

Comparative susceptibility of fluridone resistant and susceptible hydrilla to four ALS inhibiting herbicides under laboratory and greenhouse conditions

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ABSTRACT

In response to the widespread presence of fluridone resistant strains of dioecious hydrilla (*Hydrilla verticillata* [L.f.] Royle) throughout Florida, several new herbicides, including acetolactate synthase (ALS) inhibitors, are being evaluated for aquatic registration. Laboratory and greenhouse studies were conducted to determine the susceptibility of different hydrilla populations in Florida to 4 ALS inhibitors. Apical shoots of fluridone-resistant and fluridone-susceptible strains of hydrilla collected from 6 Florida lakes were placed in growth chambers and exposed to the ALS inhibitors bensulfuron-methyl, bispyribac-sodium, imazamox, and penoxsulam at concentrations of 0, 2.5, 5, 10, 25, 50, and 100 µg active ingredient (a.i.) per liter. Two of these hydrilla accessions were then established in 90 L tanks in a greenhouse and exposed for 8 weeks to the 4 ALS herbicides at concentrations of 0, 5, 10, 25, 50, and 100 µg L⁻¹. Results from both the laboratory and greenhouse studies suggest that different strains of dioecious hydrilla collected throughout Florida (including fluridone-resistant strains) show similar susceptibility to each individual ALS herbicide. Bensulfuron methyl and penoxsulam were the most active compounds with a significant increase in activity noted between 5 and 10 µg L⁻¹. Activity of bispyribac sodium showed a strong increase between 10 and 25 µg L⁻¹. No differences in activity were detect-

ed within or between these 3 herbicides at concentrations of 25, 50, and 100 µg L⁻¹. In contrast, imazamox efficacy generally increased with concentration. The 4 ALS herbicides differed in the concentration required to elicit a threshold or phytotoxic response by hydrilla, but all showed similar activity against different accessions of hydrilla. While treatment symptoms and hydrilla response could not be distinguished between bensulfuron methyl, bispyribac sodium, and penoxsulam, imazamox consistently resulted in different symptoms and rate response. Results from the laboratory assays were predictive of the results obtained in the longer term and larger scale greenhouse trials. This baseline susceptibility data can be used to determine if increased tolerance to ALS herbicides occurs over time. These studies suggest that ALS inhibitors can result in rapid growth cessation, but generally slow control of existing hydrilla biomass.

Key words: aquatic herbicides, bensulfuron-methyl, bispyribac-sodium, chemical control, imazamox, penoxsulam, submersed invasive plants.

INTRODUCTION

Hydrilla has been described as “the perfect aquatic weed” with unique physiological characteristics, numerous mechanisms for spread, and formation of persistent vegetative propagules that allow the plant to rapidly cover large areas and persist over long periods of time (Langeland 1996). The dioecious biotype of hydrilla was present in more than 50,000 ha of Florida’s public waters in 2007, with an approximate management cost of \$16 million (FDEP 2007). In the mid-1990s large infestations of hydrilla in Florida were pri-

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marily controlled using the systemic herbicide fluridone; however, poor performance was observed following numerous treatments in 2000, and presence of fluridone resistance in hydrilla was ultimately confirmed (Michel et al. 2004, Arias et al. 2005, Dayan and Netherland 2005, Puri et al. 2009).

A strong shift away from use of fluridone as a hydrilla control tool for large-scale hydrilla control in Florida and the heavy current reliance on the contact herbicide endothall suggests that new modes of action are needed. In response to the onset and rapid spread of fluridone-resistant hydrilla throughout numerous large public water bodies in Florida, new herbicide modes of action have been evaluated for control of hydrilla and other invasive aquatic plants (Glomski and Netherland 2008, Emerine et al. 2010, Mudge et al. 2010). The ALS inhibitors penoxsulam and imazamox were registered by the US Environmental Protection Agency (EPA) for aquatic use in 2007 and 2008, respectively, and bispyribac-sodium (hereafter called bispyribac) and bensulfuron-methyl (hereafter called bensulfuron) are currently being evaluated under EPA Experimental Use Permits. Penoxsulam (Galleon™) and imazamox (Clearcast™) represent the first new active ingredients with activity against hydrilla since the registration of fluridone 21 years earlier in 1986. While these products have been registered and are being used in operational programs, there is limited literature regarding their potential efficacy against hydrilla.

Penoxsulam, imazamox, bispyribac, and bensulfuron are classified into 4 herbicide families (triazolopyrimidines, imidazolinones, pyrimidinylthiobenzoates, and sulfonyleureas respectively), yet all of these families target the ALS enzyme. ALS herbicides inhibit production of the branched chain amino acids valine, leucine, and isoleucine in plants by binding to the ALS enzyme, and the subsequent inability of a sensitive plant to synthesize protein results in rapid cessation of new growth (Tranel and Wright 2002).

The ALS inhibitors have been identified as candidates for aquatic use due to favorable toxicology profiles that preclude consumptive use restrictions (potable uses, fishing, or swimming), projected use rates and use patterns similar to fluridone, potential for selective use, and slow control of target vegetation that will prevent rapid changes in water quality (Koschnick et al. 2007). Prior research has described the mechanism of action for bensulfuron against hydrilla (Ratray et al. 1993) as well as impacts on hydrilla biomass reduction and tuber inhibition (Haller et al. 1992, Langeland and LaRoche 1992, Langeland 1994, and Van and Vandiver 1994). Exposures of weeks to months to bensulfuron at concentrations from 5 to 50 $\mu\text{g L}^{-1}$ were required to control plants such as hydrilla and Eurasian watermilfoil (Nelson et al. 1993).

While the different ALS herbicides impact the same target enzyme, terrestrial experience indicates these compounds have different use rate recommendations, different weed spectrums and selectivity profiles for nontarget plants, and potential for resistance development by the target plant (Tranel and Wright 2002). Slight changes in the molecular structure of ALS-inhibiting herbicides greatly affect the potency and weed spectrum (Ladner 1991, Ren et al. 2000). A recent evaluation of penoxsulam, bispyribac, and imazamox

against variable watermilfoil (*Myriophyllum heterophyllum*) demonstrated strong activity by penoxsulam, limited activity of imazamox, and no activity of bispyribac over a fairly wide range of target use rates (Glomski and Netherland 2008). The variability in response to different ALS herbicides suggests that both target species as well as nontarget species may respond to these compounds in a unique manner.

Based on the experience with fluridone, ALS herbicides have similar properties such as low use rates, a single enzyme site of action, likely use on a large scale with significant exposure times, and ability of hydrilla biomass to remain intact for months following initial exposure. Therefore resistance development is a significant concern with the ALS herbicides, and establishing baseline susceptibility for several hydrilla populations is an important initial step to help determine if resistance or cross-resistance ultimately occurs in hydrilla. Data generated on lethal versus sublethal concentrations for each of the ALS compounds will also help ensure that phytotoxic concentrations are maintained when these products are used. To develop field-use patterns for large-scale, cost-effective, and selective control of hydrilla, laboratory and greenhouse trials can provide information on the target herbicide concentrations and exposures (Netherland and Getsinger 1995). The objective of this research was to document the response of 6 populations of hydrilla following continuous exposures to ALS herbicides and to compare the activity of 4 different ALS inhibitors being considered for aquatic use.

MATERIALS AND METHODS

Laboratory assays

Susceptibility studies were conducted on 6 populations of hydrilla in Florida, including documented fluridone-resistant strains collected from lakes Okahumpka, Seminole (Gadsden County), Tohopekaliga, and Istokpoga, as well as susceptible strains from Lake Sampson and the Rainbow River. Assays were conducted March through April 2007 and repeated May through June 2008 at the University of Florida Center for Aquatic and Invasive Plants, Gainesville, Florida. Following field collection, healthy 4 cm apical shoots of hydrilla were excised, thoroughly rinsed to remove epiphytes, and placed in 250 mL Erlenmeyer flasks containing 150 mL of a 10% Hoagland's medium buffered with sodium bicarbonate (20 mg L^{-1}). Flasks containing 4 cm apical shoot sections of healthy hydrilla were treated with bensulfuron, bispyribac, imazamox, and penoxsulam at nominal concentrations of 0, 2.5, 5, 10, 25, 50, and 100 $\mu\text{g L}^{-1}$. Flasks were placed in Percival E-36L Growth Chambers set to 27 C and a 14L:10D photoperiod. To prevent confounding issues with algae, culture solutions were exchanged at 10 d, plants were thoroughly rinsed to remove any epiphytic algae, and flasks were treated with the herbicide concentrations noted above. Light intensity measured at 8 spots in the chambers was $320 \pm 37 \mu\text{moles m}^{-2} \text{sec}^{-1}$. Hydrilla was exposed to the herbicides for 3 weeks, and flasks were then removed from the chambers and total stem length (main + laterals) and dry weight biomass were recorded.

Each treatment was replicated 4 times using a completely randomized design. Data were converted to percent biomass reduction and are presented as mean values $\pm 95\%$ confidence intervals (C.I.). An ANOVA ($p = 0.05$) indicated there was no difference in the response between fluridone-tolerant and -susceptible strains of hydrilla between studies. Moreover, no response differences were noted between any of the 6 hydrilla accessions; therefore all hydrilla data for the studies were pooled for presentation.

Greenhouse trials

Greenhouse studies were conducted using the fluridone-resistant strain of hydrilla from Lake Tohopekaliga and the fluridone-susceptible strain from Rainbow River. Studies were initiated in June 2007 and repeated in June 2008 at the University of Florida Center for Aquatic and Invasive Plants. Four apical meristems of hydrilla (about 12 cm) from each accession were planted in 500 mL pots filled with potting soil amended with 1 g per Kg Osmocote (15:9:9), covered with a 1 cm layer of builder's sand, and placed in 90 L containers in the greenhouse. Three pots of each hydrilla accession were placed in tanks filled with well water (pH 7.7, alkalinity, 1.5 mEq L⁻¹). The greenhouses were covered with 50% shade cloth, and doors and upper vents were opened to simulate ambient outdoor temperature fluctuations (22 to 31 C). The greenhouse environment prevented any confounding problems with precipitation and photolytic degradation. Due to the shading effect, daytime temperatures in the greenhouse were typically 2 to 5 C cooler than outdoor temperatures. During a 3 week pretreatment period, hydrilla grew rapidly and plants were forming multiple branches at the water surface at the time of application. Hydrilla was treated with bensulfuron, bispyribac, imazamox, and penoxsulam at concentrations of 5, 10, 25, and 100 $\mu\text{g L}^{-1}$. During the course of the treatments, a 1 cm apical tip from both hydrilla accessions was selected from the treatment tanks at 2 and 6 weeks after treatment (WAT), and net photosynthetic rates were recorded to determine comparative photosynthetic competence of apical shoots using methods described by Netherland and Lembi (1992) and Bultemeier et al. (2009). For these assays, apical shoots were placed in a buffered 10% Hoaglands solution overnight and then moved to a 300 mL BOD bottle containing the buffered Hoagland's for assay reading. Initial dissolved oxygen readings were recorded for each BOD bottle and plants were allowed to incubate in the chamber for approximately 1 h. Final dissolved oxygen readings and fresh biomass were recorded. While ALS herbicides are not known for specific impacts on photosynthesis, prior evaluations had indicated that photosynthetic capacity of new hydrilla growth was significantly compromised following exposure to ALS herbicides.

Biomass changes were compared to both pretreatment biomass and to untreated controls at the end of the study to determine growth regulating versus phytotoxic (or biomass reducing) concentrations. Studies were harvested 8 WAT and final shoot dry weight biomass was recorded. Each treatment was replicated 4 times; data were converted to percent reduction in photosynthesis and percent reduction in biomass and presented as means $\pm 95\%$ C.I. An ANOVA ($p = 0.05$)

indicated no differences in the response of fluridone-susceptible and fluridone-tolerant hydrilla (photosynthesis and biomass). In addition, there were no differences noted between studies 1 and 2, and therefore all response data were pooled for presentation.

RESULTS AND DISCUSSION

Laboratory assays

The 6 hydrilla accessions all responded in a similar manner to the individual ALS inhibitors. A major emphasis of this study was to compare the response of fluridone-resistant and fluridone-susceptible accessions; population data were pooled (Table 1). The lack of any response difference among 6 independent populations is important because it suggests that different hydrilla accessions should respond in a similar manner to each individual ALS herbicide. Combining the data for 6 different hydrilla accessions resulted in 48 readings (2 studies by 4 replicates by 6 accessions) for each ALS herbicide rate evaluated. Bensulfuron, bispyribac, and penoxsulam resulted in near complete cessation of shoot growth at concentrations of $\geq 25 \mu\text{g L}^{-1}$ (Table 1). Penoxsulam and bensulfuron reduced shoot growth by 60 and 67%, respectively, following exposure to the concentration of 5 $\mu\text{g L}^{-1}$. Plants treated with bispyribac were not different from the untreated control at the 5 $\mu\text{g L}^{-1}$ concentration but reduced shoot growth by 67% at the 10 $\mu\text{g L}^{-1}$ rate. Imazamox exposure resulted in different visual symptoms compared to the other ALS herbicides, with the formation of numerous small lateral meristems noted at concentrations $\geq 25 \mu\text{g L}^{-1}$. These meristems were very thin and abnormal in appearance, but they did result in data showing a greater increase in total stem length when compared to penoxsulam and bensulfuron at 5 to 50 $\mu\text{g L}^{-1}$ and bispyribac at 10 and 25 $\mu\text{g L}^{-1}$.

With the exception of imazamox, biomass data suggest a limited benefit for increasing treatment rates from 25 to 100 $\mu\text{g L}^{-1}$; however, biomass reduction almost doubled between concentrations of 5 and 10 $\mu\text{g L}^{-1}$ for penoxsulam and bensulfuron and between 10 and 25 $\mu\text{g L}^{-1}$ for bispyribac (Table 1). As observed with fluridone, these data suggest that differences between growth regulating and phytotoxic concentrations of the ALS herbicides may be separated by just a few $\mu\text{g L}^{-1}$ (Netherland and Getsinger 1995). Imazamox showed a different pattern compared to the other compounds with biomass reduction steadily increasing as treatment concentrations increased (Table 1).

While these short-term and small-scale laboratory studies are often limited in their ability to directly predict a response at the field scale, these trials can be helpful in determining potential for efficacy and dose ranges for larger-scale trials. These data suggest strong similarities in terms of the dose-response between penoxsulam and bensulfuron. Moreover, these data also indicate that imazamox is likely to result in a different response by hydrilla at the field scale when compared to the other ALS inhibitors evaluated. The ability to screen 6 different hydrilla accessions over a broad range of concentrations with 4 different herbicides would require a significant investment in time, resources, and space if conducted via traditional mesocosm testing.

TABLE 1. MEAN TOTAL INCREASE IN HYDRILLA SHOOT LENGTH AND PERCENT REDUCTION IN SHOOT BIOMASS DRY WEIGHT COMPARED TO THE UNTREATED CONTROL ($\pm 95\%$ C.I.) FOR FLURIDONE RESISTANT AND FLURIDONE SUSCEPTIBLE ACCESSIONS OF HYDRILLA FOLLOWING A 21 D LABORATORY EXPOSURE TO 6 CONCENTRATIONS OF PENOXsulAM, BENSulfURON, BISpyRIBAC, AND IMAZAMOX (N = 48).

Herbicide	Herbicide Concentration ($\mu\text{g L}^{-1}$)	Total Increase in Shoot Length (cm)	% Shoot Biomass Reduction	Total Increase in Shoot Length (cm)	% Shoot Biomass Reduction
		($\pm 95\%$ C.I.) ¹	($\pm 95\%$ C.I.) ¹	($\pm 95\%$ C.I.) ²	($\pm 95\%$ C.I.) ²
		Fluridone Resistant		Fluridone Susceptible	
Penoxsulam	0	21.9 (2.4)	—	20.6 (3.1)	
	2.5	15.7 (5.3)	9.8 (5.3)	17.1 (4.2)	9.5 (3.5)
	5	8.6 (1.8)	40.1 (12.8)	7.6 (2.0)	45.6 (9.0)
	10	3.1 (0.7)	67.8 (7.1)	2.5 (0.7)	71.2 (5.6)
	25	0.8 (0.6)	74.2 (8.0)	0.8 (0.7)	79.1 (6.5)
	50	0.8 (0.3)	83.4 (7.1)	0.6 (0.2)	80.3 (5.8)
Bensulfuron	0	21.9 (2.4)	—	20.6 (3.1)	
	2.5	17.7 (2.8)	14.7 (5.9)	18.4 (2.9)	14.6 (4.8)
	5	7.3 (1.4)	35.5 (8.8)	6.5 (1.3)	40 (7.5)
	10	3.6 (1.5)	70.4 (7.2)	3.3 (1.3)	76.1 (5.3)
	25	0.9 (0.6)	79.4 (6.6)	0.8 (0.5)	83.7 (4.8)
	50	0	87.8 (5.1)	0	90.1 (6.9)
Bispyribac	0	21.9 (2.4)	—	20.6 (3.1)	
	2.5	19.4 (4.0)	1.3 (1.4)	21.2 (2.8)	1.5 (1.6)
	5	16.2 (3.1)	12.0 (5.1)	13.8 (3.9)	10.4 (4.2)
	10	6.6 (2.6)	34.8 (5.1)	7.8 (2.5)	39.4 (9.2)
	25	1.1 (0.8)	73.1 (6.8)	2.0 (1.6)	78.4 (5.7)
	50	0.8 (0.6)	82.1 (6.6)	0.5 (0.6)	85.2 (5.7)
Imazamox	0	21.9 (2.4)	—	20.6 (3.1)	
	2.5	23.2 (3.4)	3.3 (3.7)	21.1 (4.2)	2.9 (3.1)
	5	19.0 (4.3)	-1.9 (1.6)	20.7 (5.4)	0.1 (0.6)
	10	17.3 (3.6)	19.3 (7.5)	19.1 (3.5)	17.4 (6.7)
	25	11.3 (2.1)	37.8 (5.3)	12.4 (1.9)	44.1 (7.6)
	50	4.4 (3.3)	63.1 (4.7)	5.2 (1.9)	69.3 (7.2)
	100	2.8 (2.2)	73.1 (5.4)	1.8 (2.0)	77.8 (5.7)

¹Data for 4 hydrilla accessions resistant to fluridone were pooled because no differences were detected between populations.

²Data for 2 hydrilla accessions susceptible to fluridone were pooled because no differences were detected between populations.

Greenhouse trials

Initial hydrilla biomass at the time of treatment was 2.54 ± 0.22 g dry weight per container and final biomass of untreated controls was 7.48 ± 0.41 g dry weight per container. Cessation of growth was visually noted within 5 d following treatment of the mesocosm tanks at concentrations $\geq 10 \mu\text{g L}^{-1}$ for penoxsulam and bensulfuron and $\geq 25 \mu\text{g L}^{-1}$ for bispyribac. At this time, leaves at the apical tips were bent back and it was not possible to discern differences in the symptoms resulting from bensulfuron, bispyribac, or penoxsulam. In contrast, imazamox treatments resulted in the formation of numerous small thin shoots that developed over several weeks at concentrations of $25 \mu\text{g L}^{-1}$ and greater (Figure 1). The development of numerous small shoots near the same apex following exposure to imidazolinone herbicides has been described as a “witch’s broom” and has been noted in trials on other aquatic species (Wersal and Madsen 2007). While the ALS compounds are reported to be slow acting

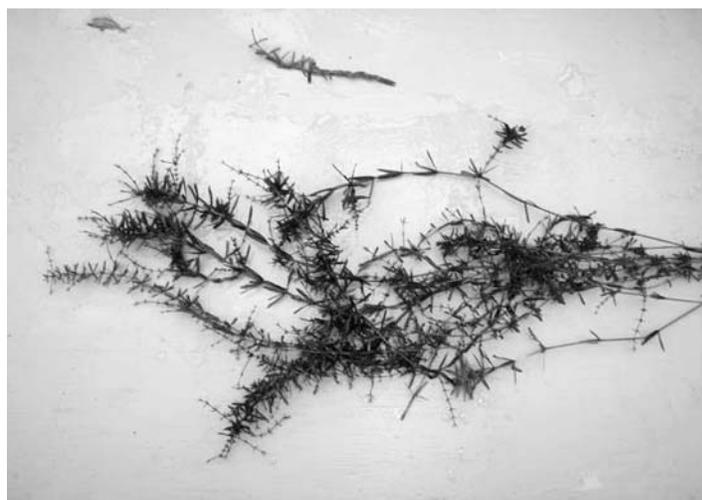


Figure 1. Numerous small axillary shoot meristems (witches broom) are noted following exposure of hydrilla to imazamox.

compared to contact herbicides (Nelson et al. 1993, Van and Vandiver 1994), impacts on new growth were observed within a week of application for all herbicides evaluated.

Following treatment with bensulfuron and penoxsulam at 10 µg L⁻¹ and greater and bispyribac at 25 µg L⁻¹ and greater, photosynthetic competence of the hydrilla apical shoots was reduced by >85% at 2 and 6 WAT (Table 2). Photosynthetic results for bensulfuron, bispyribac, and penoxsulam suggest that growth cessation occurred very quickly (within 2 weeks), and no differences were noted between 2 and 6 week readings at the higher treatment concentrations evaluated. Imazamox also reduced photosynthetic capacity of hydrilla, but it was not as active as the other 3 compounds and required concentrations of 25 and 100 µg L⁻¹ to reduce photosynthesis by 50% and more.

Penoxsulam and bensulfuron reduced biomass to below the initial levels at concentrations >5 µg L⁻¹ (Table 3). In comparison, bispyribac showed a reduced level of activity at 5 to 10 µg L⁻¹ with a significant increase in activity as concentrations were increased to 25 µg L⁻¹. Applications of bensulfuron, bispyribac, and penoxsulam at 25, 50, and 100 µg L⁻¹ reduced biomass below the initial levels of 2.5 g dry weight per container, and at 8 WAT there were no biomass differences detected between any of the herbicides at concentrations from 25 to 100 µg L⁻¹. Visual assessments of hydrilla during the course of the study support the biomass data. Despite biomass reductions compared to untreated controls, plant biomass increased over the initial biomass levels following the penoxsulam (5 µg L⁻¹), bensulfuron (5 µg L⁻¹), and bispyribac (5 and 10 µg L⁻¹) treatments (Table 3). These concentrations would be better described as providing strong growth regulating activity versus control of the hydrilla biomass. The decision to harvest at 8 weeks posttreatment was based on

control plants largely reaching the carrying capacity of the tanks between 5 and 7 weeks. Moreover, the slow activity of the ALS compounds in mesocosm trials can result in biomass persisting 16+ weeks (M. Netherland, pers. observ.). The trends for phytotoxicity versus growth-regulating concentrations were well established between 4 and 8 weeks posttreatment.

Following exposure to imazamox, hydrilla biomass increased compared to initial biomass at concentrations of 5, 10, and 25 µg L⁻¹ (Table 3). Biomass was reduced at concentrations >25 µg L⁻¹, and there were no visual or biomass differences noted between 50 and 100 µg L⁻¹ treatments. As noted with the laboratory assays, imazamox treatment resulted in a witches' broom effect typical of the imidazolinone herbicides (Figure 1). These trials definitely suggest that imazamox not only has a different rate spectrum compared to the other ALS herbicides but generally has a very different impact on hydrilla growth.

The laboratory assays predicted that fluridone-resistant and fluridone-susceptible accessions were likely to respond in a similar manner to the individual ALS herbicides, and the greenhouse studies supported this prediction. Furthermore, as determined in the laboratory assays, hydrilla was sensitive to all 4 of the ALS herbicides in the greenhouse trials, but the dose response relationships were different.

Overall, these results suggest populations of hydrilla collected from water bodies across Florida (fluridone resistant and fluridone susceptible) respond to individual ALS inhibitors in a similar manner. In addition to providing comparative efficacy data, these trials allow establishment of baseline response data that could potentially be used to determine if a given population is showing increased tolerance or resistance to ALS herbicides. Recent work on the potential for

TABLE 2. MEAN PHOTOSYNTHETIC RESPONSE ($\pm 95\%$ C.I.) OF HYDRILLA APICAL TIPS (1 CM) COMPARED TO UNTREATED CONTROLS OF HYDRILLA FOLLOWING 2 AND 6 WEEKS OF CONTINUOUS EXPOSURE TO THE ALS HERBICIDES, PENOXsulAM, BENSulfURON, BISpyRIBAC, AND IMAZAMOX IN GREENHOUSE MESOCOSM TRIALS.

Herbicide	Herbicide Concentration (µg L ⁻¹)	% Photosynthetic Reduction of apical tips at 2 WAT ($\pm 95\%$ C.I.) ¹	% Photosynthetic Reduction of apical tips at 6 WAT ($\pm 95\%$ C.I.) ¹
Penoxsulam	5	45 (7)	58 (9)
	10	85 (5)	89 (8)
	25	88 (6)	91 (6)
	100	93 (4)	95 (4)
Bensulfuron	5	57 (6)	66 (11)
	10	87 (4)	88 (12)
	25	85 (6)	90 (6)
	100	92 (5)	91 (4)
Bispyribac	5	20 (8)	26 (13)
	10	58 (11)	67 (9)
	25	87 (6)	94 (8)
	100	91 (8)	91 (6)
Imazamox	5	-6 (5)	4 (6)
	10	7 (4)	-11 (7)
	25	50 (9)	77 (5)
	100	76 (11)	83 (7)

¹Data for 6 hydrilla accessions (fluridone susceptible and resistant) were pooled because no differences were detected between populations.

TABLE 3. MEAN PERCENT BIOMASS REDUCTION ($\pm 95\%$ C.I.) OF HYDRILLA AT 56 D COMPARED TO UNTREATED CONTROLS FOR HYDRILLA FOLLOWING CONTINUOUS EXPOSURE TO THE ALS HERBICIDES, PENOX SULAM, BENSULFURON, BISPYRIBAC, AND IMAZAMOX IN GREENHOUSE MESOCOSM TRIALS.

Herbicide	Treatment Rate $\mu\text{g/L}$	% Biomass Reduction ($\pm 95\%$ C.I.) Compared to Initial Biomass ¹	% Biomass Reduction ($\pm 95\%$ C.I.) Compared to Untreated Controls at 8 WAT ¹
Penoxsulam	5	+185 (24)	38 (8)
	10	17 (11)	71 (4)
	25	50 (16)	83 (6)
	50	45 (19)	81 (6)
	100	57 (21)	85 (7)
Bensulfuron	5	+137 (31)	47 (10)
	10	9.4 (16)	69 (5)
	25	59 (11)	86 (4)
	50	57 (23)	85 (6)
	100	50 (16)	83 (5)
Bispyribac	5	+248 (24)	15 (8)
	10	+135 (21)	54 (7)
	25	38 (26)	79 (9)
	50	57 (19)	85 (6)
	100	50 (23)	83 (8)
Imazamox	5	+285 (19)	3 (6)
	10	+271 (34)	8 (11)
	25	+173 (26)	41 (9)
	50	9.4 (20)	69 (7)
	100	38 (23)	79 (8)

¹Data for a fluridone sensitive and a fluridone susceptible accession were pooled because no differences were detected between hydrilla populations.

fluridone cross-resistance shows the value of good baseline information for different accessions of hydrilla to different ALS herbicides (Puri et al. 2009). This baseline information will be especially important as use of ALS herbicides increases and the potential for resistance development increases (Richardson 2008).

These dose-response data can provide important information regarding potential use patterns for these herbicides. While penoxsulam, bensulfuron, and bispyribac treatments resulted in a fairly similar rate response and symptoms, imazamox was quite different both in terms of the hydrilla rate response and the visual symptoms. The slight differences in activity noted between penoxsulam, bensulfuron, and bispyribac do not suggest superiority of one product over another; other factors such as label use restrictions, nontarget plant selectivity, and economics will play a key role in management decisions.

Note that these laboratory treatments with imazamox resulted in continuous exposure to the target treatment concentration. Evidence from field evaluations suggests that photolytic degradation of imazamox is much faster than with penoxsulam, and therefore maintaining long-term exposures to phytotoxic concentrations may not be practical for imazamox. All research and field data suggest that use patterns for penoxsulam, bensulfuron, and bispyribac will require sustained exposures similar to fluridone use patterns (Netherland et al. 1993, Netherland and Getsinger 1995). Recent research with imazamox has focused on short-term exposures, and preliminary information suggests that unlike the other ALS herbicides, a short-term exposure (4 to 14 d) may result in ALS symptoms being manifested for an

extended period of 2 to 4 months following treatment (unpublished data). This observation is in contrast to other trials conducted with herbicides such as fluridone and bensulfuron that result in a rapid recovery of the hydrilla if the plants are removed from the treatment at 30 or 60 d (Nelson et al. 1993, Netherland et al. 1993). Based on these data as well as field observations, use patterns for imazamox are likely to differ from those of penoxsulam, bensulfuron, and bispyribac.

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