SOYBEAN

Plant Growth and Nitrogenase Activity of Glyphosate-Tolerant Soybean in Response to Foliar Glyphosate Applications

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ABSTRACT

Glyphosate [*N*-(phosphonomethyl)glycine] inhibits 5-enolpyruvylshikimate-3-phosphate synthase, EC 2.5.1.19 (EPSPS), thereby blocking aromatic amino acid synthesis. While glyphosate-tolerant (GT) soybean [*Glycine max* (L.) Merr.] contains resistant EPSPS, the N_2-fixing symbiont in soybean root nodules, *Bradyrhizobium japonicum*, does not contain a resistant enzyme, and glyphosate spray to GT soybean may interfere with the symbiotic relationship. Glyphosate-tolerant soybean was treated with glyphosate at several different stages of development to evaluate N_2 fixation, growth, and yield in a series of greenhouse, growth chamber, and field experiments. Early applications of glyphosate generally delayed N_2 fixation and decreased biomass and N accumulation in the cultivar Terral TV5866RR (TV5866RR) harvested at 19 d after emergence (DAE), but plants had recovered by 40 DAE. The biomass and N content of GT soybean were also decreased by glyphosate in plants that were grown with available soil N. There were differences in sensitivity to glyphosate among GT cultivars, with biomass decreases in response to glyphosate ranging from 0 to 30% at 40 DAE for the most tolerant and sensitive cultivars that were evaluated. In growth chamber studies, N_2 fixation was more sensitive to water deficits in glyphosate-treated plants. In field studies, there was no measured effect of glyphosate on GT soybean at Fayetteville, AR where there was adequate soil water throughout the growing season. However, glyphosate tended to decrease biomass and seed yields under conditions of limited soil water at Keiser, AR.

Glyphosate is the active ingredient in the nonselective herbicide Roundup (Monsanto, St. Louis, MO). Advances in biotechnology have resulted in GT soybean cultivars, providing an effective broad-spectrum postemergence weed control option. Glyphosate competitively inhibits EPSPS, an enzyme in the shikimate pathway, leading to the synthesis of aromatic amino acids (Duke, 1988). Glyphosate-tolerant soybean contains an EPSPS that was originally isolated from *Agrobacterium* sp. (Padgette et al., 1995) and is resistant to glyphosate. Although the EPSPS in GT soybean is tolerant to glyphosate, the N_2-fixing symbiont, *Bradyrhizobium japonicum*, has a sensitive form of the enzyme (Jaworski, 1972; Moorman et al., 1992).

The sensitivity of *B. japonicum* to glyphosate is influenced by the herbicide concentration and bacterial strain. The growth of strain USDA 110 was inhibited 41 to 100% in culture at glyphosate concentrations of 0.5 to 5 m*M* (Moorman et al., 1992). The strains USDA 123 and 138 were less sensitive at 0.5 to 1 m*M* of glyphosate, with inhibition of only 10 to 20%, but they were inhibited 100% at a 5 m*M* concentration. The bacterial strain USDA 71 was very sensitive, with bacterial growth decreased 69 to 92% by glyphosate concentrations of 0.01 to 1 m*M* (Jaworski, 1972). Despite the recognition of *B. japonicum* sensitivity to glyphosate, there have been no reports of the effect of glyphosate on N_2 fixation in GT soybean.

Glyphosate is not readily degraded in soybean, and it concentrates in metabolic sinks such as young roots and developing and mature nodules (Duke, 1988). Previous research indicates that a single foliar application of glyphosate at 0.5 kg ha^{-1} can result in concentrations up to 0.3 m*M* in the bulk root tissue of susceptible plant species (Honegger et al., 1986). Higher rates of glyphosate use or repeated applications could result in even greater concentrations, especially in the stronger metabolic sinks such as soybean root nodules compared with the bulk root system (McWhorter et al., 1980). The combination of *B. japonicum* sensitivity and potential glyphosate concentration in soybean roots and nodules could impact the symbiotic relationship that results in N_2 fixation in soybean.

Symbiotic N_2 fixation is critical for obtaining high yields in soybean grown on soils without large amounts of available N (Cooper and Jeffers, 1984). Importantly, N_2 fixation in soybean is more sensitive to water-deficit stress than are other processes such as gas exchange (Durand et al., 1987), transpiration (Sall and Sinclair, 1991), and uptake and assimilation of inorganic soil N (Purcell and King, 1996). Any conditions that adversely affect the symbiotic relationship between soybean and *B. japonicum*, such as glyphosate in the soybean root system, may also influence the sensitivity of N_2 fixation to water deficits.

Extensive research under high-yield environments (2000–4000 kg ha^{-1} seed yield) indicated that there was generally no effect of glyphosate on soybean yield compared with untreated plants under weed-free conditions (Delannay et al., 1995). Therefore, it is unlikely that glyphosate has any long-term effects on N_2 fixation or other processes that are critical for yield formation in

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Abbreviations: ARA, acetylene reduction activity; DAE, days after emergence; DI, deionized; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; FTSW, fraction of transpirable soil water; GT, glyphosate tolerant.

these environments. The response of glyphosate-treated GT soybean to water-deficit conditions, however, has not been determined. Our hypothesis was that glyphosate may affect the growth *B. japonicum* in the early stages of nodule development, leading to an increased sensitivity of N_2 fixation to water deficits. The objectives of this research were to: (i) evaluate the influence of glyphosate applications on the development of symbiotic N_2 fixation and the subsequent sensitivity of N_2 fixation to water-deficit stress in GT soybean; (ii) compare GT soybean growth with and without soil N in response to glyphosate; (iii) compare several GT soybean cultivars for sensitivity to glyphosate; and (iv) evaluate the effects of glyphosate on the growth and yield of field-grown soybean under late-planted, potentially low-yielding conditions.

MATERIALS AND METHODS

Greenhouse Experiments

Greenhouse Experiment 1

Experiments were conducted to evaluate the growth of the GT soybean cultivar TV5866RR in response to postemergence glyphosate applications. Seeds were planted in 15-cm pots with an approximate soil volume of 1.9 L. The potting medium was an N-free mixture of peat, vermiculite, and perlite (LB2, Sun Gro Hortic., Garland, TX). Soil was saturated with deionized (DI) water and then 500 mL of full-strength, N-free nutrient solution (de Silva et al., 1996) was added before planting. Pots were inoculated with approximately 1×10^7 cells of *B. japonicum* (USDA 110) at planting. After emergence, the plants were thinned to one per pot and reinoculated with *B. japonicum*. Plants were well watered by adding DI water daily, and each pot received 250 mL of N-free nutrients weekly after emergence. Day and night temperatures were approximately 30 and 24°C, respectively, and natural illumination was supplemented with 1000 W metal-halide lamps for a day length of 16 h.

Glyphosate (Roundup Ultra Herbicide, Monsanto, St. Louis, MO) was applied at 1.68 kg a.i. ha^{-1} in a carrier volume of 93 L ha^{-1} and at a spray pressure of 260 kPa. Each application was as a postemergence over-the-top spray to soybean plants using a moving-nozzle spray chamber. Herbicide application and harvest schedules are given in Table 1. At each harvest, plants were separated into shoots, roots, and nodules. Nodules were photocopied, and the total number of nodules per plant was determined from the photocopies. Plant sections were oven-dried at 65°C for 48 h, weighed, and ground to pass a 1-mm sieve. Total N was determined for the shoots and root-plus-nodule dry matter by the Agriculture Services Laboratory, University of Arkansas, with a Leco FP-228 Determinator (Leco, St. Joseph, MI). The experiment consisted of six replications and was repeated. Seeds for the first and second runs of the experiment were planted on 25 Feb. and 14 Apr. 1998, respectively. Biomass, total N, and nodule data were analyzed by harvest using analysis of variance with glyphosate treatment as a fixed effect and replication as a random effect. Treatment means were compared using Fisher's protected LSD ($P \leq 0.05$).

Table 1. Glyphosate treatments applied to TV5866RR in Greenhouse Experiment 1.

		Application timing†	
Treatment	Harvest	DAE‡	Growth stage§
none	1¶	–	–
early	1	5, 10	V1, V2
none	2	–	–
early	2	5, 10	V1, V2
late	2	18, 25, 32	V4, V5, V7

† Each glyphosate application was at 1.68 kg ha^{-1}.
‡ DAE, days after emergence.
§ Soybean growth stages at each application timing.
¶ Harvest 1 and 2 were at 19 and 40 DAE, respectively.

Greenhouse Experiment 2

An additional experiment was conducted to evaluate differences in the sensitivity to postemergence applications of glyphosate among five GT soybean cultivars from maturity groups IV and V. The cultivars that were evaluated were Asgrow A4501RR (A4501RR), Asgrow A5901RR (A590 1RR), Delta King 5961RR (DK5961RR), Hartz H5164RR (H5164RR), and TV5866RR. Growing conditions, plant developmental stages at glyphosate application timings, and data recorded were the same as in Greenhouse Experiment 1. Glyphosate was applied at 1.68 kg ha^{-1} on 5, 10, 18, 25, and 32 DAE for a total of 8.4 kg ha^{-1}, and plants were harvested at 40 DAE. Although the maximum recommended rate for glyphosate within a single soybean crop is 3.36 kg ha^{-1}, this high application-rate total was applied to accentuate any potential differences in the cultivar sensitivity to glyphosate. The experiment was a completely random design with six replications.

Greenhouse Experiment 3

The response of DK5961RR to glyphosate with and without available soil N was evaluated. Treatments were a factorial arrangement of two harvest dates, plus and minus soil N, and plus and minus glyphosate application. Experimental conditions were similar to those described for Greenhouse Experiments 1 and 2, including the timing of nutrient applications. At 5 and 12 DAE when soybean was at the V1 and V2 developmental stages (Fehr and Caviness, 1977), respectively, glyphosate was applied at 1.68 kg ha^{-1} to plants of the plus glyphosate treatment. Plants were harvested at 19 and 40 DAE as described previously. For the plus N treatment, plants harvested at 19 and 40 DAE received a total of 150 and 300 mg of N as NO_3^{-1}, respectively, in the nutrient solution. The experiment was a randomized complete block with six replications and was repeated.

Growth Chamber Experiments

The effect of glyphosate on the development of nitrogenase activity and the subsequent response of nitrogenase activity to soil water deficits in the cultivar TV5866RR were evaluated in a growth chamber. Plants were established in flow-through pots (Purcell et al., 1997) for the nondestructive measurement of acetylene (C_2H_2) reduction activity (ARA) as an indicator of the relative nitrogenase activity. Pots were constructed from polyvinyl chloride pipe that was 10-cm diam. and 40-cm long and sealed at the bottom and top with end caps. Fittings were inserted into the bottom for drainage and the introduction of air samples during ARA measurements and into the top for air exhaust and sampling. Pot tare weights were recorded before adding the potting mixture. Potting media and nutrients were the same as described in the greenhouse studies. Pots were filled with potting media, saturated with DI water, and 1 L of N-free nutrients was added to each pot. After allowing excess water to drain for 12 h, pot capacity weights were recorded.

A single plant was grown through a hole in the top of each

pot. Inoculum was added to the soil at planting and after emergence as described for greenhouse studies. The growth chamber was maintained at 24°C, and light was supplied by fluorescent and incandescent lamps with an intensity at the top of the plant of approximately 500 μmol m^{-2} s^{-1} photosynthetically active radiation. Treatments consisted of control and glyphosate-treated plants. Glyphosate was applied to glyphosate-treated plants at 1.68 kg ha^{-1} on 5, 12, and 19 DAE at the V1, V2, and V4 developmental stages, respectively, as described for the greenhouse experiments.

The development of nitrogenase activity was monitored by measuring ARA at 14, 21, and 28 DAE. Acetylene reduction activity was measured by introducing a 9:1 air/acetylene mixture at 0.5 L min^{-1} into the bottom of the pots that were sealed around the plant root system. The acetylene mixture was exhausted from the fitting in the top of the pot. After 8 min, when the ethylene (C_2H_4) concentration in the gas exhausted from the pots was constant, samples were collected from the efflux of each pot with 1-mL syringes. The ethylene concentration in the gas samples was quantified by gas chromatography using a flame ionization detector and a Porapak N column (de Silva et al., 1996). After the 8-min acetylene exposure, acetylene was removed from the gas stream, and pots were flushed with air for approximately 60 min. Previous research (data not shown) showed that this assay system does not result in an acetylene-induced decline of nitrogenase activity (Minchin et al., 1983). Ethylene concentrations were expressed as μmol $plant^{-1}$ h^{-1} C_2H_4, and the means within an experiment and sample date were compared using standard errors. The experiment was repeated four times using a completely random design, with Runs 1 and 4 having six replicates and Runs 2 and 3 having four replicates each of glyphosate-treated and untreated control plants.

The nitrogenase activity in TV5866RR in response to water deficits in the glyphosate-treated and control plants was evaluated following the ARA measurement at 28 DAE. At this time, half of the glyphosate-treated and control plants from ARA experiments 2, 3, and 4 were designated as either well watered or water deficit. The pots were weighed and watered daily at 0800 h. Well-watered plants were maintained at 0.7 of the pot capacity weight. The daily target weights for water-deficit plants were progressively decreased over a 7-d period, until on the final day of measurement, the soil was 0.33 of the pot capacity weight.

Soil water data were converted from pot capacity weight to a fraction of the transpirable soil water (FTSW) (de Silva et al., 1996). Transpirable water was calculated as the difference between the soil capacity weight and the soil weight when the transpiration for water-deficit plants was <10% of the well-watered plants (Ritchie, 1981). The soil weight at zero transpirable water was determined to be 0.24 of the soil weight at pot capacity. Daily target weights were converted to FTSW according to the equation:

$$\text{FTSW} = (1.32 \times \text{fraction of pot capacity soil weight}) - 0.32$$

Acetylene reduction activity was measured daily between 1000 and 1200 h for all plants during the drying period. Acetylene reduction activity values were double-normalized according to Ray and Sinclair (1997). The first normalization corrected for differences in activity among individual plants before initiation of the water-deficit treatments, and the second normalization minimized the effects of fluctuations in ARA for control plants (well watered, no glyphosate) among days. As a result, relative ARA was 1 for all plants at the beginning of the experiment and approximately 1 for the control plants throughout the experiment. Relative ARA values among glyphosate and water treatments within a day were compared using standard errors.

Field Experiments

Soybean was planted on 9 July and 17 June 1999 at Fayetteville and Keiser, AR, respectively, to evaluate the response of GT soybean cultivars to foliar applications of glyphosate under field conditions. The Fayetteville experiment was conducted on a Pembroke silt loam (Ultic Paleudalfs) on 15-cm-high beds that were spaced 1 m apart. Water was applied as needed with sprinkle irrigation until midreproductive development (R5). At Keiser, the soil was a Sharkey silty-clay (Vertic Haplaquepts), and soybean was planted on a flat seedbed with rows 0.96 m apart. Irrigation was applied with an overhead, lateral-move system.

The experiments at both locations were a factorial of two cultivars and five herbicide treatments in a randomized complete block design with four replications. Plots were four rows wide by 9 m long. A5901RR and DK5961RR were chosen to represent the least and most sensitive to glyphosate of the GT soybean cultivars evaluated in Greenhouse Experiment 2. The seeding rate was 370 000 ha^{-1} at both locations.

Herbicide treatments were (i) weed-free check, (ii) glyphosate at 7 and 21 DAE, (iii) glyphosate at 7 DAE and at R2, (iv) glyphosate at 21 and 35 DAE, and (v) acifluorfen {5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid} plus bentazon [3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide] at 7 DAE (included as a standard herbicide comparison). Herbicides were applied as a broadcast treatment, sprayed over the top of the canopy with a CO_2-powered backpack sprayer in a volume of 93 L ha^{-1}. Glyphosate was applied at 1.68 kg ha^{-1} at each timing, and acifluorfen and bentazon were applied at 0.42 and 0.84 kg a.i. ha^{-1}, respectively. All of the herbicide treatments contained appropriate surfactants. Plants were at V1, V4, and V9 development stages at 7, 21, and 35 DAE application timings, respectively, at both locations. R2 applications were made at 45 and 48 DAE at Fayetteville and Keiser, respectively. Plots were maintained weed free by hand-weeding as needed. Four replications of the nonnodulating isoline of the cultivar Lee were included at each location as an indicator of the plant-available soil N.

Aboveground biomass was collected from 1 m^2 from the center two rows of each plot at 14, 35, 49, and 70 DAE at both locations and at 91 DAE at Keiser. Samples were prepared and analyzed for total N as previously described. Seed yield was determined by harvesting 4.5 m from each of the center two rows. Average seed mass was determined from a random sample of 100 seeds. Biomass data were evaluated by comparing the means ± the standard errors between herbicide treatments within a cultivar and sample date. Seed yield and 100-seed weight data were analyzed by location and cultivar using analysis of variance, and significant differences were based on an LSD ($P \leq 0.05$).

RESULTS

Greenhouse Experiments

Greenhouse Experiment 1

Glyphosate affected the biomass and N accumulation of TV5866RR at Harvest 1 (19 DAE) in Runs 1 and 2 (Table 2). Shoot biomass was decreased 17%, but the root biomass was not decreased by glyphosate at Harvest 1. Nitrogen accumulation was decreased 35% in both the roots and shoots of glyphosate-treated plants at Harvest 1 (19 DAE) when averaged across runs.

Table 2. Biomass and N content of TV5866RR in response to glyphosate at Harvest 1 [19 d after emergence (DAE)] in Greenhouse Experiment 1.

Glyphosate treatment	Biomass				N	
			Nodules			
	Root	Shoot	Run 1	Run 2	Root	Shoot
	g plant^{-1}				mg plant^{-1}	
none	0.19	0.48	0.065	0.085	6.4	14.0
early†	0.17	0.40	0.043	0.088	4.2	8.9
LSD (0.05)‡	NS	0.07	–	–	1.5	5.0
within a run§	–		0.016		–	
between runs	–		0.017		–	

† Early treatment consisted of glyphosate applied foliarly at 1.68 kg ha^{-1} 5 and 12 DAE.
‡ Main effect of the treatment was significant for shoot biomass. Root biomass was not affected by glyphosate treatment at Harvest 1.
§ There was a significant run × treatment interaction for nodule biomass.

There was no difference in the number of nodules per plant (data not shown), but total nodule weight was decreased 34% in Run 1 of the experiment. In Run 2, however, nodule mass was not affected at 19 DAE.

Visual observations were consistent with a greater effect of glyphosate on N accumulation than on biomass accumulation at 19 DAE. Between 12 and 20 DAE, plants treated with glyphosate were yellow in comparison to the control plants. The plants from the control treatment were also yellow from approximately 12 to 14 DAE, corresponding to a depletion of N from seed reserves and before active N_2 fixation, but the control plants recovered from N-deficiency symptoms more rapidly than did glyphosate-treated plants.

By Harvest 2, at 40 DAE, the only evident effect of glyphosate was on soybean nodules (Table 3). In Run 1 of the experiment, the untreated plants had fewer nodules that were larger than the nodules of glyphosate-treated plants. The increased number of nodules for glyphosate-treated plants offset the decrease in individual nodule size, resulting in a similar total nodule mass among treatments. Glyphosate had no effect on soybean plants by 40 DAE in Run 2. Although greenhouse temperature and day length were similar between runs of the experiment, there were probably differences in light intensity that were associated with the February and April planting dates for Runs 1 and 2, respectively.

Table 3. TV5866RR root nodule response to glyphosate at Harvest 2 [40 d after emergence (DAE)] in Greenhouse Experiment 1.

Glyphosate treatment†	Individual nodule mass		Nodules per plant		Total nodule mass	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
	mg nodule^{-1}		no.		g plant^{-1}	
none	3.57	1.76	111	226	396	398
early	2.73	1.70	138	256	377	435
late	2.79	2.00	150	209	418	418
LSD (0.05)‡	–	–	23	NS	–	–
within a run§	0.36		–		NS	
between runs	0.60		–		NS	

† Glyphosate was applied at 1.68 kg ha^{-1} 5 and 12 DAE for the early treatment and 18, 25, and 32 DAE for the late treatment.
‡ Number of nodules per plant was analyzed by experiment because of a lack of homogeneity of variance between experiments.
§ There was a significant run by glyphosate treatment interaction for individual nodule mass and no significant difference among treatments for total nodule mass.

Table 4. Biomass and N content of glyphosate tolerant (GT) soybean cultivars at 40 d after emergence (DAE) in response to glyphosate in Greenhouse Experiment 2.

Cultivar	Glyphosate treatment	Biomass			N	
		Nodule	Root	Shoot	Root	Shoot
		g plant^{-1}			mg plant^{-1}	
TV5866RR	none	0.43	1.20†	4.25†	49†	160
	E+L‡	0.34	0.71	3.07	35	131
A4501RR	none	0.51	1.84*	5.41	60†	201
	E+L	0.47	1.22	4.55	45	185
A5901RR	none	0.47†	1.14	3.65	39	163
	E+L	0.54	0.93	4.03	45	163
DK5961RR	none	0.50*	1.39*	4.31	52*	163
	E+L	0.40	0.74	3.41	31	142
H5164RR	none	0.48	1.35	4.52	48	158
	E+L	0.47	1.18	4.36	46	170

* Indicates a significant difference with a two-sided *t*-test at the 0.05 level between control and glyphosate-treated plants within a cultivar and measurement parameter.
† Indicates a significant difference with a two-sided *t*-test at the 0.1 level between control and glyphosate-treated plants within a cultivar and measurement parameter.
‡ Glyphosate at 1.68 kg a.i. ha^{-1} was applied 5, 10, 18, 25, and 32 DAE in the early plus late (E + L) treatment.

This could account for differences in the response to glyphosate between Run 1 and 2 for the total nodule mass at Harvest 1 and the individual nodule mass at Harvest 2.

Greenhouse Experiment 2

Soybean cultivars responded differently to glyphosate applications (Table 4). Root biomass and N content at 40 DAE were decreased by 25 to 47% for the glyphosate-treated plants of the cultivars TV5866RR, A4501RR, and DK5961RR compared with the control-treatment plants. The nodule mass in DK5961RR and the shoot mass in TV5901RR were also decreased by glyphosate treatment. None of the measured biomass or N parameters were decreased by glyphosate for the cultivars A5901RR or H5164RR, and in fact, the nodule biomass for A5901RR was increased for plants in the glyphosate treatment relative to plants from the control treatment.

Although glyphosate application timings were different between Greenhouse Experiments 1 and 2, the response of GT soybean was similar. In Experiment 1, plants were treated with glyphosate early and showed a decreased biomass and N content at 19 DAE but had recovered by 40 DAE. In Greenhouse Experiment 2, the continued use of glyphosate between 19 and 40 DAE prevented the recovery seen at 40 DAE in Experiment 1.

Greenhouse Experiment 3

Dinitrogen fixation did not appear to be the only, or perhaps even the primary, physiological process that was inhibited by glyphosate in soybean. Early applications of glyphosate decreased biomass and N content by 20 to 47% for the cultivar DK5961RR by 19 DAE when it was grown in the presence of soil N (Table 5). In the treatments without soil N, glyphosate did not decrease the plant biomass or N content at 19 DAE.

Table 5. Effect of glyphosate and soil N on DK5961RR at Harvest 1 [19 d after emergence (DAE)] and Harvest 2 (40 DAE) in Greenhouse Experiment 3.

Harvest	Soil N	Glyphosate†	Biomass		N	
			Root	Shoot	Root	Shoot
			— g plant^{-1} —		— mg plant^{-1} —	
1	yes	no	0.38a‡	0.76a	9.66a	38.8a
		yes	0.20c	0.58c	5.77c	31.0b
1	no	no	0.30b	0.72ab	7.31bc	20.9c
		yes	0.30b	0.63bc	7.53b	22.2c
2	avg§	no	0.60*	2.17*	13.3	81.6*
	avg	yes	0.48	1.81	11.1	70.7
2	yes	avg	0.49	2.50*	10.8*	104.6*
	no	avg	0.58	1.48	13.6	47.8

* Indicates a significant difference within a column between N means or glyphosate means as determined by an *F*-test ($P = 0.05$).
† Glyphosate was applied at 1.68 kg ha^{-1} 5 and 12 DAE to plus glyphosate treatments.
‡ Means followed by the same letter within a column for Harvest 1 are not significantly different as determined by an LSD ($P = 0.05$).
§ Values were averaged over the treatment effects when an analysis of variance indicated the interaction term was NS.

At Harvest 2 (40 DAE), the soil N × glyphosate treatment interaction was not significant ($P = 0.66$) for plant biomass or N content, indicating that plants responded similarly to glyphosate with or without soil N. Glyphosate decreased root and shoot biomass 17 to 20% and shoot N content 13% when averaged across soil N treatments (Table 5). Plants that were grown on nutrients containing N were larger than those dependent upon N_2 fixation, and the root/shoot ratio was very different. Soil N increased shoot weight by 70% over plants that received no soil N, but the root weight was not significantly different between N treatments. Similarly, total shoot N for plants from the plus soil N treatment was more than double that from the minus N treatment, whereas root N content was greater for plants grown without soil N. These results indicate a preferential allocation of plant resources to support the root growth in plants dependent upon N_2 fixation compared with soybean plants supplemented with soil N.

Growth Chamber Experiments

In growth chamber experiments, ARA was measured at 14, 21, and 28 DAE to evaluate the development of nitrogenase activity in response to glyphosate treatment. The nitrogenase activity for glyphosate-treated and control plants was similar at 14 DAE, except in Run 4, when ARA was lower for glyphosate-treated plants (Table 6). Acetylene reduction activity was 12 to 20% lower for glyphosate-treated plants at 21 DAE in three of the four experiments. By 28 DAE, ARA was lower in glyphosate-treated plants only in Run 2 and was greater than the control plants in Run 1. These results from the growth chamber agree with Greenhouse Experiment 1 in that, under well-watered conditions, N_2 fixation was generally decreased by early application of glyphosate but subsequently recovered.

Acetylene reduction activity was more sensitive to water deficits for glyphosate-treated plants than for untreated plants (Fig. 1). The relative ARA for water-deficit plants was not different from well-watered plants until FTSW was below 0.27. The decrease in the relative ARA in response to water deficit was substantially greater for glyphosate-treated plants than for untreated plants. At an FTSW of 0.21, untreated plants had nitrogenase activity that was 88% of the well-watered plants while glyphosate-treated plants with the same amount of available water had nitrogenase activity that was 71% of the well-watered control. This relationship of a greater sensitivity of nitrogenase activity to water deficits of glyphosate-treated plants compared with untreated plants was also evident at FTSW values of 0.14 and 0.11. The relative transpiration was not different between glyphosate-treated and untreated plants (data not shown), indicating that differences in water extraction did not account for the increased sensitivity to water deficit in glyphosate-treated plants.

Table 7. Soybean seed yield as influenced by herbicide treatment and cultivar in field experiments at Keiser and Fayetteville, Arkansas in 1999.

Herbicide treatment	Application dates	Keiser		Fayetteville	
		A5901RR	DK5961RR	A5901RR	DK5961RR
	DAE†	kg ha^{-1}			
none	–	1291a*	1296a	2084a	1874a
glyphosate 1‡	7 & 21	974b	1197ab	2053a	1854a
glyphosate 2	7 & 49	1138ab	989b	2057a	1798a
glyphosate 3	21 & 35	1050ab	1291a	1999a	1992a
standard§	7	1075ab	1362a	1936a	1917a

* Means within a column followed by the same letter are not significantly different as determined by an LSD ($P = 0.05$).
† DAE, days after emergence.
‡ Glyphosate treatments consisted of an application of 1.68 kg ha^{-1} at each of the indicated timings.
§ The standard herbicide treatment consisted of acifluorfen plus bentazon applied at 0.42 plus 0.84 kg ha^{-1}.

Field Experiments

Growth of the Lee nonnodulating cultivar indicated differences in plant available soil N between the Fay-

Table 6. Acetylene reduction activity (ARA) (mean ± SE) at 14, 21, and 28 d after emergence (DAE) of TV5866RR as influenced by glyphosate in four growth chamber experiments.

DAE	Run 1		Run 2		Run 3		Run 4	
	None	Glyph†	None	Glyph	None	Glyph	None	Glyph
	μmol C_2H_4 plant^{-1} h^{-1}							
14	32 ± 2	33 ± 2	27 ± 4	23 ± 3	59 ± 10	57 ± 13	38 ± 2	28 ± 4‡
21	40 ± 3	45 ± 3	66 ± 4	53 ± 6‡	90 ± 1	75 ± 9‡	64 ± 3	56 ± 3‡
28	61 ± 7	76 ± 7‡	91 ± 10	70 ± 4‡	131 ± 5	115 ± 13	116 ± 9	107 ± 14

† Plants were treated with 1.68 kg ha^{-1} of glyphosate at 5, 12, and 19 DAE.
‡ Indicates a significant difference (± two SEs) between glyphosate-treated and untreated plants, within a run of the experiment and within a measurement date.

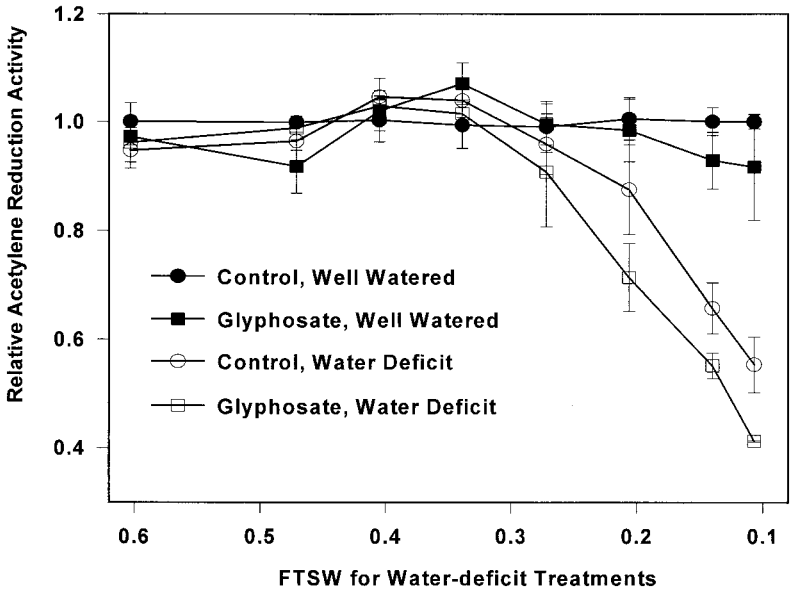

Fig. 1. Relative acetylene reduction activity (ARA) of TV5866RR soybean in the growth chamber in response to glyphosate treatment under well-watered and water-deficit conditions. Glyphosate was applied at 1.68 kg ha^{-1} at 5, 12, and 19 DAE for the glyphosate-treated plants. Well-watered plants were maintained at a fraction of transpirable soil water (FTSW) of 0.6, and water-deficit plants were watered daily to the indicated FTSW during the drying cycle. Values are the mean ± SE (n = 7).

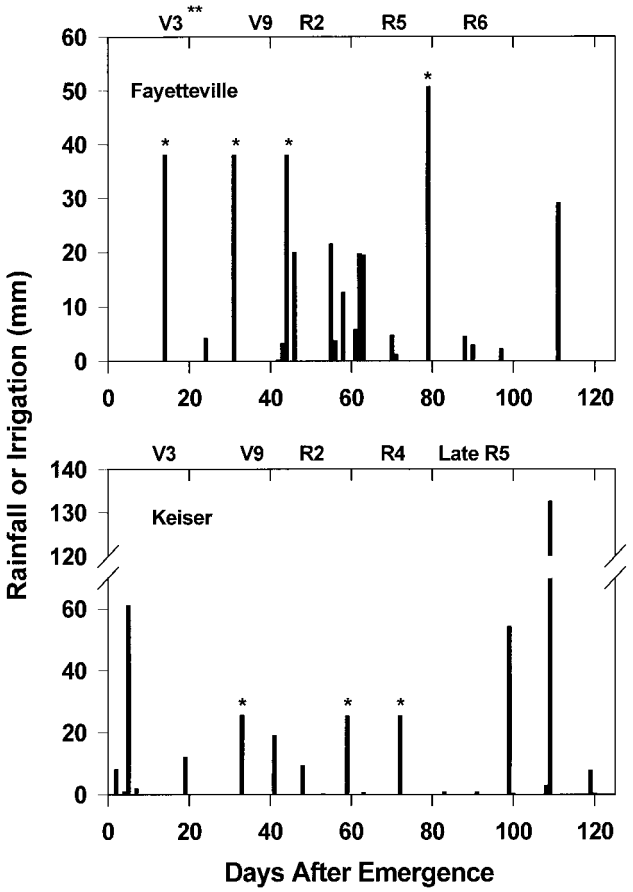

Fig. 2. Rainfall, irrigation (*), and soybean growth stages (**) for Fayetteville and Keiser field experiments. At Fayetteville, the growing season ended at 104 d after emergence (DAE) due to freezing temperatures.

etteville and Keiser locations. At the final biomass harvest, Lee nonnodulating had accumulated 40 to 50% as much N and 63 to 70% as much biomass as the weed-free check for the other two cultivars at Keiser (data not shown). At Fayetteville, N and biomass accumulation by Lee nonnodulating was approximately 65 and 76%, respectively, of the weed-free checks for the N-fixing cultivars. These data indicate that available soil N was greater at the Fayetteville location, decreasing the dependence of soybean on N_2 fixation as a N source compared with the Keiser location.

None of the herbicide treatments affected biomass or N accumulation, 100-seed weights (data not shown), or yield (Table 7) at Fayetteville where there was adequate soil water from rainfall or irrigation throughout the season (Fig. 2). At Keiser, herbicide treatments did affect N and biomass accumulation. There was no effect of herbicide treatment on the shoot N concentration within a sample date; therefore, only biomass data are presented. The standard herbicide treatment of acifluorfen plus bentazon slightly decreased the biomass of both cultivars during vegetative growth at 35 and 48 DAE (Fig. 3). By early reproductive growth, at 70 DAE, the biomass for all herbicide-treated plots was equal to the untreated check.

There was adequate soil water at the Keiser location until the biomass harvest 70 DAE (Fig. 2). Following a 25-mm irrigation applied 72 DAE, there was no significant rainfall or irrigation for 27 d, until 99 DAE. During this time, the untreated checks generally continued to increase biomass at a greater rate than herbicide-treated plants (Fig. 3). For A5901RR, all glyphosate-treated and standard herbicide-treated plots had significantly less biomass at 92 DAE than the untreated check. Although there were no significant differences in biomass

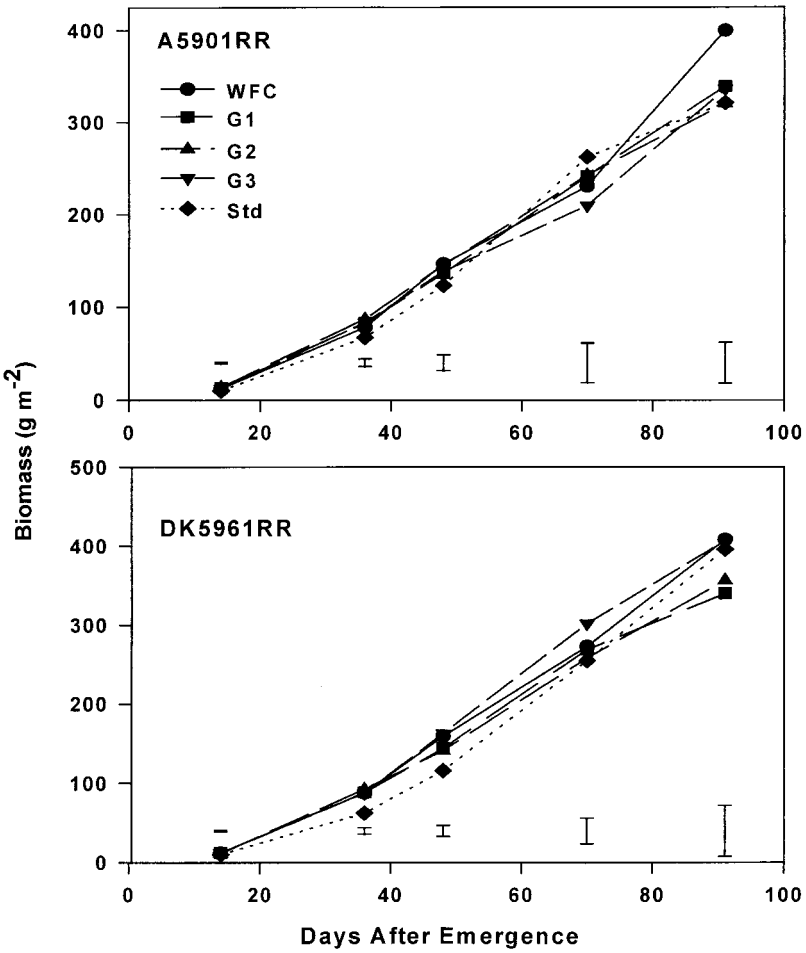

Fig. 3. Biomass of GT soybean cultivars in response to herbicide treatments in the Keiser field experiment. Treatments were: WFC, weed-free check; G1, glyphosate applied 7 and 21 d after emergence (DAE); G2, glyphosate at 7 DAE and R2; G3, glyphosate at 21 and 35 DAE; and Std, acifluorfen plus bentazon standard at 0.42 plus 0.84 kg ha^{-1} at 7 DAE. Each glyphosate application consisted of glyphosate at 1.68 kg ha^{-1}. Error bars represent two standard errors for comparison among herbicide treatments within a sample date.

for DK5961RR at 92 DAE, the biomass tended to be lower than other treatments for plots that were treated with glyphosate at 7 followed by 21 DAE or 7 followed by 49 DAE.

At Keiser, seed yields tended to reflect the differences in biomass that were seen at 92 DAE (Table 7). The A5901RR yields from herbicide-treated plots were 12 to 25% lower than from the untreated check, but only the glyphosate treatment at 7 followed by 21 DAE had a significantly lower yield than the check. The DK5961RR yield was significantly decreased (24%) by glyphosate applied at 7 followed by 49 DAE, and the yield from plots sprayed with glyphosate at 7 followed by 21 DAE was numerically lower (8%) than the untreated check. These two treatments also had a tendency for decreased biomass, though not significantly, at the biomass harvest 92 DAE (Fig. 3). The seed yields of DK5961RR from other glyphosate and standard herbicide treatments were numerically equal to the untreated check (Table 7).

Although these cultivars responded differently to multiple glyphosate applications when grown under well-watered conditions in the greenhouse, they responded similarly under field conditions. This indicates that genotypic differences in the sensitivity to glyphosate may depend on the number of applications, the application rate and timing, and the subsequent plant-growth environment.

DISCUSSION

Previous research (Jaworski, 1972; Moorman et al., 1992) established that the growth of *B. japonicum* in culture was inhibited by glyphosate at concentrations likely to be found in the roots and nodules of glyphosate-treated plants (Honegger et al., 1986; McWhorter et al., 1980). Our data indicate that applications of glyphosate to young soybean delays N_2 fixation and increases the sensitivity of N_2 fixation to water deficits, but the detrimental effects of glyphosate on plant growth are not limited to symbiotic N_2 fixation. Indeed, glyphosate decreased root and shoot growth proportionately the same for plants supplemented with N fertilizer as for plants that were totally dependent on N_2 fixation for N.

The delay in N_2 fixation in the growth chamber experiments and the decreased N accumulation in Greenhouse Experiments 1 and 2 are consistent with the disruption of normal nodulation. Roots continue to form nodules until the plant has an adequate supply of N, which appar-

ently inhibits further nodule initiation (Parsons et al., 1993). In the infected cells of a nodule, *B. japonicum* is enclosed within the symbiosome membrane, which is highly selective and separates the bacteria from the cytosol in mature nodules (Udvardi and Day, 1997). In the early stages of nodule development, the symbiosome membrane may not selectively restrict glyphosate movement. In this case, glyphosate may restrict bacterial division, as was observed with cultured *B. japonicum* in the presence of glyphosate (Jaworski, 1972). This would explain the delay in N_2 fixation for glyphosate-treated plants.

Under well-watered conditions, it appears that the delay in active N_2 fixation has no long-term impact on biomass and N accumulation. We found no difference in total biomass or N content between glyphosate-treated and untreated plants at 40 DAE following two applications of glyphosate in greenhouse studies. This response is consistent with results from our field experiment at the Fayetteville location and with numerous field studies comparing GT cultivars, treated and untreated with glyphosate under weed-free conditions, which showed no yield response to glyphosate under relatively high-yielding conditions (Delannay et al., 1995).

In addition to the delay in N_2 fixation and the increased sensitivity of N_2 fixation to water deficits by glyphosate treatment, soybean root growth was also inhibited by repeated applications of glyphosate in Greenhouse Experiment 2 (Table 4). Root mass was not measured in field studies, but decreased root growth and soil-water extraction may have contributed to the increased sensitivity of glyphosate-treated plants to water deficits that occurred in the field study at Keiser.

Although glyphosate affects GT soybean growth, N_2 fixation, and yield in water-limited environments, this response may not be consistent across genetic backgrounds. A broad range of GT cultivars evaluated in water-limited environments with and without glyphosate treatments would provide useful information on the identification of genetic backgrounds that are more suitable for nonirrigated conditions with GT production systems.

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