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J. Aquat. Plant Manage. 48: 25-30

Evaluating the Influence of pH-Dependent Hydrolysis on the Efficacy of Flumioxazin for Hydrilla Control

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ABSTRACT

Hydrilla (*Hydrilla verticillata* [L.f.] Royle) is a submersed aquatic weed that continues to spread and create significant management problems in waters throughout the United States. Management tools are limited, and several new herbicide modes of action are being evaluated for control, including 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), a rapid-acting contact herbicide that targets the plant enzyme protoporphyrinogen oxidase (PPO). Despite the rapid onset of flumioxazin injury symptoms and activity at low concentrations (<100 µg L^{-1}), regrowth of hydrilla from lateral buds was noted within 5 to 13 d after treatment at all concentrations. The lack of a consistent dose-response relationship in prior laboratory and field trials led to the hypothesis that pH-dependent hydrolysis of flumioxazin likely influenced efficacy. We evaluated the response of hydrilla exposed to an initial flumioxazin treatment of 400 µg L^{-1} in low (6.0 to 6.2), medium (7.0 to 7.2), and high (>8.5) pH water. Water samples were collected and the half-life of flumioxazin in low, medium, and high pH water was 39.0, 18.6, and 1.7 h, respectively. The results from these studies indicate that efficacy of flumioxazin, especially when applied to higher pH water, may be reduced via rapid hydrolysis.

Key Words: hydrolysis, protoporphyrinogen oxidase inhibitor.

INTRODUCTION

Hydrilla (family Hydrocharitaceae; *Hydrilla verticillata* [L.f.] Royle) is a submersed aquatic fresh-water angiosperm native to Asia or Africa that has become a serious weed problem in the United States and many other countries (Haller and Sutton 1975, Cook 1985, Van and Vandiver 1992, USDA 2007). Hydrilla has been described as "the perfect aquatic weed" due to its specialized growth habit, physiological characteristics, and various means of asexual reproduction (Langeland 1996). The dioecious biotype of hydrilla was present in more than 50,000 ha of Florida's public waters in 2007, with an approximate management cost of $16 million (FDEP 2007). In the 1960s and 1970s, hydrilla was primarily controlled with contact herbicides including diquat, endothall, and diquat plus copper combinations (Brian et al. 1958,

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Received for publication August 25, 2009 and in revised form January 5, 2010.

Hiltibran 1963, Sutton et al. 1972, Simsiman et al. 1976). During the next two decades (1980 to 2000), large areas of hydrilla in Florida were being controlled with the systemic herbicide fluridone (Haller et al. 1990, MacDonald et al. 2001); however, poor performance was observed in the late 1990s and the development of fluridone resistance in hydrilla was characterized in 2004 (Michel et al. 2004, Arias et al. 2005, Puri et al. 2007). Since then, fluridone-resistant populations of hydrilla have expanded in the waterways of Florida, and new effective herbicides are being evaluated for possible aquatic registration.

Flumioxazin is registered for pre-emergence weed control in peanut (*Arachis hypogaea* L.) and soybean (*Glycine max* L.), and for post-direct weed control in cotton (*Gossypium hirsutum* L.; Grichar and Colburn 1996, Askew et al. 1999, Burke et al. 2002, Clewis et al. 2002, Main et al. 2003). Following flumioxazin treatment, precursors to chlorophyll react with light to produce toxic singlet oxygen radicals leading to lipid peroxidation and the destruction of cellular components (Duke et al. 1991, Gupta and Tripathy 2000). Irreversible damage to the plasmalemma and tonoplast membrane lipids is followed by swelling of organelles, rupture of organelle membranes, and eventually rupture of the cellular membranes in susceptible plants (Duke et al. 1989). Entire cell contents (both cytoplasmic and vacuolar) are released after extensive membrane destruction, resulting in cell desiccation and electrolyte leakage (Becerril and Duke 1989, Duke and Kenyon 1993).

Flumioxazin affects hydrilla, has low nontarget toxicity, and is rapidly degraded in the aquatic environment. Initial data indicated hydrilla dry weight was reduced by 63% and 99% in static tests when treated with flumioxazin at 50 and 400 µg L^{-1}, respectively (Mudge and Haller 2006). It has been noted that pH can have a significant influence on flumioxazin degradation in an aqueous environment, with half-lives noted from just minutes (pH ≥ 9.0) to >4 d (pH ≤ 5.0; Katagi 2003, Senseman 2007). In hydrilla-infested waters, the pH may fluctuate by several units due to utilization of free CO_2 and HCO^-_3 (Van et al. 1976). High pH waters likely influence the efficacy of flumioxazin by shortened exposure through rapid hydrolysis. Flumioxazin field trials for hydrilla control conducted throughout Florida in 2006 and 2007 resulted in inconsistent results. While some early season treatments were effective, late season applications in water bodies with pH ranging from 7.5 to 9.5 failed to provide adequate control. The impact of pH on flumioxazin efficacy on hydrilla has not been determined for hydrilla; therefore, the objective of these studies was to determine if flumioxazin efficacy could be impacted by varying the pH and treating hydrilla under a series of concentration and exposure time scenarios.

MATERIALS AND METHODS

Initial Determination of EC_{50} Values

An initial mesocosm study was conducted and repeated at the Center for Aquatic and Invasive Plants (CAIP) at the University of Florida to determine the effect of different doses of flumioxazin on hydrilla. Hydrilla was collected from Rodman Reservoir near Interlachen, Florida in June 2005 and February 2007. Four sprigs of hydrilla (15 cm) were planted in each 10 by 10 by 12 cm (1-L) pot filled with masonry sand amended with Osmocote®[5] 15-9-12 fertilizer at a rate of 1g kg^{-1} soil. Five pots were placed in each 95-L high-density polyethylene (HDPE) tank filled with tap water (pH 7.5 at planting). The experiment was a completely randomized design with five replications (tanks). The initial study was conducted outside in a shade house (70% sunlight) in July 2005, while the repeated study was conducted in a greenhouse (70% sunlight) in April 2007. Hydrilla was allowed to acclimate for 2 weeks in 2005 and 6 weeks in 2007 before herbicide treatment. Hydrilla was immature (actively growing) and had just began to branch at the water surface (pH 8.5 to 9.5) at the time of treatment. Flumioxazin was applied as a subsurface treatment at 0, 50, 100, 200, 400, 800, and 1600 µg L^{-1} and thoroughly mixed in the water column.

All plants were harvested 21 d after treatment (DAT) by clipping biomass at the soil line; shoots were placed in a drying oven at 70 C for about 1 week and then weighed. Plant dry weight data were analyzed using nonlinear regression (exponential decay; PROC NLIN, SAS Institute 2002), and regression models were used to determine the effective concentration 50 (EC_{50}), the concentration of flumioxazin in water that resulted in a 50% reduction in dry weight compared to control plants. Data from both studies were pooled because there was no difference between the slopes of regression lines for both studies at the 95% confidence interval.

Impact of pH on Efficacy

Hydrilla was collected from 900-L concrete mesocosm stock tanks at CAIP, and one 15-cm sprig was planted in a 50-ml plastic tube (13.5 by 4 cm dia). Tubes were filled with potting media[6], amended with Osmocote fertilizer at a rate of 1g kg^{-1} soil, and topped with a 1-cm sand cap. A total of 144 tubes were planted, and hydrilla was acclimated in 900-L concrete tanks (pH 8.0) for 3 weeks prior to herbicide treatment. The initial study was conducted in September 2006 and repeated in May 2007 in an outdoor mesocosm system under a shade house (70% sunlight). Prior to herbicide treatment, one tube planted with hydrilla was placed into a 95-L HDPE tank filled with 83 L of well water (pH 8.0). Efficacy of flumioxazin was evaluated in low (6.0 to 6.2), medium (7.0 to 7.2), and high (>8.5) pH water. Muriatic acid (20.1% HCl) was added at a rate of 10 ml or 25 ml per tub to establish the initial medium or low pH treatments, respectively. Hydrilla was 45 ± 3.6 cm long with an average dry weight of 0.51 ± 0.3 g at treatment. Flumioxazin was applied as a subsurface treatment at a rate of 400 µg L^{-1} and thoroughly mixed in the water column. The pH was monitored daily, and small amounts of acid (1 to 3 ml) were added as needed to maintain desired pH levels in the medium and low pH treatments. Following the initial treatment, an additional tube of hydrilla was added every 24 h to the treated tanks at 1, 2, 3, 4, and 5 DAT to assess if pH or a pH mediated degradation of flumioxazin impacted efficacy. Day 0 plants were in the tubs at the time of treatment (day 0) and removed from the treated solutions after 4 d of ex-

posure, placed in a 900-L tank with flowing water (two exchanges per day, pH 7.5) and allowed to recover for 21 d prior to harvest. Similarly, all other treated plants (day 1 to 5) followed the same exposure and post-treatment regime as the day 0 plants. Throughout the course of the study (day 0 to day 5), control plants were also placed in and removed from untreated tanks with low, medium, and high pH water for comparative purposes. This experiment was conducted as a completely randomized design with four replications (treatment tubs). Water samples were collected from the low and medium pH treatment at 0.5, 1, 24, 48, 72, 96, and 120 h after treatment (HAT). Due to anticipated rapid degradation of flumioxazin under high pH conditions, water samples in the high pH treatment were collected at 0.25, 0.5, 1, 4, 7, 19, 24, 48, 72, 96, and 120 HAT. All water samples were immediately acidified with 0.5 ml of muriatic acid at the time of collection to a pH <4.0 to prevent hydrolysis. The samples were frozen and shipped to the Valent U.S.A. Corporation laboratory (Walnut Creek, CA) for flumioxazin analysis.

Flumioxazin was analyzed using a gas chromatograph (GC) equipped with a nitrogen-phosphorus specific flame-ionization detector (NPD) after the water was partitioned with dichloromethane. Sample extracts were quantified using a Hewlett-Packard (HP) Model 5890 GC/NPD, equipped with a packed column inlet (RTX-1, 30 m by 0.53 mm by 0.25 µm) and an HP series 530 µm column adapter (HP part # 19244-80540) with a glass insert (HP Part # 5080-8732, packed with approximately 5 mm of silanized glass wool). Agilent Chemstation computer software was used for data collection and reporting. Instrument parameters were as follows: Oven temperature was 250 C isothermal with a run time of 6.25 min and detector temperature of 280 C. The limit of quantitation (LOQ) was 5 µg L^{-1} and the limit of detection (LOD) was 2 µg L^{-1}. Concurrent laboratory fortified control water samples were fortified with flumioxazin at levels from 5 to 400 µg L^{-1}, and recoveries ranged from 77 to 111%. The overall average method recoveries in water were 93 ± 12% (n = 8).

All hydrilla biomass above the soil line was harvested 21 d after removal from the treated solutions. Shoots were placed in a drying oven at 70 C for about 1 week and then weighed. Plant weight data were converted to percent of the respective nontreated plants at each pH for each day and analyzed as a mixed model (PROC MIXED, SAS Institute 2002) with experiment used as a random factor. The water pH was considered a fixed effect, while experiment, replication (nested within experiment), and all interactions containing either of these effects were considered random effects. Classification of experiment (or the combination of experiment and location) as an environmental or random effect, permits inferences about pH to be made over a range of environments (Carmer et al. 1989, Hager et al. 2003). Type III statistics were used to test all possible effects of fixed factors. Least square means were used for mean separation at $p \leq 0.05$, and data from both studies were pooled because there were no differences between studies. Water residue data were analyzed using nonlinear regression (PROC NLIN, SAS Institute 2002) to calculate flumioxazin half-life at each pH.

RESULTS AND DISCUSSION

Initial Determination of EC_{50} Values

Flumioxazin applied at 50 to 1600 µg L^{-1} to actively growing immature hydrilla in mesocosms resulted in bleaching of the upper 5 cm of all apical tips within 3 DAT, and stems began to redden (probably due to anthocyanin production) from about 3 cm below the tip to the soil surface. Bleached apical tips began to abscise and decay within 3 to 7 DAT, when the plants began to lose cellular integrity and decay 1 to 2 weeks after treatment (WAT). Despite rapid bleaching, loss of integrity, and reduced biomass, hydrilla in all treatments began to regrow from treated tissue and formed new lateral shoots at the axillary buds on damaged stems within 5 to 13 DAT depending on flumioxazin concentration. The calculated EC_{50} of flumioxazin was 56 µg L^{-1} (Figure 1), while the EC_{90} for flumioxazin was 186 µg L^{-1} (data not shown). While the low EC_{50} value suggests a high level of initial hydrilla sensitivity to flumioxazin, the ability of the plant to form new healthy meristems via lateral buds following an exposure to rates 28 times greater than the EC_{50} value, would suggest that concentration exposure time relationships for flumioxazin and hydrilla are not likely to be as straightforward as observed for other aquatic herbicides. The confounding effect of differential rates of hydrolysis in different pH water challenges conventional concepts of determining concentration and exposure time relationships.

Impact of pH on Efficacy

The data show the effect of a 4-d exposure to flumioxazin in tanks of low, medium, and high pH water on the biomass of hydrilla based on the percent of nontreated control (Figure 2).

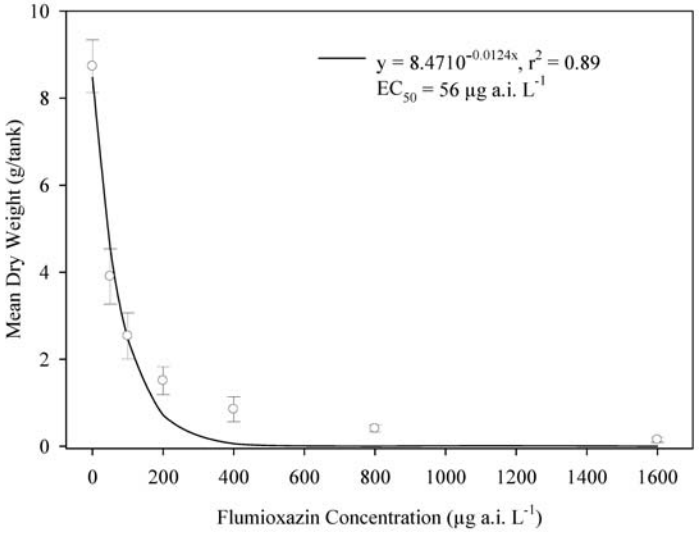

Figure 1. The effect of flumioxazin concentration on hydrilla dry weight 21 d after exposure. Flumioxazin applied as a single application to hydrilla cultured in 95-L tubs (pH 9.0 to 9.5) under 70% sunlight. Data are shown as dry weight means ± standard error (n = 10). EC_{50} = effective concentration 50, concentration of flumioxazin in water required to reduce hydrilla biomass by 50%.

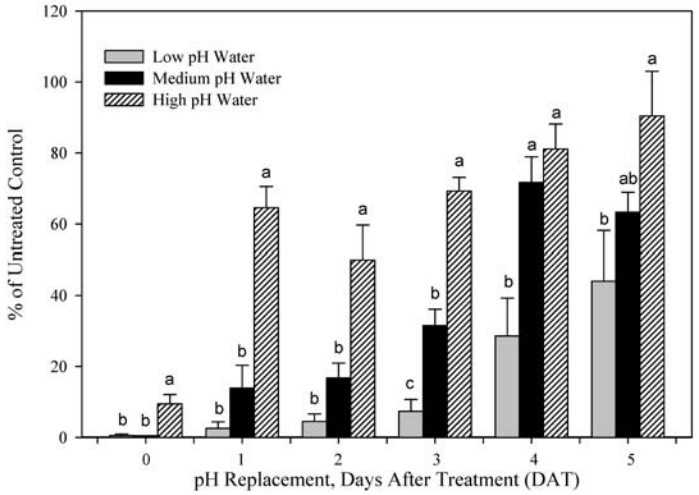

Figure 2. The effect of flumioxazin at 400 µg L^{-1} on hydrilla dry weight as influenced by low (6.0 to 6.2), medium (7.0 to 7.2), and high (>8.5) water pH under 70% sunlight. Hydrilla plants were added to low, medium, and high pH water treated with flumioxazin 0 to 5 d after initial treatment, exposed for 96 h, removed and allowed to grow in flowing, herbicide-free water (pH 8.0) for 21 d after treatment until harvest. Data are shown as percent of untreated control of each pH ± standard error (n = 8). Treatment means within a particular day were separated using least square means ($p \leq 0.05$), and means with the same letter within a particular day are not significant.

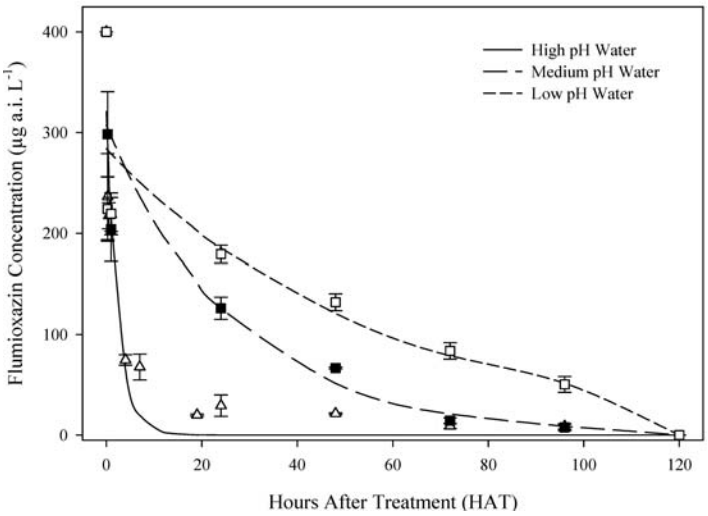

Figure 3. Dissipation of flumioxazin applied at 400 µg L^{-1} to low (6.0 to 6.2), medium (7.0 to 7.2) and high pH (>8.5) water in 95-L tubs under 70% sunlight. The dissipation of flumioxazin was calculated using nonlinear regression (exponential decay) for the low ($y = 0.0178e^{-0.0178x}$; $r^2 = 0.92$; half-life 39.0 h), medium ($y = 0.3074e^{-0.0373x}$; $r^2 = 0.93$; half-life 18.6 h), and high ($y = 0.3209e^{-0.3991x}$; $r^2 = 0.94$; half-life 1.7 h) pH treatments. All residues are reported as the mean ± standard error (n = 6).

Hydrilla plants that were in the tanks at the time of treatment (day 0), that remained in the treatment solution for 4 d, and were then removed to clean water for 21 d were either dead or about 10% of the control dry weight after the grow-out period. The recovery of hydrilla in the high pH treatment exposed at day 0 was greater than the medium and low pH treatments. Hydrilla plants that were placed in the treatment solution on day 1, that remained there for 4 d, and were then removed to herbicide-free water for 21 days showed greater recovery than day 0 plants; plants in the high pH treatment recovered to >60% of the untreated control plants. Hydrilla placed in high treatment solutions on day 5 for 4 d recovered to about 90% of control plant biomass. These data show that pH of the treatment solutions has a large impact on hydrilla control and regrowth, presumably due to rapid flumioxazin breakdown.

Hydrilla biomass as a percent of the untreated control generally increased daily, and this increase in biomass (or reduction in injury) corresponded with a decrease in flumioxazin residues (Figure 3). Plants in tanks treated with flumioxazin at medium and low pH levels were still exposed to herbicide through the 96-h exposure period, while about 98% of flumioxazin in the high pH treatment was hydrolyzed by 3 DAT (Figure 3). Although the residue analysis detected <10 µg L^{-1} of flumioxazin in the high pH solution, hydrilla biomass was still reduced by about 10 to 20% on the last 2 days of exposure. These data and the EC_{50} data calculated from the mesocosm efficacy study indicate flumioxazin is active at low concentrations (<10 µg L^{-1}).

The half-life of flumioxazin in low, medium, and high pH water (6.0 to 6.2, 7.0 to 7.2 and >8.5, respectively) was 39, 18.6, and 1.7 h, respectively (Figure 3). Katagi (2003) reported that the half-life of flumioxazin at pH 5.0, 7.0, and 9.0 under controlled laboratory conditions, was 98.4, 16.1, and 0.3 h, respectively. It was not possible to completely control pH in these tanks, but flumioxazin degradation in both of our studies followed a similar trend to the reported laboratory trials. Under field conditions, pH can fluctuate significantly during the course of a day, and consequently, flumioxazin may be exposed to different levels of pH within the same water body depending on factors such as treatment timing, density of plant growth, and background pH levels**.** Note that rapid disruption of hydrilla photosynthesis following flumioxazin exposure has been demonstrated at the laboratory scale, and therefore a treatment could potentially increase the longevity of flumioxazin by reducing the photosynthetic-driven increase in pH (Mudge 2007). Several aquatic herbicides are metabolized by either photolysis or microbial activity (Senseman 2007), and pH is not typically a water quality parameter associated with aquatic herbicide efficacy. In contrast, diurnal pH cycling may limit when flumioxazin can be applied to successfully control hydrilla (Mudge 2007).

These mesocosm studies document the impact of pH on flumioxazin efficacy. The data indicate that pH does not directly influence flumioxazin activity, as verified by the >90% control achieved following initial exposure of the hydrilla. These data provided evidence of rapid flumioxazin uptake in hydrilla, as exhibited by biomass reduction in high pH treatments. If plants treated at the high pH did not absorb flumioxazin within the first few minutes or hours after treatment, these plants would not become chlorotic and biomass would not be reduced by 90% as observed. From an operations perspective, it is likely that a pH-mediated degradation would influence efficacy by altering the concentration and exposure time to hydrilla. This study dem-

onstrated that treatments in higher pH water can provide control of hydrilla biomass if rapid mixing of flumioxazin can be insured. Conversely, field treatments in water with a high pH and high density of plant matter may prevent rapid mixing and result in degradation of flumioxazin residues prior to the majority of the plant tissue receiving an adequate exposure. While other contact herbicides such as endothall require longer exposures (24+ h) to achieve control of hydrilla (Netherland et al. 1991), degradation rates also tend to be much slower (several days) than observed for flumioxazin. Flumioxazin is active following very short exposure times; however, it also benefits from the increased exposure provided by a low pH environment. The rapid degradation of flumioxazin in higher pH conditions will likely prevent acceptable lateral and vertical movement of residues in the target treatment area.

Hydrilla treated with flumioxazin in Experimental Use Permit (EUP) ponds in Florida showed a high degree of variation in treatment results (Mudge 2007). Symptoms on field-treated plants (bleaching of tips and reddening of the stem below the tip) looked similar to results observed in mesocosm studies. In addition to a potential for pH to impact efficacy, the impact of light on flumioxazin has also been hypothesized. Upon forming a dense surface canopy, hydrilla can significantly limit light penetration. Flumioxazin and other protoporphyrinogen oxidase (PPO) inhibitors are more active in the presence of light and may require full sunlight for optimal activity (Sherman et al. 1991, Wright et al. 1995). Additionally, oxygen-derived free radicals have very short half-lives ranging from milliseconds to microseconds (Kobayashi et al. 1989), and the short half-life of flumioxazin in higher pH water potentially reduces radical formation. By the time sufficient light reaches the lower apical tips and stem segments, flumioxazin presumably has been degraded by hydrolysis and is no longer present to inhibit the PPO (Matringe et al. 1989, Cobb 1992, Aizawa and Brown 1999). High light conditions (>1000 µmol m^{-2} s^{-1}) in all greenhouse experiments facilitated bleaching as a result of inhibition of chlorophyll and membrane disruption through radical production (Duke et al. 1989).

These data provide evidence that water pH does not directly influence flumioxazin activity on hydrilla, but through an impact on aqueous flumioxazin degradation rates, pH of the treated water can have a profound impact on efficacy. Depending on the ability to achieve rapid mixing of the herbicide, flumioxazin treatments in high pH water (>9.0) may result in rapid degradation of the molecule, resulting in poor control of hydrilla.

ACKNOWLEDGMENTS

The authors thank Valent U.S.A. Corporation for proving product and partial funding of this research as well as the Aquatic Ecosystem Restoration Foundation for providing partial funding. Additionally, appreciation is extended to M. S. Glenn, D. G. Mayo, B. W. Bultemeier, C. Hulon, and L. A. Gettys for plant maintenance and harvest. 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.

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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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Received for publication May 19, 2009 and in revised form October 28, 2009.