Effect of Glyphosate on Growth, Chlorophyll, and Nodulation in Glyphosate-Resistant and Susceptible Soybean (*Glycine max*) Varieties

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ABSTRACT. Greenhouse and growth chamber experiments were conducted to examine glyphosate [isopropylamine salt of *N*-(phosphonomethyl) glycine] effects on growth, chlorophyll content, nodulation, and nodule leghemoglobin content of glyphosate-resistant and susceptible soybean (*Glycine max* [L.] Merr.) varieties. In susceptible soybean, a single application of 0.28 kg/ha reduced chlorophyll content (49%), and shoot and root dry weight (50 and 57%, respectively) at 2 wk after treatment. In glyphosate-resistant soybean, there were no significant effects on these parameters by single application up to 1.12 kg/ha, but 2.24 kg/ha reduced shoot and root dry weight by 25 to 30%. Application of glyphosate 1.12 kg/ha, followed by sequential applications at 0.56 or 1.12 kg/ha, did not affect plant growth and chlorophyll content, but application of 2.24 kg/ha followed by sequential application of 2.24 kg/ha reduced root growth. In glyphosate-resistant soybean, an application of 1.12 kg/ha 3 wk after planting did not affect nodule number or

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mass, but 2.24 kg/ha reduced these parameters by 30 and 39%, respectively, compared to untreated. Leghemoglobin content of nodules was reduced (6 to 18%) by both glyphosate rates, but effects were inconsistent with rate. At post-treatment temperatures of 18/13°C (day/night), glyphosate at 1.12 kg/ha or 2.24 kg/ha did not affect chlorophyll and growth of glyphosate-resistant soybean. However, at 25/20 and 32/27°C (day/night), glyphosate at 2.24 kg/ha reduced both chlorophyll content and growth of glyphosate-resistant soybean. Overall, treatment of glyphosate-resistant soybean with glyphosate at 1.12 had little or no effect on chlorophyll content and dry weight of shoots and roots in five of five trials. But treatment of glyphosate at 2.24 kg/ha reduced these parameters in three of five trials, suggesting potential for soybean injury at higher rates. Results showed subtle reductions of nodulation in glyphosate-resistant soybean using label rates of glyphosate, but these effects may be of minimal consequence due to the potential of soybean to compensate after short durations of stress.

KEYWORDS. Leghemoglobin, glyphosate, herbicide-resistant crops, nodule, rhizobium, transgenic crops

INTRODUCTION

Herbicide-resistant crops represent advances in plant biotechnology that may offer strategies for efficient control of weeds without injury to crops. Although herbicide resistance in crops has been achieved using traditional plant breeding methods, most recent herbicide-resistant cultivars have been created via stable integration of a foreign gene using molecular biological techniques and plant transformation (Padgette et al. 1995).

Glyphosate (isopropylamine salt of *N*-(phosphonomethyl) glycine) is a nonselective, broad-spectrum herbicide that is widely-used, but causes crop injury when applied directly to foliage. Glyphosate is toxicologically and environmentally benign (low toxicity to organisms, low or no groundwater movement, and limited persistence). Thus, glyphosate is considered an environmentally safe herbicide (Duke 1988; Franz et al. 1997). Despite extensive use of glyphosate for over 25 years, weed resistance to this herbicide has not occurred until recently. A population of glyphosate-resistant rigid ryegrass *(Lolium* *rigidum* Guad.) has been reported after 15 years of consecutive use of this herbicide (Powles et al. 1998).

Several crops that possess genes rendering them resistant to glyphosate have recently been marketed (Thayer 1999; WSSA 1998). The development of transgenic crops with resistance to glyphosate is a promising weed control strategy. Such cultivars can enable growers to utilize glyphosate in direct-spray applications to control a wide spectrum of weeds. Although these genetically transformed crops are resistant to glyphosate, application of glyphosate to some cultivars under certain environmental conditions can cause injury, including decreased chlorophyll content in soybean (*Glycine max* [L.] Merr.) (Gertz and Vencill 1999; Pline et al. 1999) and reduced boll retention in cotton (*Gossypium hirsutum* L.) (File 1999). Generally, the herbicide-resistant crops outgrow or overcome this injury (King and Purcell 1998), but stress conditions such as high or low temperature, water availability, nutritional status may exacerbate or extend injury that could affect yield. Boll abscission and reduced yield of glyphosate-resistant cotton due to glyphosate treatment has been reported by producers in the Mississippi Delta in 1997 and 1998 (File 1999). This reduction prompted an inquiry as to whether these effects were environmentally induced or due to an intolerance to glyphosate application.

No significant yield reductions due to the glyphosate tolerance gene occurred in extensive field trials of transgenic soybean (Delannay et al. 1995; Reddy and Whiting 2000; Scott et al. 1998). But the physiological effects associated with injury caused by glyphosate application to glyphosate-resistant soybean are not fully understood. Glyphosate at 0.5 mM decreased chlorophyll content in hypocotyls of soybean grown in liquid culture (Hoagland 1980). Glyphosate applications can reduce plant growth, and concomitantly reduce nodulation, in glyphosate-resistant soybean (King and Purcell 1998). Reductions in nodulation can be due to an indirect result of glyphosate injury to the plant, from direct action of glyphosate on rhizobial populations, or from action against both soybean and rhizobial populations (Moorman 1986). However, glyphosate added to soil at 2 or 20 mg/kg soil or to yeast-extract mannitol broth at 2 or 20 mg/L had no effect on two strains of *Bradyrhizobium japonicum* (Moorman 1986). Glyphosate can also affect the bacterial symbiont (*B. japonicum*) of soybean via accumulation of hydroxybenzoic acids within the plant (Moorman et al. 1992). Because little information has been published on these interactions, we examined the effects of glyphosate on plant growth, chlorophyll content, nodulation, and nodule leghemoglobin content in glyphosate-resistant and susceptible soybean varieties. Several experimental factors such as: glyphosate dose-response, single vs. sequential application of glyphosate, rhizobium inoculation with and without nitrogen, soybean growth stages, and post-application temperature on glyphosate interactions in soybean were investigated.

MATERIALS AND METHODS

General Procedures

Soybean varieties (DP 3588, susceptible variety; DP 5806RR, glyphosate-resistant variety) used were determinant, highly adaptive to the Mississippi Delta and belonged to the late V maturity group. Five soybean seeds were planted in 15-cm diameter plastic pots containing Bosket sandy loam (fine-loamy, mixed thermic Mollic Hapludalfs). After emergence, soybean plants were thinned to two uniform plants per pot. Plants were grown in the greenhouse maintained at 30/22° (± 3) C day/night temperature. Natural daylight was supplemented in the early morning and the early evening hours with sodium vapor lamps to provide a total of 14 h of illumination. The minimum daily photosynthetic photon flux density was at least 900 ± 20 μmol/m/s. Plants were watered as needed and fertilized only in the nitrogen and rhizobium study.

Herbicide solutions were prepared using a commercial formulation of glyphosate (Roundup Ultra®, isopropylamine salt of glyphosate with surfactant, Monsanto Agricultural Company, St. Louis, MO 63167, USA). Spray solutions were applied using an indoor spray chamber equipped with an air-pressurized system at a volume of 187 L/ha at 138 kPa using Teejet 8002E nozzle. The suggested label use rate of glyphosate is 1.1 to 1.7 kg ai/ha initial application, and 0.8 kg ai/ha sequential application. We selected two rates of glyphosate in our studies; 1.12 kg/ha, represented the low end of the suggested use rate and 2.24 kg/ha, represented the high end.

At 2 or 4 weeks after treatment (WAT), distal leaflets of the second or third trifoliate leaves from two plants/pot in a given treatment were sampled for chlorophyll determination. Chlorophyll was extracted with 5 ml dimethyl sulfoxide and chlorophyll concentrations were determined spectrophotometrically (Barnes et al. 1992; Hiscox and Israelstam 1979). Total chlorophyll content was expressed as mg/L/g fresh weight. At 2 or 4 WAT, soybean plants (two plants/pot) were excised at the soil surface, oven dried (60°C, 48 h), and the weights recorded. Roots were collected by washing off soil with water, then oven dried, and the weights recorded. Oven-dried shoots from each replication of each treatment were ground and total nitrogen was determined in samples using the Kjeldahl method (Baker and Thompson 1992). Nitrogen analysis was made at the Soil Testing Laboratory, University of Arkansas at Mariana, Arkansas. Total nitrogen was expressed as mg nitrogen per plant shoot. After plants were harvested for shoot dry weight, the roots were washed with water to remove soil. Nodules were harvested, counted, and then fresh weights recorded. Nodule samples were bulked for each treatment, homogenized in aliquots of Drabkin's reagent (Drabkin's reagent, Sigma Chemical Company, St. Louis, MO 63178, USA) (1:10 to 1:20 ratio: w/v) and leghemoglobin quantified spectrophotometrically at A_{540} (Wilson and Reisenauer 1963). Human hemoglobin (Human hemoglobin, Sigma Chemical Company, St. Louis, MO 63178, USA) was used as a standard and values are expressed as mg/g nodule mass.

Effect of Single and Sequential Application of Glyphosate on Glyphosate-Resistant and Susceptible Soybean Varieties

Dose-response tests were conducted in a greenhouse on 2-wk-old glyphosate-resistant and susceptible soybean plants, treated with one application of glyphosate at 0, 0.28, 0.56, 1.12, 2.24, 4.48, 6.72, and 13.44 kg ai/ha. Since glyphosate is toxic to susceptible soybean, susceptible soybean was included merely as a reference, and not to make comparisons with glyphosate-resistant soybean. Chlorophyll content and dry weights of shoot and roots were determined at 2 and 4 WAT as described earlier. In a separate test, only glyphosate-resistant soybean plants (2-wk-old) were treated with single application of glyphosate at 1.12 or 2.24 kg ai/ha. Two weeks after the first application of 1.12 kg ai/ha, sequential applications of glyphosate at 0.56 or 1.12 kg ai/ha were applied. In another treatment, plants that received 2.24 kg ai/ha initially, were treated with a sequential application of 2.24 kg/ha to simulate the high end use rate. Chlorophyll content and dry weights of shoots and roots were determined 4 wk after the first application as described earlier. Treatments were arranged in a randomized complete block design with five replications. Data for each soybean variety were subjected to analysis of variance and means were separated using Fisher's protected LSD test at $P = 0.05$.

Nitrogen and Rhizobium Interactions with Glyphosate in Glyphosate-Resistant and Susceptible Soybean Varieties

In the above study, no nodules were present due to low native rhizobial populations. To study the effect of glyphosate on nodulation, soybean seeds were treated with *Bradyrhizobium japonicum* culture. Seeds were inoculated with commercial cultures of *B. japonicum* ($\sim 10^8$ cells/seed) at planting. *B. japonicum* concentrate (*Rhizobium* concentrate for inculation, Jimmy Sanders, Inc., Hollandale, MS 38748, USA) purchased locally was diluted in water and 1 ml inoculum was placed on each soybean seed. One set of plants was supplied once with nitrogen (ammonium nitrate, 34% N, 4 g/L, 200 ml/pot) 1 wk before glyphosate application and another set was used as a no-nitrogen control. Two-wk-old soybean plants were treated with glyphosate at 1.12 or 2.24 kg/ha. Nodule number and fresh weight were recorded 2 wk after herbicide treatment. Chlorophyll content and dry weight of shoots and roots were determined and analyzed as described earlier. The experiment was conducted in a greenhouse using a randomized complete block design with five replications. Data for each soybean variety was subjected to analysis of variance and means were separated using Fisher's protected LSD test at $P = 0.05$.

Growth Stage and Glyphosate Interactions in Glyphosate-Resistant Soybean

Under field conditions, the first application of glyphosate is usually made around the second or third week after planting. This is also the critical time for nodule development and associated leghemoglobin production in nodules. To study the effect of glyphosate on nodulation, rhizobium-treated soybean plants at two growth stages were used. Two- and 3-wk-old glyphosate-resistant soybean plants were treated with glyphosate at 1.12 or 2.24 kg/ha. Chlorophyll content, nodulation parameters, and leghemoglobin concentration in nodules were determined as described earlier. The experiment was conducted in a randomized complete block design with eight replications in a greenhouse. Data for each growth stage was subjected to analysis of variance and means were separated using Fisher's protected LSD test at $P = 0.05$.

Temperature and Glyphosate Interactions in Glyphosate-Resistant Soybean

Three temperature regimes (18/13; 25/20, 32/27°C day/night, 14/10 h) were used to study the interactions of glyphosate and temperature in glyphosate-resistant soybean. Plants were grown in the greenhouse for 14 d and then moved to respective growth chambers for acclimatization 2 days before glyphosate treatment. Glyphosate was applied at 1.12 or 2.24 kg/ha to 16-d-old soybean plants. The experiment was conducted in a randomized complete block design and treatments were replicated nine times. Chlorophyll content and dry weight of shoots and roots were determined and analyzed as described earlier. Data was subjected to analysis of variance and means were separated using Fisher's protected LSD test at $P = 0.05$.

RESULTS AND DISCUSSION

Effect of Single and Sequential Application of Glyphosate on Glyphosate-Resistant and Susceptible Soybean Varieties

Single application of glyphosate at rates less than 1.12 kg/ha did not effect chlorophyll content in glyphosate-resistant soybean at 2 and 4 WAT (Table 1). Application of glyphosate at 2.24 kg/ha reduced chlorophyll content 25% at 2 WAT, but the level was not different 4 WAT when compared to the untreated control. However, glyphosate applied at rates above 4.48 kg/ha reduced chlorophyll content in glyphosate-resistant soybean to less than 27% of control at 2 WAT and the plants recovered partially by 4 WAT (66%). At 4 WAT, shoot and root dry weights of glyphosate-resistant soybean were unaffected by glyphosate at 1.12 kg/ha; however, glyphosate at 2.24 kg/ha reduced both shoot and root dry weights compared to untreated controls. In susceptible soybean, glyphosate applied as low as 0.28 kg/ha reduced chloro-

TABLE 1. Effect of glyphosate rate on chlorophyll content and growth of glyphosate-resistant and susceptible soybean.[a,b]

Soybean type	Glyphosate rate	Chlorophyll 2 WAT[c]	Chlorophyll 4 WAT	Shoot dry weight 2 WAT	Shoot dry weight 4 WAT	Root dry weight 2 WAT	Root dry weight 4 WAT
	kg/ha	% of control					
Resistant[d]	0	100 a	100 a	100 a	100 ab	100 a	100 a
	0.28	95 a	90 a	96 ab	101 a	97 a	87 ab
	0.56	107 a	103 a	86 abc	90 ab	94 a	81 ab
	1.12	104 a	94 a	82 bc	91 ab	84 ab	87 ab
	2.24	75 b	105 a	75 c	84 b	70 b	69 bc
	4.48	26 c	61 b	50 d	57 c	42 c	50 cd
	6.72	27 c	51 b	53 d	50 cd	33 c	52 cd
	13.44	26 c	66 b	31 e	36 d	31 c	36 d
Susceptible[d]	0	100 a	100 a	100 a	100 a	100 a	100 a
	0.28	51 b	103 a	50 b	68 b	43 b	73 b
	0.56	42 b	67 b	28 c	33 c	14 c	22 c
	1.12	5 c	49 b	24 c	10 d	10 c	6 c

[a] Means within a column and soybean type followed by the same letter are not significantly different at the 5% level as determined by Fisher's protected LSD test.
[b] Glyphosate was applied 2 wk after planting.
[c] WAT, weeks after glyphosate treatment.
[d] Chlorophyll, shoot dry weight, and root dry weight of untreated control plants were 286 mg/L/g fresh weight, 1.04 g/plant, and 0.3 g/plant, respectively, at 2 WAT; 204 mg/L/g fresh weight, 2.5 g/plant, and 0.7 g/plant, respectively, at 4 WAT in glyphosate-resistant soybean; 334 mg/L/g fresh weight, 1.2 g/plant, and 0.4 g/plant, respectively, at 2 WAT; 252 mg/L/g fresh weight, 2.7 g/plant, and 0.6 g/plant, respectively, at 4 WAT in susceptible soybean.

phyll content and plant growth (Table 1). In susceptible soybean, chlorophyll content in hypocotyls decreased when grown in liquid culture containing 0.5 mM glyphosate (Hoagland 1980). However, in glyphosate-resistant soybean, glyphosate at 1.12 or 2.24 kg/ha had no effect on leaf chlorophyll content (Hoagland et al. 1999).

Application of glyphosate at 1.12 kg/ha followed by 0.56 or 1.12 kg/ha at 2 wk after the first application, had no effect on chlorophyll content and growth of glyphosate-resistant soybean (Table 2). Similarly, glyphosate applied at 2.24 kg/ha followed by 2.24 kg/ha had no effect on chlorophyll content and shoot dry weight, but root dry weight was reduced to 61% of control.

TABLE 2. Effect of single and sequential applications of glyphosate on chlorophyll content and growth of glyphosate-resistant soybean 4 wk after the initial glyphosate application.[a,b,c]

Rate	Chlorophyll	Shoot dry weight	Root dry weight
kg/ha	% of control		
0	100 a	100 a	100 a
1.12	104 a	103 a	86 ab
2.24	97 a	102 a	70 ab
1.12 + 0.56	91 a	99 a	68 ab
1.12 + 1.12	98 a	89 a	65 ab
2.24 + 2.24	97 a	92 a	61 b

[a] Initial application was made 2 wk after planting; sequential application made 2 wk after initial application.
[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.
[c] Chlorophyll, shoot dry weight, and root dry weight of untreated control plants were 443 mg/L/g fresh weight, 6.1 g/plant, and 1.7 g/plant, respectively, 4 wk after initial treatment of glyphosate in glyphosate-resistant soybean.

Nitrogen and Rhizobium Interactions with Glyphosate in Glyphosate-Resistant and Susceptible Soybean Varieties

Glyphosate at 1.12 kg/ha had no effect on chlorophyll content of inoculated (*B. japonicum*) glyphosate-resistant soybean, regardless of nitrogen treatment. However, in the presence of nitrogen (70 ppm), chlorophyll content was reduced in glyphosate-resistant soybean treated with glyphosate at 2.24 kg/ha (Table 3). Glyphosate at 1.12 kg/ha or 2.24 kg/ha had no effect on shoot and root dry weights, except for decreased root dry weight at 2.24 kg/ha in the presence of nitrogen. In susceptible soybean treated with glyphosate at 0.28 kg/ha, there was no effect on chlorophyll content, but dry weights of shoot and root were greatly reduced in the absence of nitrogen (Table 3).

Nodule number and fresh weight were unaffected by glyphosate at 2.24 kg/ha in the absence of nitrogen. However, glyphosate at 1.12 kg/ha reduced nodule number and fresh weight compared to untreated control (Table 3). A similar trend was observed for leghemoglobin content. We have no explanation for this trend; however, a 25 to 35% reduction in nodule number and weight, and total plant biomass in glyphosate-resistant soybean applied with 1.7 kg/ha has been reported (King and Purcell 1998). The presence of nitrate (0.5 mM, hydroponic

TABLE 3. Effect of nitrogen and glyphosate on chlorophyll content and growth of *Bradyrhizobium japonicum*-treated glyphosate-resistant and susceptible soybean, 2 wk after glyphosate treatment.[a,b]

Soybean type	Nitrogen	Glyphosate rate	Chlorophyll	Shoot dry weight	Root dry weight	Nodules: Number	Nodules: Fresh weight	Nodules: Leghemoglobin
		kg/ha	% of control	% of control	% of control	no./plant	mg/plant	mg/g tissue
Resistant[c]	No nitrogen	0	100 a	100 a	100 a	18 a	64 a	1.6 a
		1.12	100 a	100 a	90 ab	13 b	34 b	1.4 b
		2.24	89 ab	95 a	99 a	17 a	66 a	1.5 ab
	Nitrogen	0	100 a	100 a	100 a	5 c	7 c	nd[d]
		1.12	97 a	95 a	89 ab	4 c	5 c	nd
		2.24	80 b	89 a	71 b	2 c	3 c	nd
Susceptible[c]	No nitrogen	0	100 a	100 a	100 a	22 a	102 a	1.6 a
		0.28	97 a	64 b	44 b	15 b	25 b	1.4 a
	Nitrogen	0	100 a	100 a	100 a	1 c	4 b	nd
		0.28	79 b	70 b	49 b	1 c	24 b	nd

[a] Means within a column and soybean type followed by the same letter are not significantly different at the 5% level as determined by Fisher's protected LSD test.
[b] Nitrogen was applied 1 wk after planting; glyphosate was applied 2 wk after planting.
[c] Chlorophyll, shoot dry weight, and root dry weight of untreated control plants were 420 mg/L/g fresh weight, 1.3 g/plant, and 0.4 g/plant, respecitvely, without nitrogen; 451 mg/L/g fresh weight, 1.4 g/plant, and 0.4 g/plant, respectively, with nitrogen in glyphosate-resistant soybean; 410 mg/L/g fresh weight, 1.8 g/plant, and 0.5 g/plant, respectively, without nitrogen; 446 mg/L/g fresh weight, 1.7 g/plant, and 0.4 g/plant, respecitvely, with nitrogen in susceptible soybean.
[d] nd, not determined due to insufficient tissue.

culture) significantly delayed and inhibited infection of soybean root hairs by *B. japonicum* resulting in reduced nodule numbers and nitrogen fixation (Gibson and Harper 1985). Furthermore, nodule development and nitrogenase activity are inhibited by high fertilizer nitrogen (100 to 200 ppm) levels in conventional soybean (Ham et al. 1976). High inorganic nitrogen can also cause premature decay of nodules in conventional soybean (Ham et al. 1976). In susceptible soybean treated with glyphosate at 0.28 kg/ha there was no effect on leghemoglobin content, but nodule number and fresh weight were greatly reduced in the absence of nitrogen (Table 3).

Growth Stage and Glyphosate Interactions in Glyphosate-Resistant Soybean

In 2-wk-old glyphosate-resistant soybean, glyphosate at 1.12 or 2.24 kg/ha had no effect on chlorophyll content or shoot dry weight, but root dry weight was reduced at 2.24 kg/ha (Table 4). However, in 3-wk-old soybean, glyphosate at 2.24 kg/ha reduced both chlorophyll content and plant growth. In 2-wk-old soybean, application of glyphosate at either rate did not affect nodule number and fresh weight, whereas in 3-wk-old soybean, nodule number and fresh weight was decreased by glyphosate at 2.24 kg/ha. Leghemoglobin content of nodules in 3-wk-old soybean was reduced when glyphosate was applied at both rates. Overall, total nitrogen in shoots was unaffected regardless of glyphosate rates in 2-wk-old soybean (Table 4). However, in 3-wk-old plants glyphosate at 2.24 kg/ha reduced total nitrogen content in plant shoots by 14% compared to untreated control. This reduction in total nitrogen content can be attributed partly to decreased leghemoglobin content (Table 4). Others have reported a 25 to 35% reduction in total nitrogen per plant in glyphosate-resistant soybean treated with glyphosate at 1.7 kg/ha (King and Purcell 1998).

Temperature and Glyphosate Interactions in Glyphosate-Resistant Soybean

At 18/13°C, day/night temperature, glyphosate at 1.12 kg/ha or 2.24 kg/ha did not affect chlorophyll content or growth of glyphosate-resistant soybean (Table 5). However, at 25/20 and 32/27°C, day/night temperature, glyphosate at 2.24 kg/ha reduced both chlorophyll con-

TABLE 4. Effect of glyphosate on chlorophyll content and growth in glyphosate-resistant soybean when applied at two growth stages.[a,b]

Soybean growth stage	Glyphosate rate	Chlorophyll	Shoot dry weight	Root dry weight	Total nitrogen	Nodules Number	Nodules Fresh weight	Nodules Leghemoglobin
	kg/ha	% of control	% of control	% of control	mg N/shoot	no./plant	mg/plant	mg/g tissue
2-wk-old[c]								
	0	100 a	100 a	100 a	14.7 a	22 a	114 a	1.5 a
	1.12	101 a	86 a	99 a	13.4 a	23 a	91 a	1.3 b
	2.24	91 a	86 a	88 b	14.5 a	20 a	90 a	1.4 ab
3-wk-old[c]								
	0	100 a	100 a	100 a	16.2 a	33 a	216 a	1.7 a
	1.12	103 a	85 b	84 b	15.0 ab	28 ab	242 a	1.5 b
	2.24	66 b	78 b	71 b	14.0 b	23 b	131 b	1.4 b

[a] Glyphosate was applied to soybean 2 and 3 wk after planting; data were collected 2 wk after glyphosate treatment.

[b] Means within a column and soybean growth stage followed by the same letter are not significantly different at the 5% level as determined by Fisher's protected LSD test.

[c] Chlorophyll, shoot dry weight, and root dry weight of untreated control plants were 372 mg/L/g fresh weight, 1.5 g/plant, and 0.6 g/plant, respectively, for 2-wk-old; 391 mg/L/g fresh weight, 1.8 g/plant, and 0.8 g/plant, respectively, for 3-wk-old glyphosate-resistant soybean.

TABLE 5. Effects of temperature and glyphosate on glyphosate-resistant soybean.[a,b,c]

Temperature	Glyphosate rate	Chlorophyll	Shoot dry weight	Root dry weight
day/night °C	kg/ha	% of control	% of control	% of control
18/13	0	100 a	100 a	100 a
	1.12	99 a	95 ab	100 a
	2.24	96 a	93 ab	89 ab
25/20	0	100 a	100 a	100 a
	1.12	86 a	91 ab	96 ab
	2.24	44 b	77 b	75 b
32/27	0	100 a	100 a	100 a
	1.12	98 a	93 ab	87 ab
	2.24	51 b	77 b	80 ab

[a] 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.

[b] Glyphosate was applied to soybean 2 wk after planting; data were collected 2 wk after glyphosate treatment.

[c] Chlorophyll, shoot dry weight, and root dry weight of untreated control plants were 254 mg/L/g fresh weight, 0.4 g/plant, and 0.1 g/plant, respectively, at 18/13°C; 315 mg/L/g fresh weight, 0.9 g/plant, and 0.2 g/plant, respectively, at 25/20°C; 250 mg/L/g fresh weight, 0.8 g/plant, and 0.3 g/plant, respectively, at 32/27°C temperature.

tent and growth in this resistant soybean cultivar. Soybean plants are more physiologically active at higher temperatures (25/20 and 32/27°C) than at low temperatures (18/13°C), thus more glyphosate may have been translocated to meristematic sites. This increased herbicide concentration in these plant tissues may partly be responsible for effects on the parameters studied. Others have reported a greater loss of chlorophyll content at 35°C, than at 15 or 25°C, in glyphosate-resistant soybean treated with glyphosate (Pline et al. 1999).

The results of this study suggest that no significant reductions in shoot and root weight of glyphosate-resistant soybean were elicited at the 1.12 kg/ha glyphosate rate; however, reductions occurred with increased rates. Sequential application of glyphosate at 0.56 or 1.12 kg/ha, following a 1.12 kg/ha application, had no effect on plant growth and chlorophyll content, but sequential application of a 2.24 kg/ha following a 2.24 kg/ha application injured root growth. Treatment of glyphosate-resistant soybean with glyphosate at 1.12 kg/ha had little or no effect on chlorophyll content in five of five trials, while treatment of glyphosate at 2.24 kg/ha reduced chlorophyll content in three of five trials. Similarly, glyphosate at 1.12 kg/ha had no effect on plant growth in five of five trials, while at 2.24 kg/ha it decreased plant growth in three of five trials. By comparison, glyphosate application rate of only 0.28 kg/ha reduced growth and chlorophyll content in susceptible soybean. Treatment of glyphosate-resistant soybean with 1.12 or 2.24 kg/ha glyphosate had mixed effects on nodulation (Tables 3 and 4). Glyphosate at 1.12 kg/ha significantly reduced nodule number and fresh weight in one of two trials. Application of 2.24 kg/ha glyphosate in resistant soybean, 3 wk after planting, had the most severe effects on nodule number, mass, and leghemoglobin content. Treatment of glyphosate-susceptible soybean with 0.28 kg/ha glyphosate significantly lowered nodule number and fresh weight, but nodule leghemoglobin content was unaffected (Table 3).

Subtle reductions in plant growth and nodulation parameters were observed using recommended use rates of glyphosate in a glyphosate-resistant soybean cultivar. These effects may be transient and of minimum consequence. Similarly, King and Purcell (1998) concluded that glyphosate applied at 1.7 kg/ha delayed nitrogen fixation and changed nodule number and size, but did not decrease biomass 6 wk after emergence in glyphosate-resistant soybean. In our study, we examined effects of glyphosate only on one glyphosate-resistant variety using a commercial inoculum which is a mixture of several *B. japonicum* strains. Currently, hundreds of glyphosate-resistant varieties from different maturity groups are commercially available. The physiological responses of these varieties to glyphosate application may vary, and may also depend on geographical location, environmental conditions, soil types, *B. japonicum* microbial ecology, etc. This phenomenon needs further investigation. However, most soybean farmers in the midsouthern U.S. do not use supplemental rhizobium culture or nitrogen fertilizer in soybean production. No yield reductions due to glyphosate applications to glyphosate-resistant soybean have been observed in extensive field trials (Delannay et al. 1995; Nelson and Renner 1999; Reddy and Whiting, 2000; Scott et al, 1998). Because soybean is a compensatory crop, it has the potential to tolerate short periods of stress and to recover.

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