MANGANESE FERTILIZER ANTAGONISM OF GLYPHOSATE EFFICACY

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Abstract

Michigan soybean producers have observed antagonism of glyphosate efficacy in tank mixtures with foliar manganese (Mn) fertilizers. The objectives of this study were to (1) evaluate four Mn fertilizer formulations for their effect on glyphosate activity, (2) evaluate the effect of Mn fertilizer application timing on glyphosate activity, (3) evaluate the efficacy of three adjuvants in overcoming the Mn fertilizer antagonism of glyphosate, (4) determine the spray solution ratio of Mn^{2+} and glyphosate where the antagonism occurs, and (5) understand why the antagonism occurs. Four formulations of Mn fertilizers were applied in tank mixes with glyphosate: Mn-ethylaminoacetate (Mn-EAA), Mn-lignin sulfonate (Mn-LS), Mn-ethylenediaminetetraacetate (Mn-EDTA), and $MnSO_4 \bullet H_2O$ ($MnSO_4$). Three of the Mn formulations (Mn-EAA, Mn-LS, and $MnSO_4 \bullet H_2O$) antagonized glyphosate efficacy in both greenhouse and field bioassays. Mn-EDTA did not antagonize glyphosate efficacy. In greenhouse studies, Mn-EAA sprayed less than 3 days prior to glyphosate application antagonized glyphosate efficacy on velvetleaf but not giant foxtail. The degree of antagonism on velvetleaf increased as the interval between applications decreased. However, the antagonism did not persist if Mn was sprayed at least 1 day after glyphosate. The adjuvants diammonium sulfate (AMS), EDTA, and citric acid each reduced some of the antagonism when included in glyphosate-Mn tank-mixtures. Regression equations developed from experiments conducted using a wide range of glyphosate and Mn concentrations show a 10-25% decrease in velvetleaf control for each additional kg Mn/ha added to the spray solutions from the Mn-EAA, Mn-LS and $MnSO_4$ fertilizer formulations. Experiments that measured the rate of ^{14}C-labeled glyphosate absorption by velvetleaf showed that two formulations, Mn-LS and $MnSO_4$ reduced glyphosate absorption significantly. Analysis with Electron Spin Resonance (ESR) spectroscopy demonstrate that a 2:1 glyphosate:Mn complex forms in spray solution. The formation of a glyphosate-Mn^{2+} complex in tankmix solution is likely responsible for the reduced absorption of glyphosate and may also interfere with glyphosate activity in the plant.

Introduction

Manganese (Mn) deficiency is the most common soybean micronutrient deficiency in Michigan . It occurs on mildly acidic or alkaline sands, high organic matter soils, and alluvial soils derived from calcareous materials where Mn availability is restricted by low Mn concentrations and/or high soil pH (Tisdale et al. 1993). Mn deficiency appears as interveinal chlorosis in newly emerging tissue. Both soil- and foliar-applied Mn fertilizers have been effective in alleviating deficiency symptoms and enhancing seed yield (Gettier et al. 1984, 1985). Much less Mn is required for foliar applications than for banded or broadcast soil applications, although multiple foliar applications are necessary during some growing seasons (Gettier et al. 1985). Typical foliar Mn fertilizers include $MnSO_4$ and several different Mn chelates, including EDTA, citric acid, glucoheptonate, and lignin sulfonate.

Because the appearance of Mn deficiency symptoms frequently coincides with time of post-emergence herbicide applications, producers have tank mixed herbicides and fertilizers to reduce application trips and costs. Heckman et al. (1999) reported that $MnSO_4$ did not affect the weed control efficacy of four soybean herbicides (aciflourfen, chlorimuron ethyl, imazethapyr, and bentazon), and the herbicides did not interfere with soybean utilization of Mn. However, with the widespread adoption of glyphosate-resistant soybean lines, reports have indicated that glyphosate efficacy is antagonized when tank mixed with some Mn fertilizers (Bailey et al. 2002).

Glyphosate is a non-selective, foliar applied herbicide that is widely used on soybeans, canola, cotton, and corn that have been engineered to contain glyphosate resistant genes. Glyphosate inhibits the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, which is a key enzyme in the pathway producing aromatic amino acids in plants. Plants die as the supply of aromatic amino acids are depleted and protein synthesis is disrupted (Vencill, 2002).

Glyphosate also functions as a chelating agent and forms stable complexes with di- and trivalent metal cations (Madsen et al. 1978; Glass 1984). Hard water cations such as Ca^{2+}, Mg^{2+}, and Fe^{3+}, antagonize glyphosate efficacy by complexing with glyphosate and forming salts that are not readily absorbed by plants (Thelen et al. 1995a). Glyphosate potency may also be reduced when plants secrete antagonistic cations, e.g., Ca^{2+}, to the leaf surface (Hall et al. 2000). The hard water antagonism may be overcome by increasing the glyphosate concentration relative to the cation content, by adding a chelate such as EDTA or citric acid, or by adding an excess of monovalent cations such as NH_4^+, K^+, or Na^+ to the spray solution (Buhler and Burnside 1983a, 1983b; Shea and Tupy 1984; Thelen et al. 1995a; Thelen et al. 1995b).

Materials and Methods

Field Trials. Field trials were conducted at the East Lansing Crops and Soils Teaching and Research Field Lab. East Lansing, MI, in 2001 and 2002. Soybeans were planted in 38 cm rows at 444,800 seeds/ha (2001, Pioneer 92B36; 2002, Mycogen 5251RR). The dominant weed species each year was common lambsquarters. Plots were treated with herbicide when weeds were 15-20 cm tall (13-16 June 2001, 22-25 June 2002). Treatments were applied using a backpack sprayer (TeeJet 8002 XR nozzles) at a volume of 187 L/ha, pressure of 292 kPa, and speed of 4.8 kph. Plots were evaluated for weed control 14, 28, and 100+ days after treatment. Tank mix treatments were a factorial of glyphosate (0.63 kg ae/ha), Mn fertilizer (Mn-EDTA, Mn-EAA, and Mn-LS were applied at 9.4 L/ha, $MnSO_4 \cdot H_2O$ was applied at 7.8 kg/ha), and AMS (0 and 20 g/L). In a separate application timing component of the study Mn-EAA was applied 2 d, 1 d, and 1 h before glyphosate, then 1 h and 1 d after glyphosate.

Greenhouse Bioassays. Velvetleaf and giant foxtail seed were planted in potting mix in 945-ml plastic pots. Natural light was supplemented by high-pressure sodium lights. Daylength was 16 h and the temperature was 23 ± 3 °C. Prior to receiving treatment, plants were thinned to one velvetleaf plant per pot and three giant foxtail plants per pot. Pots were randomly assigned to treatments. Each experiment consisted of four replications and was repeated. At the time of treatment, velvetleaf plants had six leaves (approximately 14 cm tall), and giant foxtail had five leaves (approximately 24 cm tall). Treatments were applied using a single tip track sprayer. Spray pressure was 172 kPa, and spray volume was applied to simulate a field rate of 187 L/ha. Plants were rated on a scale of 0 (healthy) to 10 (dead plant) 7 and 14 days after treatment. Two commercial formulations of the isopropylamine (IPA) salt of glyphosate (Roundup Ultra™ and Roundup UltraMAX™) and four formulations of Mn fertilizer were used in the bioassays: $MnSO_4$-ethylaminoacetate. Mn-EDTA, $MnSO_4$-lignin sulfonate and $MnSO_4 \cdot H_2O$. All Mn fertilizer-glyphosate treatment combinations were applied with and without AMS at 20 g/L. Unless noted. treatment solutions were prepared with tap water. The glyphosate rates (0.28 kg ae/ha or 0.45 kg ae/ha) used in these bioassays were significantly lower than those recommended for field use, and Mn rates (9.4 L/ha or 7.8 kg/ha) were relatively high for foliar applications. These rates were selected to maximize the potential for the antagonism to occur. Separate applications of Mn and glyphosate studies were conducted by applying Mn-EAA to velvetleaf 3 d, 2 d, 1 d, and 1 h before glyphosate, in tank mix with glyphosate, and 1 h, 1 d, and 2 d after glyphosate. Adjuvant experiments analyzed three different adjuvants: AMS (154 mM or 20 g/L), citric acid (100 mM or 21g/L), and the tetrasodium salt of EDTA (100 mM or 38 g/L). Variable rates of Mn and glyphosate tested six rates of IPA-glyphosate (0.00, 0.21, 0.41, 0.83, 1.66, and 3.32 kg ae/ha) in tank mixes with six rates of each Mn fertilizer (0.00, 0.11, 0.22, 0.45, 0.90, 1.8 kg Mn/ha). Distilled water was used in the variable rates study.

^{14}C-glyphosate uptake experiments. Velvetleaf was seeded in 945-mL plastic pots and grown in a greenhouse for 19-20 days, at which point they were moved into an environmental growth chamber. Two 1 µL drops were applied to the adaxial side of the second leaf. Treatment solutions contained formulated glyphosate (Roundup Ultra), spiked with 127 Bq/µL of. ^{14}C-glyphosate, labeled at the methyl carbon of N-phosphonomethyl glycine. Total glyphosate concentration was equivalent to 280 g/ha in 187 L/ha carrier. Manganese fertilizers were added at rates of 9.4 L/ha (Mn-EAA, Mn-EDTA, Mn-LS) or 7.8 kg/ha ($MnSO_4$), and AMS at 20 g/L. Treated leaves were excised and rinsed at 4, 24, and 48 h after treatment using 4 mL of an acid solution (1 part 0.1 M HCl and 1 part methanol) (Hall et al., 2000). The rinsate was radioassayed by liquid scintillation spectrophotometry.

Statistical Analysis. Data were analyzed using proc mixed and proc glm of SAS (SAS, 2001).

Results and Discussion

Tankmix antagonism. Three of the four Mn fertilizer formulations caused significant antagonism of glyphosate efficacy on the weed spectrum tested. In tankmix solutions without AMS, Mn-EAA, Mn-LS, and $MnSO_4$ significantly reduced weed control compared to the glyphosate check. Mn-EAA and Mn-LS reduced efficacy on all three species (Tables 1 and 2). In solutions with AMS, weed control was significantly less in Mn-EAA and $MnSO_4$ tankmixes than in the glyphosate+AMS check. The Mn-LS antagonism was ameliorated by the addition of AMS for velvetleaf and common lambsquarters control. but not for giant foxtail control (Tables 1 and 2). Mn-EDTA (6%) did not antagonize glyphosate efficacy (Tables 1 and 2). EDTA functions as a hexavalent chelate with high stability constants for most di- and trivalent metals (Martell and Smith 1974). Excess EDTA in the Mn fertilizer may have complexed hard water ions present in tap water that otherwise would have complexed with glyphosate and interfered with its efficacy. In addition, the Mn^{2+} stability constant of EDTA (log K = 13.81) is much higher than that of glyphosate (log K = 5.53) and may have prevented Mn^{2+} from dissociating in solution and complexing with glyphosate (Martell and Smith 1974; Madsen et al. 1978).

Some fertilizer labels advocate a specific order of adding Mn and herbicide to the spray solution. However, when the spray solution was well agitated the degree of antagonism was equal regardless of whether $MnSO_4$ was added to the spray solution before or after glyphosate (data not shown).

Timing of separate applications. After establishing that there was a significant antagonism of glyphosate activity in tankmix solutions with Mn, experiments were conducted to determine if the antagonism occurred when Mn and glyphosate were applied in separate applications. Mn-EAA was chosen as the Mn source because it consistently antagonized glyphosate when combined in solutions with and without AMS. Mn-EAA was applied 3, 2, 1 d and 1 h before glyphosate, and then 1 h, 1 and 2 d after glyphosate. The degree of antagonism increased as the period of time between applications decreased when Mn was sprayed before glyphosate. There was also a slight but significant antagonism relative to glyphosate+AMS when Mn was sprayed one hour (+1h) after glyphosate+AMS (Table 4). This phenomenon is species-dependant. It did not occur on giant foxtail in the greenhouse, nor was it observed on common lambsquarters in field trials.

There are several potential reasons why the timing antagonism occurred in velvetleaf. First, velvetleaf is pubescent and some Mn may have adhered to the leaf hairs. Subsequent applications of glyphosate would have washed the suspended Mn along with it, allowing the glyphosate and Mn to complex. Second, when velvetleaf is misted with water, calcium rich substances are released to the leaf surface from specialized trichomes known as chalk glands (Hall et al. 2000). Treating the plants with foliar Mn-EAA, e.g. misting them prior to spraying glyphosate, may have increased the amount of Ca^{2+} on the leaf surface, thereby allowing Ca^{2+}-glyphosate complexes to form and increase the antagonism. Third, with only a 1 h gap between applications, the Mn and glyphosate may have penetrated the cuticle together and complexed during that process.

Tankmix adjuvants. We observed in the initial tankmix bioassays that AMS ameliorated some, but not all, of the Mn antagonism of glyphosate activity (Tables 1 and 2). The adjuvants EDTA and citric acid, which previously were shown to have a positive effect in overcoming the hard water antagonism of glyphosate, were tested to determine if they might more completely overcome the antagonism (Shea and Tupy 1984; Thelen et al. 1995b). Citric acid and EDTA were added to spray solutions at a concentration of 100 mM (21 and 38 g/L, respectively), and AMS was added at 154 mM (or 20 g/L). Concentrations of Mn^{2+} for each of the fertilizers were: Mn-EAA – 52 mM, Mn-EDTA – 72 mM, Mn-LS – 57 mM, and $MnSO_4$ – 244 mM (values calculated from the fertilizer analysis published on labels).

Including AMS, EDTA, or citric acid increased weed control for each glyphosate-Mn fertilizer combination, except Mn-EDTA (Table 5). However, the degree of increase varied significantly depending upon the specific combination of Mn fertilizer, adjuvant, and species. The antagonism was considered present if the degree of control did not match that of 'glyphosate alone + AMS.' The specific interactions may be observed in Table 5. It is our conclusion that neither EDTA nor citric acid were sufficiently more effective than AMS with the fertilizers tested to justify the additional cost of using them to overcome the Mn antagonism of glyphosate efficacy.

Variable rates of glyphosate and manganese fertilizer. In the work described above relatively low rates of glyphosate were tankmixed with relatively high rates of Mn fertilizer to increase the likelihood of identifying any antagonisms that may exist. Bailey et al. (2002) reported that when the concentration of Mn^{2+} relative to glyphosate reached a critical threshold, control of large crabgrass decreased significantly. Growers often choose to spray the lowest rate of glyphosate possible to save money. We wanted to obtain an estimate that would predict what effect reducing manganese and/or glyphosate rates would have on velvetleaf control. We conducted experiments with four Mn fertilizers (Mn-EAA, Mn-EDTA, Mn-LS, and $MnSO_4$) at six rates (0.00, 0.11, 0.22, 0.45, 0.90, 1.8 kg Mn/ha) in a factorial combination with six rates of glyphosate (0.00, 0.21, 0.41, 0.83, 1.66, and 3.32 kg ae/ha), both with and without AMS. Based on weed control data from visual observations, we developed regression equations to describe the relationship of the rate of Mn to the rate of glyphosate (Table 5). The Mn-EAA, Mn-LS, and $MnSO_4$ terms were each negative and significant in the equations (Table 5). Thus, one may expect approximately 10-25% reduction in velvetleaf control for each kg/ha of Mn^{2+} that is included in the tankmix from these formulations.

Glyphosate absorption. Significantly less ^{14}C-labeled glyphosate was absorbed from the Mn-LS and $MnSO_4$ solutions at 48 h than from the glyphosate check (Table 6). Absorption from the Mn-EDTA tankmixes paralleled those of the glyphosate check. Mn-EAA solutions were absorbed very rapidly, 42% within 4 h, but no additionally significant absorption occurred thereafter. Adding AMS improved the absorption of ^{14}C-glyphosate in the glyphosate alone and tankmix solutions with Mn-LS and $MnSO_4$ (data not shown). The absorption data reflect the efficacy data for glyphosate, Mn-LS, $MnSO_4$, and Mn-EDTA solutions, but not those of Mn-EAA. Glyphosate in Mn-EAA solutions was absorbed at rates equal to the glyphosate check, but weed control efficacy was significantly less.

Cause of the antagonism. Using NMR spectroscopy, Thelen et al. (1995) reported that hard water cations, such as Ca^{2+}, complex with glyphosate, causing reduced glyphosate absorption and the observed hard water antagonism. $MnSO_4$ in solution dissociates into Mn^{2+} and SO_4^{2-}. Manganese in Mn-LS and Mn-EAA formulations is weakly chelated and may also dissociate and bind to a stronger ligand in solution, in this case, glyphosate. Analysis of $MnSO_4$ and glyphosate acid in solution have demonstrated that each Mn^{2+} will form a complex with two glyphosate molecules. We believe that this interaction is the primary cause for the reduced efficacy of glyphosate in glyphosate-Mn fertilizer tankmixes.

Summary

The data reported in this paper shows that caution should be used when tankmixing glyphosate and Mn fertilizers, particularly when environmental conditions favor poor weed control (e.g., large weeds, drought, etc.) or when reduced rates of glyphosate are used. In addition, fertilizers with strong chelates, such as EDTA, may be preferable for use in tankmixes.

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Table 1. Control of velvetleaf (ABUTH) and giant foxtail (SETFA) 14 d after treatment with glyphosate and glyphosate-Mn fertilizer[a] tankmixes in greenhouse bioassays.

		Weed Control				
Weed Species	Adjuvant	glyphosate alone	Mn-EAA + glyphosate	Mn-EDTA + glyphosate	Mn-LS + glyphosate	$MnSO_4 \cdot H_2O$ + glyphosate
ABUTH				-----%-----		
	none[b]	28b	8c	82a	1c	23b
	AMS	88a	66bc	88a	78ab	49c
SETFA						
	none	63b	12c	96a	19c	53b
	AMS	97a	58c	93a	78b	71b

[a] Glyphosate was applied at 0.28 kg ae/ha, Mn-EAA, Mn-EDTA, and Mn-LS at 9.4 L/ha, and $MnSO_4$ at 7.8 kg/ha. AMS was added at 20 g/L.
[b] Means within a row followed by the same letter are not statistically different, p = 0.05, Tukey adjusted.

Table 2. Control of common lambsquarters (CHEAL) 28 d after treatment with glyphosate and glyphosate-Mn fertilizer[a] tankmixes in field trials.

		Weed Control				
Year	Adjuvant	glyphosate alone	Mn-EAA + glyphosate	Mn-EDTA + glyphosate	Mn-LS + glyphosate	$MnSO_4 \cdot H_2O$ + glyphosate
2001				-----%-----		
	none[b]	98a	40c	99a	73b	70b
	AMS	99a	85a	99a	98a	86a
2002						
	none	90a	10b	73a	33b	13b
	AMS	81a	35bc	60a	69a	30c

[a] Glyphosate was applied at 0.63 kg ae/ha, Mn-EAA, Mn-EDTA, and Mn-LS at 9.4 L/ha, and $MnSO_4$ at 7.8 kg/ha. AMS was added at 20 g/L.
[b] Means within a row followed by the same letter are not statistically different, p = 0.05, Tukey adjusted.

Table 3. Control of velvetleaf (ABUTH) 14 d after treatment with glyphosate or a glyphosate-Mn-EAA tankmix[a]. Plants were treated separately with Mn-EAA 3, 2, or 1 day before (-3 d, -2 d, -1 d), 1 hour before (-1 h), 1 hour after (+1 h), and 1 or 2 days after the glyphosate application.

	Weed Control								
Adjuvant	no Mn	-3 d	-2 d	-1 d	-1 h	tankmix	+1 h	+1 d	+2 d
none	30a	18ab	12c	14bc	5c	2c	22ab	26ab	30a
AMS	89a	79abc	76bc	70cd	62d	32e	76bc	84ab	93a

[a] Glyphosate was applied at 0.28 kg ae/ha, Mn-EAA at 9.4 L/ha. AMS was added at 20 g/L.
[b] Means within a row with the same letter are not statistically different, p=0.05, Tukey adjusted.

Table 4. Control of velvetleaf (ABUTH) and giant foxtail (SETFA) 14 days after treatment with tankmixes of glyphosate. four Mn fertilizer formulations, and three adjuvants[a].

Weed species	Adjuvant	Weed Control				
		glyphosate alone	Mn-EAA + glyphosate	Mn-EDTA + glyphosate	Mn-LS + glyphosate	$MnSO_4$ + glyphosate
ABUTH		%				
	AMS[b]	93a	64a	88a	83a	54b
	citric acid	-	46b	66b	51b	74a
	EDTA	-	55ab	81ab	45b	36c
	None	58b	2c	81ab	11c	13d
SETFA						
	AMS	98a	61b	90a	70a	83ab
	citric acid	-	65b	92a	48b	89a
	EDTA	-	93a	99a	81a	71b
	None	62b	10c	92a	8c	56c

[a] Glyphosate was applied at 0.45 kg ae/ha, Mn-EAA, Mn-EDTA. and Mn-LS at 9.4 L/ha, and $MnSO_4$ at 7.8 kg/ha. AMS was added at 20 g/L.

[b] Means within a column for a given species followed by the same letter are not significantly different. p=0.05, Tukey adjusted. The standard errors were 3.0 (ABUTH) and 2.9 (SETFA).

Table 5. Regression equation describing control of velvetleaf 14 days after treatment with tankmix solutions of glyphosate (kg ae/ha) and Mn (kg Mn^{2+}/ha). Regression equations were developed from visual ratings of velvetleaf control. Six rates of glyphosate and six rates of each Mn fertilizer were used in the experiment.

Mn	Adjuvant	Regression equation	adj R^2
Mn-EAA	None	23.05 + 27.17(glyphosate) – 11.94(Mn-EAA)	0.6774
Mn-EAA	AMS[a]	54.75 + 20.61(glyphosate) – 23.13(Mn-EAA)	0.5221
Mn-EDTA	None	23.76 + 26.40(glyphosate) + 3.98[b](Mn-EDTA)	0.6888
Mn-EDTA	AMS	46.14 + 21.30(glyphosate) – 1.52[b](Mn-EDTA)	0.4476
Mn-LS	None	19.85 + 28.91(glyphosate) – 9.75(Mn-LS)	0.7060
Mn-LS	AMS	55.42 + 20.58(glyphosate) – 16.48(Mn-LS)	0.4612
$MnSO_4$	None	11.91 + 29.93(glyphosate) – 12.46($MnSO_4$)	0.8133
$MnSO_4$	AMS	44.53 + 24.34(glyphosate) – 16.00($MnSO_4$)	0.5649

[a] AMS was added at 20 g/L.

[b] Term was not statistically significant, p =0.05.

Table 6. Velvetleaf absorption of ^{14}C-labeled glyphosate from glyphosate-Mn fertilizer tankmixes at 4, 24, and 48 h. Data represent the average of with and without AMS treatments.

Rinse time (h)	^{14}C-labeled glyphosate absorption				
	glyphosate alone[a]	Mn-EAA + glyphosate	Mn-EDTA + glyphosate	Mn-LS + glyphosate	$MnSO_4$ + glyphosate
	% of applied				
4	19b	42a	24b	16b	12b
24	49a	45a	48a	34a	22a
48	53a	53a	56a	33a	24a

[a] Means within a column with the same letter are not statistically different. p=0.05.

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