Effective Manganese Management for Corn and Soybean in Glyphosate-Dominant Cropping Systems: Year 1 Report

Tony J. Vyn, Yanbing Xia and Jim Camberato
Agronomy Department, Purdue University, 915 W State Street, West Lafayette, IN 47907-2054

A. Executive Summary

The Fluid Fertilizer Foundation graciously provided financial support in 2009 to Purdue University research related to improving plant Mn availability to RR corn and RR soybean when the fields on which these crops are grown receive various intensities of glyphosate application. We also welcomed in-kind support (in terms of plant and soil sample analyses) from Waters Agricultural Laboratory and Western Laboratory. This project builds on prior support received from the Indiana Soybean Alliance. We have not completed our soil and plant analyses from the 2009 experiments, but we provide some of the background context of our earlier RR soybean investigations as well as some preliminary results from our 2009 corn research in this preliminary report. The 2009 soybean results will be available later, and all 2009 results should be interpreted with caution until statistical analyses are completed. In general terms, positive leaf nutrient concentration or crop yield responses to foliar or soil micronutrient applications are more difficult to observe when the relative spatial variability of soil properties (e.g. pH, soil micronutrient concentrations) at our on-farm and research station experimental sites is high.

B. Introduction

Glyphosate [N-(phosphonomethyl)glycine] is a non-selective, broad-spectrum, foliar applied herbicide that affects the shikimic acid pathway by the inhibition of 5-enolpyruvylshikimic-3-phosphate synthase (EPSPS), thus preventing the synthesis of the aromatic amino acids and other secondary products causing plant death (Moldes et al. 2007). Glyphosate use is more and more extensive with the widespread cultivation of glyphosate-resistant (GR) transgenic crops and the adoption of no-tillage cropping systems (Ozturk et al. 2007; Cerdeira and Duke, 2006). In the USA, GR soybean acreage has increased from 2% in 1996 to over 90% in 2008 (USDA, 2008).

Although GR soybean is resistant to glyphosate, glyphosate application may result in significant injury or yield reduction with certain conditions. Field observations in many part of the USA show Mn deficiency chlorosis is associated with frequent glyphosate applications. The possible reasons for this symptom are: 1) glyphosate applied to plant foliage may form insoluble complexes with some micronutrients, like Mn or Zn and thus interfere with nutrient uptake or metabolism, and 2) glyphosate remaining on the soil surface and exudated from plant roots may have toxic effects on certain soil micro-organisms which normally reduce soil Mn to the plant-available form (Mn^{2+}) (Kremer et al. 2005).

Manganese deficiency may cause a significant yield loss. Manganese deficiency may also decrease soybean seed oil and increase seed protein content (Wilson et al. 1982). Application of Mn fertilizer has been shown to reduce the severity of chlorosis and improve grain yield. Gordon (2007) observed soybean yield increases up to 12% in response to starter-banded and foliar Mn applications in Kansas. Banded and foliar Mn fertilizer applications are two general methods of micronutrient supplementation. Banded application has the possible advantages of reducing the cost, time, and crop-trampling injury associated with post-emergence foliar applications, and the opportunity to enhance soil Mn availability in close proximity to soybean tap root over the entire period of plant nutrient uptake. Foliar application has the advantage of selective application to the Mn deficient areas during the growing season since Mn deficiency often in patchy sections of fields rather than uniformly (Henkens and Jongman, 1965). The relatively short time required for leaves to accumulate high concentrations of Mn may account for the effectiveness of foliar Mn application. Environmental conditions, such as precipitation and temperature during a particular growing season, and cultivars, are important factors affecting crop response to Mn fertilizer. Soil Mn availability is strongly influenced by soil pH and soil moisture. As soil pH increases, plant-available Mn decreases. Manganese deficiency is more likely to occur with dry soil conditions.

Concerns about suspected Mn deficiency following glyphosate application have often been expressed by Indiana farmers to Purdue Extension specialists over the last 13 years of RR soybean production. Some of the early concerns were in suspected low soil Mn soils (perhaps because of unusually high organic content, high pH, or sandy textures). But increasingly, Mn deficiency symptoms are being reported from some of the most productive soils in Indiana. Earlier trials by Dr. Huber (Professor Emeritus at Purdue University) and others have shown large soybean yield increases (up to 18 bushels per acre) by applying foliar Mn at least 8 days following glyphosate application (Table 1), by applying manganese sulfate at planting (Gordon, 2007), or by adding gypsum at planting (the latter in attempts to immobilize exuded glyphosate).

Table 1. Effect of Mn sources on herbicidal efficacy of glyphosate on RR soybeans in 2006 at Wanatah, IN. Source: Dr. D. Huber, Purdue University, 2007.

Treatment/Nutrient source	Rate	Yield	% weed control
No herbicide*	None	46 a**	0 a
Glyphosate***	24 oz/a	57 b	100 e
Glyphosate + MnCO3	0.5 # Mn/a	75 d	91 de
Glyphosate + MnSO4	0.5 # Mn/a	70 cd	93 e
Glyphosate + Mn EDTA chelate	0.25 # Mn/a	72 cd	100 e
Glyphosate + Mn AA chelate	0.15 # Mn/a	67 c	85 d

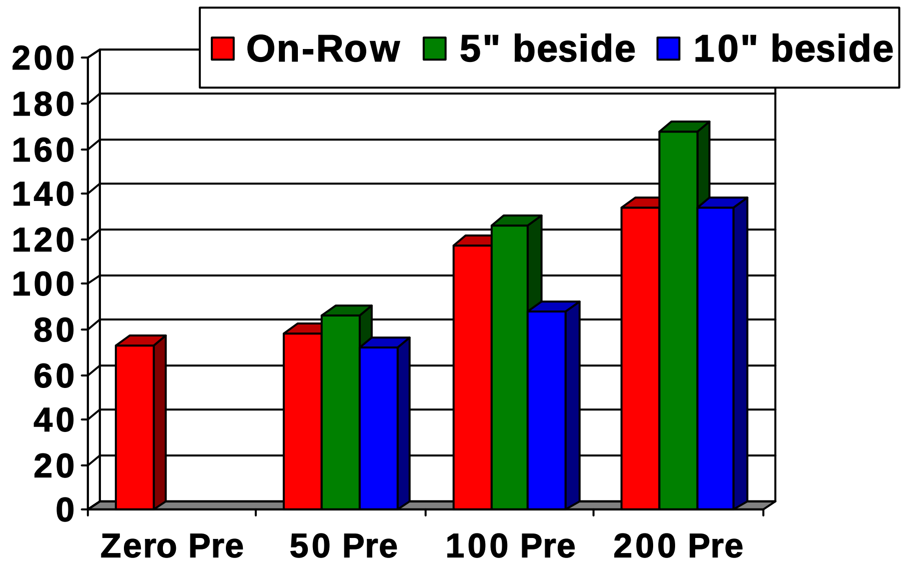
* Heavy weed pressure

**Similar letters behind the means indicate non-significant differences

*** Applied as the WeatherMax® formulation at 24 oz/a + ammonium sulfate

There are new concerns about the availability of Mn to RR corn as cumulative applications of glyphosate to both RR corn and RR soybean exceed 3-4 applications each 2-year cycle. Indiana N placement research with RTK automatic guidance equipment (funded by the Fluid Fertilizer Foundation from 2006-2008) reported that no-till corn plant Mn uptake (V-8 stage) has been observed to be much higher when RR corn has banded N fertilizer in or near the row area (Figure 1). Manganese uptake may be most limiting in RR corn production on soils with inherently low Mn when glyphosate applications are consistent and numerous (2 or more applications every year). There is little research on Mn supplementation for corn by either starter or foliar applications in North American, and it seems that such is now warranted in view of the large RR corn acreage. Furthermore, there are additional concerns for RR soybean that follow RR corn and where both crops receive multiple glyphosate applications. Crop rotations were known to affect Mn availability even before RR corn and soybean varieties were utilized (Smith, 2006).

Figure 1. Corn plant Mn concentrations (ppm) at the V-8 stage following pre-plant UAN applications of zero, 50, 100, and 200 pounds N per acre and corn row positions 0, 5, or 10 inches beside the pre-plant UAN band (Lafayette, IN, 2007).



The long-term goals of this research over the next 3 years are to improve the management of micronutrients such as Mn and Zn to help achieve the highest corn and soybean yields possible in cropping systems that are ever more reliant on glyphosate for weed control.

C. Prior Soybean Research

Materials and Methods for Soybean Mn Trials

The research was conducted in 2007 and 2008 at three locations in Northwest Indiana; Pinney-Purdue Agricultural Center near Wanatah, IN (PPAC), Rice Farm near LaCrosse, IN (Rice), and White Farm near Reynolds, IN (White). Inherent soil Mn concentrations averaged 10, 4, 13 ppm and soil pH averaged 6.3, 6.3, 7.1 at these locations, respectively, but there was considerable variability among individual plots in both available Mn and soil pH within each 4-acre experimental area. The field study was a split-plot design in all six experiments with five blocks. There were thirteen treatments/plots in each block. The main-plot treatments were glyphosate frequency: 1) no glyphosate applied (Control), 2) pre-emergence glyphosate application only (Pre only), 3) pre-emergence plus one post-emergence glyphosate applications (Pre + 1 Post), and 4) pre-emergence plus two post-emergence glyphosate applications (Pre + 2 Post). The sub-plot treatments were Mn applications: 1) no Mn applied (0), 2) 2.5 lbs/a banded Mn (BL), 3) 5.0 lbs/a banded Mn (BH), and 4) 0.5 lb/a foliar Mn application (F) which was only applied to treatment 3 (Pre + 1 Post). The glyphosate formulation was WeatherMax^{TM} and the application rate was 0.7 lb/acre per application.

Soybean was planted with the same 4-row planter in 30" (76-cm) row widths following prior corn and full-width tillage at all locations. Pre-emergence glyphosate was applied immediately after planting. The first and second post-emergence glyphosate treatments were applied roughly at V3 and V6 growth stages. The Mn form for all applications was liquid $MnSO_4$; Manganese fertilizer was banded 5 cm below and to the side of seed at planting. Foliar application occurred six weeks after planting in 2007 and eight weeks after planting in 2008.

Soybean cultivars were Beck 321NRR in 2007 and Pioneer 93M10 in 2008. They were Roundup Ready (glyphosate-resistant) soybean cultivars with maturity group 3.2 for Beck's 321NRR and 3.1 for Pioneer 93M10. Full weed control was achieved by pre-emergence application of residual herbicides. Additional hand hoeing was done in July at Rice both years. Individual plots were 20' or 30' wide (dependent on location) and 70' in length.

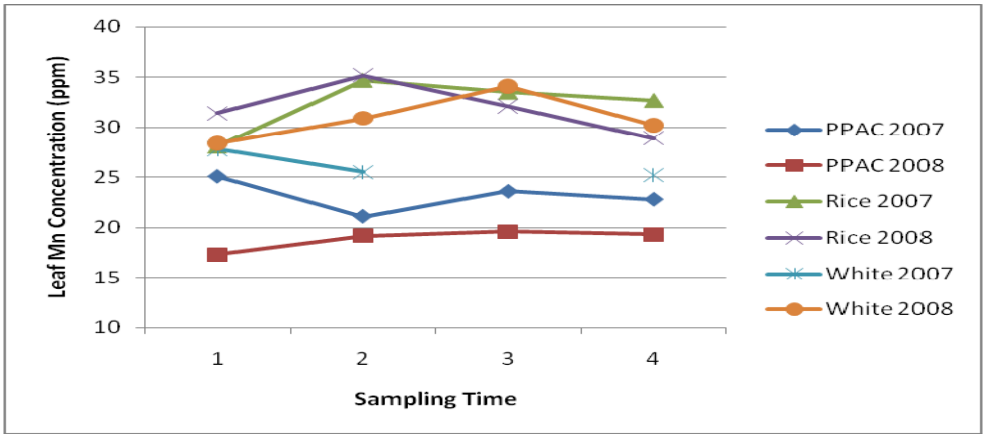
Twenty randomly chosen upper fully expanded trifoliate leaves were sampled in the center two rows of each plot four times (three times at White 2007). First and second leaf sampling was done 7~11 or 8~14 days after first or second post emergence glyphosate application to test the short-term effect of glyphosate application on soybean nutrient uptake. The third leaf sampling time was one week after second sampling, and the last sampling was one or two weeks after third sampling. The soybean was approximately at V4, R1, R2 and R3 growth stage at the first, second, third and fourth leaf sampling time. Leaf samples were dried three to five days at 60°C and then ground in preparation for tissue analysis. The center two rows in each plot were harvested by a 2-row plot combine to determine soybean yield. Years and locations were analyzed separately due to the lack of homogeneity when testing the possibility of combining years and locations. Appropriate transformation was determined by SAS PROC GLM. Analysis of variance (ANOVA) was performed using SAS PROC MIXED on all data. When treatment effects were significant at the 0.05 probability level, least-squares mean separation tests were performed.

Results and Discussion for Soybean Mn Trials

Leaf Mn concentrations always changed significantly among different leaf sampling times in our six experiments (Figure 2). But the time frame in which the leaf Mn concentration reached its maximum was inconsistent, and thus very specific to year and location.

Glyphosate application(s) did not have significant effects on leaf Mn concentrations in the upper fully expanded trifoliate leaf at any sampling time (Table 2). The first leaf sample was taken 7 – 11 days after the first post glyphosate application at about the V4 growth stage. Leaf Mn concentrations in first leaf sampling time (first column in each sub-table in Table 1) showed no statistical difference between 'Pre only' and 'Pre + 1Post' treatments. Second leaf sampling was done 8- 14 days after second post glyphosate application at about R1 growth stage. When using the values of first leaf sampling time as control, leaf Mn concentrations of second sampling time in 'Pre + 2 Post' Glyphosate treatment increased more or decreased less than that of 'Pre + 1 Post' Glyphosate treatment. These results indicate glyphosate applications did not have negative effects on glyphosate-resistant soybean leaf Mn concentrations in the absence of weed pressure. Furthermore, the tissue analysis data did not show evidence that Mn uptake and translocation in soybean was blocked several days after glyphosate application.

Figure 2. Time effects on Leaf Mn concentrations (ppm) averaged across glyphosate and Mn application treatments.



Leaf Mn Concentration (ppm)
40
35
30
25
20
15
10
1
2
3
4
Sampling Time
PPAC 2007
PPAC 2008
Rice 2007
Rice 2008
White 2007
White 2008

Table 2. Effect of sampling time on soybean trifoliate leaf Mn concentrations (ppm) within each glyphosate treatment (1. no glyphosate applied [Control], 2. pre-emergence application only [Pre only], 3. pre-emergence plus one post-emergence applications [Pre + 1 Post], and 4. pre-emergence plus two post-emergence applications [Pre + 2 Post]), averaged across Mn treatments for each site and year.

(a) PPAC 2007

Time\Gly	1st	2nd	3rd	4th
Control	22.0	18.4	20.1	21.2
Pre only	24.7	21.1	22.0	21.4
Pre+1 Post	29.3	24.1	28.1	25.7
Pre+2 Post	24.6	21.0	24.1	23.0

(b)PPAC 2008

Time\Gly	1st	2nd	3rd	4^{th}
Control	18.8	21.1	20.9	21.1
Pre only	16.1	17.1	18.1	17.1
Pre+1 Post	17.6	19.9	20.7	20.1
Pre+2 Post	16.8	18.5	18.8	18.7

(c) Rice 2007

Time\Gly	1st	2nd	3rd	4th
Control	29.3	36.3	36.7	35.7
Pre only	27.3	35.1	35.5	31.3
Pre+1 Post	28.1	33	29.3	31.3
Pre+2 Post	27.6	34.5	32.4	32.6

(d) Rice 2008

Time\Gly	1st	2nd	3rd	4^{th}
Control	29.4	34.2	30.1	27.3
Pre only	32.1	36.5	31.9	27.7
Pre+1 Post	30.7	32.9	31.2	28.5
Pre+2 Post	33.5	37.3	35.1	32.1

(e) White 2007

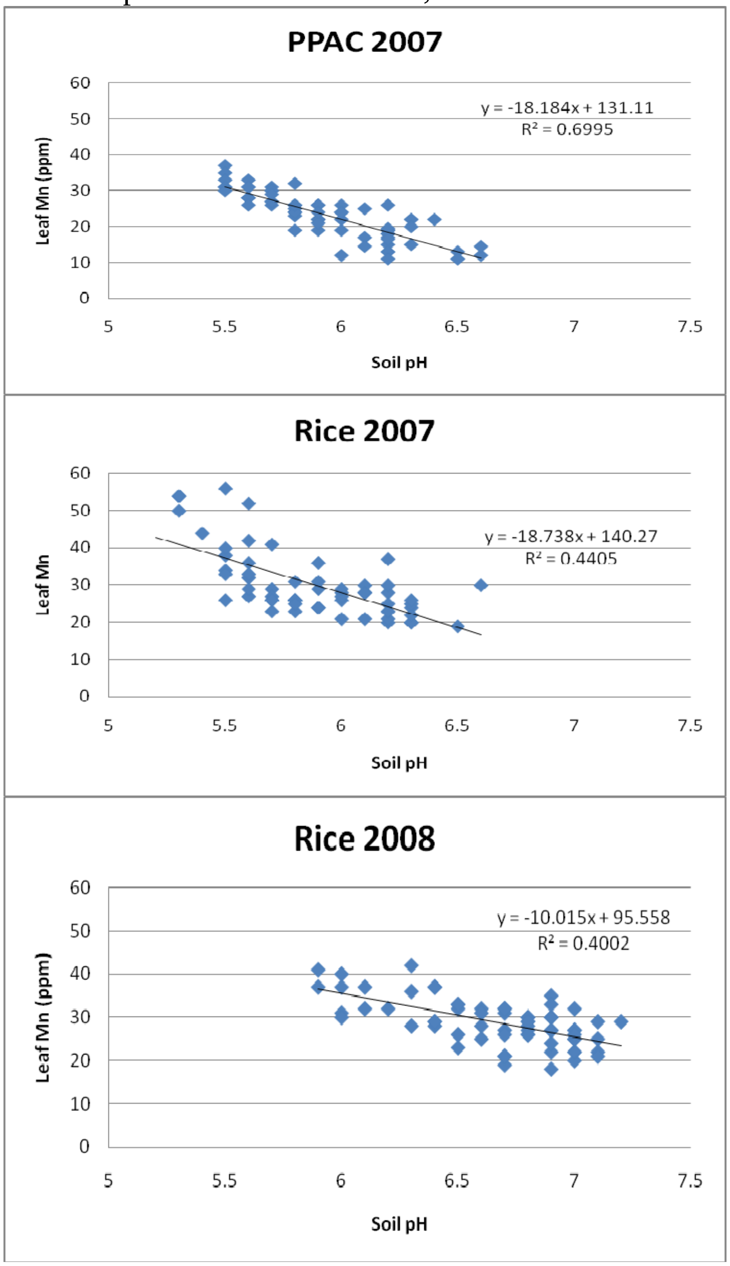
Time\Gly	1st	2nd	3rd	4th
Control	27.1	23.9	-	22.5
Pre only	27.9	26.4	-	26.3
Pre+1 Post	28.5	25.2	-	24.9
Pre+2 Post	27.5	26.5	-	27.0

(f) White 2008

Time\Gly	1st	2nd	3rd	4^{th}
Control	29.2	29.0	33.5	29.1
Pre only	26.9	29.4	33.3	29.3
Pre+1 Post	24.8	28.5	31.3	26.3
Pre+2 Post	32.5	36.5	38.4	36.0

The variation in leaf Mn concentration among experiments exceeded 10 ppm on each sampling date (Figure 2, e.g. PPAC 2008 versus White 2008), but there was also considerable variation in leaf Mn concentration among glyphosate treatments within an experiment was (e.g. leaf Mn variation of about 10 ppm at White 2008). The year, location, soil, environment and soybean variety all had considerable influence on leaf Mn concentrations. Leaf Mn concentrations had significant linear correlations with soil available Mn at PPAC 2008, Rice 2007 and both years at White; and also had significant linear correlations with soil pH at PPAC 2007 and both years at Rice (Figure 3). The within experimental area variation in soil pH and available Mn at all locations made it more difficult to detect the glyphosate and Mn treatment effects.

Figure 3. Relationship between soil pH and soybean leaf Mn concentrations for the 65 individual plots at the PPAC 2007, Rice 2007 and Rice 2008 locations.



When considering the leaf Mn concentrations averaged over four leaf sampling times, the low rate of banded Mn did not have significant effects on leaf Mn concentrations, while the high rate of banded Mn application only increased leaf Mn concentrations at Rice 2007. The second leaf sampling time was two weeks after foliar Mn application in 2007 and just one week after in 2008. Compared to all other Mn treatments, foliar Mn increased leaf Mn concentrations in all six experiments; statistically significant concentration gains were evident in four of the six environments (Table 3). In 2007, the influence of foliar Mn on leaf Mn concentrations diminished at the third leaf sampling time (three weeks after foliar Mn application). In 2008, the positive effect of foliar Mn application only persisted until the third sampling time (two weeks after foliar Mn application) at PPAC. At the last leaf sampling time, foliar Mn application did not affect leaf Mn concentrations at all. The results indicated foliar Mn fertilizer could cause a significant increase in leaf Mn concentrations for a few days, and possibly a week or two after application, but that the beneficial effect was never apparent three weeks after foliar Mn application. The increase of leaf Mn concentrations could be as high as 36 ppm due to foliar Mn application; however, the range of increase varied considerably between years or among locations.

Previous studies showed that the critical value for leaf Mn concentration was about 20 ppm, but that it may vary among cultivars (Ohki et al., 1979, Parker et al. 1981, Mills and Jones, 1991). Since the studies establishing critical value were done on conventional soybean varieties, and not on the GR soybeans, and because the GR gene insertion may alter the Mn metabolism and other metabolic processes in soybean plants, (e.g. Huber 2007 indicated that GR soybean required the application of almost 50 percent more Mn to meet their physiological sufficiency than conventional soybean varieties); the critical leaf Mn concentration values for modern soybean varieties may be higher. In our results, PPAC in 2008 had the lowest leaf Mn concentrations overall; this was the only case where leaf Mn concentrations were below the old critical value. However, locations with lowest leaf Mn concentrations did not demonstrate that the Glyphosate and Mn treatments effects were any stronger. There was no interaction between Glyphosate and Mn treatments on leaf Mn concentrations at any location, and, in any case, the leaf Mn concentrations did not predict the yield performance very well. Although some of the correlations between leaf Mn and yield were significant, the highest R square value was only 0.27 (data not shown). Those meant either the soil provided enough Mn for glyphosate-treated soybean, or that glyphosate applications did not result in higher plant demand for Mn.

Table 3. Change in trifoliate leaf Mn (ppm) concentrations between 1st and 2nd sampling times within each Mn treatment (1. no Mn applied [0], 2. 2.5 lbs/a banded Mn [BL], and 3. 5.0 lbs/a banded Mn [BH]). Different letters within a location and year indicate significant differences at 0.05 probability level between Mn treatments.

Mn Treatment	PPAC 2007	PPAC 2008	Rice 2007	Rice 2008	White 2007	White 2008
0	-4.5b	1.6b	4.4b	0.8b	-4.2	3.8
BL	-4.4b	2.6b	4.4b	2.6b	-1.8	3.8
BH	-6.6b	2.8b	5.8b	3.2b	-3.8	3.7
F	35.5a	20.0a	36.6a	11.4a	5.8	5.4

The overall impact of glyphosate application(s) on glyphosate-resistant soybean yield was not consistent in our six experiments (Table 4). For unknown reasons, glyphosate application(s) had positive effects on GR soybean yield at Rice in both years, and it was significantly positive at Rice in 2008. At PPAC and White, glyphosate application(s) caused about 4 bushel/acre or 9% yield loss at most, but the glyphosate effect on yield was not significant statistically at these two locations. Based on our results, we conclude that the glyphosate application(s) did not have any negative effect on yield of GR soybeans.

Banded and Foliar Mn treatments never significantly affected soybean yield. All mean yield values were so close to each other that there appeared to be no yield benefits with banded Mn application. However, research under a high yielding environment in Kansas indicated a 10% yield reduction of GR soybean comparing with its non-GR conventional near-isoline when no Mn was applied (Gordon, 20070. The yield gap was diminished when 2.5 lbs/a or 5.0 lbs/a banded Mn was applied. The different responses to Mn fertilization between cultivars and production systems emphasize the need for more research on optimum micro-nutrient management systems for GR soybean. Foliar Mn fertilizer application was a good treatment to temporarily relieve soybean Mn deficiency, but a single application did not increase yield.

Table 4. Glyphosate treatment effects on yield (bu/a), averaged across three Mn treatments. Different letters indicate significant differences at 0.05 probability level among glyphosate treatments.

Glyphosate Treatment	PPAC 2007	PPAC 2008	Rice 2007	Rice 2008	White 2007	White 2008
Control	57.8	47.1	47.8	45.8b	48.5	54.3
Pre only	56.6	44.3	46.9	47.5b	46.9	52.5
Pre+1 Post	58.7	44.5	48.9	51.9ab	44.3	55.0
Pre+2 Posts	54.8	44.4	51.1	55.6a	48.1	54.2

In summary, trifoliate leaf Mn concentrations in glyphosate-resistant soybean changed significantly with time but rarely in response to the applied treatments. Glyphosate application did not cause the leaf Mn concentration to decease when there was no weed glyphosate absorption or subsequent root exudation of glyphosate-based products. Banded Mn fertilizer did not impact leaf Mn concentrations significantly. Leaf Mn concentrations were always higher in the week or two after foliar Mn application than they were with either of the two banded Mn treatments. However, the beneficial effects of foliar Mn on leaf Mn concentrations in the upper canopy of soybean plants was short lived. Glyphosate application did not have negative effects on GR soybean yields in the absence of weeds and both banded and foliar Mn applications had no yield benefit in our study. More details about the project are available in a thesis (Xia, 2009).

D. Preliminary 2009 Research on Corn Response to Micronutrients

We compared various foliar micronutrient combinations in a farmer's field near La Crosse, IN. The soil was a sandy loam and the soil Zn concentrations were near 1.0 ppm in the spring of 2008 and 2009. Pioneer Hybrid 33F88 (a stacked hybrid with glyphosate resistance, BT corn borer and rootworm protection) was planted on May 29, and starter fertilizer was applied in a 2" by 2" position at a rate of 125 pounds of 9-17-0 product per acre for treatment numbers 1 through 9 below. No starter fertilizer was applied to treatments 10 and 11. Roundup Original Max was both pre (burn-down) and post applied, and foliar micronutrients were applied about a week after the Roundup when the corn plants were approximately knee high. Leaf Zn deficiencies were very obvious in several plots during vegetative growth, but were not apparent during reproductive growth.

Micronutrient Treatments applied in 2009:

1. Control WS…..No micronutrients, just H2O, planter with starter fertilizer (9-17-0).
2. Mn1…. Low rate (Brandt Chemical EDTA Mn 6% at 32 oz/acre)
3. Mn2…. Low rate (Tetra Micronutrients Pro Mn 5% at 38 oz/acre)
4. Mn3…. High rate (Tetra Micronutrients Pro Mn 5% at 76 oz/acre)
5. Zn1…. Low rate (Tetra Micronutrients Super Tel Zn Powder 35.5% at 0.25 pounds Zn/acre)
6. Zn2….. Medium rate (Tetra Micro. Super Tel Zn Powder 35.5% at 0.5 pounds Zn/ acre)
7. Zn3……High rate (Tetra Micro. Super Tel Zn Powder 35.5% at 1.0 pounds Zn/acre)
8. Mn1Zn1..... Low rate combination: (EDTA Mn 6% at 32 oz/acre plus Super Tel Zn 35% at 0.25 pounds Zn/acre)
9. Mn2Zn2…. Medium rate combination: (Pro Mn 5% at 76 oz/acre + Super Tel Zn at 0.25 pounds Zn/acre)
10. Control NS….. No micronutrients, just water, and no starter fertilizer at planting
11. Zn2, NS….. Medium rate (no starter at planting; Tetra Micronutrients Super Tel Zn Powder 35.5% at 0.5 pounds Zn/ acre)

Table 5. Effects of various foliar micronutrient products and rates on whole-plant (1 week after application) and ear-leaf (R2 stage) micronutrient concentrations, plus grain moisture and yields, for glyphosate-resistant corn planted near La Crosse, IN, 2009.†

Treatment	Whole Plant Zn	Whole Plant Mn	Ear-leaf Zn	Ear-leaf Mn	Grain Moisture at Harvest	Grain Yield
	ppm	ppm	ppm	ppm	%	Bu/ac
With Starter (WS) Fertilizer						
Control WS	113bc‡	91b	32	60	22.4bc‡	139.4
Mn1	32c	148ab	28	63	22.8b	138.7
Mn2	26c	117ab	30	48	22.3bc	165.0
Mn3	30c	235a	33	49	22.3bc	160.5
Zn1	183abc	66b	32	53	21.9c	169.6
Zn2	222abc	60b	32	55	22.8b	148.7
Zn3	428a	55b	32	59	22.4bc	136.3
Mn1Zn1	140bc	173ab	33	60	22.5bc	148.9
Mn2Zn2	390ab	132ab	32	54	22.4bc	163.3
Without Starter (NS) Fertilizer						
Control NS	29c	82b	34	68	23.9a	154.1
Zn2, NS	210abc	74b	30	63	22.9b	147.2
LSD (0.05)	286	142	38	54	0.8	82.8

† Average of 3 replications
‡ Means with the same letter are not significantly different.

Whole-plant Mn and Zn concentrations both responded positively to foliar Mn and Zn applications, but plant Mn and Zn concentrations were only significantly higher following the highest application rates of each product (Table 5). There were no residual treatment differences apparent in ear-leaf micronutrient concentrations following the micronutrient applications, and grain yield responses were also not significantly affected. Spatial variability in this trial was high, and we will present regression figures at a later time to display the dependency of plant nutrient concentrations and yields on the soil micronutrient concentrations and soil pH in individual plots. In the control plots with no starter fertilizer, whole-plant Zn concentrations trended lower and grain moisture contents were higher (the latter as expected).

Despite the low frequency of significant treatment effects, it is rather interesting to observe that that whole-plant Mn concentrations trended lower following application of Zn alone, and whole-plant Zn trended lower following application of Mn alone at these rates (Table 5). Co-applications of micronutrients may be affecting the relative balance of micronutrients in leaves.

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