CHARACTERIZATION OF FLUMIOXAZIN AS AN AQUATIC HERBICIDE

By

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To my patient and loving wife, Erin, as well as my parents Alvin and Wanda whose love and support has never ended, and to my departed grandparents Houston and Hazel Doucet; I miss you both.

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CHARACTERIZATION OF FLUMIOXAZIN AS AN AQUATIC HERBICIDE

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The suitability of flumioxazin as an aquatic herbicide for control of hydrilla and other invasive aquatic plant species was evaluated in field, greenhouse, and laboratories studies. Flumioxazin is a protoporphyrinogen oxidase inhibitor in plants, which is a precursor to production of chlorophyll. It is degraded by hydrolysis and has a half-life of 17.5 min in water at pH 9.0 compared to a half-life of 16.1 h and 4.1 d at pH 7.0 and 5.0, respectively. Flumioxazin efficacy was evaluated at various concentrations, pH, and light levels to determine the impact on hydrilla biomass, net photosynthesis, and chlorophyll content. The effective concentration of flumioxazin required to reduce hydrilla dry weight by 50% (EC_{50}) was 56 μg L^{-1} in mesocosm studies; however, regrowth was noted in concentrations as high as 1600 μg L^{-1} under high pH conditions. Apical hydrilla tips treated under high pH (>9.0) with flumioxazin at 100 μg L^{-1} failed to reduce net photosynthesis by 20% of the nontreated control. All concentrations >100 μg L^{-1} at high pH and ≥100 μg L^{-1} at low pH reduced net photosynthesis by at least 60% 168 HAT. Under low light quantity (20 μmol m^{-2} s^{-1}), flumioxazin failed to reduce net photosynthesis of apical hydrilla tips compared to medium (170 μmol m^{-2} s^{-1}), and high light (400 μmol m^{-2} s^{-1}) quantities. Non-target emergent plants appeared to tolerate flumioxazin at high pH (>9.0), whereas selectivity decreased in waters of lower pH (7.0). Phytotoxicity to non-

target ornamental and row crop plants irrigated with flumioxazin treated water was dependent on maturity as immature, actively growing plants were highly susceptible. Additionally, water lettuce and landoltia were more susceptible to submersed flumioxazin applications than foliar applications. These data provide evidence that flumioxazin has the potential for use as an herbicide with submersed and foliar applications to control hydrilla and other weeds and that application methods, environmental conditions, and concentrations influence non-target plant selectivity.

CHAPTER 1
INTRODUCTION

Currently, there are ten herbicides labeled (FIFRA-Section 3) by the U.S. Environmental Protection Agency (EPA) for aquatic use in the U.S. including 2,4-D [(2,4-dichlorophenoxy) acetic acid], carfentrazone {X,2-dichloro-5-[4-(difluromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanic acid}, copper (copper sulfate or copper chelate), diquat (1,1'-ethylene-2,2'-bipyridylium dibromide), endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid), fluridone {1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl]-4(1H)-pyridinone}, glyphosate N-(phosphonomethyl)glycine, imazapyr {(±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid}, penoxsulam {2-(2,2-difluoroethoxy)-6-(trifluoromethyl-N-(5,8-dimethoxy[1,2,4]triazolo[1,5-c]pyrimidin-2-yl))benzenesulfonamide}, and triclopyr {[(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid}. Of these, only copper, diquat, endothall, and fluridone have historically been used for hydrilla *(Hydrilla verticillata* (L. f.) Royle) control (Vandiver 2002).

Hydrilla is a submersed aquatic fresh-water angiosperm in the family Hydrocharitaceae native to Asia or Africa that has become a serious weed problem in the United States and many other countries (Cook 1985; Haller and Sutton 1975; USDA 2007; Van and Vandiver 1992). Once established, hydrilla readily dominates and replaces native submersed species by forming a canopy and reducing light penetration (Haller and Sutton 1975).

There are two biotypes of hydrilla in the U.S., which are the dioecious (plants produce only female flowers) and monoecious (male and female flowers on the same plants) (Cook and Lüönd 1982; Langeland 1996). The dioecious female biotype was introduced from Sri Lanka to the Tampa Bay, Florida area (Schmitz et al. 1990) in the early 1950's and was first observed growing outside of nursery conditions in a canal in Miami and in a spring near Crystal River, FL

in 1960 (Blackburn et al. 1969). The dioecious female plant has spread throughout the southern U.S. and as far west as California (Yeo and McHenry 1977; Yeo et al. 1984). The first population of the monoecious biotype of hydrilla was discovered in Delaware in 1976 with a second discovery in 1980 in the Potomac River (Haller 1982; Steward et al. 1984).

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 is especially problematic since its response to management efforts results in rapid regrowth from vegetative propagules such as tubers, turions, and plant fragments (Van and Vandiver 1992). Turions are compact dormant buds produced in leaf axils which detach from the plant upon maturation, while tubers (subterranean turions) are formed terminally on subterranean rhizomes (Langeland 1996). The Florida Department of Natural Resources (now the Florida Department of Environmental Protection) estimated over 20,000 ha of water in Florida contained hydrilla in 1988 (Schardt and Nall 1988), with hydrilla spreading to 40,000 ha of water in 43% of public lakes in Florida by 1995 (Langeland 1996). Rapid hydrilla growth and expansion is favored by its low light and CO2 compensation points, reduced photorespiration due to a C4 like photosynthetic mechanism and its prolific reproductive capacity (Van et al. 1976; Holaday et al. 1983; Magnin et al. 1997). Hydrilla is typically rooted in the hydrosol, although fragments frequently break loose and survive in a free-floating state (Langeland 1996). The stems may be quite long especially when the plant grows in deep clear water and branching usually does not occur until the plant grows near the water surface (Langeland 1996). Upon reaching the surface, stems begin to branch profusely forming a surface mat. This density of stem biomass causes pH in the upper 0.3 m of water during the summer to increase ≥10.0 during the day by utilization of CO_2 and HCO_3^- and to fall below 7.0 in the evening (Spencer et al.

1994; Van et al. 1976). Surface matted hydrilla can also cause dissolved oxygen levels to fall below the air-saturated level during the night, but by noon levels may reach 16 mg L^{-1}, equivalent to over 200% air saturation (Van et al. 1976).

Various forms of weed control have been evaluated for hydrilla control in Florida including biological, cultural, chemical, mechanical, and preventative techniques. Biological control agents such as grass carp are usually unpredictable forms of control (Martyn 1985). Cultural management techniques such as drawdowns have had limited success, due to regrowth from subterranean turions (Haller et al. 1976). Mechanical removal of hydrilla is not practical for large lakes, costing as much as $2500 ha yr^{-1} (SE-EPPC 2005). Due to the high costs or limited effectiveness of mechanical, biological, and cultural techniques, early successful control of hydrilla in the 1960's and 1970's was through the use of herbicides. These included the contact herbicides diquat, endothall, and diquat plus copper combinations (Brian et al. 1958; Hiltibrand 1963; Sutton et al. 1972; Simsiman 1976; Vencill 2002). Due to the rapid activity of these herbicides, it is recommended that no more than half of an infested water body be treated at one time due to potential reduced oxygen supply and fish toxicity (Anonymous 2007a; Anonymous 2003). In addition, these herbicides possess a relatively short half-life (Simsiman and Chesters 1975; Langeland et al. 1994); this usually results in rapid control but also encourages rapid regrowth, so season long weed control has not been possible with single applications of these contact herbicides.

The first residual herbicide for hydrilla control, fluridone, received a Section 3 EPA registration for aquatic use in 1986 (Dayan and Netherland 2005). Although the maximum labeled rate is 150 µg L^{-1} active ingredient (a.i.), fluridone is commonly applied at 8 to 12 µg L^{-1} with concentrations as low as 4 to 7 µg L^{-1} providing hydrilla control if the dosage was

maintained for several weeks (Van and Steward 1985). Large areas of hydrilla in Florida were being controlled at 6 to 10 μg L^{-1} in the late 1980's at costs of $250 ha^{-1} or less, (Haller et al. 1990). Several factors, including low use rates, favorable native plant selectivity, slow activity (reduced oxygen depletion), and often more than one year of hydrilla control have resulted in the reliance on fluridone for hydrilla control in large, shallow Florida lakes (Puri et al. 2006). However, poor performance was observed in the late 1990's and the development of fluridone resistance in hydrilla was characterized in 2004 (Puri et al. 2007). Since then, fluridone resistant populations of hydrilla have expanded in the waterways of Florida. This is likely a result of continuous use of fluridone, low application rates (<20 μg L^{-1}) and persistent fluridone residue (MacDonald et al. 2001; Arias et al. 2005). The loss of fluridone from an already limited number of aquatic herbicides has resulted in the search for new and effective herbicides that can be applied to aquatic systems.

Beginning in 2004, herbicide efficacy studies have been conducted at the Center for Aquatic and Invasive Plants at the University of Florida to evaluate and identify herbicides that are relatively non toxic to aquatic organisms, possess a short half-life, and show native plant selectivity[1], which are characteristics necessary for aquatic registration. Of the herbicides evaluated, 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} met the desired criteria. Hydrilla dry weight was reduced by 63% and 99% in static tests when treated with 50 and 400 μg L^{-1} a.i., respectively, in 2005 (Mudge and Haller 2006). Based upon these initial evaluations, Valent U.S.A. Corporation applied for an Experimental Use Permit (EUP) from the U.S. Environmental Protection Agency (EPA) and the Florida Dept. of Agriculture and Consumer Services (FDACS)

[1] Chiconela, T. F. and W. T. Haller. 2007. Personal Communication.

in 2006 to evaluate control of the submersed aquatic weed hydrilla with flumioxazin (FDACS 2006; Fishel 2006).

Flumioxazin is an *N*-phenylphthalimide herbicide that is registered for preemergence weed control in peanut (*Arachis hypogaea* L.) and soybean (*Glycine max* L.) and for post-direct weed control in cotton (*Gossypium hirsutum* L.) (Anonymous 2005; Askew et al. 1999; Burke et al. 2002; Clewis et al. 2002; Grichar and Colburn 1996; Main et al. 2003). Direct foliar contact (postemergence) with flumioxazin results in unacceptable crop injury regardless of plant species (Yoshida et al. 1991). Flumioxazin is a protoporphyrinogen oxidase (PPO) (protoporphyrin IX:oxygen oxidoreductase, EC 1.3.3.4) inhibiting herbicide with both soil and foliar activity (Cranmer et al. 2000; Hartzler 2004; Price et al. 2002; Price et al. 2004) and is a strong inhibitor of chlorophyll synthesis. It prevents the transformation of protoporphyrinogen IX into protoporphyrin IX which is a precursor to heme and chlorophyll production (Aizawa and Brown 1999; Matringe et al. 1989; Cobb 1992). Protoporphyrinogen IX accumulates in plastids due to inhibition of the PPO enzyme and then diffuses through plastid membrane into the cytosol, where it is oxidized to protoporphyrin IX by a plasma membrane-bound protox (Dayan and Duke 1997; Duke et al. 1991). Protoporphyrin IX reacts 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 generally provides 4 to 6 wk of residual broadleaf control when applied to the soil and there is low potential for soil carryover to subsequent rotational crops (Vencill 2002). The potential for phytotoxicity increases with high soil moisture (Sakaki et al. 1991). The half-life of flumioxazin in a sandy clay loam soil was 17.9 and 16.0 d compared to 13.6 and 12.9 d in a loamy sand soil (Ferrell and Vencill 2003a). The flumioxazin molecule is non-ionic and has a water solubility of 1.79 mg L^{-1} (Vencill 2002); due to its low water solubility, flumioxazin has a greater affinity for organic matter than for silicate clay, which prevents it from binding tightly to the soil matrix and allows it to be readily removed from soil adsorption sites by soil water (Ferrell et al. 2004). Flumioxazin use in row crops is environmentally beneficial because of low use rates (71 to 107 g ha^{-1} a.i.) and rapid soil dissipation (Lovell et al. 2001). Flumioxazin possesses favorable characteristics for use in aquatic systems since half of the herbicide is degraded by hydrolysis in 4.1 d and 16.1 h at pH 5.0 and 7.0, respectively; however, at pH 9.0, the half-life decreases to 17.5 min (Katagi 2003) which may limit aquatic weed control in water with high pH. Heavy hydrilla infestations have been shown to raise water pH to >9.0 by utilizing free CO_2 and HCO^-_3 (Van et al. 1976) which has the potential to reduce the efficacy of this herbicide by causing rapid breakdown. Kwon et al. (2004) found the photolytic degradation rate of flumioxazin increased as a function of pH with a half-life of 41.5 and 4.9 h at pH 5.0 and 7.0, respectively, after correction for the effects of hydrolysis (dark conditions).

Other forms of degradation can influence flumioxazin activity in the aquatic environment. Flumioxazin showed maximum absorbance at 217 and 286 nm but could absorb UV energy of sunlight at wavelengths greater than 290 nm (Kwon et al. 2004). In aquatic systems, natural sunlight would cause the direct breakdown by photolysis near the surface of the water, but degradation is more likely a function of hydrolysis and water pH.

Carfentrazone received federal registration in 2004 as the only PPO inhibiting aquatic herbicide (FMC 2005; Iverson and Vandiver 2005). Carfentrazone is similar to flumioxazin because of its rapid degradation by hydrolysis at high pH (Elmarakby et al. 2001; Ngim and Crosby 2001). It is effective in controlling the submersed aquatic plant Eurasian watermilfoil (*Myriophyllum spicatum* L.) and floating plants such as water lettuce (*Pistia stratiotes* L.), water fern (*Salvinia minima*) watermeal (*Wolffia* spp.), and water hyacinth (*Eichhornia crassipes* (Mart.) Solms) (FMC 2004; Koschnick et al. 2004).

Species of duckweed in the *Lemnaceae* family are commonly used in biochemical and toxicity tests because of their small size, high reproductive rate and ease of culture (Gensemer et al., 1999; Geoffroy et al. 2004; Lewis 1995; Ma et al. 2002; Parr et al. 2002). Studies evaluating pigment content (chlorophyll *a*, *b*, and carotenoids) and oxygen evolution are often reliable indicators of herbicide toxicity (Wang and Freemark 1995). Previous research has shown that flumioxazin at 1, 10, and 50 μg L^{-1} decreased photosynthetic capacity of common duckweed (*Lemna minor* L.) by 23, 62, and 64%, respectively (Frankart et al. 2002). In these studies photosynthetic capacity of common duckweed was inhibited more when plants were exposed to 200 μg L^{-1} of copper mixed with flumioxazin than all rates of flumioxazin applied alone. A very slight synergistic or additive effect was observed between the two chemicals.

One of the primary goals of aquatic weed control in public and private waters is to eliminate invasive species while maintaining a diversity of native submersed and emergent species. Native aquatic plants have been shown to improve water clarity and quality, provide valuable fish and wildlife habitat, reduce rate of sediment resuspension, and help prevent the spread of invasive plants (Dibble et al. 1996b; Heitmeyer and Vohs 1984; Savino and Stein 1982; Smart 1995). 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 has been shown to increase when canopy forming exotic plants are removed (Getsinger et al. 1997) and diversity of invertebrate and fish habitats are maintained (Dibble et al. 1996a).

Damage to non-target native plants species is a major consideration in herbicide selection, with favorable aquatic herbicides being able to selectively remove unwanted plants while minimizing damage to non-target native plants. Various mesocosm studies have been conducted to evaluate the sensitivity of native plant species to registered aquatic herbicides. Netherland et al. (1997) evaluated the effects of fluridone on the submersed species elodea (*Elodea Canadensis* Michaux), American pondweed (*Potamogeton nodosus* Poiret), sago pondweed (*Potamogeton pectinatus* L), and vallisneria (*Vallisneria americana* Michaux). Emergent natives such as soft-stem bulrush (*Scirpus validus* Vahl.), Egyptian panicgrass (*Paspalidium geminatum* Forssk), maidencane (*Panicum hemitomon* Schult.), pickerelweed (*Pontederia cordata* L.), and sagittaria (*Sagittaria lancifolia* L.) have been evaluated for tolerance to the systemic acetolactate synthase (ALS) inhibiting herbicides bispyribac-sodium {sodium 2,6-bis[(4,6-dimethoxypyrimidin-2-yl)oxy]benzoate}, imazamox {2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid}, and penoxsulam (Koschnick et al. 2007). These ALS herbicides (as well as fluridone) usually have relatively long half-lives, while contact herbicides have a relatively short half-life. Emergent, submersed, and floating species have also been evaluated for sensitivity to the contact herbicide dipotassium salt of endothall (Skogerboe and Getsinger 2002). Those species evaluated included Eurasian watermilfoil, curlyleaf

pondweed (*Potamogeton crispus* L.), Illinois pondweed (*Potamogeton illinoensis* Morong.), sago pondweed, coontail (*Ceratophyllum demersum* L.*)*, elodea, vallisneria, cattail (*Typha latifolia* L.*)*, smartweed (*Polygonum hydropiperoides* Michx.), pickerelweed and spatterdock (*Nuphar advena* Aiton).

Homeowners, commercial nurseries, and farmers in Florida often irrigate plants from surface waters (canals, ponds, lakes, etc.) (Hassell et al. 20004; Hodges and Haydu 2006); non-target plants may be affected if these waters are treated with herbicides for aquatic weed control. The use of herbicide treated irrigation water before herbicide residues dissipate below phytotoxic levels will result in injury or death of irrigated plants. Similarly, farmers may irrigate food crops from treated water, so the aquatic herbicides must have established tolerances or acceptable levels of residue on or in food crops. Tolerances of flumioxazin on certain food crops have been established by the EPA by determining the maximum amount of pesticide residue that can remain in or on a treated food commodity to ensure food safety (EPA 2003), but no such tolerances are required for ornamental plants (non-food crops). Phytotoxicity is a major concern when water with aquatic herbicide residues is used for irrigation of both food and non-food crops. Previous research has been conducted to evaluate the phytotoxic effects of irrigation water containing 2,4-D, copper, diquat, endothall, and fluridone on non-target turf and ornamental species (Andrew et al. 2003; Hiltibran and Turgeon 1977; Koschnick et al. 2005a; Koschnick et al. 2005b; Mudge et al. 2007; Riemer and Motto 1980), but similar studies have not been reported for flumioxazin.

Therefore, the objectives of this research were to determine the effect of flumioxazin on hydrilla with respect to efficacy, photosynthesis, and chlorophyll content as influenced by rates of application, water pH, and light. Further research was conducted to determine the impact of

flumioxazin on other aquatic invasive plants, non-target aquatic plants, non-target row crops and non-target ornamental plants. An additional objective of this research was to judge the suitability of flumioxazin as an aquatic herbicide, establish herbicide use patterns, and determine irrigation restriction parameters through development of possible aquatic use directions.

CHAPTER 2
THE EFFECT OF FLUMIOXAZIN ON HYDRILLA CONTROL IN NATURAL SYSTEMS AND THE INFLUENCE OF WATER PH ON HYDRILLA CONTROL IN MESOCOSMS

Introduction

Flumioxazin is fast acting herbicide which inhibits chlorophyll production and results in the production of toxic singlet oxygen radicals leading to lipid peroxidation and the destruction of cellular components (Duke et al. 1991; Gupta and Tripathy 2000). Injury symptoms may occur within 1 d after plants are treated with flumioxazin and other herbicides in the N-phenylphthalimide family (Ferrell et al. 2007b). Although flumioxazin acts rapidly to inhibit chlorophyll and destroy membranes, this herbicide is rapidly hydrolyzed 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; Vencill 2002). Hydrilla infested waters may have pH in excess of 9.0 (Van et al. 1976) and will likely influence the efficacy of flumioxazin since water with a pH >9.0 results in rapid breakdown of this herbicide by hydrolysis with an average half-life of 17.5 min under laboratory conditions (Katagi 2003). Most aquatic herbicides are degraded by either photolysis or microbial activity (Vencill 2002) so efficacy of these herbicides is unaffected by time of day during application; however, higher pH waters (due to pH cycling throughout the day) may limit when flumioxazin can be applied to successfully control hydrilla. Flumioxazin treatments at rates less than 400 µg L^{-1} in water with a pH >8.0 may not be adequate for successful hydrilla control; therefore, the objectives of these studies were to determine flumioxazin use rates and the effect of water pH.

Materials and Methods

Efficacy in Ponds

Eight ponds infested with hydrilla in Florida were treated in 2006 with submersed flumioxazin applications to determine herbicide efficacy under an Experimental Use Permit (EUP). Flumioxazin was applied at 100, 200, or 400 µg L^{-1} to each pond (0.10 to 10.12 ha)

having water pH ranging from 6.7 to 10.0. Two of the ponds were treated with flumioxazin (400 μg L^{-1}) plus copper (chelated copper, EarthTec®[2], 200 μg L^{-1}). One or more herbicide applications were made to each pond between February and October. All ponds were treated with the appropriate amount of herbicide in an equivalent of 935.0 L water ha^{-1} in a 378.5 L spray tank with hydraulic agitation. Most herbicide treatments were applied using a boat equipped with 3 weighted hoses mounted on the bow of the boat and connected to the spray tank. The weighted hoses were 1.2 to 3.7 m in length on the left, center, and right side of the boat, respectively. Some ponds were treated with hoses (0.3 to 0.6 m long) that trailed behind the boat, which applied the herbicide treatment to the surface of the water, while other treatments were applied using a handgun sprayed at the water surface. All data were based on visual observations (including injury symptoms, time of hydrilla regrowth and recovery time) prior to and after herbicide treatments. Efficacy of flumioxazin treatments was primarily based on how quickly hydrilla produced new apical tips from treated tissue and the amount of time hydrilla needed to reach the water surface following treatments.

Efficacy in Mesocosms

A mesocosm study was conducted and repeated at the Center for Aquatic and Invasive Plants at the University of Florida to determine the effect of flumioxazin on hydrilla. Hydrilla was collected from Rodman Reservoir near Interlachen, FL in June 2005 and February 2007. Four sprigs of hydrilla (15 cm) were planted in each 10 x 10 x 12 cm (1 L) pot filled with masonry sand amended with Osmocote®[3,4] 15-9-12 fertilizer at a rate of 1g kg^{-1} soil. Five pots

[2] Registered trademark of Earth Sciences Laboratories. Rogers, AZ. 72756.

[3] The Scotts Company. Marysville, OH. 43041.

[4] Mention of a trademark or a proprietary product throughout this document does not constitute a guarantee, warranty, or endorsement of the product by the author or the University of Florida, and does not imply its approval to the exclusion of other products that may also be suitable.

were placed in each 95 L HDPE (high-density polyethylene) tub filled with tap water (pH 7.5 at planting). The experiment was a completely randomized design with five replications (tubs). 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 wk in 2005 and 6 wk 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 submersed treatment at 0, 50, 100, 200, 400, 800, and 1600 μg L^{-1} a.i. and the dipotasium salt of endothall was applied to other tubs at 3000 μg L^{-1} a.i. as a comparison treatment.

All plants were harvested 21 d after treatment (DAT) by clipping biomass at the soil line; shoots were placed in a drying oven at 90 C for ca. 1 wk and then weighed. Plant dry weight data were analyzed using non-linear regression (PROC NLIN, SAS Institute 2002) and regression models were used to determine the effective concentration 50 (EC_{50}), which is 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 as 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 and one 15 cm sprig was planted in a 50 mL plastic tube (13.5-cm by 4-cm in diameter). Tubes were filled with potting media[5] amended with Osmocote fertilizer at a rate of 1g kg^{-1} soil and topped off with a 1 cm sand cap. A total of 144 tubes were planted and hydrilla was acclimated in 900 L concrete tanks for 2 wk prior to herbicide treatment. The initial study was conducted in September 2006

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

and repeated in May 2007 under a shadehouse (70% sunlight). Efficacy of flumioxazin was evaluated in low (6.0 to 6.2), medium (7.0 to 7.2), and high (>8.5) pH water. Prior to herbicide treatment, one tube planted with hydrilla was placed into each 95 L HDPE tub that had been filled with 83 L of tap water (pH 8.0). Hydrilla was 45 ± 3.6 cm long with an average dry weight of 0.51 ± 0.3 g at treatment. Muriatic acid was added at a rate of 10 mL or 25 mL per tub to establish medium or low pH treatments as needed. The high pH treatment was achieved by placing extra pots filled with hydrilla in tubs 24 h before herbicide application. These additional plants were kept in high pH treatment tubs so that photosynthesis would maintain water pH at a level above 8.5.

Flumioxazin was applied at a rate of 400 μg L^{-1} and mixed in the water as a submersed treatment. The pH was monitored daily and muriatic acid was added as needed to maintain desired pH levels in the medium and low pH treatments. Following initial treatment, an additional tube of hydrilla was added every 24 h to the treated water at 1, 2, 3, 4, and 5 DAT. Day 0 plants were in the tubs at the time of treatment (Day 0) and removed from the treated solutions after 4 d of exposure and harvested 21 d later. Plants were placed in the tubs at 1, 2, 3, 4, and 5 days after initial treatment to assess if pH or a pH mediated degradation of flumioxazin impacted efficacy. All treated plants were given a 96 h exposure and were then removed from treatment and placed into 900 L mesocosms with flowing tap water (pH 7.5) for 3 wk. This experiment was conducted as a completely randomized design with four replications (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 breakdown under high pH, water samples 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 further breakdown by reducing hydrolysis, frozen and shipped to the Valent U.S.A. Corporation laboratory (Walnut Creek, CA) for flumioxazin analysis by gas chromatography/mass spectrometry (GC/MS) using methods reported by Hirota et al. (1992).

All biomass above the soil line was harvested 21 DAT. Shoots were placed in a drying oven at 90 C for ca. 1 wk and then weighed. Plant weight data were converted to percent of the respective non-treated 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 pH of the water 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 $p \leq 0.05$. Data from both studies were pooled. Water residue data were analyzed using non-linear regression (PROC NLIN, SAS Institute 2002) to calculate flumioxazin half-life at each pH.

Results and Discussion

Efficacy in Ponds

The maximum EUP rate of 400 µg L^{-1} was chosen to treat all the ponds until results suggested lower rates could successfully control hydrilla. Preliminary studies conducted at the Center for Aquatic and Invasive Plants indicated flumioxazin controlled hydrilla at concentrations as low as 50 µg L^{-1}, but higher rates may be required in the field due to the rapid hydrolysis of flumioxazin, especially in high pH waters where hydrilla infestations commonly occur.

The Micanopy pond treated in February 2006 was the most successful of all flumioxazin treatments (Table 2-1). Hydrilla began to exhibit apical tip bleaching within 3 DAT followed by tip abscission and decay within 5 to 7 DAT. The stem segment immediately below the bleached apical tip became chlorotic/necrotic and the tissue eventually reddened before the stem lost cellular integrity and buoyancy. Hydrilla height and dry weight were measured weekly for 2 mo and periodically sampled for 6 mo in the Micanopy pond (data not shown). In addition, a non-replicated residue study was conducted in the Micanopy Pond and data suggested flumioxazin possessed a 25 to 35 h half-life at pH 6.7 (data not shown). Flumioxazin provided >95% control for at least 6 mo after treatment, as few new apical tips could be found throughout the pond; however, flumioxazin was not solely responsible for the extensive hydrilla control due to very low water levels 7 mo after herbicide treatment. Factors that could have influenced this particular treatment included low water pH (6.7), clear water, lower water temperature, time of year, and actively growing plants that were 0.6 to 1.0 m from the surface (non-matted).

The Apopka pond possessed a higher water pH at treatment (7.4), but flumioxazin still provided >80% reduction in hydrilla biomass for 8 wk when treated at 400 μg L^{-1} (Table 2-1). The first flumioxazin treatments in February and March 2006 controlled hydrilla with minimal regrowth in the first two mo after treatment (MAT). Due to the success of these initial treatments at the maximum EUP rate, it appeared that flumioxazin at lower application rates could be as effective in controlling hydrilla as the higher application rates. Three ponds were treated in Kissimmee, FL with three rates of flumioxazin (100, 200, and 400 μg L^{-1}) in May 2006 (Table2-1). These were the first EUP ponds treated that had water with a pH > 9.0, whereas the Micanopy and Apopka ponds had <7.5 pH. Hydrilla in all Kissimmee ponds (A-C) demonstrated similar injury symptoms as the Micanopy and Apopka ponds; however, hydrilla

began to sprout new shoots from treated tissue within 3 to 5 wk after treatment (WAT) and began to reach the surface by 7 WAT. These particular ponds were treated with a hand gun at the water surface, whereas the Micanopy and Apopka ponds were treated with weighted and trailing hoses, respectively. The Kissimmee ponds averaged 2.5 to 4.0 m in depth and it is possible that the majority of the herbicide treatment remained in the upper 1.0 m due to thermal stratification. Thermal stratification can occur in the summer when less dense warmer waters overly colder more dense waters (Wetzel 1975). Thermal stratification is common in surface-matted submersed plants, especially on sunny days in hydrilla stands (Getsinger et al 1990). This thermal layer can create a physical barrier, isolating layers in the water column and preventing surface-applied herbicides from immediately reaching the target vegetation. The upper layer of these infested water bodies may be in excess of 10 C more than the layers below the hydrilla mat. Consequently, the hand gun application technique may have failed to evenly distribute the herbicide into the water column. In addition, these three ponds were more turbid and heavily infested with hydrilla, permitting less light to reach the bottom. Although hydrilla in the Kissimmee ponds had not begun to form a surface mat, the hydrilla was beginning to branch near the water surface at the time of herbicide treatment.

The Apopka pond was retreated in June with only 200 $\mu g\ L^{-1}$ flumioxazin (Table 2-1); however, hydrilla began to form new apical tips within 1 WAT of herbicide application and was surface matted 3 WAT (Table 2-1). At treatment, this pond was shallow (0.5-1.3 m deep) compared to other ponds, so hydrilla did not require a long period of time to once again reach the water surface. The failure of this treatment and the low efficacy in the Kissimmee ponds at 100 and 200 $\mu g\ L^{-1}$ suggested that flumioxazin may not control hydrilla when rates $<400\ \mu g\ L^{-1}$ are

used in the warmer months when hydrilla may cause pH in the water to increase to ≥10.0 during the day by utilization of CO_2 and HCO_3^- (Spencer et al. 1994; Van et al. 1976).

Flumioxazin treatments in the latter portion of June and throughout the rest of the year were applied only with weighted hoses to ensure thorough herbicide distribution in the water column. When Kissimmee pond C was retreated with flumioxazin, the pH was measured throughout the water column; the pH was 6.5 to 7.0 near the bottom (2.5 m) compared to >9.0 at and near the water surface. Weighted hose applications placed approximately one-third of the flumioxazin treatment in direct contact with the lower portion of the hydrilla stand where it would mix with low pH water and consequently degrade at a slower rate than the flumioxazin applied in the upper 1 m of the water.

Due to reduced flumioxazin efficacy in the summer, this herbicide has too short of a half-life in high pH water and does not provide acceptable control of mature hydrilla. Therefore, flumioxazin treatments should be restricted to early season for hydrilla control or should be used in combination with other herbicides as a tank mixture. Combinations of herbicides can result in increased efficacy when used for aquatic weed management (Gray et al. 2007; Nelson et al. 2001). Copper was chosen because of increased efficacy when mixed with diquat for hydrilla and common duckweed control (Langeland et al. 2002; Sutton et al. 1972). This flumioxazin plus copper combination was evaluated as a fall treatment (September and October) in the Interlachen and Kissimmee C pond and provided ca. 6 mo of control. These treatments were successful because of potential synergism between copper and flumioxazin, later season applications (when hydrilla began tuber production and may have been more susceptible to flumioxazin), or lower water pH (7.2 to 8.5) (Haller et al. 1976; Sutton et al. 1972; Van et al. 1978).

These field studies indicate flumioxazin efficacy in ponds is highly variable. Early season applications (February and March) provided longer hydrilla control than treatments in warmer months. The level of control observed with flumioxazin varied according to factors such as water pH, water temperature, maturity/growth stage of hydrilla, time of year, and placement of herbicide in water column. Hydrilla is exposed to higher concentrations of flumioxazin for a longer period of time at low pH than at higher pH. Flumioxazin applied to hydrilla in May through August failed to provide more than a few weeks to a couple of months of control as new apical shoots sprouted from treated tissue on most plants and began to reach the surface within 3 to 7 WAT (Table 2-1). Consequently, several of these ponds were treated with flumioxazin multiple times. Although hydrilla recovered within a few WAT, flumioxazin at 400 μg L^{-1} usually provided an additional 2 to 4 wk of control compared to 100 and 200 μg L^{-1} treatments.

Efficacy in Mesocosms

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 3 cm below the bleached 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. Despite rapid bleaching, reddening, and loss of integrity, hydrilla in all treatments began to regrow from treated tissue and formed new apical shoots at the internodes within 5 to 13 DAT depending on flumioxazin concentration. The calculated EC_{50} of flumioxazin was 56 μg L^{-1}, while the EC_{90} for flumioxazin was 186 μg L^{-1} (Figure 2-1). Low herbicide use rates are desirable in aquatic ecosystems and the proposed flumioxazin labeled rate of 400 μg L^{-1} reduced dry weight by 90% of the nontreated control plants. As a comparison, 3000 μg L^{-1} endothall reduced hydrilla dry weight by 91% in the same time period.

Impact of pH on Efficacy

Dry weights of hydrilla treated at high pH differed from those treated at low and medium pH on all days except for hydrilla added 4 and 5 DAT (Figure 2-2). Although statistically different from the other treatments at 0 DAT, hydrilla placed in the high pH flumioxazin treated water was still reduced by approximately 90% of the nontreated control. There was no difference in dry weight at low and medium pH treatments except for hydrilla placed in treatment solutions 3 or 4 DAT. Hydrilla biomass generally increased daily following exposure by the plants from 1 to 5 d after exposure as percent of nontreated control. This increase in biomass corresponded with a decrease in flumioxazin residue (Figure 2-3). Plants in tubs treated with flumioxazin at medium and low pH levels were still exposed to herbicide through the 96 h exposure period, while approximately 98% of flumioxazin in the high pH treatment was hydrolyzed by 3 DAT (Figure 2-3). Although the residue analysis detected less than 10 $\mu g\ L^{-1}$ of flumioxazin in the high pH solution, hydrilla biomass was still reduced by ca. 10 to 20% on the last 2 d of exposure. These data indicate flumioxazin is active at low concentrations ($<10\ \mu g\ L^{-1}$) and/or secondary metabolites possess activity on hydrilla as well.

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 2-3). 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. Environmental factors in our studies (e.g., higher light levels, potting media, water quality, and plants which possibly absorbed and/or metabolized the flumioxazin molecule) most likely influenced the half-life of flumioxazin. The half-life data in these studies is not directly comparable to previous research by Katagi (2003) since the water pH evaluated differed between studies. However, flumioxazin degradation in both studies followed a similar trend, as pH increased, half-life decreased. Under field conditions, pH fluctuates and

can not be controlled, whereas previously reported data (Katagi 2003) was conducted under stable conditions. Consequently flumioxazin may be exposed to different pHs within the same water body; therefore, degrading at a faster rate within the same system. As a result, some plants would be exposed to higher herbicide concentrations than other plants

These field EUP trials and mesocosm studies provided further evidence of the impact of pH on flumioxazin efficacy. Water pH does not directly influence flumioxazin efficacy; pH influences degradation, which in turn reduces flumioxazin concentration and exposure time to hydrilla. The pH efficacy study data indicated high pH treatments were successful, possibly due to ample mixing and hydrilla exposure to higher concentrations for an extended period of time. Conversely, field treatments usually don't provide adequate mixing and adequate exposure at higher concentrations.

Flumioxazin applied to hydrilla in ponds under high pH conditions were less effective since flumioxazin was rapidly hydrolyzed before it could control the weed; however, application of flumioxazin to water with pH levels less than 8.0 generally provided adequate to complete control of 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 have become chlorotic and would not have been reduced by 90% as in the 0 d treatments. Other contact herbicides such as endothall require exposures of at least 48 h at 2 mg L^{-1} acid equivalent (a.e.) to provide greater than 85% reduction in biomass of hydrilla (Netherland et al. 1991). Flumioxazin appears to require greatly reduced exposure times and has increased activity in lower pH water. The degradation of flumioxazin within minutes of application in the high pH treatment prevented a significant reduction in hydrilla biomass 2 to 5 DAT. Flumioxazin is not

highly water soluble (1.79 mg L^{-1}) (Vencill 2002), so uptake into hydrilla is probably rapid due to the lipophilic nature of this molecule.

Hydrilla treated with flumioxazin in EUP ponds in Florida showed similar bleaching of apical stem segments regardless of stem length or water depth. Field treated plants followed the same bleaching pattern and reddening of the stem below the tip as noted in the preliminary mesocosm study that demonstrated flumioxazin activity on hydrilla. However, symptoms of hydrilla treated at depths greater than 1.5 m were less pronounced and stems had only 5 to 10 cm of reddened tissue below the apical tip. Submersed plants growing at these depths are often light limited (Haller and Sutton 1975) and net photosynthesis is restricted to the apical portions of the plant. Upon forming a dense mat on and just below the water surface, hydrilla can further limit light penetration. Flumioxazin and other 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 has been degraded by hydrolysis and is no longer present to inhibit the PPO (Aizawa and Brown 1999; Matringe et al. 1989; Cobb 1992). 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). Lower efficacy observed in EUP pond treatments was likely due to high pH, low light intensity in these deeper waters, and growth stage. Most treatments resulted in >95% control of hydrilla within 1.5 m of shore, where water was shallow, hydrilla was less than 0.5 m in length and plants were not shaded by emergent or floating vegetation. This evidence further supports previous

studies (Sherman et al. 1991; Wright et al. 1995) that show higher light levels increase flumioxazin activity.

Table 2-1. Hydrilla infested ponds in Florida treated with flumioxazin under an Experimental Use Permit in 2006.

Location	Date	pH	Comment/Results
Micanopy	Feb 2006	6.7	WH application at 400 µg L^{-1}; no regrowth for 6 MAT; >95% hydrilla control for 6-8 MAT
Apopka	March 2006	7.4	TH application at 400 µg L^{-1}; >80% hydrilla control for 8 wk; 30% hydrilla surface matted by 3 MAT
Kissimmee A	May 2006	9.7	HG application at 100 µg L^{-1}; hydrilla regrowth 3 WAT and near surface 7 WAT
Kissimmee B	May 2006	9.7	HG application at 200 µg L^{-1}; hydrilla regrowth 3 WAT and near surface 7 WAT
Kissimmee C	May 2006	9.7	HG application at 400 µg L^{-1} on approximately one third of pond; bleaching and control in whole pond but better control in treated area; hydrilla regrowth 5 WAT and near surface 7 WAT
Apopka	June 2006	7.2	TH application at 200 µg L^{-1}; hydrilla regrowth at 1 WAT was surface matted 3 WAT
Eustis	June 2006	9.5	TH application at 400 µg L^{-1}; minimum injury symptoms and hydrilla reached surface 1 MAT
Interlachen	June 2006	7.2	HG application at 200 µg L^{-1}; hydrilla controlled for 1 month but began to regrow and reached surface 12 WAT
Kissimmee C	June 2006	9.4	WH application at 400 µg L^{-1}; hydrilla regrowth 3 WAT, had not reached surface by 10 WAT
Gainesville	June 2006	10.0	WH application at 400 µg L^{-1}; minimum hydrilla injury and reached surface 1 MAT
Interlachen	July 2006	7.2	WH application at 400 µg L^{-1}; hydrilla regrowth at 1MAT and surfaced 3 MAT
Kissimmee C	Sept 2006	8.5	WH application at 400 µg L^{-1} plus 200 µg L^{-1} copper; hydrilla regrowth 2 MAT, but had not reached surface by March 2007
Interlachen	October 2006	7.2	WH application at 400 µg L^{-1} on whole pond, but half of pond received copper at 200 µg L^{-1}; >6 months of control; hydrilla began to reach surface in March 2007

Abbreviations: WAT, weeks after treatment; MAT, months after treatment; WH = weighted hose, 1.2 to 3.7 m long; TH = trailing hose, 0.3 to 0.6 m long injected at rear of boat; HG = hand gun, using pressure to inject herbicide 0.6-0.9 m into the water ahead of boat.

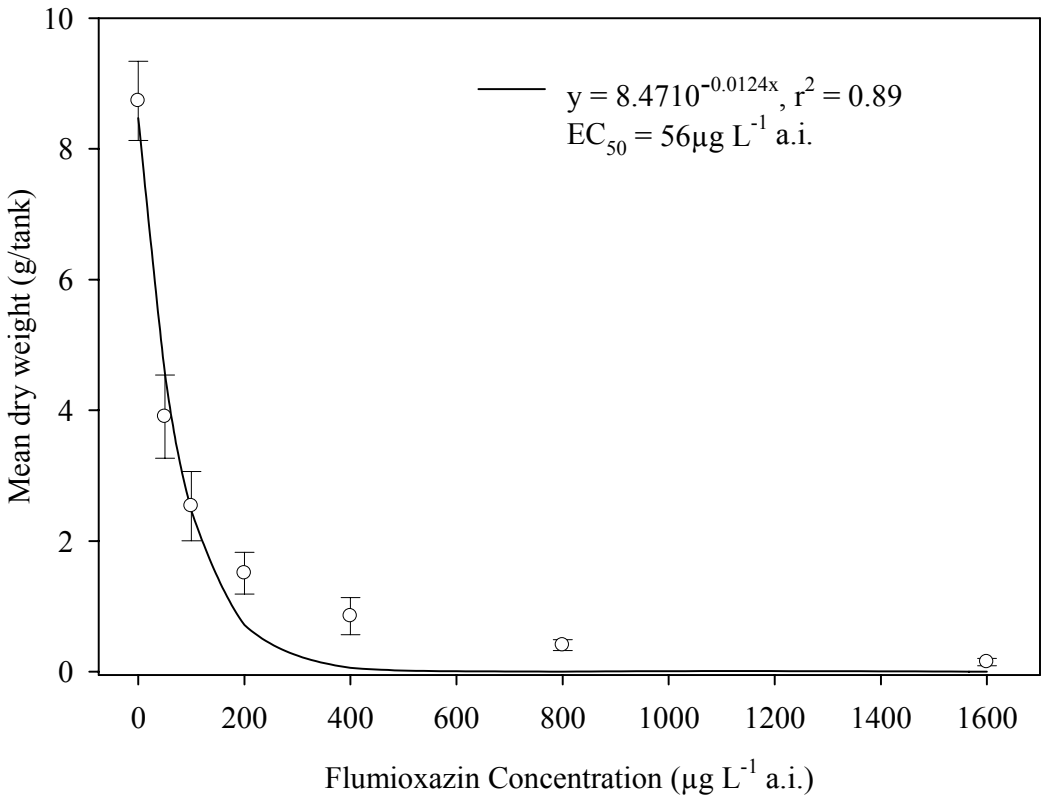

Figure 2-1. The effect of flumioxazin concentration on hydrilla dry weight 21 d after exposure under 70% sunlight. Flumioxazin applied as a single application to hydrilla cultured in 95 L tubs (pH 9.0-9.5). Data are shown as dry weight means ± standard error (n=10). EC_{50} = effective concentration 50, concentration of flumioxazin in water required to reduce hydrilla biomass by 50%.

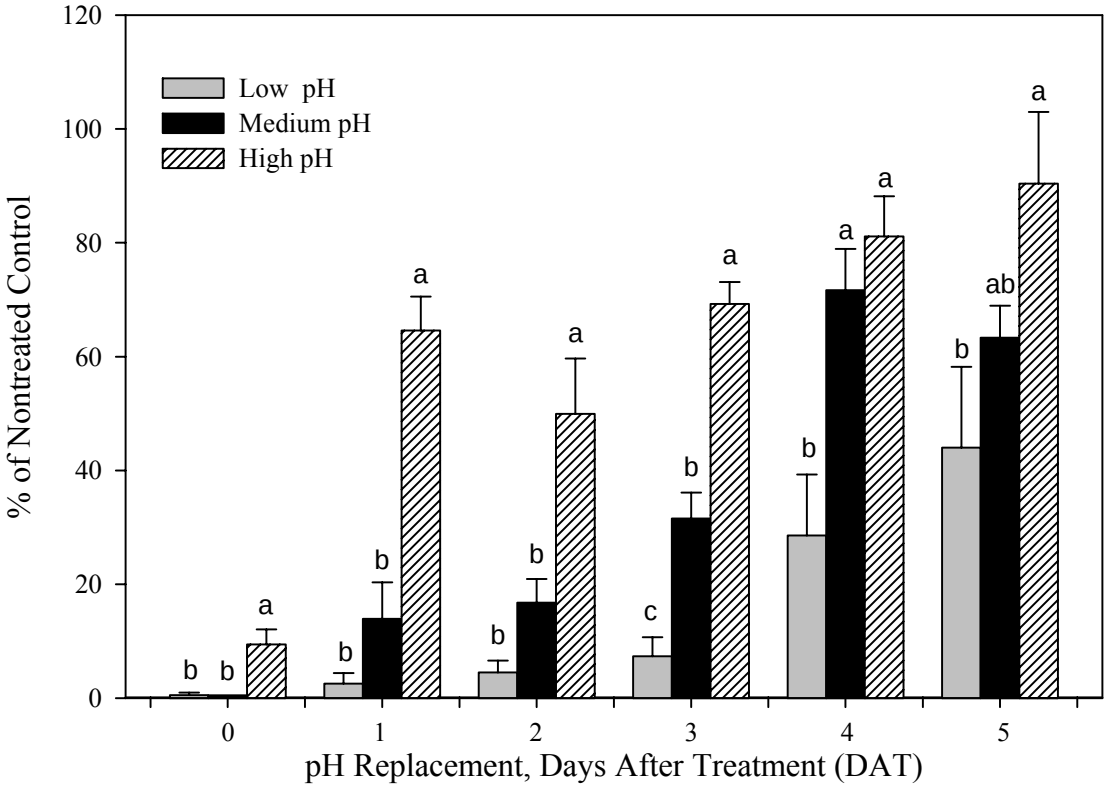

Figure 2-2. The effect of flumioxazin at 400 µg L^{-1} on hydrilla dry weight as influenced by low (6.0 to 6.2), medium (7.0 to 7.2), and high (>8.5) water pH under 70% sunlight. Hydrilla plants were added to low, medium, and high pH water treated with flumioxazin 0 to 4 d after initial treatment and allowed to grow for 21 d after treatment until harvest. Data are shown as percent of nontreated control of each pH ± standard error (n=8). Treatment means within a particular day were separated using least square means ($p<0.05$).

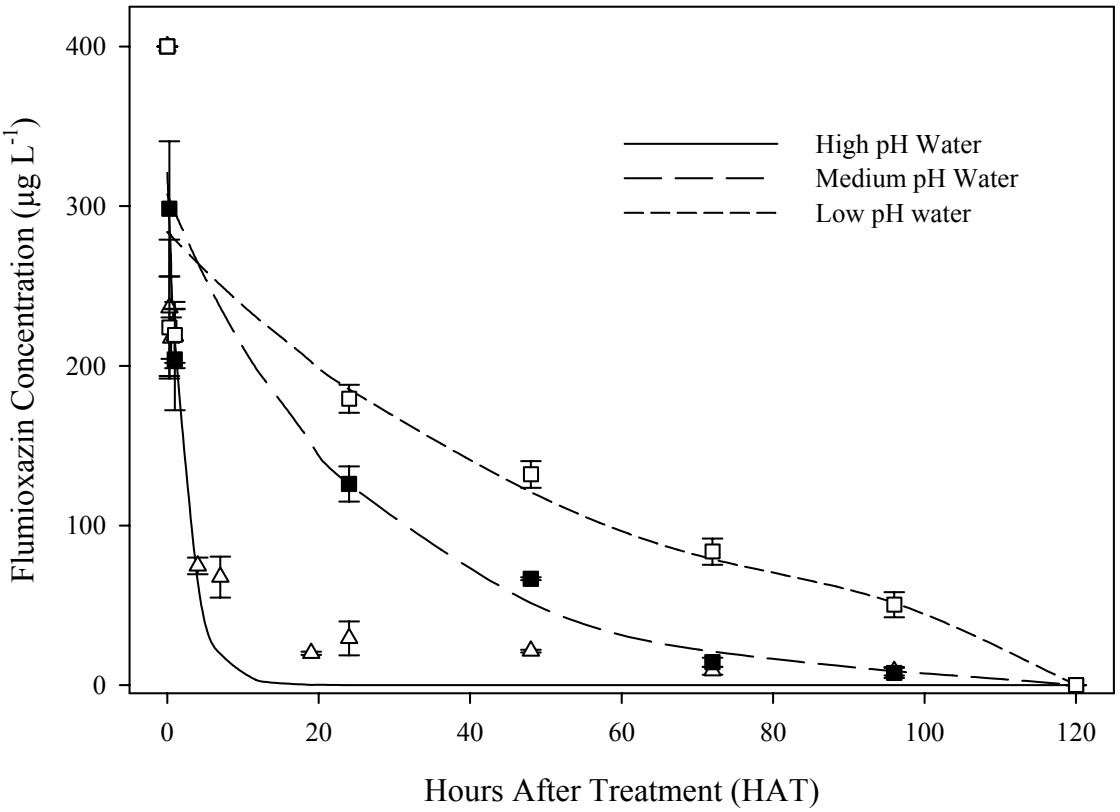

Figure 2-3. Dissipation of flumioxazin applied at 400 µg L^{-1} to low (6.0 to 6.2), medium (7.0 to 7.2) and high pH (>8.5) tap water in 95 L tubs under 70% sunlight. The dissipation of flumioxazin was calculated using non-linear regression (exponential decay) for the low ($y = 0.0178e^{-0.0178x}$; $r^2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

CHAPTER 3
THE EFFECT OF FLUMIOXAZIN ON HYDRILLA PHOTOSYNTHESIS AND CHLOROPHYLL CONTENT

Introduction

Flumioxazin was evaluated for hydrilla control in EUP field trials throughout Florida in 2006 and 2007 in water bodies ranging in pH from 6.7 to 10.0 at rates of 100 to 400 μg L^{-1} (see Chapter 2). Despite early season success, summer applications of flumioxazin failed to provide adequate season-long control. Most summer EUP trials resulted in rapid injury to the hydrilla canopy followed by re-infestation within 1 to 4 months.

Higher water pH (either due to hydrilla infestations or pH cycling throughout the day) may limit when flumioxazin can be applied for successful hydrilla control. Flumioxazin applied to water with a pH >9.0 is rapidly degraded by hydrolysis and submersed aquatic plants growing at greater depths are often light limited (Haller and Sutton 1975). Flumioxazin and other PPO inhibitors require full sunlight for optimal activity (Sherman et al. 1991; Wright et al. 1995), so the limited quantity of light at greater water depths may reduce flumioxazin activity on hydrilla.

Measurement of the net photosynthetic rate of submersed aquatic plants has been utilized to study the effects of aquatic herbicides on submersed plants (MacDonald et al. 2003; Netherland and Getsinger 1995a; Netherland and Getsinger 1995b). Carbon dioxide is absorbed by plants and fixed as one of the primary products of photosynthesis, while O_2 is evolved from the splitting of water (Oja et al. 2007). Submersed aquatic plants treated with herbicides that interfere with photosynthesis or the production of light capturing pigments (chlorophyll and carotenoids) typically evolve less O_2 compared to nontreated plants (MacDonald et al. 2002; Netherland and Getsinger 1995a). Measuring total chlorophyll content in aquatic plants provides further evidence of the impact of these herbicides on plant status (Doong et al. 1993; Netherland and Getsinger 1995a). Such studies provide information regarding the health of plants treated

with herbicides that may not show visual damage for several days. Therefore, the objective of these studies was to determine the effect of water pH, light, and flumioxazin rate on net photosynthesis and chlorophyll content of hydrilla.

Materials and Methods

Photosynthesis and pH

The effects of flumioxazin at low (6.0) and high (9.0) pH on the net photosynthetic rates of hydrilla were determined by measuring oxygen evolution (dissolved oxygen, DO) over time. Hydrilla was collected at Rodman Reservoir near Interlachen, FL in January 2007. All DO techniques were similar to those reported by Netherland and Lembi (1992) and Netherland and Getsinger (1995b). The plants evaluated in those previously cited studies remained continuously in biological oxygen demand (BOD) bottles as static exposures, whereas plants in these studies were removed every 24 h from herbicide treatments to prevent oxygen saturation. Apical hydrilla tips (4 cm) were excised from freshly collected plants and placed in clear plastic cups (473 mL) with 350 mL deionized water (DI) and allowed to acclimate for 24 h before treatment. Tips were acclimated and treated in a growth chamber[6] at a constant temperature of 27 C with a 14 h light/10 h dark photoperiod. The daytime light intensity was 380 µmol m^{-2} s^{-1}.

Preliminary studies were conducted to determine appropriate concentrations of nutrients and buffer (data not shown) to attain active growth and hold pH. Nutrients were supplied by 1% v/v Hoagland's solution and 4.7mM $NaHCO_3$ was added as a carbon source. The experimental design was a randomized design with 4 replications. The culture solution of plants treated at the low pH also received 10 µL of muriatic acid (diluted HCl) and MES buffer (5 mM) to maintain a pH of 6.0. Hydrilla tips were treated with flumioxazin at 0, 100, 200, 400, and 800 µg L^{-1} in the

[6] Percival Scientific, Inc. Perry, IA. 50220.

same plastic culture cups and the same hydrilla tip was reused daily to measure DO over time. DO was measured pre-treatment (0), 24, 48, 72, 96, 120, 144, and 168 h after treatment (HAT) using a dissolved oxygen meter[7]. To eliminate possible oxygen saturation, treated and nontreated tips were removed from the plastic cups, rinsed twice in DI water to ensure no herbicide residue transfer and placed in 300 mL BOD bottles[8] filled with fresh DI water and the same nutrient solution as the plastic culture cups for DO daily measurements. Initial DO measurements were recorded for each bottle prior to tip placement in the bottles, then tips were placed in BOD bottles and allowed to incubate for 1 h. Final DO was measured in each bottle and tips were removed, gently blotted with a dry paper towel, and weighed (fresh weight). Tips were immediately returned to their original cups after fresh weights were obtained. BOD bottles were emptied each day and rinsed after completion of DO measurements. The formula for calculating the net photosynthetic rate was:

(Final DO-Initial DO)/weight(g)/time(min)*1000 = μg O_2 g fresh weight^{-1} min^{-1}.

Many of the treated hydrilla tips lost turgor and became defoliated during the course of the experiments and disintegrated by the conclusion of the study. Decayed tips were discarded when they occurred and a value of 0 for net photosynthesis was assigned to that treatment for the remainder of the experiment.

All data were normalized to the control to account for differences in photosynthetic rates at each pH. Percent data were analyzed using non-linear regression (exponential decay) (PROC NLIN, SAS Institute 2002) and regression models were used to determine an ET_{50}, which is the amount of time hydrilla was exposed to flumioxazin before a 50% reduction in photosynthesis

[7] Accumet Excel XL40 Dissolved Oxygen/BOD/OUR/SOUR Temperature Meter. Fisher Scientific. Pittsburgh, PA 15275.

[8] Wheaton Science Products. Millville, NJ. 08332.

was reached for each rate and pH. The ET_{50} values were calculated using the slope of the line based on the following formula: $\ln(0.5)/-b_1$. Additionally, SAS produced upper and lower 95% confidence intervals (CI) for each line. When placing an upper and lower 95% confidence interval on the slope of a non-linear equation, calculated ET_{50} values ± 95% CI will not be ± a single value. This is because a non-linear equation will not always fit exactly between the range of data values. This can then require differing 95% CI values for the upper or lower limits, depending on which direction the equation trends toward. For these experiments, data were pooled because were no difference between the slopes of regression equations for both experiments at the 95% level of confidence.

Photosynthesis and Light

The effects of light quantity on net photosynthetic rates of apical hydrilla tips treated with flumioxazin were evaluated in May and June 2007. Hydrilla was collected from the Withlacoochee River near Dunnellon, FL in May 2007. Methods used were as described above for the DO pH experiment except plants were only cultured at pH 9.0. Apical tips were acclimated for 24 h and treated at low (20), medium (170), and high (400 μmol m^{-2} s^{-1}) light quantities in a growth chamber. The low light quantity chosen in this study is near the upper threshold of the light compensation point for hydrilla and is typical of the quantity of light found near the bottom of a pond/lake, whereas the high light quantity is less than the light saturation point of hydrilla and is near the light quantity found near the surface of matted hydrilla (Van et al. 1976; Bowes et al. 1979; Steward 1991). Hydrilla DO was measured every 24 h and the experiment was concluded 168 HAT. Light levels in the chambers were adjusted by removing incandescent and fluorescent bulbs to obtain desired light levels. Each light quantity treatment consisted of 10 hydrilla tips (cups); 5 control plants and 5 receiving flumioxazin at 400 μg L^{-1} for

a total of 30 tips (5 reps/treatment). Cups were placed in a randomized design in each of the growth chambers.

All data were normalized to the control to account for differences in photosynthetic rates at each light level. Percent data were analyzed using non-linear regression (exponential decay) (PROC NLIN, SAS Institute 2002) and regression models were used to calculate ET_{50} values at each light quantity.

Effect of Contact Herbicides on Photosynthesis

The effect of flumioxazin on hydrilla photosynthesis was compared to the effect of other registered contact aquatic herbicides in April 2007. Most culture and treatment techniques used in this experiment were the same as those described above in the DO pH study but this experiment had 5 replications, plants were treated at pH 9.0, and this experiment was concluded 96 HAT. Hydrilla was cultured and treated with a light quantity of 380 μmol m^{-2} s^{-1}. Herbicide treatments applied to hydrilla apical tips in this experiment included flumioxazin at 400 μg L^{-1} (all flumioxazin treatment combinations were applied at this rate), copper (copper-ethylenediamine complex, Komeen®[9], 50 and 200 μg L^{-1} a.i., hereinafter referred to as K50 and K200), flumioxazin plus K50, flumioxazin plus K200, and the dipotassium salt of endothall (5000 μg L^{-1}). All data were normalized to the control and analyzed using non-linear regression (exponential decay) (PROC NLIN, SAS Institute 2002) and regression models were used to calculate ET_{50} values for each herbicide treatment.

Chlorophyll Content

The effect of flumioxazin on chlorophyll content of hydrilla was determined using hydrilla collected from the Withlacoochee River near Dunnellon, FL in April and May 2007. Four 15 cm

[9] Registered trademark of SePRO Corporation. Carmel, IN. 46032.

sprigs were planted in each 10 x 10 x 12 cm (1 L) pot filled with masonry sand amended with Osmocote (15-9-12) fertilizer at a rate of 1g kg^{-1} soil. Two pots of hydrilla were placed in each 18.9 L bucket (28 cm diameter by 31 cm deep) filled with tap water (pH 7.5 at planting) under 70% sunlight. Hydrilla was allowed to acclimate for 4 and 3 wk for the initial and repeated study, respectively, at which time plants had reached the water surface and begun to branch. The pH ranged from 9.0 to 9.5 when flumioxazin was applied at 0, 50, 100, 200, 400, 800, and 1600 µg L^{-1}. This experiment was a randomized design with 3 replicates. Apical tips (ca. 2.5 cm, 0.09-0.11 g fresh weight) were collected from each treatment 1, 2, 3, and 4 DAT for chlorophyll analysis. Bleaching of hydrilla continued beyond 4 DAT, but no apical tips could be used for chlorophyll analysis as they had abscised the stems and were disintegrating. Excess water was removed by gentle blotting with paper towels, then tips were immediately weighed (fresh weight), placed in 20 mL scintillation vials, and frozen until chlorophyll analysis. Total chlorophyll was extracted by placing the apical tips in tubes containing dimethylsulfoxide (DMSO) (Hiscox and Israelstam 1979) in a water bath at 60 C for 6 h. Chlorophyll content was determined spectrophotometrically (Arnon 1949) and expressed as mg chlorophyll kg^{-1} of fresh weight. Non-linear regression (PROC NLIN, SAS Institute 2002) was used to determine the effect of flumioxazin on chlorophyll content.

Results and Discussion

Photosynthesis and pH

All flumioxazin treatments of 100 to 800 µg L^{-1} showed similar trends with respect to reduction in photosynthesis except 100 µg L^{-1} at high pH (Figure 3-1) which only reduced photosynthesis by approximately 20% of the nontreated control plants 168 HAT. The amount of time required to reduce photosynthesis by 50% (ET_{50}) of the control plants was 737 h for flumioxazin applied at 100 µg L^{-1} in the high pH solution (Table 3-1). All other treatments

reduced photosynthesis by 50% of the nontreated control between 68 and 118 HAT (2 to 5 DAT). Flumioxazin applied at 800 μg L^{-1} in low pH was different from 200 μg L^{-1} in the high pH treatment and from 100 μg L^{-1} in low pH treatment based on ET_{50} (± 95% confidence interval) values. No other treatment differences were observed at low or high pH. Photosynthesis was reduced more rapidly as a function of flumioxazin concentration at both high and low pH. Net photosynthesis of nontreated control plants prior to herbicide treatment (0 HAT) was 174.6 ± 16.9 (low pH) and 155.9 ± 8.7 μg O_2 g fresh $weight^{-1}$ min^{-1} (mean ± standard error) (high pH), respectively, compared to 61.2 ± 10.9 (low pH) and 65.3 ± 10.4 μg O_2 g fresh $weight^{-1}$ min^{-1} (high pH) 168 HAT. The low and high pH nontreated control plants decreased in net photosynthesis by 64.9 and 58.1%, respectively. This gradual decline in net photosynthesis over the course of the experiment was possibly attributed to decline in $NaHCO_3$ and Hoagland's solution in the plastic cups in addition to stress caused by blotting them dry to obtain fresh weight.

These data indicate a minimal pH effect on photosynthesis except for flumioxazin applied at 100 μg L^{-1} to apical tips in high pH treatment solutions. Based upon these data, flumioxazin should be applied at rates higher than 100 μg L^{-1} in high pH waters to over come the effects of rapid breakdown. Regardless of pH, flumioxazin at 400 and 800 μg L^{-1} had similar ET_{50} values and DO measurements at 168 HAT. The light quantity in this study (380 μmol m^{-2} s^{-1}) in combination with low pH or flumioxazin at rates above 100 μg L^{-1} appears to be sufficient to reduce photosynthesis and kill apical tips exposed for 168 h. Apical hydrilla tips in this particular study began to bleach at ca. 48 HAT followed by whole tip disintegration within 120 HAT in all treatments except high pH 100 and 200 μg L^{-1} treatments (data not shown). These data suggest that flumioxazin is rapidly absorbed into hydrilla despite the half-life being very

short in high pH water (9.0) and is similar to the pH efficacy data (see Chapter 2). However, these studies provide evidence that flumioxazin concentration greater than 200 μg L^{-1} may be required to overcome the rapid degradation when applied in high pH water.

Flumioxazin does not halt hydrilla photosynthesis as quickly as other herbicides such as diquat. MacDonald et al. (2002) demonstrated diquat at 344 μg L^{-1} decreased net photosynthesis in apical hydrilla tips by 44% 10 min after treatment and completely inhibited net photosynthesis 2 HAT. Although diquat quickly inhibits photosynthesis under controlled conditions, it is typically applied with copper to improve efficacy, due to difficulty of controlling hydrilla when applied alone (Sutton et al. 1970; Sutton et al. 1972; Anonymous 2003).

Photosynthesis and Light

The effect of 400 μg L^{-1} flumioxazin on photosynthesis of apical hydrilla tips at low (20), medium (170), and high (400 μmol m^{-2} s^{-1}) light levels showed apical hydrilla tips treated at medium and high light levels followed a similar trend (Figure 3-2). The photosynthetic rates of hydrilla tips treated under high light conditions were different from those at the medium light by the conclusion of the study. Low light treated tips were still photosynthesizing at approximately 73% of the nontreated control plants by the conclusion of the study. The amount of time required by flumioxazin to reduce photosynthesis by 50% (ET_{50}) at medium light was not different from the low or high light quantities (Table 3-2). The high and low light quantities differed with respect to the calculated ET_{50} values. Low light treated hydrilla would require an estimated 303 h to achieve a 50% reduction, while high light plants only required 99 h. Low light treated tips did not visually appear to be different from the low light nontreated control plants. There was no bleaching or chlorosis of the apical tips in either treatment. In addition, low light treated apical tips elongated more than 5 cm during the course of the study, whereas those treated at medium and high light quantities elongated less than 1 cm (data not shown).

Low light conditions such as those used in this study are similar to those found at the mid-depths or bottom of water bodies infested with hydrilla.

Previous research demonstrated that the light compensation point of hydrilla ranged from 7 to 20 μmol m^{-2} s^{-1} (Van et al. 1976; Bowes et al. 1979; Steward 1991). The low light quantities in these studies were ample for hydrilla to produce O_2, but were not sufficient enough for flumioxazin to completely halt photosynthesis or bleach the apical tips. Conversely, the high light levels reduced photosynthesis to about 30% of the nontreated control plants by the conclusion of the study. Flumioxazin is a strong inhibitor of chlorophyll synthesis and prevents the transformation of protoporphyrinogen IX into protoporphyrin IX. (Aizawa and Brown 1999; Matringe et al. 1989; Cobb 1992). Protoporphyrinogen IX accumulates in plastids due to inhibition of the PPO enzyme and then diffuses through the plastid membrane into the cytosol, where it is oxidized to protoporphyrin IX by a plasma membrane-bound protox (Dayan and Duke 1997; Duke et al. 1991). Protoporphyrin IX reacts 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). The low light treated plants did not exhibit bleaching within the course of these studies. However, chlorophyll turnover is continuously occurring as the average half-life of chlorophyll is relatively short (minutes to days) (Grumbach et al. 1978; Hendry and Stobart 1986); therefore, protoporphyrinogen IX should have been inhibited during the course of the experiment and result in bleaching of the apical tip. Apparently, chlorophyll turnover in hydrilla tips grown under low light was reduced, but not rapid enough to result in bleaching.

Hydrilla infested lakes form dense canopies and restrict light penetration to the lower portions of the plant (Haller and Sutton 1975). This study provides a possible explanation for the

observations noted at EUP treatment sites where the surface canopy of hydrilla was injured, the lower stems received minimal injury, and rapid regrowth occurred from the lower stem segments (see Chapter 2). These data provide evidence that low light levels hinder flumioxazin activity and explains why rapid regrowth occurs from apical tips growing under the hydrilla canopy.

Effect of Contact Herbicides on Photosynthesis

Flumioxazin applied alone to apical hydrilla tips at 400 μg L^{-1} required 156 h to reduce photosynthesis by 50% (ET_{50}) of the nontreated control plants (Table 3-3). Copper applied alone at 50 μg L^{-1} had minimal impact on photosynthesis of hydrilla, whereas copper at 200 μg L^{-1} reduced DO by 50% within 35 HAT. Flumioxazin plus K200 reduced photosynthesis by 50% more rapidly than any other treatments evaluated; however, this increase in activity was minimal compared to K200 applied alone. The combination of flumioxazin and copper may be beneficial for control of surface matted hydrilla. Flumioxazin activity has been variable in EUP field research (see Chapter 2) and the combination of these products could aid in overcoming the rapid breakdown in high pH water. Copper alone has activity on submersed and floating aquatic weeds such as elodea (*Elodea densis*), coontail, giant salvinia (*Salvinia molesta* D. S. Mitchell), and duckweed (Angelo et al. 1998; Filbin and Hough 1979, Nelson et al. 2001; Ware 1966). In addition, copper has been used successfully in combination with diquat for hydrilla control (Sutton et al. 1972; Langeland et al. 2002) and overcomes diquat resistance in landoltia [*Landoltia punctata* (G. Meyer) D.H. Les and D.J. Crawford] (Koschnick and Haller 2006).

Previous research has shown that flumioxazin at 1, 10, and 50 μg L^{-1} decreased photosynthetic capacity of common duckweed (Lemna minor L.) by 23, 62, and 64%, respectively (Frankart et al. 2002). In these studies photosynthetic capacity of common duckweed was inhibited more when plants were exposed to 200 μg L^{-1} of copper mixed with flumioxazin than all rates of flumioxazin applied alone.

Photosynthesis in this study was only reduced to 60% of the nontreated control with 5000 μg L^{-1} of endothall after a 96 h exposure. MacDonald et al. (2002) reported technical grade endothall acid applied at 372 mg L^{-1} stopped photosynthesis 120 min after treatment; however, this rate of endothall is approximately 75x higher than the maximum label rate of the dipotassium salt of endothall (Anonymous 2007a). Previous research has shown that this formulation of endothall kills various terrestrial and aquatic plants by inhibiting respiration (MacDonald et al. 1993), photosynthesis (Turgeon et al. 1972), lipid synthesis (Mann and Pu 1968), and protein synthesis (Mann et al. 1965) as well as causing cellular disruption (Keckemet 1968; Keckemet and Nelson 1968). According to the calculated photosynthesis ET_{50} values, flumioxazin alone is slower than the dipotassium salt of endothall at reducing DO by 50%.

Flumioxazin and endothall were applied at the maximum labeled rate compared to 5 and 20% of maximum labeled rate for copper at 50 and 200 μg L^{-1}, respectively. These data indicate that copper at 200μg L^{-1} reduced hydrilla photosynthesis faster than flumioxazin or the dipotasium salt of endothall. The addition of 50 μg L^{-1} copper to 400 μg L^{-1} flumioxazin did not decrease the calculated ET_{50} value from 400 μg L^{-1} flumioxazin alone. However, the addition of 200 μg L^{-1} copper to the flumioxazin did provide a faster reduction in photosynthesis.

Chlorophyll Content

Flumioxazin applied to hydrilla in a mesocosm study at rates of 50 to 1600 μg L^{-1} showed that chlorophyll content was reduced as function of increased flumioxazin concentrations (Figure 3-3). Hydrilla chlorophyll content 3 and 4 d after the flumioxazin treatment reflected more of a flumioxazin rate response than 1 and 2 DAT, as indicated by the trend of the regression lines. These data demonstrate the ability of flumioxazin to rapidly bleach apical hydrilla tips due to the inhibition of protoporphyrinogen oxidase , which is a precursor to chlorophyll (Aizawa and Brown 1999; Matringe et al. 1989; Cobb 1992). Hydrilla treated with flumioxazin in EUP trials

(see Chapter 2) showed similar apical tip bleaching in the field within 1 to 3 DAT followed by reddening of the stem 5 cm below the apical tip. These results are similar to those observed in these studies. Some of the red coloration in treated plants was likely due to anthocyanin formation or destruction of other secondary chlorophyll protecting pigments which were visible because of chlorophyll inhibition or were produced in response to the stress of the herbicide treatment (Hrazdina 1982; Sandmann et al. 1991; Spencer and Ksander 1990). Susceptible terrestrial crops and weeds exposed to other PPO inhibiting herbicides rapidly show injury symptoms including chlorosis, leaf crinkling, and stunting followed by necrosis (Lovell et al. 2001; Wilcut et al. 2001; Vencill 2002). Hydrilla may grow as much as an inch per day (Langeland 1996) and new tissue and chlorophyll is being produced in the tip much more quickly than in the lower stem segments. PPO inhibitors would inhibit chlorophyll and consequently produce more toxic radicals in the tip region than in the more mature and shaded lower stem segments. Lower stem segments of plants treated with flumioxazin were not tested for chlorophyll content in these studies since flumioxazin primarily causes bleaching in the upper apical tip (1 to 3 cm) where chlorophyll turnover is occurring more rapidly. In these chlorophyll studies, flumioxazin was applied to hydrilla cultured in high pH water. Further studies could be conducted to evaluate the effects of flumioxazin on chlorophyll content in low pH water; however, regardless of pH, only the apical portion of the plant will bleach since chlorophyll is being produced more rapidly in this region and consequently flumioxazin toxicity symptoms are more pronounced.

In conclusion, these data once again show flumioxazin is rate dependent up to 400 $\mu g\ L^{-1}$ when applied to hydrilla in high pH solutions. Most of the treatments applied to apical hydrilla tips reduced photosynthesis before the conclusion of the experiments. Approximately half of

hydrilla's biomass occurs in the upper 0.5 m of the water column when the plant forms a dense surface mat (Haller and Sutton 1975). Most of the apical tips can be found in the upper surface of these mats; hence, flumioxazin treatments tend to work exceptionally well on the upper canopy because of ample light. Treated apical tips growing >1.5 m from the surface under low light conditions at and near the lake bottom are bleached, but the lower stem sections typically don't become chlorotic and disintegrate in the same manner as the upper stem segments which receive high light intensity. Stem segments treated with flumioxazin responded by reduced photosynthesis but didn't disintegrate similar to the apical tips. The light study confirmed the lack of flumioxazin efficacy at light levels near hydrilla's light compensation point which is typically found below hydrilla mats in greater water depths. These studies provide further evidence that pH is not the only factor influencing flumioxazin efficacy at 400 $\mu g\ L^{-1}$. Light quantity and tissue type can reduce efficacy in field treatments. If flumioxazin received a Section 3 label for aquatic use, treatments applied to high pH water (>9.0) may result in failure. Based on these data and EUP research, applications should be made early in the morning before the pH is cycled to above 9.0, early in the year, or before hydrilla forms dense canopies. Flumioxazin is most effective in rapidly growing tissue where chlorophyll turnover is high. Lower pH water results in a longer half-life and increases exposure of plants to higher flumioxazin concentrations for a longer period of time. Non-matted hydrilla allows more light to penetrate to the lower canopy, where flumioxazin will more injury to lower portions of the hydrilla plant. Thus, optimum conditions for aquatic weed control with flumioxazin included rapid growth, low pH, and high light intensity.

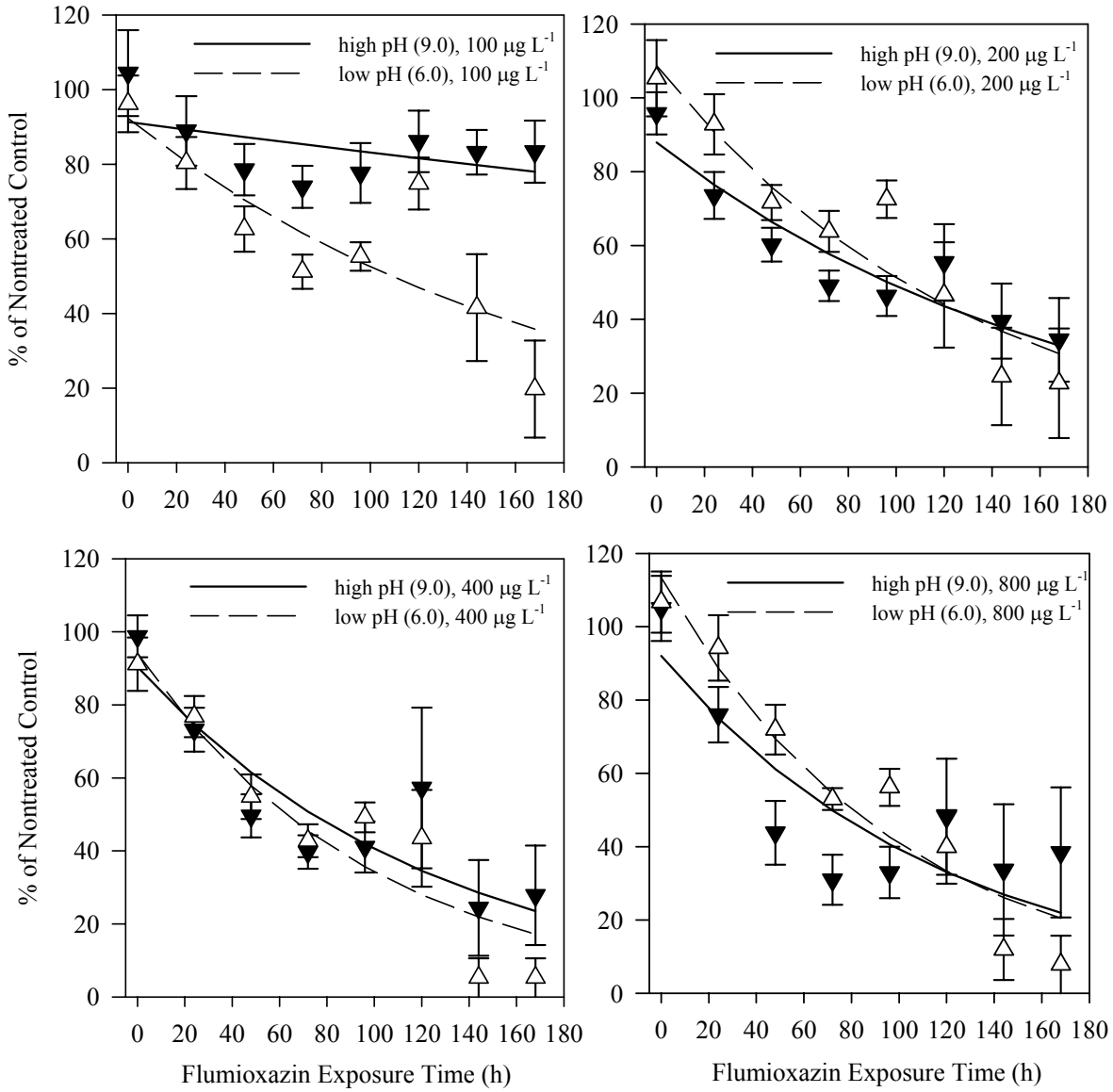

Figure 3-1. The effect of flumioxazin rate at high (9.0) and low pH (6.0) on photosynthesis of apical hydrilla tips cultured in a growth chamber for 168 h at 380 µmol m^{-2} s^{-1} of light quantity. Data are normalized to the control at each respective pH and shown as means ± standard error (n=8).

Table 3-1. The effect of flumioxazin on photosynthesis of apical hydrilla tips at high (9.0) and low (6.0) pH[a].

pH 9.0[b]	ET_{50}[c] (95% CI[d])	Regression equation	r^2
100	737 (315-2166)	y = 91.3356e-0.000940x	0.93
200	118 (90-175)	y = 87.9257e-0.00585x	0.88
400	87 (61-148)	y = 90.3356e-0.00801x	0.75
800	81 (57-144)	y = 92.0237e-0.00852x	0.71
pH 6.0[e]			
100	123 (89-197)	y = 92.3011e-0.00564x	0.85
200	92 (70-134)	y = 108.8e-0.00753x	0.85
400	69 (55-91)	y = 94.0648e-0.0101x	0.86
800	68 (56-87)	y = 113.4e-0.0102x	0.89

[a] Hydrilla cultured in a growth chamber for 168 h at 380 µmol m^{-2} s^{-1} of light quantity at high and low pH[a]. Data are normalized to the control at each respective pH.
[b] High pH: 9.0.
[c] Effective time 50: ET_{50} = time required by flumioxazin (h) to reduce hydrilla photosynthesis by 50%. Each value is a mean of two experiments with a total of 8 replications.
[d] 95% CI = 95% Confidence Interval.
[e] Low pH: 6.0, pH reduced with muriatic acid.

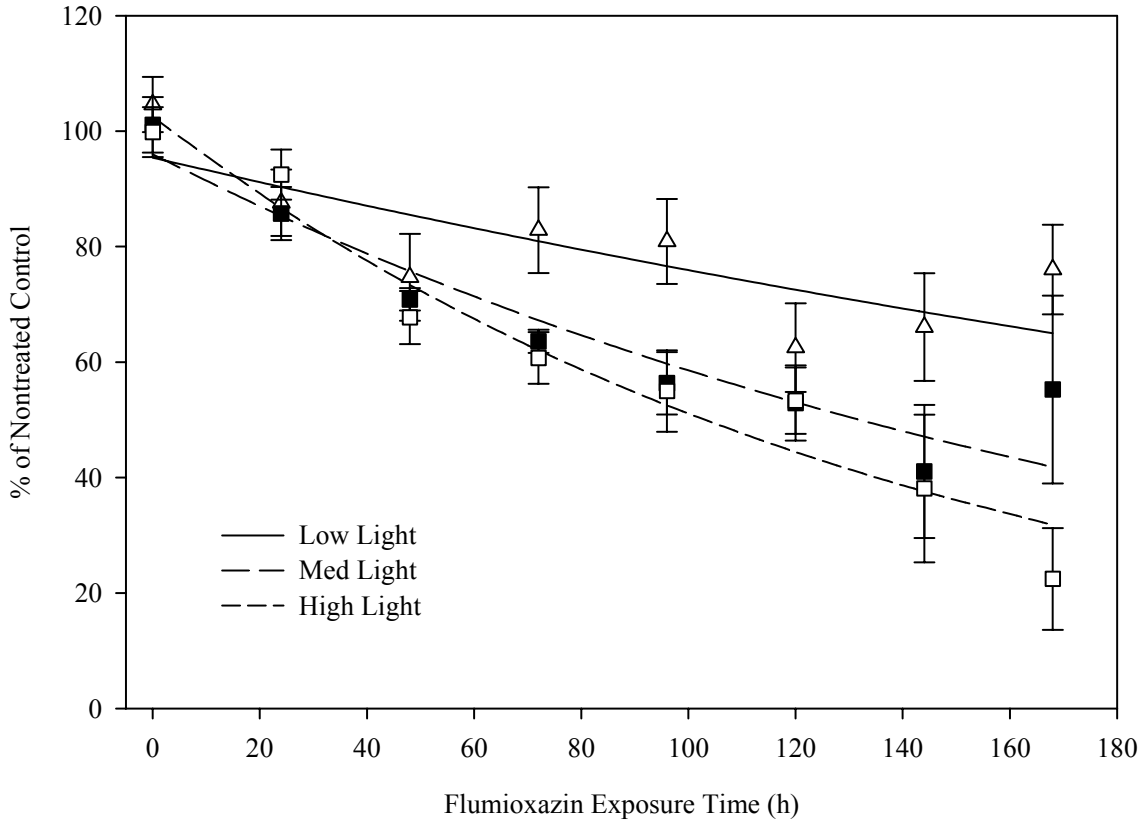

Figure 3-2. The effect of flumioxazin (400µg L^{-1}) at pH 9.0 on photosynthesis of apical hydrilla tips cultured in a growth chamber for 96 h at low (20 µmol m^2 s^1), medium (170 µmol m^2 s^1), and high light (400 µmol m^2 s^1) quantity levels. Data are normalized to the control at each respective light quantity and shown as means ± standard error (n=10).

Table 3-2. The effect of flumioxazin (400 µg L^{-1} a.i.) at pH 9.0 on photosynthesis of apical hydrilla tips cultured in a growth chamber for 168 h at low, medium, and high light quantities[a].

Light Quantity[b]	ET_{50}[c] (95% CI[d])	Regression equation	r^2
Low	303 (198-648)	y = 95.4156e-0.00229x	0.92
Medium	140 (105-209)	y = 95.9852e-0.00495x	0.88
High	99 (81-130)	y = 102.4e-0.00697x	0.90

[a] Data are normalized to the control at each respective light quantity.
[b] Low: 20µmol m^2 s^1; Medium 170µmol m^2 s^1; High 400µmol m^2 s^1.
[c] Effective time 50: ET_{50} = time required by flumioxazin (h) to reduce hydrilla photosynthesis by 50%. Each value is a mean of two experiments with a total of 10 replications (sprigs).
[d] 95% CI = 95% Confidence Interval.

Table 3-3. The effect of select contact herbicides at pH 9.0 on photosynthesis of apical hydrilla tips cultured in a growth chamber for 96 h at 380 µmol m^{-2} s^{-1a} light quantity.

Herbicide[b]	% Label rate	ET_{50}[c] (95% CI[d])	Regression equation	r^2
Flumi 400[e]	100	156 (117-233)	y = 88.9311e-0.00444x	0.99
K50	5	1568 (291-6931)	y = 103.8e-0.000442x	0.98
K200	20	35 (26-53)	y = 93.2636e-0.0198x	0.92
Flumi 400 + K50	100 + 5	126 (100-171)	y = 93.0193e-0.00549x	0.99
Flumi 400 + K200	100 + 20	20 (17-25)	y = 96.3408e-0.0340x	0.97
Endothall 5000	100	71 (62-82)	y = 101.4e-0.00978x	0.98

[a] Data are normalized to the control.
[b]Herbicide rate: µg L^{-1} a.i.
[c] Effective time 50: ET_{50} = time required by the herbicide (h) to reduce hydrilla photosynthesis by 50%. Each value is a mean of two experiments with a total of 10 replications (sprigs).
[d] 95% CI = 95% Confidence Interval.
[e] Abbreviations: Flumi, flumioxazin; K, Komeen (copper chelate); endothall = dipotasium salt.

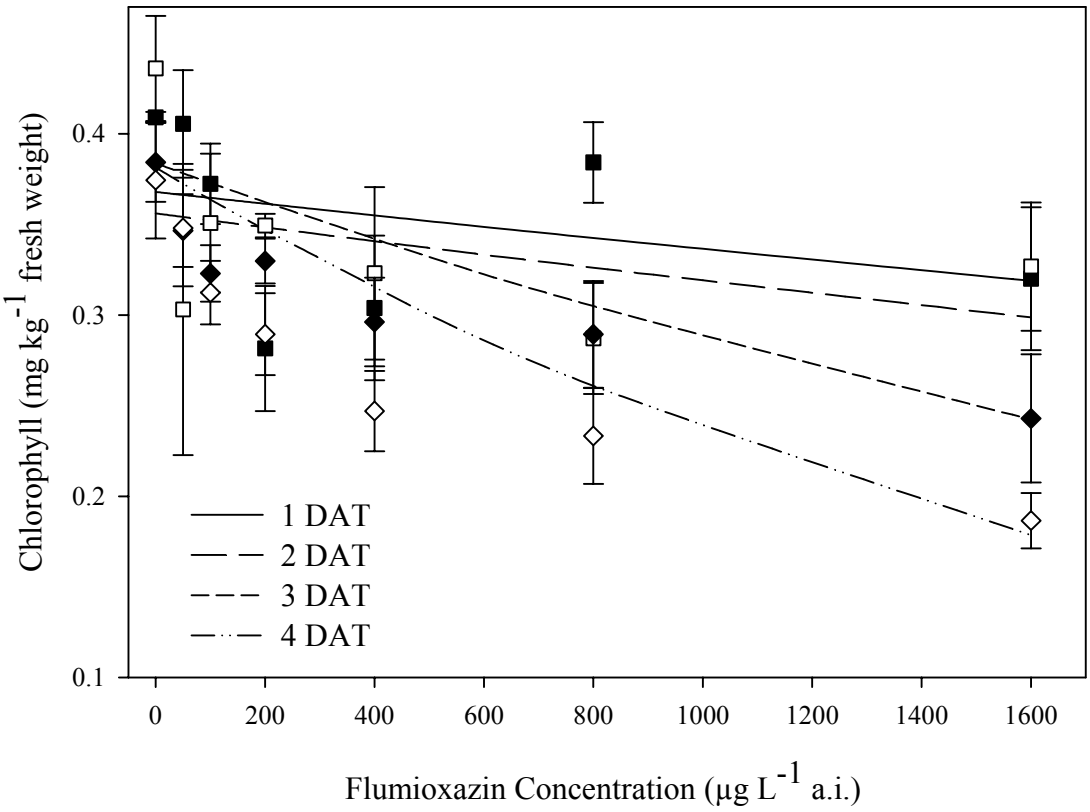

Figure 3-3. The effect of flumioxazin concentration on hydrilla chlorophyll content (mg kg^{-1} fresh weight) 1 to 4 d after treatment (DAT) under 70% sunlight. Flumioxazin was applied as a single application to hydrilla cultured in 18.9 L buckets filled with tap water (pH 9.0-9.5). Data are shown as actual means ± standard error (n=6).

CHAPTER 4
THE EFFECT OF FLUMIOXAZIN ON SUBMERSED, EMERGENT, AND FLOATING AQUATIC PLANT PLANTS

Introduction

One of the primary goals of aquatic weed control in public and private waters is to eliminate invasive plants while maintaining a diversity of native submersed and emergent species. Native aquatic plants may improve water clarity and quality, provide valuable fish and wildlife habitat, reduce rates of sediment resuspension, and help prevent the spread of invasive plants (Savino and Stein 1982; Heitmeyer and Vohs 1984; Smart 1995; Dibble et al. 1996b). Hydrilla is a submersed aquatic fresh water angiosperm that is considered native to Asia or Africa and has become a serious weed problem in the United States and many other countries (Cook 1985; Haller and Sutton 1975; USDA 2007; Van and Vandiver 1992). Once established in a body of water, hydrilla readily dominates and replaces native submersed species by forming a canopy that reduces light penetration, increases surface water temperature, and reduces dissolved oxygen (Bowes et al. 1979; Haller and Sutton 1975; Honnell et al. 1993).

Floating aquatic plants such as water hyacinth, water lettuce, and duckweed form dense, free floating mats which can interfere with navigation, hydroelectric generation, and irrigation (Harley et al. 1984). They may also harbor mosquitoes, which are vectors for diseases like malaria and encephalitis (Holm et al. 1977).

Selective removal of hydrilla and other invasive species with aquatic herbicides is beneficial for retention of native vegetation. Native plant density and diversity can increase considerably if canopy forming exotic plants are removed (Getsinger et al. 1997). Non-target

damage to native species can result from submersed and foliar applications of herbicides and is a consideration in herbicide selection[10].

Flumioxazin is being evaluated by Valent U.S.A. Corporation for control of aquatic weeds. The high costs associated with registering an herbicide for a new market (i.e., aquatics) may be overcome by maximizing the market potential. Therefore, the first objective of this research was to determine if flumioxazin has utility as a foliar and submersed treatment to control floating aquatic weeds. Both submersed and emergent non-target aquatic plants could be impacted by flumioxazin applications, so the second objective of these studies was to quantify the effects of foliar and submersed flumioxazin treatments on submersed and emergent aquatic plants.

Materials and Methods

Floating Aquatic Plants

Water hyacinth and water lettuce were collected from Rodman Reservoir near Interlachen, FL and established in 95 L HDPE tubs filled with 80 L of tap water (pH 8.0) in April 2006 at the University of Florida Center for Aquatic and Invasive Plants in Gainesville, FL. Tap water was supplemented with 1 mL of chelated iron[11] 12-0-0 plus Miracle-Gro®[12] (150 mg L^{-1}) prior to herbicide treatment and added again at 1 and 2 wk after treatment. Water hyacinth (5 plants per tub) and water lettuce (20 plants per tub) were allowed to acclimate for 3 wk before treatment. This study was repeated in August 2006 near the University of Florida in Gainesville, FL with water from Biven's Arm Lake (pH 7.5). Both studies were completely randomized designs with 4 replications (tubs).

[10] W. T. Haller. 2007. Personal Communication.

[11] Lesco, Inc. Cleveland, OH. 44114.

[12] The Scotts Company. Marysville, OH.

Flumioxazin was applied to water lettuce and water hyacinth as a foliar treatment using a forced air CO_2-powered sprayer at an equivalent of 935 L ha^{-1} diluent delivered through a single TeeJet®[13] 80-0067 nozzle at 10 psi. Flumioxazin was applied at 0, 36, 72, 143, 286, 572, and 1144 g ha^{-1} plus a non-ionic surfactant (0.25% v/v). An additional study was conducted concurrently to determine the effects of submersed flumioxazin treatment on these floating invasive species with collection and setup procedures as described above for foliar treatments. Water hyacinth and water lettuce were treated with flumioxazin as a submersed treatment at 0, 100, 200, 400, 800, and 1600 μg L^{-1}. All studies were conducted under full sunlight.

All live water hyacinth and water lettuce biomass was harvested 34 DAT, placed in a drying oven at 90 C for ca. 1 wk, and then weighed. Plant dry weight data were analyzed using non-linear regression (PROC NLIN, SAS Institute 2002). Regression models were used to determine the effective concentration 50 (EC_{50}), which is the concentration of flumioxazin that is required to cause a 50% reduction in dry weight compared to control plants.

A population of landoltia was collected from a pond with no history of herbicide treatments in Alachua County, FL and maintained in 266 L fiberglass tanks in a greenhouse (70% sunlight). The landoltia was cultured in tap water (pH 8.2) amended with topsoil and Miracle-Gro in a greenhouse (1200 μmol m^{-2} s^{-1}). Plants were treated with carbaryl insecticide[14] weekly and allowed to acclimate in the tanks for 2 wk before treatment. A 10 g aliquot (fresh weight) (1.3 ± 0.07 g dry weight) of landoltia was placed in each 3 L HDPE (17.1 cm diameter by 13.3 cm deep) pot filled with tap water (pH 8.0). Plants were allowed to acclimate in the pots for 2 d prior to herbicide treatment. All pots were amended with the same Miracle-Gro rate as

[13] TeeJet Technologies. Wheaton, IL 60189.

[14] Sevin insecticide label. Bayer CropScience. Research Triangle Park, NC 27709.

the previous studies 2 and 14 DAT. Landoltia was treated with a submersed treatment of flumioxazin at 0, 10, 25, 50, 100, 200, 400, 800, and 1600 µg L^{-1}. As a comparison treatment, diquat was applied as a foliar treatment at 1.1 kg ha^{-1} a.i. using the described methods for foliar flumioxazin treatments. The initial experiment was conducted in April 2007 and was repeated in May 2007. This experiment was a randomized design with 5 replicates.

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

Foliar flumioxazin treatments were also applied to landoltia in October 2005, April 2007, and May 2007. Flumioxazin was applied to landoltia at 0, 36, 72, 143, 286, 572, and 1144 g ha^{-1} plus a non-ionic surfactant (0.25% v/v) using the equipment described in the water lettuce and water hyacinth study.

Submersed Aquatic Plants

The submersed aquatic plants coontail, egeria (*Egeria densa* Planch.), hydrilla, southern naiad, and vallisneria were evaluated for sensitivity to flumioxazin at a high (9.0) and low (7.0) pH in 2006 and 2007. Hydrilla was collected from Rodman Reservoir near Interlachen, FL in July, September, and December 2006, while all other species were purchased from a local plant nursery. The high pH study was only conducted once (August 2006); the low pH study was conducted in September 2006 and repeated in January 2007. The high pH and initial low pH experiments were conducted in a shade house (70% sunlight), whereas the repeated low pH experiment was conducted in a greenhouse with 70% sunlight. Two sprigs of each species were

planted in each 10 by 10 by 12 cm (1 L) pot which were filled with masonry sand amended with Osmocote (15-9-12) fertilizer at a rate of 1g kg^{-1} soil and placed in 95 L HDPE tubs filled with tap water (pH 7.5 at planting). Each tub contained all five species (2 pots/species/tub). Plants were allowed to acclimate for 2 wk prior to herbicide application. This experiment was a randomized design with 4 replications (tubs). Flumioxazin was applied as a submersed treatment at 0, 50, 100, 200, 400, 800, and 1600 µg L^{-1} in high and low pH water. Water pH in all tubs was ≥9.0 prior to treatment so each low pH treatment tub was treated with ca. 15 mL of muriatic acid to lower pH to 7.0. The pH was monitored to determine if and when the pH would return to pH ≥9.0. Tub pH was not maintained at 7 since pH beyond 24 HAT was not relevant in this study since flumioxazin is taken up within minutes to hours after treatment (see Chapter 2 and 3). All living plant tissue was harvested at the soil line 28 DAT, placed in a drying oven at 90 C for ca. 1 wk and weighed.

Plant dry weight data were analyzed using non-linear regression (PROC NLIN, SAS Institute 2002). Regression models were used to determine the effective concentration 50 (EC_{50}), which is the concentration of flumioxazin that is required to cause a 50% reduction in dry weight compared to control plants.

Data from both low pH experiments were pooled for coontail, egeria, and hydrilla because there was no difference between the slopes of regression lines for both experiments at the 95% confidence interval level. There were notable differences in the pre-treatment dry weight and growth of naiad and vallisneria between the initial and repeated study; plants in the second study (January) did not grow as rapidly as those in the first study (September), so data from these experiments were analyzed separately by species.

Emergent Aquatic Plants

The sensitivity of the emergent aquatic plants eleocharis (*Eleocharis interstincta* (Vahl) Roemer & J.A. Schultes), maidencane, pickerelweed, and sagittaria were evaluated in submersed and foliar flumioxazin treatments. All plants were purchased from a local plant nursery in August 2006 and April 2007 for the submersed and foliar studies, respectively. Two sprigs of each species were planted in a mixture of 2:1 potting soil:masonry sand in 3 L HDPE pots (17.1 cm diameter by 13.3 cm deep) amended with Osmocote (15-9-12) fertilizer at a rate of 1g kg^{-1} soil. The submersed flumioxazin experiment was a randomized design with five replicates (tubs). One pot of each species was grown for 4 wk in 95 L HDPE tubs placed in a shade house (70% sunlight) for the submersed flumioxazin study. Water level in the tubs was maintained at 25 cm, and pH remained at or near 7.5. Plants were grown for 1 mo when flumioxazin was applied at 0, 50, 100, 200, 400, 800, and 1600 μg L^{-1} as a submersed treatment. Prior to treatment with a foliar application (1 mo after planting), all 5 emergent replicates (pots) were placed in one 266 L fiberglass tank (72 cm by 82 cm by 45 cm) for a total of 7 treatments (tanks). Flumioxazin was applied at 0, 36, 72, 143, 286, 572, and 1144 g ha^{-1} a.i. plus a non-ion surfactant (0.25% v/v) as a foliar treatment. Plants were treated using a forced air CO_2-powered sprayer at an equivalent of 935 L ha^{-1} diluent and delivered through a single TeeJet®[15] 80-0067 nozzle at 10 psi. Water level was maintained at 25 cm with a drain pipe and water was exchanged in the tanks for 10 min after each foliar treatment and again 24 h after treatment to ensure no herbicide would bind to the soil or remain in the water column for underwater uptake.

[15] TeeJet Technologies. Wheaton, IL 60189.

Plants were harvested 40 DAT and plant height from the soil surface to the tip of the tallest leaf was recorded. All emergent plants were harvested in the manner described for submersed plants with plant height and dry weight subjected to the same statistical procedures. Data were pooled across experimental runs when no statistical difference between the slopes of regression lines were observed.

Results and Discussion

Floating Aquatic Plants

Water lettuce was more sensitive to foliar applications of flumioxazin at 36 to 1144 g ha^{-1} than water hyacinth as indicated by calculated EC_{50} values of 69 and 1435 g ha^{-1}, respectively (Figure 4-1). Flumioxazin applied as a foliar treatment at rates greater than 143 g ha^{-1} resulted in complete control of water lettuce. Treated water lettuce plants exhibited chlorosis and necrosis on the leaves (3 to 5 DAT) and defoliation (12 to 15 DAT); also, plants completely decayed at herbicide rates ≥286 g ha^{-1} 21 DAT. Sublethal rates resulted in regrowth of young plants (ramets) from the meristematic region of water lettuce. The highest flumioxazin rate evaluated (1144 g ha^{-1}) reduced water hyacinth biomass by only 41% of the nontreated control 34 DAT. Treated water hyacinth plants exhibited blackening on younger leaves only, which is similar to injury symptoms reported in water lettuce and water hyacinth treated with the PPO inhibitor carfentrazone (Koschnick et al. 2004).

Water lettuce treated with a submersed application of flumioxazin was controlled at all concentrations evaluated in this study (data not shown) and these results suggest that water lettuce is more sensitive to flumioxazin applied in the water than applied to the foliage. In contrast, flumioxazin did not reduce water hyacinth biomass by more than 30% of the nontreated control plants at any rate evaluated in this study and confirms that water hyacinth is more

tolerant to flumioxazin than water lettuce (data not shown), similar to results with the PPO inhibitor carfentrazone (Koschnick et al. 2004).

Water hyacinth plants treated in these studies were large and mature (45 ± 8 cm in height) and may have been more tolerant of foliar treatments than smaller and immature plants; therefore, younger, more actively growing plants (10 ± 3 cm) were also tested for flumioxazin sensitivity, but these immature plants did not respond differently to treatments (data not shown). Immature and mature water hyacinths treated with foliar flumioxazin treatments in field EUP trials (data not shown) have shown tolerance levels similar to those noted in these studies.

The effects of a submersed application of flumioxazin from 10 to 1600 µg L^{-1} are presented for landoltia in Figure 4-2. Most flumioxazin treatments caused foliar bleaching within 7 to 10 DAT, but none of the treatments resulted in complete control of landoltia. Each flumioxazin treatment was different as indicated by no overlapping of 95% confidence interval bars. Landoltia colonies treated at rates above 25 µg L^{-1} began to separate and roots became detached from individual fronds. Koschnick (2005) found landoltia treated in the dark with diquat underwent root detachment without chlorosis. The primary function of roots of plants in the *Lemnaceae* family is stabilization of fronds (Landolt 1986). Diquat applied as a comparison treatment resulted in 100% control less than 5 DAT when applied at 1.1 kg ha^{-1} as a foliar treatment. Duckweed is extremely sensitive to diquat with an EC_{50} of 4 µg L^{-1} (Peterson et al. 1997) and is the current industry standard for duckweed control.

The foliar applied flumioxazin landoltia study was conducted 3 times; treated plants were visually similar to control plants at all rates showing no dose response and therefore were not harvested (data not shown). The foliar treatment to landoltia and water hyacinth was unsuccessful and high foliar rates were needed to control water lettuce. These results suggest

that flumioxazin uptake is limited by the leaf cuticle or occurs primarily through the underside or roots of the plant.

Koschnick et al. (2004) reported that the calculated EC_{50} for a foliar application of carfentrazone to reduce water lettuce was 8 to 10 g ha^{-1}, approximately 7 to 9 times less than flumioxazin's calculated EC_{50} for water lettuce. Higher concentrations (30 and 35 g ha^{-1}) of carfentrazone were required to control water hyacinth (Koschnick et al. 2004), but flumioxazin in our study failed to reduce water hyacinth biomass by more than 30%. Results from these studies show that roots or the underside of the plant is effective at absorbing flumioxazin when applied as a submersed treatment.

If registered as an aquatic herbicide, flumioxazin will likely be used primarily as a treatment for submersed weed control. Although most floating aquatic weeds are controlled by foliar herbicide applications, these results indicate that water lettuce and landoltia may be controlled with submersed treatments. Diquat and 2,4-D are the primary aquatic herbicides used for control of water lettuce, water hyacinth and duckweed (Westerdahl and Getsinger 1988; Langeland et al. 2002) and are more efficacious on water lettuce, water hyacinth, and duckweed as foliar treatments. Flumioxazin in these studies provided control only at higher application rates (water lettuce) or provided <30% control (water hyacinth and landoltia).

Further research is needed to determine if flumioxazin as a submersed treatment is as efficacious to water lettuce in higher pH water (9.0). Efficacy data for flumioxazin is also needed on additional floating species such as S*alvina* spp. and *Wolffia* spp. Additionally, the effect of flumioxazin used in conjunction with surfactants should be examined in both immature and mature water hyacinths to determine whether surfactants increase flumioxazin uptake and activity in these species.

Submersed Aquatic Plants

Water pH in control tanks returned to 9.0 within 24 HAT. The pH of water in the 100, 200, and 400 μg L^{-1} low pH treatments returned to 9.0 ca. 36 HAT, while pH of water in the two highest herbicide rates (800 and 1600 μg L^{-1}) never exceeded 8.5.

Flumioxazin applied in low (7.0) pH water at 50 to 1600 μg L^{-1} caused greater injury to coontail, hydrilla, naiad, and vallisneria than flumioxazin applied in high pH (9.0) water (Figure 4-3). All species with the exception of egeria were more tolerant to flumioxazin applied in high pH water compared to those in low pH water according to the calculated EC_{50} values for dry weight (Table 4-1). Coontail treated in high pH water was the only susceptible species to flumioxazin at the current EUP label rate of 400 μg L^{-1}. All other species treated with flumioxazin in high pH water would require an estimated flumioxazin concentration of >3194 μg L^{-1} to reduce biomass by 50%.

The pH of Florida lakes infested with surface matted hydrilla may be greater than 8.0 and likely in excess of 9.0 as a result of the hydrilla utilizing free CO_2 and HCO^-_3 during daily photosynthesis (Van et al. 1976), so egeria, naiad, and vallisneria would be only slightly injured from flumioxazin treatment in higher pH water based on these data. However, hydrilla was less affected by the treatment in high pH water, in contrast to observations from the general efficacy study (see Chapter 2). Hydrilla in this study responded immediately to flumioxazin exposure by bleaching and undergoing rapid decline, but new apical tips sprouted <1 WAT from treated tissue and flumioxazin application at 1600 μg L^{-1} reduced dry weight by only ca. 40% of the nontreated control plants. These data and the pond efficacy data outlined in Chapter 2 indicate flumioxazin produces highly variable results when applied to hydrilla growing in high pH water.

Coontail, hydrilla, and naiad in were highly susceptible to flumioxazin in low pH water and would likely be injured or controlled at the proposed label rate of 400 μg L^{-1} (Table 4-1).

The calculated EC_{50} value for naiad in experiment 1 was 10x greater than the EC_{50} in the repeated experiment (517 vs. 51, respectively), but flumioxazin at 400 μg L^{-1} would still significantly reduce biomass.

In comparison to these data, previous research has shown that coontail is sensitive to the contact herbicide dipotasium salt of endothall at concentrations as low as 0.5 mg L^{-1} a.i. (Hofstra and Clayton 2001) and endothall has been shown to reduce vallisneria biomass at concentrations greater than 0.5 mg L^{-1}, but plants recovered 8 WAT (Skogerboe and Getsinger 2002). In this study, egeria and vallisneria from the 2nd low pH experiment were not affected by flumioxazin; also, egeria was the only plant unaffected by flumioxazin treatments at either pH. Most plants exposed to flumioxazin in the high and some in the low pH treatments, with the exception of coontail, were beginning to regrow prior to harvest. Similarly, many of the non-target species treated with flumioxazin in EUP ponds recovered within a few weeks after treatment (data not shown). Non-target submersed aquatic species will only be exposed to flumioxazin for short exposures due to the rapid degradation of this herbicide (Katagi 2003), especially when applied to water with a pH >8.0. Those species with marginal tolerance should be able to overcome a flumioxazin treatment in high pH water since flumioxazin will not be present in the water for more than a couple of days. In contrast, non-target plants exposed to herbicides with a longer half-life, such as fluridone or penoxsulam, have the potential of being severely injured or killed because of longer exposures (Koschnick et al. 2007; Langeland and Warner 1986). Development and use of herbicides that selectivity control non-target aquatic plants is a priority of most state agencies involved in aquatic weed management (Anonymous 2007b; Koschnick et al. 2007).

Symptomology of hydrilla treated with flumioxazin in previous EUP studies (see Chapters 2 and 3) was bleaching in the apical tip and reddening in the stem followed by chlorosis and necrosis. Visual symptoms of other flumioxazin-treated plants in these studies included bleached apical tips followed by reddening of the stem (egeria), defoliation and darkening of tissue (naiad), defoliation and loss of stem/leaf integrity (coontail), and transparent appearing leaves (possibly due to loss of chlorophyll) (vallisneria). Plants treated with flumioxazin at pH 7.0 were glossy and darker green in color and were treated with muriatic acid to maintain pH <7.0 for 24 HAT but control plants were not discolored, so muriatic acid did not contribute any injury symptoms. Flumioxazin is hydrolyzed at a much slower rate in lower pH water (Vencill 2002) having a half-life of 16.1 h at pH 7.0 compared to 17.5 min at pH 9.0 (Katagi 2003), so the increased exposure time of these submersed aquatic plants to flumioxazin at pH 7.0 resulted in greater injury and reduction in biomass to all species except egeria. These data clearly show flumioxazin in high pH water is more selective with regard to non-target plant injury than flumioxazin in low pH water.

Emergent Aquatic Plants

Emergent aquatic plants had varying levels of sensitivity to flumioxazin concentrations ≤800 μg L^{-1}; however, there were minimal differences among plant species at concentrations of 1600 μg L^{-1} (Figure 4-5). Sagittaria dry weight was reduced by 100% at the highest concentration compared to a 73 to 83% dry weight reduction in all other emergent plants. Sagittaria was the most sensitive species followed by maidencane, eleocharis, and pickerelweed based on calculated EC_{50} values for dry weight and height (Table 4-2). Elocharis and pickerelweed were more tolerant of a submersed flumioxazin application than sagittaria and maidencane, but these species would likely be injured by flumioxazin at 400 μg L^{-1}. Although dry weight data is often a more reliable indicator to assess herbicide effectiveness, plant height

data in this study provided additional evidence of the selectivity of submersed flumioxazin treatments on emergent plants.

Visual injury symptoms observed 2 WAT included interveinal chlorosis (sagittaria and pickerelweed), reddening on leaf margins (maidencane), and minor chlorosis (eleocharis). Flumioxazin and other protox-inhibiting herbicides are absorbed primarily by plant roots with some absorbance in the shoots, but translocation is limited once herbicides are absorbed into foliar tissue (Fadayomi and Warren 1977; Ritter and Coble 1981; Unland et al. 1999; Vencill 2002). Pots without holes were used in these studies and few roots were visible above the soil line, so herbicide uptake directly from the root zone was unlikely as flumioxazin was mixed directly into the water column. Flumioxazin uptake occurred either through the underwater stem or submersed leaves. Previous research found little flumioxazin translocation in plants, but submersed treatment of emergent aquatic plants in this study suggested movement of flumioxazin from the soil or lower stem into the leaves. If translocation of flumioxazin was limited, this herbicide should have girdled the plant at the soil line and produced injury symptoms such as necrosis throughout the stem and leaves without veinal chlorosis first appearing in the leaves**.** Ferrell et al. (2007a) showed flumioxazin + MSMA (monosodium salt of MAA) resulted in a 94% yield reduction when applied as a high post-direct treatment to 20 cm tall cotton. Symptomology of flumioxazin treated cotton included necrotic lesions on leaves, reddening stems, stem girdling, and eventual lodging. Previous research also found that as cotton matures, plants become more tolerant to flumioxazin because of greater bark development and metabolic capacity (Ferrell and Vencill 2003b).

Foliar flumioxazin treatments were less injurious to emergent aquatic plants than submersed treatments (Figure 4-5). Maidencane and sagittaria would require foliar application

rates greater than 1320 and 6478 g $ha^{-1}$to reduce dry weight and height by 50% (EC_{50}), respectively (Table 4-3). An EC_{50} value could not be calculated for dry weight and height for both eleocharis and pickerelweed since increased flumioxazin concentrations resulted in an increase in dry weight (positive regression slope). Postemergent applications of flumioxazin are generally recommended for actively growing weeds less than 5 cm in height (Anonymous 2006), so the minimal foliar injury and substantial lack of reduction in biomass observed in this study to eleocharis, maidencane, pickerelweed, and sagittaria were probably due to the maturity of these plants. Injury symptoms (including chlorotic and necrotic lesions on the leaves) were similar to those described for other protox-inhibiting herbicides (Peterson et al. 2001). Tolerant species have reduced or no symptoms, whereas the leaves of susceptible species rapidly desiccate and die (Peterson et al. 2001).

Selective control of invasive weed species has always been a goal of aquatic weed managers. Herbicide applicators target specific non-native plants through the use of specifically formulated herbicides, seasonally timed herbicide application, and/or preemptive spot treatments before weeds become a problem (Cervone and Schardt 2003). Flumioxazin provided selective weed control when applied as a foliar treatment or selectivity could be attributed to the maturity of these plants. In contrast, submersed applications resulted in more injury to nontarget plants especially when treatments were made in low pH water. 2,4-D selectively controls broadleaf weed species (Cervone and Schardt 2003), whereas fluridone selectivity is based on initial treatment rate, length of exposure, and initial plant biomass (Netherland et al. 2007).

These data indicate that coontail, sagittaria, and maidencane are adversely affected by flumioxazin at concentrations <400 μg L^{-1} in water with a pH of 7.0. Most emergent and submersed plants evaluated in these studies could be injured or killed if flumioxazin is applied to

low pH water (<7.5). These studies provided "worst-case" scenarios where emergent and submersed plants were continuously exposed to flumioxazin. Herbicide concentrations in lakes are influenced by factors such as wind, flow, dilution, and pH, which minimize direct contact of native plants with herbicides such as flumioxazin when applied as a submersed contact application. Direct foliar applications to these native emergent plants would occur if they grow among targeted emergent or floating plants. Most of these non-target emergent plants will be minimally affected by the maximum proposed foliar label rate[16] of 286 g ha^{-1} a.i.

Submersed aquatic plants are often found among or near hydrilla infestations, whereas emergent plants usually grow along the shoreline. Whole lake treatments with flumioxazin are unlikely since this product will be used primarily as a contact herbicide for submersed weed control. Partial lake treatments allow for dilution of the herbicide to further reduce exposure of non-target plants.

Although several submersed and emergent species were evaluated for sensitivity to flumioxazin, further research should be conducted to determine the sensitivity of other non-target and invasive plants at various water pHs. All emergent plants in this study were mature and consequently more tolerant of both foliar and submersed flumioxazin treatments; however, most flumioxazin treatments would likely occur in early spring or summer when hydrilla can be controlled more easily due to rapid growth, lower pH, and less hydrilla biomass. Most emergent and submersed non-target plants will be immature and actively growing and could possibly be injured by foliar and submersed flumioxazin treatments.

[16] M. S. Riffle. 2007. Personal Communication.

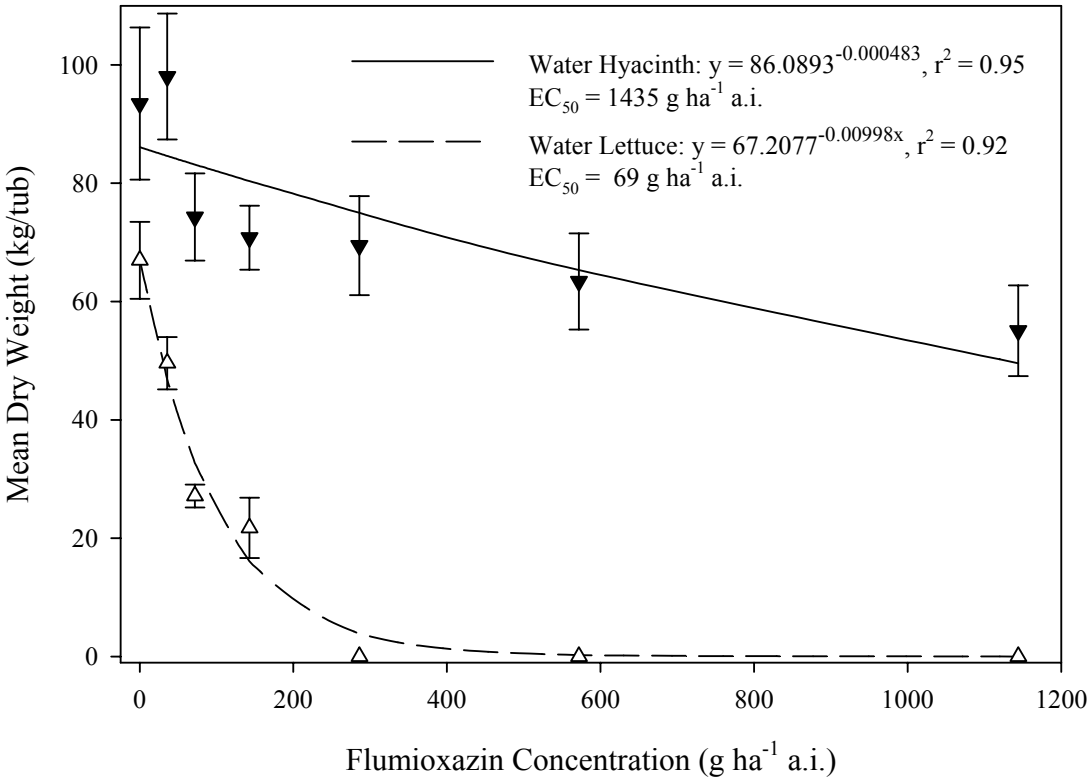

Figure 4-1. The effect of a foliar flumioxazin application (g ha^{-1} a.i.) on water lettuce and water lettuce dry weight 34 d after treatment under 100% sunlight. Flumioxazin was applied as a single application by a CO_2-powered sprayer at an equivalent of 379 L ha^{-1} diluent with a non-ionic surfactant (0.25% v/v) to water lettuce and water hyacinth grown in 95 L tubs (pH 7.5 to 8.0). Data are shown as dry weight means ± standard error (n=10). EC_{50} = effective concentration 50, concentration of flumioxazin (g ha^{-1} a.i.) that is required to reduce water lettuce and water lettuce biomass by 50%.

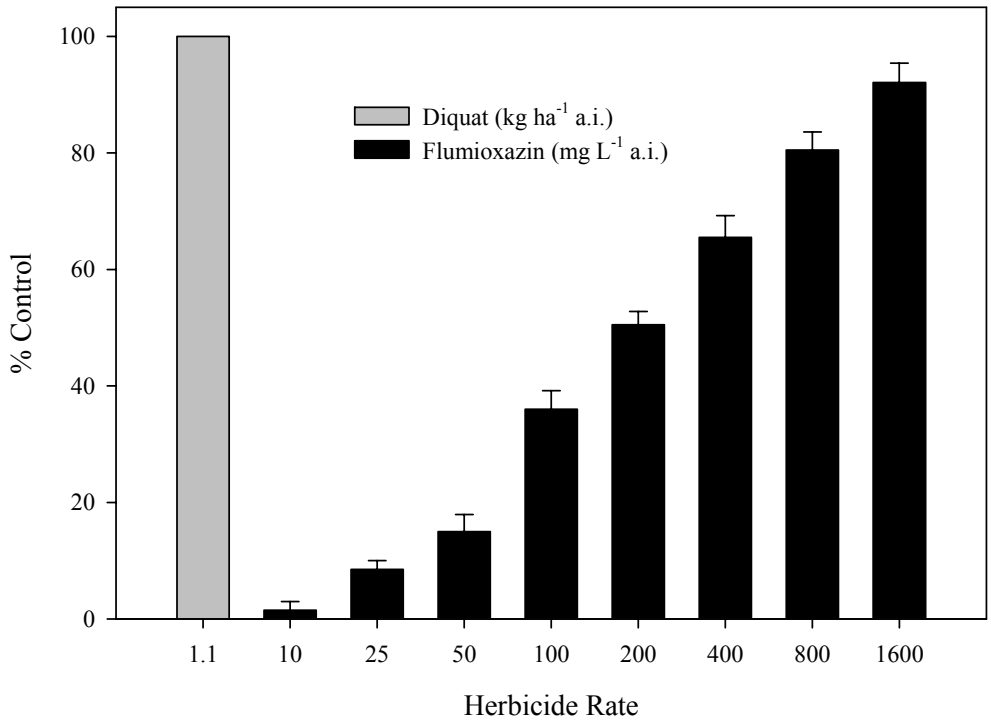

Figure 4-2. Percent control (visual) of landoltia 21 d after a foliar diquat (g ha^{-1} a.i.) and submersed flumioxazin application (µg L^{-1} a.i.). Diquat and flumioxazin each applied as a single application to landoltia cultured in 1 L pots (water pH 8.0) under 70% sunlight. Diquat was applied by a CO_2-powered sprayer at an equivalent of 379 L ha^{-1} diluent with a non-ionic surfactant (0.25% v/v). Percent control ± 95% confidence interval (CI) (n=10). Overlapping CI bars indicate no significant difference.

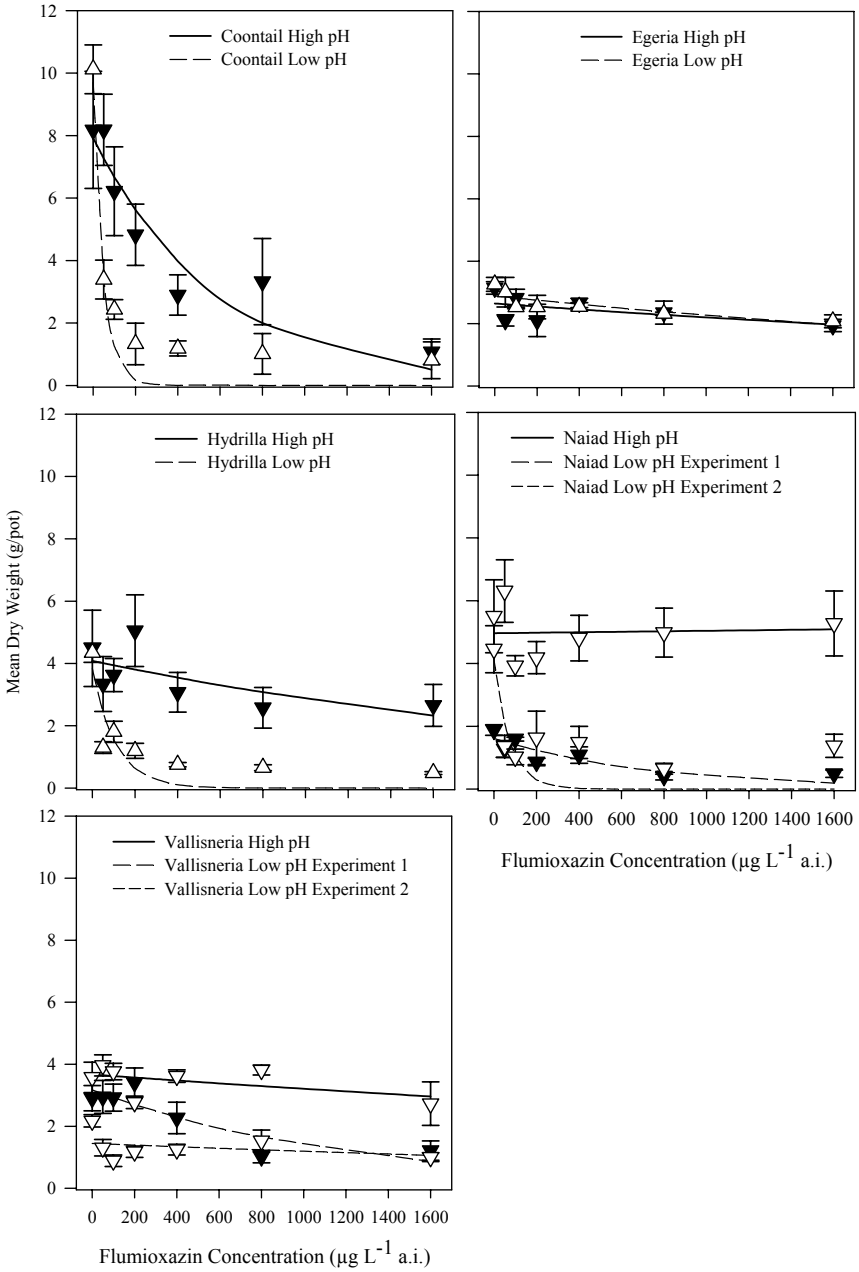

Figure 4-3. The effect of flumioxazin concentration on the dry weight of submersed aquatic plants 28 d after exposure. Flumioxazin applied as a single application to submersed aquatic species cultured in low (7.0) and high (9.0) pH water in 95 L tubs under 70% sunlight. Data are shown as actual dry weight means ± standard error (n=10 for low pH, except for naiad and vallisneria n=5; n=5 for high pH). Dry weight means ± standard error (n=10).

Table 4-1. The effect of a single submersed flumioxazin application on dry weight of submersed aquatic plants 28 d after exposure[a].

High pH[b]	EC_{50}[c] (95% CI[d])	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
Naiad	NA[e]	y = 4.9646e0.0002x	0.90
Vallisneria	5172 (2173-13863)	y = 3.6688e-0.000134x	0.95
Low pH[f]			
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
Naiad #1[g]	517 (338-1093)	y = 1.6128e-0.00134x	0.90
Naiad #2	51 (30-204)	y = 4.1424e-0.0133x	0.64
Vallisneria #1	853 (533-2120)	y = 3.1724e-0.000813x	0.90
Vallisneria #2	3536 (1270-4621)	y = 1.4483e-0.000196x	0.86

[a] Submersed aquatic species cultured under 70% sunlight.
[b] High pH: 9.0.
[c] Effective concentration 50: EC_{50} = concentration of flumioxazin (µg L^{-1} a.i.) in water required to reduce plant dry weight by 50%. Each value is a mean of two experiments with a total of 5 replications (pots) for high pH; 10 reps for coontail, egeria, and hydrilla at low pH; and 5 reps for naiad #1, naiad # 2, vallisneria #1, and vallisneria #2 at low pH.
[d] 95% CI = 95% Confidence Interval.
[e] NA = not applicable due to positive regression slope.
[f] Low pH: 7.0, pH reduced with muriatic acid.
[g] #1 and #2: experiment 1 and experiment 2.

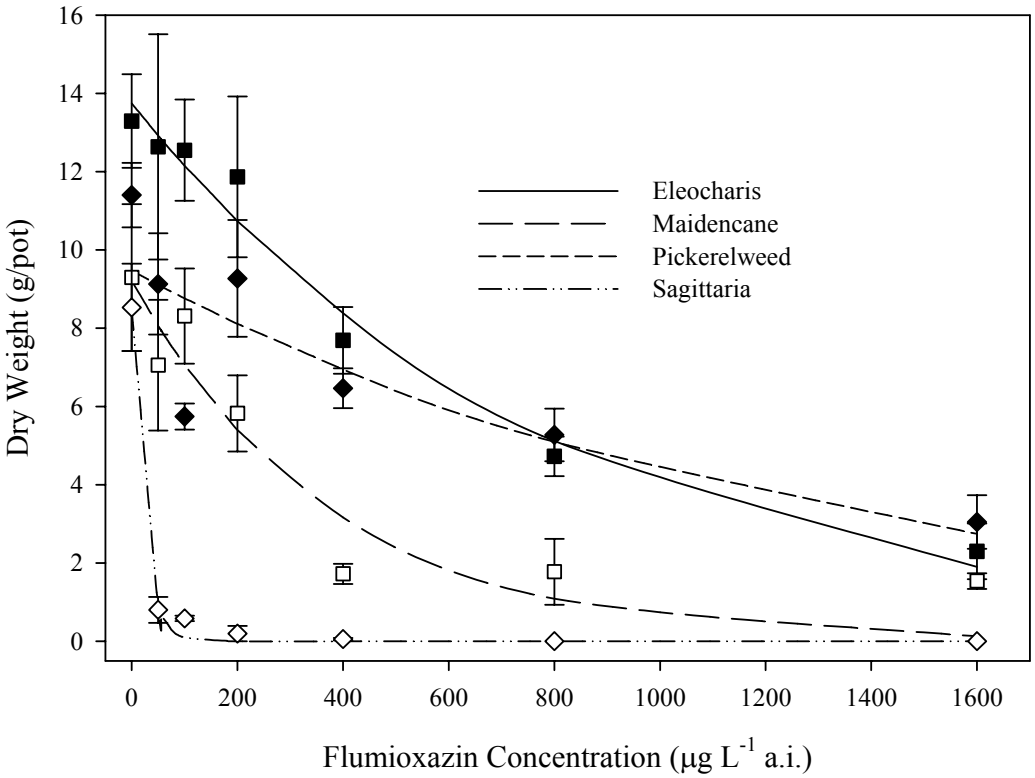

Figure 4-4. The effect of flumioxazin concentration on dry weight of emergent aquatic plants 40 d after exposure. Flumioxazin applied as a single application to emergent aquatic species cultured in 95 L tubs (pH 7.5) under 70% sunlight. Data are shown as actual dry weight means ± standard error (n=5).

Table 4-2. The effect of a single submersed flumioxazin application on dry weight of emergent aquatic species 40 d after exposure[a].

Dry Weight	EC_{50}[b] (95% CI[c])	Regression equation	r^2
Eleocharis	559 (389-1009)	y = 13.7460e-0.00124x	0.92
Maidencane	259 (168-564)	y = 9.2236e-0.00268x	0.84
Pickerelweed	894 (598-1777)	y = 9.4660e-0.000775x	0.91
Sagittaria	15 (11-26)	y = 8.5266e-0.0448x	0.93
Height			
Eleocharis	2295 (1513-4780)	y = 77.5573e-0.000302x	0.97
Maidencane	1764 (1208-3285)	y =74.5654e-0.000393x	0.96
Pickerelweed	13591 (5590-34657)	y = 64.0690e-0.000051x	0.99
Sagittaria	38 (32-47)	y = 64.3085e-0.0182x	0.95

[a] Emergent aquatic species cultured at pH 7.5 under 70% sunlight.
[b] Effective concentration 50: EC_{50} = concentration of flumioxazin (µg L^{-1} a.i.) in water required to reduce plant dry weight or height by 50%. Each value is a mean of two experiments with a total of 10 replications (pots).
[c] 95% CI = 95% Confidence Interval.

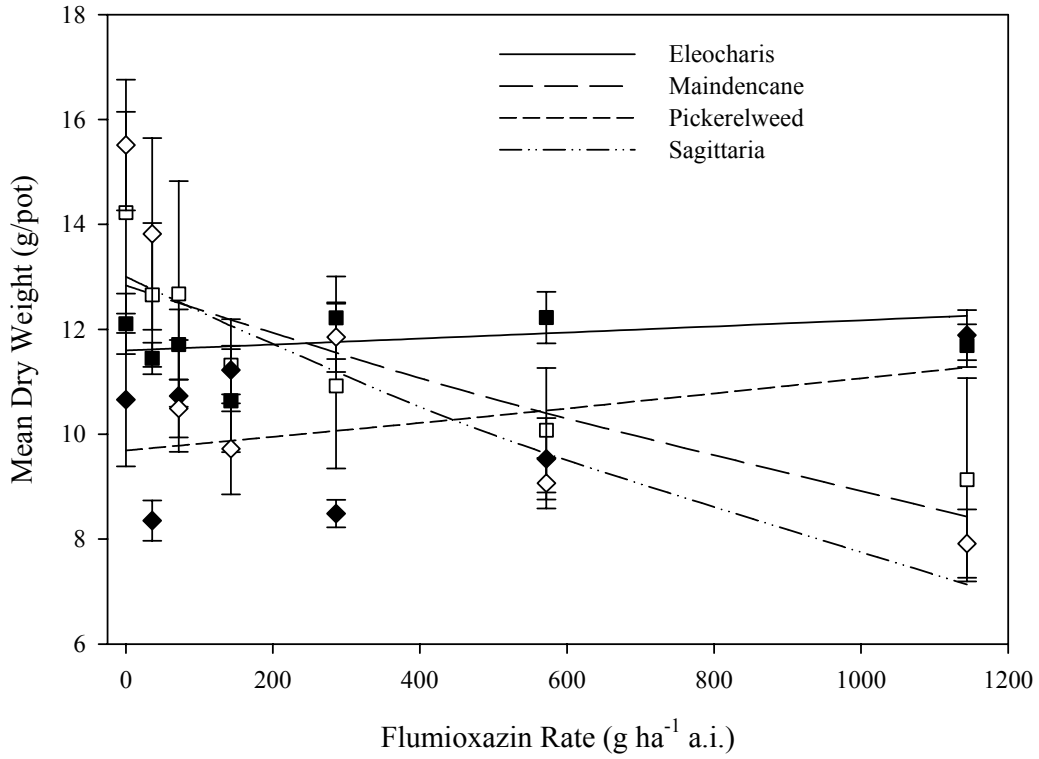

Figure 4-5. The effect of flumioxazin rate on dry weight of emergent aquatic species 40 d after treatment. Flumioxazin applied as a single application by a CO2-powered sprayer at an equivalent of 379 L ha^{-1} diluent with a non-ionic surfactant (0.25% v/v) to emergent aquatic species cultured in 277 L tanks (pH 7.5) under 70% sunlight. Data are shown as actual dry weight means ± standard error (n=5).

Table 4-3. The effect of a single foliar flumioxazin application on dry weight and height of emergent aquatic species 40 d after treatment[a].

Dry Weight	EC_{50}[b] (95% CI[c])	Regression equation	r^2
Eleocharis	NA[d]	y = 11.5964e0.00005x	0.99
Maidencane	1884 (1002-15753)	y = 12.8338e-0.000368x	0.92
Pickerelweed	NA	y = 9.6895e0.00013x	0.97
Sagittaria	1320 (859-2852)	y = 13.0027e-0.000525x	0.95
Height			
Eleocharis	NA	y = 64.8843e0.00001x	0.99
Maidencane	6478 (3938-18734)	y = 88.3085e- 0.000107x	0.99
Pickerelweed	NA	y = 65.9479e-0.000144x	0.99
Sagittaria	12160 (5501-18887)	y = 76.3892e-0.000057x	0.99

[a] Flumioxazin applied by a CO_2-powered sprayer at an equivalent of 379 L/ha diluent with a non-ionic surfactant (0.25% v/v). Emergent aquatic species cultured in 277 L tanks at pH 7.5 under 70% sunlight.
[b] Effective concentration 50: EC_{50} = concentration of flumioxazin (g ha^{-1} a.i.) to reduce plant dry weight or height by 50%. Each value is a mean of one experiments with a total of 5 replications (pots).
[c] 95% CI = 95% Confidence Interval.
[d] NA = not applicable due to positive regression slope.

CHAPTER 5
ORNAMENTAL AND ROW CROP SUSCEPTIBILITY TO FLUMIOXAZIN IN IRRIGATION WATER

Introduction

Homeowners, commercial nurseries, and farmers in Florida often irrigate plants from surface waters (canals, ponds, lakes, etc.) (Hassell et al. 20004; Hodges and Haydu 2006); non-target plants may be affected if these waters are treated with herbicides for aquatic weed control. The use of herbicide treated irrigation water before herbicide residues dissipate below phytotoxic levels will result in injury or death of irrigated plants. Previous research has evaluated the phytotoxic effects of irrigation water containing copper, 2,4-D, fluridone, diquat, and endothall on non-target turf and ornamental species (Andrew et al. 2003; Hiltibran and Turgeon 1977; Koschnick et al. 2005a; Koschnick et al. 2005b; Mudge et al. 2007; Reimer and Motto 1980), but similar studies have not been conducted with flumioxazin. Tolerances of flumioxazin on certain food crops have been established by the EPA by determining the maximum amount of pesticide residue that can remain in or on a treated food commodity to ensure food safety (EPA 2003), but no such tolerances are required for ornamental plants (non-food crops). Phytotoxicity is a major concern when water with aquatic herbicide residues is used for irrigation of both food and non-food crops. This study was conducted to assist in determining the minimum time required before treated water may be used for irrigation of row crops and ornamental species by evaluating phytotoxicity of flumioxazin-treated irrigation water on three ornamental and four row crop plants.

Materials and Methods

Ornamental Susceptibility

Greenhouse studies were conducted in July and August 2006 at the University of Florida Center for Aquatic and Invasive Plants in Gainesville, FL to evaluate the sensitivity of the

ornamental plant species begonia (*Begonia x semperflorens-cultorum* 'Senator'), impatiens (*Impatiens wallerana* **'**Super Elfin Red'), and snapdragon (*Antirrhinum majus* 'LaBella Pink') to flumioxazin. These three common ornamental plants were purchased from local nurseries in Gainesville and grown in 9.0 x 9.0 x 9.0 cm pots as purchased from the growers. Pots contained an organic commercial potting medium and were top-dressed with Osmocote (15-9-12) fertilizer at a rate of 1g kg^{-1} soil upon arrival. Plants were subjected to a 14 h photoperiod with maximum daytime temperatures of 31 ± 2 C and minimum nighttime temperatures of 21 ± 2 C. Experimental plants were selected based on uniform height to minimize variation in initial height and weight then grown for 1 wk to allow acclimation before treatment. At treatment, plant height (cm ± standard error) for each species was as follows: begonia 16.9 ± 0.8, impatiens 17.9 ± 1.1, and snapdragon 41.0 ± 4.3 cm. Snapdragons were mature, hardy, and flowering at time of treatment, while begonias and impatiens were immature with no floral production at the time of treatment.

The experiment was a completely randomized design with five replications (pots) per treatment. Plants were overhead irrigated with herbicide treated water once with a sprinkle can (equivalent to 1.27 cm of irrigation water). This volume was sufficient to cover plants and saturate the soil. The pH of the irrigation water was 7.5 and flumioxazin was mixed with the irrigation water immediately prior to irrigation. Flumioxazin concentrations of 0, 10, 25, 50, 100, 200, 400, 800, 1600, and 3200 μg L^{-1} were applied to all ornamental plant species. Plants were subsequently irrigated daily for 14 d with 1.27 cm of well water (containing no herbicide) applied overhead via a sprinkle can. Plant height was recorded from the soil surface to the tip of the tallest leaf 14 d after treatment (DAT). Pants were harvested 14 DAT by collecting all

aboveground biomass excluding dead tissue. Aboveground tissue was placed in a drying oven at 90 C for ca. 1 wk then weighed.

Plant dry weight and height data were analyzed using non-linear regression (PROC NLIN, SAS Institute 2002). Regression models were used to determine the effective concentration 10 (EC_{10}), which is the concentration of flumioxazin in irrigation water that caused a 10% reduction in dry weight compared to control plants. Koschnick et al. (2005a) reported this value to be conservative but near the threshold where an observant homeowner might detect adverse effects on plant growth. All dry weight and height data were pooled for each ornamental species as there was no difference between the slopes of regression lines for both experiments at the 95% confidence interval level.

Crop Susceptibility

Corn (*Zea mays* L. 'Garst 8346 LL'), cotton ('Stoneville 6611 B2RF'), soybean ('NG2328R) and wheat (*Triticum aestivum* L., 'Wakefield') were evaluated for flumioxazin sensitivity in 2006, with the initial study conducted in January and repeated in July. All crop seeds were planted and grown in 10 x 10 x 12 cm (1 L) pots filled with masonry sand amended with Osmocote (15-9-12) fertilizer at a rate of 1g kg^{-1} soil. Plants were kept in a greenhouse under a 14 h photoperiod with maximum daytime temperatures of 29 ± 2 C and minimum nighttime temperatures of 15 ± 2 C. Plants for this experiment were selected based on uniform height to minimize variation in initial height and weight. At treatment, plant height (cm ± standard error) for each species was as follows: corn (experiment 1) 42.8 ± 1.0, corn (experiment 2) 59.8 ± 2.0, cotton 20.5 ± 1.2, soybean 18.6 ± 0.8, and wheat 26.7 ± 0.4 cm. All crops were subjected to the same herbicide rates, overhead irrigation, harvest techniques, and statistical procedures as the ornamental species.

All row crop data (except corn) were pooled across experiments because there was no differences between the slopes of regression lines for both experiments at the 95% confidence interval level. Corn grew more quickly in the summer study and was ready for treatment 1 wk after planting, but all other crop species needed an additional week to be of similar size to plants treated in the winter study. Initial corn dry weight and height differed between summer and winter studies so corn data were not pooled across experiments.

Results and Discussion

Ornamental Susceptibility

Dry weights of impatiens and begonia differed at flumioxazin concentrations greater than 50 µg L^{-1} (Figure 5.1). Snapdragons displayed minimal necrosis and chlorosis at all flumioxazin rates throughout the course of the study, were more tolerant to flumioxazin in irrigation water than begonia or impatiens, and had greater EC_{10} values for dry weight and plant height (Table 5.1). Snapdragon EC_{10} value for dry weight (7024 µg L^{-1}) was 68 and 175 times more than the begonia and impatiens, respectively and begonia was less sensitive to flumioxazin than impatiens. Higher sensitivity levels of impatiens and begonia to flumioxazin in irrigation water was probably related to plant size and maturity at the time of treatment. Initial mean dry weights (g ± standard error) of begonia and impatiens were 1.7 ± 0.1 and 1.6 ± 0.1 and impatiens and begonia control plants increased in dry weight by as much as 119 and 135% by the conclusion of experiments. In contrast, initial mean dry weight of snapdragon was 13.4 ± 0.3 g and only increased by 32% during the course of experiments. Both preemergence and postemergence applications of flumioxazin are generally recommended for actively growing weeds less than 5 cm in height (Anonymous 2006), so the lack of injury to mature snapdragons was likely due to slow growth and resulted in reduced activity by flumioxazin on these mature plants.

Flumioxazin is ideal for use as a contact herbicide in aquatic systems since the half-life is relatively short and it is degraded by hydrolysis in 4.1 d, 16.1 h, and 17.5 min at pH 5.0, 7.0, and 9.0, respectively (Katagi 2003). The half-life of flumioxazin is dependent on pH and is similar to the aquatic herbicides endothall and diquat. Endothall reduced begonia and impatiens dry weight by 10% at concentrations of 2 to 4 mg L^{-1} a.i. when applied in irrigation water (Koschnick et al. 2005b) and diquat reduced dry weight by 10% at concentrations of 5.1 and 2.8 mg L^{-1} a.i. for begonia and impatiens, respectively (Mudge et al. 2007). The 10% reduction in biomass of these two ornamental species with endothall was within the labeled use rate, but the rate required to reduce impatiens and begonia biomass with diquat was more than 7 and 13 times the labeled use rate, respectively. The EC_{10} dry weight values for impatiens and begonia are well within the proposed flumioxazin label rate of 400 µg L^{-1} a.i. High pH water bodies treated with this herbicide should cause rapid breakdown of flumioxazin and consequently permit shorter irrigation restrictions; however, medium and low pH aquatic systems may need longer irrigation restrictions to ensure sensitive species are not injured or killed by higher herbicide rates.

Crop Susceptibility

Wheat was more sensitive to flumioxazin in irrigation water than all other crops evaluated (Figure 5.2, Table 5.2). The sensitivity of these plants to flumioxazin is indicated by dry weight EC_{10} values as follows: wheat (35), corn experiment #1 (53), cotton (106), corn experiment #2 (181), and soybean (193) (Table 5.2). Similar results were also observed with plant height data. Corn in experiment 2 and soybean were generally more tolerant of flumioxazin than other species, which was expected since flumioxazin is registered for preemergence control of broadleaf weeds in soybean (Anonymous 2005).

Although the immature ornamental species and row crops evaluated in this study displayed higher levels of sensitivity to flumioxazin compared to other short-lived contact herbicides such

as endothall and diquat, the short half-life of flumioxazin in water would partially ameliorate any damage that may occur if homeowners irrigate soon after flumioxazin application. This may be advantageous in pond or lake situations where pH exceeds 9.0, since homeowners could irrigate sooner than if a longer residual herbicide was applied. Flumioxazin is still being evaluated under an EUP and must be granted a full EPA Section 3 Label before this product can be applied to public aquatic systems, since water bodies treated with flumioxazin under the EUP may not be used for irrigation, swimming, drinking, or fish consumption. This study indicates that flumioxazin has the potential to injure and kill immature ornamental and crop species and that these plants may be injured when flumioxazin is applied at the potential maximum label use rate of 400 $\mu g\ L^{-1}$. Based on these data, if homeowners or farmers irrigate with water treated with flumioxazin soon after treatment, 10% or more injury may occur on young, actively growing plants. Flumioxazin is generally more effective on hydrilla in the early spring when it is immature and actively growing and this is the same time most crops and ornamental species are planted by farmers and homeowners. Thus, irrigation restrictions will be variable due to the significant effect of water pH on flumioxazin half-life, application rate, and differences in non-target plant susceptibility as a result of the stage of maturity.

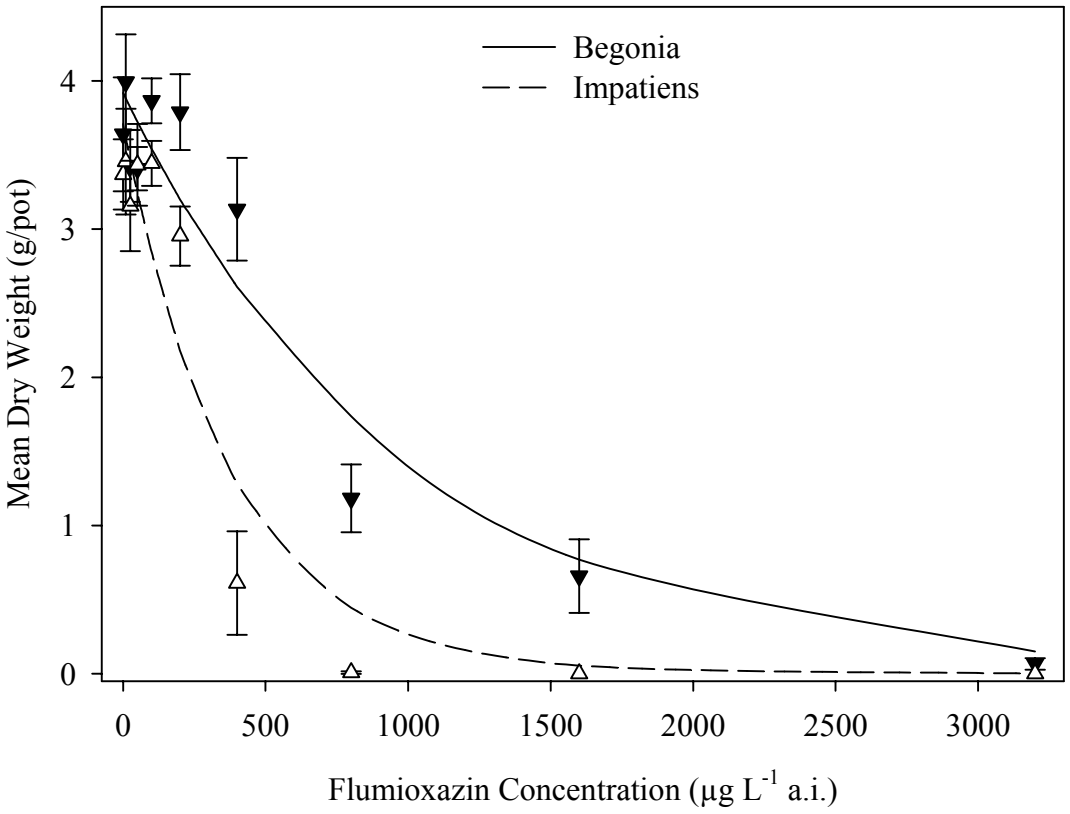

Figure 5-1. The effect of flumioxazin concentration in irrigation water (pH 7.5) on ornamental species dry weight 14 d after treatment. Flumioxazin was applied once to the plants as an overhead irrigation with a sprinkle can (equivalent to 1.27 cm of irrigation water). Data are shown as actual dry weight means ± standard error (n=10). Data for snapdragon were not included as flumioxazin resulted in minimum effects at all rates.

Table 5-1. The effect of a single overhead irrigation with 1.27 cm water containing flumioxazin on ornamental species dry weight and height 14 d after treatment.[a]

Dry weight	EC_{10}[b] (95% CI[c])	Regression equation	r^2
Begonia	103 (84-136)	y = 3.9194e-0.00102x	0.94
Impatiens	40 (32-53)	y = 3.7097e-0.00265x	0.92
Snapdragon	7024 (2450-10536)	y = 13.6060e-0.000015x	0.94
Height			
Begonia	144 (125-171)	y = 23.2842e-0.000731x	0.97
Impatiens	50 (40-67)	y = 24.3930e-0.00209x	0.93
Snapdragon	2395 (1463-7024)	y = 50.3696e-0.000044x	0.98

[a] Flumioxazin was applied in water (pH 7.5) with a sprinkle can.
[b] Effective concentration 10: EC_{10} = concentration of flumioxazin (µg L^{-1} a.i.) in irrigation water required to reduce plant dry weight or height by 10%. Each value is a mean of two experiments with a total of 10 replications (pots).
[c] 95% CI = 95% Confidence Interval.

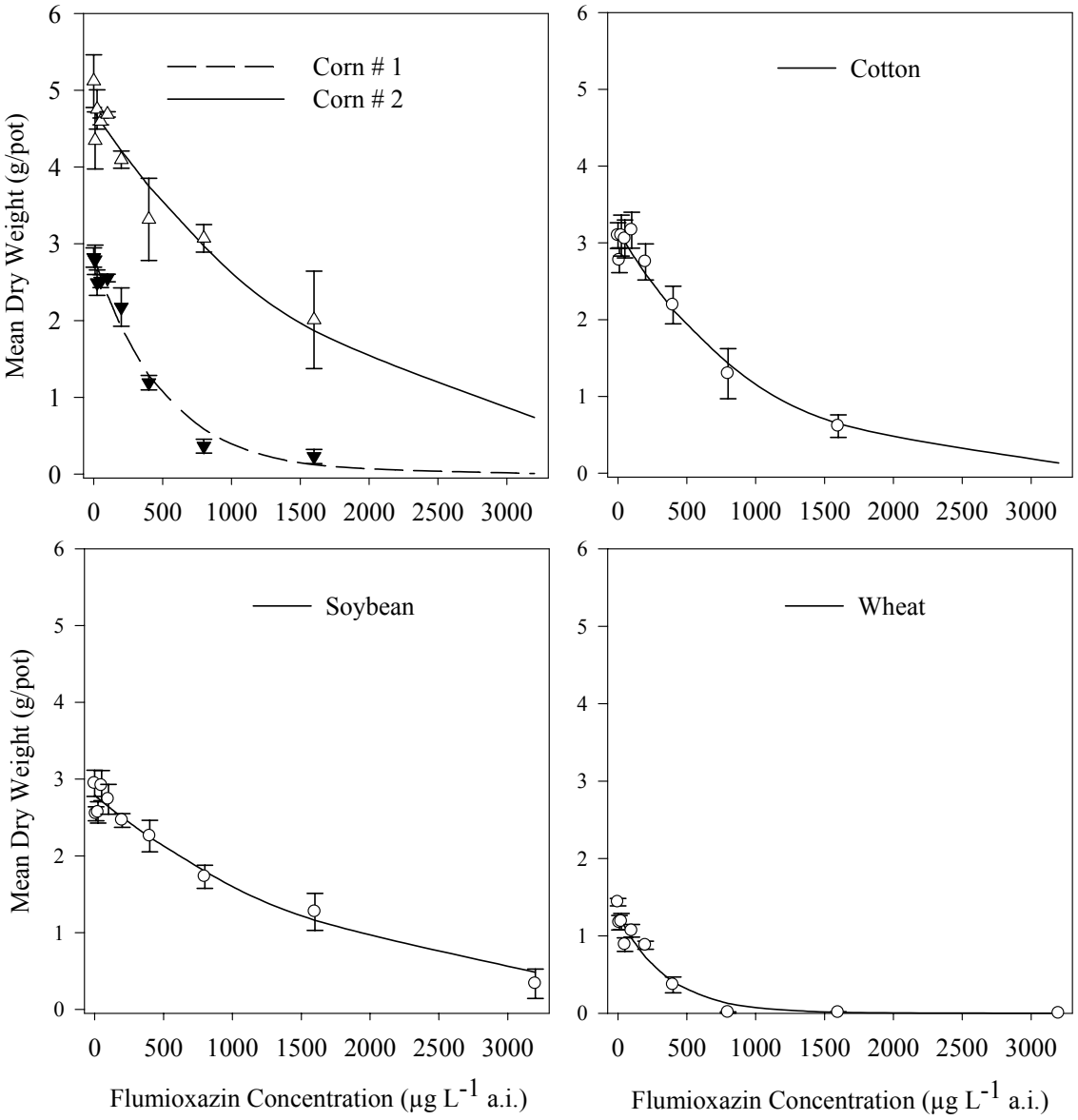

Figure 5-2. The effect of flumioxazin concentration in irrigation water (pH 7.5) on crop species dry weight 14 d after treatment. Flumioxazin was applied once to the plants as an overhead irrigation with a sprinkle can (equivalent to 1.27 cm of irrigation water). Data are shown as actual dry weight means ± standard error (n=10), except for corn (n=5, for each experiment).

Table 5-2. The effect of single overhead irrigation with 1.27 cm water containing flumioxazin on crop species dry weight and height 14 DAT. [a]

Dry weight	EC_{10}[b] (95% CI[c])	Regression equation	r^2
Corn #1[d]	53 (45-66)	y = 2.8429e-0.00198x	0.98
Corn #2	181 (135-279)	y = 4.7306e-0.000581x	0.97
Cotton	106 (84-142)	y = 3.1634e-0.000993x	0.94
Soybean	193 (158-247)	y = 2.7891e-0.000547x	0.97
Wheat	35 (30-47)	y = 1.2876e-0.00289x	0.95
Height			
Corn #1	56 (49-64)	y = 67.2884e-0.00189x	0.99
Corn #2	221 (160-357)	y = 68.2482e-0.000476x	0.97
Cotton	120 (100-150)	y = 33.1105 e-0.000875x	0.97
Soybean	206 (176-248)	y = 42.6972e-0.000511x	0.98
Wheat	46 (40-55)	y = 38.1144e-0.00229x	0.97

[a] Flumioxazin was applied in water (pH 7.5) with a sprinkle can.
[b] Effective concentration 10: EC_{10} = concentration of flumioxazin (µg L^{-1} a.i.) in irrigation water required to reduce plant dry weight or height by 10%. Each value is a mean of two experiments with a total of 10 replications (pots).
[c] 95% CI = 95% Confidence Interval.
[d] #1 and #2: experiment 1 and experiment 2.

CHAPTER 6
THE EFFECT OF FLUMIOXAZIN AND DIQUAT ON MEMBRANE PERMEABILIITY AND CHLOROPHYLL CONTENT OF LANDOLTIA

Introduction

Species of duckweed in the *Lemnaceae* family are commonly used in biochemical and toxicity tests because of their small size, high reproductive rate and the ease with which they are cultivated (Gensemer et al. 1999; Geoffroy et al. 2004; Lewis 1995; Ma et al. 2002; Parr et al. 2002). Studies evaluating pigment content (chlorophyll *a*, *b*, and carotenoids), oxygen emission, and ion leakage are often reliable indicators of herbicide toxicity (Koschnick et al. 2006; Wang and Freemark 1995).

Although hydrilla is the primary target weed in flumioxazin EUP research, invasive floating plants such as landoltia are being evaluated for sensitivity to this compound. Members of the *Lemnaceae* family are extremely sensitive to diquat as common duckweed possesses an EC_{50} of 4 μg L^{-1} (Peterson et al. 1997). It has been controlled with diquat for many years; however, landoltia plants have been discovered in Lake County FL with a resistance factor of 50x for diquat (Koschnick et al. 2006). Herbicides such as flumioxazin possess a different mode of action and can be utilized to help prevent further development of resistance and may control resistant plants. Previous research has shown flumioxazin at 1, 10, and 50 μg L^{-1} decreased photosynthetic capacity of common duckweed (*Lemna minor* L.) by 23, 62, and 64%, respectively (Frankart et al. 2002). Therefore, the objective of this research was to compare the effects of flumioxazin and diquat on ion leakage and chlorophyll content in landoltia.

Materials and Methods

Landoltia was collected from a pond with no history of herbicide treatments in Alachua County, FL in April 2007 and was cultured in 9.5-L aquaria containing a standard growth medium (Wang 1990) at the University of Florida's Center for Aquatic and Invasive Plants,

Gainesville, FL. Plants were maintained in a growth room with a 16 h photoperiod at a temperature of 26 ± 4 C. Agitation was continuously supplied to each culture with forced air via small aquarium air pumps. Aquaria and plants were rinsed and growth media were refreshed ca. every 3 to 6 wk and light levels were maintained at 150 ± 10 μmol m^{-2} s^{-1} to minimize algal growth.

Ion Leakage

The effect of flumioxazin on landoltia was measured by comparing non-specific ion leakage (conductance in μmhos cm^{-1}) over time using a conductivity bridge[17] since conductance may be used to measure non-specific ion leakage resulting from loss of membrane integrity (Koschnick et al. 2006; MacDonald et al. 1993; O'Brien and Prendeville 1978). All ion leakage techniques were modeled after research conducted by Koschnick et al. (2006). Ten colonies of landoltia (4 to 6 fronds per colony) were placed into individual 20-ml high-density polyethylene (HDPE) scintillation vials containing 15 ml DI water. Flumioxazin was added to each of the vials to achieve concentrations of 0, 10, 25, 50, 100, 200, 400, 800, and 1600 μg L^{-1}. Diquat at 10 μg L^{-1} was applied as a comparison treatment. The experiment was conducted and repeated in May 2007 as a randomized design with 5 replications. Vials were covered with Parafilm M[18] and inverted 3x after addition of herbicide. Vials containing only flumioxazin or diquat (duplicate treatment solutions) at each herbicide concentration and no plants were immediately measured for initial conductance (Ci) (conductance contributed by addition of herbicides) and appropriate corrections were made to determine total ion leakage. Vials were placed on a shaker table (100 oscillations min^{-1}) in a growth chamber and temperature of treatment solutions was

[17] Fisher Scientific Conductivity Meter. Pittsburgh, PA.

[18] Trademark of Pechiney Plastic Packaging. Menasha, WI. 54952.

maintained at 27 C with a 14 h photoperiod at 380 ± 10 μmol m^{-2} s^{-1}. Conductivity of the treatment solutions was measured at 0, 1, 3, 6, 9, 12, 24, 30, 48, and 72 h after treatment (HAT). The study was terminated 96 HAT due to the decline in conductivity resulting from algal growth in vials. Final conductivity measurements were recorded, then treatment vials containing landoltia were frozen and thawed 3x to ensure 100% ion leakage (Ct). Ion leakage at each time (Cx) is reported as percent conductivity according to the following formula used by MacDonald et al. (1993) and Koschnick et al. (2006):

% conductivity = [(Cx–Ci)/(Ct-Ci)]*100.

A repeated measures analysis was performed and means were separated using 95% confidence intervals (95% CI) (PROC GLM, SAS Institute 2002).

Chlorophyll

The effect of flumioxazin on chlorophyll content was determined on colonies of landoltia placed into scintillation vials and treated as described above for the ion leakage experiments. As a comparison treatment, landoltia was treated with diquat at 10 μg L^{-1}. This experiment was conducted and repeated in May 2007 as a randomized design with 5 replications. Treated plants were placed in a growth chamber for a 96 h exposure period then plants were removed, excess water was blotted with a paper towel, and fresh weights were recorded. Total chlorophyll was extracted by placing plants from each treatment vial into polystyrene test tubes (12 x 75 mm) containing dimethylsulfoxide (DMSO) (Hiscox and Israelstam 1979) in a water bath (65 C) for 3 h. Chlorophyll content was determined spectrophotometrically (Arnon 1949) and expressed as mg chlorophyll kg^{-1} of fresh weight. Data were analyzed using non-linear regression (PROC NLIN, SAS Institute 2002) and EC_{50} values (flumioxazin concentration required to cause a 50% reduction in chlorophyll content) were derived.

Results and Discussion

Ion Leakage

Control plants did not produce more than 10% conductivity throughout the course of these studies (data not shown). There were no differences in conductivity between flumioxazin at any concentration and diquat 1 and 3 HAT (Figure 6-1), but differences in conductivity were noted between flumioxazin at 10, 25, 50 and 1600 μg L^{-1} and diquat 6 HAT. Leakage due to diquat ≥9 HAT was greater than leakage due to flumioxazin at all concentrations. These results are similar to those reported for landoltia treated with diquat at 10 μg L^{-1} when conductivity exceeded 80% by 18 HAT (Koschnick et al. 2006). All flumioxazin treated plants displayed bleaching to a certain extent 24 HAT, but none of the treatments resulted in more than ca. 50% bleaching; in contrast, diquat treated plants were 100% chlorotic 24 HAT. Conductivity in the diquat treatment was ≥90% 30 HAT, while flumioxazin conductivity never exceeded 50% at any concentration throughout the course of the experiments. At the conclusion of these studies (96 HAT), most flumioxazin treatments began to show signs of decreasing conductivity, probably due to algal growth in the scintillation vials. If these studies were continued for a few more days, similar to the landoltia treated with flumioxazin in Chapter 4, it is likely the plants would have continued to bleach and completely leak 100% of the ions. Based on these studies, flumioxazin is not as fast acting as diquat with respect to bleaching and ion leakage; however, mesocosm data in Chapter 4 demonstrated that flumioxazin did not exhibit significant bleaching until 7-10 DAT but provided 65% control 21 DAT when applied at 400 μg L^{-1}.

Chlorophyll

An estimated flumioxazin concentration of 944 μg L^{-1} is required to reduce landoltia chlorophyll content by 50% (EC_{50}) after a 96 h exposure (Figure 6-2), but plants were not completely bleached even at the highest flumioxazin concentration (1600 μg L^{-1}). In contrast,

diquat at 10 μg L^{-1} resulted in a 99% reduction of chlorophyll content 96 HAT (data not shown). These data provide evidence that flumioxazin may have slower activity on landoltia than diquat, and suggests that landoltia may not have been exposed to flumioxazin for a sufficient amount of time in these experiments. Submersed flumioxazin applications required >1 wk to cause significant chlorosis in landoltia in mesocosm experiments (see Chapter 4) and the time allotted in these studies (96 h) was not sufficient for flumioxazin to cause significant bleaching.

Protox-inhibiting herbicides are more active in the presence of full sunlight (Sherman et al. 1991; Wright et al. 1995) and landoltia was exposed to flumioxazin in a growth chamber under low light conditions (380 ± 10 μmol m^{-2} s^{-1}) in these experiments. Also, these studies were conducted under a 14 h day length and not continuous light as in previous research (Koschnick et al. 2006). Although flumioxazin did not cause the same level of injury to landoltia as diquat, flumioxazin treatments still resulted in significant levels of ion leakage and bleaching. Continuous and higher light levels may result in greater leakage similar to that observed in diquat; however, the environment in the growth chambers was similar to field conditions (14 h day length) and not artificially altered by providing continuous light. These data indicate that flumioxazin causes slower, less severe injury to landoltia than diquat under short exposure times.

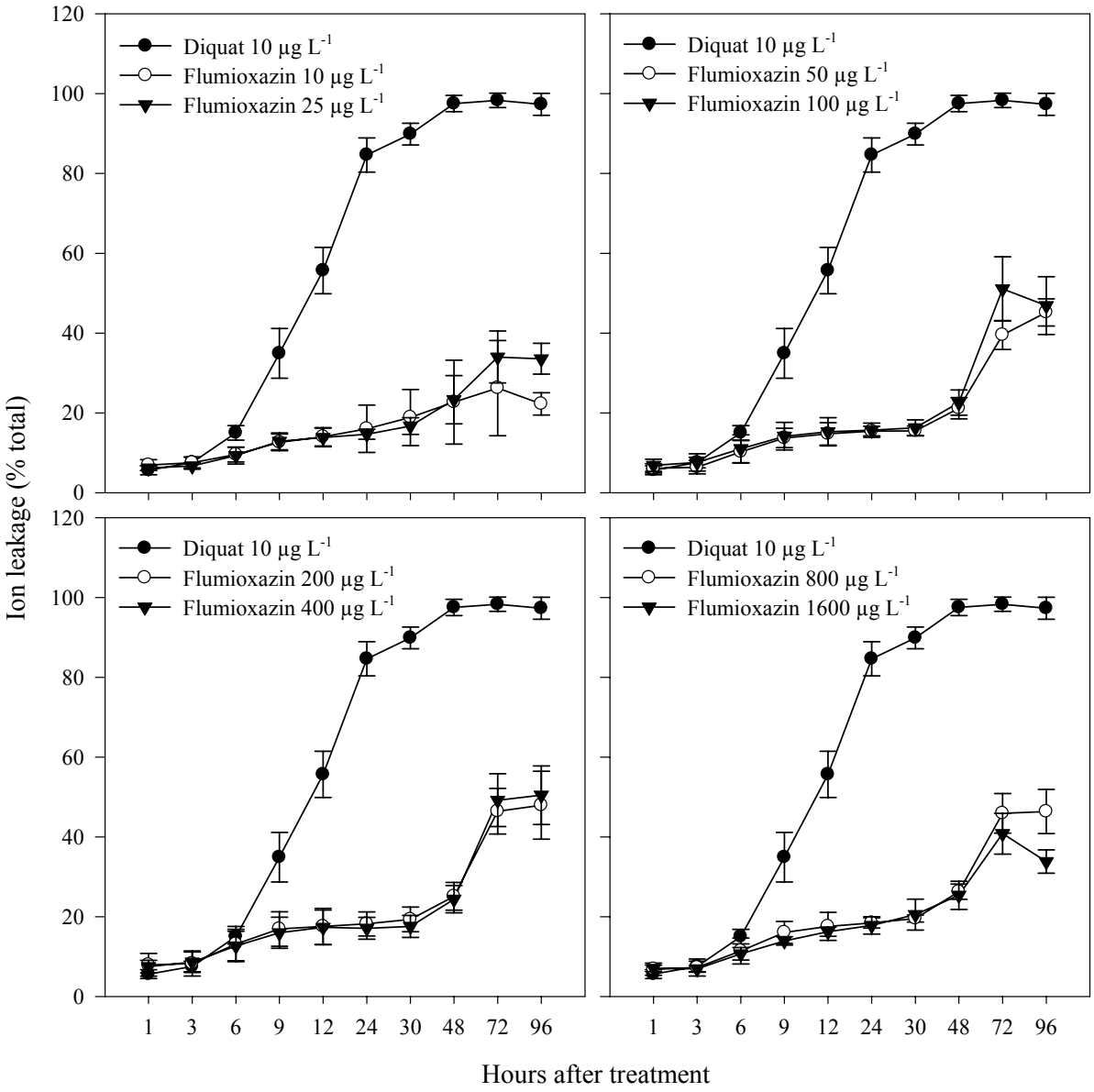

Figure 6-1. The effect of diquat and flumioxazin on ion leakage from landoltia cultured in DI water (pH 8.5) in a growth chamber for 96 h. Values are presented as means ± 95% confidence interval (CI) (n=10). Overlapping CI bars indicate no significant difference at a given time.

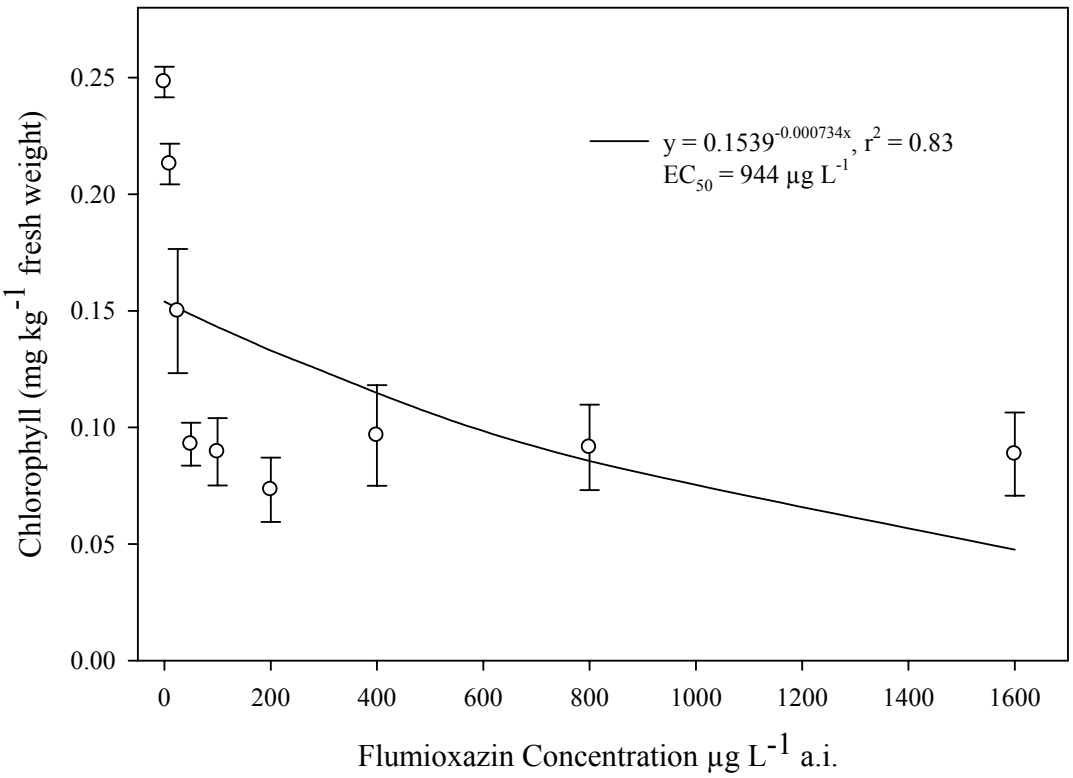

Figure 6-2. The effect of flumioxazin concentration on landoltia chlorophyll content 96 h after treatment. Landoltia was cultured in 20 mL vials containg DI water (pH 8.5) in a growth chamber. Data are shown as actual means ± standard error (n=6). EC_{50} = effective concentration 50, concentration of flumioxazin in water required to reduce landoltia chlorophyll content by 50%.

CHAPTER 7
SUMMARY AND DRAFT AQUATIC USE DIRECTIONS

Summary

These experiments provided information regarding the effectiveness of flumioxazin as an aquatic herbicide and will aid in the registration of flumioxazin for use in aquatic ecosystems to control invasive aquatic species such as hydrilla and water lettuce. Flumioxazin was screened for hydrilla control in 2005 and significantly reduced hydrilla biomass. Through a cooperative agreement with Valent U.S. A. Corporation, The Center for Aquatic and Invasive Plants at the University of Florida began evaluation of flumioxazin as a potential aquatic herbicide.

Various EUP ponds throughout Florida ranging in pH from 6.7 to 10.0 were treated with flumioxazin at concentrations of 100 to 400 μg L^{-1}. Early season treatments were successful due to lower pH, less hydrilla biomass, and/or time of year; however, flumioxazin failed to provide more than 1to 4 months of control in ponds with pH >8.5. These high pH ponds were generally infested with mature hydrilla that was near the surface. Early and late season treatments are recommended since hydrilla grows more actively and water pH is likely to be lower than in summer treatments.

Due to the lack of efficacy in EUP ponds treated with flumioxazin in the summer when the water pH was in excess of 8.5 or when hydrilla was near the surface of the water, greenhouse and laboratory studies were initiated to determine why flumioxazin was not as efficacious under these conditions. Outdoor mesocosm studies showed that flumioxazin reduces hydrilla biomass by 90% at concentrations of 186 μg L^{-1}, but possesses activity at concentrations as low as 50 μg L^{-1}. Hydrilla treated with flumioxazin underwent chlorosis in apical tips followed by reddening of lower stems. Hydrilla treated at 50 to 1600 μg L^{-1} began to lose integrity and fall to the bottom of the tanks within 5 to 7 DAT; however, new apical tips from adventitious buds in the

leaf axils soon sprouted from treated rooted and floating tissue. Flumioxazin is rapidly hydrolyzed in high pH water and has an average half-life of 17.5 min under laboratory conditions at pH 9.0 or greater. The effects of pH on flumioxazin half-life and hydrilla efficacy were evaluated in a pH efficacy study. Flumioxazin reduced hydrilla dry weight by 90% in the high (>8.5) pH treatment when hydrilla was placed into mesocosms the same day as treatment; however, hydrilla placed in high pH water 2 to 5 DAT was reduced in biomass by no more than 50%. Biomass of hydrilla in the low (6.0 to 6.2) and medium (7.0 to7.2) pH was reduced by 93 and 68%, respectively, of the nontreated control plants 3 DAT. 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. These data indicate flumioxazin is rapidly taken up by hydrilla and the short half-life of flumioxazin in water with pH >9.0 can be overcome by higher application rates.

The net photosynthetic rates of apical hydrilla tips treated with flumioxazin were measured to determine the effects of rate, pH, stem type, and light quantity. Flumioxazin applied in high pH water (9.0) at ≥200 µg L^{-1} or in low pH water (6.0) at ≥100 µg L^{-1} required less than 124 h to reduce hydrilla net photosynthesis by 50% (ET_{50}) of the non-treated control plants and only the 100 µg L^{-1} treatment applied in high pH water failed to have a significant impact on photosynthesis. Since activity of flumioxazin is influenced by light, it was hypothesized that hydrilla at the bottom of water bodies were not being controlled due to the low quantity of light reaching plants. Growth chamber studies indicated 170 and 400 µmol m^{-2} s^{-1} of light was sufficient to reduce net photosynthesis of hydrilla by 45 and 78% of the nontreated control plants, respectively, 168 HAT; however, hydrilla treated with flumioxazin at light levels of 20 µmol m^{-2} s^{-1} resulted in less than a 30% reduction in net photosynthesis 168 HAT. Furthermore,

plants treated at the lowest light level did not appear to be injured and maintained a healthy appearance throughout the course of the experiments.

Due to the high costs associated with registering a pesticide in a new market like aquatics, other invasive species were evaluated for sensitivity to flumioxazin. Water lettuce was highly sensitive to submersed and foliar flumioxazin applications, whereas water hyacinth was impacted only slightly by either application technique. Landoltia was sensitive to submersed treatments, but was non-responsive to foliar treatments.

If flumioxazin is approved for aquatic use, non-target aquatic plants may be affected by submersed and foliar flumioxazin applications and terrestrial plants may be injured from irrigation with treated water. The impact of a submersed flumioxazin application on submersed aquatic plant species including coontail, egeria, hydrilla, southern naiad, and vallisneria was evaluated at high (9.0) and low pH (7.0). Coontail, naiad, and hydrilla dry weight were reduced by 50% (EC_{50}) when flumioxazin was applied at 34, 51, and 77 μg L^{-1} in low pH water. Only coontail dry weight was reduced by 50% with flumioxazin concentrations near the proposed label maximum rate of 400 μg L^{-1}in high pH water. The emergent aquatic plants maidencane and sagittaria were reduced by 50% with flumioxazin application of less than 400 μg L^{-1}. Foliar application of flumioxazin reduced sagittaria dry weight by 50% at 147 g ha^{-1}, which is approximately half of the proposed maximum foliar application rate of 286 g ha^{-1}. Other emergent species (eleocharis, maidencane, and pickerelweed) were more tolerant to applications within the proposed maximum labeled rate. Non-target ornamental and row crop plants were overhead irrigated once with irrigation water containing flumioxazin. Immature begonia and impatiens were highly sensitive compared to mature snapdragons and were reduced by 10% in dry weight with flumioxazin in irrigation water at rates of 103 and 44 μg L^{-1}, respectively. All

row crops (corn, cotton, soybeans, and wheat) were reduced by 10% in dry weight with flumioxazin at less than 200 μg L^{-1} in irrigation water. These data clearly indicate that irrigation restrictions will be required on the label; however, since flumioxazin is rapidly degraded (especially in high pH water), shorter water use restrictions should be possible for homeowners and farmers who use flumioxazin treated water for irrigation; however, treated water with a pH of less than 8.0 will likely be restricted for irrigation for several days.

These data indicate flumioxazin has potential utility as an aquatic herbicide for control of hydrilla and other aquatic weeds. Flumioxazin possesses several desirable traits including activity at low use rates, a short half-life, and non-target selectivity. Hydrilla control will depend on several factors including water pH, growth stage of hydrilla, and light availability to stems near the bottom of a water body. Summer applications or situations where hydrilla has already surface matted (resulting in high pH) will be less effective than early and late season applications, so treatments under these conditions should be avoided since these treatments will result in a "burndown" or removal of the upper canopy and hydrilla will regrow quickly within 1 to 2 months.

Draft Aquatic Use Directions

General information

Flumioxazin is a 51 percent water dispersible granule that controls weeds by inhibiting protoporphyrinogen oxidase (Protox), an essential enzyme required by plants for chlorophyll biosynthesis. This herbicide is rapidly absorbed by aquatic plants and breaks down by pH dependent hydrolysis. Flumioxazin is a fast-acting, contact herbicide that can be applied directly into water for control of submersed aquatic weeds or directly to the foliage of emergent or floating weeds. The most effective flumioxazin applications occur when applied to young, actively growing weeds in water with a pH <8.0.

Mixing Guidelines

If the diluent water (tank mix) is pH >7.0, add an agent to lower the pH in the tank. Agitation may be applied to the spray tank, but the addition of more than 1 lb of product per gallon at a high agitation rate will likely result in large quantities of foam in the tank. The herbicide solution should be applied no longer than 2 h after mixing to prevent hydrolysis.

Control of Submersed Weeds

Apply flumioxazin for control of hydrilla and other susceptible submersed weeds in lakes, ponds, non-irrigation canals and other water bodies with limited water exchange. Total concentration of flumioxazin in a single treatment should not exceed 400 μg L^{-1} in the treated water area. For best results, apply in spring or early summer when submersed weeds are actively growing but have not reached the water surface. Water pH at a depth of 1 ft below the surface should be measured at the time of application. Flumioxazin may be applied at concentrations as low as 50 μg L^{-1}, but concentrations above 100 μg L^{-1} provide better efficacy, especially in water with a pH >8.5. Treatments should be applied as early in the morning as possible to minimize the effect of hydrolysis in high pH water since infested waters have a tendency to cycle pH from as low as 7.0 (6 A.M.) to as high as 10.0 (6 P.M.) in the upper 10 to 25 cm of a water body. If the water pH is >8.5 or hydrilla is surface matted, flumioxazin should be applied at 400 ppb to ensure best efficacy. Repeat applications of 400 ppb within 1 to 2 months after treatment may be necessary due to the rapid breakdown of this product in water with high pH. If the water pH is 7.0 to 8.4, flumioxazin may be applied at 200 to 400 ppb for control of hydrilla. Additional applications may be necessary for complete control at this pH range. Flumioxazin applied to water with a lower pH (≤6.9) will result in better efficacy due to slower breakdown in low pH water. Flumioxazin may be applied at rates of 50 to 400 ppb in these lower pH waters.

Subsurface Application

Flumioxazin should be applied with long weighted hoses to ensure proper mixing of herbicide in the water column. Thermal stratification is common in lakes with surface-matted hydrilla and this thermal layer can create a physical barrier, isolating layers in the water column and preventing surface-applied herbicides from reaching the target vegetation below the thermocline. If weighted hoses are not available, every effort should be made to ensure herbicide is uniformly mixed below the water surface to assure all plant parts are exposed to the herbicide. Inadequately mixed flumioxazin will likely break down before it comes in sufficient contact with plants near the bottom, allowing for more rapid regrowth.

Surface/Foliar Application

Flumioxazin may be applied up to 8 ounces of formulated product per acre. Control of water lettuce and other floating or emergent weeds require the addition of a spray adjuvant for foliar applications. For best results, use nonionic surfactants or methylated seed oils at manufacturer's recommended rates. Mix in sufficient diluent (50 to 100 GPA) to ensure adequate coverage.

Plant Susceptibility

Submersed, emergent, and floating vascular aquatic plants as well as macrophytic algae vary in susceptibility to flumioxazin (Table 7-1). Plants are more susceptible to submersed flumioxazin applications in lower pH water (<8.0) where half-lives are longer. For best results, treatments with this herbicide should be applied when plants are actively growing and before they are surface matted. If plants are surface matted, an initial application may be required to control the surfaced plants and a subsequent application may be necessary to provide season long control. Lower pH, actively growing weeds, less biomass, and high light penetration into the water colum favor increased flumioxazin efficacy on hydrilla and other submersed species.

Irrigation Restrictions

In addition to efficacy on weeds and impact on non-target plants, the focus of this research was to determine possible irrigation restrictions following flumioxazin applications. Chapters 2, 5, and 8 provide data on the half-life of flumioxazin under various pH situations and the sensitivity of non-target plants to flumioxazin in irrigation water. Water use restrictions for flumioxazin will be dependent on a number of factors including herbicide placement in the water column, herbicide rate, pH, and maturity of the plants being irrigated with flumioxazin treated water (Table 7-2). Submersed applications will be more restrictive than foliar applications due to higher concentrations of flumioxazin in the water. For example, ornamental species are usually planted during the spring when hydrilla is actively growing and highly susceptible to flumioxazin; therefore, irrigation water containing flumioxazin may also severely injure these immature plants. Water with a pH of 6.0 to 8.0 that has received a submersed flumioxazin application (200 to 400 μg L^{-1}) should not be used for irrigation for up to 7 d after herbicide application compared to a 2 d restriction if the pH is ≥9.0. The shorter half-life at pH 9.0 (see Chapter 2) prevents injurious levels of this herbicide from being present in the water beyond 1 DAT. Immature plants are more susceptible to submersed flumioxazin applications as pH decreases and the use rate increases. On the other hand, foliar applications pose less of a threat to these young plants. The majority of the herbicide solution will come in contact with the foliage of the emergent or floating weed and less will be available in the water to harm irrigated plants.

Table 7-1. Aquatic plant and algae control[a] with flumioxazin in water with a pH of 7.0 to 9.5[b]

Common name	Scientific name	Submersed[c]	Foliar[d]
alligatorweed	*Alternanthera philoxeroides*	F-G	G-E
baby's-tears	*Micranthemum* spp.	F	NA
broadleaf arrowhead	*Sagittaria latifolia*	E	NA
Carolina mosquito fern	*Azolla caroliniana*	E	NA
cattail	*Typha* spp.	P	P
coontail	*Ceratophyllum demersum*	E	NA
duck potato	*Sagittaria lancifolia*	E	F-G
duckweed	*Lemna minor*	G-E	G-E
egeria	*Egeria densa*	P	NA
Eurasian water milfoil	*Myriophyllum spicatum*	E	NA
fanwort	*Cabomba caroliniana*	E	NA
frog's-bit	*Limnobium spongia*	E	NA
hydrilla	*Hydrilla verticillata*	F-E	NA
jointed spikerush	*Eleocharis interstincta*	F	F
landoltia	*Landoltia punctata*	G-E	P
maidencane	*Panicum hemitomon*	G	F
muskgrass	*Chara* spp.	P-G	NA
pennywort	*Hydrocotyle* spp.	F-G	NA
pickerelweed	*Pontederia cordata*	F	F
southern naiad	*Najas guadalupensis*	F-E	NA
stonewort	*Nitella* spp.	P-F	NA
torpedo grass	*Panicum repens*	P-F	P-F
vallisneria	*Vallisneria americana*	P-F	NA
variable-leaf milfoil	*Myriophyllum heterophyllum*	E	NA
water fern	*Salvinia minima*	G-E	NA
water hyacinth	*Eichhornia crassipes*	P	P
water lettuce	*Pistia stratiotes*	E	E
water meal	*Wolffia columbiana*	G-E	NA
water shield	*Brasenia schreberi*	G-E	NA
willow	*Salix* spp.	P	P

[a]Control: NA = not applicable or not evaluated, P = poor, F= fair, G = good, E = excellent; based on EUP field and mesocosm observations.
[b]Improved efficacy at lower pH for most species.
[c]Submersed: treatment applied by weighted hoses.
[d]Foliar treatment applied with 0.25% v/v non-ionic surfactant by handgun at 935 L ha^{-1} diluent.

Table 7-2. Proposed water use restrictions to overhead irrigated crop and ornamental species following submersed and foliar flumioxazin applications.

Flumioxazin Application	Use Rate	Maturity of Irrigated Plants[a]	Water pH	Days[b]
Submersed	200-400 µg L^{-1}	Immature	≥8.5	1
	200-400 µg L^{-1}	Mature	≥8.5	1
	200-400 µg L^{-1}	Immature	7.0-8.4	7
	200-400 µg L^{-1}	Mature	7.0-8.4	1
	200-400 µg L^{-1}	Immature	≤6.9	14
	200-400 µg L^{-1}	Mature	≤6.9	2
	<200 µg L^{-1}	Immature	≥8.5	1
	<200 µg L^{-1}	Mature	≥8.5	1
	<200 µg L^{-1}	Immature	7.0-8.4	6
	<200 µg L^{-1}	Mature	7.0-8.4	1
	<200 µg L^{-1}	Immature	≤6.9	12
	<200 µg L^{-1}	Mature	≤6.9	1
Foliar	143-286 g ha^{-1}	Immature	≥8.5	1
	143-286 g ha^{-1}	Mature	≥8.5	0
	143-286 g ha^{-1}	Immature	7.0-8.4	1
	143-286 g ha^{-1}	Mature	7.0-8.4	0
	143-286 g ha^{-1}	Immature	≤6.9	1
	143-286 g ha^{-1}	Mature	≤6.9	0

[a] Growth stage of plants irrigated with water treated with flumioxazin.

[b] Number of days before water treated with flumioxazin may be used for irrigation of crop and ornamental species. Based on the half-life of flumioxazin at a given pH and the growth stage of irrigated species.

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BIOGRAPHICAL SKETCH

Christopher Ray Mudge, the son of Alvin and Wanda Mudge, was born in Alexandria, LA and was raised in the rural rice and soybean farming community of Branch, LA. Upon graduation from Iota High School in 1997, he enrolled at Louisiana State University A&M in Baton Rouge in the fall of 1997. While attending LSU, he was active in the agronomy and collegiate 4-H clubs.

After earning a B.S. in agronomy (crop management) in December 2001, he began his graduate career under the direction of Dr. Eric P. Webster, working in rice weed management. While working on his master's degree, Chris was an active participant in the Southern Weed Contest. He graduated from LSU in the spring of 2004 with a M.S. in agronomy and the title of his thesis was *Water-seeded Rice Response to Clomazone*.

Chris moved to Gainesville, FL in 2004 to work as a biological scientist for the University of Florida. In 2005, he enrolled at the University of Florida to study for a PhD in aquatic weed science under the direction of Dr. William T. Haller. During his graduate career, he presented many talks and posters at the Southern Weed Science, Aquatic Plant Management Society, Florida Weed Science, and Florida Aquatic Plant Management Society annual meetings. In 2005, Chris married the former Miss Erin Gravois of Vacherie, LA. Upon graduation, he will work in the field of aquatic weed management and continue his involvement in weed science and aquatic societies.