Compositional Analysis of Glyphosate-Tolerant Soybeans Treated with Glyphosate

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The compositional analyses and safety assessment of glyphosate-tolerant soybeans (GTS) were previously described. These analyses were extensive and included addressing the potential effects on seed composition from the genetic modification. Detailed compositional analyses established that GTS, which had not been treated with glyphosate, were comparable to the parental soybean line and to other conventional soybeans. In this study, two GTS lines, 40-3-2 and 61-67-1, were treated with commercial levels of glyphosate, the active ingredient in Roundup herbicide. The composition of the seed from soybeans sprayed with glyphosate was compared to that of a nonsprayed parental control cultivar, A5403. The nutrients measured in the seed included protein, oil, ash, fiber, carbohydrates, and amino acids. The concentration of isoflavones (also referred to as phytoestrogens) was also measured as these compounds are derived from the same biochemical pathway that was engineered for glyphosate tolerance. The analytical results from these studies demonstrate that the GTS soybeans treated with glyphosate were comparable to the parental soybean cultivar, A5403, and other conventional soybean varieties.

Keywords: *Soybean; glyphosate-tolerant; compositional analysis; glyphosate*

INTRODUCTION

The composition of glyphosate-tolerant soybean (GTS) lines 40-3-2 and 61-67-1 has been thoroughly characterized, and the results have been previously reported (Delannay et al., 1995; Padgette et al., 1995, 1996). These evaluations led to the conclusion that, except for tolerance to glyphosate herbicide, the GTS lines are substantially equivalent to the parental cultivar, A5403, and other soybean cultivars currently being marketed. Because these compositional analyses were designed to determine whether the genetic change that confers glyphosate tolerance altered the levels of key nutrients and antinutrients, the GTS lines were not sprayed with Roundup herbicide, of which glyphosate is the active ingredient.

A compositional change of GTS seed upon application of glyphosate was considered to be unlikely due to several factors: (1) There is no effect on the composition of the GTS compared to the parental cultivar, A5403, in the absence of glyphosate application. (2) Glyphosate is a specific inhibitor of the enzyme 5-enolpyruvlyshikimate-3-phosphate synthase (EPSPS), a key enzyme in the shikimate pathway of aromatic amino acid biosynthesis (Haslam, 1993), and thus nonspecific effects on the soybean composition are not anticipated. (3) The presence of the introduced glyphosate-tolerant CP4 EPSPS in glyphosate-treated GTS is sufficient to provide the required amounts of EPSPS reaction products for normal plant growth and development. On the basis of extensive yield data for glyphosate-treated GTS (Delannay et al., 1995), the application of glyphosate has no deleterious effects and should therefore not elicit any stress responses in the GTS plants or seeds. To provide experimental data to support these hypotheses, two GTS lines (40-3-2 and 61-67-1) were sprayed with glyphosate at commercial levels typically used for weed control. Several key seed components were measured in the glyphosate-treated GTS lines and compared to the levels in unsprayed, nontransgenic control soybean seed samples collected across multiple U.S. locations in 1992 and 1993.

If any affects on GTS were to be observed upon glyphosate treatment, it would most likely be expected to effect aromatic amino acid biosynthesis, because glyphosate inhibits this pathway in conventional soybeans (Sikorski et al., 1998). Initially, the CP4 EPSPS expression levels in glyphosate-treated GTS lines were measured as compared to nontreated GTS lines (Padgette et al., 1995). Second, the proximate analyses, amino acid levels (including aromatic amino acids), and isoflavone levels (secondary products of the aromatic pathway) were compared in glyphosate-treated GTS and conventional soybeans. The results of these analyses established that there were no significant differences observed in proximate, amino acid, or isoflavone compositions of glyphosate-treated GTS lines as compared to the conventional soybean line, A5403, or other commercial soybean varieties.

MATERIALS AND METHODS

GTS Lines. The GTS lines, 40-3-2 and 61-67-1, were generated through particle gun bombardment of Asgrow (Kalamazoo, MI) line A5403 with vectors PV-GMGT04 and PV-GMGT05, respectively. The plasmids PV-GMGT04 and PV-

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GMGT05 contained the CP4 EPSPS gene and a gene encoding β-glucuronidase (GUS) from *Escherichia coli* (Jefferson et al., 1986). The development and characterization of these two lines have been previously described (Delannay et al., 1995; Padgette et al., 1995, 1996). Seeds from the R_5 and R_6 generations were planted in the 1992 and 1993 field trials, respectively, to generate R_6 and R_7 seeds for analytical evaluation. There was one exception in 1992, in Macon, MO, where R_4 seed was planted. The parental control, Asgrow A5403, was grown side by side at the same field sites as the GTS lines described above. The GTS lines, 40-3-2 and 61-67-1, and A5403 lines were grown at the seven following sites in 1992: Macon, MO; Washington, LA; Greenville, MS; Newport, AR; Proctor, AR; Winterville, GA; and Seven Springs, NC. One plot of 1000 square feet (±5%) was planted for the GTS lines, 40-3-2 and 61-67-1, and the control, A5403. The soybean seed samples were collected by harvesting the whole plot using commercial equipment. In 1993, four sites were used to grow GTS line 40-3-2 and A5403: Gordon, AL; Salisbury, MD; Steele, MO; and Marion, AR. The test plots for the 1993 field studies ranged from 800 ft^2 (Marion, AR) to 3000 ft^2 (Gordon, AL). Plots for the Maryland and Missouri sites were 2000 ft^2 (±5%). The seeds were harvested as whole plots using commercial equipment. GTS line 61-67-1 was dropped from further analysis at this time for commercial reasons. These field studies have been described elsewhere (Padgette et al., 1995).

Glyphosate Application Rates and Timing. A pre-emergence application of 17.8 L/ha Roundup herbicide [6.4 kg of acid equivalent (ae)/ha of glyphosate] was applied to the 1992 GTS field plots as a conventional broadcast spray application after planting but prior to emergence. The 1992 GTS field plots were also sprayed with 1.75 L/ha of Roundup herbicide (0.63 kg of ae/ha of glyphosate) at early postemergence as a topical broadcast spray application made 40 days (±2 days) after planting. The spray regime for the 1993 GTS field plot (40-3-2 only) included a pre-emergence application of Roundup herbicide of 17.8 L/ha (6.4 kg of ae/ha of glyphosate) and an early postemergence application as well as a late postemergence application of 2.34 L/ha Roundup herbicide (0.84 kg of ae/ha of glyphosate). The late postemergence application was a topical broadcast spray application performed at the formation of the first flower buds just prior to the R1 growth stage. There were no Roundup applications, either pre- or postemergent, to the control A5403 field plots.

Enzyme-Linked Immunosorbent Assay (ELISA). ELISAs were developed and validated for CP4 EPSPS and β-glucuronidase (GUS) protein accumulation as previously described (Padgette et al., 1995). The 1992 seed samples from GTS lines 40-3-2 and 61-67-1 were extracted and evaluated for CP4 EPSPS and GUS accumulation. GTS line 40-3-2 was previously shown to contain only the CP4 EPSPS gene (Padgette et al., 1995), whereas GTS line 61-67-1 contained the genes for both CP4 EPSPS and GUS (unpublished data). Extracts of the A5403 control seed were not evaluated for protein expression because it had been previously determined by protein and genetic analysis that neither the CP4 EPSPS nor the GUS proteins are present (Padgette et al., 1995). In 1993, both leaf and seed extracts were prepared from samples collected at each site for GTS line 40-3-2 and the control line A5403. The extracts were evaluated for CP4 EPSPS accumulation only.

Analytical Assays. Soybean seed compositional assays were performed at Ralston Analytical Laboratories (St. Louis, MO). Specified reagents were obtained from Sigma Chemical (St. Louis, MO) unless otherwise noted. Seed samples from the 1992 and 1993 studies were shipped and stored at ambient temperature. Proximate analysis, which included protein, ash, moisture, oil, fiber, and carbohydrates, was performed on the 1992 seed samples of GTS lines 40-3-2 and 61-67-1 and the control line A5403. In addition to proximate analysis, the 1993 seed samples for GTS line 40-3-2 and the A5403 control were analyzed for amino acids and isoflavones. Analytical methods used to determine proximate composition, amino acids, and isoflavones have been previously described by Padgette et al. (1996).

Statistical Analysis. The compositional results (proximate analysis and amino acid and isoflavone levels) for glyphosate-treated GTS line 40-3-2 and control line A5403 were analyzed by the SAS statistical program (SAS Institute Inc., 1990). Each observation in the data consisted of one plot value per site for each of the soybean lines evaluated. Individual plot values were converted from a fresh weight basis to a dry weight basis using percent moisture values (value, dry wt) = [value, fresh wt]/[(1 − moisture)/100]. This calculation was performed on all of the characteristics except moisture. A randomized complete block model, with line as a treatment effect and location as a blocking effect, was used to test for the difference between GTS line 40-3-2 and the A5403 control. For each line and analyzed constituent, the model was fit to the data using the GLM procedure in SAS. Each line mean and its standard error were computed using the LSMEANS statement in the GLM procedure. The mean difference, the *t* test of the difference in means, and the *p* value of the difference in means were computed using the ESTIMATE statement in the GLM procedure. This *t* test is analogous to a paired *t* test except a pooled error estimate is used [previously described by Padgette et al. (1996)].

RESULTS

ELISA Results. Accumulations of the CP4 EPSPS and GUS proteins in the GTS lines and the A5403 line were estimated by ELISA. Samples were collected and evaluated from 1992 and 1993 field tests (Table 1). As shown in Table 1, CP4 EPSPS is detected in the seed of both GTS lines (1992 results) and in the leaf and seed of GTS line 40-3-2 (1993 results). As expected, only line 61-67-1 had detectable levels of GUS. Neither CP4 EPSPS nor GUS expression was detectable in extracts of the control A5403 leaf and seed. These data are consistent with the ELISA results reported by Padgette et al. (1995) for samples of GTS line 40-3-2 and control A5403 line that were not treated with glyphosate. The overall levels of accumulation of CP4 EPSPS in the GTS 40-3-2 seed treated with glyphosate were 0.301 and 0.218 μg of CP4 EPSPS/mg of fresh tissue for the 1992 and 1993 field trials, respectively. These values are comparable to those levels reported for GTS 40-3-2 seed not treated with glyphosate: 0.288 μg of CP4 EPSPS/mg of fresh tissue in 1992 and 0.201 μg of CP4 EPSPS/mg of fresh tissue in 1993 (Padgette et al., 1995).

Proximate Analysis. Proximate analyses were performed on the soybean seeds from the GTS lines treated with glyphosate and the A5403 control from the 1992 and 1993 field trials (Table 2). The 1992 analyses show that there was no statistical difference between the protein levels of the GTS lines treated with glyphosate and the A5403 control line. In addition, there were no statistical differences in oil, ash, fiber, or carbohydrates between the glyphosate-treated GTS and the control A5403 soybean line. These results are supported by additional proximate data generated in 1993 (Table 2) comparing glyphosate-treated GTS 40-3-2 and the control A5403 (note that line 61-67-1 was not included in the 1993 study due to discontinuation of commercial development of the line). There were no statistically significant differences in the proximate analysis results for glyphosate-treated GTS line 40-3-2 versus the control A5403 at the 5% confidence level for either year. These results confirm that the levels of these components for glyphosate-treated GTS are comparable to those of traditional soybeans.

Amino Acid Analysis. Amino acid analysis was performed on glyphosate-treated GTS line 40-3-2 and control A5403 soybeans from the 1993 field trials (Table

Table 1. Expression of CP4 EPSPS in GTS and A5403 Soybean Lines[a]

		μg of protein/mg of fresh wt of tissue							
		control	treated with glyphosate				control	not treated with glyphosate	
characteristic		A5403	40-3-2 (GTS)	61-67-1 (GTS)			A5403	40-3-2 (GTS)	61-67-1 (GTS)
1992 (6 sites)[b]					1992 (9 sites)				
seed (CP4)	mean	ND[e]	0.301	0.346	seed (CP4)	mean	ND	0.288	0.335
	range	NA	0.258−0.378	0.274−0.454		range	NA	0.186−0.395	0.259−0.467
	SD	NA	0.03	0.07		SD	NA	0.066	0.061
seed (GUS)	mean	ND	ND	0.022	seed (GUS)	mean	ND	ND	0.008
	range	NA	NA	0.010−0.052		range	NA	NA	0.003−0.013
	SD	NA	NA	0.014		SD	NA	NA	0.007
1993 (4 sites)					1993 (4 sites)				
seed (CP4)	mean	ND	0.218	NT[d]	seed (CP4)	mean	ND	0.201	NT
	range	NA	0.166−0.287	NA		range	NA	0.127−0.277	NA
	SD	NA	0.042	NA		SD	NA	0.017	NA
leaf (CP4)[c]	mean	ND	0.489	NT	leaf (CP4)[c]	mean	ND	0.415	NT
	range	NA	0.308−0.856	NA		range	NA	0.299−0.601	NA
	SD	NA	0.239	NA		SD	NA	0.153	NA

[a] Data previously reported for GTS 40-3-2 and A5403 by Padgette et al. (1995) and included in Table 1 for ease of comparison. [b] Only six sites were included in the statistical analysis. Samples from one site, Louisiana, were dropped due to a sample mix-up that could not be resolved. [c] Leaf samples were not collected from one of the four sites (Marion, AR) in 1993. [d] NT, not tested. The A5403 seeds were not retested for the 1992 sprayed study because these samples were previously extracted and analyzed, and the results were reported by Padgette et al. (1995). The GTS line 61-67-1 was not analyzed in 1993 because it had been deprioritized and was no longer a commercial line. [e] ND, nondetectable. The mean and standard deviation (SD) were not calculated because neither GUS nor CP4 was detected in the A5403 extracts (same for GUS expression in 40-3-2 extracts). [f] NA, not applicable. [g] Range denotes the lowest and highest individual results for each tissue assayed.

Table 2. Summary of Proximate Analyses from 1992 and 1993 of Glyphosate-Treated GTS and A5403 Control[a]

	A5403 (control)			40-3-2 (GTS)[f]			61-67-1 (GTS)[f]		
characteristic[b]	mean	range[e]	SEM	mean	range	SEM	mean	range	SEM
1992 (6 sites)[g]									
protein	41.01	37.46−44.90	0.37	40.35	36.42−44.71	0.37	41.46	38.17−47.40	0.37
ash	5.18	4.61−5.52	0.07	5.34	4.73−5.91	0.07	5.31	4.77−5.82	0.07
moisture	12.68	11.10−14.30	1.28	10.56	7.67−22.65	1.28	8.49	7.79−9.10	1.28
oil	19.8	17.40−21.84	0.23	20.41	18.19−22.19	0.23	19.39	15.90−21.17	0.23
fiber	6.35	5.86−6.52	0.15	6.44	6.13−7.11	0.15	6.28	5.76−6.88	0.15
carbohydrates	34.01	32.36−35.26	0.24	33.86	32.11−35.73	0.24	33.82	31.57−35.62	0.24
1993 (4 sites)[h]									
protein	41.4	40.39−42.32	0.451	41.43	39.35−44.14	0.451	NT[c]	NA[d]	NA
ash	5.31	5.01−5.94	0.052	5.35	5.04−5.81	0.052	NT	NA	NA
moisture	5.73	5.18−6.19	0.137	5.74	5.32−6.20	0.137	NT	NA	NA
oil	19.89	18.67−20.57	0.353	20.53	19.01−22.17	0.353	NT	NA	NA
fiber	7.36	6.63−8.10	0.145	6.86	5.59−7.66	0.147	NT	NA	NA
carbohydrates	33.38	31.57−35.08	0.712	32.67	27.86−35.32	0.711	NT	NA	NA

[a] Means reported are from single assays on single samples from six sites in 1992 and four sites in 1993. [b] All values reported as percent dry weight, except for moisture. [c] NT, not tested; GTS line 61-67-1 was deprioritized and no longer a commercial line. [d] NA, not applicable. [e] Range denotes the lowest and highest value reported for each assay. [f] No statistical differences were observed between the GTS proximate values and the control A5403 proximate values at the 5% confidence level (SAS GLM procedure) for both 1992 and 1993 results. [g] The 1992 GTS field plots were treated with a pre-emergence application of 17.8 L/ha and an early postemergence application of 1.75 L/ha Roundup herbicide. [h] The 1993 GTS field plots were treated with a pre-emergence application of 17.8 L/ha and early and late postemergence applications of 2.34 L/ha of Roundup herbicide.

3). As shown in Table 3, levels of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) as well as all amino acids measured were comparable between glyphosate-treated GTS line 40-3-2 and the control A5403 line. There were no significant differences observed at the 5% confidence level for any amino acid analyzed.

Isoflavone (Phytoestrogen) Analysis. The concentrations of the total and free forms of the isoflavones genistein, daidzein, bound coumestrol, and biochanin A were determined as shown in Table 4. These analyses show that the quantities of biochanin A (<1 ppm) and coumestrol (<4 ppm) were below the limit of detection of the assay. The limit of detection of these assays is 10 ppm (10 μg/g). As reported in Table 4, the range of total genistein and total daidzein was variable across sites and is reflected in the ranges for both A5403 and GTS 40-3-2. However, the mean levels of total genistein and total daidzein in the glyphosate-treated 40-3-2 line were comparable to those in the control A5403 line. There were no significant statistical differences observed at the 5% confidence level. The results were comparable to isoflavone levels previously reported for field-grown soybeans (Wang et al., 1990).

DISCUSSION

The data demonstrated the substantial equivalence of GTS to the parental control line A5403 and to other conventional soybeans as previously reported (Padgette et al., 1995, 1996). Those studies were designed to focus on any effects a genetic change might have on seed composition; therefore, the seeds were not sprayed with glyphosate. Approximately 150 independent field tests in which GTS line 40-3-2 was sprayed with glyphosate were evaluated and no deleterious effects were observed at the whole plant level (Delannay et al., 1995). The studies reported in this paper were conducted to assess whether the treatment of GTS with glyphosate affects

Table 3. Summary of Amino Acid Analyses of Glyphosate-Treated GTS and A5403 Control Soybean Seeds from the 1993 Field Tests[a]

	g/100 g[b]						
	A5403 (control)			40-3-2 (GTS)[c,d]			literature
amino acid	mean	range[e]	SEM	mean	range	SEM	range[f]
aspartic acid	4.50	4.30−4.59	0.063	4.51	4.21−4.75	0.063	3.87−4.98
threonine	1.57	1.54−1.60	0.013	1.57	1.52−1.63	0.013	1.33−1.79
serine	2.03	1.95−2.06	0.024	2.03	1.92−2.11	0.024	1.81−2.32
glutamic acid	7.48	7.06−7.81	0.112	7.44	6.84−7.97	0.112	6.10−8.72
proline	2.07	1.97−2.16	0.023	2.06	1.91−2.16	0.023	1.88−2.61
glycine	1.73	1.65−1.83	0.013	1.71	1.61−1.78	0.013	1.88−2.02
alanine	1.73	1.66−1.79	0.012	1.72	1.67−1.76	0.012	1.49−1.87
valine	1.94	1.87−1.97	0.019	1.93	1.83−2.00	0.019	1.52−2.24
isoleucine	1.83	1.75−1.88	0.022	1.82	1.71−1.91	0.022	1.46−2.12
leucine	3.09	2.96−3.16	0.037	3.06	2.90−3.19	0.037	2.71−3.20
tyrosine	1.39	1.34−1.41	0.012	1.38	1.32−1.44	0.012	1.12−1.62
phenyalanine	2.01	1.92−2.06	0.015	1.98	1.86−2.08	0.015	1.70−2.08
histidine	1.10	1.07−1.12	0.015	1.10	1.05−1.15	0.015	0.89−1.08
lysine	2.63	2.53−2.69	0.021	2.64	2.53−2.76	0.021	2.35−2.86
arginine	2.88	2.70−2.97	0.051	2.89	2.64−3.09	0.051	2.45−3.49
cysteine	0.57	0.50−0.61	0.008	0.59	0.54−0.60	0.008	0.56−0.66
methionine	0.54	0.48−0.57	0.009	0.54	0.51−0.55	0.009	0.49−0.66
tryptophan	0.49	0.48−0.50	0.012	0.49	0.47−0.53	0.012	0.53−0.54

[a] Means are from single assays on single samples from four sites in 1993. [b] Dry weight basis for g/100 g. [c] No significant differences from the control line were observed at the 5% confidence level (SAS GLM procedure). [d] The 1993 GTS field plots were treated with a pre-emergence application of 17.8 L/ha and early and late postemergence applications of 2.34 L/ha of Roundup herbicide. [e] Range denotes the lowest and highest value reported for each assay. [f] Han et al. (1991); Orthoefer (1978).

Table 4. Summary of Isoflavone Analyses of Glyphosate-Treated GTS and A5403 Control Soybean Seeds from the 1993 Field Trials[a]

	μg/g of dry wt						
	A5403 (control)			40-3-2 (GTS)			literautre
isoflavone	mean	range[j]	SEM	mean	range[j]	SEM	range[k]
total genistein[b]	681	230−1086	38.152	742	196−1231	38.152	461.1−1000
free genistein[c]	20	14−28	NT[h]	23	12−38	NT	
bound genistein[c]	662	210−1058	NT	719	172−1193	NT	
total daidzein[b]	521	161−931	36.323	578	106−1064	36.323	330.6−706
free diadzein[c]	42	19−70	NT	42	20−86	NT	
bound diadzein[c]	479	142−862	NT	536	85−978	NT	
total coumestrol[d]	ND[f]	NA[g]	NT	ND	NA	NT	
total biochanin[e]	ND	NA	NT	ND	NA	NT	

[a] Means are from single assays on single samples from four sites in 1993. [b] No statistical differences were observed between GTS 40−3−2 and A5403 total genistein and total daidzein concentrations at the 5% confidence level (SAS GLM Procedure). [c] No statistical comparisons were made between the GTS 40−3−2 and A5403 free and bound genistein and daidzein. [d] Coumestrol levels less than 4 ppm. This is considered below the limit of quantitation (10 ppm) for the HPLC assay. [e] Biochanin A levels less than 1 ppm. This is considered below the limit of quantitation (10 ppm) for the HPLC assay. [f] ND = not detectable. [g] NA = not applicable. [h] NT = not tested. [i] The 1993 GTS field plots were treated with a preemergence application of 17.8 L/ha and an early post emergence and late post emergence application of 2.34 L/ha of Roundup herbicide. [j] "Range" denotes the lowest and highest value reported for each assay. [k] Pettersson and Kiessling, 1984; Wang, G. et al., 1990.

the seed composition for these soybean lines. The experiments focused primarily on downstream products of the shikimate pathway, due to the fact that if the application of glyphosate had any subtle effects on GTS, the effects would expect to be coupled to aromatic amino acid biosynthesis, the biochemical pathway target of glyphosate. Therefore, CP4 EPSPS accumulation, proximates, amino acid composition, and isoflavone levels were evaluated.

The only difference between GTS soybeans and conventional soybeans is their tolerance to glyphosate. Glyphosate tolerance was conferred by transforming A5403 with the CP4 EPSPS gene (Padgette et al., 1995). The overall accumulation of CP4 EPSPS in soybean leaf and seed for GTS line 40-3-2 was previously reported, but the plants from which these samples were collected had not been treated with glyphosate (Padgette et al., 1995). It is expected that the overall level of CP4 EPSPS should not change with the application of glyphosate because the expression of the gene is constitutive and controlled by the cauliflower mosaic virus (CaMV) 35S promoter (Odell et al., 1985) with the duplicated enhancer region (Kay et al., 1987; Padgette et al., 1995). ELISA analysis of the CP4 EPSPS levels in non-glyphosate-treated GTS line 40-3-2 (Padgette et al., 1995) and glyphosate-treated GTS line 40-3-2 confirmed that the accumulations of CP4 EPSPS protein are comparable. This supports the hypothesis that the application of glyphosate does not induce expression of CP4 EPSPS to levels higher than expected for unsprayed plants.

Proximate analysis (protein, ash, moisture, oil, fiber, and carbohydrates) is the standard method for determining the base chemical composition of soybean seeds. Therefore, any significant reduction in protein content resulting from potential inhibition of protein synthesis due to the application of glyphosate should be detected. Proximate data generated from field samples collected in both 1992 (GTS lines 40-3-2 and 61-67-1) and 1993 (GTS line 40-3-2 only) showed no differences at the protein level or any other proximate component of

soybean seed between glyphosate-treated GTS and the conventional soybean line, A5403.

A more specific and sensitive measurement of any influence of glyphosate treatment on GTS would be to measure the direct products of the aromatic amino acid biosynthetic pathway, namely, aromatic amino acids themselves. There were no significant differences detected in the amino acid content, including aromatic amino acids, between glyphosate-treated 40-3-2 and control A5403 soybean seeds. These results confirm that CP4 EPSPS provides sufficient activity, in the absence or presence of glyphosate, to produce the aromatic amino acid pathway intermediate, 5-enolpyruvylshikimate 3-phosphate (EPSP) and, indirectly, the expected level of aromatic amino acids.

Another class of compounds derived from the aromatic biosynthetic pathway is the isoflavones. The isoflavones genistein, daidzein, and coumestrol are naturally present in soybeans and have been reported to possess a number of biochemical activities in mammalian species, including estrogenic and hypocholestrolemic activities (Wang et al., 1990; Murphy, 1982). More recently, soybean isoflavones have been linked as contributing factors to reducing the risk of several types of cancer, including breast and prostate cancer (Liu, 1997). In contrast, it has been postulated that these compounds may contribute to deleterious health effects in animals fed soybean meal (Setchell et al., 1987). Because of their potential impact on health and nutrition, isoflavones were measured in this study.

The levels of coumestrol and biochanin A in both the GTS seeds, which had been treated with glyphosate, and the control seeds were below the assay's limit of detection. The mean levels of total genistein and total daidzein were not statistically different for glyphosate-treated GTS 40-3-2 and the control A5403 line. However, the range of total genistein and total daidzein for both soybean lines was variable across multiple sites. These results are not unexpected because the level of isoflavones in soybean is dependent upon the genetics of the soybean variety, the location where the soybeans are grown, and crop year (Wang and Murphy, 1994; Carrao-Panizzi and Kitamura, 1995; Eldridge and Kwolek, 1983; Fukutake et al., 1996; Choi et al., 1996).

A previous study (Sanderman et al., 1988) reported that the level of isoflavones in non-glyphosate-tolerant plants may be increased when treated with glyphosate. Our results suggest that the increase in isoflavones is a result of a stress-induced response to the application of glyphosate to non-glyphosate-tolerant soybeans. The application of glyphosate to the GTS line 40-3-2 does not cause a stress response as demonstrated by the yield (Delannay et al., 1995) and compositional data. The levels of isoflavones would therefore not be expected to change in the GTS line 40-3-2, which has now been analytically confirmed.

The results of the analyses reported here demonstrate that the application of glyphosate to GTS does not affect the levels of oil, ash, fiber, carbohydrates, or protein, the amino acid composition (including aromatic amino acids), or other related downstream products (levels of isoflavones), as compared to conventional soybeans. In summary, the composition of glyphosate-treated GTS is substantially equivalent to the composition of soybean seeds from plants that have not been treated with glyphosate.

LITERATURE CITED

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