Impact of Glyphosate on the *Bradyrhizobium japonicum* Symbiosis with Glyphosate-Resistant Transgenic Soybean: A Minireview

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ABSTRACT

Glyphosate-resistant (GR) soybean [*Glycine max* (L.) Merr.] expressing an insensitive 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS) gene has revolutionized weed control in soybean production. The soybean nitrogen fixing symbiont, *Bradyrhizobium japonicum*, possesses a glyphosate-sensitive enzyme and upon exposure to glyphosate accumulates shikimic acid and hydroxybenzoic acids such as protocatechuic acid (PCA), accompanied with *B. japonicum* growth inhibition and death at high concentrations. In a series of greenhouse and field experiments, glyphosate inhibited nodulation and nodule leghemoglobin content of GR soybean. Glyphosate accumulated in nodules of field-grown GR soybean, but its effect on nitrogenase activity of GR soybean was inconsistent in field studies. In greenhouse studies, nitrogenase activity of GR soybean following glyphosate application was transiently inhibited especially in early growth stages, with the greatest inhibition occurring under moisture stress. Studies using bacteroid preparations showed that the level of glyphosate inhibition of bacteroid nitrogenase activity was related to in vitro glyphosate sensitivity of the *B. japonicum* strains. These studies indicate the potential for reduced nitrogen fixation in the GR soybean system; however, yield reductions due to this reduced N_2 fixation in early stages of growth have not been demonstrated.

SYMBIOTIC N_2 FIXATION in soybean can provide from 65 to more than 160 kg fixed nitrogen ha^{-1} (Klubeck et al., 1988) in a soybean crop, representing about 40 to 70% of the nitrogen requirement. Maintaining this significant nitrogen input can be important for economically sustainable soybean yields, 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). Thus, understanding the impacts of herbicides on the crop and the symbiont is essential. Several research groups have conducted experiments to assess the effect of glyphosate on the *B. japonicum*–soybean symbiosis to address potential implications for risk assessment on the GR soybean cropping system.

Glyphosate [*N*-(phosphonomethyl)glycine; Roundup (Monsanto, St. Louis, MO)] is a foliar-applied, broad-spectrum, nonselective herbicide that controls a wide range of weeds (e.g., grasses, sedges, and broadleaf weeds) (Franz et al., 1997; Weed Science Society of America, 2002). This herbicide inhibits the synthesis of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) in plants and microorganisms (Jaworski, 1972; Fisher et al., 1986). The mechanism of action of glypho-

sate is unique since it is the only herbicide that specifically inhibits the enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase EC 2.5.1.19 (EPSPS) (Steinrucken and Amrhein, 1980), which catalyzes the condensation of shikimic acid and phosphoenolpyruvate (Fig. 1). Inhibition of the shikimic acid pathway 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 *B. japonicum* (Moorman et al., 1992; Hernandez et al., 1999). Toxic effects of glyphosate may be attributed to (i) the inability of the organism to synthesize aromatic amino acids; (ii) an energy drain on the organism resulting from adenosine triphosphate and phosphoenolpyruvate (PEP) spent in the accumulation of shikimate, 3-deoxy-D-arabino-heptulose-7-phosphate (DAHP), and hydroxybenzoic acids; and (iii) toxicity of accumulated intermediates of the shikimic acid pathway (Fisher et al., 1986).

The introduction of transgenic soybean resistant to glyphosate has provided new opportunities for weed control in soybean that can replace or reduce the use of preemergence herbicides and tillage (Carpenter and Gianessi, 2001; Reddy, 2001a). The basis of resistance to glyphosate in soybeans is the insertion of an insensitive EPSPS gene from an *Agrobacterium* strain CP4 allowing expression of a functional shikimic acid pathway (Padgette et al., 1995). In the USA, GR soybean was first commercialized in 1996 and has been widely adopted by farmers since its introduction. The USA soybean production area planted with GR soybean has increased from 2% in 1996 to 81% in 2003 (Carpenter and Gianessi, 2001; Council for Biotechnology Information, 2002; USDA, 2003). One of the benefits of postemergence application of glyphosate is the facilitation of conservation management practices such as no-till or minimum-tillage management practices that conserve energy inputs and reduce soil erosion (Barnes, 2000).

Glyphosate is generally considered a relatively short-lived herbicide in the soil environment (Torstensson and Hamissepp, 1977; Franz et al., 1997; Weed Science Society of America, 2002). Several species of soil bacteria can metabolize glyphosate, for example, *Pseudomonas* sp. (Jacob et al., 1988), *Arthrobacter* sp. (Pipke et al., 1987), and certain members of the Rhizobiaceae (Liu et al., 1991), including *Sinorhizobium meliloti*, *Rhizobium trifolii*, *R. leguminosarurm*, *Agrobacterium rhizogenes*, and *A. tumefaciens*. These bacteria possess a carbon–phosphate lyase that hydrolyzes glyphosate to form sarcosine and inorganic phosphate, allowing them

Abbreviations: AE, acid equivalent; ARA, acetylene reduction activity; DAE, days after emergence; EPSPS, 5-enolpyruvylshikimic acid-3-phosphate synthase; GR, gyphosate-resistant; PCA, protocatechuic acid.
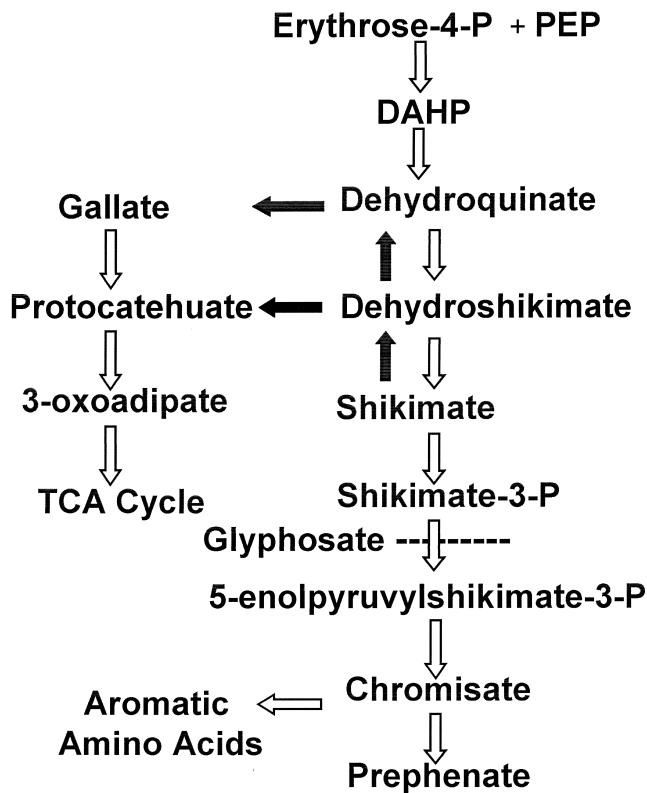
Fig. 1. Shikimic acid pathway and the inhibition by glyphosate in plants and microorganisms. Dark arrows indicate the overall effects of glyphosate inhibition of 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS) and pathways for accumulation of hydroxybenzoic acids (Moorman et al., 1992).

Table 1. Effect of glyphosate on the growth of three *Bradyrhizobium japonicum* strains and accumulation of protocatechuic acid in the culture media (Moorman et al., 1992).

Glyphosate concentration	*B. japonicum* strain		
	USDA 110	USDA 123	USDA 138
mM			
	Growth inhibition (%)		
0	0	0	0
0.5	41	10	15
1.0	47	12	19
5.0	100	100	100
	Protocatechuic acid (μM), 72 h after treatment		
0	8	10	10
1.0	4	1150	875
5.0	4	1300	1000

risk of this technology on symbiotic nitrogen fixation. This minireview will summarize results from available studies.

GLYPHOSATE EFFECTS ON *Bradyrhizobium japonicum* GROWTH AND PRODUCTION OF HYDROXBENZOIC ACIDS

Initial studies by Jaworski (1972) demonstrated that glyphosate inhibited growth of *B. japonicum* strain USDA 71 by 69 and 92% at relatively low concentrations of 0.01 and 1.0 mM, respectively. Further studies by Moorman et al. (1992) demonstrated differential growth inhibition sensitivity among *B. japonicum* strains USDA 110, 123, and 138 in a defined mannitol glutamine broth (Table 1). Growth of USDA 110 (the most sensitive strain) was inhibited 41 and 47% at 0.1 and 0.5 mM glyphosate, respectively. But the growth of strains USDA 123 and 138 was only moderately inhibited by 0.5 mM glyphosate (12 and 19%, respectively). Growth of all three strains was completely inhibited at 5 mM, and 10 mM glyphosate caused rapid cell death. The addition of aromatic amino acids to the culture media did not reverse growth inhibition by glyphosate in strain USDA 138. However, addition of aromatic amino acids in the absence of glyphosate also inhibited the growth rate of strain USDA 138. Moorman et al. (1992) observed that protocatechuic acid (PCA) accumulated in the culture media of strains USDA 123 and 138 in response to glyphosate, but not in the most sensitive strain USDA 110 (Table 1). Levels of shikimic acid were much lower than that of PCA, indicating that shikimic acid was being metabolized to PCA in these strains. In the absence of glyphosate these three *B. japonicum* strains metabolized PCA (Moorman et al., 1992), as was also reported for *B. japonicum* and other members of the Rhizobiaceae (Hussein et al., 1974; Parke and Ornston, 1984).

In studies on other bacterial species, glyphosate inhibited growth of *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* with different patterns of metabolite accumulation (Fisher et al., 1986). However, these bacteria grew and survived at glyphosate concentrations typically lethal to *B. japonicum* (>5 mM). Addition of a mixture of aromatic acids (L-phenylalanine, L-tryptophan, and L-tyrosine) reversed glyphosate inhibition of growth and accumulation of shikimate-3-phos-

to utilize glyphosate as their sole source of phosphorous. Growth of *S. meliloti* was inhibited at concentrations exceeding 1 mM glyphosate and growth was improved by the addition of aromatic acids in the presence of glyphosate (Liu et al., 1991). Despite the ability of certain *R. trifolii* strains to detoxify glyphosate, application of glyphosate to the root zone inhibited the nodulation of red clover (*Trifolium pratense* L.) (Mårtensson, 1992) and also inhibited nodulation and acetylene reduction activity (ARA) in sub clover (*Trifolium subterraneum* L.) (Eberbach and Douglas, 1989). A single foliar application of a sublethal treatment (0.21 kg acid equivalent [AE] ha^{-1} glyphosate) to a glyphosate-sensitive soybean cultivar reduced nodule number by 32%, nodule mass accumulation by 75%, and leghemoglobin content by 13%, two weeks after treatment (Reddy et al., 2000). In this study, 0.21 kg AE ha^{-1} glyphosate reduced shoot and root growth by 36 and 54%, respectively, compared with untreated control soybean. Growth, respiration, and nitrogen fixation activity of the nonsymbiotic nitrogen fixing bacteria *Azotobacter chrococcum* and *A. vinlandeii* were inhibited by glyphosate in vitro; however, relatively high concentrations were required for inhibition (Santos and Flores, 1995). These studies indicate a sensitivity of nodulation and symbiotic nitrogen fixation to glyphosate.

As GR soybean has only been commercialized since 1996, only a few studies have attempted to assess the

phate in *E. coli* and *P. aeruginosa*. In *B. subtilis*, the magnitude of glyphosate growth inhibition was reduced by addition of aromatic amino acids; however, DHAP, shikimate, and shikimate-3-phosphate continued to accumulate in the presence of glyphosate and aromatic amino acids. Aromatic amino acids directly regulate an early step in the synthetic pathway, DHAP synthetase, in *E. coli* and *P. aeruginosa*. In *B. subtilis* precursors of aromatic amino acids, prephenate, and chromisic acid were responsible for the feedback inhibition of DHAP synthetase. Thus, exogenous aromatic amino acids are unable to prevent an energy drain of a blocked EPSPS enzyme in *B. subtilis*. The deregulation of the shikimic acid pathway by glyphosate and subsequent energy drain contributes to glyphosate-mediated growth inhibition of *B. japonicum* (Moorman et al., 1992). Addition of aromatic amino acids was unable to reverse glyphosate inhibition in *B. japonicum*, thus its regulation may be similar to that of *B. subtilis*.

Studies by Hernandez et al. (1999) confirmed a differential growth inhibition by glyphosate among three *B. japonicum* strains with a 50% inhibition at 30 μM in the most sensitive strain (ISJ-32), and a 50% inhibition at >1 mM in the most tolerant strain (ISJ-33), and strain ISJ-48 was intermediate. However, all three of these strains accumulated relatively high levels of shikimate when exposed to 0.3 mM glyphosate, with the most tolerant strain ISJ-33 accumulating about three- to fourfold greater shikimate levels than the two more sensitive strains. In the presence of glyphosate, about 5 to 19% of the carbon utilized by these *B. japonicum* strains was transformed into shikimate (Hernandez et al., 1999). Thus, accumulation of shikimate and other hydroxybenzoic acids represents a significant loss of energy and may be a significant factor responsible for a reduced growth yield.

GLYPHOSATE EFFECTS ON GLYPHOSATE-RESISTANT SOYBEAN NODULATION

The effects of glyphosate on the nodulation of GR soybean have been critically assessed in a series of greenhouse experiments by Reddy et al. (2000) and King et al. (2001). In both studies various nodulation parameters of GR soybean were significantly reduced by label field application rates of glyphosate; however, results were inconsistent among all experiments. The studies by Reddy et al. (2000) evaluating foliar applications of two rates of the isopropylamine salt (IPA) of glyphosate on nodulation parameters of GR soybean (DP5806RR) treated with a commercial inoculant are summarized in Table 2. In Study 1, application of 0.84 kg AE ha^{-1} significantly reduced nodule number (28%), nodule mass (47%), and leghemoglobin content (13%); however, application of 1.68 kg AE ha^{-1} elicited no effect on nodulation. In Study 2, early glyphosate application had no effect on nodulation regardless of application rate; however, delayed application of 1.68 kg AE ha^{-1} at 3 wk after planting reduced nodule number (30%), nodule mass (39%), leghemoglobin content (18%), and total nitrogen content of shoots (14%). In the studies by King et al. (2001), early glyphosate application (1.26 kg AE ha^{-1}) at 5 and 12 days after emergence (DAE) significantly reduced nodule biomass accumulation by 33% compared with untreated plants of TV5866RR soybean at 19 DAE in one of two studies, but total nitrogen content of roots and shoots was reduced by 34 and 36% in both studies. However, late application at 18, 25, and 32 DAE had no effect on nodule biomass.

The effects of one (early postemergence, V2 stage) or two applications (early and late postemergence; V4 stage) of four salt formulations of glyphosate on the nodulation of GR soybeans under field conditions has been studied (Reddy and Zablotowicz, 2003). In this 2-yr study, nodule number was unaffected 28 d after the early (V2) postemergence treatment; however, one (V2) or two applications (V2 and V4) of all formulations significantly reduced nodule mass (fresh weight) by 21 to 28% compared with the untreated control (Table 3). Leghemoglobin content was significantly reduced by 8 to 10% compared with untreated control plants by two applications of all glyphosate formulations or the early postemergence application of the aminomethanamide dihydrogen tetraoxosulfate (ADT) glyphosate salt. A

Table 2. Effect of two rates of glyphosate application at 14 or 21 d after planting on soybean shoot nitrogen content, nodulation, and nodule leghemoglobin content, determined 14 d after application in greenhouse experiments (Reddy et al., 2000).

Glyphosate rate	Nitrogen content shoots	Nodule number	Nodule mass	Leghemoglobin content
kg AE ha^{-1}	mg N plant^{-1}	nodules plant^{-1}	mg plant^{-1}, fresh wt.	mg g^{-1} nodule fresh wt.
		Applied at 14 d after planting (Study 1)		
0	ND†	18a‡	64a	1.6a
0.84	ND	13b	34b	1.4b
1.68	ND	17a	66a	1.5ab
		Applied at 14 d after planting (Study 2)		
0	14.7a	22a	114a	1.5a
0.84	13.4a	23a	91a	1.3b
1.68	14.5a	20a	90a	1.4ab
		Applied at 21 d after planting (Study 2)		
0	16.2a	33a	216a	1.7a
0.84	15.0ab	28ab	242a	1.5b
1.68	14.0b	23b	131b	1.4b

† Not determined

‡ Means within a column for a given experiment followed by the same letter do not differ significantly at the 0.05 probability level as determined by Fisher's protected LSD test.

Table 3. Effect of various salt formulations of glyphosate (0.84 kg AE ha^{-1}) and number of applications on nodulation parameters, and glyphosate content of nodules in field grown glyphosate-resistant soybeans, 28 d after first application (Reddy and Zablotowicz, 2003).

Glyphosate formulation and applications†	Nodule number	Nodule mass fresh weight	Leghemoglobin content	Glyphosate concentration
	number plant^{-1}	mg plant^{-1}	mg g^{-1} fresh wt.	ng g^{-1} dry wt.
Untreated	41	706	9.65	9
IPA 1	33	544	9.54	58
IPA 2	35	524	8.68	147
TMS 1	30	521	9.40	79
TMS 2	35	508	8.84	39
DIA 1	39	524	9.47	67
DIA 2	35	536	8.79	123
ADT 1	36	556	8.81	75
ADT 2	34	529	8.82	47
LSD (0.05)	NS‡	146	0.80	78

† IPA, isopropylamine salt; TMS, trimethylsulfonium salt; DIA, diammonium salt; ADT, aminomethanamide dihydrogen tetraoxosulfate salt. The term 1 represents application at V2 soybean growth stage, while 2 represents application at V2 and V4 soybean growth stage.
‡ Not significant at the 0.05 probability level.

reduction in nodule mass without reducing nodule number and a reduction in leghemoglobin content suggested that glyphosate was inhibiting nodule development but not nodule formation.

GLYPHOSATE EFFECTS ON GLYPHOSATE-RESISTANT SOYBEAN

Glyphosate has been considered to undergo little or no metabolism in most plants and is readily translocated into metabolic sinks such as plant roots (Duke, 1988). Considering the demand for photosynthate in nodules it is apparent that glyphosate may also accumulate in nodules. Nodule glyphosate concentrations were determined by enzyme linked immunosorbent assay (ELISA) in a Mississippi field study (Reddy and Zablotowicz, 2003). Glyphosate concentrations in nodules of treated plants ranged from 39 to 147 ng g^{-1} nodule fresh weight (Table 3), and the highest levels associated with soybean receiving two applications of the diammonium or isopropylamine salts of glyphosate. Low concentrations (<10 ng g^{-1} nodule fresh weight) were observed in untreated plants (one of four replicates), which may have been due to potential glyphosate drift. Glyphosate residues and one of its metabolites (aminomethylphosphonic acid) were found in seeds (Duke et al., 2003) of GR soybean treated with glyphosate at label use rates. Therefore, it is possible that glyphosate could affect GR soybean growth and yield.

Since glyphosate is readily translocated to plant roots, reduced root growth of GR soybean has been observed in several greenhouse studies in soybeans dependent upon symbiotic nitrogen fixation and in soybean receiving nitrogen fertilization (King et al., 2001; Reddy et al., 2000). The accumulation of shikimic acid and several hydroxybenzoic acids was compared in leaves and nodules of conventional soybean inoculated with three *B. japonicum* strains, or in leaves of nitrate-grown soybeans as affected by two rates of glyphosate (Hernandez et al., 1999). No shikimic acid was observed in leaves of untreated soybean, while shikimic acid accumulated at levels of 62 to 108 and 109 to 184 μmol g^{-1} (2–3% of the plant's dry weight) in nodulated and nitrate dependent soybean treated with 5 and 10 mM glyphosate, respectively. Shikimic acid was observed in low concentrations (2–3 μmol g^{-1}) in nodules of untreated plants and its concentration increased with increasing rate of glyphosate. Levels of shikimic acid found in nodules of treated plants were about three- to fourfold higher than that observed in untreated plants. Protocatechuic acid was the dominant hydroxybenzoic acid found in leaves and nodules of glyphosate-treated soybean accumulating in concentrations of about 2 to 5 μmol g^{-1} in both leaves and nodules of treated soybean.

Application of glyphosate to GR soybean has caused injury, including decreased chlorophyll content under certain environmental conditions and with certain salt formulations of glyphosate (Reddy et al., 2000; Reddy and Zablotowicz, 2003). Chlorophyll loss in glyphosate-treated GR soybean was rate- and temperature-dependent, with greater loss at higher rates and higher temperatures (Pline et al., 1999). Pline et al. (1999) conjectured that glyphosate injury to GR soybean at 35°C may have resulted from increased translocation of glyphosate to new meristematic areas and could be due to secondary effects caused by glyphosate. Reddy et al. (2000) examined glyphosate effects on GR soybean under greenhouse conditions and found that glyphosate at 0.84 kg AE ha^{-1} had little or no effect on chlorophyll content and dry weight of shoot and roots in five of five trials. But treatment of glyphosate at 1.68 kg AE ha^{-1} reduced these parameters in three of five trials, indicating potential for soybean injury at higher rates. In a 2-yr field study, one and two applications of trimethylsulfonium (TMS) and ADT salt formulations of glyphosate injured GR soybean and visible injury (yellowing, speckling, and necrosis) ranged from 8 to 38%, two days after treatment. However, soybean completely recovered from injury over time, and chlorophyll content and dry weight of shoot and root growth of GR soybean were unaffected by glyphosate at 14 d after treatment (Reddy and Zablotowicz, 2003).

GLYPHOSATE EFFECTS ON NITROGEN FIXATION IN GLYPHOSATE-RESISTANT SOYBEAN

King et al. (2001) evaluated the effects of multiple foliar applications of glyphosate on nitrogen fixation activity of GR soybeans (TV5866RR) in four growth

chamber studies using the acetylene reduction assay (ARA). Soybean received three applications of glyphosate at 5, 12, and 19 DAE and ARA was determined at 14, 21, and 28 DAE. Significant reductions in ARA (12–20%) were observed in three of four studies at 21 DAE, but only in one of four studies at 14 or 28 DAE. These studies suggested that both nodulation and nitrogen fixation activity was more sensitive in the early stages of soybean development. King et al. (2001) also assessed the effects of moisture deficit on ARA activity of glyphosate-treated plants. Acetylene reduction activity was more sensitive to moisture deficits for glyphosate-treated soybean than for untreated plants.

The susceptibility of symbiotic N_2 fixation to glyphosate inhibition by *B. japonicum* strains was studied by Hernandez et al. (1999) using bacteroid preparations incubated in the presence of succinate. Using this technique, glyphosate effects on symbiotic N_2 fixation due to inhibition of photosynthesis and carbon substrate availability were minimized. These studies demonstrated that ARA in bacteroids isolated from treated conventional soybeans was 10 to 30% lower than that of untreated plants. The level of ARA inhibition corresponded to glyphosate sensitivity of the *B. japonicum* strain under in vitro conditions (Fig. 2), as the glyphosate sensitive strain (ISJ-32) was most affected by glyphosate and the glyphosate tolerant strain (ISJ-33) was least affected. In another experiment (Hernandez et al., 1999), bacteroids of these *B. japonicum* strains were isolated from untreated conventional soybean, and the bacteroids were treated with glyphosate. Glyphosate concentrations of 0.5 and 1.0 m*M* inhibited ARA of the most sensitive strain (ISJ-32) by 20 and 28%, respectively, while the most tolerant strain (ISJ-33) was inhibited by about 8 and 23%, respectively. The moderately tolerant strain ISJ-48 exhibited the least degree of inhibition (4 and 8%, respectively). The relatively low degree of ARA inhibition in bacteroids treated with glyphosate indicated that bacteroids from treated plants responded differently than bacteroids from untreated plants. Treatment of bacteroids with either shikimate (3–10 m*M*) or

PCA (0.75–1.5 m*M*) had minimal or negligible effects on either ARA or respiration, indicating that toxicity of these metabolites was not a factor in inhibition of nitrogen fixation.

A preliminary assessment of effects of glyphosate (isopropylamine salt) applications on ARA activity in GR soybean (AG4702RR) was conducted under Mississippi field conditions in 2002 (unpublished data) at the USDA Southern Weed Science Research Unit farm in Stoneville (Fig. 3). A significant reduction in ARA activity of glyphosate-treated soybean compared with untreated soybean was observed at only one of six sample times following glyphosate application, at 48 d after planting. There was no rainfall during the first 20 d following glyphosate application, and it was evident that soybean plants were exhibiting moisture stress. There was a large variance in ARA measured and differences in moisture deficit among plots may have been responsible for the large variance observed in this study.

GLYPHOSATE EFFECTS ON GLYPHOSATE-RESISTANT SOYBEAN YIELD

Currently, hundreds of GR soybean varieties from different maturity groups are commercially available. The physiological responses of these varieties to glyphosate application may vary, and the responses may also depend on geographical location, environmental conditions, soil types, *B. japonicum* populations, and other factors. This phenomenon needs further investigation. Most soybean farmers in the USA do not use supplemental *Bradyrhizobium* inoculation or nitrogen fertilizer in soybean production. No yield reductions due to glyphosate applications to GR soybean have been observed in extensive field trials (e.g., Delannay et al., 1995; Elmore et al., 2001a; Gonzini et al., 1999; Krausz and Young, 2001; Nelson and Renner 1999; Reddy, 2001b; Reddy and Whiting, 2000). Recently, Elmore
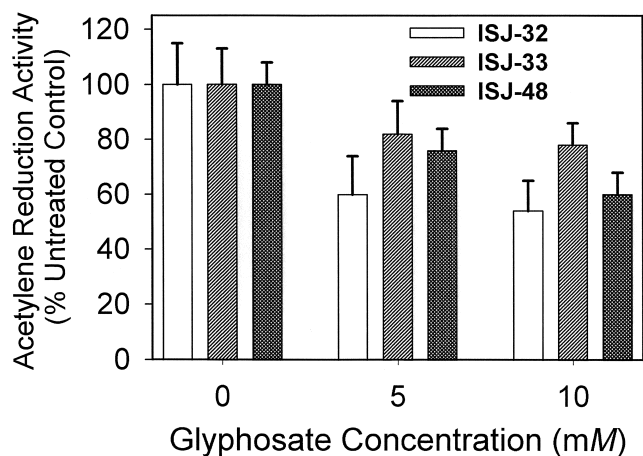

Fig. 2. Inhibition of acetylene reduction activity in bacteroid preparations of three strains of *Bradyrhizobium japonicum* extracted from nodules of conventional soybean 7 d after application of 0, 5, and 10 m*M* glyphosate (Hernandez et al., 1999).
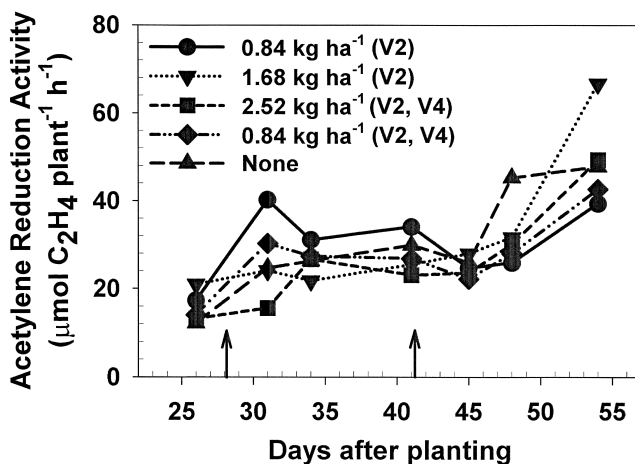

Fig. 3. Effect of different glyphosate applications on acetylene reduction activity of field grown soybeans. Arrows indicate date of glyphosate application (unpublished data). Each point represents a mean of six replicates. Significant differences (LSD values at the 0.05 probability level are indicated in parentheses) were observed at Day 31 (11.5), Day 48 (13.6), and Day 54 (13.3).

et al. (2001b) have demonstrated that GR sister lines yielded 5% less than the non-GR sisters. These authors suggest that yield suppression appears to be associated with the GR gene or its insertion process rather than to glyphosate. The effects of several herbicide regimes on biomass accumulation and seed yield of two GR soybean cultivars were evaluated at two Arkansas field sites (King et al., 2001). In the Fayetteville site, which received more abundant irrigation and rainfall, no effect of glyphosate was observed. However, in the Kaiser site, which had undergone moisture stress, significant reductions in shoot biomass (92 DAE) were observed in all three glyphosate treatments and a standard herbicide regime (acifluorfen and bentazon) in the AR5901RR cultivar. A significant reduction in yield was only observed in soybean treated with glyphosate at 7 and 21 DAE (24.6% compared with untreated control). A significant reduction in yield was observed in DK5961RR treated with glyphosate at 7 and 49 DAE (23.6% compared with the untreated control), but no effect on shoot biomass was observed.

CONCLUSIONS

Deleterious effects of glyphosate on *B. japonicum* and its inhibition of the nodulation and/or nitrogen fixation processes have been observed in GR soybean. Although the effects of GR soybean genotype has been assessed in terms of yield potential, the magnitude of inhibition of N_2 fixation in soybeans due to glyphosate application has not been critically assessed under field conditions. Soybean productivity and N_2 fixation have the potential to compensate for short periods of stress. However, even a small reduction in N_2 fixation potential may have long-term effects on sustainable soil nitrogen pools, considering the widespread adoption of the GR soybean system. The effects of glyphosate on N_2 fixation potential of GR soybean should be especially evaluated on sandy soils with limited nitrogen availability. It may also be appropriate to implement techniques such as the ^{15}N natural abundance technique (Amarager et al., 1979) and uriede content of xylem sap (McNeil, 1981; Herridge and Peoples, 1990) in field studies to assess the magnitude of N_2 fixation inhibition by glyphosate in GR soybean.

It may be also feasible to genetically construct *B. japonicum* strains that may have greater tolerance to glyphosate. *Bradyrhizobium japonicum* strains can be engineered to metabolize glyphosate, by introducing a gene for a glyphosate C–P lyase from fast growing members of the Rhizobiaceae, for example, *Sinorhizobium meliloti* (Liu et al., 1991), and thus enable the symbiont to directly detoxify glyphosate. The *phn* genes from *S. meliloti*, homologous to *phnG*, *-H*, *-I*, and *-J* of *Escherichia coli*, encoding for the structural genes of the C–P lyase that hydrolyzes glyphosate, have been cloned (Parker et al., 1999). However, the expression of *phn* is regulated by availability of phosphate, and regulatory genes in this operon may need to be modified to inactivate repression by phosphate. A second strategy may consider incorporating a glyphosate insensitive EPSPS,

for example, *A. tumefaciences* CP4, as utilized in GR resistant crops. These proposed genetic constructs can add to our basic understanding of glyphosate-mediated energy drain in *B. japonicum*, and its implications on symbiotic nitrogen fixation. However, introduction of improved *B. japonicum* strains is typically hindered by an inability to compete with indigenous strains for nodule occupancy (Berg et al., 1988; Klubeck et al., 1988), and may have limited commercial utility.

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