\mu\text{g L}^{-1}$ (P5Ex4Fb20). Means with the same letter are not significant according to Fisher's Protected LSD test at $p = 0.05$; $n = 4$.

the study; however, the low dose followed by the high dose treatment resulted in an additional 50% decrease in biomass compared to the aforementioned treatment (Figure 2). These results indicate additional penoxsulam may be applied at higher concentrations a few weeks after initial treatment to increase control. The threshold for initial and secondary treatments will need to be further researched to determine the effectiveness of high dose follow up treatments.

Both fluridone treatments were applied at 20 µg L⁻¹ followed by 5 µg L⁻¹ at 4 or 8 WAT (Table 4). Although initial and follow up treatments were the same concentration, the additional 4 wk exposure at the higher concentration resulted in an additional 87% control. These data indicate the extra 4 weeks of fluridone exposure at the higher concentration are necessary to control this weed, compared to the temporary growth regulation observed with the low dose follow up treatment at 4 WAT.

Penoxsulam concentrations were 4 and 21.5 µg L⁻¹ for the penoxsulam 5 and 20 µg L⁻¹ static treatments, respectively, 1 DAT (Table 5). Herbicide concentrations remained relatively stable throughout the course of the study for the 5 µg L⁻¹ static treatment, whereas the 20 µg L⁻¹ static concentrations declined at a much faster rate. The shorter half-life of the 20 µg L⁻¹ treatment could be attributed to greater efficacy of the higher dose as plants were controlled and desiccated at a much faster rate, increasing UV light penetration into the water column and aiding herbicide degradation. Fluridone concentrations decreased to <1 µg L⁻¹ by 8 WAT for the F20Ex4Fb5 treatment; therefore, experimental units in this treatment received an additional 3 µg L⁻¹ of fluridone 9 WAT to supplement the loss of herbicide (data not shown). The fluridone re-treatment concentration was chosen based on the half-life of fluridone in the CET experiment. Significant rainfall occurred in Vicksburg throughout the month of July, which may have contributed to the rapid dilution of the herbicide. However, during this time the P5Ex16 treatment concentration (2.8 ± 0.27) was less than the F20Ex4Fb5 concentration 4 WAT, and by 8 WAT, penoxsulam remained stable while fluridone decreased to <1 µg L⁻¹. The giant salvinia mat remained intact for the F20Ex4Fb5 treatment from 4 to 8 WAT; thus, increased photolytic degradation was unlikely to be the cause of rapid fluridone loss.

Results from this experiment indicate giant salvinia control can be achieved by implementing multiple applications

TABLE 4. HERBICIDE CONCENTRATIONS MEASURED FROM GIANT SALVINIA TREATED WITH SUBSURFACE APPLICATIONS OF PENOX SULAM AND FLURIDONE AT 20 µg L⁻¹ IN THE CET EXPERIMENT.

Herbicide	Sampling Period	Concentration (µg a.i. L ⁻¹ ± S.E.)
Penoxsulam	1 DAT ^a	26.0 ± 0.08
	1 WAT	24.6 ± 0.11
	2 WAT	20.0 ± 3.37
	4 WAT	20.3 ± 0.74
	8 WAT	13.7 ± 0.46
Fluridone	1 DAT	10.8 ± 0.23
	1 WAT	17.0 ± 0.27
	2 WAT	15.6 ± 0.20
	4 WAT	12.4 ± 0.68

TABLE 5. HERBICIDE CONCENTRATIONS MEASURED IN TREATED WATER FOLLOWING SUBSURFACE APPLICATIONS OF PENOX SULAM TO GIANT SALVINIA IN THE SINGLE STATIC VS. MULTIPLE APPLICATION EXPERIMENT.

Herbicide Treatment ^a	Sampling Period	Concentration (µg a.i. L ⁻¹ ± S.E.)
Penoxsulam 5 µg a.i. L ⁻¹	1 DAT ^b	4.0 ± 0.17
	1 WAT	4.4 ± 0.16
	2 WAT	3.3 ± 0.03
	4 WAT	2.8 ± 0.27
	8 WAT	3.7 ± 0.11
	10 WAT	3.4 ± 0.25
	12 WAT	2.7 ± 0.25
Penoxsulam 20 µg a.i. L ⁻¹	1 DAT	21.5 ± 1.64
	1 WAT	21.2 ± 1.69
	2 WAT	18.3 ± 0.90
	4 WAT	17.4 ± 0.73
	8 WAT	11.8 ± 0.29
	10 WAT	9.1 ± 0.79
	12 WAT	7.3 ± 0.71
	16 WAT	1.9 ± 1.85

^aPenoxsulam applied as a onetime treatment and plants exposed for 16 wk; n = 4.

^bAbbreviations: DAT, days after treatment; WAT, weeks after treatment.

or maintaining penoxsulam or fluridone concentrations for an extended period of time (>12 wk). Low dose repeat applications are commonly used to manage hydrilla and Eurasian watermilfoil (*Myriophyllum spicatum* L.) with fluridone or penoxsulam (Getsinger et al. 2001, Koschnick et al. 2003). Low dose static penoxsulam treatments (P5Ex16) can result in 76% control and completely suppress growth during the exposure period. Increased control can be attained by exposing plants to higher penoxsulam concentrations (10 or 20 µg L⁻¹) for longer periods of time (16 wk). Low concentrations of slow-acting herbicides such as penoxsulam and fluridone may temporarily growth regulate or stunt giant salvinia for several weeks, but plants will ultimately recover once concentrations fall below this threshold. Herbicides and plant growth regulators have been proposed and investigated to achieve a balance of controlling invasive aquatic plants while preventing negative ecological effects from unchecked growth of these species (Lembi and Chand 1992, Netherland and Lembi 1992, Nelson 1996, 1997, 2012 forthcoming). Growth regulating concentrations of slow-acting herbicides may also be beneficial for aquatic plant management, including giant salvinia. This form of management may aid in preventing development of dense infestations and their negative effects, or by slowing the recovery of target invasive plants from other required forms of management. This could include foliar herbicide applications or stress from biocontrol agents, such as the giant salvinia weevil (*Cyrtobagous salvinae*), in an integrated pest management program (Mudge and Harms 2012). Recent work has shown positive response to integrating penoxsulam treatments and multiple biocontrol agents on water hyacinth (Moran 2012).

Subsurface vs. foliar experiment. At the conclusion of the study (11 WAT), all subsurface and foliar herbicide treatments reduced giant salvinia dry weight 27 to 67% of the nontreated control (Figure 3). In particular, penoxsulam fo-

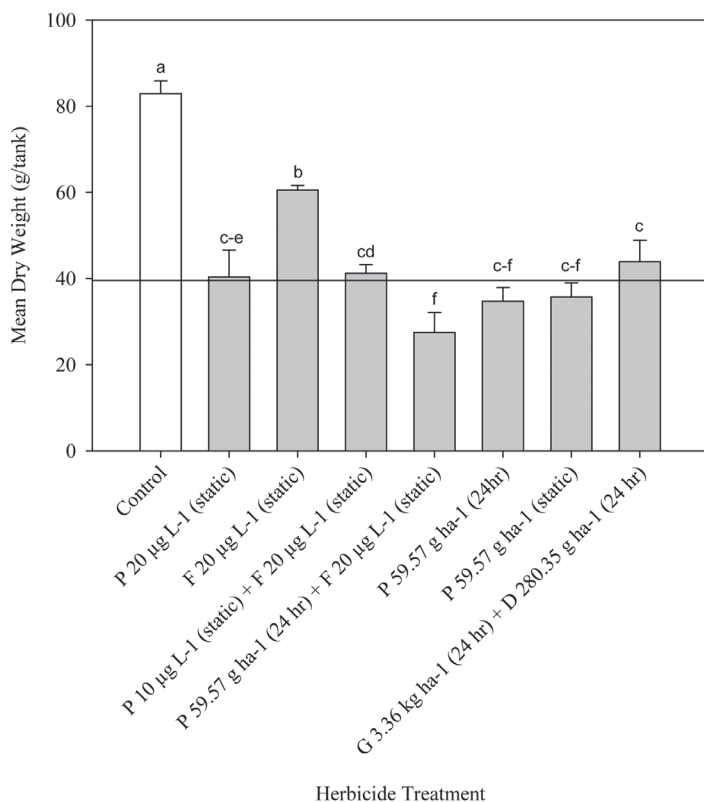


Figure 3. Effect of subsurface and foliar penoxsulam (P), fluridone (F), glyphosate (G), and diquat (D) applications on mature giant salvinia mean dry weight (\pm S.E.) 11 weeks after treatment (WAT). Plants exposed to static treatments remained for the duration of the experiment, while 24 h indicates plants were removed from treatment and placed in fresh water. Horizontal line represents pretreatment biomass for giant salvinia. Means with the same letter are not significant according to Fisher's protected LSD test at $p = 0.05$; $n = 4$.

liar (24 h) plus fluridone subsurface, penoxsulam foliar (24 h), and penoxsulam foliar (static) reduced mean plant dry weight to below pretreatment level. The penoxsulam foliar (24 h) plus fluridone subsurface treatment provided better control than all penoxsulam or fluridone stand alone or combination subsurface treatments. Although the glyphosate plus diquat mix was initially highly efficacious, plants began to recover within 3 WAT and displayed no injury symptoms by 11 WAT. Previous research demonstrated diquat at a much higher foliar rate (1.12 kg ha^{-1}) plus a methylated seed oil and organosilicone surfactant blend provided 100% control to a single layer of giant salvinia 6 WAT (Nelson et al. 2001). The amount of plant material and density of the mat in the subsurface versus foliar experiment was much greater (i.e., thicker) at the inception of the experiment compared to the single layer of giant salvinia treated in previous research by Nelson et al. (2001). The additional layers of plant material in this study likely prevented some of the herbicide spray solution from reaching the plant material below the water surface.

Fluridone concentrations failed to reach the target dose of $20 \mu\text{g L}^{-1}$ and never exceeded $6.1 \mu\text{g L}^{-1}$ throughout the course of the experiment (Table 6). The reason for the low concentration, despite the "bump" at 5 WAT is unknown. Conversely, penoxsulam concentrations remained above 30

TABLE 6. HERBICIDE CONCENTRATIONS MEASURED FOLLOWING STATIC SUBSURFACE OR FOLIAR APPLICATIONS OF PENOXsulAM AND FLURIDONE TO MATURE GIANT SALVINIA IN THE SUBSURFACE VS. FOLIAR EXPERIMENT.

Herbicide Treatment	Sampling Period	Concentration ($\mu\text{g a.i. L}^{-1} \pm \text{S.E.}$)
Fluridone ^a ($20 \mu\text{g a.i. L}^{-1}$)	1 DAT ^b	5.9 ± 0.62
	1 WAT	5.2 ± 0.28
	2 WAT	4.6 ± 0.24
	4 WAT	3.2 ± 0.20
	8 WAT	6.1 ± 0.53
	11 WAT	4.6 ± 0.50
Penoxsulam (20 a.i. L^{-1})	1 DAT	30.3 ± 1.81
	1 WAT	30.9 ± 3.55
	2 WAT	22.8 ± 1.76
	4 WAT	19.4 ± 4.79
	8 WAT	16.2 ± 1.39
	11 WAT	14.1 ± 1.32
Penoxsulam ($59.57 \text{ g a.i. ha}^{-1}$)	1 DAT	7.3 ± 0.36
	1 WAT	12.0 ± 1.14
	2 WAT	13.2 ± 0.78
	4 WAT	14.8 ± 1.63
	8 WAT	10.6 ± 0.86
	11 WAT	7.8 ± 1.26

^aFluridone applied on day of treatment and reapplied 5 WAT to increase concentration to $20 \mu\text{g a.i. L}^{-1}$.

^bAbbreviations: DAT, days after treatment; WAT, weeks after treatment; $n = 4$.

$\mu\text{g L}^{-1}$ for the first week of the study; however, concentrations decreased to $22.8 \mu\text{g L}^{-1}$ at 2 WAT and remained relatively stable throughout the remainder of the study (Table 6). The penoxsulam 59.57 g ha^{-1} foliar rate (24 h and static) was equivalent in amount of active ingredient to a $20 \mu\text{g L}^{-1}$ subsurface penoxsulam treatment. Partial migration of herbicide into underlying water was a planned effect of the static foliar treatment. Although the theoretical in-water concentration of $20 \mu\text{g L}^{-1}$ was never achieved by this static foliar treatment, some of the herbicide solution was absorbed by the foliage, and the remainder reached the water column and was available for uptake by the submersed foliage. The penoxsulam static foliar treatment was $12.0 \mu\text{g L}^{-1}$ at 1 WAT and decreased to $7.8 \mu\text{g L}^{-1}$ by 11 WAT (Table 4).

Giant salvinia treated with subsurface or foliar static penoxsulam treatments began to exhibit similar injury symptoms as early as 2 WAT. Plants treated with penoxsulam at $20 \mu\text{g L}^{-1}$ and 59.57 g ha^{-1} (static) exhibited growth regulation, and older tissue became necrotic through 6 WAT. The penoxsulam foliar (24 h) application resulted in witches broom symptoms on all new tissue in addition to necrosis of older tissue 2 WAT. Fluridone-treated plants exhibited chlorosis by 1 WAT; however, minimal bleaching of the foliage remained by 4 WAT, and plants were symptom free by 6 WAT. The rapid decrease in injury symptoms was probably due to the low fluridone concentrations in the water. Although the fluridone treatment was targeted at $20 \mu\text{g L}^{-1}$, the concentration was 5.9 ± 0.62 to 3.2 ± 0.20 between 1 DAT and 4 WAT, respectively (Table 6). The decrease in injury symptoms and concentrations prompted a bump treatment 5 WAT to increase the dose to $20 \mu\text{g L}^{-1}$ in all experimental units containing fluri-

done. The fluridone bump resulted in an increase in bleaching symptoms through the remainder of the study.

The subsurface penoxsulam plus subsurface fluridone treatment resulted in a variety of injury symptoms throughout the course of the experiment. Plant injury symptoms included necrosis and growth regulation (1 to 2 WAT) along with minimal chlorosis and witches broom. The combination of glyphosate plus diquat resulted in faster and more intense injury symptoms than any other herbicide treatment in this trial. Plants treated with this herbicide combination exhibited necrosis <1 WAT. The rapid activity of this combination was not surprising because diquat injures giant salvinia as early as 1 DAT (Nelson et al. 2001). Although this combination seemed to be highly efficacious at quickly desiccating older tissue, new plant growth was observed as early as 3 WAT.

The single static versus multiple application study demonstrated penoxsulam at 20 $\mu\text{g L}^{-1}$ reduced giant salvinia biomass 100% when plants were exposed for 16 wk, whereas plants in this study were continuing to die and lose buoyancy at 11 WAT. An additional 3 to 5 wks of exposure should have resulted in near complete to complete control based on the response of giant salvinia to the penoxsulam in the single static versus multiple applications study. This notion is supported by previous research, which indicated that ALS- and PDS-inhibiting herbicides penoxsulam and fluridone, respectively, are relatively slow acting and require long exposures (60+ d) to effectively control target species (Netherlands and Getsinger 1995, Koschnick et al. 2007b). The intent was to conclude the experiment 16 WAT, but it was shortened because control plants began to decline in health after temperatures were unusually cooler than normal in September 2010.

Although fluridone-treated plants were minimally controlled in this study (Fig. 3), the CET and single static versus multiple application studies demonstrated the effectiveness of this product (Fig. 1 and 2). Fluridone was highly efficacious (99% control) when plants were exposed to 20 $\mu\text{g L}^{-1}$ for at least 8 wk (Fig. 2). The focus of the third year of research was to extend the exposure time beyond 8 wk to achieve 100% control, but fluridone concentrations failed to reach or be maintained at the target concentration (Table 6).

The tank mix of glyphosate plus diquat plus two surfactants (nonionic and buffering agent + nonionic organo-silicone) is currently one of the recommended foliar treatments for giant salvinia in Louisiana (D. E. Sanders and A. J. Perret, 2012, pers. comm.). One or two plant layers of giant salvinia are controlled with this mixture; however, multiple levels of plant material are difficult to penetrate with a single application of any foliar applied herbicide or herbicide combination; therefore, multiple applications are often necessary to effectively control or eradicate dense giant salvinia infestation (Nelson et al. 2007). Both foliar penoxsulam treatments (static and 24 h exposure) provided similar control to the glyphosate plus diquat tank mix evaluated in this study. The penoxsulam 59.57 g ha^{-1} foliar rate was equivalent to a 20 $\mu\text{g L}^{-1}$ subsurface treatment if all the herbicide spray reached the water column and failed to come in contact with the plant canopy. In comparison, the 24 h foliar penoxsulam treatment was designed to limit any potential herbicide uptake from the water column. Although the dry weight data reflected no differences, many new healthy leaves were developing from

plants exposed for 24 h, whereas only a few healthy fronds were witnessed with the static foliar penoxsulam treatment. Because penoxsulam was still present 11WAT ($7.8 \pm 1.26 \mu\text{g L}^{-1}$), an additional few weeks of herbicide exposure may have separated these treatments, allowing older plant tissue in the static penoxsulam foliar treatment to desiccate and allow more new plant growth in the 24 h penoxsulam foliar and glyphosate plus diquat treatments. In addition, penoxsulam is recommended at 35.04 to 98.12 g ha^{-1} as a foliar application, which is equivalent to 2.0 to 5.6 oz product A^{-1} . Future research should be conducted to determine if a higher foliar rate can provide greater efficacy as well as faster activity.

Previous research has shown that penoxsulam is an effective herbicide when applied subsurface to control hydrilla and variable-leaf watermilfoil (*Myriophyllum heterophyllum* Michx.; Koschnick et al. 2007a, Glomski and Netherland 2008). Our data indicate penoxsulam as a foliar or subsurface application can be a viable alternative to the standard tank mix of glyphosate plus diquat plus two surfactants for controlling various sized infestations of giant salvinia. Penoxsulam may have the potential to provide improved, longer-term control over previous standard foliar treatments under certain use scenarios, particularly for large, dense infestations with high recovery potential. Subsurface applications should be maintained for a minimum of 8 wks to provide acceptable control, but 12+ wk of exposure generally provided excellent control. Penoxsulam applied at 5 to 20 $\mu\text{g L}^{-1}$ under extended exposures, can provide growth regulation or control of giant salvinia. Along with lethal control outcomes, the ability to use low-dose penoxsulam for growth regulation is an additional use characteristic that may complement other control techniques such as biological control or foliar applications where otherwise re-growth potential would preclude effective management. Depending on potential for dilution or other forms of dissipation, multiple applications or bump treatments may be necessary to maintain effective concentrations of penoxsulam in the water column. A foliar or subsurface penoxsulam treatment may be a beneficial treatment depending on the locale of plants (open water vs. backwater), presence of nontarget plant species, or the number of layers/thickness of the giant salvinia mat. The penoxsulam and fluridone data generated in these three experiments need to be further investigated on an operational level in field sites infested with giant salvinia.

SOURCES OF MATERIALS

¹Miracle-Gro® 36-6-6, The Scott's Company, Marysville, Ohio

²Aquashade®, Applied Biochemists, Germantown, Wisconsin

³Galleon SC®, SePRO Corporation, Carmel, Indiana

⁴Sonar AS®, SePRO Corporation, Carmel, Indiana

⁵AquaPro®, SePRO Corporation, Carmel, Indiana

⁶Reward® Landscape and Aquatic Herbicide, Syngenta Professional Products, Greensboro, NC

⁷Aqua-King Plus®, Winfield Solutions, LLC, St. Paul, Minnesota

⁸Thoroughbred®, Winfield Solutions, LLC, St. Paul, Minnesota

⁹TeeJet Technologies, Wheaton, IL

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