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Nitrogenase activity, nitrogen content, and yield responses to glyphosate in glyphosate-resistant soybean

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Abstract

Transgenic glyphosate-resistant (GR) soybean [*Glycine max* (L.) Merr.] expressing a glyphosate-insensitive 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme has provided new opportunities for weed control in soybean production. However, glyphosate is toxic to the soybean nitrogen-fixing symbiont, *Bradyrhizobium japonicum,* as its EPSPS enzyme is sensitive to glyphosate. The effects of glyphosate on symbiotic parameters, nitrogen accumulation, and yield in GR soybean under weed-free conditions were determined in a 3-yr field study during 2002–2004. Four glyphosate (0.84, 1.68, 2.52 + 2.52, and 0.84 + 0.84 kg ae/ha) treatments applied at 4 and 6 weeks after planting (WAP) soybean were compared to a no glyphosate, hand weeded (weed-free) control. In 2002 and 2003, soybean plants were harvested at 5, 6, 7, and 8 WAP, and roots assessed for nitrogenase activity (acetylene reduction assay, ARA), root respiration, nodulation, and root biomass. Soybean seed yield, leaf and seed nitrogen content were determined in all three years. No consistent effect of glyphosate was observed on either ARA or root respiration. In 2002, both ARA and respiration were about a third of that in 2003, attributed to early-season drought in 2002. All glyphosate treatments reduced foliar nitrogen content (26–42%) in 2002. In 2003 and 2004, three and two glyphosate treatments, respectively, reduced foliar nitrogen content (8–13%), with the greatest reduction when glyphosate was applied at the highest rate. Soybean yield was reduced by 11% with two applications of 2.52 kg ae/ha glyphosate compared to hand weeded control in 2002, but seed yield was not affected in 2003 and 2004. Total seed nitrogen harvested in 2002 and 2003 was reduced by 32% and 17%, respectively, when two applications of 2.52 kg ae/ha glyphosate were applied compared to hand weeded soybean. These studies indicate that nitrogen fixation and/or assimilation in GR soybean was only slightly affected at label use rate, but was consistently reduced at above label use rates of glyphosate and the greatest reductions occurred with soil moisture stress following glyphosate application.

Keywords: Nitrogen fixation; Nitrogen assimilation; Protein nitrogen; Transgenic soybean

1. Introduction

Glyphosate [*N*-(phosphonomethyl)glycine] is a non-selective, broad-spectrum, foliar-applied herbicide that controls a wide range of weeds, e.g. grasses, sedges, and broadleaf weeds (Franz et al., 1997; WSSA, 2002). It inhibits the biosynthesis of aromatic amino acids (phenylalanine, tyrosine, and tryptophan), which leads to several metabolic disturbances, including the arrest of protein synthesis and prevention of secondary product formation (Franz et al., 1997) and the deregulation of the shikimate pathway, leading to general metabolic disruption (Duke, 1988). Glyphosate-resistant (GR) soybean was engineered by stable integration of a transgene from *Agrobacterium* species that codes for a herbicide-insensitive 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme in the shikimate pathway (Delannay et al., 1995; Padgette et al., 1995). Transgenic soybean, resistant to glyphosate, represents a revolutionary breakthrough in weed control technology. In the USA, GR soybean acreage has steadily increased from 2% in 1996 to 85% in 2004 (USDA, 2004).

Although GR soybean is resistant to glyphosate, application of glyphosate has resulted in significant soybean injury under certain conditions and with certain formulations. Glyphosate has decreased chlorophyll

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content (Pline et al., 1999; Reddy et al., 2000), nodule biomass and leghemoglobin content (Reddy et al., 2000), and nitrogen fixation and nitrogen accumulation (King et al., 2001) in some glyphosate-resistant soybean cultivars. No significant yield reductions due to the glyphosate resistant gene occurred in extensive field trials of transgenic soybeans (Delannay et al., 1995; Elmore et al., 2001a; Krausz and Young, 2001; Norsworthy, 2004; Reddy, 2001; Reddy and Whiting, 2000). However, recently a few studies reported reduced soybean yield under stress conditions such as low water availability and in certain glyphosate-resistant soybean cultivars (Elmore et al., 2001b; King et al., 2001).

Symbiotic nitrogen fixation in soybean can provide from 65 to over 160 kg fixed nitrogen/ha (Klubeck et al., 1988) in a soybean crop, representing about 40–70% of the nitrogen requirement. Maintaining this significant nitrogen input can be important for profitable soybean yields, and sustaining long-term soil productivity, especially in soils containing low available soil nitrogen. Symbiotic nitrogen fixation can be affected by herbicides due to direct effects on the rhizobial symbiont as well as indirect effects on the physiology of the host plant (Moorman, 1989). Aside from plants, autotrophic microorganisms also possess EPSPS enzymes and are therefore susceptible to glyphosate (Jaworski, 1972; Fisher et al., 1986). EPSPS catalyzes the condensation of shikimic acid and phosphoenolpyruvate (Steinrucken and Amrhein, 1980). Inhibition of EPSPS by glyphosate results in the accumulation of shikimic acid and/or certain hydroxybenzoic acids such as protocatechuic and/or gallic acid in sensitive plant species (Becerril et al., 1989; Lydon and Duke, 1988), and in *B. japonicum* (Moorman et al., 1992; Hernandez et al., 1999). Toxic effects of glyphosate in prokaryotes may be attributed to: (1) the inability of the organism to synthesize aromatic amino acids, (2) an energy drain on the organism resulting from adenosine triphosphate and phosphoenolpyruvate spent in the accumulation of shikimate, 3-deoxy-D-arabino-heptulose-7-phosphate and hydroxybenzoic acids, and (3) toxicity of accumulated intermediates of the shikimic acid pathway (Fisher et al., 1986). In other studies, glyphosate reduced the nitrogenase activity of *B. japonicum* bacteroids with the inhibition proportional to the in vitro sensitivity of these strains under culture conditions (Hernandez et al., 1999). These researchers also demonstrated the accumulation of protocatechuic acid in soybean nodules of glyphosate-treated soybean plants suggesting that glyphosate was translocated to nodules. Recently, we have detected glyphosate residues in nodules of GR soybean following routine glyphosate applications under field conditions (Reddy and Zablotowicz, 2003).

Glyphosate effects on the bacterial symbiont (*B. japonicum*) of soybean, nodule parameters, and acetylene reduction activity have been studied under culture and greenhouse conditions as reviewed by Zablotowicz and Reddy (2004). However, only a few studies have attempted to assess the risk of this technology on symbiotic nitrogen fixation under field conditions. Furthermore, under field conditions, the presence of weeds in plots may confound the effects of glyphosate on soybean physiology. Competition for resources between soybean and weeds until weeds are killed by routine glyphosate postemergence applications may limit photosynthesis and subsequent yield potential. Glyphosate effects on both the *Bradyrhizobium* and endomycorrhizal symbiosis in GR soybean in the absence of weeds under field conditions merits investigation.

Therefore, the focus of this study was to assess physiological parameters and yield of GR soybean in response to glyphosate applications under weed-free conditions. The specific objectives of this study were to determine the effects of glyphosate on nodules, acetylene reduction activity, nitrogen content of leaf and seed, and yield of GR soybean under weed-free conditions.

2. Materials and methods

Research was conducted from 2002 through 2004 at the USDA Southern Weed Science Research Unit farm, Stoneville, MS. The soil was a Dundee silt loam (fine-silty, mixed, thermic Aeric Ochraqualf) with pH 6.3, 1.1% organic matter, a CEC of 15 cmol/kg, and soil textural fractions of 26% sand, 56% silt, and 18% clay, and contained an abundant native population of *B. japonicum*. The experimental area was tilled with a disk harrow followed by a field cultivator in the fall of each year. Experimental area was treated with paraquat at 1.1 kg ai/ha 1–4 days prior to soybean planting to kill existing vegetation. Soybean cultivar 'Asgrow AG4702RR' at 355,000 seeds/ha was planted on April 19, 2002, April 21, 2003, and April 27, 2004. A different experimental site with similar soil conditions was used in each year.

Glyphosate treatments included single and sequential applications beginning at 4 weeks after planting (WAP). The single application treatments consisted of glyphosate applied 4 WAP at rates of 0.84 or 1.68 kg ae/ha. The sequential application treatments consisted of glyphosate applied 4 and 6 WAP at rates of 0.84 + 0.84 or 2.52 + 2.52 kg ae/ha. At 4 WAP, soybean was in the two- to three-trifoliolate leaf stage and 6 WAP soybean was in the six- to seven-trifoliolate leaf stage. Glyphosate at 2.52 fb 2.52 kg ae/ha was higher than suggested label use rate for single and total in-crop application of glyphosate. This rate was selected to 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 in the study. Herbicide treatments were applied with a tractor-mounted sprayer with TeeJet 8004 standard flat spray tips delivering 187 L/ha water at 179 kPa. The commercial formulation of glyphosate was used with no additional adjuvant.

Ten to 15 soybean plants were randomly sampled from the middle two rows of each plot at 5, 6, 7, and 8 WAP. Plants were excavated with roots and shoot intact, immediately transported to the laboratory and assayed within 30 min of collection. Nitrogenase activity was assayed as described elsewhere (Hardy et al., 1968; Zablotowicz et al., 1981). Roots with nodules intact were excised, and incubated in 60 mL plastic syringes (5 and 6 WAP) or 1 L Mason jars (7 and 8 WAP). Two roots were placed in the syringes and six roots in the Mason jars, sealed, 10% volume of air was removed and replaced with an equal volume of acetylene. After one hour incubation at room temperature, duplicate 1.0 mL gas samples were removed and analyzed by gas chromatography for ethylene formation and carbon dioxide evolution. An Agilent HP6960 (Agilent Technologies, Wilmington, DE) gas chromatograph equipped with manual injector, injector loop, sample splitter and flame ionization detector (FID) and thermal conductivity detector (TCD) was used. Using the sample loop and splitter 0.25 mL of gas was directed into a 30 m length × 0.53 mm i.d. alumina megabore column (115-3532) connected to the FID detector, and 0.25 mL of sample was injected into a HP-PLOT D column (30 m length × 0.53 mm i.d. megabore with 40 μm film; 1905D-Q04) connected to the TCD detector using helium as a carrier gas. Chromatographs were integrated using Chem Station software. Standard curves for ethylene and carbon dioxide were developed for each day of analysis and used to determine ARA activity and carbon dioxide evolved. Samples having less than 9% acetylene were not used in the analysis. In 2002, two replicate samples were assayed from each plot, and in 2003 three replicate samples were assayed from each plot. Following the incubation, roots were washed, the nodules removed from the roots, and the dry weight of nodules and roots determined following oven-drying at 60 °C for 4–5 days.

The seventh-trifoliolate leaf at 6 WAP was sampled from twenty five randomly selected soybean plants in each plot each year. At harvest about 200 soybean pods were randomly hand sampled from middle two rows for seed nitrogen content. The leaf and grain samples were oven-dried (60 °C), finely ground twice in a Wiley Mill (0.42 mm sieve). Samples were re-dried the night before nitrogen analysis to remove any moisture that may have been absorbed prior to analysis. Total nitrogen was determined from triplicate samples (10–15 mg) using a Flash EA 112 elemental analyzer (CE Elantech, Lakewood, NJ). Nitrogen was expressed as percent of leaf or seed weight. Total seed nitrogen per hectare (kg/ha) was calculated as the product of yield and nitrogen content.

Soybean was grown nonirrigated for at least 8 WAP and was irrigated thereafter as needed because of late season dry weather. All plots including glyphosate-treated were hand weeded periodically throughout the season to keep them weed-free. Soybean was harvested from each plot using a combine on September 9, 2002, September 4, 2003, and August 27, 2004, and grain yield was adjusted to 13% moisture. Each treatment consisted of four soybean rows spaced 102 cm apart and 12.2 m long. Treatments were arranged in a randomized complete block design with four replications. The data for each variable were analyzed by analysis of variance (SAS, 2001). Treatment means were separated at the 5% level of significance using Fisher's LSD test.

3. Results and discussion

Soybean root biomass accumulation was relatively unaffected by glyphosate (Table 1). In 2002, root biomass in soybean treated with two applications of 2.52 kg/ha glyphosate was 20–25% less than untreated hand-weeded control at 7 ($P > 0.10$) and 8 ($P > 0.07$) WAP. In 2003, there was no effect of glyphosate on root biomass at any sample date. Overall, soybean root dry weight increased from 5 to 8 WAP in all treatments, and root biomass accumulation was relatively lower in 2002 compared to 2003. In other studies, glyphosate at 1.68 kg/ha (Reddy et al., 2000) and 6.3 kg/ha (King et al., 2001) rates has reduced shoot and root dry weights of glyphosate-resistant soybean under greenhouse conditions.

Table 1
Glyphosate effects on root biomass accumulation in glyphosate-resistant soybean in 2002 and 2003[a]

Treatment Herbicide	Rate (kg ae/ha)	Applied (WAP)	Soybean root dry weight (mg/plant)							
			2002				2003			
			5 WAP	6 WAP	7 WAP	8 WAP	5 WAP	6 WAP	7 WAP	8 WAP
Glyphosate	0.84	4	282	479	642	1192	331	646	918	1649
Glyphosate	1.68	4	253	464	626	1277	339	655	863	1682
Glyphosate fb	2.52	4	189	434	554	961	324	626	957	1815
Glyphosate	2.52	6								
Glyphosate fb	0.84	4	252	536	601	1114	301	565	933	1806
Glyphosate	0.84	6								
Hand-weeded	—	—	254	452	694	1277	303	647	982	1761
F-test ($P > F$)			0.12	0.38	0.10	0.07	0.50	0.29	0.48	0.59

[a]Abbreviations: fb, followed by; WAP, weeks after planting soybean.

All plants evaluated were relatively well nodulated as would be expected in fields having a long history of soybean production. Nodule biomass was relatively unaffected by glyphosate (Table 2). Nodule biomass accumulation in soybean treated with two applications of 2.52 kg/ha glyphosate was slightly reduced in 2002 ($P>0.07$) and increased in 2003 ($P>0.09$) compared to untreated hand-weeded control at 8 WAP. In 2003, nodule biomass accumulation was dramatically (about 100%) greater in all treatments compared to 2002 especially at 7 and 8 WAP. In another study, glyphosate at 0.84 kg/ha applied as single or sequential reduced nodule biomass compared to untreated control (Reddy et al., 2000). In this study, plots were kept weed-free compared to presence of weeds in their study (Reddy et al., 2000). The potential competition for soil nitrogen by weeds may have forced soybean plants to compensate by developing greater nodule mass in untreated control. Consequently, soybean plants in untreated plots may have had higher nodule biomass compared to soybean plants in glyphosate-treated plots that were relatively weed-free.

Nitrogen fixation potential measured as acetylene reduction activity was greater in 2003 compared to 2002 (Table 3), obviously reduced by water deficit in 2002 (Table 4). Amount of daily rainfall received during sampling period (5–8 WAP) was lower and distribution was erratic in 2002 compared to 2003. In 2002, significant effects of treatments were only observed at 7 and 8 WAP. At 7 WAP, the untreated hand-weeded control had greater ARA activity compared to the glyphosate treatments, while at 8 WAP, glyphosate at 1.68 kg/ha rate had the highest ARA activity. In 2003, no significant effect on ARA attributable to treatment was observed. Thus, using ARA as an estimate of nitrogen fixation potential only a minor effect of glyphosate was observed in 2002, when soybean plots were under moisture deficiency. As limited information on the effects of glyphosate on root metabolism has been reported, root respiration also was measured during the ARA assay. Overall, soybean root respiration was unaffected by glyphosate in both years with the exception of slight reduction in root respiration by two applications of 0.84 or 2.52 kg/ha glyphosate

Table 2
Glyphosate effects on nodule biomass accumulation in glyphosate-resistant soybean in 2002 and 2003[a]

Treatment Herbicide	Rate (kg ae/ha)	Applied (WAP)	Soybean nodule dry weight (mg/plant)							
			2002				2003			
			5 WAP	6 WAP	7 WAP	8 WAP	5 WAP	6 WAP	7 WAP	8 WAP
Glyphosate	0.84	4	119	140	124	238	57	267	432	522
Glyphosate	1.68	4	91	126	167	255	59	211	377	514
Glyphosate fb	2.52	4	85	119	133	192	62	299	438	650
Glyphosate	2.52	6								
Glyphosate fb	0.84	4	132	135	167	222	70	246	446	502
Glyphosate	0.84	6								
Hand-weeded	—	—	116	143	192	251	62	252	411	578
F-test ($P>F$)			0.66	0.57	0.44	0.07	0.40	0.15	0.40	0.09

[a]Abbreviations: fb, followed by; WAP, weeks after planting soybean.

Table 3
Glyphosate effects on acetylene reduction activity in glyphosate-resistant soybean in 2002 and 2003[a,b]

Treatment Herbicide	Rate (kg ae/ha)	Applied (WAP)	Acetylene reduction activity (nmole ethylene formed/plant/h)							
			2002				2003			
			5 WAP	6 WAP	7 WAP	8 WAP	5 WAP	6 WAP	7 WAP	8 WAP
Glyphosate	0.84	4	31	25	26 b	39 b	34	38	77	135
Glyphosate	1.68	4	22	28	32 b	67 a	29	33	67	143
Glyphosate fb	2.52	4	27	24	30 b	49 b	33	42	66	163
Glyphosate	2.52	6								
Glyphosate fb	0.84	4	27	22	32 b	43 b	36	35	68	123
Glyphosate	0.84	6								
Hand-weeded	—	—	26	25	45 a	48 b	30	34	65	158
F-test ($P>F$)			0.44	0.34	—	—	0.53	0.12	0.64	0.18

[a]Abbreviations: fb, followed by; WAP, weeks after planting soybean.
[b]Means within a column followed by the same letter are not significantly different at the 5% level as determined by Fisher's protected LSD test.

Table 4
Daily rainfall and sampling date of glyphosate-resistant soybean at Stoneville, MS in 2002 and 2003

Year	Month	Day	Rainfall (mm)	Weeks after planting
2002	May	3	11	
		4	13	
		10	10	
		11	8	
		13	3	
		18	20	
		23	0	5
		27	5	
		30	0	6
	June	6	0	7
		10	12	
		13	0	8
		14	3	
		23	2	
		25	6	
		28	43	
		29	39	
2003	May	3	2	
		5	1	
		6	1	
		11	24	
		15	2	
		17	26	
		19	1	
		21	2	
		25	6	
		26	1	
		27	0	5
	June	3	18	6
		6	12	
		7	2	
		10	0	7
		12	53	
		13	5	
		14	13	
		17	0	8
		18	51	
		19	7	
		27	15	
		30	9	

compared to untreated hand-weeded control at 8 WAP in 2002 (Table 5).

The nitrogen content of the fully expanded seventh-trifoliolate leaf at time of the second glyphosate application (6 WAP) was reduced in glyphosate treated plots compared with untreated hand-weeded plot in 2002 and 2003 (Table 6). In 2004, glyphosate at 2.52 fb 2.52 kg/ha reduced leaf nitrogen content compared to hand-weeded control. The highest leaf nitrogen content was observed in 2004 with the greatest moisture availability during the early stages of soybean development. Soybean nitrogen fixation is more sensitive to drought compared to assimilation of inorganic soil nitrogen (Purcell et al., 2004).

Glyphosate did not affect soybean yield regardless of rate compared with untreated hand-weeded (Table 7) with one exception. Glyphosate at 2.52 fb 2.52 kg/ha reduced soybean yield by 11% in 2002. However, it should be stressed that this rate is above the label use rate for GR soybean. Field studies conducted in Arkansas indicated that glyphosate-resistant soybean yield was reduced under drought conditions with two applications of glyphosate (King et al., 2001). As previously discussed with other parameters, this may have been due to early-season dry weather in 2002. Total rainfall amounts received during May and June of 2002 was 20% less than the normal monthly rainfall compared to 13 and 126% higher rainfall in 2003 and 2004, respectively.

Nitrogen content of soybean grain was significantly higher for the hand-weeded treatment than the glyphosate treatments in 2002 and 2003 (Table 7). Glyphosate at 1.68, 0.84 fb 0.84, and 2.52 fb 2.52 kg/ha reduced seed nitrogen content in 2002 and 2003. However, seed nitrogen content was unaffected in 2004. Total seed nitrogen (protein nitrogen) calculated as product of seed nitrogen and soybean yield was reduced with glyphosate at 2.52 fb 2.52 kg/ha in 2002 and 2003 and glyphosate at 0.84 fb 0.84 kg/ha in 2002 compared to untreated hand-weeded plot. Protein nitrogen was unaffected by glyphosate in 2004. In another study, despite the narrow range, nitrogen

Table 5
Glyphosate effects on root respiration in glyphosate-resistant soybean in 2002 and 2003[a,b]

Treatment Herbicide	Rate (kg ae/ha)	Applied (WAP)	Soybean root respiration (nmole CO_2 evolved/plant/h)							
			2002				2003			
			5 WAP	6 WAP	7 WAP	8 WAP	5 WAP	6 WAP	7 WAP	8 WAP
Glyphosate	0.84	4	370	484	443	747 ab	610	1015	1333	2107
Glyphosate	1.68	4	345	434	480	820 a	613	879	1200	2084
Glyphosate fb	2.52	4	302	433	465	617 b	609	968	1248	2417
Glyphosate	2.52	6								
Glyphosate fb	0.84	4	305	485	475	623 b	548	866	1201	2043
Glyphosate	0.84	6								
Hand-weeded	—	—	304	458	559	722 ab	585	840	1246	2321
F-test ($P>F$)			0.09	0.74	0.42	—	0.92	0.06	0.50	0.21

[a] Abbreviations: fb, followed by; WAP, weeks after planting soybean.
[b] Means within a column followed by the same letter are not significantly different at the 5% level as determined by Fisher's protected LSD test.

Table 6
Glyphosate effects on leaf nitrogen 6 WAP in glyphosate-resistant soybean in 2002–2004[a,b]

Treatment Herbicide	Rate (kg ae/ha)	Applied (WAP)	Soybean leaf nitrogen content (%)		
			2002	2003	2004
Glyphosate	0.84	4	3.7 c	3.5 b	6.1 b
Glyphosate	1.68	4	3.8 bc	3.6 ab	6.6 a
Glyphosate fb	2.52	4	3.2 d	3.3 c	6.1 b
Glyphosate	2.52	6			
Glyphosate fb	0.84	4	4.1 b	3.5 b	6.8 a
Glyphosate	0.84	6			
Hand-weeded	—	—	5.6 a	3.8 a	6.9 a

[a]Abbreviations: fb, followed by; WAP, weeks after planting soybean.
[b]Means within a column followed by the same letter are not significantly different at the 5% level as determined by Fisher's protected LSD test.

Table 7
Glyphosate effects on yield, seed nitrogen content, and protein nitrogen in glyphosate-resistant soybean in 2002–2004[a,b]

Treatment Herbicide	Rate (kg ae/ha)	Applied (WAP)	Soybean yield (kg/ha)			Seed nitrogen (%)			Total seed nitrogen (kg/ha)		
			2002	2003	2004	2002	2003	2004	2002	2003	2004
Glyphosate	0.84	4	3697 a	4417 a	3874 a	5.5 b	7.2 ab	6.9 a	204 a	321 a	266 a
Glyphosate	1.68	4	3265 bc	4443 a	3915 a	6.1 a	7.2 ab	6.9 a	198 a	320 a	271 a
Glyphosate fb	2.52	4	3035 c	4079 a	3805 a	4.7 c	6.6 c	7.0 a	142 c	271 b	266 a
Glyphosate	2.52	6									
Glyphosate fb	0.84	4	3462 ab	4404 a	3974 a	5.0 bc	7.3 ab	6.8 a	174 b	323 a	271 a
Glyphosate	0.84	6									
Hand-weeded	—	—	3397 b	4325 a	3828 a	6.2 a	7.6 a	6.8 a	211 a	328 a	261 a

[a]Abbreviations: fb, followed by; WAP, weeks after planting soybean.
[b]Means within a column followed by the same letter are not significantly different at the 5% level as determined by Fisher's protected LSD test.

content was slightly lower with two applications of glyphosate at 0.84 kg/ha compared to a single application (Reddy and Zablotowicz, 2003).

This study demonstrated that label use rates of glyphosate in a GR soybean cultivar had minimal effects on nitrogen assimilation (leaf nitrogen, seed nitrogen) and that GR soybean yield was unaffected by glyphosate at label use rate. The greatest and more consistent reductions in nitrogen assimilation were observed when glyphosate was applied above the recommended rate. Currently, hundreds of GR soybean cultivars from different maturity groups are commercially available. The physiological and yield responses of these cultivars to glyphosate may vary, and may also depend on geographical location, environmental conditions, soil types, sensitivity of native populations of *B. japonicum*, etc. However, most soybean farmers in the midsouthern US do not use supplemental rhizobium culture or nitrogen fertilizer in soybean production. The potential for GR soybean injury from glyphosate treatments exists (Reddy et al., 2004). It has been shown that injury in GR soybean is caused by aminomethylphosphonic acid (AMPA) formed from glyphosate degradation. The extent of injury in glyphosate-treated GR soybean is largely dependent on levels of AMPA formed within the plant. Under field conditions, the extent of AMPA formation may depend on glyphosate rate, genotype, and environmental conditions. Dependence of AMPA formation on these various factors explains why some soybean farmers observe glyphosate injury in GR soybean and others do not, and why the same farmer often observes injury in one year and not in other years. The effect of AMPA on nodulation, nitrogen fixation, and nitrogen assimilation in GR soybean needs further investigation. Inconsistent effects of glyphosate on nodulation and nitrogen fixation observed in several studies may be related to extent of AMPA formation in GR soybean.

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