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Effect of pH on Submersed Aquatic Plant Response to Flumioxazin

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ABSTRACT

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) was applied to the submersed aquatic plant species coontail (*Ceratophyllum demersum* L.), egeria (*Egeria densa* Planch.), hydrilla (*Hydrilla verticillata* [L.f.] Royle), and vallisneria (*Vallisneria americana* Michaux) at concentrations of 0, 100, 200, 400, 800, and 1600 µg active ingredient (a.i.) L^{-1} under high (9.0) and neutral (7.0) pH. Flumioxazin was more efficacious when applied to plants growing in neutral pH conditions than when applied under high pH conditions. Coontail was the only submersed species to be controlled in high pH conditions at the maximum label Experimental Use Permit (EUP) concentration of 400 µg L^{-1}. Other species evaluated in this study required concentrations >3194 µg L^{-1} to reduce biomass by 50% when applied to high pH water. In contrast, plants exposed in neutral pH water conditions, were often severely injured following exposure to flumioxazin. Increasing tolerance of species treated in neutral pH water based on dry-weight calculated effective concentration 50% (EC$_{50}$) values were (in µg L^{-1}) coontail (34), hydrilla (77), vallisneria (1244), and egeria (3285). Flumioxazin concentrations as low as 50 µg L^{-1} initially injured (bleaching, reddening, and defoliation) most plant species at both pHs; however, plants generally began to produce some healthy new growth prior to harvest. Results of these studies demonstrated a differential species tolerance to flumioxazin and a potential for a strong influence of pH to impact treatment efficacy as well as selectivity.

Key words: Ceratophyllum demersum, chemical control, EC$_{50}$: Effective Concentration 50, *Egeria densa*, *Hydrilla verticillata*, protoporphyrinogen oxidase inhibitor, *Vallisneria americana*.

INTRODUCTION

One of the primary goals of aquatic weed management in public and private waters is to control growth of invasive plant species while maintaining a diversity of native submersed and emergent species. Native aquatic plants can improve water clarity and quality, provide valuable fish and wildlife habitat, reduce sediment resuspension, and help prevent the spread of invasive plants (Savino and Stein 1982, Heitmeyer and Vohs 1984, Smart 1995, Dibble et al. 1996). Selective removal of invasive species is beneficial for continued existence and diversity of native vegetation. Invasive submersed aquatic species often form dense canopies that significantly increase surface water temperature, reduce dissolved oxygen, and decrease light penetration for native species (Bowes et al. 1979, Honnell et al. 1993). Native plant density and diversity have been shown to increase when canopy-forming exotic plants are removed (Getsinger et al.

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1997) and continued presence of native vegetation allows diversity of invertebrate and fish habitats to be maintained (Dibble et al. 1996). Selective removal of hydrilla (*Hydrilla verticillata* [L.f.] Royle) and other invasive species with aquatic herbicides can be beneficial for retention of native vegetation. However, damage to native species can result from subsurface and foliar applications of herbicides and is an important factor in herbicide selection.

The submersed aquatic weed hydrilla has been a serious problem in the United States, especially in Florida, for many years (Haller and Sutton 1975, Cook 1985). In 2005, there were more than 20,000 ac of hydrilla in Florida lakes, while 312,000 ac of hydrilla have been controlled since 1981 (FWC 2005). There are a limited number of herbicides available for aquatic use in the United States with even fewer available for hydrilla control. Flumioxazin a protoporphyrinogen oxidase (PPO; protoporphyrin IX:oxygen oxidoreductase, EC 1.3.3.4) inhibiting herbicide, has been evaluated in greenhouse and field studies for control of hydrilla and other invasive species (Cranmer et al. 2000, Price et al. 2002, 2004, Hartzler 2004, FDACS 2006, Mossler et al. 2006, Mudge 2007). 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).

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). Field trials have demonstrated flumioxazin efficacy has been highly dependent on water pH especially when applied to water with pH >8.0 (Mudge 2007). The majority of these trials were conducted in water bodies where hydrilla was the dominant or only species present. Development and use of herbicides that selectivity control nontarget aquatic plants is a priority of many state agencies involved in aquatic weed management (Koschnick et al. 2007, UF 2007). Submersed nontarget aquatic plants could be impacted by flumioxazin applications; therefore, the objective of these studies was to quantify the effects of flumioxazin on native and invasive submersed aquatic plant species under neutral and higher pH conditions.

MATERIALS AND METHODS

The submersed aquatic species coontail (*Ceratophyllum demersum* L.), egeria (*Egeria densa* Planch.), hydrilla, and vallisneria (*Vallisneria americana* Michaux) were evaluated for sensitivity to flumioxazin at a high (9.0) and neutral (7.0) pH in 2006 and 2007. The high pH study was only conducted once (August 2006); the neutral pH study was conducted in September 2006 and repeated in January 2007. The high pH and initial neutral pH experiments were conducted outside under a shade house (70% sunlight), whereas the repeated neutral pH experiment was conducted inside a greenhouse (70% sunlight).

Hydrilla was collected from Rodman Reservoir near Interlachen, Florida in July, September, and December 2006, while all other species were purchased from a local plant nursery. Two sprigs of each species were planted in 1-L pots (10 by 10 by 12 cm) that were filled with masonry sand amended with Osmocote®[3] (15-9-12) fertilizer at a rate of 1g kg^{-1} soil and placed in 95-L plastic tanks lined with polyethylene bags and filled with well water (pH 7.5 at planting). Each tub contained all four species (two pots of each species/tub). Plants were allowed to acclimate for 2 weeks prior to herbicide application. This experiment was a randomized design with four replicates (tanks). Flumioxazin was applied as a subsurface treatment at concentrations of 0, 50, 100, 200, 400, 800, and 1600 µg L^{-1} under high and neutral pH conditions. Water pH in all tanks was ≥9.0 (9.4 ± 0.4) prior to treatment so each neutral pH treatment tub was treated with about 15 ml of muriatic acid (20.1% HCl) to lower pH to 7.0 at around 7:00 AM. The pH was monitored in the neutral pH treatments and remained stable through 24 h after treatment (HAT). Water pH was not maintained at 7.0 beyond 24 HAT because flumioxazin has been reported to degrade to <1 µg L^{-1} within about 4 HAT once the pH increases to 9.0. (Katagi 2003, Senseman 2007). Although the amount of time required for flumioxazin uptake into hydrilla and other submersed species has not been determined, previous research has shown an exposure of <24 h was sufficient to reduce hydrilla biomass by 90%, regardless of water pH at treatment (Mudge 2007). In addition, once hydrilla has established in

TABLE 1. CALCULATED EC$_{50}$ (µG A.I. L^{-1}) AND REGRESSION EQUATIONS FOR FOUR SUBMERSED AQUATIC PLANT SPECIES EXPOSED TO SUBSURFACE FLUMIOXAZIN APPLICATION 4 WEEKS AFTER TREATMENT.

High pH (9.0)	EC$_{50}$[a] (95%CI[b])	Regression equation	r^2
Coontail	403 (248-1081)	y = 7.9148e-0.00172x	0.86
Egeria	3747 (1720-23104)	y = 2.6419e-0.000185x	0.94
Hydrilla	3194 (869-6931)	y = 4.0822e-0.000351x	0.83
Vallisneria	5172 (2173-13863)	y = 3.6688e-0.000134x	0.95
Neutral pH (7.0)			
Coontail	34 (27-46)	y = 9.6997e-0.0204x	0.87
Egeria	3285 (1925-11179)	y = 2.8606e-0.000211x	0.94
Hydrilla	77 (53-138)	y = 3.8329e-0.00902x	0.86
Vallisneria	1244 (732-4151)	y = 2.2940e-0.000557x	0.90

[a]Effective concentration 50: EC$_{50}$ = concentration of flumioxazin (µg a.i. L^{-1}) in water required to reduce plant dry weight by 50%. Each value is a mean of one experiment with a total of four replications (pots) for high pH; eight replications for coontail, egeria, hydrilla and vallisneria at neutral pH.
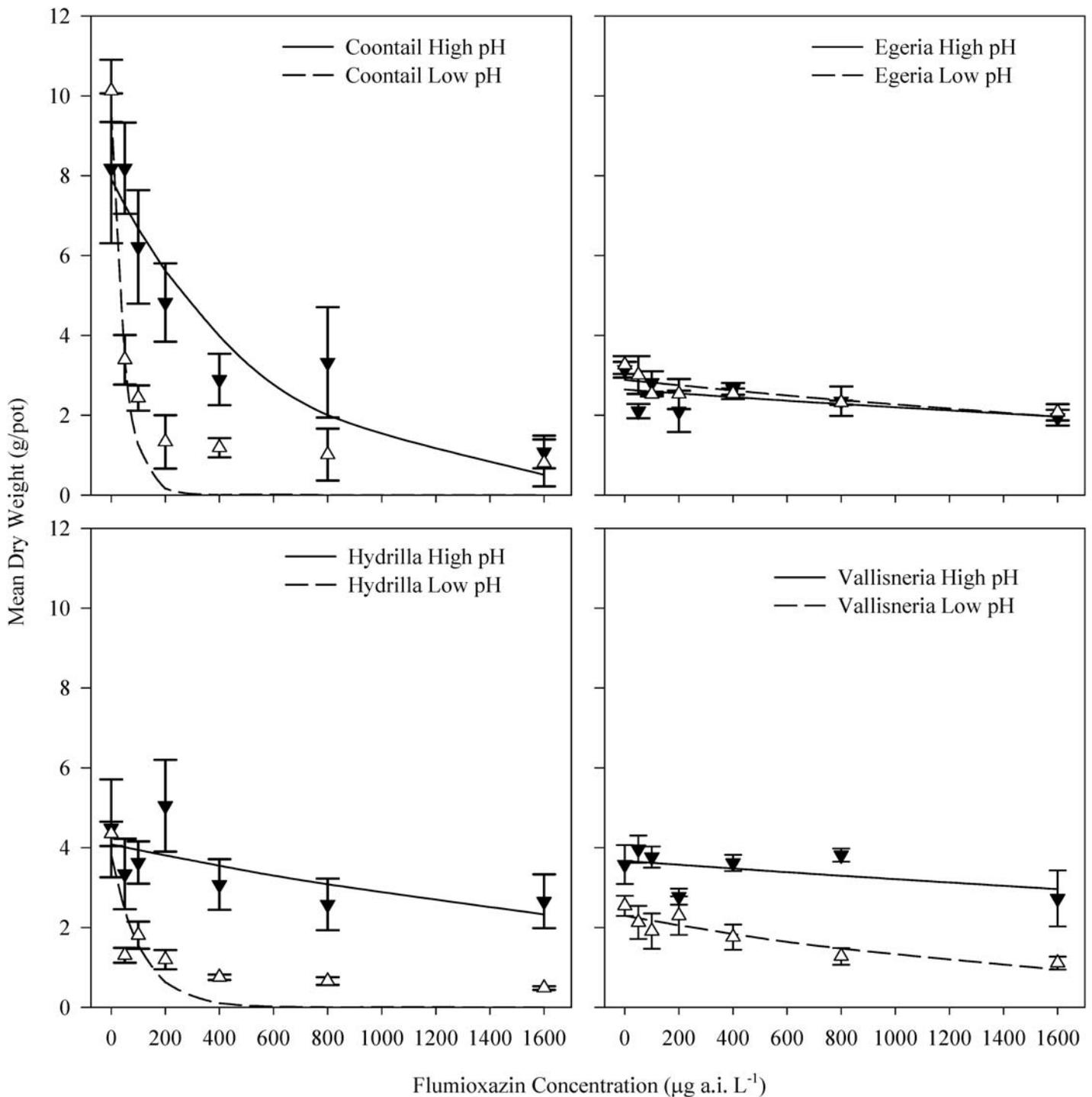[b]Abbreviations: 95% CI = 95% Confidence Interval.

Figure 1. The effect of flumioxazin concentration on the dry weight of submersed aquatic plant species 4 weeks after exposure. Flumioxazin applied as a single subsurface application to submersed aquatic species cultured in neutral (7.0) and high (9.0) pH water in 95-L tubs. Data are shown as actual dry weight means ± standard error (n = 4 for high pH; n = 8 for neutral pH).

ponds and lakes, the pH will typically rise during the day and reach peak levels by mid morning to early afternoon (Van et al. 1976).

All living plant tissue was harvested at the soil line 4 weeks after treatment (WAT), placed in a drying oven at 90 C for 1

week and weighed. Plant dry weight data were analyzed by nonlinear regression (exponential decay; PROC NLIN, SAS Institute 2002). Regression models were used to determine the effective concentration 50 (EC_{50}), the concentration of flumioxazin required to cause a 50% reduction in dry weight

compared to untreated control plants. Data from both neutral pH experiments were pooled for coontail, egeria, hydrilla, and vallisneria because there was no difference between the slopes of regression lines at the 95% confidence interval level.

RESULTS AND DISCUSSION

At treatment, plant dry weights (g ± standard error; high pH/neutral pH) were as follows: coontail (7.0 ± 0.6/6.7 ± 1.1), egeria (2.4 ± 0.2/3.0 ± 0.6), hydrilla (1.8 ± 1.1/3.2 ± 0.1), and vallisneria (2.7 ± 0.8/1.7 ± 0.4). Final dry weights for control plants (high pH/neutral pH) were as follows: coontail (8.2 ± 1.9/9.8 ± 0.9), egeria (3.1 ± 0.2/3.3 ± 0.2), hydrilla (4.5 ± 1.2/4.3 ± 1.0), and vallisneria (3.6 ± 0.5/2.5 ± 0.3). These data indicate plants grown under high and neutral pH conditions were actively growing at the time of flumioxazin treatment. Symptoms of hydrilla treated with flumioxazin were bleaching in the apical tip and reddening in the stem followed by necrosis. Visual symptoms of other treated plants in these studies included bleached apical tips followed by reddening of the stem (egeria), defoliation and loss of stem/leaf integrity (coontail), and leaf transparency (vallisneria).

The pH in the control tanks (pH 7.0) returned to 9.0 within 24 HAT. The pH of water in the 100, 200, and 400 µg L^{-1} neutral pH treatments returned to 9.0 at approximately 36 HAT, while pH of water in the two highest herbicide concentrations (800 and 1600 µg L^{-1}) never exceeded 8.5. All flumioxazin treatments applied in neutral pH water injured coontail and hydrilla to a greater extent than flumioxazin applied under high initial pH conditions (Table 1; Figure 1). Coontail and hydrilla required flumioxazin concentrations of 34 and 77 µg L^{-1}, respectively, to reduce dry weight by 50% (EC$_{50}$) when exposed at pH 7.0, whereas all other species exposed to flumioxazin at this pH required concentrations ≥517 µg L^{-1}. Based on EC$_{50}$ values, egeria and vallisneria were the only species relatively tolerant of flumioxazin regardless of pH (Table 1).

Coontail treated under high pH conditions was the only species to be injured at the maximum flumioxazin concentration of 400 µg L^{-1}. All other species treated in high pH water would require an estimated flumioxazin concentration of >3194 µg L^{-1} to reduce biomass by 50%. These data indicate the necessary flumioxazin concentrations to significantly injure or control these species at pH 9.0 were not present for a long enough period when compared to the neutral pH treatment. Flumioxazin is hydrolyzed at a much slower rate in the neutral pH water compared to the high pH water (Senseman 2007). The reported half-life of flumioxazin at pH 5.0, 7.0, and 9.0 under controlled laboratory conditions is 4.1 d, 16.1 h, and 17.5 min, respectively (Katagi 2003). As the water pH increases, flumioxazin degradation increases from hours (neutral pH) to minutes (high pH). These data suggest only a few hours of exposure are required to injure or control submersed aquatic plants; therefore, it is important that flumioxazin is applied when the pH is at its lowest point (morning or early season). The pH in hydrilla-infested water will likely increase up to 3 units as the day progresses, but these data indicate only short exposure requirements may be necessary.

The pH of Florida lakes infested with surface matted hydrilla may be >8.0 and likely >9.0 as a result of the hydrilla utilizing free CO_2 and HCO^-_3 during photosynthesis (Van et al. 1976). Nontarget species vallisneria and coontail should be slightly injured by flumioxazin in high pH water based on these data. Hydrilla demonstrated initial flumioxazin injury symptoms when applied under high pH conditions; nonetheless, several new apical tips sprouted <1 WAT from treated tissue and the 1600 µg L$^-$ treatment reduced dry weight by only 40% of the nontreated control plants. Based on these data, single flumioxazin applications will likely provide less than desirable hydrilla control when applied to high pH water. Conversely, if applied to neutral pH water, this herbicide could be beneficial to aquatic weed managers.

Coontail were highly susceptible to flumioxazin in neutral pH water and would likely be injured or controlled at the proposed label rate of 400 µg L^{-1} (Table 1). Egeria and vallisneria were minimally affected by flumioxazin. Most species exposed to flumioxazin in the high pH treatment and several in the neutral pH treatments were beginning to recover from the treatment as new leaves and shoots had begun to develop prior to harvest. A pH-mediated degradation of flumioxazin via hydrolysis will typically result in short exposure periods in higher pH waters (Katagi 2003). This study demonstrated a differential species tolerance to flumioxazin and a potential for a strong influence of pH to impact treatment efficacy as well as selectivity. Further research should be conducted to determine the sensitivity of other non-target plant species in waters of varying pH.

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Comparative Aquatic Dissipation Rates Following Applications of Renovate OTF Granular Herbicide and Rhodamine WT Liquid

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ABSTRACT

A field study was conducted in October 2008 to compare the dissipation rates of concurrent applications of a granular formulation of triclopyr herbicide (Renovate® OTF) and the inert dye Rhodamine WT, acting as a surrogate for a liquid herbicide. Applications were made to a relatively deep 4-ha plot (mean depth = 4.75 m) in a cove of Grandview Lake, Indiana. Renovate OTF was applied using boat-mounted, forced-air spreaders at a dose of 800 µg L^{-1} triclopyr. Rhodamine WT was applied through two long, trailing, weighted-hoses at a dose of 14 µg L^{-1} dye. Following application, both compounds rapidly mixed within the water column. Monitoring of water concentrations demonstrated relatively rapid dissipation patterns due to water exchange; 26.7 h half-life for triclopyr and 12.4 h for the dye. The results indicate the granular formulation would have a 2.2x longer exposure time and a different vertical residue profile than a subsurface injection of a liquid formulation, suggesting the potential for greater plant efficacy.

Key words: dye, formulation, granule, triclopyr.

INTRODUCTION

Rapid dissipation of aquatic herbicides due to various water exchange processes can lead to poor submersed weed control in a variety of situations. The ability to target herbicide placement and maintain the concentration in the plant mass within the 3-dimensional aquatic environment can be critical to maximize efficacy. Additional variables such as

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