Chemosphere 83 (2011) 593–598

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Physiological responses of glyphosate-resistant and glyphosate-sensitive soybean to aminomethylphosphonic acid, a metabolite of glyphosate

Wei Ding [a,c], Krishna N. Reddy [a,*], Robert M. Zablotowicz [a], Nacer Bellaloui [b], H. Arnold Bruns [a]

[a] US Department of Agriculture, Agricultural Research Service, Crop Production Systems Research Unit, P.O. Box 350, 141 Experiment Station Road, Stoneville, MS 38776, USA
[b] US Department of Agriculture, Agricultural Research Service, Crop Genetics Research Unit, P.O. Box 345, 141 Experiment Station Road, Stoneville, MS 38776, USA
[c] Northeast Agricultural University, Agronomy College, Department of Plant Protection and Key Laboratory of Soybean Biology in Chinese Ministry of Education, Harbin 150030, China

ARTICLE INFO

Article history:
Received 18 May 2010
Received in revised form 21 November 2010
Accepted 3 December 2010
Available online 28 December 2010

Keywords:
Aminomethylphosphonic acid
AMPA
Glyphosate
Soybean injury
Photosynthesis
Nitrogen fixation

ABSTRACT

Aminomethylphosphonic acid (AMPA) is formed in glyphosate-treated glyphosate-resistant (GR) and glyphosate-sensitive (GS) soybean [*Glycine max* (L.) Merr.] plants and is known to cause yellowing in soybean. Although, AMPA is less phytotoxic than glyphosate, its mode of action is different from that of glyphosate and is still unknown. Greenhouse studies were conducted at Stoneville, MS to determine the effects of AMPA on plant growth, chlorophyll content, photosynthesis, nodulation, nitrogenase activity, nitrate reductase activity, and shoot nitrogen content in GR and GS soybeans. AMPA was applied to one- to two-trifoliolate leaf stage soybeans at 0.1 and 1.0 kg ha^{-1}, representing a scenario of 10% and 100% degradation of glyphosate (1.0 kg ae ha^{-1} use rate) to AMPA, respectively. Overall, AMPA effects were more pronounced at 1.0 kg ha^{-1} than at 0.1 kg ha^{-1} rate. Visual plant injury (18–27%) was observed on young leaves within 3 d after treatment (DAT) with AMPA at the higher rate regardless of soybean type. AMPA injury peaked to 46–49% at 14 DAT and decreased to 17–18% by 28 DAT, in both soybean types. AMPA reduced the chlorophyll content by 37%, 48%, 66%, and 23% in GR soybean, and 17%, 48%, 57%, and 22% in GS soybean at 3, 7, 14, and 28 DAT, respectively. AMPA reduced the photosynthesis rate by 65%, 85%, and 77% in GR soybean and 59%, 88%, and 69% in GS soybean at 3, 7, and 14 DAT, respectively, compared to non-treated plants. Similarly, AMPA reduced stomatal conductance to water vapor and transpiration rates at 3, 7, and 14 DAT compared to non-treated plants in both soybean types. Photosynthesis rate, stomatal conductance, and transpiration rate recovered to the levels of non-treated plants by 28 DAT. Plant height and shoot dry weight at 28 DAT; nodulation, nitrogenase activity at 10 DAT, and nitrate reductase activity at 3 and 14 DAT were unaffected by AMPA. AMPA reduced root respiration and shoot nitrogen content at 10 DAT. These results suggest that a foliar application of AMPA could indirectly reduce photosynthesis through decreased chlorophyll content in GR and GS soybean up to 14 DAT, but affected plants can recover to normal growth by 28 DAT.

Published by Elsevier Ltd.

1. Introduction

Glyphosate-resistant (GR) soybean was created by stable integration of a transgene that encodes for a glyphosate-insensitive 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme (Padgette et al., 1996). Expression of glyphosate-resistant EPSPS enzyme helps GR soybean to survive glyphosate treatment. Nevertheless, decreased chlorophyll content, plant growth, nodule biomass, leghemoglobin content, and nitrogen fixation and accumulation have been observed following glyphosate treatment in GR soybean under certain conditions (Reddy et al., 2000; King et al., 2001; Reddy and Zablotowicz, 2003; Zablotowicz and Reddy, 2007; Bellaloui et al., 2008). AMPA is the most frequently detected metabolite of glyphosate in plants (Reddy et al., 2004, 2008). Glyphosate may be metabolized by plants via two pathways similar to those in certain microorganisms (Franz et al., 1997). One involves oxidative cleavage of the C–N bond to yield AMPA and the other, involves breaking of the C–P bond by C–P lyase to generate sarcosine. AMPA, the main metabolite of glyphosate has been found in both GR and GS soybean treated with glyphosate (Duke et al., 2003; Reddy et al., 2004, 2008; Sammons and Tran, 2008). AMPA formation from glyphosate degradation in soybean has been correlated with the appearance of yellowing in GR soybean (Reddy et al., 2004).

Glyphosate is rapidly degraded in soils (Accinelli et al., 2005; Gimsing et al., 2004; Zablotowicz et al., 2009) with over 20–70% of the glyphosate mineralized to CO_2 in about 5 weeks, depending

* Corresponding author. Tel.: +1 662 686 5298; fax: +1 662 686 5422.
E-mail address: krishna.reddy@ars.usda.gov (K.N. Reddy).

0045-6535/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.chemosphere.2010.12.008

on the soil type. The metabolite, AMPA may accumulate in the soil corresponding to 10–20% of the initial glyphosate applied (Gimsing et al., 2004). In Danish soils, the half-life of glyphosate was 9 d, while AMPA was 32 d, indicating the potential for accumulation of the metabolite (Simonsen et al., 2008). In other studies (Mamy et al., 2005), AMPA exhibited a half-life of 25–75 d. Glyphosate residues in-crop plants exposed to the herbicide are also more resilient in the soil as these residues are generally present as AMPA and bound residues (Doublet et al., 2009).

AMPA has also been detected in cowpea [*Vigna unguiculata* (L.) Walpers], sicklepod [*Senna obtusifolia* (L.) H.S. Irwin & Barneby], coffee senna (*Cassia occidentalis* L.), Illinois bundleflower [*Desmanthus illinoensis* (Michx.) MacM. ex B.L. Robins. & Fern.], kudzu [*Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen & S.M. Almeida], and horseweed [*Conyza canadensis* (L.) Cronq.] following glyphosate application (Reddy et al., 2008). Detection of AMPA following glyphosate treatment indicates that glyphosate oxidoreductase may be responsible for the herbicide's degradation in plants upon application. It has been observed that chlorosis ('yellow flashes') occur in GR soybean expressing glyphosate oxidase following the treatment with glyphosate (Sammons and Tran, 2008). Glyphosate inhibits EPSPS in the shikimate pathway (Amrhein et al., 1980). By blocking EPSPS, glyphosate causes many-fold increases in the shikimate levels in treated plants (Lydon and Duke, 1988). An elevated shikimate level is unique to glyphosate exposure and is used as an early and highly sensitive indicator of glyphosate effects on plants (Harring et al., 1998; Pline et al., 2002). However, shikimate levels were not affected by AMPA treatment in both GR and GS soybeans indicating that the mode of action of AMPA is apparently different from that of glyphosate (Reddy et al., 2004). Since AMPA is phytotoxic to soybean by an unknown mechanism, we have investigated physiological effects of AMPA on GR and GS soybean in this study. The objective of this study was to determine the effects of AMPA on visual plant injury, plant growth, chlorophyll content, photosynthesis, nodulation, nitrogenase activity, nitrate reductase activity, and shoot nitrogen content in GR and GS soybeans under greenhouse conditions.

2. Materials and methods

Three greenhouse experiments were conducted during May 2009–March 2010 at the USDA-ARS, Jamie Whitten Delta States Research Center, Stoneville, MS. A GR soybean cultivar (AG4605RR/S) and a GS cultivar (Williams 82) were grown in 15-cm diameter plastic pots containing 1.7 kg of 1:1 (v/v) mixture of Bosket sandy loam soil (fine-loamy, mixed, thermic Mollic Hapludalfs) and Dundee silty clay loam soil (fine-silty, mixed, thermic Aeric Ochraqualf). The Dundee silty clay loam was from a field under continuous soybean production for 4 years and contained an abundant native population of *Bradyrhizobium japonicum*. The greenhouse was maintained at 28/22 ± 3 °C, day/night temperature, with natural light supplemented by sodium vapor lamps to provide a 13-h photoperiod. Soybeans were seeded and later thinned to two uniform plants per pot after emergence and sub irrigated with distilled water as needed. Soybean plants at the one- to two-trifoliolate leaf (about 20 d from sowing) growth stage were used for AMPA treatment. AMPA was applied at rates of 0.1 and 1.0 kg ha^{-1}, representing a scenario of 10% and 100% glyphosate (single application at 1.0 kg ha^{-1} use rate) degradation to AMPA, respectively. AMPA spray solutions were prepared using technical grade AMPA (>99% purity, Sigma–Aldrich, Allentown, PA) and Tween 20 (Sigma–Aldrich, St. Louis, MO) at 0.5%, v/v. The control plants were sprayed with water and Tween 20 (0.5%, v/v). Spray solutions were applied using an indoor spray chamber equipped with an air-pressurized system delivering 190 L ha^{-1} at 140 kPa using 8002E flat-fan nozzles.

2.1. Experiment 1. Plant injury, plant height, shoot dry weight, chlorophyll and photosynthesis

AMPA was applied to the soybean at 0.1 and 1.0 kg ha^{-1}. Tween 20-treated plants were included as the control. Soybean injury was visually estimated at 3, 7, 14, 28 d after the treatment (DAT) on a relative scale of 0 (no soybean injury) to 100% (soybean death). Chlorophyll content was determined at 3, 7, 14, and 28 DAT using the youngest fully expanded leaf from two plants in each pot. Chlorophyll was extracted with 10 mL dimethyl sulfoxide and chlorophyll concentrations were quantified spectrophotometrically as described by Hiscox and Israelstam (1979). Total chlorophyll content was expressed as mg g^{-1} leaf fresh weight.

Photosynthesis rate was measured using Li-6400XT portable photosynthesis system (Li-COR Biosciences, Lincoln, NE) equipped with a 6400–02 LED light source. Measurements were made on both plants in each pot. Net photosynthesis rate (a), stomatal conductance (g_{sw}), intercellular CO_2 (C_i), and transpiration (E) were measured at 3, 7, 14, and 28 DAT. When measuring photosynthesis, the cuvette chamber conditions were set to provide photosynthetic photon flux density of 1000 μmol m^{-2} s^{-1} and cuvette block temperature was maintained at 24 °C, and concentration of the CO_2 was set at 350 μmol mol^{-1} with a flow rate of 500 mL s^{-1}. Humidity levels of the reference and sample chambers were set at 30 g kg^{-1}. Stomatal ratio was set at 0.5 and measurements made on a 6 cm^2 segment of the top most center leaflet of each plant in a pot. The chamber was attached to a leaflet, the photosynthesis allowed to stabilize and the data recorded.

After photosynthesis and chlorophyll measurements at 28 DAT, soybean plant height was measured on both plants in each pot. Soybean plants were then excised at the soil surface, dried at 60 °C for 72 h, and dry weights were recorded.

2.2. Experiment 2. Nodulation, acetylene reduction assay, respiration, shoot and root biomass, and shoot nitrogen

Both soybean plants were sampled from each pot at 10 DAT. Shoots were excised at the soil surface, oven dried, and dry weights recorded. Total N was determined from duplicate samples (10–15 mg) using a Flash EA 112 elemental analyzer (CE Elantech, Lakewood, NJ). Shoot N was expressed as per cent of shoot dry weight. Total shoot N (mg N shoot^{-1}) was calculated as the product of percent N and shoot dry weight.

Nitrogenase activity was assayed using the acetylene reduction assay described by Hardy et al. (1968) and Zablotowicz et al. (1981). Roots were excavated with nodules intact and incubated in 60 mL plastic syringes. Two roots were placed in a syringe, sealed, 10% volume of air was removed and replaced with an equal volume of acetylene. After one hour incubation at room temperature, duplicate 1 mL gas samples were removed and analyzed by gas chromatography for ethylene formation and CO_2 evolution as described previously (Bellaloui et al., 2008; Zablotowicz and Reddy, 2007). An Agilent HP6960 (Agilent Technologies, Wilmington, DE) gas chromatograph equipped with a manual injector, injector loop, sample splitter and flame ionization detector (FID), and thermal conductivity detector (TCD) was used for the quantification of ethylene formation and CO_2 accumulation, respectively. Following the incubation, roots were washed, the nodules removed from the roots, and numbers were recorded. The nodules and roots were oven dried and their dry weights recorded.

2.3. Experiment 3. Leaf in vivo nitrate reductase activity

Two soybean plants were excised from each pot at 3 and 14 DAT and immediately transported to the laboratory for nitrate reductase activity. Nitrate reductase activity (NRA) was measured based

on an indigenous NO_3^- using methods described by Klepper and Hageman (1969) and later by Bellaloui et al. (2008). Approximately 0.4 g of fresh leaf tissue was placed in 5 mL of potassium phosphate buffer (19.171 g L^{-1} of K_2HPO_4 $3H_2O$ + 2.177 g L^{-1} of KH_2PO_4 + 10 mL of 1% (v/v) 1-propanol) at a concentration of 100 mM, pH 7.5 in the test tube. The incubation solution was vacuum filtered for 1 min, and the test tube and contents were flashed with nitrogen gas for 30 s and incubated at 30 °C. Aliquots of 1.0 mL were taken at intervals of 0, 60, 120, 180, and 300 min after flashing for NO_2^- determination. Then, 4 mL of deionized water and reagents of 1.0 mL of 1% (w/v) sulfanilamide in 10% v/v HCl and 1.0 mL of *N*-naphthyl-(1)-ethylenediamine dihydrochloride (0.1%) were added to the samples. After 30 min, the samples were read at 540 nm using a Beckman Coulter DU 800 spectrophotometer. The concentration of NO_2^- was calculated from a standard calibration curve using KNO_2. To determine NRA under conditions of adequate nutrition, soybean plants were supplied once with 20 mL 18% (w/v) solution of the fertilizer containing 20% nitrogen, 20% phosphate, and 20% potash to each pot at 8 d after sowing.

2.4. Statistics

AMPA treatments were arranged in a randomized complete block design and the experiments were repeated once. There were six replications in all the experiments with the exception of photosynthesis that had nine replications. Separate experiments were conducted for GR and GS soybean. Data were subjected to the analysis of variance using SAS PROC GLM (SAS Institute, Cary, North Carolina) and treatment means were separated at the 5% level of significance using Fisher's Protected LSD test.

3. Results and discussion

3.1. Experiment 1. Plant injury, plant height, shoot dry weight, chlorophyll and photosynthesis

The yellowing of leaves (injury) in the upper canopy was apparent within 3 DAT regardless of AMPA rate and soybean type (Table 1). Effects of AMPA were more pronounced in 1.0 kg ha^{-1} than in 0.1 kg ha^{-1} rate. Visual plant injury increased from 18% to 27% at 3 DAT to 46–49% at 14 DAT, and injury decreased to about 18% by 28 DAT in both soybean types. AMPA at 0.1 kg ha^{-1} did not affect chlorophyll content at 3, 7, 14, and 28 DAT in both soybean types (Table 1). However, AMPA at 1.0 kg ha^{-1} reduced the chlorophyll content by 37%, 48%, 66%, and 23% in GR soybean and 17%, 48%, 57%, and 22% in GS soybean at 3, 7, 14, and 28 DAT, respectively. AMPA had no significant effects on plant height and shoot dry weight of GR and GS soybean regardless of the use rates at 28 DAT (Table 1). Although, GR soybean exhibited higher sensitivity to AMPA than GS soybean for plant injury, chlorophyll content, and photosynthesis rate at 3 DAT, the results of all the four intervals taken together suggest that both soybean types regardless of the glyphosate-resistant trait are sensitive to AMPA. Based on visual observations, we observed that greening of leaves was delayed in AMPA-treated plants, but leaf growth appeared to be comparable to that of non-treated plants. Chlorosis in glyphosate-treated GR soybean has been attributed to AMPA formed by glyphosate degradation (Reddy et al., 2004).

AMPA at 0.1 kg ha^{-1} did not affect the photosynthesis rate at 3 and 7 DAT in GR soybean (Table 2). However, AMPA at a higher rate reduced the photosynthesis rate by 65% and 85% at 3 and 7 DAT, respectively in GR soybean compared to non-treated plants. At 14 DAT, AMPA at both low and high rates reduced the photosynthesis rate by 27–77% in GR soybean, and the affected plants recovered from the loss of photosynthesis by 28 DAT. AMPA at a higher rate reduced stomatal conductance (50%) to water vapor and transpiration rate (31%) only at 14 DAT. Internal concentration of CO_2 in the leaf was generally higher in GR soybean treated with AMPA at 3, 7, and 14 DAT, but unaffected at 28 DAT.

In GS soybean, observations were similar to GR soybean for photosynthesis rates and were unaffected by AMPA at 0.1 kg ha^{-1} at 3 and 7 DAT (Table 3). AMPA at 1.0 kg ha^{-1} reduced photosynthesis rate by 59% and 88% at 3 and 7 DAT, respectively in GS soybean. At 14 DAT, AMPA at both 0.1 and 1.0 kg ha^{-1} rates reduced photosynthesis by 15–69% in GS soybean and by 28 DAT photosynthesis in treated plants recovered to levels similar to non-treated plants. AMPA at 1.0 kg ha^{-1} reduced stomatal conductance (40%) to water vapor and transpiration rate (23%) only at 3 DAT. Similar to GR soybean, in GS soybean, internal CO_2 concentration of the leaf was generally higher in GS soybean treated with AMPA at 3, 7, and 14 DAT and unaffected at 28 DAT.

AMPA effect on photosynthesis rate and internal CO_2 concentration in the leaf were similar in both GR and GS soybean types. Apparently, photosynthesis rate and leaf internal CO_2 concentration exhibit a negative relationship: a decrease in CO_2 fixation may have contributed to higher internal CO_2 concentration in leaf tissue. Overall, the affected plants recovered by 28 DAT.

Overall, AMPA effects were more pronounced at 1.0 kg ha^{-1} rate than at a lower rate. The high rate was selected to represent the 'worst case scenario' to promote soybean injury. Glyphosate suggested label use rate for single or multiple in-crop applications range from 0.87 to 2.52 kg ha^{-1}. AMPA was detected in seeds of GR soybean treated with glyphosate at 3–8 weeks after planting (Arregui et al., 2003; Duke et al., 2003) suggesting that AMPA levels can buildup within the plant. Plant injury and chlorophyll reduction peaked at 14 DAT. Photosynthesis, stomatal conductance, internal concentration of CO_2 and transpiration were affected at 14 DAT. These parameters were unaffected at 28 DAT in both soybean types suggesting that AMPA effects peaked at 14 DAT and the affected plants recovered from injury by 28 DAT. This supports our

Table 1
Aminomethylphosphonic acid effect on plant injury, chlorophyll content, plant height, and shoot dry weight in glyphosate-resistant and glyphosate-sensitive soybean plants.[a,b]

AMPA rate (kg ha^{-1})	Injury (%)				Chlorophyll (mg g^{-1} leaf tissue)				Plant height (cm)	Shoot dry weight (g $plant^{-1}$)
	3 DAT	7 DAT	14 DAT	28 DAT	3 DAT	7 DAT	14 DAT	28 DAT	28 DAT	28 DAT
Glyphosate-resistant soybean										
0	0 c	0 c	0 b	0 b	1.78 a	1.37 a	1.32 a	1.47 a	44 a	9.8 a
0.1	8 b	6 b	3 b	0 b	1.69 a	1.27 a	1.23 a	1.46 a	46 a	9.9 a
1.0	27 a	42 a	46 a	17 a	1.13 b	0.71 b	0.45 b	1.13 b	42 a	8.1 a
Glyphosate-sensitive soybean										
0	0 c	0 b	0 b	0 b	1.29 a	1.34 a	1.40 a	1.45 a	54 a	10.0 a
0.1	5 b	3 b	0 b	0 b	1.20 a	1.20 a	1.41 a	1.31 a	54 a	10.0 a
1.0	18 a	35 a	49 a	18 a	1.06 b	0.70 b	0.60 b	1.14 b	51 a	8.0 a

[a] Abbreviations: DAT, days after treatment.
[b] Means within a column and soybean type followed by same letter are not significantly different at the 5% level as determined by Fisher's LSD test.

Table 2
Effect of aminomethylphosphonic acid on photosynthesis (a), stomatal conductance (g_{sw}), intercellular CO_2 (C_i), and transpiration (E) at 3, 7, 14, and 28 d after treatment in glyphosate-resistant soybean.[a]

AMPA rate (kg ha^{-1})	a (μmol CO_2 m^{-2} s^{-1})	g_{sw} (mol H_2O m^{-2} s^{-1})	C_i (μmol CO_2 mol^{-1} air)	E (mmol H_2O m^{-2} s^{-1})
3 d after treatment				
0	6.83 a	−9.98 b	200.80 ab	12.07 a
0.1	6.81 a	2.47 ab	189.93 b	8.49 a
1.0	2.39 b	−3.14 a	213.60 a	7.68 a
7 d after treatment				
0	13.86 a	0.55 a	288.84 c	9.05 a
0.1	11.72 a	0.55 a	315.84 b	8.53 a
1.0	2.07 b	0.36 a	367.09 a	8.00 a
14 d after treatment				
0	18.16 a	0.79 a	318.31 c	7.80 a
0.1	13.25 b	0.64 b	331.25 b	7.23 a
1.0	4.25 c	0.39 c	360.90 a	5.34 b
28 d after treatment				
0	13.26 a	0.28 a	150.25 a	4.32 a
0.1	14.83 a	0.33 a	190.71 a	4.84 a
1.0	14.76 a	0.30 a	185.69 a	4.70 a

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

Table 3
Effect of aminomethylphosphonic acid on photosynthesis (a), stomatal conductance (g_{sw}), intercellular CO_2 (C_i), and transpiration (E) at 3, 7, 14, and 28 d after treatment in glyphosate-sensitive soybean.[a]

AMPA rate (kg ha^{-1})	a (μmol CO_2 m^{-2} s^{-1})	g_{sw} (mol H_2O m^{-2} s^{-1})	C_i (μmol CO_2 mol^{-1} air)	E (mmol H_2O m^{-2} s^{-1})
3 d after treatment				
0	5.48 a	0.46 a	201.27 b	7.18 a
0.1	6.08 a	0.50 a	189.50 b	7.65 a
1.0	2.24 b	0.28 b	225.93 a	5.52 b
7 d after treatment				
0	12.99 a	0.57 a	299.31 b	9.03 a
0.1	11.29 a	0.60 a	305.33 b	8.94 a
1.0	1.60 b	0.33 a	359.21 a	6.56 a
14 d after treatment				
0	19.17 a	0.70 a	294.60 b	6.47 a
0.1	16.35 b	0.92 a	316.48 b	6.73 a
1.0	5.87 c	0.48 a	357.56 a	5.87 a
28 d after treatment				
0	13.89 a	0.23 a	−162.40 a	3.94 a
0.1	15.13 a	0.29 a	−324.40 a	4.43 a
1.0	15.18 a	0.26 a	23.00 a	4.24 a

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

visual observation that chlorophyll synthesis was delayed in AMPA-treated plants, but leaf growth appeared to be similar to that in non-treated plant.

3.2. Experiment 2. Nodulation, acetylene reduction assay, respiration, shoot and root biomass, and shoot nitrogen

The GR and GS soybean plants were well nodulated as would be expected since Dundee silty clay loam having a long history of soybean production was used in the growth medium to ensure native population of *Bradyrhizobium japonicum*. AMPA had no significant effect on nodule number and nodule dry weight at 10 DAT regardless of rate and soybean types, though there was a decrease in nodule number and their dry weight with a higher AMPA rate over the control (Table 4). Similarly, nitrogen fixation potential measured as acetylene reduction activity at 10 DAT was unaffected in both GR and GS soybean by AMPA regardless of the rate. To assess effects of AMPA on root metabolism, CO_2 accumulation (root respiration) was also measured during acetylene reduction assay. Overall, root respiration was unaffected by AMPA at the lower rate, but the higher rate reduced root respiration in both GR and GS soybeans. AMPA at 1.0 kg ha^{-1} rate reduced shoot and root dry biomass by 20% and 28%, respectively in GR soybean and by 26% and 37%, respectively in GS soybean. The combined effect of lower root biomass and reduced photosynthesis can be factor in lower root respiration.

3.3. Experiment 3. Leaf in vivo nitrate reductase activity (NRA)

There was no direct effect of AMPA on nodulation parameters (nodule number and dry weight) and nitrogenase activity in both soybean types in this experiment (Table 4). However, AMPA at 1.0 kg ha^{-1} reduced root dry weight and respiration that may be through an indirect effect on nitrogen uptake and assimilation (Table 4). Leaf NRA indicated that AMPA at 0.1 and 1.0 kg ha^{-1} had no effect on leaf NRA at 3 and 14 DAT in both soybean types (Table 5). Nitrate must be reduced to NO_2^- for plants to assimilate N into various nitrogenous compounds. Nitrate reductase (NR, EC 1.6.6.1) is the enzyme that catalyses the first step of NO_3^- reduction into NO_2^-. Nitrate reductase is continuously synthesized and degraded so these measurements at 3 and 14 DAT are a snap shot of AMPA effect on NRA. Shoot N content 10 DAT was significantly higher in AMPA-treated compared to non-treated plants regardless of rate and soybean type (Table 5). However, total shoot N was

Table 4
Aminomethylphosphonic acid effects on nodule number and dry weight, nitrogenase activity, root respiration, shoot and root dry biomass, and shoot nitrogen content at 10 d after treatment in glyphosate-resistant and glyphosate-sensitive soybean.[a]

AMPA rate (kg ha^{-1})	Nodule number (no. plant^{-1})	Nodule dry weight (mg plant^{-1})	Nitrogenase activity (μmol ethylene formed plant^{-1} h^{-1})	Root respiration (μmol CO_2 evolved plant^{-1} h^{-1})	Shoot dry biomass (g plant^{-1})	Root dry biomass (g plant^{-1})
Glyphosate-resistant soybean						
0	24.9 a	150 a	3.44 a	84.1 a	1.35 a	0.45 a
0.1	24.2 a	129 a	3.29 a	72.2 ab	1.25 ab	0.41 a
1.0	23.7 a	135 a	2.84 a	61.0 b	1.09 b	0.32 b
Glyphosate-sensitive soybean						
0	31.1 a	184 a	4.60 a	82.4 a	1.27 a	0.40 a
0.1	30.5 a	183 a	5.78 a	74.5 a	1.18 a	0.31 ab
1.0	29.1 a	166 a	4.38 a	54.3 b	0.95 b	0.25 b

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

Table 5
Aminomethylphosphonic acid effects on leaf nitrate reductase activity (NRA), shoot nitrogen, and total shoot nitrogen in glyphosate-resistant and glyphosate-sensitive soybean.[a]

AMPA rate (kg ha^{-1})	NRA (μmol nitrite g^{-1} h^{-1})		Shoot N (%)	Total shoot N (mg N shoot^{-1})
	3 d after treatment	14 d after treatment	10 d after treatment	
Glyphosate-resistant soybean				
0	6.98 a	8.17 a	2.7 c	35.3 a
0.1	9.88 a	13.08 a	3.0 b	35.5 a
1.0	8.03 a	12.93 a	3.4 a	36.9 a
Glyphosate-sensitive soybean				
0	11.03 a	7.15 a	2.4 c	30.1 a
0.1	9.14 a	6.46 a	2.7 b	31.6 a
1.0	6.53 a	9.76 a	3.0 a	28.4 a

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

non-significant regardless of AMPA rate and soybean type (Table 5). Nitrate reductase activity was only measured in newly developed leaves, and there may have been sufficient NO_3^- assimilated in other leaves, and shoot N content represented the entire shoot biomass. Furthermore, more biomass from normal growth in non-treated plants may have resulted in dilution of N in tissue compared to AMPA-treated plants (Table 4).

4. Conclusions

Visual plant injury (5–27%) was observed on young leaves within 3 DAT regardless of AMPA rate and soybean type. Overall, AMPA effects were more pronounced at 1.0 kg ha^{-1} than at 0.1 kg ha^{-1} rate suggesting that a 10% degradation of glyphosate (at 1.0 kg ha^{-1} use rate) would not have a deleterious effect on soybean. AMPA injury peaked at 14 DAT and recovered to levels of non-treated plants by 28 DAT in both soybean types. AMPA at 1.0 kg ha^{-1} decreased significantly the chlorophyll content, photosynthesis, and root respiration in both soybean types. AMPA had no effect on nodulation and NRA at 10 DAT, and plant height and shoot dry weight at 28 DAT in GR and GS soybeans. AMPA increased shoot N at 10 DAT in both soybean types. The fact that AMPA decreases chlorophyll content prior to reducing photosynthesis rate suggests that AMPA might interfere in chlorophyll biosynthesis in soybean.

Generally, drift exposure or root uptake from soil carryover from previous applications will be less than 10% of that of a commercial application rate of glyphosate. These data suggest that exposure to relatively high rates of AMPA may impair chlorophyll synthesis, photosynthesis, and shoot/root biomass accumulation. However, the probability of soybean exposure to greater than one-tenths of the use rate is unlikely. From an environmental toxicity perspective, it is unlikely that inadvertent exposure of soybean to AMPA from either soil carryover or through glyphosate conversion in plant will have a significant effect on plant growth and development.

Acknowledgments

We thank Efren Ford, Earl Gordon, and Sandra Mosley for technical assistance.

References

Accinelli, C., Koskinen, W.C., Seebinger, J.D., Vicari, A., Sadowsky, M.J., 2005. Effects of incorporated corn residues on glyphosate mineralization and sorption in soil. J. Agric. Food Chem. 53, 4110–4117.
Amrhein, N., Schab, J., Steinrücken, H.C., 1980. The mode of action of the herbicide glyphosate. Naturwissenschaften 67, 356–357.
Arregui, M.C., Lenardón, A., Sanchez, D., Maitre, M.I., Scotta, R., Enrique, S., 2003. Monitoring glyphosate residues in transgenic glyphosate-resistant soybean. Pest Manage. Sci. 60, 163–166.
Bellaloui, N., Zablotowicz, R.M., Reddy, K.N., Abel, C.A., 2008. Nitrogen metabolism and seed composition as influenced by glyphosate application in glyphosate-resistant soybean. J. Agric. Food Chem. 56, 2765–2772.
Doublet, J., Mamy, L., Barriuso, E., 2009. Delayed degradation in soil of foliar herbicides glyphosate and sulcotrione previously absorbed by plants: consequences on herbicide fate and rick assessment. Chemosphere 77, 582–589.
Duke, S.O., Rimando, A.M., Pace, P.F., Reddy, K.N., Smeda, R.J., 2003. Isoflavone, glyphosate, and aminomethylphosphonic acid levels in seeds of glyphosate-treated, glyphosate-resistant soybean. J. Agric. Food Chem. 51, 340–344.
Franz, J.E., Mao, M.K., Sikorski, J.A., 1997. Glyphosate: a unique global herbicide. ACS Monograph 189. American Chemical Society, Washington, DC, p. 653.
Gimsing, A.L., Borggaard, O.K., Jacobsen, O.S., Aamand, J., Sorenson, J., 2004. Chemical and microbiological soil characteristics controlling glyphosate mineralisation in Danish surface soils. Appl. Soil Ecol. 27, 233–242.
Hardy, R.W.F., Holsten, D., Jackson, E.K., Burns, R.C., 1968. The acetylene–ethylene assay for nitrogen fixation: laboratory and field evaluation. Plant Physiol. 43, 1185–1205.
Harring, T., Streibig, J.C., Husted, S., 1998. Accumulation of shikimic acid: a technique for screening glyphosate efficiency. J. Agric. Food Chem. 46, 4406–4412.
Hiscox, J.D., Israelstam, G.F., 1979. A method for the extraction of chlorophyll from leaf tissues without maceration. Can. J. Bot. 57, 1332–1334.

King, C.A., Purcell, L.C., Vories, E.D., 2001. Plant growth and nitrogenase activity of glyphosate-tolerant soybean in response to foliar glyphosate applications. Agron. J. 93, 179–186.

Klepper, L., Hageman, R.H., 1969. The occurrence of nitrate reductase in apple leaves. Plant Physiol. 44, 110–114.

Lydon, J., Duke, S.O., 1988. Glyphosate induction of elevated levels of hydroxybenzoic acids in higher plants. J. Agric. Food Chem. 36, 813–818.

Mamy, L., Barriuso, E., Gabrielle, B., 2005. Environmental fate of herbicides trifluralin, metazachlor, metamitron, and sulcotrione compared with fate of glyphosate, a substitute broad spectrum herbicide for different glyphosate-resistant crops. Pest Manage. Sci. 61, 905–916.

Padgette, S.R., Re, D.B., Barry, G.F., Eichholtz, D.E., Delannay, X., Fuchs, R.L., Kishore, G.M., Fraley, R.T., 1996. New weed control opportunities: development of soybeans with a Roundup Ready gene. In: Duke, S.O. (Ed.), Herbicide-Resistant Crops: Agricultural, Environmental, Economic, Regulatory, and Technical Aspects. CRC Press, Boca Raton, FL, pp. 53–84.

Pline, W.A., Wilcut, J.W., Duke, S.O., Edmisten, K.L., Wells, R., 2002. Accumulation of shikimic acid in response to glyphosate applications in glyphosate-resistant and conventional cotton (*Gossypium hirsutum* L.). J. Agric. Food Chem. 50, 506–512.

Reddy, K.N., Zablotowicz, R.M., 2003. Glyphosate-resistant soybean response to various salts of glyphosate and glyphosate accumulation in soybean nodules. Weed Sci. 51, 496–502.

Reddy, K.N., Hoagland, R.E., Zablotowicz, R.M., 2000. Effect of glyphosate on growth, chlorophyll, and nodulation in glyphosate-resistant and susceptible soybean (*Glycine max*) varieties. J. New Seeds 2, 37–52.

Reddy, K.N., Rimando, A.M., Duke, S.O., 2004. Aminomethylphosphonic acid, a metabolite of glyphosate, causes injury in glyphosate-treated, glyphosate-resistant soybean. J. Agric. Food Chem. 52, 5139–5143.

Reddy, K.N., Rimando, A.M., Duke, S.O., Nandula, V.K., 2008. Aminomethylphosphonic acid accumulation in plant species treated with glyphosate. J. Agric. Food Chem. 56, 2125–2130.

Sammons, D., Tran, M., 2008. Examining yellow flash in roundup Ready soybean. North Central Weed Sci. Soc. Proc. 63, 120.

Simonsen, L., Fomsgaard, I.S., Svensmark, B., Splid, N.H., 2008. Fate and availability of glyphosate and AMPA in agricultural soil. J. Environ. Sci. Health – Part B 43, 365–375.

Zablotowicz, R.M., Reddy, K.N., 2007. Nitrogenase activity, nitrogen content, and yield responses to glyphosate in glyphosate-resistant soybean. Crop Prot. 26, 370–376.

Zablotowicz, R.M., Focht, D.D., Cannell, G.H., 1981. Nodulation and N fixation of field-grown California cowpeas as influenced by irrigated and droughted conditions. Agron. J. 73, 9–12.

Zablotowicz, R.M., Accinelli, C., Krutz, L.J., Reddy, K.N., 2009. Soil depth and tillage effects on glyphosate degradation. J. Agric. Food Chem. 57, 4867–4871.