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Effect of Carrier Water Hardness and Ammonium Sulfate on Efficacy of 2,4-D Choline and Premixed 2,4-D Choline Plus Glyphosate

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Spray water quality is an important consideration for optimizing herbicide efficacy. Hard water cations in the carrier water can reduce herbicide performance. Greenhouse studies were conducted to evaluate the influence of hard water cations and the use of ammonium sulfate (AMS) on the efficacy of 2,4-D choline and premixed 2,4-D choline plus glyphosate for giant ragweed, horseweed, and Palmer amaranth control. Carrier water hardness was established at 0, 200, 400, 600, 800, or 1,000 mg L^{-1} using $CaCl_2$ and $MgSO_4$, and each hardness level consisted of without or with AMS at 10.2 g L^{-1}. One-third of the proposed use rates of 2,4-D choline at 280 g ae ha^{-1} and 2,4-D choline plus glyphosate at 266 plus 283 g ae ha^{-1}, respectively, were applied in the study. An increase in carrier water hardness showed a linear trend for reducing 2,4-D choline and 2,4-D choline plus glyphosate efficacy on all weed species evaluated in both studies. The increase in water hardness level reduced giant ragweed control with 2,4-D choline and the premix formulation of 2,4-D choline plus glyphosate to a greater extent without AMS than it did with AMS in the spray solution. Increases in water hardness from 0 to 1,000 mg L^{-1} reduced weed control 20% or greater with 2,4-D choline. Likewise, the efficacy of the premixed 2,4-D choline plus glyphosate was reduced 21% or greater with increased water hardness from 0 to 1,000 mg L^{-1}. The addition of AMS improved giant ragweed, horseweed, and Palmer amaranth control $\geq$ 17% and $\geq$ 10% for 2,4-D choline and 2,4-D choline plus glyphosate application, respectively. The biomass of all weed species was reduced by $\geq$ 8% and $\geq$ 5% with 2,4-D choline and 2,4-D choline plus glyphosate application, respectively, when AMS was added to hard water.

Nomenclature: 2,4-D choline; ammonium sulfate; glyphosate; giant ragweed, *Ambrosia trifida* L. AMBTR; horseweed, *Conyza canadensis* (L.) Cronq. ERICA; Palmer amaranth, *Amaranthus palmeri* S. Wats. AMAPA.

Key words: 2,4-D choline and glyphosate formulation, Enlist Duo herbicide, Enlist weed control system, water quality.

La calidad del agua de aplicación es una consideración importante para optimizar la eficacia del herbicida. La presencia de cationes de agua pesada en el agua de mezcla puede reducir el desempeño del herbicida. Se realizaron estudios de invernadero para evaluar la influencia de los cationes de agua pesada y el uso de ammonium sulfate (AMS) sobre la eficacia de 2,4-D choline y la premezcla de 2,4-D más glyphosate para el control de *Ambrosia trifida*, *Conyza canadensis,* y *Amaranthus palmeri*. La dureza del agua de mezcla se estableció en 0, 200, 400, 600, 800, ó 1,000 mg L^{-1} usando $CaCl_2$ y $MgSO_4$, y cada nivel de dureza fue analizado con y sin AMS a 10.2 g L^{-1}. En el estudio se aplicó un tercio de las dosis propuestas para 2,4-D choline a 280 g ae ha^{-1} y 2,4-D choline más glyphosate a 266 más 283 g ae ha^{-1}, respectivamente. El incremento en la dureza del agua de mezcla mostró una tendencia lineal a reducir la eficacia de 2,4-D choline y 2,4-D choline más glyphosate sobre todas las especies de malezas evaluadas en ambos estudios. El incremento en el nivel de dureza del agua redujo más el control de *A. trifida* con 2,4-D choline y con la formulación en premezcla de 2,4-D choline más glyphosate en soluciones de aplicación sin AMS que en las que tenían AMS. El aumentar la dureza del agua de 0 a 1,000 mg L^{-1} redujo el control de malezas con 2,4-D choline 20% o más. De la misma forma, la eficacia de la premezcla de 2,4-D más glyphosate fue reducida 21% o más al incrementarse la dureza del agua de 0 a 1,000 mg L^{-1}. La adición de AMS mejoró el control de *A. trifida*, *C. canadensis*, y *A. palmeri* $\geq$17% y $\geq$10% con aplicaciones de 2,4-D choline y 2,4-D choline más glyphosate, respectivamente. La biomasa de todas las especies de malezas fue reducida $\geq$8% y $\geq$5% con aplicaciones de 2,4-D choline y 2,4-D choline más glyphosate, respectivamente, cuando se agregó AMS a agua dura.

Water is the primary carrier for most of the herbicide applications. About 99% of herbicide spray solutions consist of water (Stahlman et al. 1997). Water quality factors, such as temperature

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and pH, have a critical role on herbicide performance (Devkota et al. 2016b; Green and Hale 2005). Herbicide carrier water is generally obtained from an underground source because it is readily available and cost effective. Constituents in underground water primarily depend on the type of aquifer through which water passes to underground reservoirs and mineral compositions of the bedrock (Freeze and Cherry 1979). Water hardness is primarily dependent on the concentration of cations, such as calcium (Ca^{2+}) and magnesium (Mg^{2+}), in water and is expressed as an equivalent of calcium carbonate ($CaCO_3$). In the midwestern United States, underground aquifers include limestone bedrock, which contribute to higher concentrations of Ca^{2+} and Mg^{2+} in underground water (IDNR 1980). In Indiana groundwater, the average concentrations of Ca^{2+} and Mg^{2+} cations are 400 mg L^{-1} and 115 mg L^{-1}, respectively (IDNR 1999). The negative influence of monovalent and divalent cations present in the spray solution on herbicide efficacy has been reported by previous researchers (Devkota et al. 2016a; Hanson and Rieck 1976; Nalewaja and Matysiak 1991; Wills and McWhorter 1985).

Most previous studies focused on the efficacy of glyphosate, a weak acid herbicide, applied with carrier water containing calcium, magnesium, iron, zinc, sodium, potassium, and aluminum (Abouziena et al. 2009; Buhler and Burnside 1983; Hanson and Rieck 1976; Sandberg et al. 1978; Shilling and Haller 1989; Wills and McWhorter 1985). These researchers concluded that the efficacy of glyphosate was reduced when applied in the presence of hard water cations. Buhler and Burnside (1983) reported that glyphosate efficacy was reduced in the presence of 400 mg L^{-1} of calcium chloride ($CaCl_2$), magnesium sulfate ($MgSO_4$), zinc sulfate ($ZnSO_4$), and iron sulfate ($FeSO_4$) in the spray solution. Moreover, glyphosate mixed in spray solutions containing 50 mg L^{-1} Ca^{2+} exhibited reduced activity on wheat (*Triticum aestivum* L.) (Shea and Tupy 1984). Glyphosate applied at 0.42 or 0.84 kg ae ha^{-1} with Ca^{2+} or Mg^{2+} cations at a concentration $>$250 mg L^{-1} reduced control of yellow nutsedge (*Cyperus esculentus* L.), broadleaf signalgrass [*Urochloa platyphylla* (Nash) R.D. Webster], pitted morningglory (*Ipomoea lacunosa* L.), and Palmer amaranth (Mueller et al. 2006). Calcium present in the spray solution reduced glyphosate absorption into the plant from Ca^{2+} associating with the carboxylic group and phosphonate group present in glyphosate (Thelen et al. 1995).

2,4-D is a weak acid herbicide and a member of auxin herbicide family (Grossmann 2010). Previous studies conducted to evaluate auxin herbicides with respect to water quality have reported antagonism with hard water cations present in the carrier water (Nalewaja et al. 1991; Patton et al. 2015; Roskamp et al. 2013; Woznica et al. 2003). Efficacy of dicamba and 2,4-D applied at reduced rates on kochia [*Kochia scoparia* (L.) Schrad.] was negatively influenced by water containing 400 to 800 mg L^{-1} of Ca^{2+} or Mg^{2+} cations (Nalewaja and Matysiak 1993; Nalewaja et al. 1991). Similarly, the presence of calcium and iron in the spray solution antagonized the effect of 2,4-D plus glyphosate on wheat (Nalewaja and Matysiak 1992).

Ammonium sulfate (AMS) or diammonium sulfate is a water-conditioning agent widely used for herbicide applications. AMS is reported to prevent the antagonistic effects of hard water cations on weak acid herbicides and to improve herbicide efficacy (Nalewaja and Matysiak 1993; O'Sullivan et al. 1981; Zollinger et al. 2010). The addition of AMS in the presence of hard water cations improved glufosinate efficacy for Palmer amaranth control (Devkota and Johnson 2016). The sulfate ion of AMS binds with cations, such as Ca^{2+}, Mg^{2+}, K^+, Na^+ in water, whereas ammonium ions of AMS form a complex with the herbicide molecule (Thelen et al. 1995). Activity of glyphosate was enhanced by the addition of AMS in spray water consisting of Ca^{2+} (Nalewaja and Matysiak 1991), and this result was attributed to increased glyphosate absorption through the leaf cuticle and cell membrane (Thelen et al. 1995).

Enlist Duo herbicide was developed for use with Enlist corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.]. Enlist Duo herbicide is a premix formulation containing 195 g ae L^{-1} of 2,4-D choline and 205 g ae L^{-1} of glyphosate dimethylamine with recommended rates of 1,640 to 2,185 g ae ha^{-1}. The formulated product of 2,4-D choline plus glyphosate (Enlist Duo) is applied to Enlist corn, soybean, and cotton (*Gossypium hirsutum* L.) for POST control of broadleaf weeds and grasses. However, current knowledge on the effects of carrier water hardness on 2,4-D choline or Enlist

Duo herbicide is limited, and there are no published reports, to our knowledge, with information on the effect of spray water hardness on these formulations. Herbicides used in Enlist weed-control systems could be applied to areas with a wide range of water hardness levels; therefore, studies evaluating the effect of carrier water hardness are needed to understand whether hard water cations has a negative effect on the performance of the new formulation of 2,4-D choline and its premixed product with glyphosate. Therefore, research was conducted with the objective of evaluating the effect of carrier water hardness and the water-conditioning agent AMS on new herbicide formulations for the Enlist crop system. Our hypothesis was that carrier water hardness would reduce the efficacy of 2,4-D choline and 2,4-D choline plus glyphosate and that use of AMS as a water-conditioning agent would minimize the negative effects of water hardness on herbicidal efficacy.

Materials and Methods

Greenhouse studies were conducted in spring and fall of 2015 to evaluate the effect of carrier water hardness and AMS on the activity of an experimental formulation of 2,4-D choline (GF-2654; 456 g ae L^{-1} formulation of 2,4-D choline salt; Dow AgroSciences, Indianapolis, IN) and the premix formulation of 2,4-D choline plus glyphosate (Enlist Duo herbicide; 195 plus 205 g ae L^{-1} of choline and dimethylammonium salt of 2,4-D and glyphosate, respectively; Dow AgroSciences). Separate studies were conducted to evaluate the treatment effect of (1) 2,4-D choline, and (2) premixed 2,4-D choline plus glyphosate for control of giant ragweed, horseweed, and Palmer amaranth.

Giant ragweed, horseweed, and Palmer amaranth seeds were planted in potting soil (Redi-Mix, Sun-Gro Redi-Earth Plug and Seedling Mix, Sun-Gro Horticulture, Bellevue, WA) using 26- by 26- by 6-cm poly-flats for seed germination. At the one to two true-leaf stage, seedlings were transplanted into 164-cm^3 cone containers (Ray Leach SC-10 Super Cell Cone-tainers, Stuewe & Sons, Tangent, OR) filled with potting soil. Transplants were watered daily and fertilized weekly (Miracle-Gro water soluble all purpose plant food [24–8–16, N–P–K], Scotts Miracle-Gro Products Inc., Marysville, OH). Plants were grown in the greenhouse with day/night temperatures of 22/28 C, under a 16-hr photoperiod regiment. Treatments were applied to horseweed at the six- to eight-cm rosette stage, to giant ragweed at the five to six true-leaf stage, and to Palmer amaranth at the 8 to 12 true-leaf stage.

Treatments consisted of two factor combinations of (1) carrier water hardness, and (2) AMS. Calcium chloride (Ca^{2+} dihydrate, granular, macron-fine chemicals, Avantor Performance Materials, Inc., Center Valley, PA) and Mg^{2+} (magnesium sulfate anhydrous, Fisher Scientific, Pittsburgh, PA) cations were used in a 3:1 ratio with deionized water to create various hardness levels. The levels of carrier water hardness prepared included 0, 200, 400, 600, 800, and 1,000 mg L^{-1}. Water samples were tested for desired hardness level using hardness test kit (Total Hardness Test Kit, HACH, Loveland, Co). Each hardness level also consisted of either no AMS or the addition of AMS (N-Pak AMS liquid, 34% ammonium sulfate, Winfield Solutions, LLC, St. Paul, MN) at 10.2 g L^{-1} (equivalent to 1% wt/wt of dry AMS) for water-conditioning purpose. In addition, a nontreated check was included for comparison of the treatments.

After preparing water samples at each hardness level, AMS was added to the appropriate treatments before mixing the herbicide. Herbicides were used at approximately one-third of lowest recommended research rate. 2,4-D choline was applied at 280 g ae ha^{-1}, and 2,4-D choline plus glyphosate was applied at 266 plus 283 g ae ha^{-1}, respectively. Immediately after mixing the herbicides, treatments were applied using a compressed air-track sprayer calibrated to deliver 140 L ha^{-1} using a TeeJet 8002EVS nozzle (TeeJet Technologies, Spraying Systems Co., North Avenue at Schmale Road, Carol Stream, IL), and at a speed of 4.8 km h^{-1}.

Data Collection and Analysis. Both studies were conducted as randomized complete blocks with five replications and were repeated over time for a total of two experimental runs. Visually assessed plant injury was recorded on a 0 to 100 scale (where 0 equals no injury or similar to the nontreated control, and 100 equals complete plant death) at weekly intervals for 3 wk after treatment (WAT). At 3 WAT, plant biomass above the soil surface was harvested and placed in a 60 C forced-air drier. Biomass was determined and converted to a biomass reduction percentage compared with the nontreated control.

Data were analyzed using PROC GLM in SAS version 9.3 (SAS Institute Inc., Cary, NC). There was no significant run effect in either study; therefore, data were pooled over experimental runs and were analyzed separately by weed species. Control and biomass data were subjected to analysis of covariance (ANCOVA) at $\alpha = 0.05$ to determine the effect of AMS as a class variable and water hardness as a regression variable. Data were also tested for ANCOVA assumptions by confirming that residuals were random, homogeneous, and followed normality. Both the linear and quadratic terms of the regression variable were included in the model to establish its relationship with the class variable. The difference between the slopes of the lines was determined by the significant interaction effect of AMS and water hardness. The relationship between treatments with and without AMS and water hardness was best fit with the linear model in Equation 1

$$y = mx + b, \quad [1]$$

where y represents control (or biomass reduction), m represents the slope, x represents the water hardness in milligrams per liter, and b represents the maximum control in the absence of water hardness.

Likewise, data were combined over water hardness levels, and mean separation between the AMS and non-AMS treatment was performed with adjusted Tukey statistics at $P \leq 0.05$.

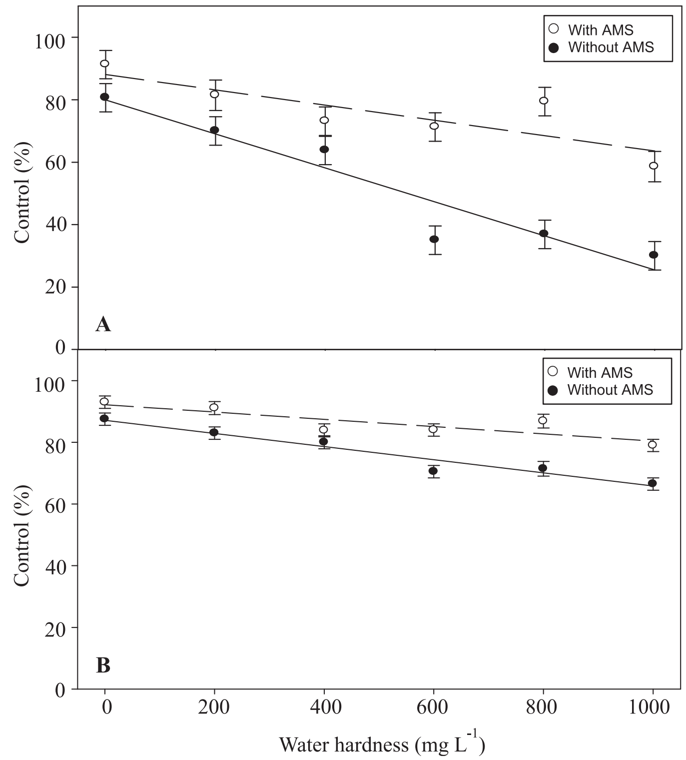

Figure 1. Giant ragweed control as a function of water hardness at 3 wk after treatment with (A) 2,4-D choline, predicted models are $y = -0.024x + 88.07$ (with AMS, $R^2 = 0.49$, $P = 0.0001$) and $y = -0.054x + 79.94$ (without AMS, $R^2 = 0.68$, $P < 0.0001$); and (B) premixed 2,4-D choline plus glyphosate, predicted models are $y = -0.011x + 92.29$ (with AMS, $R^2 = 0.56$, $P < 0.0001$) and $y = -0.021x + 87.14$ (without AMS, $R^2 = 0.63$, $P < 0.0001$). In the equations, y = control (%), and x = water hardness (mg L^{-1}).

Results and Discussion

Giant Ragweed Control. The ANCOVA for control of giant ragweed showed a significant ($P = 0.0004$) interaction between AMS and water hardness. Increasing spray water hardness reduced giant ragweed control linearly with 2,4-D choline applied at 280 g ae ha^{-1} but at different rates based on whether or not AMS was present. Increased water hardness reduced giant ragweed control with 2,4-D choline at a greater rate in the absence than in the presence of AMS (Figure 1). The giant ragweed control was reduced 55% when water hardness increased from 0 to 1,000 mg L^{-1}. However, with the addition of AMS, giant ragweed control was reduced 24% with increased water hardness from 0 to 1,000 mg L^{-1}. Data averaged over the hardness levels showed that giant ragweed control with addition of AMS was 23% or greater compared with without AMS (Table 1). There was no interaction ($P = 0.375$) of water hardness and AMS on giant ragweed biomass (Figure 2). The giant ragweed biomass reductions were 7 and 13% in the presence and absence of AMS, respectively, when water hardness increased from 0 to 1,000 mg L^{-1}. This result illustrates that biomass increased with increasing levels of water hardness in the presence or absence of AMS. Data pooled across water hardness treatments illustrated that the difference on giant ragweed biomass was 9% or greater with AMS than without AMS for 2,4-D choline (Table 1).

There was a significant interaction ($P = 0.0093$) between water hardness and AMS for giant ragweed control with the premixed formulation of 2,4-D choline plus glyphosate. This interaction was primarily driven by the difference in the slope of the predicted model for giant ragweed control

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Table 1. Control and biomass reduction of giant ragweed, horseweed, and Palmer amaranth at 3 wk after 2,4-D choline and premixed 2,4-D choline plus glyphosate application as affected by ammonium sulfate (AMS).[a]

Herbicide[b]	AMS[c]	Giant ragweed		Horseweed		Palmer amaranth	
		Control[d]	Biomass reduction	Control	Biomass reduction	Control	Biomass reduction
	% v/v	%					
2,4-D choline[e]	0	53 b	54 b	20 b	33 b	41 b	47 b
	2.5	76 a	63 a	40 a	41 a	58 a	65 a
2,4-D choline plus glyphosate	0	76 b	49 b	48 b	38 b	69 b	56 b
	2.5	86 a	55 a	61 a	43 a	83 a	65 a

[a] Data were combined over experimental runs and water hardness treatments.

[b] 2,4-D was applied as choline salt formulation at 280 g ae ha^{-1}, and premixed 2,4-D plus glyphosate was applied at 266 plus 283 g ae ha^{-1}, respectively.

[c] AMS was applied at 0 or 10.2 g L^{-1} in the spray solution.

[d] Means within a column and for each herbicide represented by same letter are not different according to adjusted Tukey at $\alpha \leq 0.05$.

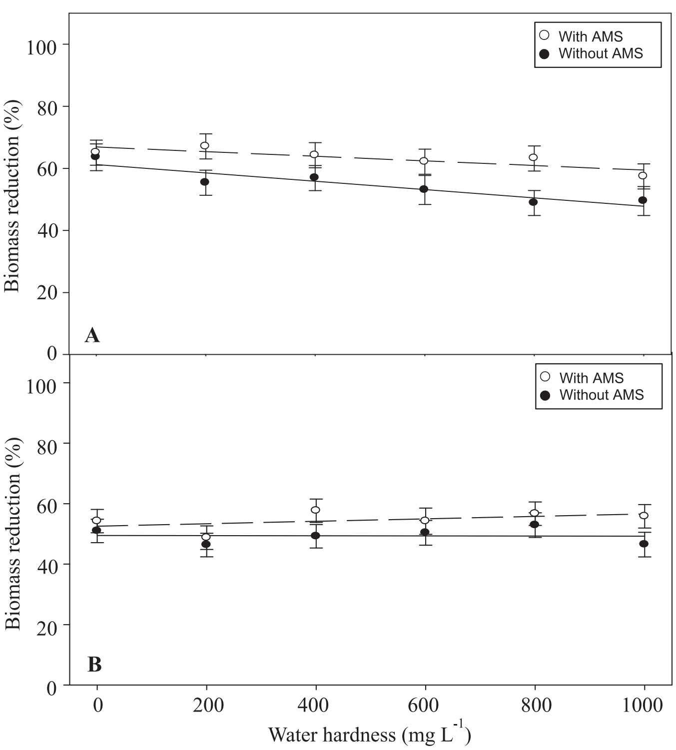

Figure 2. Giant ragweed biomass reduction as a function of water hardness at 3 wk after treatment with (A) 2,4-D choline, predicted models are $y = -0.0073x + 66.9$ (with AMS, $R^2 = 0.29$, $P = 0.048$) and $y = -0.0134x + 61.24$ (without AMS, $R^2 = 0.35$, $P = 0.0084$); and (B) premixed 2,4-D choline plus glyphosate, predicted models are $y = 0.0041x + 52.5$ (with AMS, $R^2 = 0.2$, $P = 0.36$) and $y = -0.00024x + 49.5$ (without AMS, $R^2 = 0.21$, $P = 0.96$). In the equations, y = biomass reduction (%), and x = water hardness (mg L^{-1}).

reduction with the increased level of water hardness in the absence or presence of AMS (Figure 1). The giant ragweed control reduction with 2,4-D choline plus glyphosate at 266 plus 283 g ae ha^{-1} was 21% with increased water hardness from 0 to 1,000 mg L^{-1} in the absence of AMS, whereas giant ragweed control was reduced 12% with increased water hardness from 0 to 1,000 mg L^{-1} in the presence of AMS. Data pooled across water hardness illustrated that giant ragweed control with premixed 2,4-D choline plus glyphosate was 10% or greater with AMS than without AMS (Table 1). There was no interaction of water hardness and AMS on premixed 2,4-D choline plus glyphosate on giant ragweed biomass. Likewise, an increase in water hardness levels did not have a negative influence on 2,4-D choline plus glyphosate activity for reduction of giant ragweed biomass (Figure 2). However, 2,4-D choline plus glyphosate showed increased activity with AMS rather than without AMS on giant ragweed biomass. Treatments without AMS resulted in 6% or less giant ragweed biomass reduction compared with the AMS treatments (Table 1).

Horseweed Control. The interaction of water hardness and AMS was not significant ($P = 0.832$) for 2,4-D choline efficacy on horseweed. Horseweed control was reduced as water hardness increased either in the presence or absence of AMS. Horseweed control was reduced 20% when water hardness increased from 0 to 1,000 mg L^{-1} in the absence of AMS (Figure 3). Likewise, in the presence of AMS, horseweed control was reduced 19% when water hardness increased from 0 to

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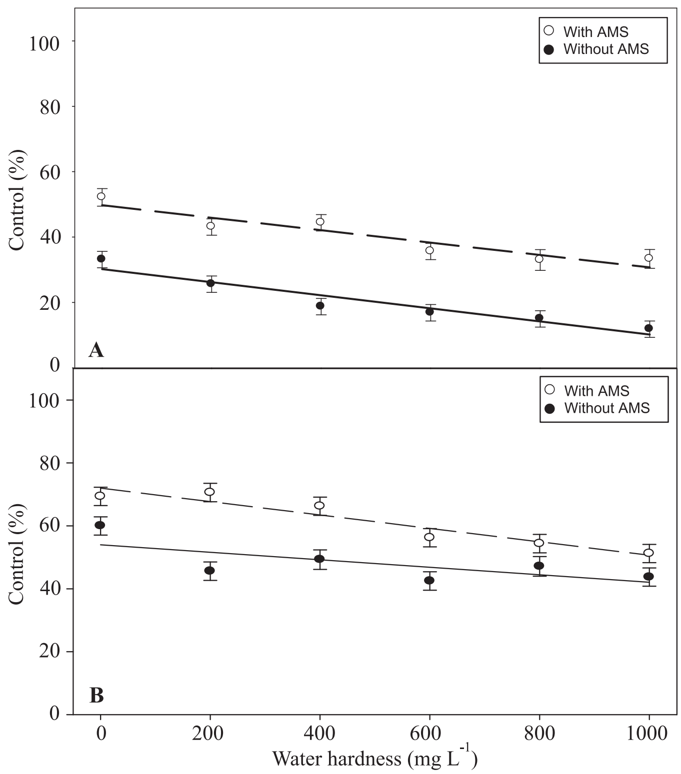

Figure 3. Horseweed control as a function of water hardness at 3 wk after treatment with (A) 2,4-D choline, predicted models are $y = -0.019x + 49.8$ (with AMS, $R^2 = 0.43$, $P = 0.0007$) and $y = -0.02x + 30.2$ (without AMS, $R^2 = 0.76$, $P < 0.0001$); and (B) premixed 2,4-D choline plus glyphosate, predicted models are $y = -0.021x + 72.02$ (with AMS, $R^2 = 0.7$, $P < 0.0001$) and $y = -0.011x + 54.01$ (without AMS, $R^2 = 0.4$, $P = 0.0016$). In the equations, y = control (%), and x = water hardness (mg L^{-1}).

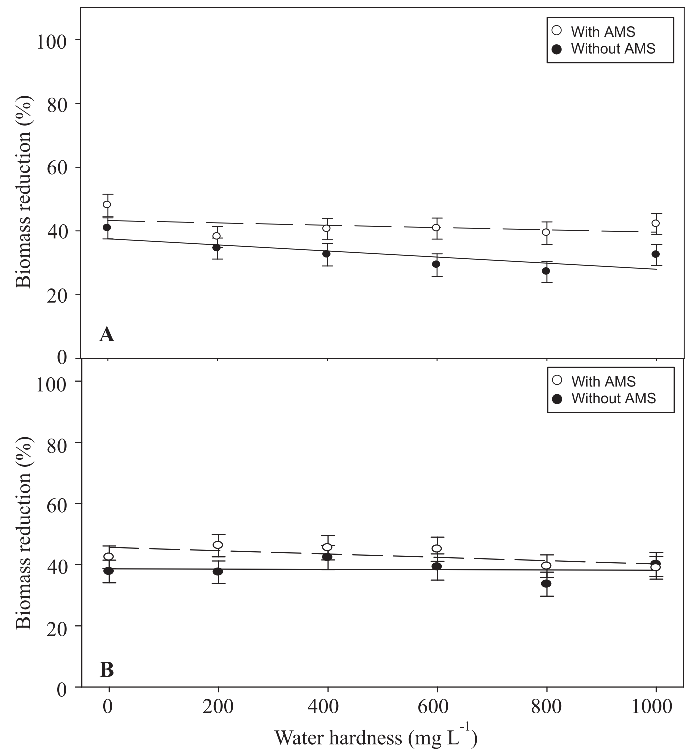

Figure 4. Horseweed biomass as a function of water hardness at 3 wk after treatment with (A) 2,4-D choline, predicted models are $y = -0.00364x + 43.28$ (with AMS, $R^2 = 0.2$, $P = 0.78$) and $y = -0.00959x + 37.56$ (without AMS, $R^2 = 0.37$, $P = 0.0047$); and (B) premixed 2,4-D choline plus glyphosate, predicted models are $y = -0.0054x + 45.7$ (with AMS, $R^2 = 0.21$, $P = 0.248$) and $y = -0.00047x + 38.7$ (without AMS, $R^2 = 0.2$, $P = 0.845$). In the equations, y = biomass reduction (%), and x = water hardness (mg L^{-1}).

1,000 mg L^{-1}. Data pooled over water hardness showed that horseweed control with 2,4-D choline was 20% or greater with the AMS than without the AMS (Table 1). Roskamp et al. (2013) reported a 48% increase in horseweed control when 2,4-D amine was applied with AMS rather than without AMS in the presence of Ca^{2+} concentration at 590 mg L^{-1}. In the absence of AMS, horseweed biomass increased 10% with increased water hardness from 0 and 1,000 mg L^{-1} (Figure 4). However, there was no incremental increase in horseweed biomass with increased water hardness from 0 and 1,000 mg L^{-1} in the presence of AMS. Data combined over water hardness levels illustrated that 2,4-D choline was more efficacious when applied with AMS than without AMS with 8% or greater horseweed biomass reduction (Table 1).

The interaction between water hardness and AMS was not significant ($P = 0.074$) for horseweed control with premixed 2,4-D choline plus glyphosate. This suggests that the rate of horseweed control reduction did not differ with the increased water hardness in the presence or absence of AMS. Increased water hardness level reduced horseweed control linearly in either the presence or absence of AMS for 2,4-D choline plus glyphosate application (Figure 3). Horseweed control with 2,4-D choline plus glyphosate decreased 21 and 12% in the presence and absence of AMS, respectively, with increased water hardness from 0 to 1,000 mg L^{-1}. Data combined across water hardness treatments showed horseweed control of 13% or greater with AMS than without AMS for premixed 2,4-D choline plus glyphosate efficacy (Table 1). The ANCOVA illustrated a nonsignificant interaction ($P = 0.416$) between water hardness and AMS on premixed 2,4-D choline plus glyphosate in horseweed biomass. Likewise, horseweed biomass was not

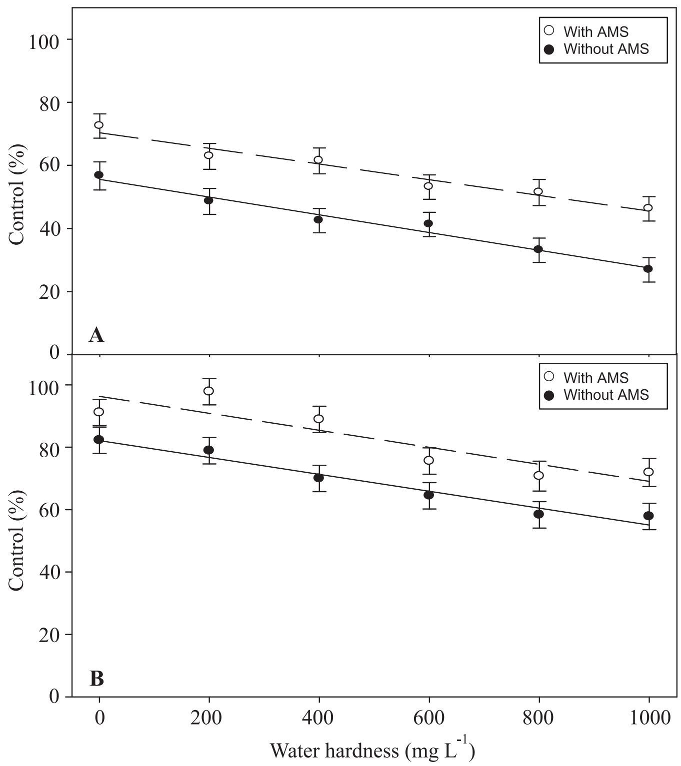

Figure 5. Palmer amaranth control as a function of water hardness at 3 wk after treatment with (A) 2,4-D choline, predicted models are $y = -0.024x + 70.34$ (with AMS, $R^2 = 0.47$, $P = 0.002$) and $y = -0.028x + 55.54$ (without AMS, $R^2 = 0.75$, $P = 0.0001$); and (B) premixed 2,4-D choline plus glyphosate, predicted models are $y = -0.027x + 96.27$ (with AMS, $R^2 = 0.51$, $P < 0.0001$) and $y = -0.027x + 82.14$ (without AMS, $R^2 = 0.6$, $P < 0.0001$). In the equations, $y =$ control (%), and $x =$ water hardness (mg L^{-1}).

influenced by increased water hardness from 0 to 1,000 mg L^{-1}. The magnitude of the predicted model was greater on horseweed biomass with the addition of AMS than it was without AMS (Figure 4). The magnitude of the regression line for horseweed biomass reduction was 7% or greater with the addition of AMS than without AMS for premixed 2,4-D choline plus glyphosate application. Likewise, the difference in horseweed biomass was 5% or greater with the addition of AMS than without AMS for 2,4-D choline plus glyphosate application (Table 1).

Palmer Amaranth Control. Palmer amaranth control with 2,4-D choline was not affected by the interaction ($P = 0.656$) of water hardness and AMS. There was a negative linear trend for Palmer amaranth control with increased water hardness in either the presence or absence of AMS. Without AMS, Palmer amaranth control was reduced 28% when water hardness increased from 0 to 1,000 mg L^{-1} (Figure 5). Similarly, with the addition of AMS, Palmer amaranth control was reduced 25% when water hardness increased from 0 to 1,000 mg L^{-1}. A comparison between the AMS treatments showed that 2,4-D choline applied with AMS controlled Palmer amaranth 17% or greater than it did without AMS (Table 1). Roskamp et al. (2013) also reported 27% greater control of redroot pigweed (*Amaranthus retroflexus* L.) with 2,4-D amine applied with AMS than without AMS in the spray solution consisting of Ca^{2+} concentration at 590 mg L^{-1}. ANCOVA showed a significant interaction ($P = 0.0042$) between water hardness and AMS on 2,4-D choline activity for Palmer amaranth biomass. Increased levels of water hardness from 0 to 1,000 mg L^{-1} increased Palmer amaranth biomass accumulation in the absence of AMS compared with the presence of AMS. When water hardness increased from 0 to 1,000 mg L^{-1} biomass reduction was lowered 16% in the presence of AMS compared with 39% in the absence of AMS (Figure 6). Likewise, when averaged over water hardness levels, the difference in reduction of Palmer amaranth biomass was 18% or greater with 2,4-D choline applied with AMS compared with no AMS (Table 1).

The interaction of water hardness and AMS was not significant ($P = 0.896$) for 2,4-D choline plus glyphosate activity on Palmer amaranth. This illustrates that the Palmer amaranth control reduction with the increased levels of water hardness did not differ with or without AMS. However, the magnitude of Palmer amaranth control was greater in the presence of AMS compared with no AMS (Figure 5). The increased water hardness from 0 to 1,000 mg L^{-1} showed a linear decrease in Palmer amaranth control of 27% and 37% in the presence and absence of AMS, respectively, for premixed 2,4-D plus glyphosate application. When averaged over water hardness, Palmer amaranth control was 14% greater with the addition of AMS compared with no addition of AMS for 2,4-D choline plus glyphosate application (Table 1). There was no interaction of water hardness and AMS on 2,4-D choline plus glyphosate activity for Palmer amaranth biomass reduction. In the presence and absence of AMS, reduction in Palmer amaranth biomass was at least

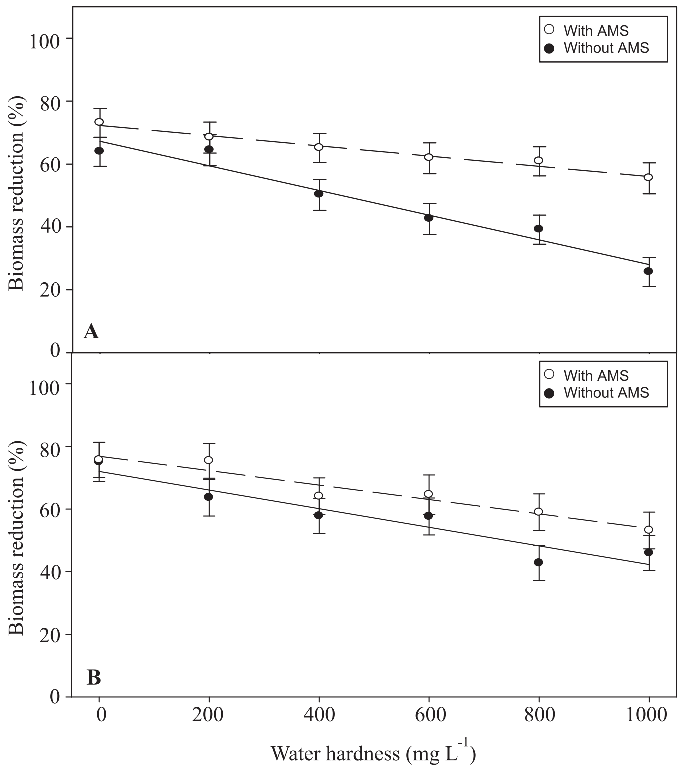

Figure 6. Palmer amaranth biomass reduction as a function of water hardness at 3 wk after treatment with (A) 2,4-D choline, predicted models are $y = -0.016x + 72.26$ (with AMS, $R^2 = 0.3$, P = 0.033) and $y = -0.039x + 67.25$ (without AMS, $R^2 = 0.7$, $P < 0.0001$); and (B) premixed 2,4-D choline plus glyphosate, predicted models are $y = -0.023x + 76.8$ (with AMS, $R^2 = 0.43$, P = 0.004) and $y = -0.029x + 72$ (without AMS, $R^2 = 0.39$, P = 0.0015). In the equations, y = biomass reduction (%), and x = water hardness (mg L^{-1}).

23 and 30% or greater, respectively, with increased water hardness from 0 to 1,000 mg L^{-1} (Figure 6). The effect of AMS was significant ($P < .0001$), which shows that Palmer amaranth biomass was reduced with 2,4-D choline plus glyphosate when applied with AMS compared with being applied without AMS. When combined across water hardness treatments, reduction in Palmer amaranth biomass was 9% or greater with AMS than without AMS (Table 1).

In the current research, the efficacy of 2,4-D choline on giant ragweed, horseweed, and Palmer amaranth was reduced with increased levels of hard water cations in the spray solution. The addition of AMS reduced the effect of hard water on 2,4-D choline efficacy for giant ragweed, horseweed, and Palmer amaranth control. Results of this study are in agreement with other studies that showed that the presence of Ca^{2+} and Mg^{2+} cations in spray water reduced the efficacy of 2,4-D on common lambsquarters (*Chenopodium album* L.), redroot pigweed, horseweed, and kochia (Nalewaja et al. 1991; and Nalewaja and Matysiak 1993; Roskamp et al. 2013). Moreover, the above-mentioned studies have reported that the addition of AMS increased 2,4-D activity. The increase in herbicide activity with AMS has also been attributed to improved herbicidal movement through the leaf cuticle because of the binding of the ammonium ion present in AMS to the anion of the weak acid herbicide (Wanamarta et al. 1989). Likewise, improved absorption of chlorimuron, imazapyr, and imazethapyr herbicides has been reported with the addition of AMS (Fielding and Stoller 1990; Gronwald et al. 1993; Kent et al. 1991).

Hard water antagonism was observed in premixed 2,4-D choline plus glyphosate for giant ragweed, horseweed, and Palmer amaranth control. Glyphosate is one of the herbicide components in the premixed formulation. Previous research studies evaluating carrier water hardness have reported that the efficacy of glyphosate decreased in the presence of hard water cations in the spray solution (Nalewaja and Matysiak 1991, 1992; Sandberg et al. 1978; Stahlman and Phillips 1979). The increased Ca^{2+} concentration from 0 to 800 mg L^{-1} increased tall morningglory [*Ipomoea purpurea* (L.) Roth] biomass from 0.6 to 1.1 g $plant^{-1}$ with glyphosate applied at 1.68 kg ha^{-1} (Sandberg et al. 1978). Nalewaja and Matysiak (1991) reported that fresh-weight reduction of wheat was 75, 49, and 0% when glyphosate was applied without hard water cations, with Mg^{2+} cations, and with Ca^{2+} cations in the spray solution, respectively. Thelen et al. (1995) reported that glyphosate absorption in sunflower (*Helianthus annuus* L.) decreased 26% in the presence of 500 mg L^{-1} of Ca^{2+} in the spray solution. In the same study, authors reported that glyphosate absorption increased 24% or greater with the addition of 0.5% wt/v AMS in the spray solution. Likewise, Zollinger et al. (2010) reported that hard water cations antagonized the efficacy of aminopyralid, tembotrione, dicamba plus diflufenzopyr, and glufosinate; however, use of AMS overcame the hard water antagonism in those herbicides.

The current study provides critical knowledge about the antagonistic effect of hard water cations

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on the efficacy of the choline salt of 2,4-D and premixed 2,4-D choline plus glyphosate formulations. The results from this study showed that an increase in the concentration of hard water cations in spray solution reduced the activity of 2,4-D choline and 2,4-D choline plus glyphosate formulation in a linear trend for horseweed, giant ragweed, and Palmer amaranth control. The addition of AMS in the spray solution has the potential to reduce the negative effect of hard water on 2,4-D choline and premixed 2,4-D choline plus glyphosate activity for giant ragweed, horseweed, and Palmer amaranth control.

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Literature Cited

Abouziena HF, Elmergawi RA, Sharma S, Omar AA, Singh M (2009) Zinc antagonizes glyphosate on yellow nutsedge (*Cyperus esculentus*). Weed Sci 57:16–20

Buhler DD, Burnside OC (1983) Effect of water quality, carrier volume, and acid on glyphosate phytotoxicity. Weed Sci 31:163–169

Devkota P, Johnson WG (2016) Glufosinate efficacy as influenced by carrier water pH, hardness, foliar fertilizer, and ammonium sulfate. Weed Technol 30:848–859

Devkota P, Spaunhorst DJ, Johnson WG (2016a) Influence of carrier water pH, hardness, foliar fertilizer, and ammonium sulfate on mesotrione efficacy. Weed Technol 30:617–628

Devkota P, Whitford F, Johnson WG (2016b) Influence of spray-solution temperature and holding duration on weed control with premixed glyphosate and dicamba. Weed Technol 30:116–122

Fielding RJ, Stoller E W (1990) Effects of additives on efficacy, uptake, and translocation of chlorimuron ethyl-ester. Weed Technol 4:264–271

Freeze RA, Cherry JA (1979) Groundwater. Englewood Cliffs, NJ: Prentice-Hall. 604 p

Green JM, Hale T (2005) Increasing the biological activity of weak acid herbicides by increasing and decreasing the pH of the spray mixture. J ASTM Int 2:62–71

Gronwald JW, Jourdan SW, Wyse DL, Somers DA, Magnusson MU (1993) Effect of ammonium sulfate on absorption of imazethapyr by quackgrass (*Elytrigia repens*) and maize (*Zea mays*) cell suspension cultures. Weed Sci 41:325–334

Grossmann K (2010) Auxin herbicides: current status of mechanism and mode of action. Pest Manag Sci 66:113–120

Hanson CL, Rieck CE (1976) The effect of iron and aluminum on glyphosate toxicity. Page 49 *in* Proceedings of the Southern Weed Science Society, Volume 29. Las Cruces, NM: SWSS

[IDNR] Indiana Department of Natural Resources (1980) Water Quality. http://www.in.gov/dnr/water/files/804_Water_Quality.pdf. Accessed August 15, 2015

[IDNR] Indiana Department of Natural Resources (1999) Ambient Ground Water Chemistry. http://www.in.gov/dnr/water/5246.htm. Accessed August 10, 2015

Kent LM, Wills GD, Shaw DR (1991) Influence of ammonium sulfate, imazapyr, temperature, and relative humidity on the absorption and translocation of imazethapyr. Weed Sci 39:412–416

Mueller TC, Main CL, Thompson MA, Steckel LE (2006) Comparison of glyphosate salts (isopropylamine, diammonium, and potassium) and calcium and magnesium concentrations on the control of various weeds. Weed Technol 20:164–171

Nalewaja JD, Matysiak R (1991) Salt antagonism of glyphosate. Weed Sci 39:622–628

Nalewaja JD, Matysiak R (1992) Species differ in response to adjuvants with glyphosate. Weed Technol 6:561–566

Nalewaja JD, Matysiak R (1993) Spray carrier salts affect herbicide toxicity to kochia (*Kochia scoparia*). Weed Technol 7:154–158

Nalewaja JD, Woznica Z, Matysiak R (1991) 2,4-D amine antagonism by salts. Weed Technol 5:873–880

O'Sullivan PA, O'Donovan JT, Hamman WM (1981) Influence of non-ionic surfactants, ammonium sulfate, and nozzle effects on glyphosate efficacy. Can J Plant Sci 61:391–400

Patton AJ, Weisenberger DV, Johnson WG (2015) Divalent cations in spray water influence 2,4-D efficacy on dandelion (*Taraxacum officinale*) and broadleaf plantain (*Plantago major*). Weed Technol 30:431–440

Roskamp JM, Chahal GS, Johnson WG (2013) The effect of cations and ammonium sulfate on the efficacy of dicamba and 2,4-D. Weed Technol 27:72–77

Sandberg C L, Meggitt WF, Penner D (1978) Influence of spray volume and calcium on glyphosate phytotoxicity. Weed Sci 26:476–479

Shea PJ, Tupy DR (1984) Reversal of cation-induced reduction in glyphosate activity with EDTA. Weed Sci 32:802–806

Shilling DG, Haller WT (1989) Interaction effects of diluent pH and calcium content of glyphosate activity on *Panicum repens* L. (torpedo grass). Weed Res 29:441–448

Stahlman PW, Phillips WM (1979) Effects of water quality and spray volume on glyphosate phytotoxicity. Weed Sci 27:38–41

Stahlman PW, Currie RS, El-Hamid MA (1997). Nitrogen carrier and surfactant increase foliar herbicide injury in winter wheat (*Triticum aestivum*). Weed Technol 11:7–12

Thelen KD, Jackson EP, Penner D (1995) The basis for the hard-water antagonism of glyphosate activity. Weed Sci 43:541–548

Wanamarta G, Penner D, Kells JJ (1989) The basis of bentazon antagonism on sethoxydim absorption and activity. Weed Sci 37:400–404

Wills GD, McWhorter CG (1985) Effect of inorganic salts on the toxicity and translocation of glyphosate and MSMA in purple nutsedge (*Cyperus rotundus*). Weed Sci 33:755–761

Woznica Z, Nalewaja JD, Messersmith CG, Milkowski P (2003) Quinclorac efficacy as affected by adjuvants and spray carrier water. Weed Technol 17:582–588

Zollinger RK, Nalewaja JD, Peterson DE, Young BG (2010) Effect of hard water and ammonium sulfate on weak acid herbicide activity. J ASTM Int 7:1–10. DOI: 10.1520/JAI102869

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