J. Aquat. Plant Manage. 50: 106-111

Mechanical and chemical control of the invasive cordgrass *Spartina densiflora* and native plant community responses in an estuarine salt marsh

ENRIQUE MATEOS-NARANJO, JESÚS CAMBROLLÉ, JUAN GARCÍA DE LOMAS, RAQUEL PARRA, AND SUSANA REDONDO-GÓMEZ*

ABSTRACT

The South American cordgrass, *Spartina densiflora,* has invaded a wide range of saltmarsh areas in southern Spain. A field experiment to examine physical and chemical control of *S. densiflora*, including mowing, herbicide (glyphosate), mowing plus herbicide combination, and the breaking of rhizomes, was conducted in low-gradient marsh invaded by *S. densiflora* to find a means of controlling this invasive species. The growth parameters of density, as well as species richness and diversity, were used to assess the efficacy of different treatments in December 2007 and 2008. All treatments reduced live tiller density of *S. densiflora* after 1 and 2 years of treatment. Compared to the control, the reductions in tiller density with rhizome breaking, mowing plus herbicide, mowing, and herbicide application were 85, 65, 56, and 38% and 66, 70, 52, and 52% after 1 and 2 years of treatment respectively. Despite a reduction in *S. densiflora* abundance, none of the treatments eradicated this species completely. However, rhizome breaking and mowing plus herbicide treatments proved to have the highest control efficiency, and plots treated with these treatments contained the highest values of native species richness and diversity.

Key words: breaking rhizomes, herbicide, invasive species, marshes, mowing

INTRODUCTION

The South American cordgrass, *Spartina densiflora* Brongn. (Poaceae), is invading salt marshes from southern Europe (Figueroa and Castellanos 1988), North Africa (Fennane and Mathez 1988), and North America (Kittelson and Boyd 1997). In its native South America, *S. densiflora* is a salt-marsh dominant over a wide latitudinal range and exhibits considerable morphological variation (Bortolus 2006). It has been postulated that this species was accidentally introduced into southwest Spain by means of lumber trade from South America. In southwest Spain, *S. densiflora* has emerged as a vigorous invader and ecosystem engineer that spreads by prolific seed production and consolidates its stands by clonal growth. It can be a formidable competitor; it produces dense tussocks with tall canopies and persistently high above- and belowground biomass (Figueroa and Castellanos 1988, Castillo et al. 2008, Mateos-Naranjo et al. 2008).

Invasion by *S. densiflora* is one of the most important conservation problems affecting the Gulf of Cadiz in the southwestern Iberian Peninsula because this species alters the composition of plant and animal communities, reducing their biological diversity (Kittelson and Boyd 1997). It has become the dominant plant species on recent tidal marsh restorations in the Doñana National Park (Gallego-Fernández and García-Novo 2007), threatening to spread to other marsh systems in southern Europe. Learning how to effectively manage populations of *S. densiflora* is vital; thus, research must be conducted to find methods that will either control or eradicate this species, as has been already suggested for other species of *Spartina* (An et al. 2007).

Chemical and mechanical methods may constitute an important tool for the control of *S. densiflora*, as they have for other species of *Spartina* (Patten 2004, Roberts and Pullin 2008). Hedge et al. (2003) explained the establishment and considerable limitations of a wide range of control techniques, including physical removal, mowing, and herbicide, for the management of nonnative *Spartina* plants (*S. alterniflora*, *S. anglica*, and *S. patens*) in Washington State. They indicated that the combination of mowing and herbicide application provided the greatest control efficacy. For *S. densiflora* only long-term flooding and glyphosate application under controlled environmental conditions have been studied as control techniques (Mateos-Naranjo et al. 2007, 2009). Therefore, the aims of this study were to (1) evaluate the effects of different methods on the control of *S. densiflora* under field conditions and (2) determine whether these control methods lead to enhanced plant diversity in the long term for restoration of invaded areas.

MATERIAL AND METHODS

Study location

This experiment was performed at Tinto Marshes, situated on the joint estuary of the Odiel and Tinto rivers near the

*First, second, fourth, and fifth authors: Departamento de Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla, Apartado 1095, 41080 Sevilla, Spain; Second author: Departamento de Biología. Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz. Rio San Pedro s/n, 11510 Cádiz, Spain. Corresponding author email: emana@us.es. Received for publication July 7, 2011 and in revised form February 7, 2012.

town of Huelva, on the Atlantic coast of southwestern Spain (37°15'N, 6°58'W). The salt marsh is subject to a Mediterranean climate with oceanic influences. Winter is wet and mild (mean temperature about 11 °C in January) and summer is long and dry (mean temperature about 25 °C). Mean annual rainfall is 510 mm, with an interannual variation coefficient of 31%. The semidiurnal tides have a mean range of 2.10 m and a mean spring tidal range of 2.97 m, representing 0.40 to 3.37 m above Spanish Hydrographic Zero (SHZ). Mean sea level is +1.85 m relative to SHZ (Mateos-Naranjo et al. 2008).

To test the efficacy of the different methods in the control of *Spartina* and the damage to native plants, we restricted our experiment to low-gradient marsh invaded by *S. densiflora* with an area of 0.5 ha with a height difference of 40 cm between its lower (+2.8 m SHZ) and upper (+3.2 m SHZ) limit of study area. In this location, *S. densiflora* shared habitat with the native species *Halimione portulacoides*, *Arthrocnemum macrostachyum*, *Sarcocornia perennis*, and *Salicornia ramosissima*. [A detailed examination of the physicochemical properties of the study site is given in Table 1.]

Environmental measurements

Environmental soil characteristics were analysed. Measurements of sediment conductivity, pH (n = 15), and redox potential (n = 10) were made in low tide on the upper 10 cm at the sediment in December 2006. Conductivity of sediment was determined in the laboratory with a conductivity meter (Crison-522, Spain) after mixing the sediment with distilled water (1:1; Redondo-Gómez et al. 2007). Redox potential and pH of the sediment were obtained with a portable meter and electrode system calibrated in the field (Crison pH/mV p-506, Spain). Soil water content was determined from samples taken from the upper 10 cm of sediment (n = 15). Samples were weighed before and after drying at 80 C for 48 h.

Experimental design and treatments

In December 2006, nine replicate plots of 2.5 by 2.5 m were positioned 2 m apart in the study area, and four treatments (mowing, herbicide, mowing plus herbicide combination, and the breaking of rhizomes) and one control were randomly assigned to each plot.

Mechanical treatments (mowing and breaking) of plots were carried out by personnel from the University of Seville using various hand-held brush cutters and shovels. All mowing plots were mown to within 10 cm of the substrate. For the breaking of rhizomes treatment, the above-ground portions of the plants in the plots were removed and then the below-ground sediments, rhizomes, and roots were dissected into 20 cm long sediments with shovels.

Herbicide was applied homogeneously to the plot surface using a backpack agricultural sprayer (Matabi 5-L, Goizper S.C., Spain) with a hand-held wand and an adjustable brass nozzle at a speed of 1 m s^{-1} and at a pressure of 200 kPa (250 mL of spray volume). The herbicide used was glyphosate at 7200 g a.i. ha^{-1} (Glialka® 36; 360 g a.i. L^{-1}, Presmar SL, Spain). In the mowing plus herbicide treatment, glyphosate was applied immediately after 24 h of mowing at the lowest tidal level to provide the necessary time for herbicide uptake and translocation. The chemical treatment was likewise performed at low tides, allowing 3 to 6 h of drying time before inundation of 50% of the plant. Weather conditions were optimal, with air temperatures ranging between 12 and 15 C and wind speeds from 0 to 5 Km h^{-1}. Also, in December the application period coincided with a period of neap tides and with plants dropping seeds and entering senescence (Nieva et al. 2005).

Data collection

To estimate the control efficacy, four 0.2 by 0.2 m quadrats were randomly selected in each plot in December 2007 and 2008, and the number of live tillers of *S. densiflora* was recorded. Native species richness and plant species diversity were measured for each treatment. Species diversity was calculated using Shannon's index (H'; Shannon and Weaver 1949) formula

$$H' = -\Sigma pi \cdot ln pi,$$

where pi = relative abundance of each species divided by the total number of species observed in each plot.

Statistical analysis

Statistical analyses were carried out using Statistica v. 6.0 (Statsoft Inc.). Data were analyzed using a one-way analysis of variance (F-test). Data were first tested for normality with the Kolmogorov-Smirnov test and for homogeneity of variance with the Brown-Forsythe test. We used a normal error distribution, so alive tiller density and native species richness were Ln (x) transformed, respectively, for one-way analysis of variance. Significant test results were followed by Tukey tests for identification of important contrasts ($P < 0.05$).

RESULTS

Control of invasive *S. densiflora*

All mechanical and chemical treatments had significant effects on the control efficacy of *S. densiflora* after 1 and 2 years of treatment, with all treated plots showing lower live tiller density than control plots (Table 2; Figure 1). Breaking rhizome treatment recorded the lowest tiller density of *S. densiflora* after 1 year of treatment, followed by mowing plus herbicide, mowing, and herbicide treatment (Figure 1). Compared to the control, the reductions in tiller density with these treatments were 85, 65, 56, and 38% respectively.

TABLE 1. PHYSICOCHEMICAL PROPERTIES OF THE THREE CONTRASTING SITES FROM TINTO MARSHES. VALUES ARE MEAN ± SE OF 15 REPLICATES. EXCEPT FOR REDOX POTENTIAL (VALUES ARE MEAN ± SE OF 5 REPLICATES IN THE UPPER AND LOWER LIMIT OF STUDY AREA).

Parameter	values	
Conductivity (mS cm^{-1})	15.6 ± 0.8	
pH	6.7 ± 0.1	
Redox potential (mV)	87 ± 6.3	-146 ± 19.9
Soil water content (%)	27 ± 0.4	

Table 2. Results of a one-way analysis of variance (ANOVA) for different control treatments on S. densiflora live tiller density and species richness and native plant diversity of treated plots (Shannon's index, H´) one and two years after treatment. Treatments: C, control plot; H, herbicide; B, breaking rhizome; M, mowing; and M+H, mowing plus herbicide combination. Asterisk indicate significant difference; Tukey test, P < 0.05).

1st year	One way Anova		d.f.	F	P	2nd year	One way Anova		d.f.	F	P
	variable	Tiller density (tiller m-2)	4	67.60	0.000		variable	Tiller density (tiller m-2)	4	37.32	0.000
	Treatment	C	H	B	M		Treatment	C	H	B	M
	H	0.000*	—	—	—		H	0.000*	—	—	—
	B	0.000*	0.000*	—	—		B	0.000*	0.025*	—	—
	M	0.000*	0.006*	0.000*	—		M	0.000*	1.000	0.018*	—
	M+H	0.000*	0.000*	0.000*	0.616		M+H	0.000*	0.000*	0.619	0.000*
1st year	One way Anova		d.f.	F	P	2nd year	One way Anova		d.f.	F	P
	variable	Species richness	4	9.59	0.000		variable	Species richness	4	8.40	0.000
	Treatment	C	H	B	M		Treatment	C	H	B	M
	H	1.000	—	—	—		H	0.998	—	—	—
	B	0.002*	0.002*	—	—		B	0.002*	0.004*	—	—
	M	0.006*	0.006*	0.986	—		M	0.133	0.234	0.341	—
	M+H	0.007*	0.007*	0.976	1.000		M+H	0.003*	0.007*	0.999	0.473
1st year	One way Anova		d.f.	F	P	2nd year	One way Anova		d.f.	F	P
	variable	Native plant diversity	4	13.04	0.000		variable	Native plant diversity	4	6.55	0.001
	Treatment	C	H	B	M		Treatment	C	H	B	M
	H	0.954	—	—	—		H	0.880	—	—	—
	B	0.000*	0.000*	—	—		B	0.005*	0.041*	—	—
	M	0.011*	0.049*	0.341	—		M	0.101	0.472	0.657	—
	M+H	0.001*	0.004*	0.941	0.803		M+H	0.004*	0.033*	1.000	0.597

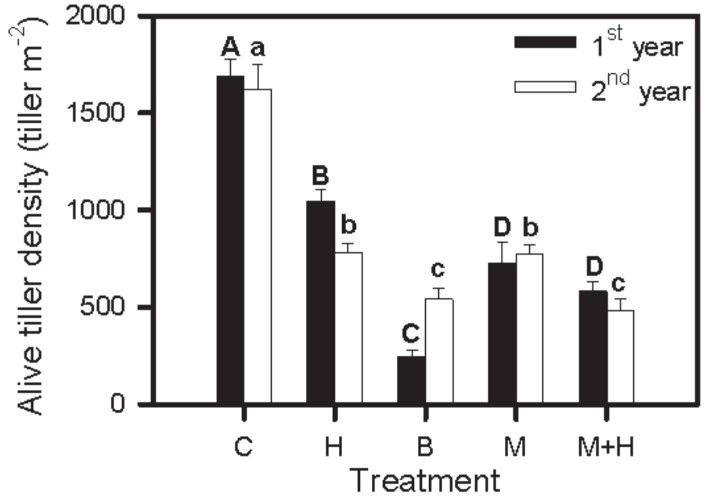

Figure 1. Live tiller density in treated plots at Tinto marshes 1 and 2 years after treatment. Treatments: C, control plot; H, herbicide; B, breaking rhizome; M, mowing; and M+H, mowing plus herbicide combination. Values represent mean ± SE, $n = 36$. Different letters indicate means that are significantly different from each other (capital letters for first year and lowercase for the second year of treatment; Tukey test, $P < 0.05$).

However, rhizome breaking treatment showed a tiller density similar to mowing plus herbicide treatment after 2 years of treatment (Figure 1). After 2 years, breaking and mowing plus herbicide treatments recorded the lowest tiller densities (Figure 1), with percentage reductions compared to the control of 66 and 70%, respectively. Herbicide and mowing treatments each showed a tiller reduction of 52% and were not statistically different (Table 2).

Recolonization by native vegetation

Breaking, mowing, and mowing plus herbicide treatments showed the highest natives species richness after 1 year of treatment (Table 2; Figure 2); however, diversity values were not similar to those of the mowing treatment due to the presence of dominant species (Table 2; Figure 3). After 2 years, rhizome breaking and mowing plus herbicide treatments showed the highest natives species richness (Figure 2). These treatments also recorded the highest diversity values (Table 2; Figure 3). Finally, species richness and diversity values of plots treated with herbicide were similar to the control plot as a consequence of the wider coverage by *S. densiflora*.

DISCUSSION

Our results show that breaking rhizomes and mowing plus herbicide combination treatments had the highest control efficacy on live tiller density of *S. densiflora* compared to the control plot. Glyphosate application was the least efficacious treatment (in terms of autochthonous plant species colonization), especially in some plots where the coverage by *S. densiflora* was 100% (as happened in the control plot). Although glyphosate did reduce live tiller density, the shading effect of the large number of dead and erect tillers of *S. densiflora* might account for the lower presence of other species in herbicide treatment plots. This species exhibits a phalanx

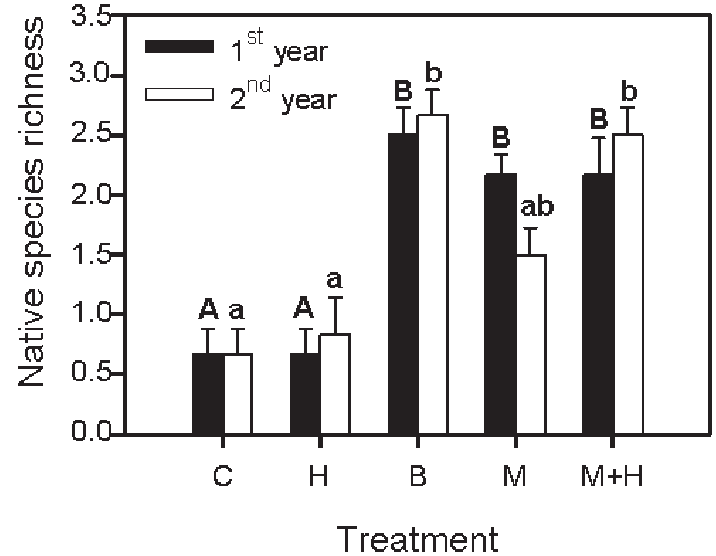

Figure 2. Mean species richness in treated plots at Tinto marshes 1 and 2 years after treatment. Treatments: C, control plot; H, herbicide; B, breaking rhizome; M, mowing; and M+H, mowing plus herbicide combination. Values represent mean ±SE, $n = 18$. Different letters indicate means that are significantly different from each other (one-way ANOVA, species × treatment, $p < 0.05$).

type of growth characterized by the production of dense tussocks, which can reduce light at soil surface and thereby inhibit colonization by native species (Castellanos et al. 1998). Mateos-Naranjo et al. (2009) demonstrated that glyphosate at doses as high as 7200 g a.i. ha^{-1} has a negative effect on the photosynthetic apparatus and growth of *S. densiflora* under controlled environmental conditions, so this may reduce

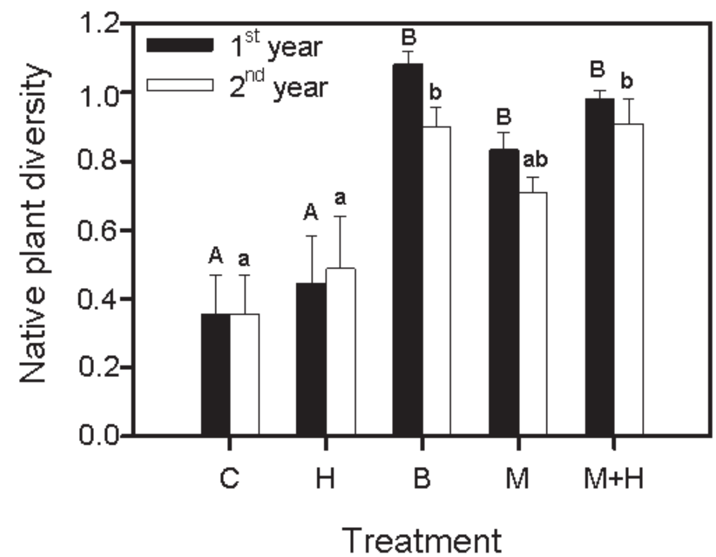

Figure 3. Native plant diversity (Shannon's index, H´) in treated plots at Tinto marshes 1 and 2 years after treatment. Treatments: C, control plot; H, herbicide; B, breaking rhizome; M, mowing; and M+H, mowing plus herbicide combination. Values represent mean ± SE, $n = 36$. Different letters indicate means that are significantly different from each other (capital letters for the first year and lowercase for the second year of treatment; Tukey test, $P < 0.05$).

its competitive ability. However, Patten (2002) found that the time between application and tidal inundation over the canopy affected the efficacy of glyphosate in the control of *S. alterniflora*, and Zanatta et al. (2007) observed that soil water content influenced the glyphosate efficacy in the control of *Euphorbia heterophylla*. Thus, the effect of environmental factors such as tidal influence and soil water content could alter the efficacy of glyphosate application and partly explain our results. In addition, to increase the efficacy of glyphosate application, repetition of the treatment in subsequent seasons is necessary, as previously suggested for different *Spartina* species (Roberts and Pullin 2008) as well as other invasive species of wetland ecosystems (Ailstock et al. 2001).

Many studies have demonstrated variable responses of *Spartina* to mowing, depending on the species (Li and Zhang 2008, Roberts and Pullin 2008). Accordingly, *S. alterniflora* showed a significant reduction in the density with an overall mean percentage decline of 68%, whereas *S. anglica* and *S. townsendii* showed an over-compensation effect with a mean increase of 42.8 and 14.7%, respectively (Roberts and Pullin 2008). For *S. densiflora*, we recorded mean percentage reductions of about 56 and 52% after 1 and 2 years of treatment, respectively. The removal of above-ground parts by mowing might have greatly reduced the energy allocation to its below-ground structures, leading to a reduced regrowth potential (Haferkamp and Karl 1999). Mowing was performed only once at the beginning of the experiment, although repeated mowing has been shown to effectively reduce growth in other *Spartina* species (Li and Zhang 2008). Major et al. (2003) showed that mowing might be the least efficacious control treatment for *Spartina alterniflora*, the most labour intensive, and the most destructive to the surrounding mudflat. Moreover, its efficacy increases with the frequency of mowing and the use of larger machinery, two aspects that add up to greater damage to the associated mudflat. In addition, mechanical control methods are costly and require highly specialized equipment (Li and Zhang 2008). Therefore, further testing of repeated mowing is needed to better assess the control of *S. densiflora* with this method and ascertain whether it constitutes a feasible option.

Finally, rhizome breaking and mowing plus herbicide treatments reduced the density of *S. densiflora* between 85 and 65% after 1 year of treatment, and between 66 and 70% after 2 years. Li and Zhang (2008) observed similar reductions for *S. alterniflora* treated with rhizome breaking in the first season, but growth had almost recovered to the control level by the end of the second growing season. Other techniques such as the use of roto-tilling, has produced >90% efficacy for the control of *S. alterniflora* during winter trials but was <70% effective during spring trials (Hedge et al. 2003). In comparison, Roberts and Pullin (2008) found that the use of mowing followed by glyphosate application decreased the density of *S. alterniflora* by 91%. In contrast, the same intervention, when used to control *S. anglica*, increased densities by 19% per plot. Major et al. (2003) found that one-time mowing seemed to yield a more consistently uniform application of the herbicide and was likely to provide an initial reduction in the plant's energy reserves before chemical treatment. Furthermore, in rhizome breaking and mowing plus herbicide plots, *S. densiflora* was replaced by a higher number of native species than in herbicide, mowing, and control plots, resulting in the highest diversity values.

CONCLUSIONS

Our data indicated that *S. densiflora* has a strong capacity to resist mechanical and chemical control interventions; a single application of the various control techniques tested in this study seemed incapable of eradicating *S. densiflora* and returning invaded marshes to a pre-invasion state. However, this study provides valuable information for the management of this exotic species in the invaded habitats. Rhizome breaking and mowing plus herbicide application could be useful for the control of the invasion of this species; both treatments decreased *S. densiflora* biomass and favored an increased diversity and native species richness.

ACKNOWLEDGMENTS

We are grateful to Antonio J. Ruiz Rico for revision of the English in the manuscript. We also thank the Spanish Environmental and Science and Technology Ministries for their support (project 042/2007 Organismo Autónomo Parques Nacionales and project CTM2008-04453).

LITERATURE CITED

Ailstock MS, Norman CM, Bushmann PJ. 2001. Common reed *Phragmites australis* control and effects upon biodiversity in freshwater nontidal wetlands. Restor. Ecol. 9:49-59.

An SQ, Gu BH, Zhou CF, Wang ZS, Deng ZF, Zhi YB, Li HL, Chen L, Yu DH, Liu YH. 2007. *Spartina* invasion in China: implications for invasive species management and future research. Weed Res. 47:183-191.

Bortolus A. 2006. The austral cordgrass *Spartina densiflora* Brong.: its taxonomy, biogeography and natural history. J. Biogeogr. 33:158-168.

Castellanos EM, Heredia C, Figueroa ME, Davy AJ. 1998. Tiller dynamics of *Spartina maritima* in successional and non-successional Mediterranean salt marsh. Plant Ecol. 137:213-225.

Castillo JM, Mateos-Naranjo E, Nieva FJ, Figueroa E. 2008. Plant zonation at salt marshes of the endangered cordgrass *Spartina maritima* invaded by *Spartina densiflora*. Hydrobiologia. 614:363-371.

Fennane M, Mathez J. 1988. Nouveaux matériaux pour la flore de Maroc. Naturalia Montspeliensia. 52:135-141.

Figueroa ME, Castellanos EM. 1988. Vertical structure of *Spartina maritima* and *Spartina densiflora* in Mediterranean marshes, pp. 105-108. In : M. J. A. Werger, P. J. M. Van der aart, H. J. During, and J. T. A. Verhoeven (*eds.*). Plant form and vegetation structure. SPB Academic Publishing, The Hague.

Gallego-Fernández JB, García-Novo F. 2007. High-intensity versus low-intensity restoration alternatives of a tidal marsh in Guadalquivir estuary, SW Spain. Ecol. Eng. 30:112-121.

Haferkamp MR, Karl MG. 1999. Clipping effects on growth dynamics of Japanese brome. J. Range Manage. 52:339-345.

Hedge P, Kriwoken LK, Patten K. 2003. A review of Spartina management in Washington State, US. J. Aquat. Plant Manage. 41:82-90.

Kittelson PM, Boyd MJ. 1997. Mechanisms of expansion for an introduced species of cordgrass, *Spartina densiflora*, in Humboldt Bay, California. Estuaries. 20:770-778.

Li H, Zhang L. 2008. An experimental study on physical controls of an exotic plant *Spartina alterniflora* in Shangai, China. Ecol. Eng. 32:11-21.

Major WW, Grue CE, Grassley JM, Conquest L. 2003. Mechanical and chemical control of smooth Cordgrass in Willapa Bay, Washington. J. Aquat. Plant Manage. 41:6-12.

Mateos-Naranjo E, Redondo-Gómez S, Silva J, Santos R, Figueroa ME. 2007. Effect of prolonged flooding on the invader *Spartina densiflora* Brong. J. Aquat. Plant Manage. 45:121-123.

Mateos-Naranjo E, Redondo-Gómez S, Luque CJ, Castellanos EM, Davy AJ, Figueroa ME. 2008. Environmental limitations on recruitment from seed in invasive *Spartina densiflora* on a southern European salt marsh. Estuar. Coast. Shelf S. 79:727-732.

Mateos-Naranjo E, Redondo-Gómez S, Cox L, Cornejo J, Figueroa ME. 2009. Effectiveness of glyphosate and imazamox on the control of the invasive cordgrass *Spartina densiflora*. Ecotox. Environ. Safe. 72:1694-1700.

Nieva FJJ, Castellanos EM, Castillo JM, Figueroa ME. 2005. Clonal growth and tiller demography of the invader cordgrass *Spartina densiflora* brongn. at two contrasting habitats in Sw European salt marshes. Wetlands. 25:122-129.

Patten K. 2002. Smooth cordgrass (*Spartina alterniflora*) control with Imazapyr. Weed Technol. 16:826-832.

Patten K. 2004. Comparison of chemical and mechanical control efforts for invasive Spartina in Willapa Bay, WA, Report. The Washington State Department of Agriculture.

Redondo-Gómez S, Mateos-Naranjo E, Davy AJ, Fernández-Muñoz F, Castellanos EM, Luque T, Figueroa ME. 2007. Growth and photosynthetic responses to salinity of the salt-marsh shrub *Atriplex portulacoides*. Ann. Bot-London 100:555-563.

Roberts PD, Pullin AS. 2008. The effectiveness of management interventions for the control of *Spatina* species: a systematic review and meta-analysis. Aquat. Conserv. 18:592-618.

Shannon CE, Weaver W. 1949. The mathematical theory of communication. University of Illinois Press, Urbana, IL.

Zanatta JF, Procopio SO, Manica R, Pauletto EA, Carnelutti A, Vargas L, Sganzerla DC, Rosenthal MDA, Pinto JJO. 2007. Soil water contents and glyphosate efficacy in controlling *Euphorbia heterophylla*. Planta Daninha. 25:799-811.

J. Aquat. Plant Manage. 50: 111-116

Response of target and nontarget floating and emergent aquatic plants to flumioxazin

CHRISTOPHER R. MUDGE AND WILLIAM T. HALLER*

ABSTRACT

The effects of subsurface and foliar flumioxazin {2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione} treatments were evaluated on the floating weeds waterhyacinth (*Eichhornia crassipes* (Mart.) Solms), water lettuce (*Pistia stratiotes* L.), and landoltia (*Landoltia punctata* [G. Mey] D.H. Les and D.J. Crawford) as well as the nontarget emergent species eleocharis (*Eleocharis interstincta* (Vahl) Roem & J.A. Schult), maidencane (*Panicum hemitomon* Schult.), pickerelweed (*Pontederia cordata* L.), and sagittaria (*Sagittaria lancifolia* L.). All subsurface treatments (≥100 µg a.i. L^{-1}) and foliar application rates >143 g a.i. ha^{-1} provided complete water lettuce control. Conversely, both flumioxazin application techniques provided <30% control of waterhyacinth. No injury symptoms were exhibited by landoltia treated with foliar flumioxazin applications, and in water, concentrations ≥200 µg a.i. L^{-1} were required to provide more than 50% control. Sagittaria was the most sensitive nontarget emergent species to subsurface flumioxazin applications, followed by maidencane, eleocharis, and pickerelweed. Sagittaria dry weight was reduced 100% at herbicide concentrations ≥800 µg a.i. L^{-1} compared to a 73 to 83% dry weight reduction in eleocharis, maidencane, and pickerelweed. Conversely, all emergent species were highly tolerant to foliar flumioxazin treatments, yielding calculated EC_{50} values ≥1320 g a.i. ha^{-1} for dry weight. Results of this study indicate differential efficacy and selectivity among floating and emergent target and nontarget aquatic plant species when treated with flumioxazin.

Key words: chemical control, dose response, EC_{50}: Effective Concentration 50, *Eichhornia crassipes*, *Eleocharis interstincta*, *Landoltia punctata*, *Panicum hemitomon*, *Pistia stratiotes*, *Pontederia cordata*, protoporphyrinogen oxidase inhibitor, *Sagittaria lancifolia*, selectivity

INTRODUCTION

Invasive floating aquatic plants, including waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) and water lettuce (*Pistia stratiotes* L.), spread by vegetative reproduction, forming extensive free floating mats that often interfere with navigation, hydroelectric generation, irrigation, and fishing as well as lowering the dissolved oxygen and pH of the water (Weldon and Blackburn 1966, Harley et al. 1984, Owens and Madsen 1995). They may also harbor mosquitoes, which are vectors for diseases like dengue fever, malaria, and encephalitis (Holm et al. 1977). Conversely, native emergent aquatic plants may provide a diverse and valuable food source and habitat for animals, can improve water clarity and quality, reinforce shorelines, and protect soil against erosion from wind and wave action (Savino and Stein 1982, Heitmeyer and Vohs 1984, Smart 1995, Dibble et al. 1996). Damage to emergent nontarget and native plants species is a major consideration in herbicide selection; favorable aquatic herbicides are able to selectively remove unwanted plants while minimizing damage to nontarget native plants.

One of the primary goals of aquatic weed management is to control invasive plants while maintaining a diverse native plant community. Native plant density and diversity have been shown to increase when canopy-forming exotic plants

*Former Graduate Research Assistant and Professor, Center for Aquatic and Invasive Plants, Institute of Food and Agricultural Sciences, University of Florida, PO Box 110610, Gainesville, FL 32611. Current address of first author: US Army Engineer Research and Development Center, Vicksburg, MS 39180. Corresponding author's E-mail: Christopher.R.Mudge@usace.army.mil. Received for publication February 10, 2012 and in revised form June 28, 2012.

are removed (Getsinger et al. 1997), and continued presence of native vegetation allows diversity of invertebrate and fish habitats to be maintained (Dibble et al. 1996). Therefore, it is beneficial to selectively remove floating invasive species to maintain native vegetation and, in turn, wildlife habitat.

In 2010, flumioxazin received FIFRA-Section 3 registration for control of submersed, emergent, and floating aquatic weeds in the United States (Valent USA Corporation 2011). Flumioxazin is a very fast-acting contact herbicide that inhibits protoporphyrinogen oxidase (PPO; protoporphyrin IX:oxygen oxidoreductase, EC 1.3.3.4). It inhibits chlorophyll biosynthesis by preventing transformation of protoporphyrinogen IX into protoporphyrin IX, a precursor to heme and chlorophyll production (Matringe et al. 1989, Cobb 1992, Aizawa and Brown 1999). It has been evaluated in greenhouse and field studies for control of hydrilla (*Hydrilla verticillata* [L.f.] Royle) and other invasive aquatic species (Mudge 2007, Richardson et al. 2008, Mudge et al. 2010).

The high costs associated with registering an herbicide for a new market (i.e., aquatics) may be overcome by maximizing the market potential; therefore, one objective of this research was to determine if flumioxazin has utility as a foliar or a subsurface treatment to control floating aquatic weeds. Emergent nontarget aquatic plants could be impacted by flumioxazin applications, so the second objective was to quantify the effects of foliar and subsurface flumioxazin treatments on common nontarget emergent aquatic plants.

MATERIALS AND METHODS

Floating aquatic plants. *Waterhyacinth and water lettuce.* Plants were collected from Rodman Reservoir near Interlachen, Florida, and established in 95 L high-density polyethylene (HDPE) tanks filled with 80 L of tap water (pH 8.0) in April 2006 at the University of Florida (Center for Aquatic and Invasive Plants) in Gainesville, Florida. Tap water was supplemented with 1 mL of Chelated Iron Plus[1] (12-0-0) and 150 mg L^{-1} Miracle-Gro®[2] (24-8-16) fertilizer prior to herbicide treatment. Nutrients were added again at 1 and 3 weeks after treatment. Waterhyacinth (5 plants per tank) and water lettuce (20 plants per tank) were allowed to acclimate for 3 weeks before treatment. This study was repeated in August 2006 on the main campus of the University of Florida with water from Biven's Arm Lake (pH 7.5). Both studies were completely randomized designs with 4 replications (tanks) for each treatment. All studies were conducted under full sunlight.

For the foliar treatments, flumioxazin[3] was applied to the foliage with a forced air CO_2-powered sprayer calibrated to deliver a spray volume of 935 L ha^{-1} through a single TeeJet®[4] 80-0067 nozzle at 0, 36, 72, 143, 286, 572, and 1144 g a.i. ha^{-1} plus a non-ionic surfactant[5] (0.25% v/v). Subsurface flumioxazin treatments were conducted concurrently at each location. Flumioxazin was applied at 0, 100, 200, 400, 800, and 1600 µg a.i. L^{-1} as static treatments.

All live waterhyacinth and water lettuce tissue was harvested 34 d after treatment (DAT), placed in a drying oven at 90 C for about 1 week, and weighed. Plant dry weight data were analyzed using nonlinear regression (exponential decay, $y = b_0 e^{-bx}$) with the PROC NLIN procedure (SAS Institute 2002), and regression models were used to determine the effective concentration 50 (EC_{50}), which is the concentration of flumioxazin required to cause a 50% reduction in dry weight compared to control plants. Because there were no differences between the slopes of the regression lines at the 95% confidence level, data were pooled for each repeated study.

Landoltia. A population of landoltia (*Landoltia punctata* [G. Mey] D.H. Les and D.J. Crawford) was collected from a pond with no history of herbicide treatments in Alachua County, Florida, and cultured at the Center for Aquatic and Invasive Plants in 266 L fiberglass tanks in a greenhouse (light intensity of 1200 µmol m^{-2} s^{-1}). Plants were cultured in tap water (pH 8.2) amended with Miracle-Gro (24-8-16, 150 mg L^{-1}) and allowed to acclimate for 2 weeks before treatment. A 10 g aliquot (fresh weight; 1.3 ± 0.07 g dry weight) of landoltia was placed in 3 L HDPE (17.1 cm diameter by 13.3 cm deep) pots filled with tap water (pH 8.0) and Miracle-Gro. Plants were allowed to acclimate in the pots for an additional 2 d prior to herbicide treatment. All pots were amended with Miracle-Gro at 2 and 14 DAT.

The subsurface experiment was conducted in April and May 2007. Landoltia was treated with flumioxazin at 0, 10, 25, 50, 100, 200, 400, 800, and 1600 µg a.i. L^{-1} as static treatments. As a comparison treatment, diquat[6] was applied as a foliar treatment at 1.1 kg a.i. ha^{-1} using the described methods for foliar flumioxazin treatments. This experiment was a randomized design with five replicates. Foliar flumioxazin trials were conducted in October 2005, April 2007, and May 2007. Flumioxazin was applied to landoltia at 0, 36, 72, 143, 286, 572, and 1144 g a.i. ha^{-1} plus a non-ionic surfactant (SunStream; 0.25% v/v) using the same methods as described in the water lettuce and waterhyacinth studies.

Due to the difficulty of removing large quantities of necrotic or chlorotic and dead landoltia plants, visual estimates of control (% control) were determined on a scale of 0 to 100%, where 0 = no chlorosis or necrosis and 100 = plant death. Percent control ratings were based on nontreated control plants. There were no differences in control between the two experiments (Fisher's Protected LSD, $p \leq 0.05$); therefore, the data from the two experiments were pooled for analysis and means were separated using 95% confidence intervals.

Emergent aquatic plants. The sensitivity of the nontarget emergent aquatic plants eleocharis, maidencane, pickerelweed, and sagittaria were similarly evaluated against subsurface and foliar flumioxazin application techniques. All plants were purchased from a local plant nursery in August 2006 and April 2007 for the subsurface and foliar studies, respectively. Two healthy stems (30 to 38 cm) of each species were planted in a mixture of 2:1 potting media[7] to masonry sand in 3 L HDPE pots amended with Osmocote®[8] (15-9-12) fertilizer at a rate of 1g kg^{-1} soil.

The subsurface flumioxazin experiment was a randomized design with five replicates (tanks). Each species was cultured outdoors under shade cloth (70% sunlight) for 4 weeks in 95 L HDPE tanks. Water level in the tanks was maintained at 25 cm, and pH remained at or near 7.5 throughout the study. Plants were cultured for 1 month when flumioxazin was applied at 0, 50, 100, 200, 400, 800, and 1600 µg a.i. L^{-1} as static treatments. In the foliar flumioxazin trial, all five emergent replicates (pots) were placed in one 266 L fiberglass tank (72 by 82 by 45 cm) prior to treatment. Flumioxazin was applied as a foliar treatment at 0, 36, 72, 143, 286, 572, and 1144 g a.i. ha^{-1} with a non-ionic surfactant (SunStream; 0.25% v/v) using the same methods as the floating plants.

At 40 DAT, all live tissue from the foliar and subsurface trials was harvested at the soil line, placed in a drying oven at 90 C for about 1 week, and weighed. Plant dry weight data was analyzed using no-linear regression (PROC NLIN, SAS Institute 2002), and EC_{50} values were determined for dry weight. Data were pooled across experimental runs when no statistical differences between the slopes of regression lines were observed.

RESULTS AND DISCUSSION

Floating aquatic plants. *Waterhyacinth and water lettuce.* Water lettuce was much more sensitive to foliar applications of flumioxazin than waterhyacinth, with EC_{50} values of 69 and 1435 g a.i. ha^{-1}, respectively (Figure 1). All foliar rates ≥286 g a.i. ha^{-1} resulted in complete control of water lettuce. Treated water lettuce plants exhibited chlorosis and necrosis on the leaves 3 to 5 DAT and defoliation 12 to 15 DAT. Foliar flumioxazin rates ≥286 g a.i. ha^{-1} resulted in complete plant decay 21 DAT, whereas sublethal rates (36 to 143 g a.i. ha^{-1}) resulted in regrowth of young plants of water lettuce (ramets) from the central meristematic region. Previous research (Richardson et al. 2008) demonstrated flumioxazin provided 97% control of water lettuce plants (9 cm diameter) with 34 g a.i. ha^{-1} and 100% with higher rates. Our research evaluated flumioxazin on larger and more mature plants (15+ cm diameter), which likely explains the lower level of flumioxazin sensitivity in our research compared to that of Richardson et al. (2008). Waterhyacinth biomass was reduced by only 41% of the nontreated control at the highest foliar flumioxazin rate evaluated (1144 g a.i. ha^{-1}) 34 DAT. The projected EC_{50} was 1435 g a.i. ha^{-1} (Figure 1), about three times the maximum label rate. Treated waterhyacinth plants exhibited blackening on younger leaves only, which is similar to injury symptoms noted on water lettuce and waterhyacinth treated with the PPO inhibitor carfentrazone-ethyl (Koschnick et al. 2004).

Subsurface flumioxazin applications provided 100% water lettuce control at concentrations ≥100 µg a.i. L^{-1} (data not shown). In contrast, flumioxazin applied to the water column failed to reduce waterhyacinth biomass by more than 30% of the nontreated control plants at any rate evaluated (data not shown) and confirms that waterhyacinth is more tolerant of flumioxazin than water lettuce. These results are similar to those reported for waterhyacinth and water lettuce treated with the PPO inhibitor carfentrazone-ethyl (Koschnick et al. 2004).

Landoltia. The effects of a subsurface application of flumioxazin to landoltia (Figure 2) show that most flumioxazin treatments caused foliar bleaching within 7 to 10 DAT, but none of the treatments resulted in complete control of landoltia. Each flumioxazin treatment was different as indicated by the 95% confidence intervals. Landoltia colonies treated at concentrations >25 µg a.i. L^{-1} began to separate, and roots became detached from individual fronds. Koschnick (2005) reported landoltia treated in the dark with diquat underwent root detachment without chlorosis. The primary function of roots of plants in the Lemnaceae family is stabilization of fronds (Landolt 1986). Diquat applied as a comparison treatment resulted in 100% control less than 5 DAT when the herbicide was foliar applied at 1.1 kg a.i. ha^{-1}. Duckweed is extremely sensitive to diquat and has a typical EC_{50} of 4 µg a.i. L^{-1} (Peterson et al. 1997), the current industry standard for duckweed control.

The foliar applied flumioxazin landoltia study was conducted three times; treated plants were visually similar to control plants at all rates up to 1144 g a.i. ha^{-1} and showed no dose response, and therefore were not harvested (data not shown). The foliar treatments to landoltia and waterhya-

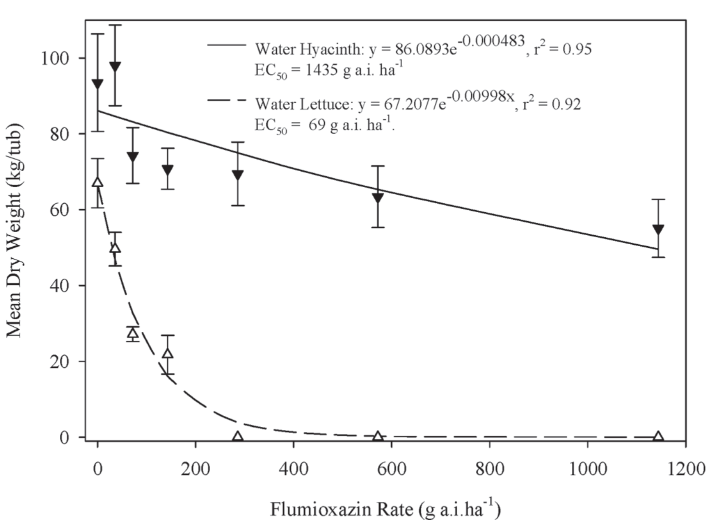

Figure 1. The effect of a foliar flumioxazin application on the dry weight of waterhyacinth and water lettuce 34 d after treatment. Data are shown as dry weight means ± standard error (n = 8). EC_{50} = effective concentration 50, concentration of flumioxazin (g a.i. ha^{-1}) required to reduce waterhyacinth and water lettuce biomass by 50%.

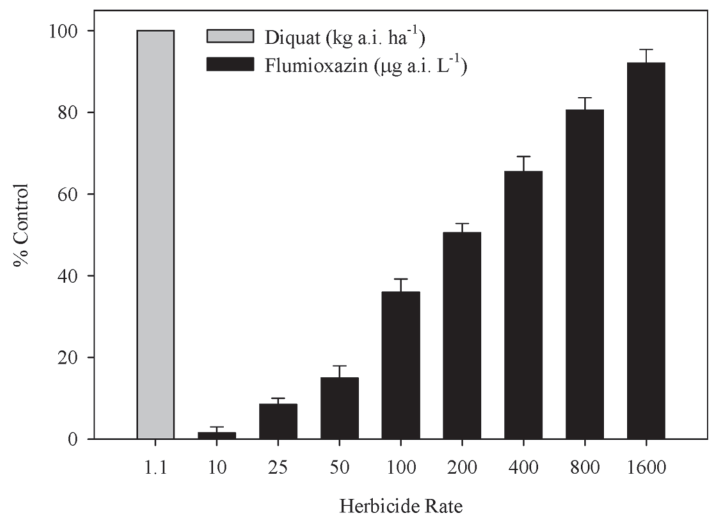

Figure 2. Percent control (visual) of landoltia 21 d after a foliar diquat (kg a.i. ha^{-1}) and subsurface flumioxazin application (µg a.i. L^{-1} a.i.). Percent control ±95% confidence interval (n = 10). Overlapping confidence interval bars indicate no significant difference.

cinth were unsuccessful, and foliar rates ≥286 g a.i. ha^{-1} were needed to control water lettuce. These results suggest that flumioxazin uptake is limited by the leaf cuticle or occurs primarily through root uptake or absorption by the underside of the plant.

Further research is needed to determine if flumioxazin applied as a subsurface treatment is as efficacious to water lettuce in higher pH water (9.0). Mudge et al. (2010) demonstrated flumioxazin in neutral pH water is more injurious to submersed aquatic plants than when applied to high pH (9.0) water.

Emergent aquatic plants. Emergent aquatic plants had highly variable sensitivity to flumioxazin aqueous concentrations up to 1600 µg a.i. L^{-1} (Figure 3). Sagittaria dry weight was reduced 100% at concentrations ≥800 µg a.i. L^{-1} compared to a 73 to 83% dry weight reduction in eleocharis, maidencane, and pickerelweed. Sagittaria was the most sensitive species to a subsurface flumioxazin treatment followed by maidencane, eleocharis, and pickerelweed based on calculated EC_{50} values for dry weight (Table 1). These data indicate eleocharis, pickerelweed, and maidencane would be injured by the maximum flumioxazin concentration of 400 µg a.i. L^{-1}; however, eleocharis and pickerelweed would likely recover from the treatment.

Visual injury symptoms observed 2 weeks after the subsurface flumioxazin application to emergent plants included interveinal chlorosis (sagittaria and pickerelweed), reddening on leaf margins (maidencane), and minor chlorosis (eleocharis). Flumioxazin and other PPO-inhibiting herbicides are absorbed primarily by plant roots with some absorbance in the shoots when applied to the soil, but translocation is limited once herbicides are absorbed into foliar tissue because of the rapid desiccation (Fadayomi and Warren 1977, Ritter and Coble 1981, Unland et al. 1999, Senseman 2007). Pots without holes were used in these studies, and few roots were visible above the soil line. Therefore, flumioxazin uptake occurred either through the underwater stem, submersed leaves, or roots. Previous research (Fadayomi and Warren 1977, Ritter and Coble 1981) found little translocation of PPO-inhibiting herbicides in plants, but the subsurface treatment of emergent aquatic plants in this study suggested movement of flumioxazin from the soil or lower stem into the leaves. If translocation of flumioxazin was limited, this herbicide should have girdled the plant at the soil line and produced injury symptoms such as necrosis of the stem and leaves without veinal chlorosis first appearing in the leaves. Ferrell et al. (2007) showed flumioxazin in combination with MSMA (monosodium salt of MAA) resulted in a 94% yield reduction when applied as a high post-direct treatment to 20 cm tall cotton. Symptomology of flumioxazin-treated cotton included necrotic lesions on leaves, reddening stems, stem girdling, and eventual lodging. Previous research also found that as cotton matures, plants become more tolerant to flumioxazin because of greater bark development and metabolic capacity (Ferrell and Vencill 2003).

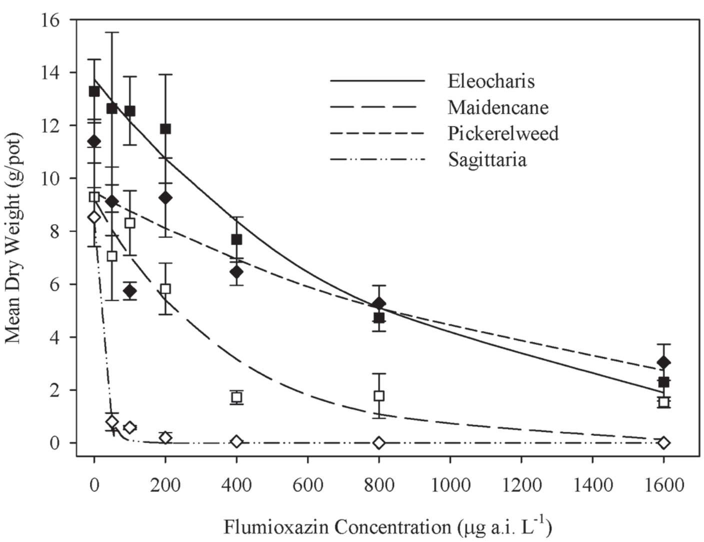

Figure 3. The effect of flumioxazin concentration on the dry weight of the nontarget emergent aquatic plants eleocharis (■), maidencane (□), pickerelweed (◆), and sagittaria (◇) 40 d after treatment. Data are shown as actual dry weight means ± standard error (n = 10).

Foliar flumioxazin treatments were much less injurious to emergent aquatic plants than subsurface treatments (Figure 4). Maidencane and sagittaria would require foliar application rates >1884 and 1320 g a.i. ha^{-1}, respectively, to reduce dry weight by 50% based on the calculated EC_{50} values (Table 1). An EC_{50} value could not be calculated for dry weight for both eleocharis and pickerelweed because increased flumioxazin concentrations resulted in an increase in dry weight (positive regression slope). Postemergent applications of flumioxazin are generally recommended for actively growing weeds <5 cm in height (Valent USA Corporation 2009), so the minimal foliar injury and substantial lack of reduction in biomass observed in this foliar study were probably due to the advanced maturity of these plants. Injury symptoms (including chlorotic and necrotic lesions on the leaves) were similar to those described for other PPO-inhibiting herbicides (Peterson et al. 2001). Tolerant species have reduced or no symptoms, whereas the leaves of susceptible species rapidly desiccate and die (Peterson et al. 2001). The limited injury symptoms exhibited by plants in the foliar experiment were similar to plants exposed to subsurface treatments.

Selective control of invasive weed species is the goal of aquatic weed managers. Herbicide applicators target invasive plants through the use of specifically formulated herbicides, seasonally timed herbicide applications, and/or preemptive spot treatments before weeds become a problem (University of Florida 2011). The native emergent plants tested in this study were tolerant to foliar flumioxazin applications, evidenced by no observed mortality. In contrast, subsurface applications resulted in more injury to nontarget plants, especially when treatments were made in low pH water. Flumioxazin is rapidly degraded by hydrolysis, with an average half-life of 4.1 d, 16.1 h, and 17.5 min at pH 5.0, 7.0, and 9.0, respectively (Katagi 2003, Senseman 2007). Mudge et al. (2010) demonstrated that water pH does not directly influence flumioxazin activity, but through an impact on aqueous flumioxazin degradation rates, pH of the treated water can have a profound impact on efficacy. Mesocosm and field trials have demonstrated differences in flumioxazin efficacy and selectivity when applied to water with a pH >8.0 (Mudge 2007, Mudge and Haller 2010).

Table 1. Calculated dry weight EC_{50} values and regression equations for four emergent nontarget plant species exposed to a subsurface (µg a.i. L^{-1}) or foliar (µg a.i. ha^{-1}) flumioxazin application 40 d after treatment[a].

Subsurface	EC_{50}[b] (95% CI[c])	Regression equation	r^2
Eleocharis	559 (389-1009)	y = 13.7460e-0.00124x	0.92
Maidencane	259 (168-564)	y = 9.2236e-0.00268x	0.84
Pickerelweed	894 (598-1777)	y = 9.4660e-0.000775x	0.91
Sagittaria	15 (11-26)	y = 8.5266e-0.0448x	0.93
Foliar			
Eleocharis	NA	y = 11.5964e0.00005x	0.99
Maidencane	1884 (1002-15753)	y = 12.8338e-0.000368x	0.92
Pickerelweed	NA	y = 9.6895e0.00013x	0.97
Sagittaria	1320 (859-2852)	y = 13.0027e-0.000525x	0.95

[a]Emergent aquatic species cultured at pH 7.5 under 70% sunlight.
[b]Effective concentration 50: EC_{50} = subsurface concentration in water (µg a.i. L^{-1}) or foliar rate (g a.i. ha^{-1}) of flumioxazin required to reduce plant dry weight or height by 50%. Each value is a mean of two experiments with a total of 10 replications (pots).
[c]Abbreviations: 95% CI = 95% Confidence Interval; NA = not applicable, due to positive regression slope.

These studies provided "worst-case" scenarios where emergent plants were continuously exposed to flumioxazin. Although residues were not collected to determine flumioxazin half-lives, the neutral pH permitted a longer plant exposure to the herbicide under static conditions compared to an herbicide application in high pH (9.0) water. In an open lake system, herbicide concentrations are influenced by factors such as wind, flow, dilution, and pH, which minimize direct contact of nontarget plants with herbicides such as flumioxazin when applied as a subsurface contact application. Direct foliar applications to these native emergent plants would occur if they grow among targeted emergent or floating plants in mixed communities. Most of these nontarget emergent plants will be minimally affected by the maximum foliar rate of 429 g a.i. ha^{-1} (Valent USA Corporation 2011).

All emergent plants in this study were relatively mature and consequently more tolerant of most foliar and subsurface flumioxazin treatments; however, most flumioxazin treatments will occur in the spring or early summer when target species, including hydrilla, can be controlled more easily due to rapid growth, lower water pH, and less target species biomass. Many nontarget emergent plants will also be immature and actively growing and could possibly be injured by foliar and subsurface flumioxazin treatments. Results of this research indicate differential efficacy and selectivity among floating and emergent target and nontarget aquatic plant species when treated with flumioxazin.

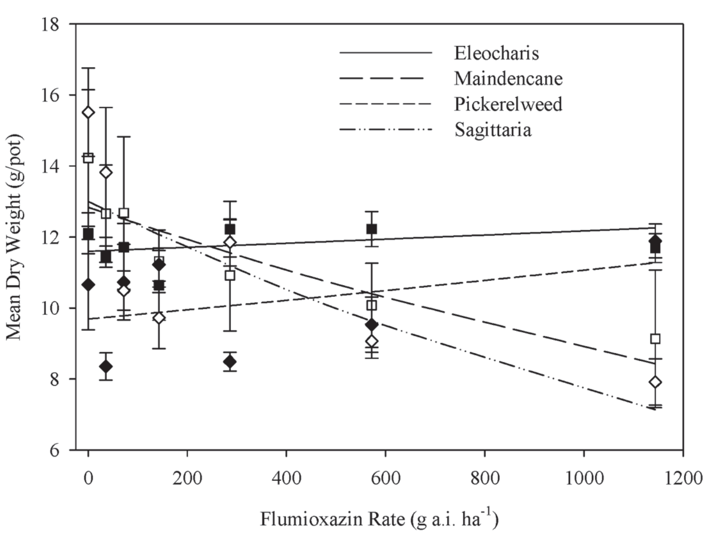

Figure 4. The effect of foliar flumioxazin applications on dry weight of the nontarget emergent aquatic plants eleocharis (■), maidencane (□), pickerelweed (◆), and sagittaria (◇) 40 d after treatment. Data are shown as actual dry weight means ± standard error (n = 10).

ACKNOWLEDGMENTS

The Aquatic Ecosystem Restoration Foundation, Valent USA Corporation, and the Florida Department of Environmental Protection Bureau of Invasive Plant Management provided funding for this research. Product was provided by Valent. Appreciation is extended to Brett Bultemeier, Margaret Glenn, David Mayo, Lyn Gettys, and Tomas Chiconela for technical assistance. Judy Shearer and Ryan Wersal 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.

SOURCES OF MATERIALS

[1]12-0-0, Lesco, Cleveland, OH
[2]Miracle-Gro®, The Scotts Company, Marysville, OH
[3]Clipper™, Valent U.S.A. Corporation, Walnut Creek, CA
[4]TeeJet Technologies, Wheaton, IL
[5]SunStream®, Brewer International, Vero Beach, FL
[6]Reward® Landscape and Aquatic Herbicide, Syngenta Crop Protection, Greensboro, NC
[7]Earthgro® Topsoil, The Scotts Company, Marysville, OH
[8]Osmocote® The Scotts Company, Marysville, OH

LITERATURE CITED

Aizawa H, Brown HM. 1999. Metabolism and degradation of porphyrin biosynthesis herbicides. Pages 348-381 *in* P. Böger, and K. Wakabayashi, ed. Peroxidizing Herbicides. Springer-Verlag, Berlin.

Cobb A. 1992. Herbicides that inhibit photosynthesis. Pages 46–80 *in* A. Cobb, ed. Herbicides and Plant Physiology. Chapman and Hall, London.

Dibble ED, Killgore KJ, Harrel SL. 1996. Assessment of fish-plant interactions. Amer. Fish. Soc. Symp. 16:357-372.

Fadayomi O, Warren GF. 1977. Uptake and translocation of nitrofen and oxyfluorfen. Weed Sci. 25:111-114.

Ferrell JA, Faircloth WH, Brecke BJ, MacDonald GE. 2007. Influence of cotton height on injury from flumioxazin and glyphosate applied post-directed. Weed Tech. 21:709-713.

Ferrell JA, Vencill WK. 2003. Flumioxazin soil persistence and mineralization in laboratory experiments. J. Agric. Food Chem. 51:4719-4721.

Getsinger KD, Madsen JD, Turner EG, Netherland MD. 1997. Restoring native vegetation in a Eurasian watermilfoil-dominated plant community using the herbicide triclopyr. Regul. Rivers Res. and Manage. 13:357-375.

Harley KLS, Forno IW, Kassulke RC, Sands DPA. 1984. Biological control of water lettuce. J. Aquat. Plant Manage. 22:101-102.

Heitmeyer ME, Vohs, PA. 1984. Distribution and habitat use of waterfowl wintering. Oklahoma. J. Wildl. Manage. 48:51-62.

Holm LG, Plucknett DL, Pancho JV, Herberger JP. 1977. The World's Worst Weeds. University Press of Hawaii, Honolulu. 609 pp.

Katagi, T. 2003. Hydrolysis of n-phenylimide herbicide flumioxazin and its anilic acid derivative in aqueous solutions. J. Pestic. Sci. 28:44-50.

Koschnick TJ. 2005. Documentation, characterization, and proposed mechanism of diquat resistance in *Landoltia punctata* (G. Meyer) D.H. Les and D.J. Crawford. PhD dissertation. Gainesville, FL: University of Florida. 123 p.

Koschnick TJ, Haller WT, Chen AW. 2004. Carfentrazone-ethyl pond dissipation and efficacy on floating plants. J. Aquat. Plant Manage. 42:103-108.

Landolt E. 1986. The family of Lemnaceae – A monographic study. Veroffentlichungen der Geobotanischen Institutes der ETH, Stiftung Rubel, Zurich.

Matringe M, Camadro JM, Labette P, Scalla R. 1989. Protoporphyrinogen oxidase as a molecular target for diphenyl ether herbicides. Biochem. J. 260:231-235.

Mudge, C. R. 2007. Characterization of Flumioxazin as an Aquatic Herbicide. PhD dissertation. Gainesville, FL: University of Florida. 120 p.

Mudge CR, Haller WT. 2010. Effect of pH on submersed aquatic plant response to flumioxazin. J. Aquat. Plant Manage. 48:30-34.

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 Manage. 48:25-30.

Owens CS, Madsen JD. 1995. Low temperature limits of water hyacinth. J. Aquat. Plant Manage. 33:63-68.

Peterson DE, Regehr DL, Thompson CR, Al-Khatib K. 2001. Herbicide Mode of Action. Publication C-715. Manhattan, KS: Kansas Cooperative Extension Service. pp. 14-15.

Peterson HG, Boutin C, Freemark KE. 1997. Toxicity of hexazinone and diquat to green algae, diatoms, cyanobacteria and duckweed. Aquat. Toxicol. 39:111-134.

Richardson RJ, Roten RL, West AM, True SL, Gardner AP. 2008. Response of selected aquatic invasive weeds to flumioxazin and carfentrazone-ethyl. J. Aquat. Plant Manage. 46:154-158.

Ritter RL, Coble HD. 1981. Penetration, translocation, and metabolism of acifluorfen in soybean (*Glycine max*), common ragweed (*Ambrosia artemisiifolia*), and common cocklebur (*Xanthium pensylvanicum*). Weed Sci. 29:474-480.

SAS Institute. 2002. SAS/STAT User's Guide. Version 9.1. Cary, NC: Statistical Analysis Systems Institute. pp. 2083-2226.

Savino JF, Stein RA. 1982. Predator-prey interactions between largemouth bass and bluegills as influenced by simulated, submerged vegetation. T. Am. Fish. Soc. 111:255-266.

Senseman SA. (editor). 2007. Herbicide Handbook. 9th ed. Lawrence, KS: Weed Science Society of America. 458 pp.

Smart RM. 1995. Preemption: An important determinant of competitive success, pp. 231-236. In: Proceedings, 29th annual meeting, Aquatic Plant Control Research Program. Miscellaneous Paper A-95-3, U. S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

University of Florida. 2011. Plant management in Florida waters: selective application of aquatic herbicides. http://plants.ifas.ufl.edu/manage/control-methods/chemical-control/selective-application-of-aquatic-herbicides. Accessed 10 Feb 2012.

Unland RD, Al-Khatib K, Peterson DE. 1999. Interactions between imazamox and diphenylethers. Weed Sci. 47:462-466.

Valent USA Corporation. 2009. SureGuard herbicide. http://www.valent.com/Data/Labels/2009-SGD-0001%20SureGuard%20-%20Form%201490-D.pdf. Accessed 10 Feb 2012.

Valent USA Corporation. 2011. Clipper Herbicide. http://www.valent.com/Data/Labels/2011-CLP-0001%20Clipper%20-%20form%201791-A.pdf. Accessed 10 Feb 2012.

Weldon LW, Blackburn RD. 1966. Waterlettuce-Nature, problem and control. Weeds. 14:5-9.

J. Aquat. Plant Manage. 50: 116-124

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

CHRISTOPHER R. MUDGE, M. A. HEILMAN, H. J. THEEL, AND K. D. GETSINGER*

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 µg 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 µg L^{-1}) decreased plant

*First, second, and third authors: Research Biologists, US Army Engineer Research and Development Center, Vicksburg, MS 39180; Second author: Aquatic Technology Leader, SePRO Corporation, Carmel, IN 46032. Corresponding author's E-mail: Christopher.R.Mudge@usace.army.mil. Received for publication March 22, 2012 and in revised form August 20, 2012.

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 fertilizer[1] 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 dye[2] 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 penoxsulam[3] and fluridone[4] treatments were applied to giant salvinia at various CET scenarios (Table 1). A nontreated reference was

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

Treatment	Concentration (µg 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 µg L^{-1}), initial exposure times (1 to 16 wk), and successive treatments (5 to 20 µg 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 µg L^{-1} exposed (Ex) for 4 wk followed by (Fb) a second application of penoxsulam at 20 µg 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 µg L^{-1} static treatments (P5Ex16 and P20Ex16) and 8 WAT for fluridone 20 µg L^{-1} exposed for 4 wk Fb 5 µg 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 µg L^{-1} (static), fluridone at 20 µg L^{-1} (static), penoxsulam at 10 µg L^{-1} (static) plus fluridone at 20 µg 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]µg a.i. L^{-1}.
[c]Abbreviations: 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 glyphosate[5] at 3.36 kg acid equivalent (a.e.) ha^{-1} (24 h exposure) plus diquat[6] at 280.35 g a.i. ha^{-1} (24 h exposure; Table 3). A nonionic + buffering agent surfactant at 0.25% v/v[7] was added to the penoxsulam foliar treatments and nonionic + buffering agent (Aqua-King Max) plus nonionic organosilicone[8] 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 CO_2-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	—	—

[a]Nonionic + buffering agent surfactant (0.25% v/v) added.
[b]Nonionic + buffering agent (0.25% v/v) and nonionic organo-silicone (0.125% v/v) surfactants added.

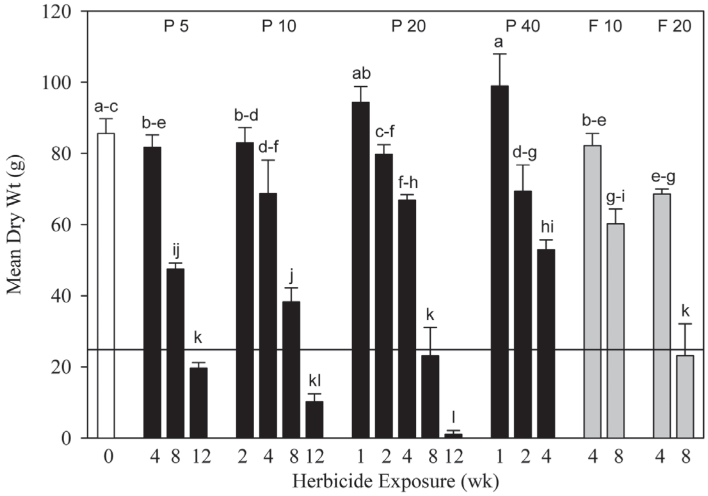

Figure 1. Effect of exposure time and concentration of penoxsulam (P) and fluridone (F) on giant salvinia dry weight (mean ± standard error) 16 weeks after treatment (WAT). Numbers behind herbicide abbreviations represent herbicide concentrations in µg a.i. L^{-1}. Horizontal line represents mean pretreatment 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 µg L^{-1} exposed for 12 wk. Fluridone at 20 µg L^{-1} (8 wk exposure) was equally effective as penoxsulam at 5 (12 wk exposure), 10 (12 wk exposure), or 20 µg 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 µg L^{-1} rate of each herbicide were 26.0 and 10.8 µg 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 µg 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 ≥75% and to less than pretreatment level (Figure 2). Static penoxsulam treatments (5, 10, and 20 µg 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 µg 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 µg L^{-1} for a 4 wk exposure followed by 20 and 5 µg L^{-1}, respectively (Table 2). Both herbicide treatments resulted in a decrease in giant salvinia biomass by the conclusion of

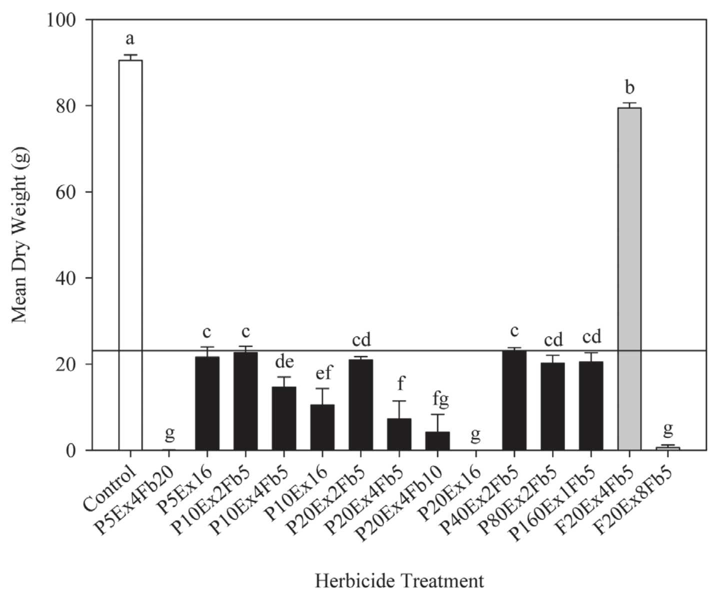

Figure 2. Effect of single static and multiple penoxsulam (P) and fluridone (F) subsurface applications (µg a.i. L^{-1}) coupled with exposure time on the growth of giant salvinia dry weight (mean) 16 weeks after treatment (WAT). Horizontal line represents mean pretreatment biomass for giant salvinia. Herbicide treatment abbreviation example as follows: penoxsulam applied at 5 µg L^{-1} exposed (Ex) for 4 wk followed (Fb) by 20 µg 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 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 salviniae*), 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-

Table 4. Herbicide concentrations measured from giant salvinia treated with subsurface applications of penoxsulam 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 penoxsulam 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
	16 WAT	1.8 ± 0.11
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

[a]Penoxsulam applied as a onetime treatment and plants exposed for 16 wk; n = 4.

[b]Abbreviations: DAT, days after treatment; WAT, weeks after treatment.

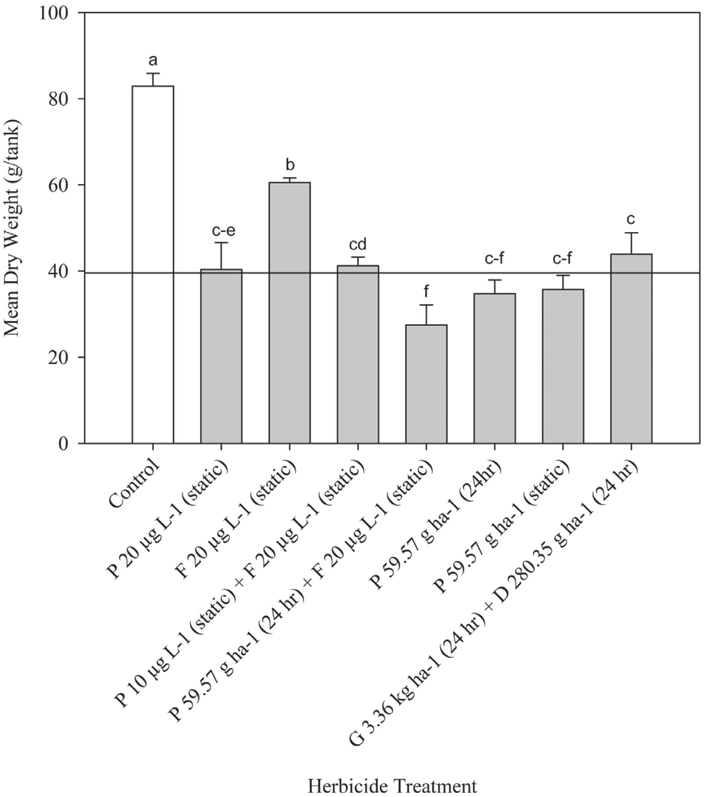

Figure 3. Effect of subsurface and foliar penoxsulam (P), fluridone (F), glyphosate (G), and diquat (D) applications on mature giant salvinia mean dry weight (± S.E.) 11 weeks after treatment (WAT). Plants exposed to static treatments remained for the duration of the experiment, while 24 h indicates 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 µg L^{-1} and never exceeded 6.1 µg 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 (µg a.i. L^{-1} ± S.E.)
Fluridone[a] (20 µg 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 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

[a]Fluridone applied on day of treatment and reapplied 5 WAT to increase concentration to 20 µg a.i. L^{-1}.
[b]Abbreviations: DAT, days after treatment; WAT, weeks after treatment; n = 4.

µg L^{-1} for the first week of the study; however, concentrations decreased to 22.8 µg 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 µg 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 µg 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 µg L^{-1} at 1 WAT and decreased to 7.8 µg 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 µg 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 µg 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 µg L^{-1} in all experimental units containing fluridone. 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 µg 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 (Netherland 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 µg 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 µg 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 ± 1.26 µg 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 µg 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

[1]Miracle-Gro® 36-6-6, The Scott's Company, Marysville, Ohio
[2]Aquashade®, Applied Biochemists, Germantown, Wisconsin
[3]Galleon SC®, SePRO Corporation, Carmel, Indiana
[4]Sonar AS®, SePRO Corporation, Carmel, Indiana
[5]AquaPro®, SePRO Corporation, Carmel, Indiana
[6]Reward® Landscape and Aquatic Herbicide, Syngenta Professional Products, Greensboro, NC
[7]Aqua-King Plus®, Winfield Solutions, LLC, St. Paul, Minnesota
[8]Thoroughbred®, Winfield Solutions, LLC, St. Paul, Minnesota
[9]TeeJet Technologies, Wheaton, IL

ACKNOWLEDGMENTS

This research was supported by SePRO Corporation/Aquatic Ecosystem Restoration Foundation and the US Army Aquatic Plant Control Research Program. Appreciation is extended to L. Nelson, C. Grodowitz, J. Namanny, M. Robertson, J. Smith, K. DeRossette, M. Sternberg, and A. Poovey for technical assistance throughout the research. D. Sanders (LSU AgCenter) and A. Perret (Louisiana Department of Wildlife and Fisheries) kindly provided recommendations on giant salvinia control in Louisiana. M. Netherland and L. Glomski kindly 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 CITED

Anderson WP. 1996. Weed Science: Principles and Applications, 3rd ed. West Publishing Company, St. Paul, MN.

Cary PR, Weerts PGJ. 1983. Growth of *Salvinia molesta* as affected by water temperature and nutrition. III. Nitrogen-phosphorus interactions and effect of pH. Aquat. Bot. 19:171-182.

Forno IW, Harley KLS. 1979. The occurrence of *Salvinia molesta* in Brazil. Aquat. Bot. 6:185-187.

Getsinger KD, Madsen JD, Koschnick TJ, Netherland MD, Stewart RM, Honnell DR, Staddon AG, Owens CS. 2001. Whole-lake applications of Sonar TM for selective control of Eurasian watermilfoil. Technical Report ERDC/EL TR-01-7, US Army Engineer Research and Development Center, Vicksburg, MS.

Glomski LM, Netherland MD. 2008. Efficacy of fluridone, penoxsulam, and bispyribac-sodium on variable-leaf milfoil. J. Aquat. Plant Manage. 46:193-196.

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. ANS Digest 4(2):13-16.

Johnson S, Sanders D, Eisenberg L, Whitehead K. 2010. Fighting the blob: efforts to control giant salvinia. Louisiana Agriculture. 5(1)3:6-9.

Koschnick TJ, Haller WT, Vandiver VV, Santra U. 2003. Efficacy and residue comparisons between two slow-release formulations of fluridone. J. Aquat. Plant Manage. 41:25-27.

Koschnick T, Heilman MA, Miller S. 2007a. Penoxsulam: a new aquatic herbicide for large-scale aquatic plant management. Proc. Aquatic Plant Management Soc. 47:41.

Koschnick TJ, Netherland MD, Haller WT. 2007b. Effects of three ALS-inhibitors on five emergent native plant species in Florida. J. Aquat Plant Manage. 45:47-51.

Lembi CA, Chand T. 1992. Response of hydrilla and Eurasian watermilfoil to flurprimidol concentrations and exposure times. J. Aquat. Plant Manage. 30:6-9.

Madsen JD, Wersal RM. 2009. Giant salvinia. Invasive Plant Atlas of the MidSouth. http://www.gri.msstate.edu/ipams/Species.php?SName= Salvinia+ molesta&CName=. Accessed 9 Aug 2010.

McFarland DG, Nelson LS, Grodowitz MJ, Smart RM, Owens CS. 2004. *Salvinia molesta* D. S. Mitchell (giant salvinia) in the United States: a review of species ecology and approaches to management. SR- 04-2. U.S. Army Engineer Research and Development Center, Environmental Laboratory, Vicksburg, MS. 40 pp.

Moran PJ. 2012. Influence of biological control damage on efficacy of penoxsulam and two other herbicides on waterhyacinth. J. Aquat. Plant Manage. 50:32-38.

Mudge CR, Harms NE. 2012. Development of an integrated pest management approach for controlling giant salvinia using herbicides and insects. US Army Engineer Research and Development Center, Vicksburg, MS. APCRP Bulletin. ERDC APCRP-A-12-1.

Murai N, Skoog F, Doyle ME, Hanson RS. 1980. Relationships between cytokinin production, presence of plasmids, and fasciation caused by strains of *Corynebacterium fascians*. Proc. Natl. Acad. Sci. USA. 77:619-623.

Nelson LS. 1996. Growth regulation of Eurasian watermilfoil with flurprimidol. J. Plant Growth Regul. 15:33-38.

Nelson LS. 1997. Response of hydrilla and American pondweed to flurprimidol. J. Aquat. Plant Manage. 35:50-54.

Nelson LS, Skogerboe JG, Getsinger KD. 2001. Herbicide evaluation against giant salvinia. J. Aquat. Plant Manage. 39:48-53.

Nelson LS, Glomski LM, Gladwin DN. 2007. Effect of glyphosate rate and spray volume on control of giant salvinia. J. Aquat. Plant Manage. 45:58-61.

Netherland MD. 2011. Comparative susceptibility of fluridone resistant and susceptible hydrilla to four ALS inhibiting herbicides under laboratory and greenhouse conditions. J. Aquat. Plant Manage. 49:100-106.

Netherland MD, Getsinger KD. 1995a. Laboratory evaluation of threshold fluridone concentrations under static conditions for controlling hydrilla and Eurasian watermilfoil. J. Aquat. Plant Manage. 33:33-36.

Netherland MD, Lembi CA. 1992. Gibberellin synthesis inhibitor effects on submersed aquatic weed species. Weed Science. 40:29-36.

Owens CS, Smart RM. 2010. Effects of nutrients, salinity, and pH on *Salvinia molesta* (Mitchell) growth. Vicksburg, MS: US Army Engineer Research and Development Center. APCRP Technical Notes Collection. ERDC/TN APCRP-EA-23.

Owens CS, Smart RM, Stewart RM. 2004. Low temperature limits of giant salvinia. J. Aquat. Plant Manage. 42:91-94.

Richardson RJ, Gardner AP. 2007. Evaluation of penoxsulam for water hyacinth [*Eichhornia crassipes* (Mart.) Solms] and giant salvinia (*Salvinia molesta* Mitchell) control. Weed Sci Soc Am Abstr. 47:58. http://wssa.net/Meetings/WSSAAbstracts/abstractsearch.php. Accessed 20 Mar 2012.

Senseman SA. 2007. Herbicide Handbook. 9th ed. Lawrence, KS: Weed Science Society of America. 458 p.

Theel HJ, Nelson LS, Mudge CR. 2012. Growth regulating hydrilla and subsequent effects on habitat complexity. J. Aquat. Plant Manage. *In press*.

Thomas P, Room PM. 1986. Taxonomy and control of *Salvinia molesta*. Nature. 320(17):581-584.

Tranel PJ, Wright TR. 2002. Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Science. 50:700-712.

Wersal RM, Madsen JD. 2007. Comparison of imazapyr and imazamox for control of parrotfeather (*Myriophyllum aquaticum* (Vell.) Verdc.). J. Aquat. Plant Manage. 45:132-136.

Wersal RM, Madsen JD. 2010. Combinations of penoxsulam and diquat as foliar applications for control of waterhyacinth and common salvinia: evidence of herbicide antagonism. J. Aquat. Plant Manage. 48:21-25.