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Can low rates of imazapyr or glyphosate improve graminicide activity on torpedograss?

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ABSTRACT

Invasive grass control remains one of the greatest challenges in aquatic plant management. High rates of glyphosate or imazapyr are commonly used but lack the selectivity desired by many aquatic managers. Recent progress with graminicides has demonstrated marked efficacy on torpedograss and excellent selectivity for many nontarget aquatic plants. However, torpedograss control has yet to be maximized with any graminicides, and regrowth can be considerable. To this end, tank mixes of reduced rates of glyphosate or imazapyr could be useful to both increase efficacy and maintain selectivity. Unfortunately, graminicides also have a long history of antagonism with several other classes of herbicides including acetolactate synthase inhibitors. The potential interactions between graminicides and glyphosate and imazapyr are unknown in aquatic settings. To address these issues, greenhouse studies were conducted to evaluate the performance of sethoxydim applied at 0.53 kg ha^{-1} and fluazifop-p-butyl applied at 0.42 kg ha^{-1} when tank mixed with glyphosate (applied at 0.84 or 1.68 kg ae ha^{-1}) or imazapyr (applied at 0.07 or 0.14 kg ai ha^{-1}). At 60 d after treatment, we found no benefit to tank mixing glyphosate or imazapyr with either graminicide because biomass reductions were not improved compared to the graminicides alone. No antagonism was found between the graminicides and imazapyr, and limited antagonism was found between glyphosate and both graminicides. This antagonism was observed as a reduced impact of the tank mix on belowground biomass when compared to the expected reduction of the tank mix. Overall, these studies indicate that reduced rates of the two most commonly used herbicides for aquatic invasive grass control do not improve graminicide activity on torpedograss.

Key words: fluazifop-p-butyl, herbicide antagonism, invasive aquatic grass, *Panicum repens* L., sethoxydim.

INTRODUCTION

Graminicides have been widely used in agricultural and forestry weed management systems over the last 40 yr, providing selective control of many weedy grasses (Clay et al. 2006, Kukorelli et al. 2013). They occur in at least three chemical families, which include aryloxyphenoxypropionates, cyclohexanediones, and phenylpyrazolins (WSSA 2014). Graminicides are foliar absorbed and translocate to meristematic regions, where they target the acetyl CoA carboxylase enzyme (ACCase), which results in inhibition of fatty acid biosynthesis. Dicotyledonous species are naturally resistant to the ACCase inhibitors because of an insensitive ACCase enzyme (Burton et al. 1989).

The selectivity of graminicides has been well established in terrestrial systems (Kukorelli et al 2013) and has recently been verified on several nongraminoid monocotyledonous aquatic plants. In mesocosm studies, Enloe and Netherland (2017) found foliar applications of sethoxydim, fluazifop-p-butyl, and clethodim did not reduce growth of California bulrush (*Schoenoplectus californicus* (C.A. Mey.) Palla), knotted spikerush (*Eleocharis interstincta* (Vahl) Roem. & Schult.), Gulf Coast spikerush (*Eleocharis cellulosa* Torr.), broadleaf cattail (*Typha latifolia* L.), pickerelweed (*Pontederia cordata* L.), or broadleaf arrowhead (*Sagittaria latifolia* Willd.). They also found that the three graminicides reduced torpedograss (*Panicum repens* L.) belowground biomass by 60 to 80%. This led to sethoxydim and fluazifop-p-butyl receiving Florida experimental use permits (EUPs) in 2015 and 2016, respectively, for emergent aquatic grass control. These and other data collected under the sethoxydim EUP resulted in a 24(c) label for TIGR® herbicide in Florida in 2017 (Anonymous 2017). Although optimal use patterns and rates have yet to fully be defined, early results from recent studies have shown good initial activity for airboat and aerial torpedograss treatments with some regrowth occurring either within or in the following growing season (S. F. Enloe, unpublished data).

Beyond direct efficacy, within the realm of aquatics additional questions need to be addressed regarding graminicides. In terrestrial systems, graminicide activity can be negatively influenced (i.e., antagonism) when tank mixed with several other classes of herbicides. Numerous studies have documented either true antagonism or reduced control with graminicides and a range of other herbicides including certain ALS inhibitors, photosynthetic inhibitors, auxin type, and PPO inhibitors (Young et al. 1996). This issue was detected very early in graminicide development in row crop agriculture (Campbell and Penner 1982) and has since remained. Herbicide antagonism has not been widely studied for emergent aquatic plants but has been documented for free-floating plants. Wersel and Madsen (2010) found antagonism between penoxsulam and diquat when applied to common salvinia (*Salvinia minima* Baker).

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From agricultural studies, Snipes and Allen (1996) examined the interactions of sethoxdim and fluazifop with pyrithiobac for johnsongrass (*Sorghum halepense* (L.) Pers.) control in cotton. Pyrithiobac is an ALS inhibitor with some grass activity. They found significant antagonism between both graminicides and this ALS herbicide. Johnsongrass control was reduced by 20, 21, and 52% over three study years when sethoxydim was applied with pyrithiobac compared to sethoxydim alone. Johnsongrass control was reduced by 13, 23, and 41% over 3 yr when fluazifop was tank mixed with pyrithiobac compared to fluazifop alone.

Holshouser and Coble (1990) found reduced control (antagonism) of fall panicum (*Panicum dichotomiflorum* Michx.), large crabgrass (*Digitaria sanguinalis* (L.) Scop.), and goosegrass (*Eleusine indica* (L.) Gaertn.) when sethoxydim was applied with either imazaquin or chlorimuron. Grichar et al. (2002) found clethodim activity on broadleaf signalgrass (*Urochloa platyphylla* (Nash) R.D. Webster) was significantly reduced in tank mixes with imazethapyr, imazapic, and several other herbicides. Barnes and Oliver (2004) found antagonism of aryloxyphenoxypropionate graminicides when mixed with chloransulam, but not with the cyclohexanediones graminicides for annual grass control. These and other studies provide clear indication that this issue needs to be examined for aquatic grass control, especially where target grasses are almost exclusively perennial and much more robust than annual grasses commonly targeted with graminicides.

Current management strategies for control of invasive aquatic grasses include glyphosate and imazapyr applied alone or in combination (Smith et al. 1993, Hanlon and Langeland 2000, Netherland and Lancaster 2014). Glyphosate is generally applied at rates of 3.36 to 4.48 kg ai ha^{-1} or higher while imazapyr is applied at rates of 0.56 to 1.12 kg ai ha^{-1}. These rates are largely nonselective. However, using these herbicides at much lower rates may increase selectivity at the cost of effective weed control. This inevitably leads to the question of tank mixing low rates of glyphosate and imazapyr with graminicides to potentially increase invasive grass control while still minimizing nontarget injury. In an attempt to achieve selectivity, Gettys and Sutton (2004) found reduced concentrations of glyphosate still provided some activity on torpedograss, which warrants additional reduced glyphosate rate work with graminicides. Previous research on glyphosate and sethoxydim tank mixes has yielded mixed results. Chuah et al. (2004) found some evidence of antagonism between the two herbicides on glyphosate resistant goosegrass. However, Jhala et al. (2013) found no evidence of antagonism on other annual grasses. Within the realm of perennial grasses, little is known regarding potential antagonism between glyphosate and graminicides. Although they have different modes of action, both are phloem mobile and readily translocate to active meristems. However, it is unclear how they may interact as tank mix partners for aquatic grass control.

Based upon known antagonism between graminicides and many other herbicides, and our interest in improving invasive aquatic grass control with more selective treatments, our objective was to evaluate the performance of two graminicides, fluazifop-p-butyl and sethoxydim, with low rates of glyphosate and imazapyr.

MATERIALS AND METHODS

Studies were conducted in 2016 at the University of Florida Center for Aquatic and Invasive Plants in Gainesville, FL. Torpedograss, which is one of the most invasive aquatic grasses in Florida, was selected as the species of study. Plants were initially established in April 2016 by planting 10 cm shoot cuttings into 3.78-L plastic pots filled with builder's sand and 10 g of Osmocote Plus[1] (15:9:12). Pots were placed in 20 concrete mixing tubs ($36 \times 90 \times 20$ cm) with 10 pots per tub. The water level was maintained at a depth of 15 cm throughout the duration of the study, approximating saturated conditions. Approximately 8 wk after establishment, 10 replicate pots were harvested for baseline measurements, oven dried to a constant weight at 65 C, and oven dry weights recorded. Following the baseline harvest, pots were moved just outside the greenhouse where herbicides were applied with a four-nozzle CO_2-pressurized backpack sprayer at 187 l ha^{-1}. Each herbicide treatment was applied to 10 replicate pots, which were allowed to dry before being carefully dipped in clean water to remove any herbicide residue from the outside of the pots. All treated torpedograss foliage was gently held above the water during this process to prevent any herbicide wash off before being randomly placed back into the 20 tubs.

Herbicide treatments for the first and second experimental runs were applied on 10 and 24 June 2016, respectively. Herbicide treatments included two graminicides (fluazifop-p-butyl[2] [0.42 kg ai ha^{-1}] and sethoxydim[3] [0.53 kg ai ha^{-1}]), each applied with and without glyphosate at two rates[4] (0.84 or 1.68 kg ae ha^{-1}) or imazapyr at two rates[5] (0.07 or 0.14 kg ai ha^{-1})]. Additionally, two commercial standard treatments were applied and included glyphosate at 3.36 kg ha^{-1}, and imazapyr (0.56 kg ai ha^{-1}). A nonionic spray adjuvant[6] was added to all treatments at 32 oz/A to 2.3 L ha^{-1}. Plant biomass was harvested at 60 d after treatment and oven dried to a constant weight at 65 C. Live biomass was separated into above- (shoot) and below-ground (root + rhizome) components.

Statistical analysis

We held two goals with respect to the analysis. The first was to compare efficacy for treatments consisting of a graminicide (fluazifop-p-butyl or sethoxydim) alone or in combination with a tank mix partner (glyphosate or imazapyr). Tank mixes using the two graminicides were not compared directly in this short-term trial because results may not relate to long-term outcomes dependent on factors such as speed of activity. The second goal was to specifically test if tank mixes were antagonistic.

To accomplish these objectives, a generalized linear mixed model approach assuming a gamma distribution and a log link function was performed using SAS PROC GLIMMIX to accommodate the finding that variance increased proportionally with plant weight (Schabenberger and Pierce 2002). This type of model allowed differences between a treatment and the untreated check to be expressed in terms of percentage reduction (see the Appendix). The analysis of variance (ANOVA) of 17 treatments was partitioned using four sets of contrast

Table 1. Pretreatment and 60 days after treatment (DAT) untreated sample averages (± standard error) by run ($n = 20$).

Sample Timing	Experimental Run	Shoot wt[1] (g)	Root + Rhizome wt (g)
Pretreatment	1	9.6 (0.6) c	9.3 (0.5) b
	2	9.3 (0.6) c	8.2 (0.5) b
60 DAT	1	23.4 (0.9) b	35.2 (1.7) a
	2	33.5 (1.9) a	38.1 (3.0) a

[1]Means followed by the same letter are not significantly different at the 5% level (LSD) based on a one-way ANOVA.

statements to test the effects of each base graminicide (sethoxydim or fluazifop-p-butyl) with each tank mix partner (glyphosate or imazapyr). Means for each of these four sets of treatments were compared at the $P = 0.05$ level of significance within each set only if the overall test of treatment differences for that set was significant using Fisher's LSD approach. Treatment means compared in each set included the base herbicide (glyphosate or imazapyr) at three rates, the graminicide at one rate, and this graminicide rate tank mixed with two rates of the base herbicide.

Tests of antagonism were performed to compare expected and actual tank mix responses as defined by Colby (1967). Blouin et al. (2004) defined the contrast (difference) of interest as the mean response to herbicide in mixture minus the product of the mean response to each herbicide used alone (accounting for the use of percentage response in their formula). Tests of antagonism were performed for two rates of the base herbicide for each of the four sets of treatments.

RESULTS AND DISCUSSION

Pretreatment shoot length averaged 77 ± 0.6 cm among baseline harvested experimental units (data not shown). Pre- and posttreatment biomass harvests from the untreated controls indicated vigorous growth during the course of the study. Shoot weights doubled or tripled from the pretreatment to the 60 days after treatment (DAT) harvests. Belowground biomass (roots + rhizomes) nearly quadrupled between the pretreatment and 60 DAT harvests (Table 1).

Table 2. Percentage reduction in torpedograss biomass compared to the untreated control for glyphosate, sethoxydim, and tank mixes. Treatment means were compared at $P = 0.05$ only if overall test of treatment differences was significant (Fisher's LSD = 0.05). Expected percentage controls with asterisks indicate a significant (LSD = 0.05) test of antagonism comparing the actual and expected tank mix percentage control.

Treatment	Rate ($kg\ ha^{-1}$)	Shoot	Root	Total
		% Reduction		
Glyphosate	0.84	73 bc	71 bc	71 bc
	1.68	90 abc	78 ab	82 ab
	3.36	96 a	80 ab	88 a
Sethoxydim	0.53	68 c	57 c	62 c
Glyphosate + sethoxydim	0.84 + 0.53	83 bc	77 ab	80 ab
	1.68 + 0.53	92 ab	76 ab	83 ab
Expected control[1]				
Glyphosate + sethoxydim	0.84 + 0.53	91	87*	89
	1.68 + 0.53	97	90*	93*

[1]Expected control is a theoretical estimate calculated using the level of control achieved by each herbicide used alone.

Table 3. Percentage reduction in torpedograss biomass compared to the untreated control for glyphosate, fluazifop-p-butyl, and tank mixes. Treatment means compared at $P = 0.05$ only if overall test of treatment differences was significant (Fisher's LSD = 0.05). Expected percentage control with asterisk indicates a significant (LSD = 0.05) test of antagonism comparing the actual and expected tank mix percentage control.

Treatment	Rate ($kg\ ha^{-1}$)	Shoot	Root	Total
		% Reduction		
Glyphosate	0.84	73 bcd	71 bc	71 cde
	1.68	90 abc	78 abc	82 bc
	3.36	96 a	80 ab	88 a
Fluazifop-p-butyl	0.42	59 d	49 d	53 e
Glyphosate + Fluazifop-p-butyl	0.84 + 0.42	81 bcd	72 bc	75 cd
	1.68 + 0.42	92 b	84 a	86 ab
Expected control[1]				
Glyphosate + Fluazifop-p-butyl	0.84 + 0.42	89	85*	87
	1.68 + 0.42	96	89	92

[1]Expected control is a theoretical estimate calculated using the level of control achieved by each herbicide used alone.

This is strongly indicative of good growing conditions with no pot-bound plant concerns.

For the sethoxydim/glyphosate study, sethoxydim resulted in similar reductions in torpedograss shoot, root + rhizome and total biomass as glyphosate at 0.84 $kg\ ha^{-1}$ (Table 2). However, it did not perform as well as glyphosate at 3.36 $kg\ ha^{-1}$ for all three parameters or glyphosate at 1.68 $kg\ ha^{-1}$ for reductions in root + rhizome or total biomass. The tank mixes of sethoxydim and glyphosate at 0.84 and 1.68 $kg\ ha^{-1}$ generally improved torpedograss control compared to sethoxydim alone. However, these tank mixes did not improve torpedograss control compared to the same rates of glyphosate alone. Additionally, actual control for the glyphosate + sethoxydim tank mixes was lower than the theoretical expected control by 10 to 14%. This technically indicates some antagonism between the two herbicides at the rates tested. However, this antagonism was not expressed as a clear reduction in control as much as it was in a failure to achieve the expected level of control. This is somewhat more subtle than classical considerations of antagonism, where a definitive reduction in efficacy is observed when two herbicides that are tank mixed result in poorer control than either herbicide applied alone (Colby 1967). However, the lack of a reduction in control or in achieving the expected increase in control would suggest the tank mixing of low rates of glyphosate with sethoxydim will not be useful for torpedograss control.

For the fluazifop-p-butyl/glyphosate study, fluazifop-p-butyl was not as effective as glyphosate at 0.84, 1.68, or 3.36 $kg\ ha^{-1}$ for almost all parameters measured (Table 3). The tank mixes of fluazifop-p-butyl + glyphosate at 0.84 or 1.68 $kg\ ha^{-1}$ improved torpedograss control compared to fluazifop-p-butyl alone. However, they did not improve torpedograss control compared to the same glyphosate rates applied alone. Overall, expected control did not generally differ from actual control for the tank mix treatments. In only one case did the tank mix result in antagonism, and that was at the lowest glyphosate tank mix rate, which resulted in a 13% decrease in root + rhizome biomass. Similar to sethoxydim, these results indicate that tank

Table 4. Percentage reduction in torpedograss biomass compared to the untreated control for imazapyr, sethoxydim, and tank mixes. Treatment means compared at $P = 0.05$ only if overall test of treatment differences was significant (Fisher's LSD = 0.05).

Treatment	Rate ($kg\ ha^{-1}$)	Shoot	Root	Total
		% Reduction		
Imazapyr	0.07	69 a	44 a	55 a
	0.14	70 a	48 a	58 a
	0.56	75 a	67 a	71 a
Sethoxydim	0.53	68 a	57 a	62 a
Imazapyr + sethoxydim	0.07 + 0.53	74 a	57 a	63 a
	0.14 + 0.53	81 a	64 a	71 a
Expected control[1]				
Imazapyr + sethoxydim	0.07 + 0.53	90	76	83
	0.14 + 0.53	90	78	84

[1]Expected control is a theoretical estimate calculated using the level of control achieved by each herbicide used alone.

mixing low rates of glyphosate with fluazifop-p-butyl were not useful to improve torpedograss control.

For the sethoxydim/imazapyr study, there were no significant differences among any treatments for shoot, root + rhizome, or total reductions in torpedograss biomass (Table 4). For shoot biomass reductions, treatment means ranged from 68 to 81%. For root + rhizome reductions, treatment means ranged from 44 to 64%. For total biomass reductions, treatment means ranged from 55 to 71%. Additionally no significant antagonism was detected for either tank mix of imazapyr + sethoxydim treatment.

For the fluazifop-p-butyl/imazapyr study, there were again no significant differences between any herbicide treatments for all parameters measured (Table 5). Treatments reduced shoot biomass 59 to 75%, root + rhizome biomass 44 to 67%, and total biomass 53 to 71%. No significant antagonism was detected between fluazifop-p-butyl and imazapyr. However, it should be noted that there was a high degree of variation in the study, and the actual tank mix results were 12 to 24% lower than the predicted.

For the single herbicides tested, our results are generally similar to previous torpedograss mesocosm studies. Enloe and Netherland (2017) found higher rates of glyphosate (4.2 $kg\ ha^{-1}$) and imazapyr (1.4 $kg\ ha^{-1}$) applied in the summer reduced torpedograss shoot biomass by approximately 85 and 90%, respectively, at 60 DAT. The same authors also found fluazifop-p-butyl (0.21 $kg\ ha^{-1}$) and sethoxydim (0.56 $kg\ ha^{-1}$) applied in the summer reduced torpedograss shoot biomass by approximately 85 and 80%, respectively, at 60 DAT. Belowground biomass reductions for all four herbicides ranged from 60 to 80% for the summer application timing.

Table 5. Percentage reduction in torpedograss biomass compared to the untreated control for imazapyr, fluazifop-p-butyl, and tank mixes. Treatment means were compared at $P = 0.05$ only if overall test of treatment differences was significant (Fisher's LSD = 0.05).

Treatment	Rate ($kg\ ha^{-1}$)	Shoot	Root	Total
		% Reduction		
Imazapyr	0.07	69 a	44 a	55 a
	0.14	70 a	48 a	58 a
	0.56	75 a	67 a	71 a
Fluazifop-p-butyl	0.42	59 a	49 a	53 a
Imazapyr + fluazifop-p-butyl	0.07 + 0.42	63 a	59 a	60 a
	0.14 + 0.42	67 a	54 a	60 a
Expected control[1]				
Imazapyr + fluazifop-p-butyl	0.07 + 0.42	87	71	79
	0.14 + 0.42	88	74	80

[1]Expected control is a theoretical estimate calculated using the level of control achieved by each herbicide used alone.

Although imazapyr works very slowly and may express its herbicidal activity over several months, in this study we allowed the plants to respond for 60 DAT. We believe this was still ample time to measure a meaningful effect between imazapyr and the two graminicides. These studies clearly indicate that low rates of either glyphosate or imazapyr do not improve torpedograss control when mixed with sethoxydim or fluazifop. Given these results with the two most effective herbicides currently used for aquatic grass control, it is unlikely that other aquatic herbicides would be useful tank mix partners to increase invasive grass control. However, it would still be useful to ensure that other herbicides used for emergent weed control such as imazamox, 2,4-D, or carfentrazone do not antagonize graminicide activity in aquatic settings.

SOURCES OF MATERIALS

[1]Osmocote Plus, Scotts Company, 14111 Scottslawn Rd., Maryville, OH 43041.

[2]Fusilade II, Syngenta Crop Protection, P.O. Box 18300, Greensboro, NC 27419.

[3]Poast, BASF Corporation, 26 Davis Dr., Research Triangle Park, NC 27709.

[4]Rodeo, Dow AgroSciences, 9330 Zionsville Rd., Indianapolis, IN 46268.

[5]Habitat, BASF Corporation, 26 Davis Dr., Research Triangle Park, NC 27709.

[6]Dyne-Amic, Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017.

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APPENDIX

The log link function for the generalized linear mixed model (McCullagh and Nelder 1989) in this analysis was of the form

$$\log(w_{ij}) = \mu + r_i + t_j + (rt_{ij}) \tag{1}$$

where w_{ij} is the conditional mean of plant weights for the n_{ij} pots in run i that received treatment j, μ is the intercept, r_i (i = 1,2) is the run random effect (∼ iid $N(0,\sigma_r^2)$), t_j is the treatment effect (t = 1,…,17), and rt_{ij} is the run × treatment random effect (∼ iid $N(0,\sigma_{rt}^2)$).

In this experiment, the untreated check establishes a baseline level. Proportional treatment response for treatment i relative to the check is estimated by $\exp(t_j - t_c)$ where t_c is the treatment effect for the untreated check in model (1) and, alternatively, percentage reduction = 100 × (1 − $\exp(t_j - t_c)$). Treatment mean comparisons are essentially comparisons of proportional (or percentage) response using model (1) because the t_c term cancels in the differencing.

Tests of antagonism could be performed directly as linear combinations of mean differences using model (1). Expected tank mix response (for two herbicides) is calculated as $\exp[(t_{h1} - t_c) + (t_{h2} - t_c)]$ where h_1 and h_2 denote treatments in which herbicides 1 and 2 are used alone. The contrast to test if the ratio of tank mix proportional response/expected proportional response differs from 1 (> 1 = antagonism, < 1 = synergy) is the test that $(t_{h1h2} - t_c) - [(t_{h1} - t_c) + (t_{h2} - t_c)] = t_{h1h2} - t_{h1} - t_{h2} + t_c$ differs from zero using the linear part of model (1).