Glyphosate effects on symbiotic relationships, nitrogen assimilation and seed composition in glyphosate-resistant soybean

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Introduction

Understanding the impact of transgenic herbicide-resistant cropping systems is needed to manage the use of these systems. Previous research has demonstrated that glyphosate can affect nitrogen fixation and/or nitrogen assimilation in glyphosate-resistant (GR) soybean (Zablotowicz and Reddy, 2007). In other studies application of glyphosate to a glyphosate-sensitive soybean generally decreased NRA activity with different patterns of inhibition in specific soybean parts (Bellaloui et al., 2006).

The objective of this study was to investigate the effects of glyphosate application of 1.12 kg and 3.36 kg ae ha-1 glyphosate (GLY) on symbiotic relationships, nitrogen metabolism and seed composition in GLY-resistant (GR) soybean.

Materials & Methods

The study was conducted in Stoneville, MS, using GR soybean cultivars AG4503RR and AG4604RR in 2006 and 2007. Glyphosate treatments consisted of two rates (1.12 and 3.36 kg ae ha^{-1}) applied postemergence twice in a sequence at 4 and 6 weeks after planting (WAP), representing the V4 and V7 growth stages. Glyphosate at 3.36 kg ae ha^{-1} was higher than suggested label use rate for single and total in-crop application of glyphosate, and represent the 'worst case scenario' to promote soybean injury. A hand-weeded control with no herbicide was included for a comparison. No preemergence herbicides were used. Each treatment consisted of four soybean rows spaced 102 cm apart and 16.8 m long. Treatments were arranged in a randomized complete block design with four replications. Yield was determined at maturity from all four rows.

Soybean plants were sampled from each plot at one week after each glyphosate treatment for nitrate reductase activity. Plants were excavated with roots and shoot intact, transported to the laboratory, and assayed for Apparent nitrate reductase activity (ANRA) using the method of Klepper and Hageman (1969). Potential NRA (PNRA) was conducted in the presence of 10 mM exogenous nitrate.

Nitrogenase activity was assayed using the acetylene reduction assay to measure acetylene reduction activity (ARA) and root respiration as described elsewhere (Bellaloui et al., 2006; Zablotowicz and Reddy, 2007). Nodule dry weight was measured and mycorrhizal colonization was determined at each sampling. The roots were washed in water to remove soil particles and 25 - 3 cm fibrous roots segments ~ 1 to 2 mm diameter from each plot cleared in KOH, and stained according to Sylvia (1994). The roots were observed under a disecting microscope 20 x magnification and the number of infection clusters per root segment counted.

Total nitrogen content was determined on a Flash EA112 elemental analyzer (CE Elantec). Delta ^{15}N ($^{15}N/^{14}N$ ratio based on natural abundance) was determined using a Thermo Finnegan Delta Plus advantage mass spectrophotometer and a isomass elemental analyzer.

Protein and fatty acids were determined using near infrared reflectance using a AD7200 diode array feed analyzer.

Table 1. Effect of glyphosate application rate on nitrate reductase activity in various soybean parts

Timing (WAP)	Glyphosate Rate (kg ae ha^{-1})	Leaf NRA Actual/Potential	Stem NRA Actual/Potential	Root NRA Actual/Potential	Nodule NRA Actual/Potential
4	0	5.9 a / 7.2 a	3.8 a / 4.4 a	3.9 a / 4.6 a	3.7 a / 4.4 a
	1.12	4.4 b / 6.1 b	3.7 a / 4.6 a	2.6 b / 4.5 a	2.0 b / 4.2 a
	3.36	3.2 c / 5.5 c	3.6 b / 4.2 b	2.3 c / 3.9 b	1.1 c / 3.0 c
6	0	5.8 a / 6.5 a	4.3 a / 5.1 a	3.4 c / 4.5 b	3.7 a / 4.6 a
	1.12	4.8 b / 5.7 b	3.8 b / 4.2 b	3.8 b / 4.4 b	1.5 c / 2.9 c
	3.36	3.3 c / 6.4 a	3.8 b / 5.6 a	5.7 a / 5.5 a	2.7 b / 3.5 b

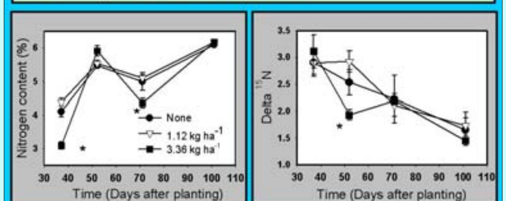
ANRA and PNRA averaged over 2006 and 2007; Means followed by same letter do not differ at 5% level

Table 2. Soybean symbiotic relationships as affected by glyphosate application rate

Timing (WAP)	Glyphosate Rate (kg ae ha^{-1})	ARA (uMol $plant^{-1}$ h^{-1})	Nodule (number $plant^{-1}$)	Mycorrhizae (Infections cm-1) 2006	Mycorrhizae (Infections cm-1) 2007
4	0	12.2 a	16.0 a	0.72 a	1.99 a
	1.12	11.0 a	15.0 a	0.64 a	1.86 a
	3.36	12.3 a	17.0 a	0.45 b	1.13 b
6	0	15.5 a	21.8 a	1.40 a	2.88 a
	1.12	15.0 a	25.0 a	1.22 a	2.70 a
	3.36	15.5 a	23.0 a	0.84 b	2.12 b

ARA and nodule number averaged over 2006 and 2007; Means followed by same letter do not differ at 5% level

Figure 1. Effect of glyphosate application on leaf and seed nitrogen and delta ^{15}N ratio



* Indicates significant difference at 5% level by Fishers LSD

Table 3. Soybean seed characteristics as affected by glyphosate application rate

Glyphosate Rate (kg ae ha^{-1})	Protein (%)	Oil (%)	Oleic (%)	Linoleic (%)
0	40.8 b	23.2 a	25.5 c	8.7 a
1.12	41.0 b	23.0 a	25.9 b	8.6 a
3.36	46.1 a	20.8 b	31.1 a	6.4 b

Seed composition averaged over 2006 and 2007; Means followed by same letter do not differ at 5% level

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Results & Discussion

➢ Glyphosate significantly reduced apparent NRA with the greatest effect in leaf and nodule tissue especially at the highest glyphosate rate (Table 1). Considering potential NRA, only specific cases of inhibition by glyphosate was observed, especially after the second application indicating nitrate in tissue was a limiting factor. Inhibition of NRA is consistent with a glyphosate effect in both resistant and sensitive soybean (Bellaloui et al., 2006).

➢There was no significant effect of glyphosate on nodulation or ARA (Table 2), previous studies indicated an inconsistent effect on these symbiotic parameters (Zablotowicz & Reddy, 2007). Mycorrhiozal infection was only reduced at high rates indicating that label rates should not affect this important symbiosis.

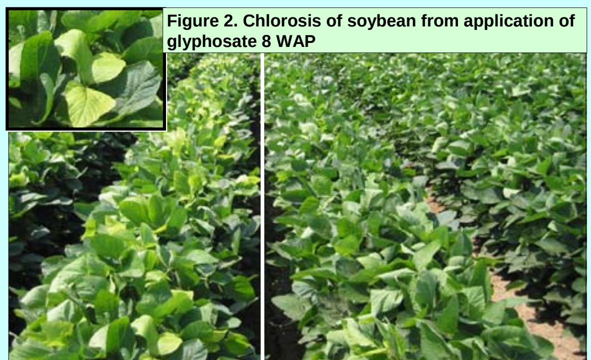
➢Leaf nitrogen content was reduced by the highest glyphosate rate in two of three leaf samples (Figure 1a). Delta 15N ratios decreased with time indicating with greater maturity an increased input from nitrogen fixation (Figure 1B). However, delta 15N ratios were lowest in leaf treated with the high glyphosate rate in the 2nd sample, indicating N2 fixation compensated for lower nitrogen uptake. These results confirm reduced nitrogen uptake in response to high glyphosate rates, as previously demonstrated with the NRA data. In final seed yield there was no effect of glyphosate on either total seed nitrogen content or delta 15N ratios.

➢Following application of high glyphosate rate, chlorosis was observed in newly expanded leaves (Figure 2). Soybean leaf chlorosis is a typical symptom due to the glyphosate metabolite aminomethylphosphonic acid (Reddy et al., 2004). These symptoms may also be a result of impeded nitrogen uptake.

➢Seed composition was significantly affected by high glyphosate rates (Table 3). Protein content was greatest in soybean treated with high rates of glyphosate, although similar concentrations of total nitrogen was observed. Conversely, oil concentration was reduced by high glyphosate. Two major oil components were also affected by high glyphosate with oleic acid increased and linolenic acid decreased. These results indicate that in addition to altering nitrogen metabolism, high rates of glyphosate can have a significant affect on carbon metabolism and partitioning among various chemical components.

➢ In both years, there was no effect of glyphosate on soybean yield (> 4600 kg ha^{-1}) regardless of glyphosate rate (data not shown). This indicates the excellent ability of soybean to recover from any physiological stress following glyphosate application.

Figure 2. Chlorosis of soybean from application of glyphosate 8 WAP



Glyphosate Rate 3.36 kg ha^{-1} | Non-Treated

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