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Effects of Three ALS-inhibitors on Five Emergent Native Plant Species in Florida

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ABSTRACT

Three acetolactate synthase (ALS) inhibiting herbicides are currently being evaluated for the control of hydrilla (*Hydrilla verticillata* L.f. Royle) and other submersed aquatic weeds. The impacts of long-term aqueous exposures of these herbicides on non-target native emergent plant species are unknown. Therefore, trials were conducted to determine the effect of aqueous applications of the ALS-inhibitors penoxsulam [2-(2,2-difluoroethoxy)-N-(5,8-dimethoxy [1,2,4]triazolo[1,5-c] pyrimidin-2-yl)-6-(trifluoromethyl) benzenesulfonamide], imazamox [2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid], and bispyribac sodium [2,6-bis(4,6-dimethoxypyrimidin-2-yloxy)benzoic acid] on established soft-stem bulrush (*Scirpus validus* Vahl.), Egyptian panicgrass (*Paspalidium geminatum* (Forssk.) Stapf), maidencane (*Panicum hemitomon* Schult.), pickerelweed (*Pontederia cordata* L.) and sagittaria (*Sagittaria lancifolia* L.). Plants were exposed to initial aqueous concentrations of each herbicide at 25, 75, 150 and 300 µg/L for 66 d. At harvest, shoot and root biomass of untreated controls by species increased from 100 to >1500%. While both grass species increased in biomass following exposure to ALS-inhibitors at rates up to 300 µg/L, the shoot biomass of panicgrass was inhibited by bispyribac and penoxsulam at concentrations of 75 µg/L and higher when compared to untreated controls. Maidencane was generally more tolerant than panicgrass to all three herbicides. Bulrush shoot growth was inhibited at all concentrations, with differential responses to the 3 herbicides. Pickerelweed shoot growth was reduced below pre-treatment weights at 25 µg/L and higher following treatment with penoxsulam and bispyribac. Sagittaria was more sensitive to penoxsulam at rates of 25 and 75ug/L compared to imazamox and bispyribac. There was inhibition of root growth for all species, but whether inhibition resulted from direct toxicity to roots or as an indirect result of shoot inhibition was not determined. The spectrum of herbicidal activity for all three herbicides was similar (in order of increasing tolerance: pickerelweed<sagittaria<bulrush<panicgrass<maidencane). Use patterns for control of submersed plants need to be established in order to determine the likelihood of impacting non-target emergent plants.

Key words: aquatic herbicides, penoxsulam, imazamox, bispyribac, chemical control, selectivity.

INTRODUCTION

The need to discover and develop new herbicides for use in Florida's hydrilla (*Hydrilla verticillata* (L.f.) Royle) management program has increased in the past 3 to 5 years as a result of hydrilla developing resistance to fluridone{1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl-4 (1H)-pyridinone} (Arias et al. 2005). There are currently three potential new herbicides being evaluated for aquatic use under Experimental Use Permits (EUPs) issued by the U.S. Environmental Protection Agency. Penoxsulam[4], imazamox[5], and bispyribac sodium[6] are classified into three herbicide families (triazolopyrimidines, imidazolinones, and pyrimidinylthiobenzoates, respectively), but all of these families have a similar mechanism of action, acetolactate synthase inhibition (ALS inhibitors). The ALS inhibitor bensulfuron-methyl was previously considered for hydrilla control (Haller et al. 1992, Langeland and Laroche 1992, Van and Vandiver 1992). Bensulfuron-methyl was effective for hydrilla control at sustained concentrations ranging from 10 to 50 µg/L, and our studies have indicated that the three ALS compounds being evaluated are active on hydrilla in the range of 5 to 100 µg/L (unpublished data).

The ALS inhibitors have been identified as candidates for aquatic use due to toxicology profiles that will allow immediate consumptive use of the water (potable uses, fishing, or swimming) and their activity on exotic plants like hydrilla at relatively low concentrations. Similar to fluridone, the ALS inhibitors will likely require long exposure periods (60 to 120 days) and will slowly reduce hydrilla biomass. Aquatic plant managers want to know the potential response of desirable native emergent vegetation.

Despite sharing common modes of action, ALS herbicides are known to have quite different effects on plant species. The triazolopyrimidines herbicides (e.g., penoxsulam) are generally very effective for control of broadleaf plants (dicots and broadleaf monocots) and sedges, while the imidazolinones (e.g., imazamox) and pyrimidinylthio-benzoates (e.g., bispyribac) in general control a broader plant spectrum. Selectivity in all families appears to be rate dependent. Slight

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Table 1. Height and weight of established plants per pot (17 cm diameter) at the time of herbicide application (initial ± standard error), and for untreated control plants 66 d after herbicide treatment (final ± standard error).

Species	Initial			Final			
	Biomass (g)		Plant height (cm)	Biomass (g)		% increase in biomass	
	Shoot	Root		Shoot	Root	Shoot	Root
Bulrush	6.8 ± 1.8	4.4 ± 0.9	118 ± 7	88 ± 11	67 ± 14	1194	1423
Panicgrass	6.3 ± 0.5	2.7 ± 0.2	98 ± 2	103 ± 14	38 ± 14	1535	1307
Maidencane	2.3 ± 0.5	1.7 ± 0.5	56 ± 2	42 ± 9	8 ± 2	1726	371
Pickerelweed	2.1 ± 0.5	3.0 ± 0.9	54 ± 2	12 ± 5	6 ± 2	471	100
Sagittaria	2.4 ± 0.5	2.0 ± 0.7	42 ± 2	21 ± 7	14 ± 5	775	600

changes in the molecular structure of ALS-inhibiting herbicides greatly affect the potency and weed spectrum (Ladner 1991, Ren et al. 2000). This, along with low use rates and low toxicity to mammals and fauna has lead to registration of more than 50 ALS inhibiting herbicides for weed control in a variety of terrestrial weed management programs (Heap 2005). The ALS inhibitor metsulfuron-methyl was recently evaluated to support a 24C-Special Local Need for use in Florida lake restoration projects following drawdown, and small-scale trials indicated that while metsulfuron-methyl was very active on the broadleaf monocots, there was limited activity on the aquatic grass species tested (Chiconela et al. 2004).

ALS compounds inhibit the production of the amino acids valine, leucine, and isoleucine in plants by binding to the ALS enzyme (Anderson 1996, Tranel and Wright 2002). This inhibition of amino acid production results in decreased protein and enzyme synthesis resulting in a rapid cessation of growth. New plant growth is more rapidly affected than older plant tissue due to lack of new protein production. ALS herbicides are slow-acting due to the delayed activity on mature plant tissue and factors such as adverse growing conditions, e.g. cool weather, may result in a lack of weed control due to reduced plant growth.

Hydrilla is sensitive to some ALS-inhibitors (Rattray et al. 2003), yet non-target emergent aquatic vegetation may also be affected by aqueous concentrations of these compounds. For example, many emergent aquatic species are targeted in weed control operations in rice production with foliar applications of ALS-inhibiting herbicides (Carey et al. 2000, Webster and Masson 2001, Jabusch and Tjeerdema 2005, Shiraishi 2005, Vasilakoglou and Dhima 2005, Kelly et al. 2006). Furthermore, foliar applications of metsulfuron-methyl as low as 17.5 g/ha reduced pickerelweed and sagittaria biomass by >90% (Chiconela et al. 2004). Maintaining a diverse community of native aquatic plants is a priority in public waters in the state of Florida. Therefore, herbicides that negatively impact a broad spectrum of native species would not likely be considered for hydrilla management. The effects of these new herbicides on emergent plant species from aqueous exposure are unknown. The objective of this research was to evaluate these herbicides on five important Florida native littoral species. All five are members of the class Monocotyledonae. Bulrush belongs to the family Cyperaceae; panicgrass and maidencane belong to the Poaceae, pickerelweed belongs to the Pontederiaceae, and sagittaria belongs to the Alismataceae.

MATERIALS AND METHODS

Bulrush, panicgrass, maidencane, pickerelweed, and sagittaria were established in 3-L (17.1-cm diameter × 13.3 cm deep) plastic pots filled with a mixture of 1/3 commercial potting soil and 2/3 coarse builder's sand amended with Osmocote® fertilizer (1 g/Kg soil of 15:9:12). One pot of each species was placed into 90-L (60-cm diameter × 60 cm deep) plastic mesocosms and allowed to grow for four weeks prior to treatment. Mesocosms were placed under a shade house (30% shade) covered with plastic to prevent rainwater from diluting the treatments and to reduce photolytic degradation of the herbicides. Water was maintained at 25.4 ± 5.1 cm depth.

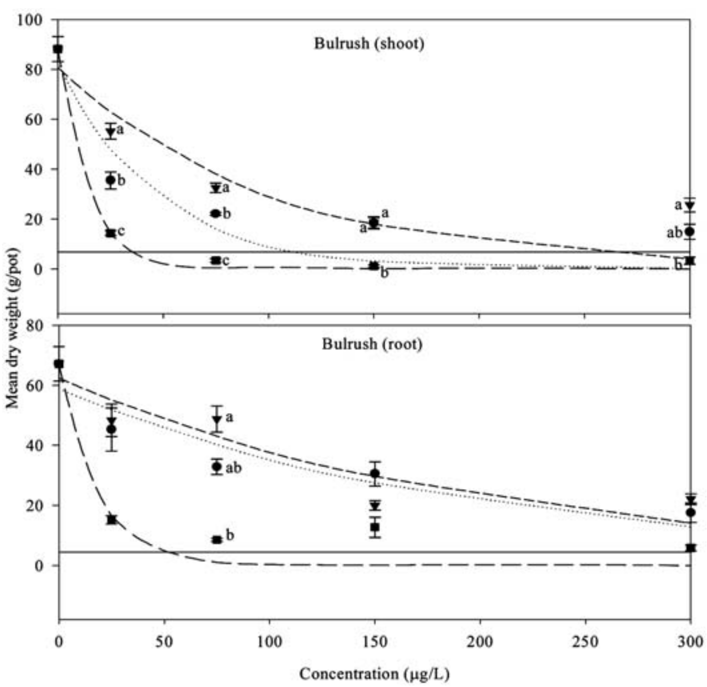

Figure 1. Regression of bulrush shoot (A) and root (B) biomass (n = 5) at 66 d after treatment versus concentration of three experimental aquatic herbicides (—— initial biomass, ··•·· bispyribac, – – ▼ – – imazamox, — — ■ — — penoxsulam). Means (± standard error) with different letters are significantly different for each rate according to Fishers Protected LSD (d.f. = 4; p = 0.05). Shoot: bispyribac: $y = 82.7097^{-0.0219x}$, $r^2 = 0.85$; imazamox: $y = 80.7901^{-0.01x}$, $r^2 = 0.88$; penoxsulam: $y = 88.1067^{-0.0719x}$, $r^2 = 0.93$. Root: bispyribac: $y = 58.813^{-0.00507x}$, $r^2 = 0.77$; imazamox: $y = 62.3803^{-0.00496x}$, $r^2 = 0.83$; penoxsulam: $y = 66.9006^{-0.0556x}$, $r^2 = 0.80$.

Table 2. Calculated EC_{50} (µg/L) for three ALS-inhibitors (bispyribac, imazamox, and penoxsulam) from non-linear regression for root and shoot biomass.

Species	Bispyribac		Imazamox[a]		Penoxsulam	
	Root	Shoot	Root	Shoot	Root	Shoot
Bulrush	137	32	140	69	12	10
Panicgrass	80	89	na	na	64	134
Maidencane	162	160	377	185	630	3067
Pickerelweed	14	8	117	12	51	8
Sagittaria	73	96	278	105	23	9

[a]na = not applicable due to positive regression slope.

Mesocosms containing each of the five species were treated with aqueous concentrations of penoxsulam, imazamox, or bispribac on 15 May 2005. Treatments consisted of each herbicide at concentrations of 25, 75, 150, and 300 µg/L, and untreated controls. Concentrations tested were up to 12 times higher than those observed to be effective on hydrilla (Haller et al. 1992) in order to determine concentrations causing a 50% inhibition of shoot and root growth (EC_{50}) and to compare relative toxicities. Treatments were assigned in a completely randomized design with five replications per treatment.

Plants were harvested on 20 July 2005 (66 days after treatment), and shoots and roots were separated at harvest to determine the effects on both above and below ground biomass production. Below ground biomass was harvested as ALS-inhibitors are known to inhibit root production (Saari et al. 1994, Ellis et al. 2004). Harvested plant material was placed into a forced air oven for several days at 80C, and dry weights determined. Data were analyzed by ANOVA and subjected to non-linear regression analysis and EC_{50}'s determined (Koschnick et al. 2005). Means within each treatment concentration were separated using Fishers Protected LSD ($p < 0.05$).

RESULTS AND DISCUSSION

Shoot biomass of untreated control plants increased by 471 to 1726% over the 66 d experiment, and root biomass increased from 100 to 1423% (Table 1). These rates of growth likely enhanced activity of the ALS-inhibiting herbicides in this study.

Bulrush

Penoxsulam caused the greatest effect on bulrush shoot production compared to imazamox and bispyribac at concentrations up to 150 µg/L, and bispyribac was more phytotoxic than imazamox at 25 and 75 µg/L (Figure 1). Increasing concentrations of all three herbicides resulted in limited growth based on root and shoot dry weight compared to initial biomass levels (Figure 1). While the herbicide exposures inhibited growth as compared to the >1000% increase in growth of untreated controls, they did not kill the plants by 66 DAT. Plants exposed to the ALS-compounds were shorter in stature, and showed little visual injury except at the highest concentrations. At concentrations of 150 and 300 µg/L, visual symptoms were limited to yellowing of bulrush stems and some necrosis, with the exception of penoxsulam at 300 µg/L, which reduced shoot weight of bulrush to 0 in 4 of the 5 replications. Penoxsulam also had the strongest impact on root production (Table 2).

Maidencane and Panicgrass

The grasses, maidencane and panicgrass, were generally more tolerant than the broadleaf monocots to the three ALS-inhibitors (Figures 2 and 3). Growth of panicgrass was inhibited by penoxsulam and bispyribac at concentrations of 75 µg/L and higher, but the average shoot biomass was greater

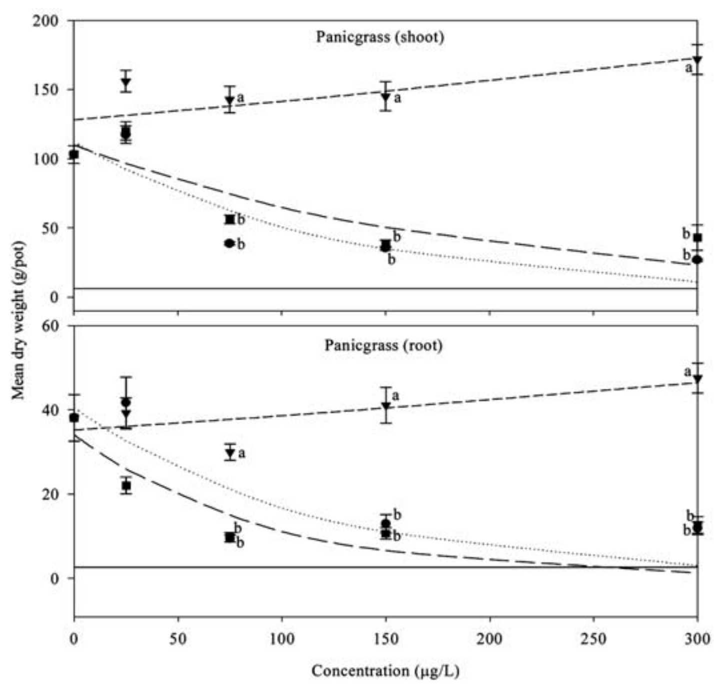

Figure 2. Regression of panicgrass shoot (A) and root (B) biomass (n = 5) at 66 d after treatment versus concentration of three experimental aquatic herbicides (—— initial biomass, ··•·· bispyribac, – – ▼ – – imazamox, — — ■ — — penoxsulam). Means (± standard error) with different letters are significantly different for each rate according to Fishers Protected LSD (d.f. = 4; p = 0.05). Shoot: bispyribac: $y = 112.1^{-0.00777x}$, $r^2 = 0.89$; imazamox: $y = 128.1^{0.001x}$, $r^2 = 0.92$; penoxsulam: $y = 109.9^{-0.00519x}$, $r^2 = 0.85$. Root: bispyribac: $y = 40.474^{-0.00865x}$, $r^2 = 0.64$; imazamox: $y = 35.2295^{0.00092x}$, $r^2 = 0.83$; penoxsulam: $y = 34.0769^{-0.0109x}$, $r^2 = 0.67$.

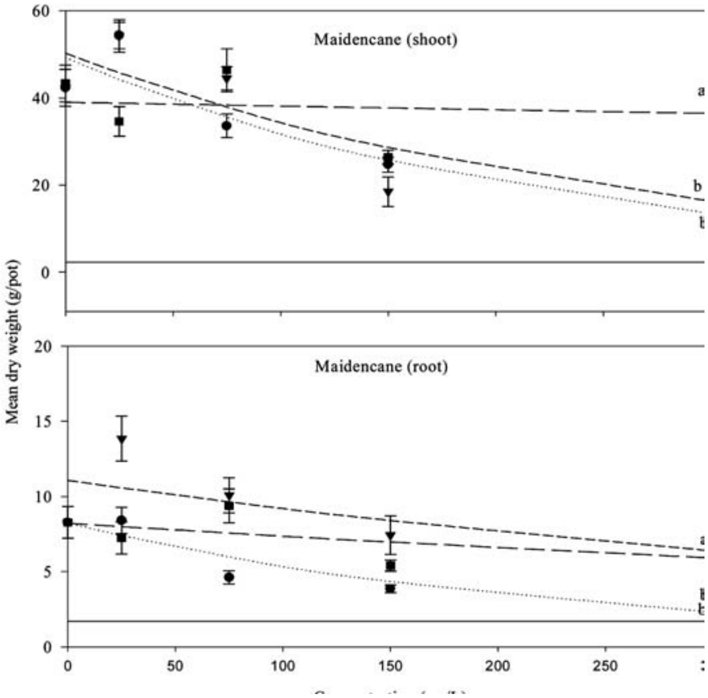

Figure 3. Regression of maidencane shoot (A) and root (B) biomass (n = 5) at 66 d after treatment versus concentration of three experimental aquatic herbicides (—— initial biomass, ··•·· bispyribac, – – ▼ – – imazamox, — — ■ — — penoxsulam). Means (± standard error) with different letters are significantly different for each rate according to Fishers Protected LSD (d.f = 4; p = 0.05). Shoot: bispyribac: $y = 49.2795^{-0.00433x}$, $r^2 = 0.87$; imazamox: $y=50.238^{-0.00375x}$, $r^2 = 0.84$; penoxsulam: $y = 38.9896^{-0.000226x}$, $r^2 = 0.82$. Root: bispyribac: $y = 8.2311^{-0.00427x}$, $r^2 = 0.79$; imazamox: $y = 11.0598^{-0.00184x}$, $r^2 = 0.74$; penoxsulam: $y = 8.2043^{-0.0011x}$, $r^2 = 0.77$.

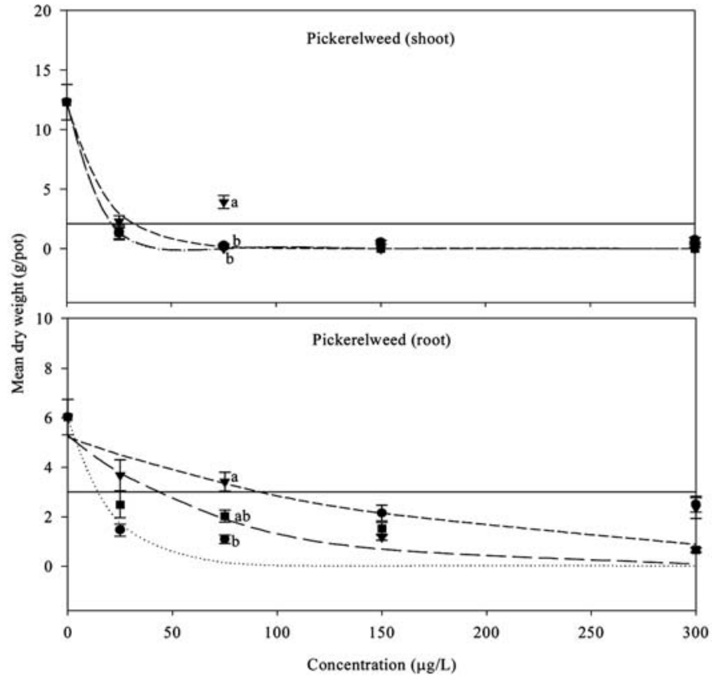

Figure 4. Regression of pickerelweed shoot (A) and root (B) biomass (n = 5) at 66 d after treatment versus concentration of 3 experimental aquatic herbicides (—— initial biomass, ··•·· bispyribac, – – ▼ – – imazamox, — — ■ — — penoxsulam). Means (± standard error) with different letters are significantly different for each rate according to Fishers Protected LSD (d.f. = 4; p = 0.05). Shoot: bispyribac: $y = 12.3065^{-0.0892x}$, $r^2 = 0.75$; imazamox: $y = 12.199^{-0.0565x}$, $r^2=0.70$; penoxsulam: $y = 12.3066^{-0.0870x}$, $r^2=0.75$. Root: bispyribac: $y = 5.9731^{-0.0497x}$, $r^2 = 0.58$; imazamox: $y = 5.2165^{-0.00593x}$, $r = 0.69$; penoxsulam: $y = 5.2878^{-0.0136x}$, $r^2 = 0.68$.

than 25 g/pot. Imazamox caused no reduction in panicgrass root or shoot biomass up to concentrations of 300 µg/L (Figure 2). Leaf tips showed signs of curling with crinkling and some necrosis following exposure to 150 and 300 µg/L of bispyribac and penoxsulam, but biomass increased and was greater than pretreatment levels at 66 DAT.

Maidencane was the more tolerant of the two grasses to all the herbicides based on EC_{50} values (Table 2.) Concentrations as high as 150 µg/L caused slight reductions in root and shoot biomass of maidencane compared to controls (Figure 3).

Pickerelweed and Sagittaria

Pickerelweed and sagittaria were generally the most susceptible of the species studied to imazamox, bispyribac, and penoxsulam (Figure 4 and 5). All three ALS-inhibitors caused similar reductions in pickerelweed root and shoot growth at 25, 150, and 300 µg/L, and pickerelweed was the most susceptible species tested to all three herbicides (Table 2). Pickerelweed was controlled below pre-treatment biomass weights by all herbicide treatments except imazamox at 25 and 75 µg/L (Figure 4). Sagittaria was generally more tolerant than pickerelweed to the ALS-inhibitors, except for penoxsulam (Table 2). Bispyribac and imazamox caused similar reductions in biomass with increasing concentrations (Figure 5).

The ALS-inhibitors tested inhibit hydrilla growth at 25 µg/L or less (non-published data). Assuming each of these herbicides was applied at equal concentrations for hydrilla control (i.e., 25 µg/L), the spectrum of activity of each of the herbicides would be similar. Pickerelweed would be the most sensitive of the five plants evaluated to all of these herbicides. Pickerelweed was the only species in which the lowest treatment rate reduced biomass to levels at or below pre-treatment values for all three ALS herbicides. Nevertheless, concentrations required to cause death of pickerelweed need to be further determined under field conditions. The data suggests the other species tested would be fairly tolerant to single applications of these three ALS-inhibitors at concentrations in the range of 25 µg/L if applied for hydrilla control.

These data allow a comparison of relative toxicities of the three ALS-herbicides to native emergent plants following a single treatment. Ultimate use patterns for hydrilla control will depend on herbicide toxicity to hydrilla (potency) and half-life or exposure in the field. Therefore, as expected, concentration and exposure times will have an impact on selectivity and will likely be different for the various ALS herbicides. Additionally, the half-life will impact use patterns of each herbicide, and multiple treatments may be necessary to sustain target concentrations to control hydrilla. Recent field sampling suggests that degradation rates can vary significantly between the three ALS inhibitors and residues following spring and summer treatments tend to degrade much quick-

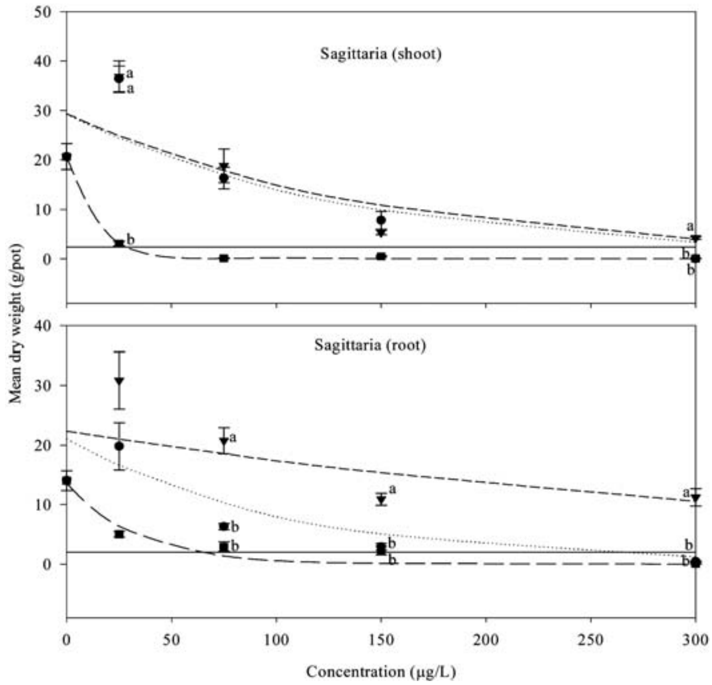

Figure 5. Regression of sagittaria shoot (A) and root (B) biomass (n = 5) at 66 d after treatment versus concentration of 3 experimental aquatic herbicides (—— initial biomass, ··•·· bispyribac, – – ▼ – – imazamox, — — ■ — — penoxsulam). Means (± standard error) with different letters are significantly different for each rate according to Fishers Protected LSD (d.f. = 4; p = 0.05). Shoot: bispyribac: $y = 29.2277^{-0.00722x}$, $r^2 = 0.73$; imazamox: $y = 29.3616^{-0.00663x}$, $r^2 = 0.70$; penoxsulam: $y = 20.6677^{-0.0757x}$, $r^2 = 0.75$. Root: bispyribac: $y = 21.0571^{-0.00949x}$, $r^2 = 0.59$; imazamox: $y = 22.3469^{-0.00249x}$, $r = 0.65$; penoxsulam: $y = 13.611^{-0.0305x}$, $r^2 = 0.71$.

er than fall and winter applications. Regardless, these results suggest that the susceptibility of the five species tested in order of increasing tolerance is: pickerelweed, sagittaria, bulrush, knotgrass and maidencane. Additional studies should be conducted to evaluate the impact of long-term exposure to sustained concentrations that would result from sequential application to control hydrilla after use rates are developed for each compound.

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