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Small-scale Primary Screening Method to Predict Impacts of the Herbicide Flumioxazin on Native and Invasive Emergent Plants

by LeeAnn M. Glomski and Michael D. Netherland

PURPOSE: The purpose of this work was to develop and utilize a rapid, small-scale primary screening method to evaluate the activity of a recently registered aquatic herbicide, flumioxazin, against 23 native emergent plant species and five invasive emergent species.

BACKGROUND: In evaluating potential use patterns of new aquatic herbicides, it is important to determine concentrations that impact target as well as non-target vegetation. The recently registered aquatic herbicide flumioxazin is efficacious against the floating weeds water lettuce (*Pistia stratiotes* L.) and giant salvinia (*Salvinia molesta* Mitchell) (Richardson et al. 2008) as well as the submersed species hydrilla (*Hydrilla verticillata* (L.f.) Royle) (Mudge and Haller 2006) and Eurasian watermilfoil (*Myriophyllum spicatum* L.) (Getsinger et al. 2011). These species are often found in close proximity to or intermixed with native emergent vegetation and information on the impact of flumioxazin to most emergent plant species is limited. Information regarding the selectivity of flumioxazin (and other aquatic herbicides) will help resource managers decide which herbicides, use rates, and timing of application are most appropriate to reduce injury to the native emergent species present. This work has become increasingly relevant as research conducted last year demonstrated a potential selective use pattern for flumioxazin in areas where water lettuce is intermixed with emergent species (Netherland 2011). The endangered snail kite (*Rosthramus sociabilis)* is utilizing habitats in areas of large-scale floating plant control operations. Current operational use of the broad-spectrum herbicide diquat, while highly effective at controlling water lettuce, generally results in significant visual injury symptoms on numerous emergent plant species. Aquatic plant managers are under increasing pressure to reduce non-target injury to plants such as bulrush (*Schoenoplectus spp*), spikerush (*Eleocharis spp*), cattail (*Typha spp*), and other emergent species. Operational treatments in 2011-2012 included expanding the use of flumioxazin for selective water lettuce control on Lake Okeechobee and the St. Johns River, Florida. Determining the response of native and invasive plants to the different use rates and timing of flumioxazin will help refine use patterns.

Flumioxazin is a protox-inhibiting herbicide originally registered for broadleaf weed control in terrestrial systems (Senseman 2007); however, it has been approved for use in aquatic sites in recent years. Protox inhibitors disrupt chlorophyll synthesis by competing with protoporphyrinogen for binding sites on the protoporphyrinogen oxidase enzyme. Without available binding sites, protoporphyrinogen leaks into the cytoplasm and is converted to protoporphyrin IX when exposed to light. Protoporphyrin IX then reacts with oxygen to form singlet oxygen radicals that damage cell membranes causing them to leak (Hess 2000, Senseman 2007). This leakage of electrolytes has been measured and used to determine herbicide injury of membrane-disrupting and photosynthesisinhibiting herbicides (Falk et al. 2006; Koo et al. 1994; Koschnick et al. 2006; Li et al. 2000; Vanstone and Stobbe 1977; Yanase et al. 1990), freezing resistance and frost tolerance (Nunes and Smith 2003; Sukumaran and Weiser 1972; Dexter et al. 1932) and seed vigor (Duke et al. 1983). More recently, it has been used to determine the impacts of flumioxazin and carfentrazone-ethyl on native and invasive submersed plants (Glomski and Netherland, in press).

In addition to emergent spray applications, flumioxazin can be applied as a submersed treatment to control invasive species such as hydrilla, Eurasian watermilfoil, water lettuce, and giant salvinia (Valent USA Corporation 2011). Many times, these species are found in close proximity to or intermixed with native emergent vegetation. Past research has shown that submersed herbicide applications can cause unintended damage to some emergent species while leaving other species undamaged. For example, submersed applications of the auxin mimics 2,4-D and triclopyr when used to control Eurasian watermilfoil, can impact white waterlily (*Nymphaea odorata* Ait.), soft-stem bulrush (*Schoenoplectus tabernaemontani* (C.C. Gmel.) Palla) and hardstem bulrush (*S. acutus* Muhl. Ex Bigelow) whereas spatterdock (*Nuphar lutea* (L.) Sm.) and American bulrush (*S. americanus* Pers.) are not impacted (Glomski and Nelson 2008; Glomski et al. 2009; Glomski and Netherland 2012). Some research has been conducted to determine the selectivity of submersed applications of the ALS-inhibiting herbicides bispyribac-sodium, imazamox, and penoxsulam on emergent vegetation (Glomski and Mudge 2009; Koschnick et al. 2007). To date, there has been no extensive species screening for flumioxazin activity following submersed and emergent use patterns.

MATERIALS AND METHODS: To determine the effect of flumioxazin on emergent plants, small-scale assays were conducted in reach-in growth chambers at the U.S. Army Engineer Research and Development Center's Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, Texas. Studies were conducted using a Percival E-36L (Perry, IA) growth chamber set at 25 °C and continuous light. Light intensity was 430 μmol photons m^{-2} s^{-1}. An incubation medium of 1 mM 2-(morpholino) ethanesulfonic acid buffer (MES) and 2% (w/v) sucrose was prepared and pH of the medium was adjusted to 6.5 with 2.1 N NaOH (Duke and Kenyon 1993, Kenyon et al. 1985). Twenty mL of medium were added to 150-mL cups along with 0.25 g of fresh leaf tissue. Species tested are listed in Table 1. The more tissue per cup, the more sensitive the assay (Duke and Kenyon 1993) and the weight used in these assays was enough to cover the surface (for most species) without over-packing the cups. Treatments included 100, 200, and 400 µg ai L^{-1} flumioxazin (Clipper, Valent USA Corporation, Walnut Creek, California) and an untreated control. Conductivity readings were taken in each cup after 2 days of herbicide exposure using an Accumet AP85 pH/conductivity meter (Fisher Scientific, Pittsburgh, Pennsylvania). Cups were then exposed to two freeze-thaw cycles before final conductivity readings were taken to determine total electroconductivity of the tissue. Percent electrolyte leakage was calculated using the following equation similar to Falk et al. (2006):

$$(\text{Conductivity before freeze-thaw}/\text{Conductivity after freeze-thaw}) * 100$$

Treatments were replicated four times and assay data were subjected to one-way analysis of variance (ANOVA) with means compared via the Student-Newman-Keuls method (SNK; $\alpha=0.05$).

Table 1. Common and scientific name of plants screened for sensitivity to flumioxazin.

Common Name	Scientific Name	Native?
Alligatorweed	*Alternanthera philoxeroides* (Mart.) Griseb.	N
American bulrush	*Schoenoplectus americanus* (Pers.) Volkart ex Schinz & R. Keller	Y
American lotus	*Nelumbo lutea* Willd.	Y
Arrowhead	*Sagittaria latifolia* Willd.	Y
Cattail	*Typha latifolia* L.	Y
Cuban bulrush	*Oxycaryum cubense* (Poepp. & Kunth) Lye	Y
Duck potato	*Sagittaria lancifolia* L.	Y
Floating water primrose	*Ludwigia peploides* (Kunth) P.H. Raven	Y
Giant bulrush	*Schoenoplectus californicus* (C.A. Mey.) Palla	Y
Giant salvinia	*Salvinia molesta* D.S. Mitchell	N
Gulf Coast spikerush	*Eleocharis cellulosa* Torr.	Y
Hardstem bulrush	*Schoenoplectus acutus* Muhl. Ex Bigelow A. Love & D. Love	Y
Horsetail	*Equisetum hyemale* L.	Y
Knotgrass	*Paspalum distichum* L.	Y
Large-flower primrose	*Ludwigia grandiflora* (Michx.) Greuter & Burdet	N
Maidencane	*Panicum hemitomon* Schult.	Y
Mexican waterlily	*Nymphaea mexicana* Zucc.	Y
Pale spikerush	*Eleocharis macrostachya* Britton	Y
Pennywort	*Hydrocotyle sp.*	Y
Pickerelweed	*Pontederia cordata* L.	Y
Slender spikerush	*Eleocharis acicularis* (L.) Roem. & Schult.	Y
Soft-stem bulrush	*Schoenoplectus tabernaemontani* (C.C. Gmel.) Palla	Y
Spatterdock	*Nuphar lutea* (L.) Sm.	Y
Squarestem spikerush	*Eleocharis quadrangulata* (Michx) Roem. & Schult.	Y
Water hyacinth	*Eichhornia crassipes* (Mart.) Solms	N
Water lettuce	*Pistia stratiotes* L.	N
Water paspalum	*Paspalum fluitans* (Elliot) Kunth	Y
White waterlily	*Nymphaea odorata* Aiton	Y

RESULTS AND DISCUSSION: Of the 28 species tested, 15 showed no change in electrolyte leakage when exposed to increasing rates of flumioxazin, whereas 13 species showed a significant change compared to untreated plants (Table 2). Species that did not show a change in electrolyte leakage were categorized as not sensitive and species that did show a change were designated as sensitive. All of the bulrush and spikerush species tested fell into the not sensitive category and all of the grass and lily species were sensitive. Other species could not be grouped as easily. For example, within the genus *Sagittaria*, duck potato was found to be sensitive to flumioxazin, whereas arrowhead was not. The same was also true for the two *Ludwigia* species; large-flower primrose was sensitive but floating water primrose was not. Of the invasive floating species tested, giant salvinia and water lettuce were sensitive but water hyacinth was not. The remaining four species included horsetail and pennywort, which were not sensitive and alligatorweed and cattail, which were sensitive.

It is interesting to note that of the sensitive plants, only maidencane and large-flower primrose showed evidence of a rate response to increased flumioxazin concentrations (Table 2). In contrast, all of the other species denoted as sensitive showed a similar level of electrolyte leakage in response to increasing rates of flumioxazin. While cattail was denoted as sensitive, the magnitude of change in electrolyte leakage was quite low compared to the other plants that were denoted as sensitive (Table 2).

Table 2. Mean (± SE) percent electrolyte leakage of emergent plants exposed to flumioxazin for two days. Each mean represents the average of four replicate treatments. Means sharing the same letter in each row do not significantly differ from each other. Data were subjected to a one-way analysis of variance and means were separated using the Student-Newman-Keuls Method (SNK; α=0.05).

	Species	Control	100 µg L^{-1}	200 µg L^{-1}	400 µg L^{-1}
Not Sensitive	American bulrush	26.6 ± 1.0	27.6 ± 0.4	32.4 ± 2.2	33.2 ± 2.5
	Arrowhead	22.9 ± 1.6	21.0 ± 2.7	24.5 ± 3.2	22.9 ± 3.6
	Cuban bulrush	73.4 ± 3.8	66.4 ± 4.5	74.0 ± 4.9	79.8 ± 2.5
	Flatstem spikerush	73.5 ± 1.0	73.2 ± 1.1	73.0 ± 2.2	70.2 ± 0.3
	Floating water primrose	51.3 ± 0.4	53.8 ± 2.3	58.2 ± 2.5	57.8 ± 1.9
	Giant bulrush	66.8 ± 3.8	62.2 ± 1.7	61.1 ± 2.8	63.5 ± 1.0
	Gulf Coast spikerush	64.6 ± 6.1	57.6 ± 7.0	55.6 ± 1.9	53.7 ± 6.2
	Hardstem bulrush	53.8 ± 3.8	64.2 ± 2.4	68.4 ± 8.4	56.7 ± 6.1
	Horsetail	50.3 ± 4.5	67.5 ± 3.8	64.6 ± 7.8	64.4 ± 6.4
	Pennywort	59.2 ± 4.9	57.2 ± 7.6	52.9 ± 3.1	52.5 ± 3.9
	Pickerelweed	33.4 ± 1.9	36.9 ± 2.3	27.8 ± 2.8	27.3 ± 2.4
	Slender spikerush	60.5 ± 6.9	58.9 ± 3.4	59.2 ± 3.8	64.5 ± 3.0
	Soft-stem bulrush	74.1 ± 3.2	67.0 ± 3.5	69.6 ± 5.5	71.7 ± 4.7
	Squarestem spikerush	61.6 ± 1.5	57.5 ± 7.4	59.0 ± 4.1	59.1 ± 1.4
	Water hyacinth	47.6 ± 6.8	42.9 ± 4.2	43.9 ± 4.6	40.3 ± 7.7
Sensitive	Alligatorweed	57.4 ± 2.6 b	87.0 ± 2.5 a	85.6 ± 2.6 a	86.3 ± 1.2 a
	American lotus	45.4 ± 3.2 b	64.9 ± 1.8 a	57.7 ± 5.0 a	62.1 ± 2.2 a
	Cattail	38.0 ± 2.6 b	44.6 ± 1.9 a	46.4 ± 1.9 a	48.4 ± 2.2 a
	Duck potato	19.5 ± 1.3 b	35.2 ± 2.9 a	27.6 ± 3.8 a	31.9 ± 4.2 a
	Giant salvinia	50.9 ± 4.3 b	68.3 ± 6.6 a	79.3 ± 1.3 a	76.2 ± 5.3 a
	Knotgrass	64.3 ± 2.6 b	80.6 ± 3.4 a	85.8 ± 2.4 a	77.6 ± 1.5 a
	Large-flower primrose	58.1 ± 3.5 b	66.6 ± 4.0 b	79.1 ± 4.0 a	81.1 ± 3.2 a
	Maidencane	67.4 ± 1.2 c	66.9 ± 2.5 c	80.1 ± 1.7 b	88.6 ± 1.5 a
	Mexican waterlily	22.4 ± 2.0 b	71.4 ± 6.6 a	71.9 ± 6.5 a	77.6 ± 5.3 a
	Spatterdock	52.6 ± 3.1 b	89.6 ± 6.7 a	95.3 ± 4.0 a	75.0 ± 7.4 a
	Water lettuce	45.5 ± 4.7 b	71.5 ± 6.7 a	75.6 ± 2.8 a	83.7 ± 4.9 a
	Water paspalum	43.0 ± 7.9 b	73.0 ± 3.5 a	65.9 ± 5.1 a	61.5 ± 2.1 a
	White waterlily	24.2 ± 3.8 b	60.3 ± 5.1 a	61.0 ± 6.1 a	55.4 ± 1.5 a

The assay results will ultimately require additional field and mesocosm corroboration and it is likely that species will be further categorized into sensitive, not sensitive, and rate-dependent sensitive categories. Similar to results from the small-scale cup assay, duck potato and maidencane were sensitive to flumioxazin in a large-scale mesocosm trial conducted by Mudge and Haller (in press), with EC_{50} values of 15 and 259 µg ai L^{-1}, respectively. Mudge and Haller (in press) also reported an EC_{50} value of 894 µg ai L^{-1} for pickerelweed, which was not found to be sensitive in this assay. Several species observed as highly sensitive following various field applications (e.g. American lotus, Mexican waterlily, spatterdock, water lettuce, and white waterlily)[1] were also sensitive in this assay. While cattails have not been particularly sensitive to flumioxazin following field applications for water lettuce

[1] Personal Observation. 2012. Dr. Mike Netherland, Research Biologist, US Army Engineer Research and Development Center, Vicksburg, MS.

control at lower use rates (140 to 420 g ha^{-1}), increasing use rates to 560 to 1120 g ha^{-1} can significantly increase visual injury.

Flumioxazin is generally not recognized as being phloem-mobile (Senseman 2007); therefore, it is likely that many emergent rooted plants denoted as "sensitive" to flumioxazin will initially display strong injury symptoms on the vegetation above the water line. This assay cannot predict whether a given species will ultimately be controlled by flumioxazin, but it should indicate if a given species is likely to be severely injured by flumioxazin.

Primary screens that provide rapid data on the relative sensitivity of a species to a given herbicide can enhance the design of larger scale studies as well as predict the response of field populations. The utility for these small assays to provide information on threatened or rare plant species has not been explored, but with the limited tissue requirements, this would be a good first step for determining potential sensitivity. Given the high cost, extensive time requirements, and limited number of replicates available in the larger systems, methods that can be used to predict response and improve study design are of significant value. As further selectivity data for the protox inhibitors is developed, managers can use this information for site-specific treatment recommendations when selective control is considered a priority.

FUTURE WORK: The results of these assays require additional confirmation with various mesocosm and field trials to determine if rate responses in the assays can be used to further refine field recommendations. Additional assay and mesocosm trials focusing on "sensitive species" may help determine which of these plants are highly sensitive versus species that are moderately sensitive. This would provide valuable information to managers who are under increasing pressure to provide selective control. Additional assay development with other active ingredients is planned, as this approach can represent a low-cost predictive tool that improves study design.

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POINTS OF CONTACT: For additional information, contact the authors, LeeAnn M. Glomski (972) 436-2215, *LeeAnn.M.Glomski@usace.army.mil*, Michael D. Netherland (352) 392-0335, *Michael.D.Netherland@usace.army.mil* or the Manager of the Aquatic Plant Control Research Program, Linda S. Nelson (601) 634-2656, *Linda.S.Nelson@usace.army.mil*. This technical note should be cited as follows:

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