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Reversal of Cation-Induced Reduction in Glyphosate Activity with EDTA[1]

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Abstract. **Glyphosate [*N*-(phosphonomethyl)glycine] phytotoxicity to greenhouse-grown wheat (*Triticum aestivum* L. 'Centurk') was greatly reduced by Ca^{2+} in the carrier solution. Addition of ethylenediaminetetraacetic acid (EDTA) restored glyphosate phytotoxicity in hard water and increased phytotoxicity in deionized water. The phytotoxicity of 0.1 kg ae/ha glyphosate plus 1.0% (w/v) EDTA was equal to that observed for 0.2 kg/ha of the herbicide alone in deionized water or 0.4 kg/ha in water containing 200 ppm Ca^{2+}. EDTA was more compatible with glyphosate in combination with X-77 or MON-0011 nonionic surfactant than with cationic surfactant (contained in a commercial formulation). Phytotoxicity could be increased by adding EDTA and nonionic surfactant to the latter formulation. EDTA increased glyphosate phytotoxicity to wheat when applied in water that contained high levels of calcium and bicarbonate. Glyphosate activity was greater at pH 4 than at pH 6 or 10, but EDTA was equally effective at pH 4, 6, or 10 for all levels of calcium tested up to 800 ppm. $NaHCO_3$ probably reduced phytotoxicity by increasing the pH of the glyphosate solution.**

Additional index words. **Additives, chelates, hard water, sequestrants.**

INTRODUCTION

Almost three-fourths of U.S. groundwaters have hardness ratings of 120 to over 1200 ppm $CaCO_3$ equivalent (3). In Nebraska, water hardness typically ranges from below 150 to over 300 ppm $CaCO_3$ equivalent. Water quality can greatly influence the activity of some pesticides, due to physical or chemical interactions between the active ingredients or other formulation components and inorganic ions in solution (12). Hard water typically contains high levels of carbonate and bicarbonate and is alkaline. This alkalinity can reduce the activity of acidic pesticides if the solution pH is above the pKa of the parent molecule (2).

Glyphosate activity has been shown to decrease significantly when applied in hard water (4, 5, 15, 17). This reduction is apparently due to the formation of chelates between glyphosate molecules and polyvalent cations, particularly Ca^{2+}, Mg^{2+}, Fe^{3+}, and Al^{3+}. The problem has been dealt with by increasing the amount of active ingredient or nonionic surfactant used, decreasing carrier volume, or adding acid or ammonium sulfate to the herbicide solution (5, 14, 17, 18). Reducing carrier volume probably has the most merit, since it not only decreases the ratio of cations/glyphosate molecules but also increases glyphosate concentration in spray droplets, both of which increase the phytotoxicity of the herbicide (1, 5, 14). However, significant reductions in carrier volume are not always desirable since optimum contact with target plant foliage may not be achieved.

In our laboratory an alternative approach utilizing the chelating properties of EDTA was investigated. Since the stability constant for the Ca-EDTA complex is 10.59 (8) compared to 3.25 for Ca-glyphosate (13), equilibrium should favor the Ca-EDTA complex and free glyphosate. The use of chelating agents and other additives in combination with pesticides is not a new concept. Turner and Loader (19) found that EDTA caused an increase in activity of glyphosate or dichlorprop [2-(2,4-dichlorophenoxy)propionic acid] on quackgrass [*Agropyron repens* (L.) Beauv. #[3] AGGRE]. Although it was suggested that competitive complexing of polyvalent cations by the chelator may be responsible for this observation, their studies did not investigate this relationship.

These studies were conducted to: a) evaluate EDTA as an additive to increase glyphosate phytotoxicity in hard water, b) establish the relationship between calcium level and EDTA concentration for optimum herbicide effectiveness, and c) investigate EDTA compatibility with glyphosate formulation components.

MATERIALS AND METHODS

These experiments were conducted in a greenhouse using wheat as the bioassay species. For each experiment 30 seed were planted in plastic pots containing Sharpsburg silty clay loam (Typic Argiustoll; fine, montmorillonitic, mesic). Halide lamps provided supplemental illumination at an average intensity of 870 $\mu E \cdot m^{-2} \cdot s^{-1}$ PAR for a total of 16 h/day. Greenhouse temperatures ranged from 22 to 33 C and relative humidity varied from 25 to 65%. Pots were subirrigated and nitrogen fertilizer was supplied by adding 1 g/L ammonium nitrate at 10-day intervals.

Natural well water was obtained from various locations across Nebraska (Table 1) or simulated hard water was prepared by adding $CaCl_2$ (reagent grade) to deionized water[4] to give the desired calcium concentration before addition of glyphosate. The effect of bicarbonate on glyphosate activity was determined by adding the herbicide to 0.005 and 0.02 M $NaHCO_3$ solutions. EDTA (trisodium salt, monohydrate 98% pure) was added, as required, to treatment solutions at 0.125, 0.25, 0.5, or 1.0% (w/v). Glyphosate

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[3] Letters following this symbol are a WSSA-approved computer code from Important Weeds of the World, 3rd ed. 1983. Available from WSSA, 309 West Clark Street, Champaign, IL 61820.

[4] Nanopure system, Sybron/Barnstead, Kirkwood, MO. Deionized water contained <5 ppm Ca^{2+}.

Table 1. Analysis of water obtained from several sites in Nebraska.

Water source	pH	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	NO_3^-	PO_4^{3-}	SO_4^{2-}	CO_3^{2-}	HCO_3^-	Hardness
		(ppm)									(ppm $CaCO_3$)
Deionized	5.1	2	0	0	0	0.1	0.0	0.0	0	7	4
Lincoln	8.6	77	17	4	49	5.3	0.1	16.0	10	354	260
Denton	8.6	88	16	5	45	0.2	0.0	19.8	9	329	284
Lamar	8.5	40	9	7	12	2.0	0.0	4.2	3	170	139
North Platte	8.6	102	19	13	77	0.5	0.0	99.4	6	219	189
Scottsbluff	8.6	98	26	10	92	3.6	0.0	78.0	10	331	288

was then added as a technical grade acid or a commercially formulated product[5] to give a final application rate of 0.05, 0.1, 0.2, or 0.4 kg ae/ha in 168 L/ha of carrier. X-77 (alkylarylpolyoxyethylene glycol, fatty acids, and isopropanol) or MON-0011[6] nonionic surfactant was added to all glyphosate acid and some commercially formulated product solutions at 0.1, 0.25, 0.4, or 0.8% (v/v). No difference in activity or compatibility with EDTA was observed between the two surfactants. In one experiment, solution pH was adjusted to 4 or 6 with HCl or to 10 with KOH.

Herbicide treatments were applied when the plants were 18-22 cm tall, which was generally after 10 days of growth. Plants were sprayed at a pressure of 138 kPa in two successive passes with a bench top sprayer (6) to obtain the desired application rate. All treatments were arranged in a completely randomized design with three replications and the experiments were repeated.

Usually, glyphosate injury symptoms first appeared after 4 days. Plants were harvested 2 weeks after treatment by cutting at soil level. Fresh weights were measured, plants were dried at 65-70 C for at least 48 h, and dry weights were determined. Statistical analysis using fresh- or dry-weight data produced the same significant separation of treatment means. Data are reported as percent reduction in fresh weight compared to untreated controls. EDTA alone did not visibly injure plants or affect harvest weight.

RESULTS AND DISCUSSION

Calcium, EDTA, and glyphosate activity. The activity of glyphosate applied at 0.1 kg/ha was significantly reduced by the presence of 50 ppm Ca^{2+} in the carrier solution (Figure 1). An analogous study indicated that Mg^{2+} had a similar effect (data not presented). At 100 ppm Ca^{2+}, phytotoxicity at the 0.1 kg/ha rate was less than 30% of that applied in water containing negligible calcium. The addition of 0.125% (w/v) (0.2 kg/ha) EDTA was sufficient to restore activity lost due to 100 ppm calcium in the treatment solution. Higher EDTA rates enhanced phytotoxicity such that the activity of 0.1 kg/ha glyphosate plus 1.0% (w/v) EDTA was similar to or greater than that of 0.4 kg/ha glyphosate alone (Table 2). The effect of EDTA appeared to be maximal at 1.0%, and further increases in the quantity the chelate added did not significantly increase phytotoxicity, even in carrier containing 800 ppm Ca^{2+} (data not presented).

The fact that EDTA increased glyphosate phytotoxicity in water containing negligible calcium indicated an additional effect of the chelator. This occurred in all experiments and suggested that EDTA either enhanced penetration of glyphosate through plant membranes or increased the activity of the herbicide inside the plant. Research using *Escherichia*

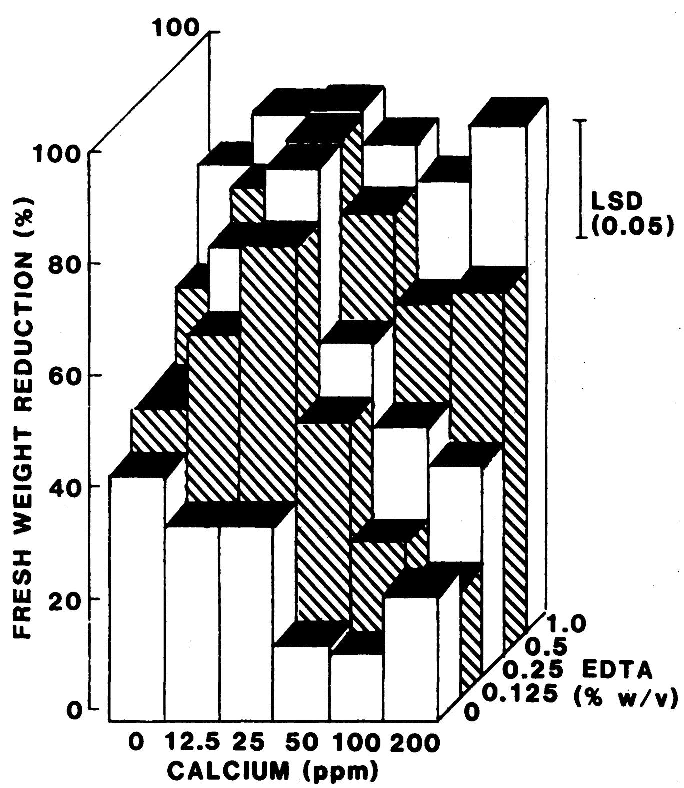

Figure 1. Reduction in wheat fresh weight 2 weeks after application of 0.1 kg ae/ha glyphosate + 0.25% v/v X-77 as influenced by calcium and EDTA in the spray solution.

[5] Roundup is a product of Monsanto, St. Louis, MO, which contains the isopropylamine salt of glyphosate as the active ingredient.
[6] Monsanto, St. Louis, MO.

Table 2. Effect of glyphosate, calcium, and EDTA on the fresh-weight reduction of wheat plants 2 weeks after herbicide application in the greenhouse. Treatment solutions contained 0.1% v/v MON-0011.

Glyphosate rate	<5 ppm Ca^{2+}			200 ppm Ca^{2+}			800 ppm Ca^{2+}		
	No EDTA	0.5% EDTA	1.0% EDTA	No EDTA	0.5% EDTA	1.0% EDTA	No EDTA	0.5% EDTA	1.0% EDTA
(kg ae/ha)	(% reduction)								
0.05	4	56	72	1	35	61	0	0	38
0.10	32	75	88	6	71	86	5	12	62
0.20	72	87	92	19	83	92	15	44	82
0.40	92	92	94	62	92	93	38	78	94
LSD (0.05)	10			19			16		

coli and higher animal membranes has shown that EDTA will effectively increase uptake of antibiotics and other organic chemicals (7, 9, 11, 21). Chelators such as EDTA have also been routinely used by plant physiologists to manipulate membrane integrity and alter calcium ion concentrations in cell preparations (3, 20).

Effect of formulation. EDTA was found to be more compatible with nonionic surfactant plus glyphosate than with the commercially formulated product which contains 1.8% cationic surfactant (Figures 2 and 3). Although this commercial formulation was equal to or better than a laboratory mixture of glyphosate acid plus nonionic surfactant in the absence of EDTA, the addition of the chelator gave superior results in the latter mixture. This was probably due to antagonism between EDTA anions and the cationic surfactant in the commercially formulated product. Free surfactant is necessary for adequate glyphosate uptake, and the addition of nonionic surfactant increased the activity of the commercially formulated product plus EDTA treatment. These observations were made either in the presence or absence of 800 ppm calcium, which show both the efficient chelating properties of EDTA and its additional effect of enhancing glyphosate activity.

Effect of EDTA in natural well water. EDTA was highly effective in reversing the inhibition of glyphosate phytotoxicity when applied in Nebraska well waters (Figure 4). As previously discussed, EDTA was more compatible with

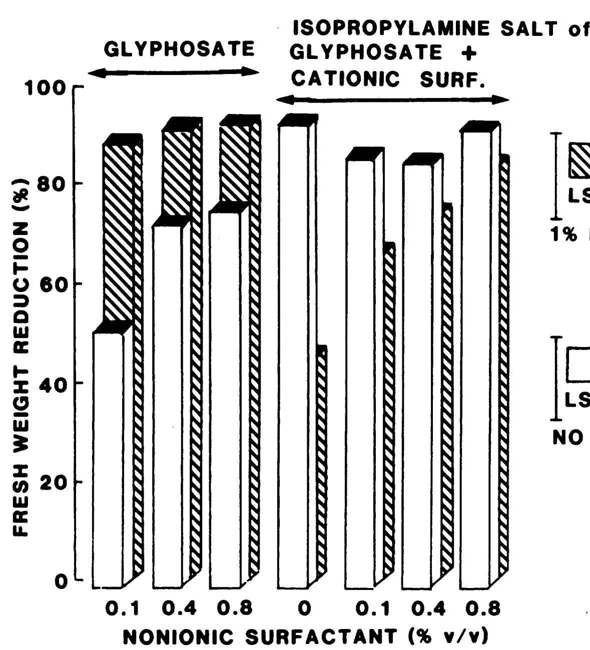

Figure 2. Influence of formulation, nonionic surfactant, and EDTA on the phytotoxicity of 0.1 kg ae/ha glyphosate to wheat 2 weeks after application in deionized water. Glyphosate = glyphosate acid, glyphosate isopropylamine salt + cationic surfactant = commercially formulated product.

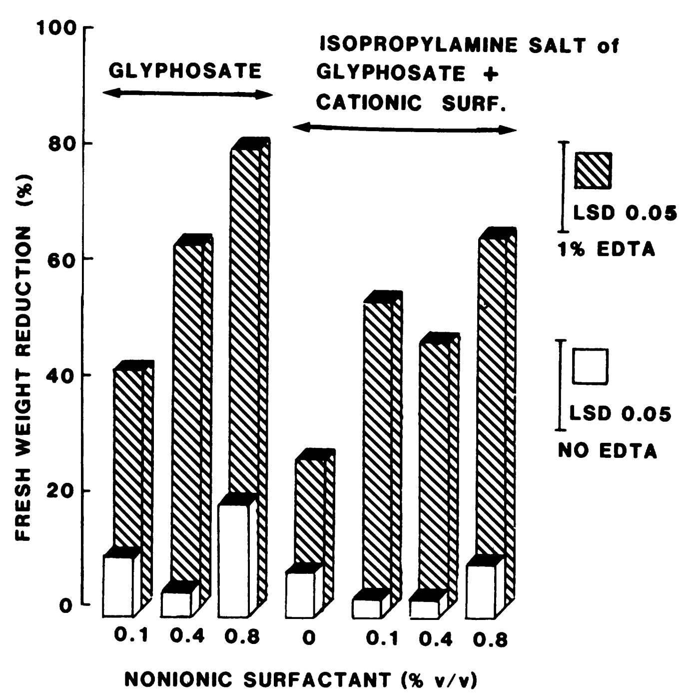

Figure 3. Influence of formulation, nonionic surfactant, and EDTA on the phytotoxicity of 0.1 kg ae/ha glyphosate to wheat 2 weeks after application in 0.020 M $CaCl_2$. Glyphosate = glyphosate acid, glyphosate isopropylamine salt + cationic surfactant = commercially formulated product.

glyphosate acid plus nonionic surfactant than with the commercially formulated product. The former treatment gave activity equivalent to that obtained with deionized water. However, the addition of 0.25% X-77 to the commercial formulation plus EDTA solution produced similar results (data not shown). Again, it is noteworthy that even in deionized water EDTA increased glyphosate phytotoxicity to wheat.

Effect of pH and bicarbonate. Glyphosate is an amino acid, with four pKa values of <2, 2.6, 5.6, and 10.6 (16). Research data indicate that glyphosate activity will increase sharply at acidic pH but will not change significantly from neutrality to more alkaline levels (5). This observation was also made in our laboratory either in the presence or absence of calcium where no EDTA had been added to the treatment solution (Figure 5). EDTA consistently reversed inhibition of glyphosate by calcium and improved glyphosate activity at all pH levels tested. The pH of a 0.004 M solution of glyphosate in deionized water was 3. The addition of $NaHCO_3$ raised the solution pH to 5.5, and the resultant decrease in glyphosate activity was not significantly different than that caused by adjusting pH to 6 or 10 with HCl or KOH. This suggests that an increase in pH may be the major cause of reduced glyphosate activity in the presence of bicarbonate. EDTA also reversed this inhibition, again demonstrating another effect of the chelator, since Na^+ will not form a chelate with glyphosate and has not been shown to significantly affect the activity of the herbicide (4, 10, 17).

These studies support previous research indicating that glyphosate activity is reduced in hard water, primarily due to its being complexed with divalent cations and that glyphosate activity increases as pH decreases (4, 5, 17, 19). Furthermore, the reduction in activity due to bicarbonate appears to be primarily a pH effect. EDTA, a common chelating agent, was found to increase glyphosate activity in simulated hard water or natural well water. Highest herbicide activity was obtained with 1.0% (1.5 kg/ha) EDTA, although less chelator would restore activity lost due to Ca^{2+} in solution. Glyphosate phytotoxicity in deionized water was also increased by adding EDTA. EDTA was effective between pH 4 and 10 but it was more compatible with nonionic than with cationic surfactant. A new glyphosate formulation[7], which does not contain surfactant, has been found in our laboratory to be compatible with EDTA and nonionic surfactant (data not presented). Observations from preliminary field research with EDTA corroborate the results of the greenhouse experiments presented here.

[7] Rodeo is a product of Monsanto, St. Louis, MO.

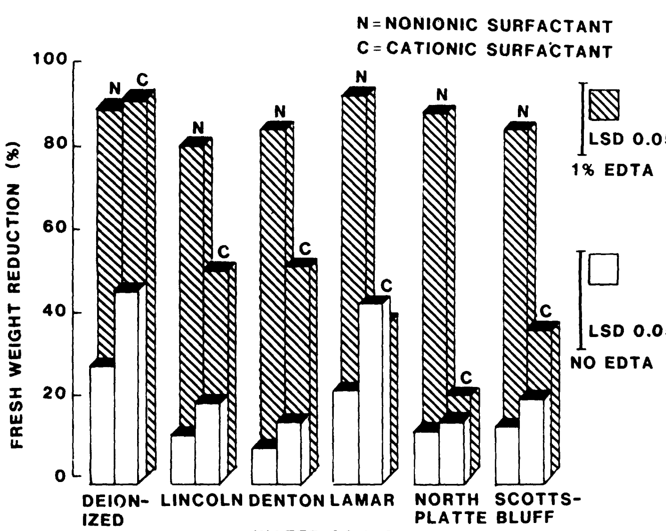

Figure 4. Reduction in wheat fresh weight 2 weeks after application of 0.1 kg ae/ha glyphosate + 0.25% v/v X-77 (N) or 0.1 kg ae/ha glyphosate isopropylamine salt + cationic surfactant (commercially formulated product) (C) as influenced by Nebraska well water source and EDTA addition to the spray solution.

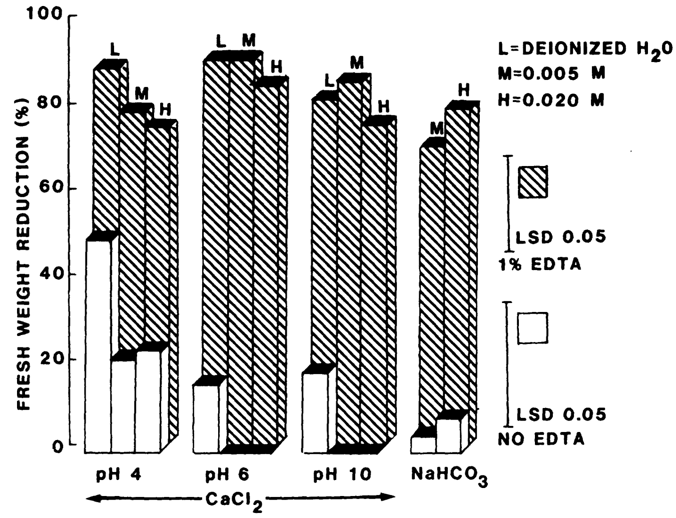

Figure 5. Reduction in wheat fresh weight 2 weeks after application of 0.1 kg ae/ha glyphosate + 0.25% v/v X-77 in deionized water as influenced by $CaCl_2$, $NaHCO_3$, pH, and EDTA.

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