EFFECTS OF GLYPHOSATE APPLICATION AND MANGANESE FERTILIZATION ON LEAF MANGANESE CONCENTRATION AND YIELD OF GLYPHOSATE-RESISTANT SOYBEAN

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Abstract

Glyphosate-resistant (GR) soybean is widely accepted in the United States. Recent research in Indiana and elsewhere has observed that post-emergence foliar applications of glyphosate may cause or exacerbate manganese (Mn) deficiency and then limit yield of GR soybeans on low Mn availability soils. The objectives of this study were to 1) better understand how glyphosate application(s) might reduce the uptake and translocation of Mn in GR soybean plants, and 2) determine the effectiveness of starter-banded and foliar Mn in GR soybean production systems with various frequencies of glyphosate application. A field study involving wide-row (30") soybean production was conducted at 3 Indiana locations with relatively low soil-test Mn in 2007 and 2008. Main-plot treatments were 4 levels of glyphosate application frequency (zero, pre-emergence only, pre-emergence plus single post-emergence, and pre-emergence plus double post-emergence). Sub-plot treatments were 3 levels of banded Mn (0, 2.5 and 5.0 lbs/a). A foliar Mn application (0.5 lb/a) was also evaluated following only the pre-emergence plus single post-emergence glyphosate application. The formulation of glyphosate herbicide was Roundup WeatherMax™. Full weed control was achieved on all plots by pre-emergence residual herbicide application and hand weeding so that possible glyphosate and Mn treatment effects on soybean would not be confounded by differential weed competition. Trifoliate leaf Mn concentrations in GR soybean cultivars changed during the growing season but rarely in response to the applied treatments. Glyphosate applications never reduced leaf Mn concentrations relative to the control plots without glyphosate. Banded Mn fertilizer did not improve leaf Mn concentrations. However, foliar Mn application increased leaf Mn concentrations significantly, although this beneficial effect was short lived. Foliar application, therefore, only temporarily relieved soybean Mn deficiency. The application of glyphosate to soybean in the absence of weed growth did not reduce yield; but neither banded nor foliar Mn supplementation increased soybean yield substantially.

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 in field often appears in patchy or streaky patterns, not 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 that determine crop responses 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.

The objectives of this research were to 1) determine leaf Mn concentrations in GR soybean at different growth stages, 2) better understand the effect of glyphosate application(s) on GR soybean plants, and 3) determine the optimum method of Mn supplementation in GR soybean production systems at various frequencies of glyphosate application.

Materials and Methods

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™ and the application rate was 0.7 lb/a 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 Discussions

Leaf Mn concentrations always changed significantly among different leaf sampling times in our six experiments (Figure 1). But the time frame in which the leaf Mn concentration reached its maximum was inconsistent, and thus very specific to year and location.

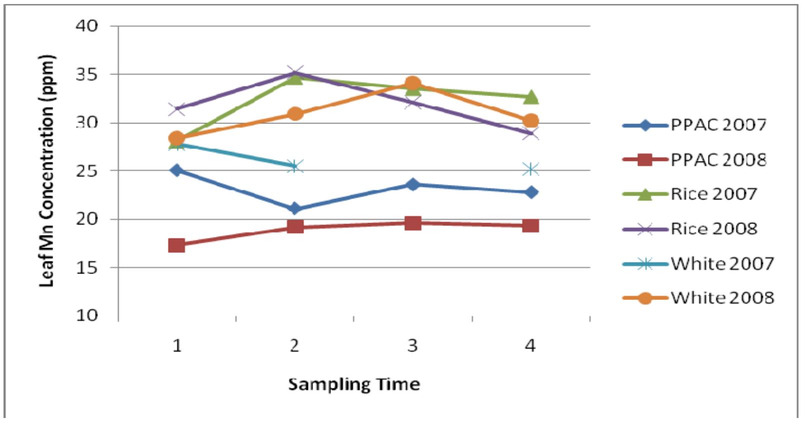

Figure 1. Time effects on Leaf Mn concentrations (ppm) averaged across glyphosate and Mn application treatments.

Glyphosate application(s) did not have significant effects on leaf Mn concentrations in the upper fully expanded trifoliate leaf at any sampling time (Table 1). 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.

The variation in leaf Mn concentration among experiments exceeded 10 ppm on each sampling date (Figure 1, 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. In our study, leaf Mn concentrations had significant linear correlations with soil available Mn at PPAC 2008, Rice 2007 and both years at White; and had significant linear correlations with soil pH at PPAC 2007 and both years at Rice. 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.

When considering the leaf Mn concentrations averaged over four leaf sampling times (three sampling times in White 2007), 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.

Table 1. 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	4th
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	4th
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	4th
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 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 2). 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 in our study, or that glyphosate applications did not result in higher plant demand for Mn.

Table 2. 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 3). 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. The yield gap was diminished when 2.5 lbs/a or 5.0 lbs/a banded Mn was applied. The different responses of Mn fertilization between cultivars and production conditions emphasizes the need for more research on the nutrient management system of 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 3. Glyphosate treatment effects on yield (bu/a), 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

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 and both banded and foliar Mn applications had no yield benefit in our study.

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References

Cerdeira, A.L., S.O. Duke. 2006. The current status and environmental impacts of glyphosate-resistant crops: A review. J. Environ. Qual. 35:1633-1658.

Gordon, B. 2007. Manganese nutrition of glyphosate-resistant and conventional soybeans. Better Crops 91/4:12-13.

Henkens, C.H., E. Jongman. 1965. The movement of manganese in the plant and the practical consequences. Neth. J. Agric. Sci. 13:392-407.

Huber, D.M. 2007. What about glyphosate-induced manganese deficiency? Fluid Journal. Fall 2007:20-22.

Jones, J.B., B. Wolf., H.A. Mills. 1991. Plant analysis handbook: a practical sampling, preparation, analysis, and interpretation guide.

Kremer, R.J., N.E. Means and K.S. Kim. 2005. Glyphosate affects soybean root exudation and rhizosphere microorganisms. Int. J. Environ. Anal. Chem. 85:1165-1174.

Moldes, C.A., L.O. Medici, O.S. Abrahao, S.M. Tsai, R.A. Azevedo. 2008. Biochemical responses of glyphosate resistant and susceptible soybean plants exposed to glyphosate. Acta Physiol Plant 30:469-479.

Ohki, K., F.C. Boswell, M.B. Parker, L.M. Shuman, D.O. Wilson. 1979. Critical manganese deficiency level of soybean related to leaf position. Agron. J. 71:233-234.

Ozturk, L., A. Yazici, S. Eker, O. Gokmen, V. Romheld, I. Cakmak. 2007. Glyphosate inhibition of ferric reductase activity in iron deficient sunflower roots. New Phytol. 177:899-906.

Parker, M.B., F.C. Boswell, K. Ohki, L. M. Shuman, D.O. Wilson. 1981. Manganese effects on yield and nutrient concentration in leaves and seed of soybean cultivars.

USDA, United States Department of Agriculture, 2008. National Agricultural Statistics Service.

Wilson, D.O., F.C. Boswell, K. Ohki, M.B. Parker, L.M. Shuman, M.D. Jellum. 1982. Changes in soybean seed oil and protein as influenced by manganese nutrition. Crop Sci. 22:948-952.

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