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Aminomethylphosphonic Acid Accumulation in Plant Species Treated with Glyphosate

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Aminomethylphosphonic acid (AMPA) is the most frequently detected metabolite of glyphosate in plants. The objective of this study was to determine if there is any correlation of metabolism of glyphosate to AMPA in different plant species and their natural level of resistance to glyphosate. Greenhouse studies were conducted to determine the glyphosate I_{50} values (rate required to cause a 50% reduction in plant growth) and to quantify AMPA and shikimate concentrations in selected leguminous and nonleguminous species treated with glyphosate at respective I_{50} rates. Coffee senna [*Cassia occidentalis* (L.) Link] was the most sensitive (I_{50} = 75 g/ha) and hemp sesbania [*Sesbania herbacea* (P.Mill.) McVaugh] was the most resistant (I_{50} = 456 g/ha) to glyphosate. Hemp sesbania was 6-fold and Illinois bundleflower [*Desmanthus illinoensis* (Michx.) MacM. ex B.L.Robins. & Fern.] was 4-fold more resistant to glyphosate than coffee senna. Glyphosate was present in all plant species, and its concentration ranged from 0.308 to 38.7 μg/g of tissue. AMPA was present in all leguminous species studied except hemp sesbania. AMPA concentration ranged from 0.119 to 4.77 μg/g of tissue. Shikimate was present in all plant species treated with glyphosate, and levels ranged from 0.053 to 16.5 mg/g of tissue. Non-glyphosate-resistant (non-GR) soybean accumulated much higher shikimate than glyphosate-resistant (GR) soybean. Although some leguminous species were found to be more resistant to glyphosate than others, and there was considerable variation between species in the glyphosate to AMPA levels found, metabolism of glyphosate to AMPA did not appear to be a common factor in explaining natural resistance levels.

KEYWORDS: Aminomethylphosphonic acid; AMPA; glyphosate; glyphosate-resistant crop; shikimic acid; soybean

INTRODUCTION

Glyphosate is a nonselective broad-spectrum herbicide used extensively throughout the world. Glyphosate inhibits the biosynthesis of aromatic amino acids (phenylalanine, tryptophan, and tyrosine), leading to reduced synthesis of proteins and secondary products (*1*). Furthermore, deregulation of the shikimate pathway leads to general metabolic disruption (*2, 3*). The molecular target site of glyphosate in the shikimate pathway is 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Inhibition of EPSPS causes greatly increased levels of shikimate accumulation (*4, 5*).

Glyphosate-resistant (GR) crops, mainly soybean [*Glycine max* (L.) Merr.], cotton (*Gossypium hirsutum* L.), canola (*Brassica napus* L.), and corn (*Zea mays* L.), were created by stable integration of a transgene that codes a glyphosate-insensitive EPSPS (*6*). Expression of the GR EPSPS helps to maintain normal aromatic amino acid levels in GR crops treated with glyphosate. GR crops are grown in several countries, and their rapid adoption has led to a large increase in the use of glyphosate. Rapid adoption of GR crops is mainly due to simplicity and flexibility of controlling most grasses and broadleaf weeds with a single herbicide at a lower cost.

Glyphosate degrades relatively rapidly in soils by microbial processes (*1, 7*). The most frequently detected degradation product in soil and water is aminomethylphosphonic acid (AMPA). Little is known about the enzyme(s) involved in the degradation of glyphosate to AMPA in plants. It has been

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conjectured that glyphosate can be metabolized by plants via two pathways similar to those present in microorganisms (*1*). One involves oxidative cleavage of the C−N bond to yield AMPA and the other breaking of C−P bond by a C−P lyase to generate sarcosine. AMPA is phytotoxic to plant species, although it is considerably less active than glyphosate (*1, 8, 9*). Although glyphosate is minimally metabolized by plants (*2*), AMPA is found as a major metabolite in seeds of canola (*10*), wheat (*Triticum aestivum* L.) (*11*), field pea (*Pisum sativum* L.), barley (*Hordeum vulgare* L.), flax (*Linum usitatissimum* L.) (*12*), and GR soybean treated with glyphosate (*13*). AMPA was also found in foliage of glyphosate-treated crested wheatgrass [*Agropyron cristatum* (L.) Gaertn.] (*14*). AMPA residues were also detected in leaves and seeds of field-grown GR soybean treated with glyphosate at label use rates, indicating metabolism of glyphosate in GR soybean (*15*).

Detection of AMPA in leaves, stems, and seeds of several crops including GR soybean following glyphosate application suggests that a plant glyphosate oxidoreductase (GOX) or similar type of enzyme catalyzes this conversion. AMPA is phytotoxic to soybean, although less active than glyphosate; its mode of action is apparently different from that of glyphosate, because GR and glyphosate-sensitive soybeans are equally sensitive to AMPA (*9*). GOX has been characterized in several genera of bacteria; however, no plant-derived GOX has been described in the literature.

Some leguminous species are more resistant [e.g., hemp sesbania; sicklepod, *Senna obtusifolia* (L.) H.S. Irwin & Barneby] to glyphosate than others [coffee senna; kudzu, *Pueraria montana* var. *lobata* (Willd.) Maesen & S.M. Almeida]. Low levels of resistance to glyphosate in certain leguminous species may be due to differences in GOX activity (rapid detoxification of glyphosate). We have previously reported that AMPA is formed from glyphosate degradation in glyphosate-treated GR soybean (*9*). In the present study, we investigated if other leguminous species also produce AMPA from glyphosate. The main objective of this study was to determine if differences in glyphosate sensitivity between species can be explained by degradation to AMPA. Because shikimate accumulation is sometimes used as an indicator of sensitivity to glyphosate, a secondary objective was to determine if this factor correlates with interspecies sensitivity to glyphosate. GR and non-GR soybean along with four nonleguminous species were included for comparison.

MATERIALS AND METHODS

General Experimental Conditions. Greenhouse experiments were conducted from July 2006 to March 2007 at USDA-ARS, Southern Weed Science Research Unit, Stoneville, MS. Eleven plant species with varying levels of sensitivity to glyphosate were used in the study (**Table 1**). The plant species were corn, coffee senna, cowpea [*Vigna unguiculata* (L.) Walpers], hemp sesbania, horseweed [*Conyza canadensis* (L.) Cronq.], Illinois bundleflower, Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot], kudzu, sicklepod, soybean, and velvetleaf (*Abutilon theophrasti* Medik.). Horseweed (from Tunica, MS), coffee senna, hemp sesbania, Illinois bundleflower, and Italian ryegrass (all from Stoneville, MS) seeds were collected from plants growing in the field. Kudzu seed was purchased from Adams-Briscoe Seed Co., Jackson, GA. Cowpea was purchased from Farmers Feed and Supply Co., Leland, MS. Sicklepod and velvetleaf seeds were purchased from Azlin Seed Service, Leland, MS. Corn and soybean was purchased from Jimmy Sanders, Inc., Hollandale, MS. DKC69-72 RR2, a GR variety, and Pioneer 32D99, a non-GR variety, of corn and Asgrow 4603RR, a GR variety, and DP5110S, a non-GR variety, of soybean were used. Plants were grown in 10 cm diameter plastic pots containing a 1:1 (v/v) mixture of soil (Bosket sandy loam, fine-loamy, mixed, thermic Mollic Hapludalfs; pH 8.2, 0.5% organic matter, cation exchange capacity = 17 cmol/kg, 51% sand, 37% silt, 12% clay) and potting mix (Jiffy Products of America Inc., Batavia, IL). The greenhouse was maintained at 28/22 °C (±3 °C) day/night temperature with natural light supplemented by sodium vapor lamps to provide a 13 h photoperiod. Plants were subirrigated with water as needed. Plant growth stage and number of plants used for glyphosate treatment are summarized in **Table 1**. Spray solutions were applied using an indoor spray chamber equipped with an air-pressurized system delivering 190 L/ha at 140 kPa using 8002E flat-fan nozzles.

Table 1. Plant Growth Stage at the Time of Glyphosate Application

plant species[a]	plants used, no./pot	leaves, no./plant	plant height, cm	plant age, weeks
soybean, GR	3	1–2	10–12	3
soybean, non-GR	3	1–2	12–15	3
cowpea	3	1–2	12–15	3
sicklepod	2	3–4	10–12	4
coffee senna	2	3–4	10–12	4
hemp sesbania	2	5–6	13–15	4
Illinois bundleflower	6	4–5	7–10	4
kudzu	5	2–3	5–8	4
velvetleaf	2	5–6	10–12	4
horseweed	1	25–30	10–13[b]	20
corn, GR	2	1–2	35–40	3
corn, non-GR	2	1–2	35–40	3
Italian ryegrass	6	3–6	10–15	6

[a] GR, glyphosate-resistant; non-GR, non-glyphosate-resistant. [b] Horseweed rosette diameter.

Glyphosate I_{50}. Glyphosate acid at 0, 0.03, 0.06, 0.12, 0.25, 0.50, 1.00, 2.00, and 4.00 kg/ha was applied to plants. Spray solutions were prepared using technical grade glyphosate acid (>97% purity, Sigma-Aldrich, Allentown, PA) and Tween 20 (Sigma-Aldrich, St. Louis, MO) at 0.5%, v/v. Technical grade glyphosate acid was used to minimize interference from various ingredients in the commercial formulations of glyphosate. Tween 20-treated plants were included as the control. At 14 days after treatment (DAT), plants were excised at the soil surface and shoot fresh weights were recorded. Data are expressed as percent shoot fresh weight reduction as compared to nontreated plants. Treatments were arranged in a randomized complete block design with five or six replications. Data were fitted to a sigmoidal logistic model 1 or 2 to relate percent shoot fresh weight reduction (y) to herbicide rate (x).

$$y = \frac{a}{1 + \exp^{-(x-x_0)/b}} \quad (1)$$

In eq 1, a is the difference of the upper and lower response limits (asymptotes), x_0 is the herbicide rate that results in a 50% reduction in y (I_{50}), and b is the slope of the curve around x_0. The regression parameters for eq 1 were computed using SigmaPlot (version 10.0, Systat Software Inc., San Jose, CA).

$$y = c + \frac{d-c}{1 + \exp\{b[\log(x) - \log(e)]\}} \quad (2)$$

In eq 2, the parameter e is also denoted I_{50}, and it is herbicide rate producing a response halfway between the upper limit, d, and lower limit, c. The parameter b is the relative slope of the curve around e. The regression parameters for eq 2 were computed using *R* software (*16*). The I_{50} rate was determined using eq 1 for non-GR soybean and eq 2 for all other species. Glyphosate levels at 170 g/ha for horseweed (*17*) and 220 g/ha for Italian ryegrass (*18*) were used as I_{50} rate.

Glyphosate, AMPA, and Shikimate Accumulation in Glyphosate-Treated Plants. Glyphosate, AMPA, and shikimate levels were quantified following treatment with I_{50} rates of glyphosate. The I_{50} rate for each plant species was selected to avoid plant death and to enable detection of AMPA residues in plants. Glyphosate I_{50} rates of non-GR corn and soybean were used for their respective GR varieties to enable comparison between GR and non-GR types. Preparation of spray solutions and application was as described in the above study. Tween

20-treated plants were included as controls. Plants were harvested at 7 DAT. Plants were clipped at the soil surface, washed with running water, rinsed with distilled water to remove glyphosate remaining on the leaf surface, and blotted dry with paper towels. Treated plant samples consisted of both stem and leaves. All plant samples were air-dried, ground, and analyzed for glyphosate, shikimate, and AMPA. There were five to seven pots per treatment, and treatment was replicated five times. Treatment means with standard deviation are presented.

Extraction of Plant Tissue and Derivatization of Extracts. Plant samples were analyzed for glyphosate, shikimic acid, and AMPA. For glyphosate and AMPA analysis, extraction and derivatization were performed according to published procedures (*19*), with modifications. One gram of ground tissue (from the combined sample of all plants in each treatment) was extracted with 10 mL of water in a 15 mL centrifuge tube, shaken, placed in a sonicating bath for 20 min, and then centrifuged (Sorvall RC 5C Plus; Kendro Laboratory Products, Asheville, NC) at 47000 rpm, 20 °C, for 20 min. Supernatant was removed. The tissue sample pellet was extracted a second time by adding 10 mL of water, and procedures were performed as in the first extraction. The volume of the combined supernatant was measured, and then 5 μL of 12.1 M HCl was added and shaken. Four milliliters was transferred to a 20 mL scintillation vial with a Teflon-lined cap, shaken with 4 mL of methylene chloride, and centrifuged (Savant speed vac model SVC 200, Savant Instruments, Inc., Holbrook, NY) for 10 min. A portion (1.8 mL) of the water layer was taken, and 200 μL of acidic modifier [16 g of KH_2PO_4, 160 mL of H_2O, 40 mL of methanol (MeOH), 13.4 mL of HCl] was added. One milliliter was loaded to a cation exchange resin column (AG 50W-X8, H^+; Bio-Rad Laboratories, Hercules, CA) previously equilibrated with two 5 mL portions of water. The sample was eluted until the level of column bed. CAX mobile phase (160 mL of H_2O, 40 mL of MeOH, 2.7 mL of HCl) (0.7 mL) was added, eluted, and discarded. Twelve milliliters of CAX mobile phase was again added to the column to elute the analytes. The eluate was collected in a 20 mL vial and evaporated to dryness using a Savant speed vac. To the dried sample was added 1.5 mL of CAX mobile phase, and then the vial was placed in a sonicating bath for 30 min. A 20 μL aliquot was taken and added to 640 μL of a solution of 2,2,3,3,4,4,4-heptafluoro-1-butanol and trifluoroacetic anhydride (1:2) in a chilled 4 mL vial. The mixture was allowed to equilibrate at room temperature for 10–15 min. The vial was transferred to a heating block at 90 °C for 1 h and then allowed to cool to room temperature. The solvent was evaporated under a stream of nitrogen, and the residue was dissolved in 80 μL of ethyl acetate containing 0.2% citral; 50 μL was transferred to a GC vial and analyzed by GC-MS. This method afforded 90 and 86% recoveries of glyphosate and AMPA, respectively, on the basis of duplicate extraction experiments in which samples were fortified with 100 ng standards per gram of sample.

For the analysis of shikimic acid, powdered sample (0.5 g) was placed in an extraction cell with 12.5 mL of deionized water (pH 2, adjusted with HCl), vortexed for 2 min, then extracted using a MARS Xpress Ultra-High Throughput Microwave Digestion System (CEM Corp., Matthews, NC) for 20 s at 400(¼) W. The extract was filtered through a 0.45 μm filter, and a 100 μL aliquot was analyzed for shikimic acid by HPLC (Agilent 1200 series HPLC system, Agilent Technologies, Inc., Santa Clara, CA) using a Gemini C18 column, 250 × 4.6 mm, 5 μm (Phenomenex, Torrance, CA). The mobile phase consisted of 98% H_2O (pH 3.0) and 2% MeOH in H_2O eluted isocratically, flow rate = 1 mL. Quantification of shikimic acid was performed from a calibration curve of standard shikimic acid (Sigma-Aldrich, St. Louis, MO). The limit of detection (LOD) and limit of quantitation (LOQ) for shikimic acid were 4 and 12.3 ng on column (10 μL injection), respectively.

GC-MS Analysis of Glyphosate and AMPA in Plant Samples. Analysis of glyphosate and AMPA by GC-MS (Agilent 6890 series GC coupled to a JEOL GCMateII mass spectrometer) was performed using a DB-5 capillary column (J&W Scientific, Inc., Folsom, CA), 30 m length by 0.25 mm i.d. by 0.25 μm film. The MS detector was a magnetic sector; spectra were acquired in the positive, low resolution, selected ion monitoring mode. The injection port, GC interface, and ionization chamber were maintained at 260, 230, and 120 °C, respectively. The carrier gas was ultrahigh-purity helium at a 1 mL/min flow rate. The sample injection volume was 1 μL. Glyphosate and AMPA in the samples were quantitated from a calibration curve of the respective derivatized standards.

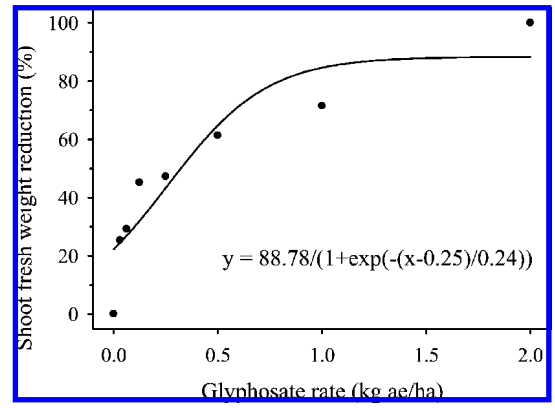

Figure 1. Response of non-glyphosate-resistant soybean to glyphosate at 2 weeks after treatment. Response is expressed as percent shoot fresh weight reduction compared to the nontreated plants. Observed mean values are plotted. Data were fitted to eq 1.

For the analysis of glyphosate and AMPA, the temperature program was as follows: initial, 70 °C, held for 3.5 min, raised to 160 at 30 °C/min rate, raised to 270 at 70 °C/min rate, raised to 310 at 35 °C/min rate, and finally held at this temperature for 3 min. AMPA derivative was observed at 7:23 min (*m/z* 571, 502, 446, 372) and glyphosate derivative was observed at 7:59 min (*m/z* 611, 584, 460). The LOD and LOQ for glyphosate were 19.9 and 160 pg on column (1 μL injection), respectively. The LOD and LOQ for AMPA were 3.71 and 11.2 pg on column (1 μL injection), respectively. Analysis was performed in duplicate.

RESULTS AND DISCUSSION

Glyphosate I_{50}. Dose–response experiments were conducted to determine glyphosate I_{50} rates, a measure of sensitivity to glyphosate. The observed data and fitted dose–response curves for non-GR soybean are shown in **Figure 1** and for eight other plant species in **Figure 2**. Regression parameters for dose–response curves fitted to eq 2 are presented in **Table 2**. Glyphosate inhibited growth in a nonlinear dose-dependent manner in all plant species. Glyphosate I_{50} rates ranged from 75 to 456 g/ha among the 11 plant species (**Tables 2** and **3**). Coffee senna was most sensitive (I_{50} = 75 g/ha) and hemp sesbania was most resistant (I_{50} = 456 g/ha) to glyphosate. Hemp sesbania was about 6-fold and Illinois bundleflower was about 4-fold more resistant to glyphosate compared to coffee senna. Cowpea, sicklepod, and non-GR soybean were about 3-fold more resistant to glyphosate compared to coffee senna. These results indicate that some leguminous species are more resistant to glyphosate than others. The differential resistance to glyphosate in certain leguminous species may be due to differences in levels of degradation of glyphosate to the much less phytotoxic metabolite of glyphosate, AMPA. To test this hypothesis, we measured glyphosate, shikimate, and AMPA levels in stems and leaves of seven leguminous species including GR soybean following glyphosate treatment at the I_{50} rate. Four nonleguminous species were included for comparison.

Glyphosate, AMPA, and Shikimate Accumulation in Glyphosate-Treated Plants. Glyphosate was present in all plant species 7 DAT, and its concentration ranged from 0.308 to 38.7 μg/g of tissue (**Table 3**). Because each species was treated using its respective I_{50} rate, comparison of glyphosate concentrations among plant species is not valid. Furthermore, differences in leaf canopy and other biological factors almost certainly

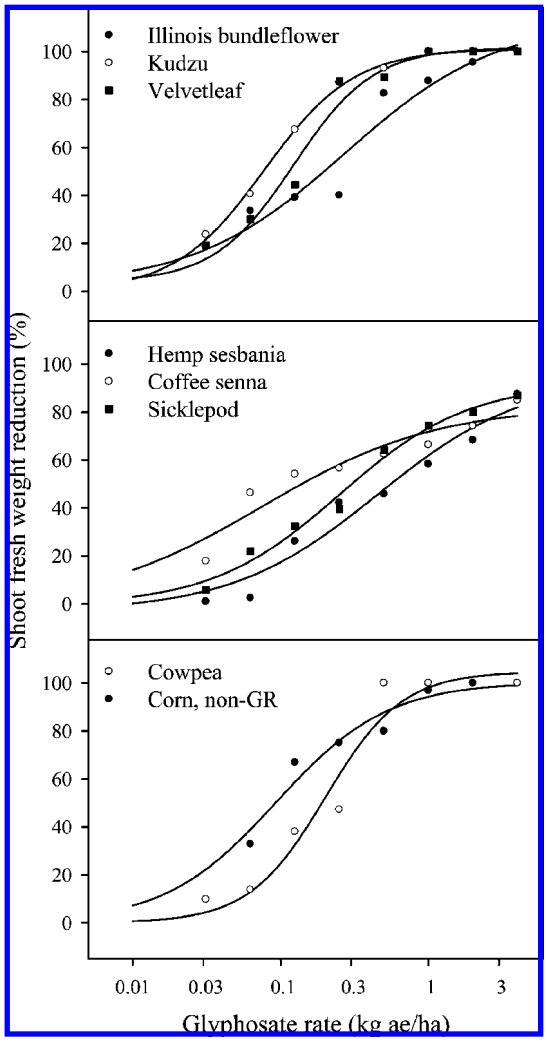

Figure 2. Response of eight plant species to glyphosate at 2 weeks after treatment. Response is expressed as percent shoot fresh weight reduction compared to the nontreated plants. Observed mean values are plotted. Data were fitted to eq 2.

Table 2. Glyphosate I_{50} Rate and Estimated Regression Parameters from Response Curves Fitted to Equation 2[a,b]

	regression parameters			
plant species	glyphosate I_{50} rate (e), g of ae/ha	slope (b)	lower limit (c)	upper limit (d)
cowpea	201	−1.6780	0.0093	104.75
sicklepod	252	−0.9978	−0.5796	92.07
coffee senna	75	−0.7415	−1.1506	82.57
hemp sesbania	456	−0.8831	−3.1637	94.71
Illinois bundleflower	272	−0.8466	2.1984	113.00
kudzu	77	−1.4331	−0.0006	101.15
velvetleaf	122	−1.6135	3.8723	101.73
corn, non-GR	93	−1.1484	−0.0033	100.50

[a] non-GR, non-glyphosate-resistant. [b] I_{50}, glyphosate rate required to cause a 50% reduction in plant growth.

influenced the differences in glyphosate levels between species. Although both GR and non-GR soybeans were treated at the same rate (250 g/ha), GR soybean had a lower glyphosate concentration compared to non-GR soybean. GR soybean possesses insensitive EPSPS enzyme and was not affected by glyphosate. More biomass production in GR soybean after glyphosate treatment resulted in dilution of glyphosate in tissue. Glyphosate translocates to roots, from which it can be exuded into the soil (*20, 21*). Differences in this process between GR and non-GR soybean could have also contributed to the differences in glyphosate content. A similar trend was observed between GR and non-GR corn.

Table 3. Effect of Glyphosate at I_{50} Rate on Glyphosate and Aminomethylphosphonic Acid (AMPA) Concentration at 7 Days after Treatment in Several Plant Species[a,b]

plant species[c]	glyphosate[d] I_{50}, g of ae/ha	glyphosate, ng/g of tissue	AMPA, ng/g of tissue	ratio, glyphosate/ AMPA
soybean, GR	250	5826 (812)	119 (118)	49
soybean, non-GR	250	25036 (5136)	668 (531)	38
cowpea	201	26763 (5872)	4765 (3589)	6
sicklepod	252	6414 (1985)	1834 (870)	4
coffee senna	75	5906 (4912)	287 (259)	21
hemp sesbania	456	38650 (9022)	nd[e]	
Illinois bundleflower	272	3274 (1471)	1513 (1446)	2
kudzu	77	5561 (2826)	297 (286)	19
velvetleaf	122	678 (153)	nd	
horseweed	170	26326 (5201)	314 (76)	84
corn, GR	93	308 (280)	nd	
corn, non-GR	93	851 (364)	nd	
Italian ryegrass	220	7432 (2724)	nd	

[a] I_{50}, glyphosate rate required to cause a 50% reduction in plant growth. [b] Numbers in parentheses indicate one standard deviation. [c] GR, glyphosate-resistant; non-GR, non-glyphosate-resistant. [d] Glyphosate at 250 and 93 g/ha was used for GR soybean and corn, respectively, to enable comparison between GR and non-GR types. Tween 20 at 0.5% (v/v) was added to all treatment solutions. [e] nd, peak not detected or peak below limit of quantitation.

AMPA was present in all leguminous species except hemp sesbania (**Table 3**). AMPA concentration ranged from 119 ng/g of tissue in GR soybean to 4765 ng/g of tissue in cowpea. In another study, 8 μg of AMPA/g of tissue was detected in GR soybean 7 DAT with glyphosate at 6.72 kg/ha (*9*). Low levels of AMPA in the present study may be due to the relatively low rate of glyphosate used. Overall, AMPA was detected in six of the seven leguminous species studied. However, there was no correlation between the glyphosate to AMPA ratio (a possible indicator of GOX activity) in the tissue with the glyphosate I_{50} values. Detection of AMPA following glyphosate treatment suggests that a plant GOX may be responsible for this conversion. However, nothing is known about the enzymology of glyphosate degradation to AMPA in plants. AMPA is phytotoxic to both GR and non-GR soybean, and its mode of action is apparently different from that of glyphosate (*9*). Among four nonleguminous species, AMPA was present in horseweed but not in velvetleaf, Italian ryegrass, and corn (both GR and non-GR).

In nontreated plants, shikimate was present in all plant species and ranged from 2 to 904 μg/g of tissue (**Table 4**). Elevated levels of shikimate were observed in all plant species except GR soybean, GR corn, and non-GR corn treated with glyphosate. Shikimate levels ranged from 53 μg/g of tissue in GR soybean to 16530 μg/g of tissue in cowpea. Non-GR soybean accumulated much higher shikimate levels than GR soybean. By blocking EPSPS, glyphosate causes many-fold increases in shikimate levels in glyphosate-treated, non-GR soybean (*5*) and oil seed rape (cv. Iris) (*4*) plants. In other research, Duke et al. (*13*) also observed that shikimate levels in GR soybean seed were unaffected by commonly used glyphosate treatments in soybean production. Elevated shikimate levels are used as an early and highly sensitive indicator of glyphosate effects on glyphosate-sensitive plant tissues (*4*). In transgenic, GR cotton, shikimate levels rise when the plants are treated with enough glyphosate to cause sublethal effects on reproductive tissues

Table 4. Effect of Glyphosate at I_{50} Rate on Shikimate Concentration at 7 Days after Treatment in Several Plant Species[a,b]

plant species[c]	glyphosate[d] I_{50}, g of ae/ha	shikimate, μg/g of tissue: glyphosate treated	shikimate, μg/g of tissue: nontreated
soybean, GR	250	53 (49)	65 (53)
soybean, non-GR	250	15251 (1132)	27 (21)
cowpea	201	16530 (1809)	64 (36)
sicklepod	252	1092 (164)	348 (313)
coffee senna	75	686 (353)	277 (241)
hemp sesbania	456	6828 (930)	266 (184)
Illinois bundleflower	272	668 (126)	21 (17)
kudzu	77	8915 (368)	188 (109)
velvetleaf	122	159 (213)	2 (2)
horseweed	170	9101 (1614)	251 (234)
corn, GR	93	245 (114)	241 (23)
corn, non-GR	93	149 (48)	161 (72)
Italian ryegrass	220	6701 (920)	904 (231)

[a] I_{50}, glyphosate rate required to cause a 50% reduction in plant growth. [b] Numbers in parentheses indicate one standard deviation. [c] GR, glyphosate-resistant; non-GR, non-glyphosate-resistant. [d] Glyphosate at 250 and 93 g/ha was used for GR soybean and corn, respectively, to enable comparison between GR and non-GR types. Tween 20 at 0.5% (v/v) was added to all treatment solutions.

(*22*). Thus, the absence of an effect on shikimate observed in this study indicated that the insensitive EPSPS was either not inhibited or that the insensitive EPSPS utilized all of the shikimate that would have accumulated from inhibition of the native EPSPS. However, there was no correlation between the absolute amount of accumulated shikimate and the fold increase in shikimate with species sensitivity (I_{50} values) to glyphosate.

These results indicate that AMPA is formed in the plant species treated with glyphosate. However, AMPA data do not support the theory that metabolism of glyphosate explains the relative sensitivities to glyphosate in the species tested. A caveat is that AMPA might be differently metabolized in the different plant species, confounding interpretation of the AMPA data. We know of no papers that have attempted to determine AMPA degradation in plants, although it is reported to degrade in soil by both biotic and abiotic processes (*23, 24*). Differential natural resistance to glyphosate in leguminous species may have been due to differential interception and retention of spray, absorption, translocation, and sensitivity of EPSPS among the species. Nevertheless, in Illinois bundleflower and, perhaps, sicklepod the ratio of glyphosate to AMPA suggests that metabolic degradation of glyphosate could play a role in the response to glyphosate.

It is also possible that a portion of the AMPA comes from degradation of glyphosate on plant surfaces, followed by uptake of the metabolite. AMPA from soil microbes in contact with root-exuded glyphosate might be translocated to shoots. Recently, an oxidative (abiotic) degradation of glyphosate involving C−P and C−N bond cleavage in the presence of manganese oxide has been suggested as another potential degradation pathway (*23*). However, the facts that AMPA was not detected in some species and relatively higher levels of AMPA were found in other plant species do not support the view that the AMPA formation is due to conversion of glyphosate to AMPA by leaf surface or root-associated microflora and oxidative degradation. AMPA residues were found in leaves, stems, and seeds of GR soybean following glyphosate applications (*9, 13, 15*). Furthermore, over time, there is more AMPA in some plant tissues than glyphosate. We found much more AMPA than glyphosate in seeds of glyphosate-treated GR soybean (*13*). Previous studies have shown axenic cell cultures of soybean to degrade glyphosate much better than cells of other crops (*25*). Taken together, these data suggest that six of seven leguminous species may have a plant GOX. However, no plant-derived GOX has been described in the literature, making this an area ripe for further investigation.

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