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Influence of Surfactants and Additives on Phytotoxicity of Glyphosate to Torpedograss[1]

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ABSTRACT

Greenhouse experiments were conducted to evaluate the effect of selected surfactants [Improve® (fatty amine ethoxylate and ethoxylated nonylphenol), MON-0818 (polyoxyethylene tallow amine) and X-77 (alkylaryl polyoxyethylene glycols, free fatty acids and isopropanol)] and additives [ethylenediamine tetraacetate (EDTA), $NH_4H_2PO_4$, $(NH_4)_2SO_4$, and KH_2PO_4] on glyphosate [(*N*-phosphonomethyl) glycine] efficacy in torpedograss (*Panicum repens* L.) when applied in either hard (5 mM calcium) or soft water (deionized). Improve provided the best herbicidal efficacy when glyphosate was applied at 0.56 kg/ha. MON-0818 provided control equal to Improve when glyphosate was applied at 1.12 kg/ha. However, optimum activity from MON-0818 was concentration dependent whereas Improve provided excellent results at various concentrations. The antagonism of glyphosate activity by hard water was ameliorated by the additives in the following order (most to least effective): $(NH_4)_2SO_4$ > EDTA > $NH_4H_2PO_4$ > KH_2PO_4. The activity of EDTA, $NH_4H_2PO_4$ and KH_2PO_4 was concentration dependent and ranged from no activity to complete elimination of hard water antagonism. The activity of $(NH_4)_2SO_4$ was concentration independent. EDTA at 100 mM was the only additive that reduced glyphosate activity.

Key words: Hard water, antagonism, adjuvants, ammonium sulfate, EDTA, chelate.

INTRODUCTION

Torpedograss is a rhizomatous, perennial grass that grows in both terrestrial and aquatic ecosystems throughout Florida (15) and other parts of the Southeastern United States (25). Glyphosate [(*N*-phosphonomethyl) glycine] is a non-selective herbicide that is used extensively for the control of torpedograss in Florida; however, results have been inconsistent (18). Glyphosate has been shown to be readily translocated to rhizomes of other perennial grasses and therefore can provide long term control of these difficult to manage species (5, 11). However, Baird *et al.*, (2) reported that 4.13 kg/ha of glyphosate did not provide long term control of torpedograss. Baird *et al.*, (1) showed that rates as high as 4.7 kg/ha of glyphosate did not control torpedograss that was partially submerged, indicating that torpedograss is more difficult to control when growing in an aquatic situation.

A number of additives and surfactants have been evaluated with glyphosate to improve herbicidal activity. Wyrill and Burnside (27) demonstrated that certain surfactants enhanced glyphosate activity more than others, and that there was some species-surfactant specificity. This indicates that if a particular species is difficult to control, evaluation of different surfactant and herbicide combinations could improve efficacy.

Wills and McWhorter (26) have also shown that certain additives can influence the activity of glyphosate. Some salts containing cations, such as calcium (cation responsible for water hardness), have been shown to reduce the activity of glyphosate (16, 26). Shea and Tupy (16) reported that certain additives reversed the negative effects that hard water had on glyphosate. Florida water sources used as diluent for glyphosate application contain calcium at concentrations as high as 25 mM (850 ppm) in ground water (7) and up to 3 mM (120 ppm) in surface water (6). Thus, inconsistent control of torpedograss with glyphosate could be caused by hard water antagonism and/or inappropriate surfactant-herbicide combinations. A number of factors could be evaluated and potentially modified to influence the efficacy achieved with glyphosate.

These studies were conducted to: 1) evaluate several surfactants and additives for enhancing the activity of glyphosate on torpedograss using soft water and 2) evaluate the ability of these additives to reduce the antagonism of glyphosate by hard water.

MATERIALS AND METHODS

Effects of surfactants on glyphosate activity in soft water. The activity of glyphosate on torpedograss as influenced by various surfactants was studied under greenhouse conditions. Four, 10 cm rhizome sections collected from East Lake Tohopeliga, FL were planted vertically in 500 ml plastic pots that contained potting medium (Metro-mix)[3]. Plants were treated six weeks after planting (averaged 30 cm in height). The experiment was conducted as a 3 by 3 by 4 factorial using a randomized complete block (RCB) design: glyphosate rates; 0, 0.56, and 1.12 kg a.e./ha, surfactants; Improve®, MON-088, and X-77 and, surfactant concentrations; 0, 0.05, 0.1, and 0.5% (v/v). Deionized water (hereafter referred to as soft water) was used to prepare all solutions in the surfactant studies. The solution combinations were applied to the plants using a CO_2-pressurized single-nozzle sprayer calibrated to deliver 860 l/ha at 276 kPa.

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Prior to treatment, root-rhizome tissue was harvested from eight plants to establish a pretreatment dry weight (DW) for the whole population. This tissue was dried at 60 C for four days and weighed. This data (pretreatment DW) was used to calculate root-rhizome weight change over the treatment period. Four weeks after treatment, aboveground tissue (shoots) was removed. After four additional weeks, shoot regrowth and composite root-rhizome tissue were separated and dried at 60 C for four days and then the following data was obtained: shoot number (regrowth), shoot DW (regrowth), and composite root-rhizome weight change based on DW. Percent change in root-rhizome weight [either inhibition (positive values) or stimulation (negative values) of growth relative to the untreated control] was calculated as follows:

$$1 - \left[\frac{\text{Final Treatment DW - Pretreatment DW}}{\text{Final Control DW - Pretreatment DW}}\right] * 100$$

This method was used so that only growth over the treatment period was considered. Considering only weight change enhanced the accuracy of appraising the treatment effects. This method was found to be necessary for evaluating the effects of a postemergence herbicide that are based on dry weight, as tissue that dies in response to a lethal treatment has weight. Thus, dead tissue would not show 100% inhibition unless only weight change was considered. Percent change in shoot regrowth (DW) and shoot number was calculated as follows:

$$1 - \left[\frac{\text{Treated Plant Variable}}{\text{Control Plant Variable}}\right] * 100$$

Plants were maintained in a glasshouse with a day to night temperature range of 36 to 22 C and a 6 hr photoperiod (maintained with artificial lighting). Plants were cultured in a water saturated condition during the experiment and fertilized with 10x nutrient solution at three and six weeks after planting (9).

Effects of additives on glyphosate activity in hard and soft water. Studies were conducted to determine the influence of various additives on glyphosate activity in soft water, and to determine if these additives reduce glyphosate antagonism caused by hard water. Torpedograss plants were established and maintained as previously described. The experiment was conducted as a 4 by 4 factorial using a RCB design: additives; ammonium phosphate ($NH_4H_2PO_4$), ethylenediamine tetraacetate (EDTA), potassium phosphate (KH_2PO_4), and ammonium sulfate $(NH_4)_2SO_4$, concentrations; 0, 5, 10, 50 and 100 mM. Each of these 16 combinations included 4 mM calcium (hard water) and glyphosate applied at 1.12 kg a.e./ha.

In addition, glyphosate was applied alone and with each additive at 100 mM in soft water. Also, each additive was applied without glyphosate to torpedograss at 100 mM both with 5 mM calcium and without. Data collection and treatment application methods were conducted as described previously.

In each study four replications were used and all experiments were conducted twice. Data were initially analyzed by analysis of variance to test for single factor effects and then to test for interactions (8). There was not a significant interaction between experiments and the treatment variables, thus data were combined across experiments (within each study). However, where treatment interactions (surfactant by concentration by glyphosate rate and additive by concentration) were significant ($P < 0.05$) the data are presented accordingly. Standard error or LSD (protected) values are presented with means.

RESULTS AND DISCUSSION

When applied alone, none of the three surfactants inhibited the growth of torpedograss (Tables 1 and 2). However, shoot and root-rhizome growth increased significantly when Improve was applied at a concentration of 0.5% (compared to the untreated control). Wyrill and Burnside (27) have shown that responses by plants to various surfactants can range from growth inhibition to stimulation. Overall, the response of root-rhizome tissue (Table 2) was greater than shoot tissue (Table 1) except when no surfactant was present in the spray solution. For example, when glyphosate was applied at 0.56 kg/ha without surfactant there was 22 and 23% inhibition of shoot and root-rhizome growth, respectively. In contrast, Improve applied at 0.05% with 0.56 kg/ha glyphosate inhibited growth of shoots and root-rhizome tissue by 17 and 61%, respectively. This indicates that glyphosate was translocated to the below ground tissue, and further, that the surfactant enhanced this movement. Glyphosate translocates readily in plants and accumulates in rhizome nodes (5, 10). How-

Table 1. Influence of surfactants and glyphosate in soft water on shoot regrowth of torpedograss.

Glyphosate (kg a.e./ha)	Surfactant Concentration (% v/v)	Surfactant		
		Improve	MON-0818	X-77
		% inhibition		
0	0	0	0	0
	0.05	−12[1]	8	−2
	0.1	−22	−8	−9
	0.5	−44*	−12	−16
LSD (0.05)[3] 36				
0.56	0	22	22	22
	0.05	17	−10	−6
	0.1	30*	19	26
	0.5	52*	22	31*
LSD (0.05) 20				
1.12	0	34*	34*	34*
	0.05	74*	38*	16
	0.1	78*	68*	38*
	0.5	80*	40*	37*
LSD (0.05) 14				

[1]Negative numbers represent % stimulation.
[2]Means followed by an asterisk are significantly different from the untreated control ($P < 0.05$).
[3]LSD values for comparing surfactants.

TABLE 2. INFLUENCE OF SURFACTANTS AND GLYPHOSATE IN SOFT WATER ON COMPOSITE ROOT-RHIZOME GROWTH OF TORPEDOGRASS.

Glyphosate (kg a.e./ha)	Surfactant Concentrate (% v/v)	Surfactant		
		Improve	MON-0818	X-77
		% inhibition		
0	0	0	0	0
	0.05	–21[1]	–11	–17
	0.1	–32	–22	–36
	0.5	–51*[2]	–23	–43
LSD (0.05)[3] 33				
0.56	0	23	23	23
	0.05	61*	26	33*
	0.1	74*	50*	39*
	0.5	83*	66*	70*
LSD (0.05) 22				
1.12	0	40*	40*	40*
	0.05	98*	92*	54*
	0.1	93*	93*	67*
	0.5	100*	92*	74*
LSD (0.05) 16				

[1]Negative numbers represent % stimulation.

[2]Means followed by an asterisk are significantly different from the untreated control ($P < 0.05$).

[3]LSD values for comparing surfactants.

ever, the type of surfactant and its concentration influence both amount and distribution of glyphosate translocated (3, 17).

At the low rate of glyphosate (0.56 kg/ha) only Improve at 0.5% significantly enhanced (i.e., increased shoot inhibition) herbicidal activity more than glyphosate without surfactant (Table 1). Glyphosate at 0.56 kg/ha in combination with either 0.1% Improve or 0.5% X-77 significantly reduced shoot growth compared to the untreated control, but not compared to glyphosate alone. All three surfactants at 0.5% increased glyphosate (0.56 kg/ha) activity on root-rhizome tissue (Table 2). Again, Improve enhanced glyphosate activity most.

At the high rate of glyphosate (1.12 kg/ha) and the low surfactant concentration (0.05%) only Improve significantly increased the activity of glyphosate on shoot regrowth (Table 1). At one concentration or more, Improve and MON-0818, but not X-77, significantly enhanced glyphosate activity on shoots. Improve provided optimum enhancement of herbicidal activity at 0.05%. MON-0818 gave better results at 0.1% than at 0.5% indicating that there is an optimum concentration. Improve and MON-0818 enhanced glyphosate activity equally when glyphosate was applied at 1.12 kg/ha by inhibiting root-rhizome growth at the lowest concentration (Table 2). In contrast, even the highest concentration of X-77 did not enhance herbicidal activity as much as the lowest concentration of the other surfactants. Specificity between surfactants and glyphosate is indicaated by these data. This type of relationship has been reported previously (3, 17, 27). Surfactants are thought to primarily influence herbicidal uptake either through the formation of a herbicide-surfactant complex or through altered plasmalemma permeability. Therefore, specificity between a herbicide and a surfactant or between and a plant seems logical (20, 22, 24, 27).

Table 3 shows the influence that hard water and the four additives (applied in soft water) had on inhibtion of shoot regrowth in torpedograss by glyphosate. Individually, hard water and EDTA reduced glyphosate activity. It should be pointed out that hard water was evaluated using 5 mM calcium and EDTA at 100 mM was evaluated in soft water. EDTA at lower concentrations (5 and 10 mM) was not antagonistic, but was beneficial in terms of reducing hard water antagonism (Table 4). Shea and Tupy (16) reported that EDTA increased glyphosate activity. Numerous studies have demonstrated that cations such as calcium are antagonistic to glyphosate (4, 13, 16, 19, 26). Research has also indicated that salts such as $NH_4H_2PO_4$ (26), KH_2PO_4 (12, 26), and $(NH_4)_2SO_4$ (14, 21, 23) can also increase glyphosate activity. Although these additives did not reduce glyphosate activity, the high level of inhibition (100%) achieved in this study precluded any conclusions about activity enhancement.

The effects of the four additives on reducing hard water antagonism are presented in Tables 4 and 5. Hard water caused 28 and 61% loss in glyphosate activity based on shoot biomass and number, respectively. All additives evaluated reduced antagonism caused by hard water, but the amount of reversal varied with the additive and its concentration. The phosphate containing salts ($NH_4H_2PO_4$ and KH_2PO_4) reduced hard water antagonism at high concentrations (50 and 100 mM), but had little or no effect at the lower concentrations (5 or 10 mM). Based on shoot biomass, $NH_4H_2PO_4$ completely reversed hard water antagonism at 100 mM while KH_2PO_4 only provided partial amelioration. These salts have been evaluated previously for improving glyphosate activity, but were not evaluated for reversing antagonism caused by hard water (12, 26).

The concentration effects of EDTA were the opposite from the other additives described heretofore. Antagonism by hard water was reduced by EDTA at the lower concentrations (5 and 10 mM) while at the higher concentrations, EDTA had no effect (Tables 3 and 4). Shea and Tupy (16) have reported previously that EDTA reverses hard water induced reduction in glyphosate activity. These

TABLE 3. INFLUENCE OF HARD WATER AND ADDITIVES APPLIED IN SOFT WATER ON GLYPHOSATE ACTIVITY IN TORPEDOGRASS.

Treatment[1]	Shoot regrowth	
	% inhibition[2,3]	% loss in glyphosate activity
Glyphosate in soft water	100 ± 0	0
Glyphosate in hard water[4]	72 ± 4	28
Glyphosate + $NH_4H_2PO_4$[5]	99 ± 1	1
Glyphosate + EDTA[5]	62 ± 9	38
Glyphosate + KH_2PO_4[5]	96 ± 1	4
Glyphosate + $(NH_4)_2SO_4$[5]	99 ± 1	1

[1]Glyphosate applied at 1.12 kg a.e./ha.

[2]All values are significantly different from the untreated control ($P < 0.05$).

[3]Mean values followed by the standard error of the mean.

[4]5 mM calcium.

[5]All additives were applied at 100 mM in soft water (deionized water).

TABLE 4. INFLUENCE OF VARIOUS ADDITIVES ON HARD WATER ANTAGONISM OF GLYPHOSATE ACTIVITY ON SHOOT REGROWTH (g) BY TORPEDOGRASS.

Additive[1]	Concentration (mM) 5	10	50	100
	% inhibition[2] (% loss in glyphosate activity)			
$NH_4H_2PO_4$	63 (37)	82 (18)	90 (10)	98 (2)
EDTA	92 (8)	92 (8)	83 (17)	71 (29)
KH_3PO_4	62 (38)	75 (25)	87 (13)	83 (17)
$(NH_4)_2SO_4$	94 (6)	100 (0)	100 (0)	98 (2)
NONE (glyphosate alone in soft water)[3]	100 (0)			
NONE (glysophate in harad water)[4]	72 (28)			
LSD (0.05)	17	20	14	10

[1]Solutions containing an additive including M,ON-0818 (0.5% v/v), glyphosate (1.12 kg a.e./ha) and were formulated using hard water (5 mM calcium).

[2]All values are significantly different from the untreated control ($P < 0.05$).

[3,4]Glyphosate applied at 1.12 kg a.e./ha and calcium at a concentration of 5 mM.

TABLE 5. INFLUENCE OF VARIOUS ADDITIVES ON HARD WATER ANTAGONISM OF GLYPHOSATE ACTIVITY ON THE NUMBER OF REGROWN SHOOTS BY TORPEDOGRASS.

Additive[1]	Concentration (mM) 5	10	50	100
	% inhibition[2] (% loss in glyphosate activity)			
$NH_4H_2PO_4$	43 (57)	57 (43)	79 (21)	78 (22)
EDTA	80 (20)	90 (10)	52 (48)	42 (58)
KH_2PO_4	35 (65)	56 (44)	72 (28)	31 (29)
$(NH_4)_2SO_4$	73 (27)	99 (1)	100 (0)	98 (2)
NONE (glyphosate alone in soft water)[3]	100 (0)			
NONE (glyphosate in hard water)[4]	39 (61)			
LSD (0.05)	34	31	25	29

[1]Solutions containing an additive included MON-0818 (0.5% v/v), glyphosate (1.12 kg a.e./ha) and were formulated using hard water (5) mM calcium).

[2]All mean values are significantly different from the untreated control ($P < 0.05$).

[3,4]Glyphosate applied at 1.12 kg a.e./ha and calcium at a concentration of 5 mM.

researchers proposed that because EDTA is a chelating agent, it complexes with calcium thus reducing its ability to interact with glyphosate. Further, they proposed that when both calcium and EDTA are in solution with glyphosate, the EDTA will preferentially interact with calcium over glyphosate thus reversing the antagonism. However, the data reported here suggest the interaction to be more complex. If EDTA only interacted with calcium then EDTA should not have reduced glyphosate activity (Table 3). Further, if EDTA only reacted with calcium, higher concentrations of EDTA should not have resulted in reduced amelioration of antagonism caused by calcium. It is therefore proposed that an interaction occurred between EDTA and glyphosate and that the interaction was dependent on both the compounds present and their concentrations. At the lower concentrations, EDTA preferentially interacted with calcium thus restoring glyphosate activity, but as concentration increased excess EDTA began to interact with glyphosate as well. Thus, at higher concentrations, reduced glyphosate activity was caused by two phenomena. Calcium was chelated by EDTA, but excess EDTA then interacted with glyphosate. This conclusion is supported by the fact that glyphosate plus EDTA caused 62% inhibition (Table 3) of shoot regrowth which is less than the 71% inhibition caused by EDTA plus calcium plus glyphosate (Table 4). Presumably, the additional calcium is preventing EDTA from interfering with glyphosate activity.

Overall, $(NH_4)_2SO_4$ provided the most consistent reversal of calcium antagonism (Tables 4 and 5). Statistically, all concentrations of $(NH_4)_2SO_4$ negated the effect of hard water on glyphosate. A number of studies have shown that $(NH_4)_2SO_4$ can enhance glyphosate activity (14, 21, 23). However, the use of $(NH_4)_2SO_4$ to reverse the antagonistic effect of hard water on glyphosate was not reported.

These data indicated that Improve was the best surfactant to use in conjunction with glyphosate for the control of torpedograss in soft water. Hard water was shown to reduce the control of torpedograss with glyphosate. Additives such as EDTA, $NH_4H_2PO_4$ or KH_2PO_4 can restore some of the lost activity, but the reversal was shown to be concentration dependent. Practically, $(NH_4)_2SO_4$ would be the best additive to use in hard water, not only because it is inexpensive (1.14 kg/860 1 to achieve a 10 mM concentration) and readily available, but because its activity was not as concentration sensitive.

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