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Glyphosate Induction of Elevated Levels of Hydroxybenzoic Acids in Higher Plants

John Lydon* and Stephen O. Duke

Glyphosate [*N*-(phosphonomethyl)glycine] effects on hydroxybenzoic acid levels in pigweed (*Amaranthus retroflexus* L.), ryegrass (*Lolium perenne* L.), soybean [*Glycine max* (L.) Merr.], velvetleaf (*Abutilon theophrasti* Medic.), and yellow nutsedge (*Cyperus esculentus* L.) were investigated. Leaves were harvested at 3 and/or 6 days after treatment with four levels of glyphosphate. Acid-hydrolyzed extracts were analyzed by HPLC. The concentration of protocatechuic acid in leaf tissue varied among species and was dependent upon the glyphosate dose, duration of exposure, and tissue assayed. Gallic acid levels were higher in four of the five species, and 4-hydroxybenzoic acid was higher in three of the five species treated with glyphosate as compared to controls. Vanillic and syringic acids, which are methylated forms of protocatechuic and gallic acids (respectively), were unaffected by glyphosphate treatment. The data indicate that some hydroxybenzoic acids such as protocatechuic, gallic, and 4-hydroxybenzoic may be directly synthesized from shikimate or shikimate precursors.

The nonselective, broad-spectrum, postemergence herbicide glyphosate [*N*-(phosphonomethyl)glycine] inhibits 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (EC 2.5.1.19), an enzyme of the shikimic acid pathway (Amrhein, 1986). This results in the cessation of aromatic amino acid synthesis, followed by reduced protein synthesis, growth, and premature cellular death (Duke, 1988). Inhibition of EPSP synthase also causes high levels of shikimic acid to accumulate (Amrhein et al., 1980; Berlin and Witte, 1980). These levels are even larger than might be expected because lowered levels of shikimic acid pathway products result in deregulation (from end product inhibition) of carbon flow into the shikimic acid pathway (Jensen, 1985).

The shikimic acid pathway is responsible for synthesis of most phenolic compounds found in higher plants. Most

Southern Weed Science Laboratory, USDA—ARS, P.O. Box 350, Stoneville, Mississippi 38776.

of these compounds are products of cinnamate, which is formed by deamination of phenylalanine by the enzyme phenylalanine ammonia-lyase (PAL) (EC 4.3.1.5). It is documented that glyphosate treatment decreases levels of cinnamate derivatives in higher plants (Duke and Hoagland, 1985; Duke, 1988). Data indicate that hydroxybenzoic acids can be derived from shikimic acid either directly or indirectly via cinnamates (Haslam, 1986). A shikimate-inducible form of 3-dehydroquinate hydrolase (DHQase 2, EC 4.2.1.10), which is associated with conversion of quinate to protocatechuate, has been isolated from higher plants (Boudet et al., 1986). Therefore, an increase of shikimic acid in plant tissues caused by glyphosate treatment could be accompanied by a concomitant increase in hydroxybenzoic acids. Glyphosate-induced increases in gallic acid have been reported in *Rhus typhina* L., *Quercus robur* L., *Geranium pyrenaicum* Burm. f., and *Camellia sinenis* L. O. Ktze. (Amrhein et al., 1982); however, no numerical data have thus far been published. All four of these species naturally produce high levels of gallic acid. Recently, Cañal et al. (1987) reported that glyphosate increased the level of gentisic, 4-hydroxybenzoic, vanillic, and syringic acids in yellow nutsedge (*Cyperus esculentus* L.); however, no data were presented for protocatechuic and gallic acids. Hydroxybenzoic acids have been implicated in many plant processes including flowering (Khurana and Maheshwari, 1983), formation of polyphenolic compounds (Haslam, 1986), allelopathy (Van Sumere et al., 1972; Wang et al., 1967), recognition by infecting bacteria (Bolton et al., 1986), host plant resistance (Reda and El-Banhawy, 1986), and utilization as phytoalexins (Franich et al., 1986). Any compound that significantly affects production of this group of secondary compounds could have many secondary effects on plants when used at sublethal levels. In this study we demonstrate that both crop and weed species that are affected by glyphosate (as determined by the accumulation of unusually high levels of shikimic acid) typically have elevated levels of hydroxybenzoic acids, particularly protocatechuic acid.

MATERIALS AND METHODS

Plant Material. Plants were grown from seed in a glasshouse. Pigweed (*Amaranthus retroflexus* L.), soybean [*Glycine max* (L.) Merr. cv Centennial], and velvetleaf (*Abutilon theophrasti* Medic.) were planted as three seeds per pot in 1.2-L pots, and beds of ryegrass (*Lolium perenne* L.) were established in 1.2-L pots. All pots were filled with Jiffy-Mix (Ball Jiffy) potting media and watered with tap water once every 4 days until seedling establishment. The established plants were watered once every other day, with every other watering consisting of a dilute solution of Peters 20-20-20 general purpose fertilizer (0.25 g L^{-1}). Yellow nutsedge (*C. esculentus* L.) plants were cultivated similarly except they were started from rhizomes. All plants were 21–25 days old at the time of treatment.

Herbicide Treatment. Pigweed, soybean, and ryegrass plants were treated with 0, 1, 5, and 10 mM glyphosate (isopropylamine salt) in 0.1% Triton-X 100, whereas velvetleaf plants were treated with 0 and 10 mM glyphosate. A 50-μL drop of test solution was spread over the leaf surface of the sixth and seventh leaf from the apex of pigweed and velvetleaf and the first two true leaves of soybean plants. Beds of ryegrass plants were sprayed at a rate of 50 mL/0.02 m^2 canopy surface area. Yellow nutsedge plants were sprayed with 2 mL of 0 or 10 mM glyphosate per pot. Leaf tissues from pigweed, ryegrass, and soybean were harvested 3 and 6 days after treatment. Leaf tissues from velvetleaf and yellow nutsedge were harvested 6 days after treatment. When harvested, leaf tissues of pigweed and soybean were separated into three categories: (1) contact-treated mature leaves that received the test solution; (2) mature leaves from the same plant that were not contact-treated; (3) developing leaves from the same plant that were not contact-treated. In the case of velvetleaf, only young non-contact-treated leaves were harvested. All leaves within replicates of ryegrass and yellow nutsedge were pooled.

Sample Preparation and Extraction. Freshly harvested leaves were dipped in liquid nitrogen, lyophilized, and stored at −10 °C over silica gel desiccant. A 150-mg dry-weight (DW) sample of powdered leaf tissue was refluxed in 25 mL of 1 N HCl at 100 °C for 1 h. The resulting extract was suction filtered through Whatman No. 1 filter paper, the pH adjusted to 2.5 with concentrated NaOH, and the volume brought to 50 mL with deionized water. A 2-mL aliquot was filtered through a Nalgene 0.2-μm PTFE syringe filter and analyzed by HPLC. An 18-mL portion of the extract was washed two times with 20 mL of HPLC-grade ethyl acetate; the ethyl acetate fractions were combined, evaporated to dryness at 35 °C on a rotary evaporator, redissolved in 2 mL of 1.7 mM phosphoric acid (pH 2.7), filtered through a 0.2-μm syringe filter, and analyzed by HPLC. Shikimic acid was not quantitated in this concentrated extract because it was inadequately extracted by ethyl acetate.

HPLC Analysis. Except for the column, which was a Custom LC, Inc., 250 × 4.6 mm (i.d.) Sherisorb 5-μm ODS-I reversed-phase column, the system was composed of Waters Associates HPLC components: two pumps (Model 510), a controller (Model 720), an autosampler (Model 710B), a variable-wavelength detector (Model 490), and a data module (Model 740). The HPLC solvent used in the shikimic acid assay was 3.5 mM phosphoric acid (pH 2.5) at 0.5 mL min^{-1}. A sample volume of 13 μL was injected, and detection was performed at 215 nm. The retention time (RT) of shikimic acid was 8.42 min. The column was washed with 100% MeOH and then allowed to equilibrate in 3.5 mM phosphoric acid before reinjection. The minimum detectable limit for shikimic acid was 25 μg/100 mg DW. Assuming 90% moisture content, approximately 1 g fresh weight was sufficient to detect average shikimic acid levels in leaf tissue of monocotyledons and herbaceous dicotyledons (Yoshida et al., 1975). Hydroxybenzoic acids were assayed as described by Lydon et al. (1987) using a solvent gradient composed of 1.7 mM phosphoric acid (pH 2.7, solvent A) and HPLC methanol (solvent B) at a flow rate of 1.5 mL min^{-1} as follows: 0–10 min, 100% A; 10–20 min, 5% B in A; 20–30 min, 5–20% B in A (linear gradient); 30–55 min, 20% B in A. A sample volume of 75 μL was injected, and detection was performed at 215 nm. The column was washed between injections as described above. The minimum detection limit for the hydroxybenzoic acids was approximately 1 μg/100 mg DW.

Compounds were identified by comparison of retention times and stopped-flow UV spectral analysis with pure standards. The system was calibrated for shikimic, gallic, protocatechuic, gentisic, 4-hydroxybenzoic, vanillic, and syringic acids against an eight-point standard curve ranging from 0.065 to 13 nmol/13-μL injection. The RTs (min) for the hydroxybenzoic acids were as follows: gallic acid, 12.13; protocatechuic acid, 20.69; gentisic acid, 28.30; 4-hydroxybenzoic acid, 30.47; vanillic acid, 39.04; syringic acid, 48.99 (Figure 1).

Stopped-Flow UV Spectral Analysis. Stopped-flow UV spectral analysis was conducted by stopping the solvent flow and trapping the eluting peak in the detector cell.

Table I. Effects of Glyphosate on the Concentration of Shikimic (Shik), Protocatechuic (Proto), Gallic (Gall), Gentisic (Gent), 4-Hydroxybenzoic (4-Hyd), Vanillic (Van), and Syringic (Syr) Acids from Young Non-Contact-Treated Velvetleaf and Pooled Yellow Nutsedge Leaves 6 Days after Treatments[a]

species	glypho-sate, mM	Shik, nmol/mg DW	Proto, nmol/mg DW × 10^{-1}	Gall, nmol/mg DW × 10^{-1}	Gent, nmol/mg DW × 10^{-1}	4-Hyd, nmol/mg DW × 10^{-1}	Van, nmol/mg DW × 10^{-1}	Syr, nmol/mg DW × 10^{-1}
velvetleaf	0	0 a	1.8 a	4.7 a	3.2	8.4	2.6	2.3
	10	211 b	112.0 b	20.4 b	2.3	9.6	3.6	2.9
yellow nutsedge	0	31 a	7.2 a	0 a	5.2	6.4	3.1	1.0
	10	135 b	12.9 b	0.4 b	7.6	5.8	3.5	1.8

[a] Values for Shik and hydroxybenzoic acids are the means of three replicates. Means in a column within a species not followed by a letter or followed by the same letter are not significantly different at $P < 0.05$ according to Newman–Keuls multiple-range test.

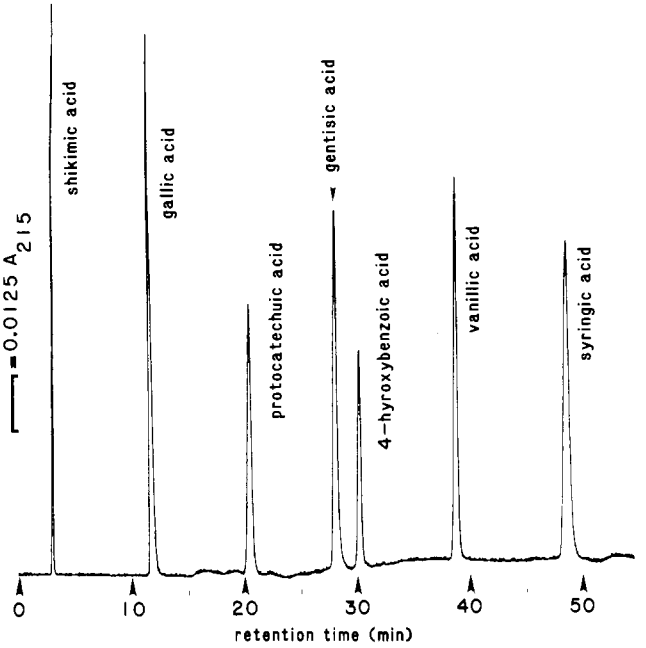

Figure 1. Elution profile of shikimic, gallic, protocatechuic, gentisic, 4-hydroxybenzoic, vanillic, and syringic acids on a Spherisorb 5-μm ODS-I reversed-phase C18 HPLC column. See Materials and Methods for a description of the elution system.

The peak of interest was then scanned from 190 to 350 nm at 1-nm increments and at a speed of 1 nm s^{-1}. Scans were corrected for solvent absorption. Sufficient sample was injected to obtain a response of 0.090–0.110 absorption unit at the wavelength of maximal absorption for each peak scanned.

Data Analysis. Data were analyzed by single-factor analysis of variance, with four treatments and two (ryegrass) or three (pigweed, soybean, velvetleaf, yellow nutsedge) replicates per treatment, with each replicate composed of leaf material from three or more plants. Significantly different means were separated at the 95% level by the Student–Newman–Keuls multiple-range test (Kleinbaum and Kupper, 1978).

RESULTS

The 10 mM glyphosate dose was lethal to all plant species tested, causing chlorosis and a gradual cessation of growth in developing tissues. Similar but less severe symptoms were displayed by most plants treated with 1 and 5 mM glyphosate; however, only the 5 mM treatment on ryegrass was lethal.

Shikimic acid levels at 3 and 6 days after treatment in young non-contact-treated leaves of pigweed and soybean, mature contact-treated leaves of soybean, and pooled leaves of ryegrass increased greatly as a result of treatment with 5 and 10 mM glyphosate as compared to controls and the 1 mM treatment (Figures 2–4). At 3 and 6 days after treatment, only the 10 mM glyphosate dose resulted in

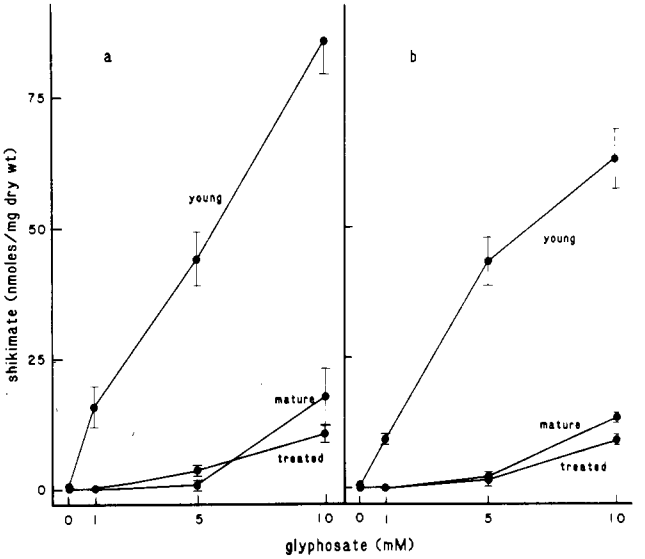

Figure 2. Effects of 0, 1, 5, and 10 mM glyphosate in 0.1% Triton X-100 on shikimic acid levels in pigweed leaf tissue (a) 3 days and (b) 6 days after treatment: treated = contact-treated mature; young = nontreated developing; mature = nontreated mature leaf tissue. Each data point represents the mean of three replicates ±1 SE.

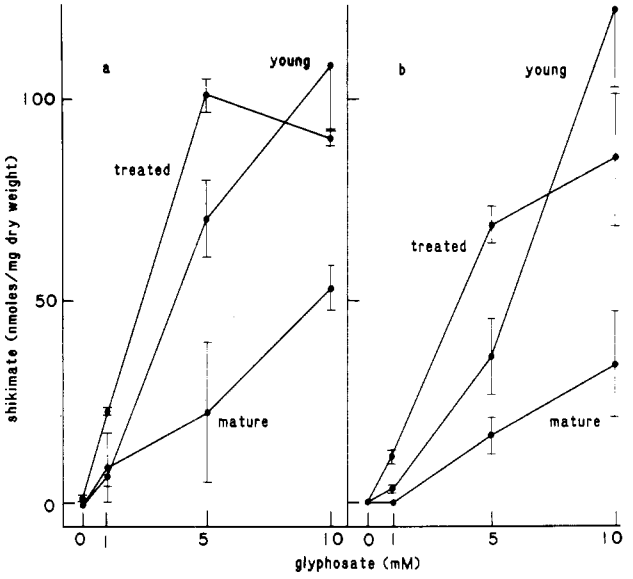

Figure 3. Effects of 0, 1, 5, and 10 mM glyphosate in 0.1% Triton X-100 on shikimic acid levels in soybean leaf tissue (a) 3 and (b) 6 days after treatment: treated = contact-treated mature; young = nontreated developing; mature = nontreated mature leaf tissue. Each data point represents the mean of three replicates ±1 SE.

significantly higher levels of shikimic acid in nontreated mature leaves of pigweed and soybean and treated leaves of pigweed as compared to controls (Figures 2–4). Treatment with 10 mM glyphosate also resulted in higher

Table II. Effects of Glyphosate on the Concentration of Protocatechuic (Proto), Gallic (Gall), Gentisic (Gent), 4-Hydroxybenzoic (4-Hyd), Vanillic (Van), and Syringe (Syr) Acids from Pigweed and Ryegrass Leaves 3 and 6 Days after Treatment[a]

species	days after treatment	tissue[b]	glyphosate, mM	Proto, nmol/mg DW × 10^{-1}	Gall, nmol/mg DW × 10^{-1}	Gent, nmol/mg DW × 10^{-1}	4-Hyd, nmol/mg DW × 10^{-1}	Van, nmol/mg DW × 10^{-1}	Syr, nm/mg DW × 10^{-1}
pigweed	3	Y	0	0.5 a	0.3 a	3.9	5.6	5.2	1.8
			5	43.1 b	1.7 b	3.4	4.3	4.6	3.3
			10	151.1 c	5.5 c	4.6	5.4	4.3	3.3
pigweed	6	Y	0	0.4 a	1.0 a	3.2 a	2.8 a	5.0	2.0
			5	53.0 b	2.0 b	3.9 a	2.2 a	4.4	2.0
			10	295.1 c	12.2 c	5.9 b	5.8 b	4.8	2.3
ryegrass	3	P	0	1.6 a	0.8	2.7	3.6	2.6	
			1	14.1 b	0.6	1.8	3.8	2.4	
			5	25.5 c	1.1	2.6	4.1	3.2	
			10	22.9 c	1.2	3.2	3.7	2.5	
ryegrass	6	P	0	3.1 a	1.8	2.4	2.1 a	2.4	
			1	23.2 b	2.5	2.9	4.0 b	3.0	
			5	34.1 c	0.8	3.2	5.0 b	3.6	
			10	30.3 c	0.8	3.1	4.1 b	2.8	

[a] Values for hydroxybenzoic acids are the means of three (pigweed) or two (ryegrass) replicates. Means in a column within a species and days after treatment not followed by a letter or followed by the same letter are not significantly different at $P < 0.05$ according to Newman–Keuls multiple-range test. [b] Key: Y = young non-contract-treated leaves; P = pooled leaf tissue; DW = dry weight.

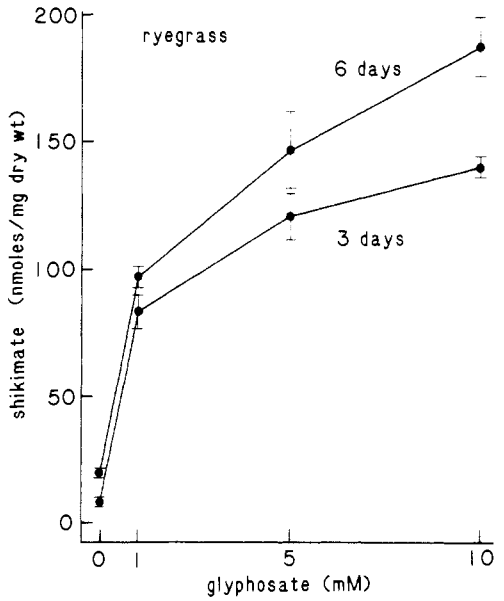

Figure 4. Effects of 0, 1, 5, and 10 mM glyphosate in 0.1% Triton X-100 on shikimic acid levels in ryegrass leaf tissue 3 and 6 days after treatment. Each data point represents the mean of two replicates ±1 SE.

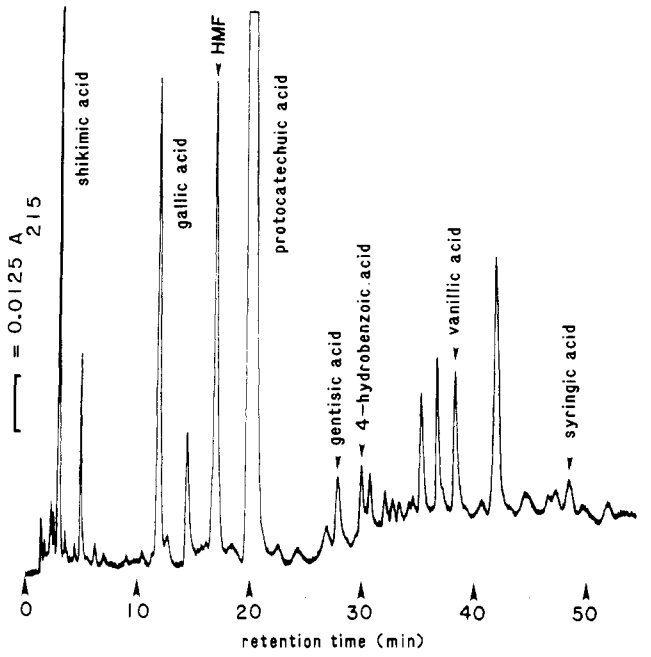

Figure 5. HPLC chromatogram of an acid-hydrolyzed leaf extract of young non-contact-treated leaves from 10 mM glyphosate-treated pigweed plants. Injection volume was 75 μL, which was equivalent to 2.03 mg DW leaf tissue. HMF = 5-(hydroxymethyl)-2-furaldehyde, a product of acid-hydrolyzed sugars. See Materials and Methods for a description of the elution system.

shikimic acid levels in young non-contact-treated velvetleaf and pooled yellow nutsedge leaf tissues as compared to controls (Table I). Leaf tissues from the various species that accumulated unusually high levels of shikimic acid as a result of glyphosate treatment were further assayed for hydroxybenzoic acids.

Most of the hydroxybenzoic acids commonly found in plants were detected in the concentrated extracts of control and glyphosate-treated young leaf tissue from pigweed. Glyphosate treatment had a pronounced effect on the concentration of hydroxybenzoic acids extracted from these tissues, particularly on protocatechuic acid (Figure 5). The concentration of protocatechuic acid in young non-contact-treated leaves from pigweed was dependent upon the concentration of glyphosate applied and the duration of exposure. Protocatechuic acid levels in these tissues 3 days after treatment were 86- and 300-fold higher in the 5 and 10 mM treatment (respectively) as compared to controls (Table II). Six days after treatment, the level of protocatechuic acid in the 10 mM glyphosphate treatment had increased 700-fold over that of controls. Although the gallic acid response to glyphosate in these tissues was similar to that of protocatechuic acid, increasing with dose and time of exposure, the magnitude of effect was considerably less. Gentisic and 4-hydroxybenzoic acid levels were unaffected by glyphosate 3 days after treatment. However, by 6 days after treatment these hydroxybenzoic acids in the 10 mM treatments were approximately double those of controls. Vanillic and syringic acids in these tissues were unaffected by glyphosate at 3 and 6 days after treatment. The mature contact-treated and non-contact-treated leaves of pigweed were not assayed for hydroxybenzoic acids.

The concentration of protocatechuic acid in ryegrass leaves 3 days after treatment increased 8- and 16-fold when treated with 1 and 5 mM glyphosate (respectively) as compared to controls (Table II). Treatment with 10 mM glyphosate had no additional effect. The glyphosate-in-

Table III. Effects of Glyphosate on the Concentration of Protocatechuic (Proto), Gentisic (Gent), 4-Hydroxybenzoic (4-Hyd), and Vanillic (Van) Acids from Soybean Leaves 3 and 6 Days after Treatment[a]

days after treatment	tissue[b]	glyphosate, mM	Proto, nmol/mg DW × 10^{-1}	Gall, nmol/mg DW × 10^{-1}	Gent, nmol/mg DW × 10^{-1}	4-Hyd, nmol/mg DW × 10^{-1}	Van, nmol/mg DW × 10^{-1}
3	Y	0	0.8 a	0.7 a	16.7 a	2.4 a	7.9
		5	26.5 b	4.3 b	11.8 b	4.5 b	8.3
		10	82.0 c	6.3 c	9.2 b	6.4 c	7.3
6	Y	0	5.9 a	0.1 a	15.3 a	2.3 a	7.6
		5	14.0 b	5.2 b	12.7 a	5.5 b	9.3
		10	44.6 c	3.9 b	5.4 b	4.8 b	6.1
3	M	0	3.0 a	0.3	4.3	2.3	7.1
		5	3.4 a	0.9	5.4	3.7	4.4
		10	14.1 b	5.5	6.5	4.2	5.1
6	M	0	6.3 a	0.2	4.2	2.3	5.1
		5	8.5 a	1.8	5.4	4.4	5.2
		10	19.3 b	4.7	4.3	4.8	6.1
3	MT	0	6.9 a	2.2	2.2 a	4.6	7.1
		5	93.9 b	5.8	4.8 b	6.7	6.6
		10	108.7 b	8.6	3.9 ab	4.6	6.6
6	MT	0	2.8 a	2.0 a	4.8 a	4.9	6.0
		5	35.8 b	4.1 b	2.9 b	5.2	5.8
		10	103.7 c	4.1 b	4.5 a	9.5	4.4

[a] Values for hydroxybenzoic acids are the means of three replicates. Means in a column within a leaf category and days after treatment not followed by a letter or followed by the same letter are not significantly different at $P < 0.05$ according to Newman–Keuls multiple-range test. [b] Key: Y = young non-contact-treated; M = mature non-contact-treated; MT = mature contact-treated; DW = dry weight.

duced protocatechuic acid accumulation was slightly higher at 6 days as compared to 3 days in all treatments. Except for the marginal effects of glyphosate on 4-hydroxybenzoic acid, the other hydroxybenzoic acids were not affected by glyphosate treatment. Syringic acid was not detected in any of the ryegrass leaf extracts.

Glyphosate treatment also dramatically affected protocatechuic acid levels in soybean leaf tissues. The least affected soybean leaf tissue was the mature non-contact-treated leaf tissue, which also accumulated the lowest levels of glyphosate-induced shikimic acid (Figure 3) in this species. Nevertheless, mature non-contact-treated leaves from plants treated with 10 mM glyphosate had significantly higher levels of protocatechuic acid 3 and 6 days after treatment as compared to controls (Table III). Protocatechuic acid levels from young non-contact-treated leaves increased with increasing dose of glyphosate at 3 and 6 days after treatment. The concentration of gallic acid and 4-hydroxybenzoic acid also increased with increasing dose of glyphosate in young non-contact-treated leaves 3 days after treatment. By 6 days after treatment, the levels of these hydroxybenzoic acids in the 5 mM glyphosate treatment were not significantly different from the 10 mM treatment but were significantly higher than controls. Glyphosate of 5 and 10 mM concentration reduced gentisic acid levels in these tissues 3 days after treatment as compared to controls, whereas by 6 days after treatment only the 10 mM dose significantly reduced gentisic acid levels.

Mature contact-treated leaves from the 10 mM glyphosate-treated soybean plants contained the highest levels of protocatechuic acid measured in this species, averaging >100 nmol mg^{-1} DW at 3 and 6 days after treatment (Table III). Although lower at 6 days as compared to 3 days after treatment, the level of protocatechuic acid for the 5 mM glyphosate treatment was significantly higher than controls. There was a trend toward increased levels of gallic acid in mature contact-treated leaves as a result of glyphosate treatment; however, it was only significantly greater in the glyphosate treatments 6 days after treatment as compared to controls. Glyphosate effects on gentisic acid levels in mature contact-treated leaves were variable. The 10 mM glyphosate treatment had no effect on gentisic acid accumulation as compared to controls at either 3 or 6 days after treatment, whereas 5 mM glyphosphate treated leaves contained higher levels of gentisic acid 3 days but lower levels 6 days after treatment.

The effects of glyphosate on hydroxybenzoic acid levels in velvetleaf and yellow nutsedge leaves were similar to those in the other species tested, with the 10 mM treatment accumulating significantly higher levels of protocatechuic and gallic acids than controls (Table I). The other hydroxybenzoic acids in either species were not affected by the treatment. A survey of the plants 12 days after treatment indicated that protocatechuic acid remained the hydroxybenzoic acid most affected by the glyphosate treatments in all species (data not shown).

DISCUSSION

All species tested showed a glyphosate-induced accumulation of unusually high levels of shikimic acid. The extent to which sink and source leaves were affected reflect the translocation pattern of glyphosate in higher plants (Honegger et al., 1986). Our results clearly demonstrate that hydroxybenzoic acids also typically accumulate in glyphosphate-treated leaf tissues. While no data were presented for protocatechuic and gallic acids, Cañal et al. (1987) reported that other hydroxybenzoic acids such as gentisic, salicylic, vanillic, and syringic were significantly higher in yellow nutsedge leaves sprayed with 10 mM glyphosate as compared to controls. Contrary to their results, we found that vanillic and syringic acids (which are methylated forms of protocatechuic and gallic acids) were not affected by glyphosate treatment in any of the species tested. The disparity between our results and those of Cañal et al. (1987) may be attributed to differences in time between treatment and havest, because in the latter study leaf tissues were harvested 14 days after treatment.

Hydroxybenzoic acids, particularly protocatechuic, gallic, and 4-hydroxybenzoic, appear to be derived not from cinnamate but from shikimic acid or shikimic acid precursors because glyphosate disrupts the shikimic acid pathway at a point before the formation of cinnamates. Therefore, our results and those of others (Cañal et al., 1987) support the view of Haslam (1986) and Boudet et al. (1986) that hydroxybenzoic acids are "overflow metabolites" of the shikimic acid pathway. In addition, these results imply that the species tested contain the

shikimate-inducible enzyme DHQase 2, which leads to the conversion of quinate to protocatechuic acid (Boudet et al., 1986).

The results also indicate that hydroxybenzoic acid accumulation in weed species (the target organisms of herbicides) is affected by glyphosate to a greater extent than in crop species. This may be explained by the fact that crop species generally have a lower capacity for phenolic biosynthesis than weed species, possibly the result of breeding out undesirable flavor properties. In addition, our results suggest that although glyphosate is considered not to be an environmentally persistent herbicide (Duke, 1988), the use of glyphosate may have long-term secondary effects. For example, the decomposition of glyphosate-sprayed plants in the field, and the release of abnormally high concentrations of compounds such as protocatechuic acid, may alter the effects of plant residues on subsequent crops and surrounding vegetation. Protocatechuic acid has been shown to have many effects on plants, such as growth reduction in cowpea (*Vigna sinensis* L.) plants and tumorigenesis induction by *Agrobacterium tumefaciens* in *Nicotiana tabacum* L. cells (Alsaadawi et al., 1986; Bolton et al., 1986). Thus, depending upon the persistence of these herbicide-induced plant metabolites in the soil, glyphosate may indirectly affect agricultural ecosystems.

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Registry No. Shik, 138-59-0; proto, 99-50-3; gall, 149-91-7; gent, 490-79-9; 4-hyd, 99-96-7; van, 121-34-6; syr, 530-57-4; glyphosate, 1071-83-6.

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