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

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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

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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, $p \le 0.05$); 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-

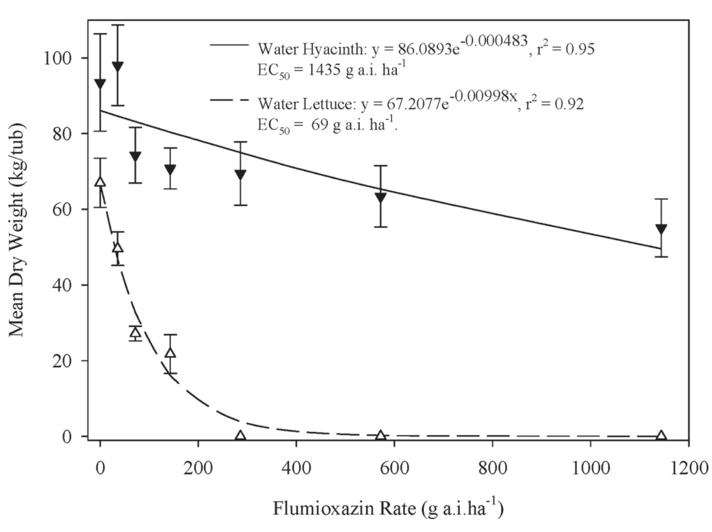

Figure 1. The effect of a foliar flumioxazin application on the dry weight of waterhyacinth and water lettuce 34 d after treatment. Data are shown as dry weight means ± standard error (n = 8). EC_{50} = effective concentration 50, concentration of flumioxazin (g a.i. ha^{-1}) required to reduce waterhyacinth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

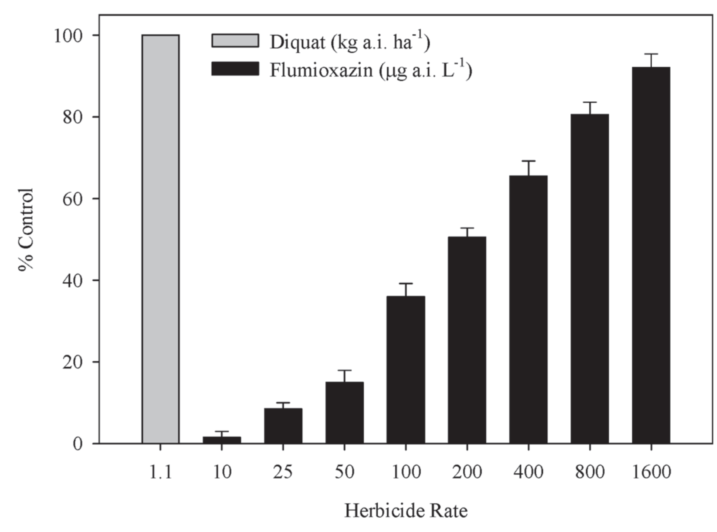

Figure 2. Percent control (visual) of landoltia 21 d after a foliar diquat (kg a.i. ha^{-1}) and subsurface flumioxazin application (µg a.i. L^{-1} a.i.). Percent control ±95% confidence interval (n = 10). Overlapping confidence interval bars indicate no significant difference.

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

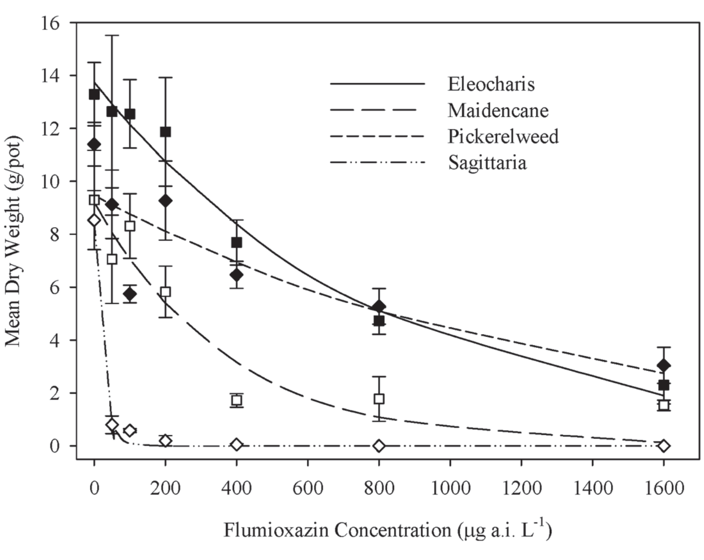

Figure 3. The effect of flumioxazin concentration on the dry weight of the nontarget emergent aquatic plants eleocharis (■), maidencane (□), pickerelweed (◆), and sagittaria (◇) 40 d after treatment. Data are shown as actual dry weight means ± standard error (n = 10).

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).

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.

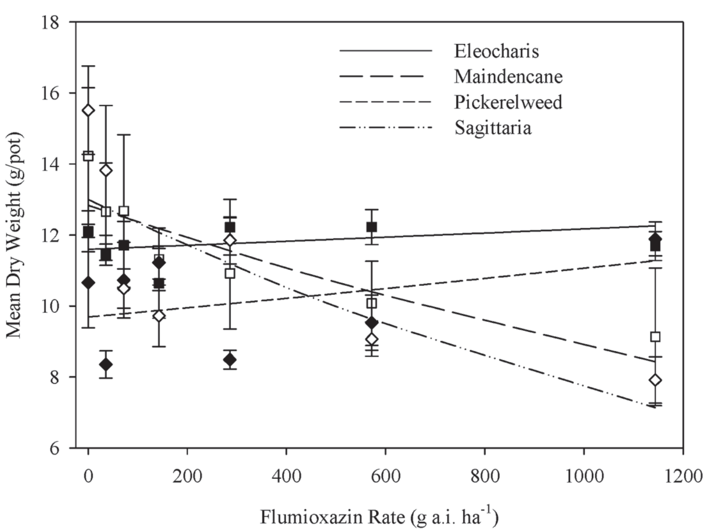

Figure 4. The effect of foliar flumioxazin applications on dry weight of the nontarget emergent aquatic plants eleocharis (■), maidencane (□), pickerelweed (◆), and sagittaria (◇) 40 d after treatment. Data are shown as actual dry weight means ± standard error (n = 10).

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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

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

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ABSTRACT

Giant salvinia (*Salvinia molesta* Mitchell) continues to be problematic and spread throughout the southern portion of the United States. Traditional management of this invasive weed has been application of the foliar herbicides diquat, glyphosate, and combinations of the two. Unfortunately, thick surface mats that limit contact with foliar sprays and fast recovery potential have resulted in mixed efficacy. Three experiments were conducted to determine the efficacy of subsurface and foliar penoxsulam and subsurface fluridone applications on giant salvinia. These studies were conducted to determine concentration exposure time (CET) relationships, determine if repeat applications can be as effective as single static applications of each respective herbicide, and if subsurface or foliar applications will control mature giant salvinia compared to standard foliar treatments used operationally. In the CET experiment, both herbicides were more effective at growth regulating or controlling giant salvinia when exposed ≥8 wk, regardless of concentration. All penoxsulam concentrations evaluated (5 to 40 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

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