**Effect of preceding crop and glyphosate application on nutrient levels in soybean (A09229)**

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**Background:**

Glyphosate is widely-used in Manitoba cropping systems, and may be applied multiple times in a single growing season as a means to control a broad spectrum of weeds. Extensive use of glyphosate has raised questions regarding the potential effects of repeated applications of glyphosate on crop productivity and growth. The issue of glyphosate residues in soil and potential effects on crop productivity is a complex one given the potential influences of various factors including management practices, crop, soil and environmental conditions.

Relationships between glyphosate application, glyphosate-tolerant soybean, and plant nutrient status have been studied under field and controlled environment conditions. In greenhouse studies in Brazil, Zobiole et al. (2010a) reported reductions in the accumulation of macro- and micronutrients in soybean leaf tissue and declines in nutrient uptake when increasing rates of glyphosate were applied to a glyphosate-resistant soybean cultivar. In a second study, declines in macro- and micronutrient accumulations were associated with increased rates and late applications of glyphosate in both RR1 and RR2 cultivars (Zobiole et al. 2011). Single and sequential applications of glyphosate were similarly reported to reduce macro- and micronutrients in the leaf tissue of different cultivar maturity groups grown on different soil types (Zobiole et al. 2010b). Bott et al. (2008) also reported declines in the micronutrient status of glyphosate-resistant soybean with recommended rates of glyphosate, and suggested that effects may be influenced by growing conditions and environmental factors.

Several papers have focussed more specifically on the relationship between Mn and glyphosate-resistant genetics. One report suggested that glyphosate-resistant soybean genotypes may be less efficient with respect to Mn uptake compared to conventional genotypes (Huber et al. 2004) and strategies have been suggested to address immobilization of Mn by glyphosate (Huber 2007). Based on field studies conducted in Kansas, Loecker et al. (2010) noted that, while soybean genetics appeared to influence soybean response to Mn, glyphosate resistance did not consistently result in Mn deficiency or greater responsiveness to the application of Mn. In studies with sunflower, Eker et al. (2006) reported that glyphosate application negatively affected the Fe and Mn status of the plant, which was attributed to the formation of glyphosate-metal complexes in the plant and/or rhizosphere effects. Relatively little information appears to be available currently regarding the potential effects of glyphosate residues present in the soil on nutrient uptake by glyphosate-resistant soybean, however.

Preceding crop is another factor that has the potential to influence not only nutrient status but also performance of soybean in production systems. As examples, N-fixing crops may provide a N benefit to subsequent crops in rotation (Manitoba Soil Fertility Guide 2007), while preceding crop has been shown to influence biological N fixation by soybean (Sanders 2016). Growing a mycorrhizal versus non-mycorrhizal pre-crop prior to a mycorrhizal crop may also influence mycorrhizal colonization and, in turn, nutrient availability, water availability and/or yield. Recent studies in Manitoba demonstrated reduced mycorrhizal colonization in soybean following canola or wheat versus soybean following soybean or corn. Mycorrhizal fungi may further be influenced by factors such as soil characteristics as well as agronomic management (Li 2016; Miller et al. 2008; Sanders 2016). Fertilizer management in the preceding crop may also influence the nutrient status of the subsequent crop in rotation.

**Objective:**

The objective of this study was to determine the effect of preceding crop and previous glyphosate application management practices on nutrient status, yield and quality of a subsequent glyphosate-tolerant soybean crop.

**Materials and Methods:**

*Background regarding the experimental site:* Field experiments were conducted at multiple sites across western Canada from 2013 through 2016 to determine the effect of repeated glyphosate applications on crop performance and nutrient uptake of major crops (wheat, canola, field pea) grown in this region. The original study was led by Dr. Bob Blackshaw, who has now retired from AAFC-Lethbridge.

When the original study concluded in the fall of 2016, the experimental sites at Brandon and Scott were left in place. This provided a unique opportunity to collect preliminary data regarding the potential effects of repeated field applications of glyphosate on macro- and micro-nutrient uptake by a subsequent glyphosate-tolerant soybean, and on soybean yield and quality.

The original experiment consisted of four replicates of five glyphosate rate treatments (0, 1, 2, 4, 8 kg ae glyphosate/ha), and was seeded to wheat, field pea and canola in 2013, 2014, and 2015, respectively. In the control treatment, Liberty/Select/Amigo was applied approximately one week prior to the crop seeding date, at the same time as glyphosate treatments were applied. In 2016, main plots were split into three subplots which were seeded to one of wheat, pea or canola for a total of 60 plots. Sub-plot dimensions were 1.8 x 10 m at Brandon and 3.65 x 14 m at Scott. Soil samples collected in-season in 2017 and submitted to a commercial lab for analysis indicated increased AMPA and glyphosate concentrations associated with increasing rates of glyphosate application (data not presented).

In 2017, a glyphosate-tolerant soybean cultivar (RR2Y, relative maturity 000.8, 2250 heat units) was seeded on May 19th at Brandon, MB and on May 23rd at Scott, SK. Soybean was direct seeded at a rate of 50 seeds m-2 using an air seeder, commercial inoculant was applied at recommended rates and a standard rate of fertilizer P (40 kg P2O5 ha-1) were applied at seeding, and soybeans were managed using generally-accepted management practices for the region. Recommended herbicides were applied at recommended rates in-crop to control the weeds present. Glyphosate was not applied in-crop to avoid confounding of the experimental treatments. At the Scott site, one application of a recommended insecticide was applied on June 25th to control cutworms, which minimized any insect damage to the plots. Soybeans were harvested on September 28th at Scott, and on October 5th at Brandon.

Soil samples were collected (0-15, 15-60 cm) from each plot, and treatments composited across replicates to determine background nutrient levels (2M KCl-extractable NO3-N, Olsen P, NH4OAc-extractable K, DTPA-extractable Cu, Mn, Zn) in each treatment (Table A1). In-crop, plant density was determined after all plants had emerged by counting 2-3m lengths of row. Crop biomass yield was determined approximately 4 weeks after crop emergence and again at midseason (R3) by hand harvesting 2-1m lengths of row at 2 locations per plot. Soybeans were harvested by plot combine at maturity to determine yield. Test weight, % oil and % protein of seed were determined using an Infratec™ 1241 Grain Analyzer (Foss North America Inc., Eden Prairie, MN), and thousand seed weight determined using a mechanized seed counter. Plant and grain samples were dried, ground and submitted to a commercial lab for nutrient analysis. Total Kjeldahl N, P and K were determined using salicylate/nitroprusside, molybdate/ANSA, and atomic absorption procedures, respectively, following a sulphuric acid digest. (The Kjeldahl method employed did not include nitrate-N.) The concentration of Cu, Mn and Zn was determined by atomic absorption, following nitric perchloric digestion. Days to emergence, flowering and crop maturity were determined based on periodic visual assessments of the experimental plots. Soil samples were collected pre-plant for nutrient analysis.

For the purpose of this report, data were analyzed as a split plot for each site using Proc Mixed in SAS, with replicate considered a random effect and treatments considered fixed effects. Tukey's multiple comparison procedure was used to further assess treatment effects, with P<0.05 considered significant. Two plots at Brandon were considered outliers due to low yields, and were excluded from the current analyses.

**Results and Discussion:**

***Days to emergence and plant density***

In 2017, preceding crop appeared to have a more frequent effect overall on soybean than did glyphosate treatments. At Brandon, the average days to emergence for soybean following wheat (6 d) was slightly less than following canola and pea (8 d), although treatment had no effect on plant stand which averaged 46 plants/m2 (Table 1). At Scott, the number of days to emergence was similar regardless of treatment (13 d); however, plant stands were slightly higher for wheat (44 plants/m2) than canola (40 plants/m2). At both Brandon and Scott, plant density would be considered to be within recommended levels regardless of treatment. No effects of glyphosate treatment were evident for these early-season measurements on at either site.

***Soybean biomass yield and nutrient concentration***

*In-crop biomass yield*

Soybean biomass yield was determined several weeks after emergence (early) and again at growth stage R3 (midseason) at both Brandon and Scott (Table 1). Previous glyphosate management had limited effects on biomass yield, having no effect on early-season biomass yield at either Brandon or Scott, or on mid-season biomass yield at Scott. At Brandon, biomass yield at R3 was higher in the treatment that had received repeated applications of 8 kg ae glyphosate/ha over preceding years than in the control treatment that received no glyphosate, with no differences noted among the remaining rates of application. The reason for this effect at Brandon is not known.

Preceding crop had no effect on early or midseason biomass yield at Brandon in 2017; however, at Scott, both early and midseason biomass yield varied as a function of preceding crop, with pea>wheat>canola for early-season biomass and pea=wheat>canola for mid-season biomass (Table 1). In part, slightly higher plant stands following wheat than canola (44 vs 40 plants m-2) may have contributed to the observed differences in biomass yield at Scott. Although mycorrhizal colonization was not measured in the current study, one possibility is that soybean biomass may have been influenced in part by whether or not the preceding crop was mycorrhizal. Because both pea and wheat are mycorrhizal while canola is non-mycorrhizal, the mycorrhizal network established under the pea and wheat crops could potentially have contributed to mycorrhizal colonization of the soybean crop thereby benefitting crop performance and nutrient uptake as discussed below.

*Plant nutrient concentrations and uptake (early, midseason, harvest)*

Early- and mid-season nutrient concentrations (N, P, K, Cu, Mn, Zn) of soybean plants were determined at both sites in 2017 (Figs. 1-8; Table 2). Previous glyphosate management had no effect on the nutrient concentrations measured in soybean plants at early- or mid-season at either Brandon or Scott. Further, no significant interactions between previous glyphosate management and preceding crop were noted under the conditions of this study (Table 2). Similarly, in-season nutrient uptake by the soybean crop was not influenced by previous glyphosate management in most cases, nor were significant interactions evident between previous glyphosate management and preceding crop. At Brandon only, Cu and Zn uptake by soybean plants at R3 varied with glyphosate treatment, reflecting differences in plant biomass production (Tables 1 and 2). Previous glyphosate management similarly had no effect on nutrient concentration or uptake in soybean seed at harvest at the Scott site (Table 2). At Brandon, previous glyphosate management had no effect on nutrient concentrations in soybean seed at harvest except in the case of K. However, differences in the uptake of all nutrients measured were noted, mirroring observed differences in grain yield (Tables 1 and 3).

Preceding crop frequently influenced soybean nutrient status (Figs. 1-8; Table 2). At both Brandon and Scott, growing soybean after pea rather than canola resulted in a higher N concentration in soybean early in the growing season, with wheat being intermediate (Figs 1 and 2). This translated into greater early season N uptake by soybean at Scott as follows: pea>wheat>canola; no difference in early-season N uptake was measured in soybean at Brandon (Figs 5 and 6). Preceding crop had no effect on N concentration or uptake of soybean at R3 at Brandon; however, N removed in harvested soybean seed was higher for canola than wheat reflecting the higher soybean yields obtained after canola than wheat at Brandon (Fig. 5; Table 1). At Scott, N uptake at R3 (pea=wheat>canola) and in harvested soybean seed (pea>wheat>canola) similarly reflected higher biomass and soybean seed yields following pea and wheat than canola (Fig. 6; Table 1). Preceding crop had no effect on plant N concentration of soybean at R3 at Brandon or Scott, but was higher in the seed of soybean following canola rather than pea or wheat likely due to biological dilution in the higher-yielding soybean treatments. Differences in N concentration and/or uptake in soybean may be related to several factors. Manitoba studies have demonstrated differences in biological N fixation by soybean as a function of preceding crop (Sanders 2016), while higher N concentration and content in soybean shoots have been associated with mycorrhizal versus non-mycorrhizal plants in some cases. In the current study, all preceding crops had been grown using generally-accepted management practices for the region including fertility management, and only commercial inoculant had been applied to soybean. Although detailed soil analysis by plot was not conducted, analysis of soil samples composited across replicates did not reveal a consistently higher soil nitrate-N after pea (Table A1). Under Manitoba conditions, an estimated N benefit equivalent to 25 lb N/ac is often ascribed to peas, which is generally expected to be detected in late fall soil nitrate tests under moist conditions; however, if N release were delayed, N derived from the preceding pea crop may have contributed to the available N supply at Scott.

Early-season P concentration in soybean plants was higher following pea and wheat than following canola at both Brandon (pea=wheat>canola) and Scott (pea>wheat>canola), with similar results evident for early-season P uptake in soybean at Brandon (wheat≥pea≥canola) and Scott (pea>wheat>canola) (Figs. 1, 2, 5, 6). This effect on both P concentration and uptake continued through midseason (pea=wheat>canola) and in the soybean seed (pea=wheat>canola) at Scott. In contrast, at Brandon, preceding crop had no effect on midseason plant or seed P concentrations, although P removal in harvested soybean seed was higher for canola than for pea or wheat reflecting the higher soybean yields following canola at this site. The differences observed in early-season soybean P levels did not appear to reflect differences in spring soil test P levels based on the data available (Table A1), and the same rate of fertilizer P had been applied in all treatments. In part, growing mycorrhizal crops such as pea and wheat prior to soybean, rather than canola which is a non-mycorrhizal crop, may have contributed to enhanced mycorrhizal colonization of the soybean crop early in the growing season and, in turn, increased P availability for the soybean crop. Higher P concentrations in soybean following wheat and pea were accompanied by increases in early and midseason biomass yield and also seed yield at Scott (Table 1).

Preceding crop had no effect on biomass or seed K concentration or uptake at Brandon, except for K removal in harvested seed which was higher for canola than wheat, reflecting the higher yields in canola (Figs. 1, 2, 5, 6; Table 1). In contrast, at Scott, K concentration was consistently higher in soybean following pea than canola in the early and midseason biomass samples, and also in soybean seed samples at harvest. Higher K concentrations combined with higher soybean biomass and seed production contributed to higher K uptake for soybean following pea than canola. The differences in K uptake observed at Scott did not appear to be associated with differences in soil test K based on the data available (Table A1), and no K fertilizer had been applied to the soybean crop. As noted in the case of phosphorus, growing a mycorrhizal crop like pea before soybean could potentially have contributed to increased K availability compared to growing a non-mycorrhizal crop like canola.

Preceding crop had a consistent effect on plant and seed Cu concentration and uptake for soybean at Scott, with Cu concentration and uptake for early- and midseason biomass and in soybean seed consistently higher in pea than canola with pea>wheat>canola (Figs. 4 and 8). At Brandon, early-season Cu concentration and uptake followed a similar trend as Scott, with pea=wheat>canola for Cu concentration and wheat≥pea≥canola for Cu uptake (Figs. 3 and 7); however, preceding crop had no effect on either Cu concentration or uptake in soybean plants at R3. Further, although Cu concentrations in soybean seed following canola was lower than following wheat, Cu uptake in harvested soybean seed was higher following canola than wheat reflecting the higher soybean yields following canola than wheat (Table 1).

Effects of preceding crop appeared to be somewhat different in the case of Mn than for other nutrients assessed (Figs. 3,4,7,8). At Brandon, Mn concentration and uptake was higher in early-season biomass for soybean following canola than wheat, with canola=pea>wheat for Mn concentration and canola≥pea≥wheat for Mn uptake (Figs. 3 and 7). Preceding crop had no effect on Mn concentration or uptake at midseason, nor on Mn concentration in soybean seed at harvest. However, Mn uptake in soybean seed was higher for soybean following canola than wheat likely reflecting higher seed yields for the canola pre-crop treatment. At Scott, also, Mn concentration was higher for canola than either pea or wheat at early- and midseason and in soybean seed at harvest. While Mn uptake was higher following canola and pea than wheat in early-season biomass, no treatments effects were evident for midseason Mn uptake. At harvest, Mn uptake in soybean seed was lowest for soybean following canola, followed by wheat then pea which followed seed yield trends (Table 1).

Effects of preceding crop on Zn generally followed a similar pattern to that observed for P at Scott (Figs. 4 and 8). Early-season Zn concentration and uptake was higher for pea than canola with pea>wheat>canola. While preceding crop had no effect on Zn concentration at midseason, Zn uptake was higher for pea and wheat than canola reflecting higher biomass production in these treatments (Table 1). Zn concentrations were also higher in soybean seed at harvest where soybean followed pea or wheat rather than canola. This, in combination with higher seed yields, contributed to higher Zn removal in harvested soybean seed where soybean followed pea or wheat rather than canola (pea>wheat>canola). Like Scott, early-season Zn concentrations were lower in soybean following canola than pea at Brandon, but this did not translate into differences in Zn uptake by the crop (Figs. 3 and 7). No treatment effects were noted at midseason; however, Zn uptake in harvested soybean seed was higher for soybean following canola than pea or wheat reflecting observed yield differences (Table 1).

***Soybean seed yield and seed quality***

At Brandon, seed yield of soybean was higher after canola than wheat, with pea being intermediate (Table 1). No effects of preceding crop on soybean seed quality (e.g. test weight, seed weight, % protein, % oil), were observed at Brandon (Table 1). In contrast, at Scott, soybean yield was highest following pea, followed by wheat, then canola (Table 1). Higher soybean biomass production at growth stage R3 had also been associated with pea and wheat as compared to canola. The wheat pre-crop resulted in soybean seed with a higher percent oil (wheat>pea>canola) and lower percent protein (canola>pea>wheat). Pea and wheat pre-crops were also associated with a slightly lower soybean test weight than canola, while a pea pre-crop produced a higher seed weight than wheat, with canola being intermediate. No effects of glyphosate treatment were evident at Scott; however, at Brandon, both soybean biomass yield at growth stage R3 and seed yield were lower in the control than at the higher glyphosate rates (Table 1).

***Summary***

The current study was overlain on established experiments at Brandon, MB and Scott, SK which provided a unique opportunity to examine the effects of preceding crop and previous glyphosate management on nutrient status, yield and quality of soybean. While caution should be exercised in drawing broad conclusions from this study given that only two site-years of data are available, this study does provide preliminary information to augment the limited body of information currently available related to soybean production in western Canada.

Under the conditions of the current study, preceding crop had a more frequent and consistent effect on soybean performance and nutrient status than did previous glyphosate management. Glyphosate management generally had no measurable effect on soybean establishment or nutrient status. At Brandon only, the highest glyphosate rates resulted in a higher soybean seed yield than the control treatment that received no glyphosate, which contributed to higher nutrient uptake in soybean seed at harvest; however, the reason for the observed effect on soybean yield is not known. This effect was not evident at the Scott site.

Preceding crop had a comparatively greater influence on soybean establishment, growth, yield and nutrient status, although effects of preceding crop on early-season soybean establishment and yield varied between experimental sites. The concentration and uptake of nutrients including N, P, K, Cu, Mn and Zn in soybean biomass and seed was frequently influenced by preceding crop, which may be the result of a number of factors. Although mycorrhizal colonization was not assessed in the current study, results suggest that growing a mycorrhizal (e.g. pea, wheat) rather than a non-mycorrhizal crop (e.g. canola) prior to soybean may have been one factor that might have contributed to enhanced crop growth and nutrient status in some cases, particularly for immobile nutrients such as P, K, Cu and Zn. These findings may have implications for soybean management. As an example, in the case of P management, soybean is a relatively heavy user of P (Manitoba Soil Fertility Guide 2007) but has shown limited responses to fertilizer P (Bardella 2016). As soybean is a mycorrhizal crop that is able to effectively access soil P (Kalra and Soper 1968), potential may exist to utilize agronomic management practices that support mycorrhiza in order to optimize its various potential benefits, as well as enhance the availability of soil P to soybean. The selection of rotation sequences that benefit soybean may provide growers with one tool to enhance soybean performance under Manitoba conditions.

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Figure 1. Concentration of N, P and K in soybean biomass (early- and mid-season) and in soybean seed at Brandon in 2017, as affected by the 2016 preceding crop. Columns within a graph labelled with the same letter are not statistically different based on Tukey’s multiple comparison procedure at P=0.05.



Figure 2. Concentration of N, P and K in soybean biomass (early- and mid-season) and in soybean seed at Scott in 2017, as affected by the 2016 preceding crop. Columns within a graph labelled with the same letter are not statistically different based on Tukey’s multiple comparison procedure at P=0.05.



Figure 3. Concentration of Cu, Mn and Zn in soybean biomass (early- and mid-season) and in soybean seed at Brandon in 2017, as affected by the 2016 preceding crop. Columns within a graph labelled with the same letter are not statistically different based on Tukey’s multiple comparison procedure at P=0.05.



Figure 4. Concentration of Cu, Mn, and Zn in soybean biomass (early- and mid-season) and in soybean seed at Scott in 2017, as affected by the 2016 preceding crop. Columns within a graph labelled with the same letter are not statistically different based on Tukey’s multiple comparison procedure at P=0.05.



Figure 5. N, P and K uptake by soybean biomass (early- and mid-season) and in soybean seed at Brandon in 2017, as affected by the 2016 preceding crop. Columns within a graph labelled with the same letter are not statistically different based on Tukey’s multiple comparison procedure at P=0.05.



Figure 6. N, P and K uptake by soybean biomass (early- and mid-season) and in soybean seed at Scott in 2017, as affected by the 2016 preceding crop. Columns within a graph labelled with the same letter are not statistically different based on Tukey’s multiple comparison procedure at P=0.05.



Figure 7. Cu, Mn and Zn uptake by soybean biomass (early- and mid-season) and in soybean seed at Brandon in 2017, as affected by the 2016 preceding crop. Columns within a graph labelled with the same letter are not statistically different based on Tukey’s multiple comparison procedure at P=0.05.



Figure 8. Cu, Mn, and Zn uptake by soybean biomass (early- and mid-season) and in soybean seed at Scott in 2017, as affected by the 2016 preceding crop. Columns within a graph labelled with the same letter are not statistically different based on Tukey’s multiple comparison procedure at P=0.05.

Appendix A.

