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Combinations of Penoxsulam and Diquat as Foliar Applications for Control of Waterhyacinth and Common Salvinia: Evidence of Herbicide Antagonism

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ABSTRACT

Waterhyacinth (*Eichhornia crassipes* [Mart.] Solms) and common salvinia (*Salvinia minima* Baker) are two floating aquatic plants that can cause wide-spread problems in the southern United States. These species can cause reductions in ecosystem function as well as the abundance of native plant species. Herbicides are often used in an attempt to control both species; however, few recommendations exist for common salvinia. Penoxsulam (2-(2,2-difluoroethoxy)-*N*-(5,8 dimethoxy [1,2,4] triazolo [1,5-*c*] pyrimidin-2-yl)-6 (trifluoromethyl) benzenesulfonamide) is newly registered for use in aquatic environments and is efficacious on floating plants as a submersed application; however, foliar application rates are largely undefined. The objectives of these studies were to determine the effect of foliar application rates of penoxsulam for waterhyacinth and common salvinia, and to evaluate the effectiveness of combinations of penoxsulam with diquat against these same plants species. A mesocosm study was conducted using foliar rates of penoxsulam (24.5, 49.1, and 98.2 g ai ha^{-1}) alone and in combination with diquat (130.8 g ai ha^{-1}). At six weeks after treatment (WAT), penoxsulam alone at all rates resulted in 100% control of waterhyacinth, and at 10 WAT control remained ≥90%. Penoxsulam was not as effective at controlling common salvinia. The combination of herbicides did not increase efficacy, and there was evidence of antagonism at the rates tested. Future studies should assess lower rates for waterhyacinth control and influences of salvinia mat thickness on application timing and herbicide efficacy.

Key words: Eichhornia crassipes, Galleon SC®, Reward®, *Salvinia minima*.

INTRODUCTION

Waterhyacinth (*Eichhornia crassipes* [Mart.] Solms) and common salvinia (*Salvinia minima* Baker) are widespread problems in waterways throughout the southern United States. Waterhyacinth is an invasive free-floating aquatic plant from the tropical and subtropical regions of South America (Holm et al. 1991). Waterhyacinth effectively doubles the number of plants within 12.5 d (Penfound and Earle 1948), increases dry biomass at a rate of 1.2% d^{-1}, and peak biomass can reach a maximum of 2.5 kg m^{-2} under optimal conditions (Center and Spencer 1981). Waterhyacinth impedes the recreational use of rivers and lakes (fishing, swimming, and boat traffic) and the generation of hydroelectric power. Furthermore, waterhyacinth increases the potential for flooding, reduces primary productivity (e.g., phytoplankton), and alters ecosystem properties (Toft et al. 2003).

Common salvinia is a free-floating aquatic fern native to central and South America (Olguin et al. 2002). While not as well known an invasive species as the congeneric giant salvinia (*Salvinia molesta* Mitchell), it is a significant nuisance weed in southern aquatic and wetland systems (Jacono and Richerson 2008). Common salvinia is capable of high growth rates and is tolerant to a wide range of environmental conditions (Olguin et al. 2002). In Louisiana, common salvinia biomass reached 1.02 kg m^{-2} and caused reductions in native plant abundance (Walley 2007).

To counteract the negative impacts often associated with non-native aquatic plants, effective control methods need to be identified. Penoxsulam (2-(2,2-difluoroethoxy)-*N*-(5,8 dimethoxy [1,2,4] triazolo [1,5-*c*] pyrimidin-2-yl)-6 (trifluoromethyl) benzenesulfonamide) was registered in 2008 for use in aquatic environments and may be effective in controlling non-native aquatic plants. Penoxsulam is an acetolactate synthase (ALS) inhibiting herbicide with a broad spectrum of grass and broadleaf weed control (Senseman 2007). Penoxsulam is readily absorbed by leaves, shoots, and roots, and is translocated to meristematic tissues via phloem and/or xylem flow (Senseman 2007). Susceptible plant injury usually results in rapid growth inhibition followed by chlorosis, vein reddening, and plant death within 4 weeks after treatment (WAT; Senseman 2007).

Penoxsulam is efficacious as a submersed application for control of waterhyacinth and giant salvinia at relatively low

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[4]Galleon SC®, SePRO Corporation, Carmel, Indiana.

[5]Reward® Landscape and Aquatic Herbicide, Syngenta Professional Products P.O. Box 18300 Greensboro, NC 27419.

[6]Sunwet®, Brewer International, P.O. Box 690037, Vero Beach, FL 32969. Received for publication August 16, 2009 and in revised form January 10, 2010.

use rates (ppb; Richardson and Gardner 2007), but foliar application rates for use as a spot treatment are largely undefined with no data available on common salvinia efficacy. Labeled rates of penoxsulam for control of floating species as a foliar application are 2 to 5.6 oz acre^{-1} (35.1 to 98.9 g ai ha^{-1}), with control taking up to 60 d or longer for some plant species (SePRO Corporation 2009). Therefore, combinations of penoxsulam with low or sublethal rates of a contact herbicide, such as diquat, may shorten the control period or provide rapid visual indication of exposure. The objectives of this study were to (1) determine foliar application rates of penoxsulam that are efficacious on waterhyacinth and common salvinia; and (2) evaluate whether tank mixing penoxsulam and diquat improves the speed and effectiveness of control for waterhyacinth and common salvinia.

MATERIALS AND METHODS

The study was conducted in 378-L mesocosms at the R. R. Foil Plant Science Research Center, Mississippi State University, for 10 weeks from August to November 2007. Waterhyacinth and common salvinia were planted from greenhouse stock at Mississippi State University by randomly placing each species into 24 mesocosms (48 mesocosms total) to cover the water surface. Plants were allowed to grow for approximately 2 weeks in respective mesocosms prior to herbicide applications. Mesocosms were amended with 30 mg L^{-1} of Miracle Gro®[3] fertilizer (24-8-16) weekly to maintain growth. A single pretreatment biomass sample was collected from every mesocosm the same day as herbicide applications using a 0.10 m^2 quadrat for waterhyacinth and a 0.05 m^2 quadrat for common salvinia.

Foliar applications of penoxsulam as Galleon® SC[4] were applied at 24.5, 49.1, and 98.2 g ai ha^{-1} (1.4, 2.8, 5.6 oz acre^{-1}) alone and in combination with diquat applied as Reward®[5] at 130.8 g ai ha^{-1} (4 oz acre^{-1}). All combination treatments were mixed in the same tank. A diquat-alone treatment was included as well as an untreated reference for statistical purposes. A 0.5% v:v methylated seed oil surfactant (Sunwet®[6]) was added to the spray solution, and the solutions applied at 935 L ha^{-1} using a CO_2 pressurized single nozzle (8002 flat fan) spray apparatus. Each treatment was replicated in 3 mesocosms. After herbicide application, mesocosms were immediately drained and refilled to remove any residual herbicide in the water. Plants were rated weekly for percent control on a scale of 0 (no control) to 100% (complete control) in 10% increments. At 6 and 10 WAT a single biomass sample was harvested in all waterhyacinth mesocosms using the 0.10 m^2 quadrat and two samples harvested in all common salvinia mesocosms using the 0.05 m^2 quadrat. The quadrats were placed into respective tanks and then live green plant material was harvested from within the quadrats.

A general linear model was used in SAS® to determine differences between control ratings within weeks, and a Fisher's Protected LSD was used to separate any differences. A similar analysis was conducted on biomass within species at 6 and 10 WAT. All analyses were conducted at a $p = 0.05$ level of significance.

Herbicide synergism or antagonism of penoxsulam and diquat was estimated using the dry weight biomass of waterhyacinth 10 WAT with the following equation outlined by Colby (1967):

$$E_1 = (X_1 + Y_1) - (X_1 Y_1 / 100) \qquad (1)$$

Waterhyacinth biomass was first converted to percent control prior to estimating herbicide response. In equation 1, E_1 is the expected control with herbicides A + B; X_1 is observed control with herbicide A; and Y_1 is control with herbicide B. When the observed plant response is greater than the expected response, the combination is synergistic; when less than expected the combination is antagonistic. When the observed and expected responses are equal the combination is considered additive. Observed and expected values were calculated for each replication (mesocosm) and values subjected to a Wilcoxin Rank Sum Test to determine statistical significance between the difference of the observed and expected values. Estimates were not computed for common salvinia due to rapid plant recovery in all treatments.

RESULTS AND DISCUSSION

Waterhyacinth

Visual waterhyacinth control was 65 to 70% at 1 WAT when applications included diquat, whereas control with penoxsulam alone was only 20 to 25% (Table 1). By 3 WAT, control was similar between the penoxsulam alone and combination treatments. All treatments that included penoxsulam were more effective than diquat alone treatments. At 4 WAT, the penoxsulam-alone treatments resulted in greater control than any treatment containing diquat. At 6 WAT, penoxsulam alone resulted in 100% control of waterhyacinth, and at 10 WAT control remained ≥90%. Applications containing diquat resulted in significant control early in the study when compared to untreated reference plants; however, there was significant antagonism between diquat and penoxsulam when used in combination (Table 2). By 8 WAT all combination treatments resulted in less control than penoxsulam alone.

Based on waterhyacinth biomass (Figure 1), there was significant antagonism between diquat and penoxsulam (Table 2). The penoxsulam alone treatments were more efficacious then the combination of penoxsulam + diquat and diquat alone. Cedergreen et al. (2007) reported that antagonism was the most common type of interaction between herbicides. Furthermore, the antagonistic response could be so severe that the effect of a single herbicide is reduced in the presence of the other herbicide. For example, the transport of the systemic herbicide glyphosate (N-(phosphonomethyl) glycine) was reduced when mixed with the photosystem II inhibitor terbuthylazine (*N*^{2}-*tert*-butyl-6-chloro-*N*^{4}-ethyl-1,3,5-triazine-2,4-diamine) (Cedergreen et al. 2007). Diquat also inhibited the translocation of glyphosate in the terrestrial plant *Phyllanthus tenellus* Roxb., where it was concluded diquat produced rapid visual symptoms but inhibited long

Table 1. Control ratings of waterhyacinth and common salvinia following foliar applications of penoxsulam and diquat alone and in combination.

Herbicide (g ai ha^{-1})	Weeks After Treatment[a]									
	One	Two	Three	Four	Five	Six	Seven	Eight	Nine	Ten
	%									
Waterhyacinth										
Untreated Reference	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
Penoxsulam 24.5	20 b	50 b	85 cd	90 e	100 d	100 d	100 e	100 e	100 f	100 e
Penoxsulam 49.1	25 b	50 b	80 c	90 e	95 d	100 d	100 e	90 de	90 ef	90 de
Penoxsulam 98.2	20 b	50 b	80 c	90 e	100 d	100 d	100 e	95 de	95 ef	95 de
Penoxsulam 24.5 + Diquat 130.8	70 c	70 c	70 c	70 c	70 c	60 bc	60 c	60 c	60 c	60 c
Penoxsulam 49.1 + Diquat 130.8	70 c	80 d	80 c	85 de	90 cd	90 bcd	85 de	80 d	80 ed	80 cd
Penoxsulam 98.2 + Diquat 130.8	70 c	70 c	80 c	80 d	80 c	70 bcd	70 cd	65 c	70 cd	70 c
Diquat 130.8	65 c	55 b	55 b	50 b	50 b	40 b	40 b	35 b	35 b	35b
LSD	8	9	12	9	12	31	18	15	16	16
Common salvinia										
Untreated reference	0 a	0 a	0a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
Penoxsulam 24.5	70 c	75 bc	70 bcd	65 c	60 c	30 ab	0 a	0 a	0 a	0 a
Penoxsulam 49.1	70 c	65 b	60 b	55 bc	45 bc	25 ab	0 a	0 a	0 a	0 a
Penoxsulam 98.2	80 c	90 bc	90 c	80 c	55 bc	30 ab	0 a	0 a	0 a	0 a
Penoxsulam 24.5 + Diquat 130.8	70 c	60 b	50 b	30 b	20 ab	5 a	0 a	0 a	0 a	0 a
Penoxsulam 49.1 + Diquat 130.8	80 c	80 bc	80 cd	80 c	70 c	45 b	20 a	20 a	0 a	0 a
Penoxsulam 98.2 + Diquat 130.8	70 c	70 bc	65 bc	60 bc	45 bc	15 ab	0 a	0 a	0 a	0 a
Diquat 130.8	25 b	5 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
LSD	11	20	23	30	37	38	24	21		

[a]Means in a column followed by the same letter do not differ significantly at p = 0.05 according to Fisher's Protected LSD. Analyses were conducted within weeks and species.

Table 2. Synergistic or antagonistic response of waterhyacinth to combinations of penoxsulam and diquat 10 weeks after treatment. An asterisk indicates significance according to a Wilcoxin Rank Sum Test.

Herbicide (g ai ha-1)	Observed Response (n = 3)	Expected Response	Difference in Response[a]
Biomass (% of Control)			
Penoxsulam 24.5 + Diquat 130.8	77	100	-23*
Penoxsulam 49.1 + Diquat 130.8	94	99	-5
Penoxsulam 98.2 + Diquat 130.8	90	100	-10*

[a]Calculated as the observed response - the expected response; a "+" represents synergism, and "-" represents antagonism from Colby (1967).

term control by glyphosate (Wehtje et al. 2008). Additionally, Wehtje stated higher glyphosate rates must be used to avoid loss of long-term efficacy in combination with diquat.

Our results show antagonism of diquat with all penoxsulam rates tested. The addition of diquat for control of waterhyacinth did not increase the efficacy of penoxsulam; excellent biomass reduction was achieved by 6 WAT with 24.5 g ai ha^{-} of penoxsulam and control maintained to 10 WAT. Because 24.5 g ai ha^{-1} was the lowest rate tested, additional tests should be conducted evaluating lower dose responses.

Common salvinia

Control of common salvinia was 70 to 80% 1 WAT for all applications with the exception of diquat alone (Table 1). By 2 WAT the 98.2 g ai ha^{-1} treatment of penoxsulam resulted in 90% control, however by 6 WAT control was only 30%. Control was similar between penoxsulam alone and the combination applications throughout the study with the exception of the 24.5 g ai ha^{-1} penoxsulam + diquat combination. This application resulted in less control by 2 WAT. Common salvinia had completely recovered by 7 WAT for all applications with the exception of diquat alone, which recovered by 3 WAT. Biomass collected at 6 and 10 WAT show significant reductions in common salvinia biomass when compared to reference plants (Figure 2). However, during both harvest times there were no distinct differences between applications with the exception of diquat alone. Biomass was similar in the diquat alone treatment and 24.5 g ai ha^{-1} penoxsulam + diquat by 10 WAT. This result with diquat alone was somewhat expected due to the low rate used. Nelson et al. (2001) reported that giant salvinia was very susceptible to diquat at rates of 2091 and 4182 g ai ha^{-1}, a >90% increase in diquat than that used in this study. Sublethal diquat rates were used in this

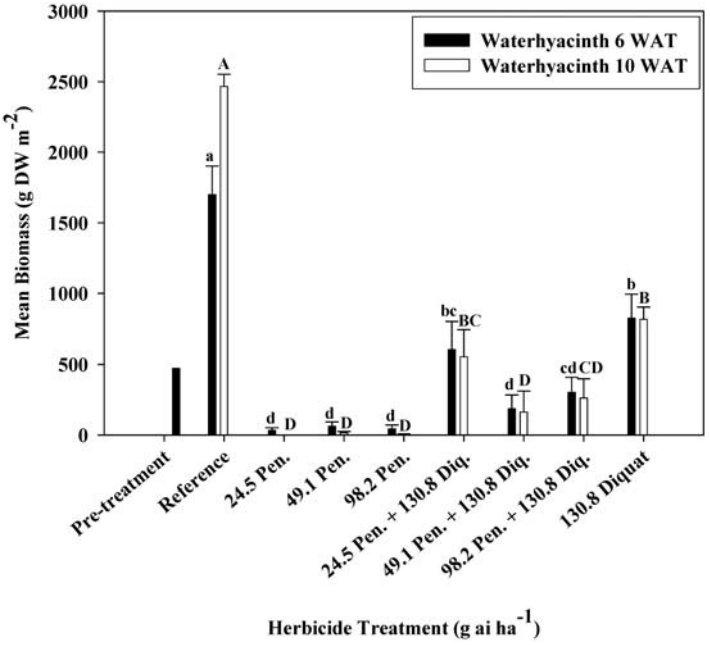

Figure 1. Mean (±1 SE) dry weight biomass of waterhyacinth harvested 6 and 10 WAT with penoxsulam and diquat. Bars sharing the same letter do not differ according to a Fisher's Protected LSD test at a p = 0.05 level of significance. All analyses were conducted within WAT; lower case letters compare means for 6 WAT, uppercase letters compare means at 10 WAT.

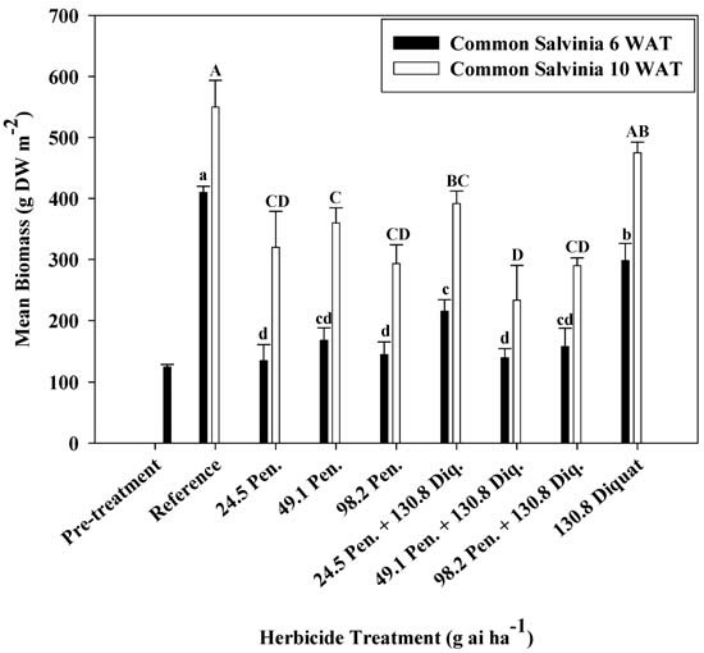

Figure 2. Mean (±1 SE) dry weight biomass of common salvinia harvested 6 and 10 WAT with penoxsulam and diquat. Bars sharing the same letter do not differ according to a Fisher's Protected LSD test at a p = 0.05 level of significance. All analyses were conducted within WAT; lower case letters compare means for 6 WAT, uppercase letters compare means at 10 WAT.

study to evaluate the potential to be used as a marker or enhance visual susceptibility of penoxsulam, or to enhance control.

Another explanation for reduced herbicide efficacy on salvinia may have been the thickness of the common salvinia mat at the time of application. Previous herbicide evaluations on giant salvinia were conducted on a single layer of plants (Nelson et al. 2001, 2007) or on individual plants (Fairchild et al. 2002). Mat thickness in the current study was 2 to 4 cm at the time of application. We speculate that plants at the surface of the mat were directly exposed to and killed by the herbicide. Plants under the surface mat did not come in contact with the spray solution and subsequently recolonized the mesocosms after the death of the exposed plants. This may explain the occasional failures of herbicide treatments on salvinia, duckweed, and watermeal under field conditions. Our data may be an indicator of herbicide efficacy on the mat phase of salvinia growth, although no research has been conducted on the direct effects of mat thickness on herbicide efficacy. We believe that having penoxsulam or diquat in the water column may result in better herbicide efficacy on salvinia species because more plants are in contact with the water.

These data show that penoxsulam applied as a foliar treatment is very efficacious on waterhyacinth. Control was achieved more rapidly, >90% 4 WAT, than the previously stated 60 days. Furthermore, greater control was achieved with penoxsulam alone at rates as low as 24.5 g ai ha^{-1} than by combining penoxsulam with diquat. Penoxsulam was not as effective at controlling common salvinia. The combination of penoxsulam with diquat did not offer any increased efficacy for either species; moreover, there was antagonism between these two herbicides. Future studies are needed to assess lower rates for waterhyacinth control and the influences of salvinia mat thickness on foliar application timing and herbicide efficacy. With the apparent susceptibility to penoxsulam, in-water treatments should also be further assessed for common salvinia as well as determining relationships between herbicide efficacy and salvinia mat thickness.

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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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[6]Earthgro Topsoil is a registered trademark of The Scotts Company. Marysville, OH. 43041.
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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).

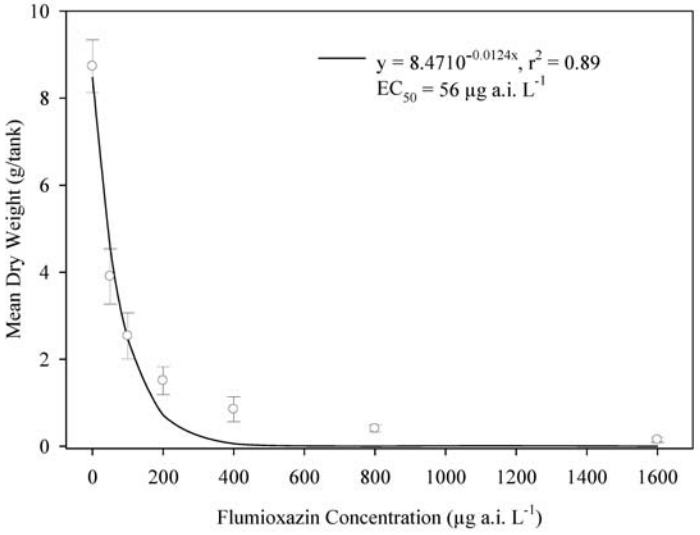

Figure 1. The effect of flumioxazin concentration on hydrilla dry weight 21 d after exposure. Flumioxazin applied as a single application to hydrilla cultured in 95-L tubs (pH 9.0 to 9.5) under 70% sunlight. Data are shown as dry weight means ± standard error (n = 10). EC_{50} = effective concentration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

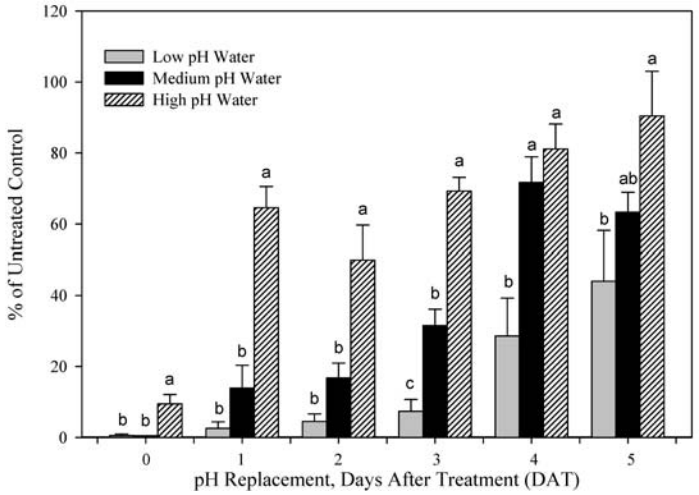

Figure 2. The effect of flumioxazin at 400 µg L^{-1} on hydrilla dry weight as influenced by low (6.0 to 6.2), medium (7.0 to 7.2), and high (>8.5) water pH under 70% sunlight. Hydrilla plants were added to low, medium, and high pH water treated with flumioxazin 0 to 5 d after initial treatment, exposed 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.

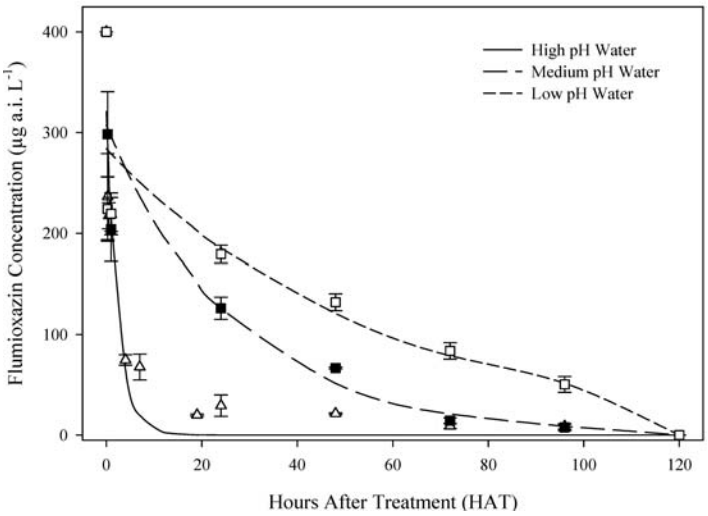

Figure 3. Dissipation of flumioxazin applied at 400 µg L^{-1} to low (6.0 to 6.2), medium (7.0 to 7.2) and high pH (>8.5) water in 95-L tubs under 70% sunlight. The dissipation of flumioxazin was calculated using nonlinear regression (exponential decay) for the low ($y = 0.0178e^{-0.0178x}$; $r^2 = 0.92$; 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.

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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.

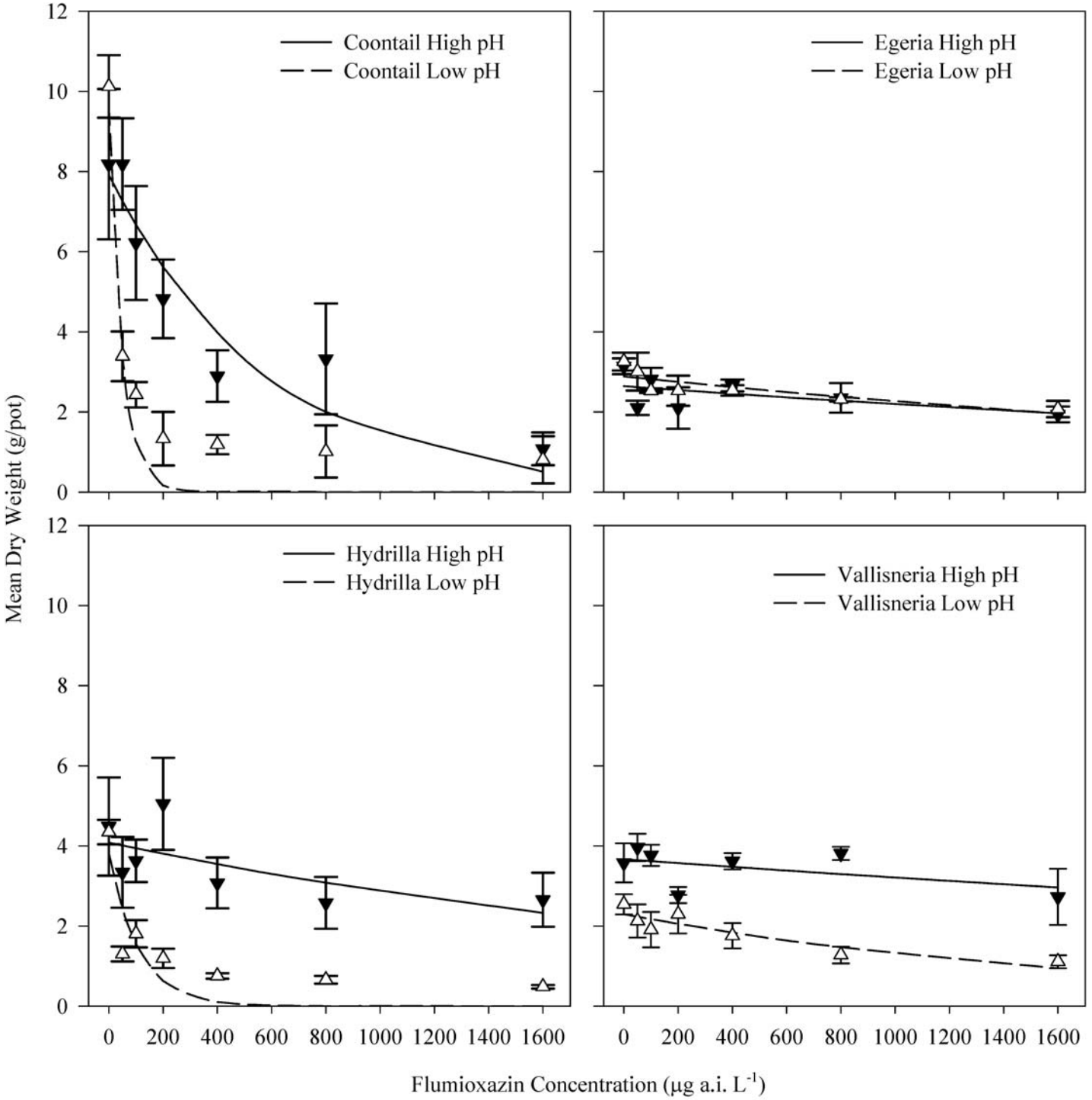

Figure 1. The effect of flumioxazin concentration on the dry weight of submersed aquatic plant species 4 weeks after exposure. Flumioxazin applied as a single subsurface application to submersed aquatic species cultured in neutral (7.0) and high (9.0) pH water in 95-L tubs. Data are shown as actual dry 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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temperature differentials and plant density can also alter herbicide distribution. Custom subsurface injection application systems with trailing hoses have been suggested for improved delivery of liquid herbicides in deeper water areas (Haller et al. 2007). Granular formulations also have been developed to assist in delivering aquatic herbicides (Aquathol® Super K; Navigate®; Sonar® Q, PR, and SRP; and more recently Renovate OTF and SonarOne). These formulations release the herbicide in and around submersed plant communities and often sink to the bottom, depending on plant density and frequency, where the herbicide is released (Steward and Nelson 1972, Applied Biochemists 2009).

Few field studies have compared dissipation rates or residue profiles following application of a liquid herbicide to a granule formulation. Fox and Haller (1992) reported that liquid applications using long weighted-hoses or the use of granules increased potential exposure time to the herbicides compared to liquid applications using unweighted hoses. Field observations suggest that under certain conditions liquid herbicide can be rapidly diluted, resulting in poor submersed weed control. Skogerboe and Netherland (2008) reported that 80 to 90% of liquid herbicide was lost from partial-lake treatment areas within 15 h following treatment. Herbicide applications in areas such as relatively deep sites with low growing vegetation, with potential for rapid water exchange, or those adjacent to or surrounded by a large percentage of untreated water could be impacted greatly by dilution. In these situations, where rapid water exchange may be a concern, the use of a granular herbicide could theoretically maintain placement of the herbicide or increase exposure times. This effect would be dependent on the vertical placement of the herbicide, the release rate of the active ingredient from the granule, and the vertical diffusion rate from the sediment-water interface. Delayed release of the herbicide from a granule could increase potential exposure time compared to a liquid formulation when 100% of the herbicide is immediately available but vulnerable to dilution. However, herbicide release from granules has to be rapid enough to build threshold concentrations that result in an herbicidal effect.

Triclopyr (3,5,6-trichloro-2-pyridinylocyacetic acid, triethylamine salt) was approved for aquatic use in 2002 by the US Environmental Protection Agency. The liquid formulation (i.e., Renovate® 3, SePRO Corporation, Carmel, IN; hereafter referred to as SePRO) was the only formulation initially registered. In 2006, a granule formulation (Renovate OTF; SePRO) was developed to aid delivery of triclopyr to the plant mass and to minimize immediate dilution or dispersion. The granule contains 14% active ingredient (ai): 10% acid equivalent (ae). Rhodamine WT (Keystone Analine Corporation, Chicago, IL) is an inert fluorescent dye containing 20%, or 240 g ai L^{-1}. This dye is used for a variety of purposes, including water movement studies, and is commonly used to predict herbicide movement in aquatic environments (Fox et al. 1991, 1992, 1993, 2002, Turner et al. 1994).

Concentration exposure-time studies have provided valuable information regarding the required duration of herbicide exposure for the effective control of many submersed weeds (Netherland et al. 1991, 1993, Netherland and Getsinger 1992). However, the influence of herbicide formulation and application technique on concentration exposure time and weed control has not been well explored, especially when comparing the effectiveness of a granular formulation to liquid application of the same herbicide. To investigate this potential difference in residue profiles due to formulation, a field dissipation study was conducted to compare the dissipation rates of concurrent applications of Renovate OTF and the inert dye Rhodamine WT. Dissipation studies investigate the movement and degradation of pesticides. They are usually conducted under "worst case" conditions, those factors that would prolong the degradation rate of the compound or maximize its off-site movement. Efficacy is not a factor in dissipation studies, which are often conducted "bare plot." Thus, for this study, a treatment scenario was chosen where herbicide would be applied to a relatively deep site with sparse vegetation found predominately near the bottom. This site was selected to represent a treatment area with potential for relatively rapid dilution from untreated water and that allowed monitoring of vertical distribution of the compounds.

MATERIALS AND METHODS

Grandview Lake is a 132-ha reservoir located in Bartholomew County, Indiana, approximately 11 km southwest of the town of Columbus and 70 km south of Indianapolis (Figure 1). This study was conducted October 2008 in a 4-ha bay in the southern portion of the lake.

A bathymetric survey was conducted along predetermined transects using a Garmin GPS 72 and a Garmin Fishfinder 160 (Olathe, KS). Depths in the bay to be treated were recorded at 366 waypoints, and an average plot depth of 4.75 m was calculated.

Four sampling locations (Figure 1; stations 1, 2, 3 and 4) were sited within the treatment plot at locations where the water depth was 5.5 m. An additional six sampling locations were sited outside the treatment area, in areas were the water

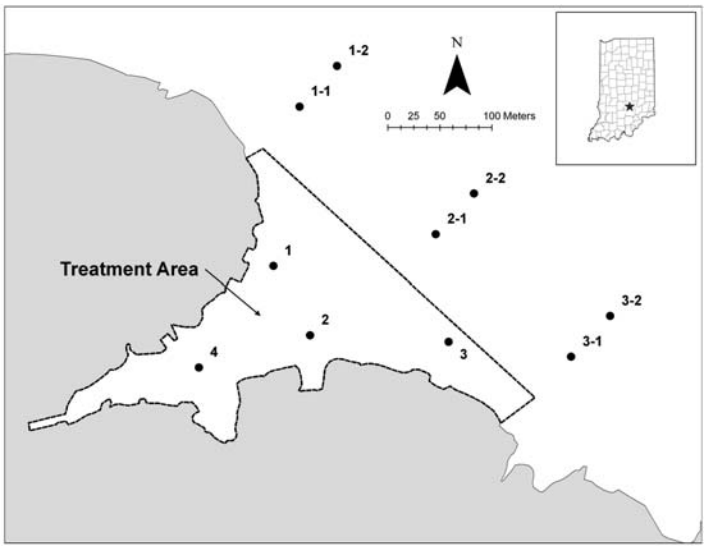

Figure 1. Plot map and location of Grandview Lake, IN.

depth was at least 12 m (Figure 1; stations 1-1, 1-2, 2-1, 2-2, 3-1, and 3-2). The off-plot sample locations were approximately 50 and 100 m from the open-water edge of the treatment area. The on-plot stations were marked with PVC pipe-mounted sampling devices (described later), and the off-plot stations were recorded as GPS waypoints.

Concurrent applications of the granular triclopyr and liquid dye took place on the morning of October 7 beginning at 08:30 and complete by 10:00. The granular product was applied from a boat with 2 Echo PB755H landscape blowers (Lake Zurich, IL) customized for granular application. The blowers were mounted on each side of the application boat and applied the granules at 45° from the bow of the boat. We applied 3400 lbs of formulated material evenly across the treatment area, which resulted in a nominal water concentration of 800 µg ae triclopyr L^{-1}. The dye was deep injected by trailing two 13.7-m weighted hoses from a boat traveling ≤8 kph equipped with a high-pressure Hypro D30 diaphragm pump (New Brighton, MN) run by a Honda Power Equipment Group DX160 motor (Alpharetta, GA). Each injection hose weighed 5.92 kg including the weighted/nozzle section, which weighed 2.02 kg. We applied 11.4 L of dye formulation in six tank mixes consisting of 1.9 L of dye in 189 L of water. This resulted in a nominal water concentration of 14 µg ai L^{-1} dye.

Water was sampled from the on-plot stations at 21 discrete events, beginning with pre-application samples collected the evening before application and continuing at approximately 0.5, 1, 2, 4, 6, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 56, 64, 72, and 96 h after treatment. The off-plot stations were sampled on the same schedule, although early events were omitted for some stations.

At the on-plot stations, water was collected from six discrete depths at each station. These depths were +0.15, +0.30, +0.60, +1.2, and +2.4 m from the bottom of the lake, representing the lower half of the column, and a near-surface sample collected at approximately +5.0 m, or about 0.5 m below the water surface. The lower 5 samples were collected via an automated sampling device consisting of submersible 12-volt bilge pumps connected at the specified depths to a PVC pipe anchored into the lake bottom. Each bilge pump had a 1.3-cm dia PVC feed pipe running to approximately 1 m above the water surface, capped with a gate valve. The power leads for the bilge pumps were taped to the feed pipes and wired into a common switch. To collect samples, a 12-volt battery was connected to the common switch, and the pumps were allowed to run for a minimum of 30 to 60 sec to purge the feed lines. The pumps were then run one at a time, starting with the lowest, and water samples of approximately 200 ml were collected into pre-labeled 250-ml amber Nalgene® bottles. The near-surface sample was collected by lowering a bottle by hand to depth and allowing water to enter the bottle.

The off-plot water samples were collected from an approximate 3-m depth by lowering a bilge pump connected to a 1.9-cm PVC feed pipe and pumping the water sample up into the pre-labeled sample bottle.

Water samples were stored in coolers with ice during the collection, holding, and shipping periods. The sample labels contained only a sample ID number keyed to the sample collection sheet, which led to blind laboratory analyses.

Water temperature and dissolved oxygen (DO) measurements were taken in 1-m increments to the bottom of the water column each morning and afternoon during the study. These measurements were taken at a marked location within the treatment plot near sample location 2.

The water samples were received at the SePRO Research and Technology Campus (Whitakers, NC) on 15 October 2008. The samples were placed into frozen storage and thawed in lots of 100 for analysis via the immunoassay (ELISA) method for triclopyr (FasTEST - SePRO; Poovey et al. 2004). The limit of detection (LOD) was 10 µg L^{-1}. When analysis of each lot was complete they were returned to the freezer and the next lot was allowed to thaw. Analyses were completed on 15 November 2008.

After completion of the ELISA testing for triclopyr residues, the samples were shipped under ambient conditions on 3 December 2008 and were received at the University of Florida Center for Aquatic and Invasive Plants (Gainesville, FL) on 8 December 2008. The samples were allowed to remain at ambient temperature, and dye concentrations were analyzed using a Turner Designs Model 10 field fluorometer and Model 8000-010 handheld fluorometer (Sunnyvale, CA). The LOD for dye was 0.1 µg L^{-1}, and the fluorometers were periodically calibrated using Rhodamine WT standards ranging from 1.2 to 9.6 µg L^{-1}.

First-order dissipation half-lives of triclopyr and dye were calculated using Microsoft Excel software statistical functions, using the full precision of the values entered into the spreadsheet. The calculations were performed using the average whole-plot concentrations.

RESULTS AND DISCUSSION

Measurements of water temperature and DO demonstrated that both parameters were nearly homogeneous through depth and time (Tables 1 and 2). This indicated there was no stratification that might inhibit distribution of the herbicide or dye through the water column.

The concentration data derived by the lab analyses were summarized by averaging the results from the on-plot stations by depth increment (Tables 3 and 4), and also by whole-plot (Figure 2), for each sampling period. There were no detectable residues of either triclopyr or dye in the pre-application samples.

The target whole-plot concentrations for triclopyr and dye were 800 µg L^{-1} and 14 µg L^{-1}, respectively. The whole-plot average concentrations (Figure 2) indicate the target concentration of triclopyr was not achieved on a whole-plot basis. Data indicate that the concentration of triclopyr at the lower depths in the early time periods did meet this targeted dose (Table 3). As expected, the granule application resulted in release of the active ingredient over time from the bottom of the water column where the majority of granules had settled. A study conducted under laboratory conditions (without sediment) demonstrated that in 4 h Renovate OTF had released 94% of the triclopyr acid equivalent and achieved 99% of the theoretical concentration after 24 h (Hahn 2006). Data indicate that the concentration of dye (applied via long-trailing hose) met the nominal application rate in the mid-depths of the water column, but as with triclopyr, vertically dissipated

Table 1. Water temperature (C) measured through the water column twice daily during the study period.

Depth (m)	Oct 6 PM	Oct 7 AM	Oct 7 PM	Oct 8 AM	Oct 8 PM	Oct 9 AM	Oct 9 PM	Oct 10 AM
0	21.2	20.1	20.3	20.0	20.2	19.6	20.7	19.6
1.0	21.2	20.2	20.3	20.0	20.2	19.7	20.7	19.6
2.0	20.5	20.2	20.3	20.0	20.2	19.6	19.8	19.5
3.0	20.3	20.2	20.1	20.0	20.0	19.6	19.8	19.5
4.0	20.2	20.2	20.1	20.0	20.0	19.6	19.7	19.5
5.0	20.2	20.2	20.1	20.0	20.0	19.5	19.6	19.5
6.0	20.1	20.1	20.1	20.0	19.9	19.5	19.6	19.5
7.0	20.1	20.1	20.0	19.9	19.9	19.5	19.5	19.5
7.5	—	20.0	19.9	19.8	19.8	19.4	19.5	19.5
8.0	20.1	—	—	—	—	—	—	—

Table 2. Dissolved oxygen (mg L^{-1}) measured through the water column twice daily during the study period.

Depth (m)	Oct 6 PM	Oct 7 AM	Oct 7 PM	Oct 8 AM	Oct 8 PM	Oct 9 AM	Oct 9 PM	Oct 10 AM
0	8.71	8.42	8.52	8.10	8.80	8.10	8.67	8.73
1.0	8.02	8.10	8.68	7.82	8.39	7.88	8.40	8.57
2.0	8.50	8.33	8.60	7.87	8.38	8.28	8.62	8.23
3.0	8.54	8.20	8.35	7.95	8.42	8.00	8.54	8.47
4.0	8.53	7.98	8.03	8.15	8.69	7.36	8.25	8.48
5.0	8.27	8.02	7.80	7.35	8.34	7.83	7.88	8.49
6.0	8.03	8.19	7.99	7.39	8.22	7.75	7.94	8.56
7.0	8.52	8.31	8.20	7.70	8.10	7.94	8.10	8.65
7.5	—	7.82	8.01	7.45	8.48	8.13	8.04	8.55
8.0	8.52	—	—	—	—	—	—	—

Table 3. Average on-plot triclopyr concentrations (µg ae L^{-1}) by depth increment (indicated by distance above the lake bottom) following a nominal 800 µg ae L^{-1} application (n = 4; HAT: hours after treatment).

		Triclopyr Concentration µg L-1					
Date	HAT	+0.15 m	+0.30 m	+0.60 m	+1.2 m	+2.4 m	+5.0 m
7-Oct	0.5	750.3	492.3	434.4	273.5	203.4	124.2
7-Oct	1	693.6	607.6	511.5	388.7	257.7	112.7
7-Oct	2	861.5	762.2	518.2	210.6	372.8	231.1
7-Oct	4	671.2	526.4	331.5	231.8	133.4	131.5
7-Oct	6	608.2	350.4	323.6	137.6	115.5	310.8
7-Oct	8	484.5	461.5	219.9	162.9	60.9	290.4
7-Oct	12	552.4	447.5	381.1	173.5	145.4	156.8
8-Oct	16	275.5	171.9	129.0	150.3	168.6	131.8
8-Oct	20	212.4	315.9	322.1	196.1	223.3	124.6
8-Oct	24	288.9	289.5	172.0	150.4	196.2	145.3
8-Oct	28	184.0	172.3	189.5	133.6	177.0	167.0
8-Oct	32	234.6	172.1	208.6	75.9	112.0	145.1
8-Oct	36	207.3	167.0	170.0	152.2	164.8	147.4
9-Oct	40	196.0	185.8	171.8	167.1	181.4	174.8
9-Oct	44	195.5	198.7	189.7	236.3	172.7	232.5
9-Oct	48	180.4	172.6	152.8	124.6	115.1	117.4
9-Oct	56	114.0	72.6	76.8	87.9	94.1	76.0
10-Oct	64	129.3	103.4	83.1	67.0	64.5	58.0
10-Oct	72	85.5	60.4	60.0	49.8	69.9	40.6
11-Oct	96	27.7	26.0	22.5	22.6	19.6	20.5

relatively rapidly (Table 4). Triclopyr concentrations demonstrate that as the triclopyr acid was released from the granules, it quickly dispersed through the water column and did not result in abnormally high or long-lived concentrations at the sediment/water interface (Table 3). There was no evidence of accumulation of triclopyr at the bottom of the lake. There was a concentration gradient by depth for the majority of the sample events. The triclopyr and dye began dispersing

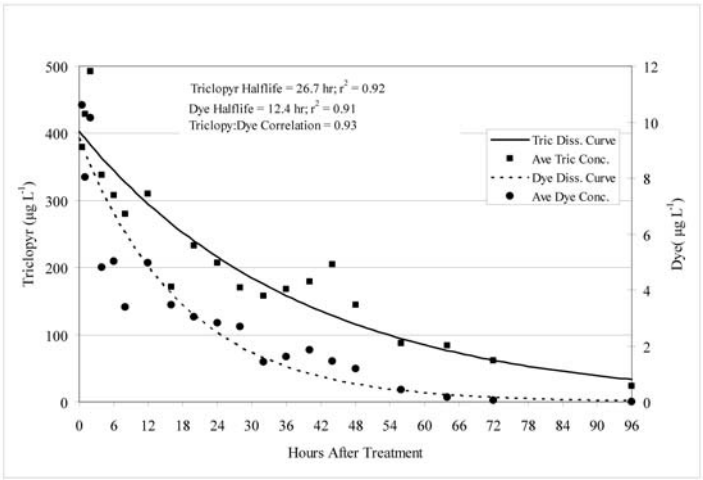

Figure 2. First-order dissipation curves of triclopyr (Tric.) and rhodamine dye (Dye; µg L^{-1}) following concurrent applications in Grandview Lake, IN.

vertically through the water column immediately after application and were well mixed by 28 h after application.

Triclopyr dissipated from the plot with a calculated half-life of 26.7 h (r^2 = 0.92), and Rhodamine WT dye dissipated with a calculated half-life of 12.4 h (r^2 = 0.91; Figure 2). The correlation coefficient between the triclopyr and dye dissipations was 0.93, which indicates that the two compounds had strongly correlated patterns of dissipation from the test plot. This result is in agreement with other lake studies in which Rhodamine WT was applied concurrently with liquid triclopyr (Turner et al. 1994, Petty et al. 1998, Fox et al. 2002). A factor influencing the post-application concentrations was the wind pattern noted during and after application. Observations recorded during the application state that the winds were calm, <8 km h^{-1}, and from the east to northeast, which would have reduced water exchange from the treatment plot bay and the rest of the lake (Figure 1). Soon after application the wind speeds increased and came from a southerly direction. These observations are confirmed by hourly weather data recorded at the nearby Columbus Municipal Airport, which recorded no wind from 8:00 to 11:00 on the day of application, followed by winds averaging 16.6 kph from the southeast to southwest from 12:00 through 18:00 the following day. These winds would increase chemical loss from the treatment plot through increased water-exchange rates, primarily pushing the material to the northwest shoreline (supported by visual observation of dye movement) and off-plot to the north. Winds remained negligible for the majority of the remainder of the study.

Overall, off-plot concentrations of triclopyr and dye were quite low. From 36 to 64 h, triclopyr and dye concentrations were <10% of the dose applied, and from 72 to 96 HAT concentrations were 3% or less of the concentration applied.

Under the conditions of this pilot study, triclopyr released from the granules remained in the treatment plot 2.2 times as long as the liquid dye, as determined by the individual half-lives. This would indicate that the exposure time of triclopyr to target plants resulting from the granular application would be twice that of an equivalent application of liquid herbicide. The factors influencing these differences are likely the release of the active ingredient from the granules and its mode of delivery, placing it at the bottom of the water column where it is likely less susceptible to wind-driven or surface-current water exchange with untreated water.

Table 4. Average on-plot dye concentrations (µg L^{-1}) by depth increment (indicated by distance above the lake bottom) following a nominal 14 µg L^{-1} application (n = 4; HAT: hours after treatment).

		Dye Concentration µg L^{-1}					
Date	HAT	+0.15 m	+0.30 m	+0.60 m	+1.2 m	+2.4 m	+5.0 m
7-Oct	0.5	11.1	8.3	8.0	9.5	9.8	17.0
7-Oct	1	6.5	5.7	5.2	6.3	13.3	11.2
7-Oct	2	4.9	4.5	7.0	12.8	20.1	11.6
7-Oct	4	4.8	1.8	0.2	5.3	9.6	7.1
7-Oct	6	1.5	2.0	4.8	5.3	5.7	11.0
7-Oct	8	1.7	3.7	1.7	1.9	0.9	10.4
7-Oct	12	6.1	6.3	5.4	4.8	4.0	3.3
8-Oct	16	3.3	3.1	3.0	3.2	3.4	4.9
8-Oct	20	2.9	3.4	2.8	2.8	3.1	3.2
8-Oct	24	0.9	3.2	3.0	3.0	3.9	3.0
8-Oct	28	2.9	2.9	2.6	2.6	2.4	2.7
8-Oct	32	1.9	1.7	1.8	0.7	0.8	1.7
8-Oct	36	1.9	1.6	1.6	1.6	1.5	1.6
9-Oct	40	2.0	1.7	1.7	1.5	1.6	2.7
9-Oct	44	1.6	1.5	1.7	1.5	1.4	1.0
9-Oct	48	1.4	1.5	1.3	1.1	1.0	0.9
9-Oct	56	0.4	0.5	0.4	0.6	0.5	0.2
10-Oct	64	0.2	0.2	0.1	0.2	0.2	0.2
10-Oct	72	0.1	0.1	0.1	0	0	0
11-Oct	96	0	0	0	0	0	0

Application techniques and/or herbicide formulations need to be further developed to maximize concentration retention under various field conditions and dilution patterns. Additional research needs to be conducted to compare the dissipation pattern of herbicides from different delivery systems and application techniques to optimize concentration and exposure times under field conditions. Research should include evaluating the influence of plant density or height on vertical herbicide distribution and dissipation from the treated area; treatment areas that are exposed to immediate dilution from untreated water (e.g., shoreline areas or open water areas) versus those that are more protected from dilution (e.g., coves); and impacts of temperature stratification. Various delivery systems should be evaluated, including controlled-release and fast- or quick-release herbicide formulations; and liquid herbicide surface application, sub-surface injection, or deep-water injection.

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