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Metabolism of [^{14}C]Quizalofop-ethyl in Soybean and Cotton Plants

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The metabolism of [*phenyl*-U-^{14}C]quizalofop-ethyl or [*quinoxaline*-^{14}C]quizalofop-ethyl was investigated in soybean and cotton plants. [^{14}C]Quizalofop-ethyl was applied to soybean or cotton plants at 4 oz of AI/acre as a postemergence spray, and plant samples were harvested initially (day 0) and at 3, 6, and 13.5 (maturity) weeks after treatment. No detectable ^{14}C residues (<0.01 ppm) were found in mature beans or pods, whereas the mature fiber and seeds from the cotton contained 0.08 and 0.09 ppm total ^{14}C residues, respectively. The proposed metabolic pathway of quizalofop-ethyl was similar in both soybean and cotton plants. In the foliage, quizalofop-ethyl was rapidly metabolized to 2-[4-[(6-chloroquinoxalin-2-yl)oxy]phenoxy]propanoic acid (quizalofop) that metabolized to the phenol metabolites 6-chloroquinoxalin-2-ol and 2-(4-hydroxyphenoxy)propanoic acid. Glucose conjugates of quizalofop possibly were formed since addition of β-glucosidase to aqueous extracts (containing polar ^{14}C residues) of soybean foliage slowly released [^{14}C]quizalofop.

Quizalofop-ethyl (ethyl 2-[4-[(6-chloro-2-quinoxalinyl)oxy]phenoxy]propanoate, the active ingredient in ASSURE herbicide, a registered product of Du Pont in the United States) is used to control both annual and perennial grass weeds in broadleaf crops. Tolerant crops include alfalfa, bean, cabbage, canola, carrot, lettuce, potato, soybean, sugar beet, tobacco, tomato, and turnip.

The metabolic pathways of phenoxyphenoxy herbicides similar to quizalofop-ethyl have been reported. Shimabukuro et al. (1979), Jacobson and Shimabukuro (1984), and Gorbach et al. (1977) studied the metabolism of diclofop in oat and wheat plants. The metabolic pathway of quizalofop in tolerant plants has not been reported previously. This study establishes the metabolic pathways of quizalofop-ethyl in two tolerant crops, soybean and cotton. The accumulation potential of quizalofop-ethyl residues in mature soybeans and cotton fibers and seeds was also determined. When either the phenyl or the phenylquinoxaline portion of the quizalofop-ethyl molecule was radiolabeled with ^{14}C, it was possible to follow the metabolic fate of both ring structures of the quizalofop-ethyl molecule.

MATERIALS

Quizalofop-ethyl (ethyl 2-[4-[(6-chloro-2-quinoxalinyl)oxy]phenoxy]propanoate) was uniformly labeled on the phenyl ring or the phenyl portion of the quinoxaline ring. In the cotton study, the specific activities of [*phenyl*-U-^{14}C]quizalofop ethyl and [*quinoxaline*-^{14}C]quizalofop-ethyl were 10.9 and 9.7 μCi/mg, respectively. In the soybean study, [*phenyl*-U-^{14}C]quizalofop-ethyl and [*quinoxaline*-^{14}C]quizalofop-ethyl each had a specific activity of 12.4 μCi/mg. The radiochemical purities were greater than 99%. Nonradiolabeled standards of quizalofop-ethyl and potential metabolites were synthesized at the Agricultural Products Department, E. I. du Pont de Nemours and Co., Inc. (Wilmington, DE).

All solvents used in the extraction and analytical procedures of both studies were high-performance liquid chromatography (HPLC) grade (Fisher Scientific, Fair Lawn, NJ), except methyl ethyl ketone, which was reagent grade. β-Glucosidase (Type II), cellulase (Type I), and protease were purchased from Sigma Chemical Co. (St. Louis, MO). All other common chemicals were reagent grade or better.

EXPERIMENTAL METHODS

Plant Growth and Treatment. Soybean plants (Williams variety) were field grown near Newark, DE. The soybean plants, at the second trifoliate leaf stage, were divided into two plots, and a postemergence spray of [*phenyl*-U-^{14}C]quizalofop-ethyl (40.0 mg; 495 μCi), dissolved in 500 μL of 4% Atlox 3408F (ICI America) in xylene and diluted with 130 mL of water, was applied to the foliage in the first plot at 4.1 oz of AI/acre. [*quinoxaline*-^{14}C]Quizalofop-ethyl (37.8 mg; 467 μCi), dissolved in 500 μL of 4% Atlox 3408F in xylene and diluted with 130 mL of water, was sprayed on the foliage in the second plot at 3.9 oz of AI/acre.

Cotton plants (Coker 310, 15 in. tall) were field grown near Fayetteville, NC, and divided into two plots. A postemergence spray of [*phenyl*-U-^{14}C]quizalofop-ethyl (47.7 mg; 520 μCi), dissolved in 25 mL of acetone and diluted with 150 mL of water, was sprayed on the foliage of the cotton plants in one plot, and [*quinoxaline*-^{14}C]quizalofop-ethyl (48.0 mg; 466 μCi), dis-

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solved in 25 mL of acetone and diluted with 150 mL of water, was sprayed on the foliage of the plants in the second plot. This application rate was equivalent to 3.7 oz of AI/acre.

In order to thin the soybean and cotton crops and obtain samples for analyses, foliage was harvested immediately after the applied spray solution dried (day 0) and at 1 (soybeans only), 3, 6, and 13.5 weeks (maturity). At maturity, the soybeans and the fiber and seeds of cotton plants were separated from harvested plant tissue.

Total ^{14}C Residue Determination. After harvesting, aliquots of foliage, beans, cotton seeds, and cotton fiber were lyophilized. Aliquots were combusted on a Packard Model 306 sample oxidizer (Packard Instrument, Co., Downers Grove, IL) to locate detectable levels of ^{14}C residues. Crop portions containing significant amounts of ^{14}C residues were extracted.

Extraction of ^{14}C Residues from Cotton and Soybean Foliage. An acetone wash (2 mL of acetone/g of tissue) was used to remove any ^{14}C residues not absorbed into the foliage. The foliage was then extracted with methylene chloride–acetone (1:1, v/v) (2 mL of solvent/g of tissue) by maceration with a Tekmar Tissumizer (Tekmar Co., Cincinnati, OH). The tissue–solvent mixture was shaken for 1 h, centrifuged at 2500 rpm for 15 min, and decanted through Whatman No. 1 filter paper. The tissue was extracted two more times with methylene chloride–acetone with use of the same procedure. The extracts were pooled, the volume was measured, and duplicate aliquots were counted by liquid scintillation counting (LSC) on a Model 6881 Mark III liquid scintillation counter (Tracor Analytic, Elk Grove Village, IL). Plant tissue was then similarly extracted three more times with acetone–ethanol–water (2:1:1, v/v/v). The extracted plant tissue was air-dried, and aliquots were combusted to determine the amounts of unextracted (bound) ^{14}C residues.

Characterization of Unextractable ^{14}C Residues in Cotton and Soybean Foliage. Since significant amounts of ^{14}C residues remained in the cotton foliage following these multisolvent extractions, additional extraction techniques were employed. The foliage was extracted with 1% phosphoric acid in acetonitrile, followed by a methanol Soxhlet extraction. Next, cellulase and protease were used to attempt to solubilize the remaining bound ^{14}C residues. A final attempt was made to release the bound ^{14}C residues by using a lignin-solubilizing procedure (AOAC, 1980).

The soybean foliage also contained significant amounts of bound ^{14}C residues following the multisolvent extractions and was continuously extracted with methanol on a Soxhlet apparatus. No additional attempts were made to extract these bound ^{14}C residues.

Extraction of ^{14}C Residues from Mature Cotton Seeds and Fibers. Cotton seeds were extracted with methylene chloride–acetone (1:1, v/v) by maceration on a Tekmar Tissumizer. The seed–solvent mixture was shaken for 1 h, centrifuged at 2500 rpm for 10 min, and decanted through filter paper. The seeds were extracted two more times with CH_2Cl_2–acetone. The three extracts were pooled and analyzed by LSC.

The cotton seeds were then similarly extracted three more times with acetone–ethanol–water (2:1:1, v/v/v) and analyzed by LSC. The amount of unextracted (bound) ^{14}C residues remaining in the extracted seeds was determined by combustion analysis and LSC.

Cotton fiber (approximately 40 g) was shaken for 1 h with CH_2Cl_2–acetone (1:1, v/v) and then extracted with acetone–ethanol–water (2:1:1, v/v/v) as described for the seeds.

EXPERIMENTAL METHODS

Thin-Layer Chromatography (TLC). Organic phases were applied to E. Merck silica gel 60 TLC plates (0.5-mm thickness, with fluorescent indicator), along with standards of [^{14}C]quizalofop-ethyl, [^{14}C]quizalofop, phenol 1 (soybean study only), phenol 2, phenol 3, and phenol 4. Each TLC plate was developed in toluene–acetone–methanol–acetic acid (150:60:12:1, v/v/v/v). R_f values of the compounds are listed in Table I. Radioactive areas on the TLC plates were located and quantitated on a Berthold Model LB 2832 linear analyzer (Berthold Instruments, Wildbad, West Germany). Nonradioactive standards were detected under ultraviolet (UV) light.

Table I. Thin-Layer Chromatography R_f Values for Quizalofop-Ethyl and Metabolites[a]

compound[b]	R_f	compound[b]	R_f
quizalofop-ethyl	0.95	P1	0.08
quizalofop	0.41	P2	0.12
phenol 1	0.81	P3	0.55
phenol 2	0.53	P4	0.26
phenol 3	0.71	P5	0.64
phenol 4	0.25	P6	0.09

[a] Chemical names and structures are shown in Figure 2. [b] Compounds were eluted to 15 cm on a silica gel plate (E. Merck, Silica Gel 60, 0.5-mm thickness) with toluene–acetone–methanol–acetic acid (150:60:12:1, v/v/v/v).

High-Pressure Liquid Chromatography (HPLC). Organic and aqueous phases were analyzed by one of the following HPLC methods:

Method 1: Hewlett-Packard Model 1090A HPLC with a Zorbax ODS column (Mac-MOD Analytical Inc., Chadds Ford, PA) (9.4 mm × 25 cm) at 40 °C, flow rate 4 mL/min, and the following mobile phase

time, min	solvent
0–15	40% pH 2.2 water–60% CH_3CN
15–17	40% pH 2.2 water–60% CH_3CN to 100% CH_3CN (linear gradient)
17–19	100% CH_3CN
19–21	100% CH_3CN to 40% pH 2.2 water–60% CH_3CN (linear gradient)

Method 2: Du Pont Model 8800 HPLC with a Zorbax ODS column (4.6 mm × 15 cm) at 45 °C, flow rate 2 mL/min, and a mobile phase with a linear gradient of 30–100% acetonitrile in pH 2.2 water over 20 min.

Method 3: Same as method 2, but with the following mobile phase

time, min	solvent
0–10	5–35% CH_3CN in pH 2.2 water
10–15	100% CH_3CN (wash)

Method 4: Same as method 2, but with a 15% CH_3CN–85% pH 2.2 water isocratic mobile phase.

Method 5: Same as model 2, but with a Zorbax C-8 column and the following mobile phase

time, min	solvent
0–10	100% pH 2.2 water to 20% CH_3CN–80% pH 2.2 water
10–15	20% CH_3CN–80% pH 2.2 water
15–20	100% CH_3CN (wash)

Method 6: Same as method 2, but with a Zorbax NH_2 column and 80% CH_3CN–20% pH 2.2 water isocratic mobile phase.

Methods 1–6: Fractions sequentially collected at 0.5-min intervals and analyzed by LSC; radiochromatograms plotted with use of RS/1 (BBN Software Products Corp., Cambridge, MA).

Characterization of ^{14}C Residues in Cotton and Soybean Foliage. The organic solvents from the CH_2Cl_2–acetone and acetone–ethanol–water extracts were evaporated on a rotary evaporator at 37 °C until only an aqueous solution remained. The aqueous phase from each extract was partitioned three times with equal volumes of *n*-hexane. The aqueous phase was then partitioned three times with equal volumes of methylene chloride. The *n*-hexane and methylene chloride phases from each extract were pooled separately and analyzed by LSC. The methylene chloride and *n*-hexane phases were concentrated with a combination of rotary and nitrogen evaporation. The methylene chloride phase was analyzed by TLC, while the *n*-hexane phase was redissolved in acetonitrile and analyzed by HPLC

(method 1). The remaining aqueous extracts were analyzed as described below. The aqueous extract from soybean foliage was freeze-dried.

Analysis of Aqueous Extracts. The aqueous extracts (after *n*-hexane and methylene chloride partitioning) were pooled, buffered to pH 5, and incubated with β-glucosidase for 24 h at 37 °C to hydrolyze glucose conjugates of [^{14}C]quizalofop-ethyl metabolites. Then, the aqueous solution was acidified, with concentrated HCl, and partitioned three times with equal volumes of ethyl acetate–methyl ethyl ketone (MEK) (2:1, v/v). The ethyl acetate–MEK phases were pooled and analyzed by LSC and TLC (after concentration). The remaining acidified aqueous solution was also analyzed by LSC.

Following enzyme treatment, the aqueous solution (at pH 1.5) was incubated for 6 h at 100 °C (to further hydrolyze glucose conjugates) and then partitioned three times with equal volumes of ethyl acetate. The aqueous and ethyl acetate (pooled) phases were analyzed by LSC. The pooled ethyl acetate phase was concentrated and analyzed by TLC.

Isolation and Identification of Metabolites. In a separate experiment, young soybean plants were treated with [*phenyl*-U-^{14}C]quizalofop-ethyl at 10 oz of AI/acre to produce sufficient quantities of ^{14}C metabolites for isolation and identification. Plants were harvested 15 days after application and extracted as previously described to obtain the methylene chloride and aqueous extracts. The aqueous extract was freeze-dried to a powder.

Purification and Identification of the Major Radiolabeled Metabolite. A portion of the concentrated methylene chloride extract was applied across six preparative silica gel TLC plates. The major radioactive area was located on each plate (R_f 0.41). The silica from this area was scraped from each plate, and radioactivity was extracted from the silica with methylene chloride–methanol (1:1, v/v) by sonication. The solvent–silica mixture was filtered through a C-2 Bond Elut column (Analytichem Int., Harbor City, CA). The silica was extracted two more times, and the extracts were pooled and analyzed by LSC. The methylene chloride–methanol extract was evaporated to near-dryness on a rotary evaporator. The radiolabeled metabolite was dissolved in ~0.5 mL of acetonitrile, and the ^{14}C metabolite was further purified by HPLC (method 1). The major radioactive peak eluted with a retention time between 8 and 9 min. The acetonitrile in the eluant containing the ^{14}C metabolite was removed by rotary evaporation. The remaining aqueous mobile phase was partitioned with an equal volume of methylene chloride. The methylene chloride, which contained greater than 99% of the total radioactivity, was concentrated to ~1 mL and submitted for mass spectral analysis. A direct chemical ionization (DCI) mass spectrum was obtained with use of methane as the reagent gas on a Finnigan Model 4500 mass spectrometer. An electron impact mass spectrum was also obtained on a Du Pont Model 492 mass spectrometer equipped with a Vespel probe (Vespec Corp., Houston, TX).

Characterization of Quizalofop–Glucose Conjugate. A portion of the freeze-dried aqueous extract from soybeans was dissolved in mobile phase and analyzed by HPLC (method 2). This aqueous extract was also treated with β-glucosidase, incubated for 3 days at 37 °C, and analyzed as described previously. Fresh enzyme was added daily during the incubation period. An additional portion of the aqueous extract, minus the enzyme, was also incubated as a control. Both the enzyme-treated sample and the control were analyzed daily by HPLC (method 2).

Characterization of Phenol 4–Glucose Conjugate. A portion of the freeze-dried aqueous extract was dissolved in an acetate buffer (pH 5.0) and analyzed by HPLC (method 3). A portion of the freeze-dried aqueous fraction that was treated with β-glucosidase for 24 h was also analyzed by HPLC (method 3).

Purification of Phenol 4 from the Freeze-Dried Aqueous Extract. One gram of the freeze-dried aqueous extract was dissolved in 0.1 M HCl and the resultant mixture heated at 100 °C for 6 h. After the mixture was cooled to room temperature, this hydrolysis reaction mixture was extracted three times with equal volumes of ethyl acetate–MEK (2:1, v/v). The organic extracts were combined and reduced in volume by rotary evaporation, followed by further solvent removal (to dryness) on a nitrogen evaporator. The resulting solid was dissolved in mobile phase and analyzed by HPLC (method 4); a radiochemical detector (Radiomatic Flo-One, Model DR; Radiomatics Instruments, Tampa, FL) was used to detect radioactivity eluting from the column. A single radioactive peak (eluting at 9 min) was collected and extracted three times with ethyl acetate. The extracts were combined and concentrated to dryness as previously described. The radioactivity was redissolved in 0.5 mL of pH 2.2 water (formic acid) and analyzed by HPLC (method 5). The single radioactive peak (eluting at 10.9 min) was collected and extracted with ethyl acetate, which was then removed by evaporation. The remaining solids were redissolved in acetonitrile–pH 2.2 water (80:20, v/v) and analyzed by HPLC (method 6). The single radiolabeled peak (eluting at 5 min) was collected, the solvent was evaporated under a stream of nitrogen, and the resulting solid was submitted for mass spectral analysis under the conditions previously described.

RESULTS AND DISCUSSION

Dissipation of ^{14}C Residues from Foliage. The dissipation of ^{14}C residues from soybean and cotton foliage after a postemergence application of [*phenyl*-U-^{14}C]- and [*quinoxaline*-^{14}C]quizalofop-ethyl is summarized in Figure 1. Levels of [^{14}C]quizalofop-ethyl residues declined rapidly in both soybean and cotton foliage.

In the soybean study, the levels of ^{14}C residues declined from approximately 18 ppm on day 0 to less than 0.1 ppm 6 weeks after the [*phenyl*-U-^{14}C]quizalofop-ethyl application and from 23.2 ppm on day 0 to less than 0.1 ppm 6 weeks after the [*quinoxaline*-^{14}C]quizalofop-ethyl application. The first half-life for ppm of total ^{14}C residues was less than 1 week.

In the cotton study, the levels of ^{14}C residues declined from 10.1 ppm on day 0 to 0.03 ppm at maturity in plants treated with [*phenyl*-U-^{14}C]quizalofop-ethyl and from 20.6 ppm on day 0 to 0.1 ppm at maturity in plants treated with [*quinoxaline*-^{14}C]quizalofop-ethyl. The first half-life for ppm of total ^{14}C residues was about 2 weeks. The rapid decline of ^{14}C residues in these plants can be attributed partially to growth dilution.

Total ^{14}C Residues in Soybeans and Cotton Fiber and Seeds. No detectable ^{14}C residues (<0.01 ppm) were found in mature soybeans or pods, whereas the mature fiber and seeds in the cotton study contained 0.08 and 0.09 ppm total ^{14}C residues, respectively. There appears to be no potential for significant accumulation of quizalofop-ethyl-derived residues in mature soybeans and cotton fiber and seeds.

Extraction of ^{14}C Residues from Soybean and Cotton Foliage. Immediately after application of the postemergence spray of [^{14}C]quizalofop-ethyl (day 0), up to 55% of the total ^{14}C residues could be washed from the surface of the cotton or soybean foliage (Tables II and III). However, within 1–3 weeks after application, only about 5% of the ^{14}C residues could be washed from the foliage, indicating that [^{14}C]quizalofop-ethyl had readily absorbed into both soybean and cotton plant tissue (Figure 1). During the same time period, the extractable ^{14}C residues ranged from 48% to 81%, while the percentage of bound ^{14}C residues increased from about 1–2% on day 0 to 22–33% within 3 weeks after treatment. If bound ^{14}C residues exceeded 0.1 ppm, an attempt was made to characterize the bound residues. These bound ^{14}C residues could not be solubilized with cellulase, protease enzymes, or by a lignin solubilizing procedure (AOAC, 1980).

Identification of the Quizalofop Conjugate in the Aqueous Phase. Three radioactive peaks were detected, eluting at 0–2 min (37% of injected radioactivity), 5–8 min (31% of injected radioactivity), and 9–10 min (7%

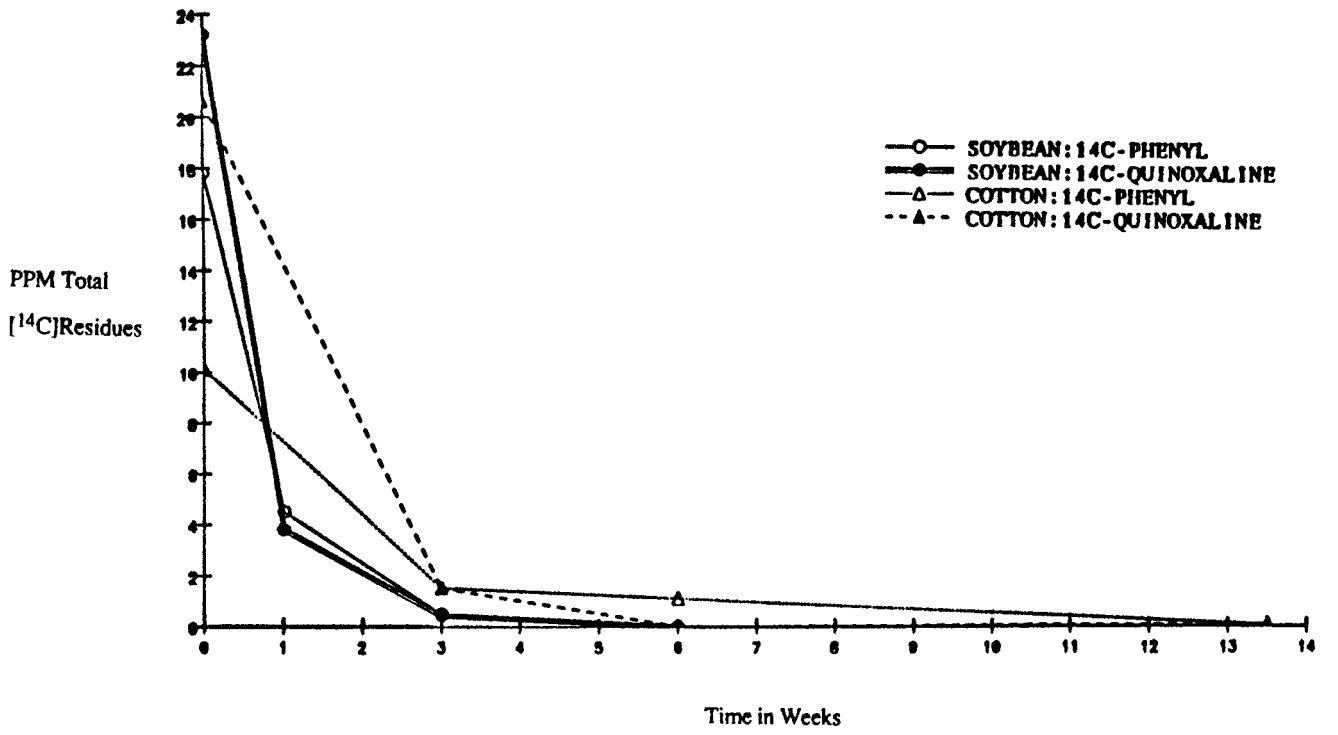

Figure 1. Dissipation of ^{14}C residues in soybean and cotton foliage after application of [^{14}C]quizalofop-ethyl.

Table II. Extraction of ^{14}C Residues from Soybean Foliage

	% of total dpm							
	day 0		week 1		week 3		week 6	
extraction phase	phenyl[a]	quinoxaline[b]	phenyl	quinoxaline	phenyl	quinoxaline	phenyl	quinoxaline
wash	39	55	3	5	1	1	2	4
extractable	60	43	64	71	74	71	86	78
bound[c]	1	2	33	24	25	28	12	18
total	100	100	100	100	100	100	100	100
ppm[d]	(17.8)	(23.2)	(4.5)	(3.8)	(0.48)	(0.43)	(0.009)	(0.009)

[a,b] Soybean foliage treated with [*phenyl*-U-^{14}C]quizalofop-ethyl or [*quinoxaline*-^{14}C]quizalofop-ethyl. [c] Bound = unextractable ^{14}C residues. [d] ppm = [^{14}C]quizalofop-ethyl equivalents.

Table III. Extraction of ^{14}C Residues from Cotton Foliage

	% of total dpm							
	day 0		week 1		week 3		week 6	
extraction phase	phenyl[a]	quinoxaline[b]	phenyl	quinoxaline	phenyl	quinoxaline	phenyl	quinoxaline
wash	41	52	4	5	3	<1	27	5
extractable	57	48	74	69	73	81	30	25
bound[c]	2	<1	22	26	24	19	43	70
total	100	100	100	100	100	100	100	100
ppm[d]	(10.1)	(20.6)	(1.5)	(1.5)	(1.1)	(0.009)	(0.03)	(0.10)

[a,b] Cotton foliage treated with [*phenyl*-U-^{14}C]quizalofop-ethyl or [*quinoxaline*-^{14}C]quizalofop-ethyl. [c] Bound = unextractable ^{14}C residues. [d] ppm = [^{14}C]quizalofop-ethyl equivalents.

of injected radioactivity), when the freeze-dried aqueous extract was analyzed by HPLC; the remainder of the radioactivity was spread diffusely throughout the chromatogram. After treatment with β-glucosidase, the radioactivity eluting between 5 and 8 min slowly decreased (to 18% of the radioactivity), while the fraction of the radioactive material eluting between 9 and 10 min increased (to 18% of the radioactivity). The relative amounts of the three radiolabeled species in the control solution did not change with time.

These results indicate that the peak eluting at 5–8 min is a carbohydrate conjugate of the peak eluting at 9–10 min. The 9–10-min peak coeluted with quizalofop in this HPLC system (elution time 9.6 min) and also coeluted with quizalofop on the same column but with an isocratic mobile phase of 40% acetonitrile in pH 2.2 water (retention time 7.2 min). Thus, chromatographic evidence indicates that the peak eluting at 5–8 min in the linear gradient HPLC system is the carbohydrate conjugate of quizalofop, which is slowly cleaved to form quizalofop upon β-glucosidase treatment.

Identification of the Phenol 4 Conjugate in the Aqueous Phase. The majority of the radioactivity eluting between 0 and 2 min in the above HPLC system eluted at 4–5 min with the nonlinear gradient. After treatment with β-glucosidase, the retention time of the radioactive species eluting at 4–5 min changed, such that it eluted between 5 and 6 min. This enzymatic hydrolysis was much quicker than observed for the carbohydrate conjugate of the quizalofop conjugate. Complete hydrolysis occurred

Table IV. Composition of ^{14}C Residues in Cotton Foliage Treated with [*phenyl*-^{14}C]- or [*quinoxaline*-^{14}C]Quizalofop-ethyl

	% of total dpm				
	day 0		week 3		
compound	phenyl	quin-oxaline	phenyl	quin-oxaline	week 6[a] phenyl
quizalofop-ethyl	68	76	8	13	2
quizalofop	28	22	17	7	6
quizalofop conjugate	ND[b]	ND	11	ND	ND
phenolic metabolites[c]	ND	ND	4	7	20
other metabolites[d]	2	1	18	15	16
polars[e]			21	32	32
bound[e]	2	1	21	26	24
total	100	100	100	100	100
ppm[g]	(10.1)	(20.6)	(1.5)	(1.5)	(1.1)

[a] [*quinoxaline*-^{14}C]Quizalofop-ethyl-treated foliage was not analyzed. [b] ND = not detected. [c] Phenolic metabolites include phenol 2, phenol 4, and their respective glucose conjugates. [d] Other metabolites include P1–P6 glucose conjugates. [e] Polars include acidified CH_3CN, CH_3OH Soxhlet, acetate buffer, cellulase, protease, aqueous, and acid hydrolysis extracts. [f] Bound = unextractable ^{14}C residues. [g] ppm = [^{14}C]quizalofop-ethyl equivalents.

after only 24 h of incubation, whereas only partial hydrolysis of the quizalofop conjugate occurred after 72 h. Mild acid hydrolysis (pH 1, 100 °C, 6 h, released exocon extracted into ethyl acetate) could effect the same transformation. When the product from the enzymatic hydrolysis was mixed with the product from the acid hydrolysis, it migrated as a single radiolabeled peak in this HPLC system. Since mild acid hydrolysis gave the same product as enzymatic hydrolysis, acid hydrolysis was used to generate this metabolite for identification purposes.

After purification, DCI mass spectrometry demonstrated that this metabolite had an M + 1 ion of m/e 183, while electron impact mass spectrometry gave a parent ion of m/e 182. These results are both consistent with the structure of phenol 4. Also, this metabolite coeluted with the authentic standard of phenol 4 in each of the HPLC systems. It was concluded that the radioactivity eluting in the 4–5-min fraction was the glucose conjugate of phenol 4.

In this present work, we indicate the presence of glucose conjugates of various soybean and cotton primary metabolites of quizalofop-ethyl. Such indications of glucose conjugation are based upon release of the various exocons (primary metabolites) upon β-glucosidase treatment. Release of the exocon by this treatment is not proof of a glucose conjugate; β-glucosidase (EC 3.2.1.21) can hydrolyze other glycosidic linkages (Heyworth and Walker, 1962). However, for simplicity we will continue to refer to these conjugates as glucose conjugates.

Composition of Radioactivity in the Cotton Foliage. Metabolism of [^{14}C]quizalofop-ethyl in cotton plants was rapid and extensive. The composition of ^{14}C residues is shown in Table IV.

Quizalofop-ethyl metabolized to quizalofop, the glucose conjugate of quizalofop, and phenolic metabolites (phenol 2 and phenol 4 and their respective glucose conjugates) within 3 weeks after application. Six weeks after application, the quizalofop conjugate was no longer detectable but the phenolic metabolites were still present.

Unidentified metabolites (other metabolites, Table IV) included the glucose conjugates of P1, P2, and P3 ([*phenyl*-^{14}C]quizalofop-ethyl study) and P4, P5, and P6 ([*quinoxaline*-^{14}C]quizalofop-ethyl study). Each of these metabolites consisted of <10% of the total radioactivity in any sample. The characterization of these glucose conjugates was based entirely on β-glucosidase treatment, resulting in the release of these free metabolites.

About half of the remaining bound ^{14}C residues were released by extraction with acidified CH_3CN, CH_3OH Soxhlet, acetate buffer, cellulase, protease, aqueous, and acid hydrolyses. The total ^{14}C residues in these extracts are listed as polars in Table IV. ^{14}C residues in each of these extracts amounted to <10% of total ^{14}C (<0.1 ppm) and were not identified (due to low residue levels and difficulties in extract cleanup). No additional attempts were made to release the remaining bound ^{14}C residues (21–26%).

Composition of Radioactivity in the Soybean Foliage. Metabolism of [^{14}C]quizalofop-ethyl in soybean plants is similar to, but slightly faster than, that observed in cotton. The composition of ^{14}C residues is shown in Table V.

Quizalofop-ethyl metabolized to quizalofop, the glucose conjugate of quizalofop, and some phenolic metabolites (phenol 1, phenol 2 and phenol 2 conjugate, phenol 3, phenol 4 and phenol 4 conjugate) 1 week after application. After 3 weeks of incubation, the quizalofop conjugate and the phenolic metabolites were no longer detectable.

As the phenolic metabolites disappeared, more polar metabolites (other metabolites, Table V) were detected at 1 and 3 weeks. At 1 week (and similarly at 3 weeks), these metabolites consisted of the following unidentified polar compounds: P2 (1–2%; ~0.06 ppm); ^{14}C remaining at the origin of the TLC plate (10–14%; 0.4–0.6 ppm); ^{14}C distributed across the TLC plate having no distinct peaks (5–9%; 0.2 ppm). No further attempts were made to release the bound ^{14}C residues, since in cotton, cellulase, protease, and acid hydrolysis released no additional ^{14}C residues.

Proposed Metabolic Pathway. Figure 2 shows the proposed metabolic pathway for quizalofop-ethyl in soybean and cotton foliage. The primary route of metabolism appears to be via rapid deesterification of quizalofop-ethyl to quizalofop. Quizalofop either becomes conjugated to glucose or undergoes cleavage at the ether linkage, producing phenol 2 (6-chloroquinoxalin-2-ol) and phenol 4 (2-(4-hydroxyphenoxy)propanoic acid). Phenol 4 also became conjugated to glucose.

Similar phenolic and ester glucose conjugates have been reported by other investigators. Shimabukuro et al. (1979) and Jacobson and Shimabukuro (1984) reported the metabolism of diclofop-methyl oats to the free acid diclofop, which became conjugated to glucose. In wheat plants, Gorbach et al. (1977), Shimabukuro et al. (1979), and Jacobson and Shimabukuro (1984) reported hydroxylation of diclofop-methyl on the phenyl moiety, which also formed a glucose conjugate. The ester glucose conjugate of 3-phenoxybenzoic acid in cotton and the ester glucose conjugate of (2,4-dichlorophenoxy)acetic acid have also been reported (More et al., 1978; Feung et al., 1976).

CONCLUSION

It is clearly evident that the metabolic pathway of quizalofop ethyl is similar in soybean and cotton foliage. ^{14}C residues of quizalofop-ethyl dissipated rapidly in both soybean and cotton plants. Terminal ^{14}C residues in mature soybeans and in mature cotton fiber and seeds were low (<0.01, 0.10, and 0.09 ppm, respectively), indicating accumulation of ^{14}C residues is minimal.

Table V. Composition of ^{14}C Residues in Soybean Foliage Treated with [*phenyl*-^{14}C]- or [*quinoxaline*-^{14}C]Quizalofop-ethyl

	% of total dpm					
	day 0		week 1		week 3	
compound	phenyl	quinoxaline	phenyl	quinoxaline	phenyl	quinoxaline
quizalofop-ethyl	44	52	6	4	5	5
quizalofop	49	39	26	35	42	36
quixalofop conjugate	ND[a]	ND	14	4	ND	ND
phenolic metabolites[b]	ND	ND	5	10	ND	2
other metabolites[c]	6	6	16	22	22	26
bound[d]	1	3	33	25	31	31
total	100	100	100	100	100	100
ppm[e]	(17.8)	(23.2)	(4.5)	(3.8)	(0.48)	(0.43)

[a] ND = not detected. [b] Phenolic metabolites include phenol 1, phenol 2, phenol 3, phenol 4, and the respective glucose conjugates of phenol 2 and phenol 4. [c] Other metabolites include P2, ^{14}C remaining at the origin of the TLC plate, and ^{14}C distributed across the TLC plate having no distinct peaks. [d] Bound = unextractable ^{14}C residues. [e] ppm = [^{14}C]quizalofop-ethyl equivalents.

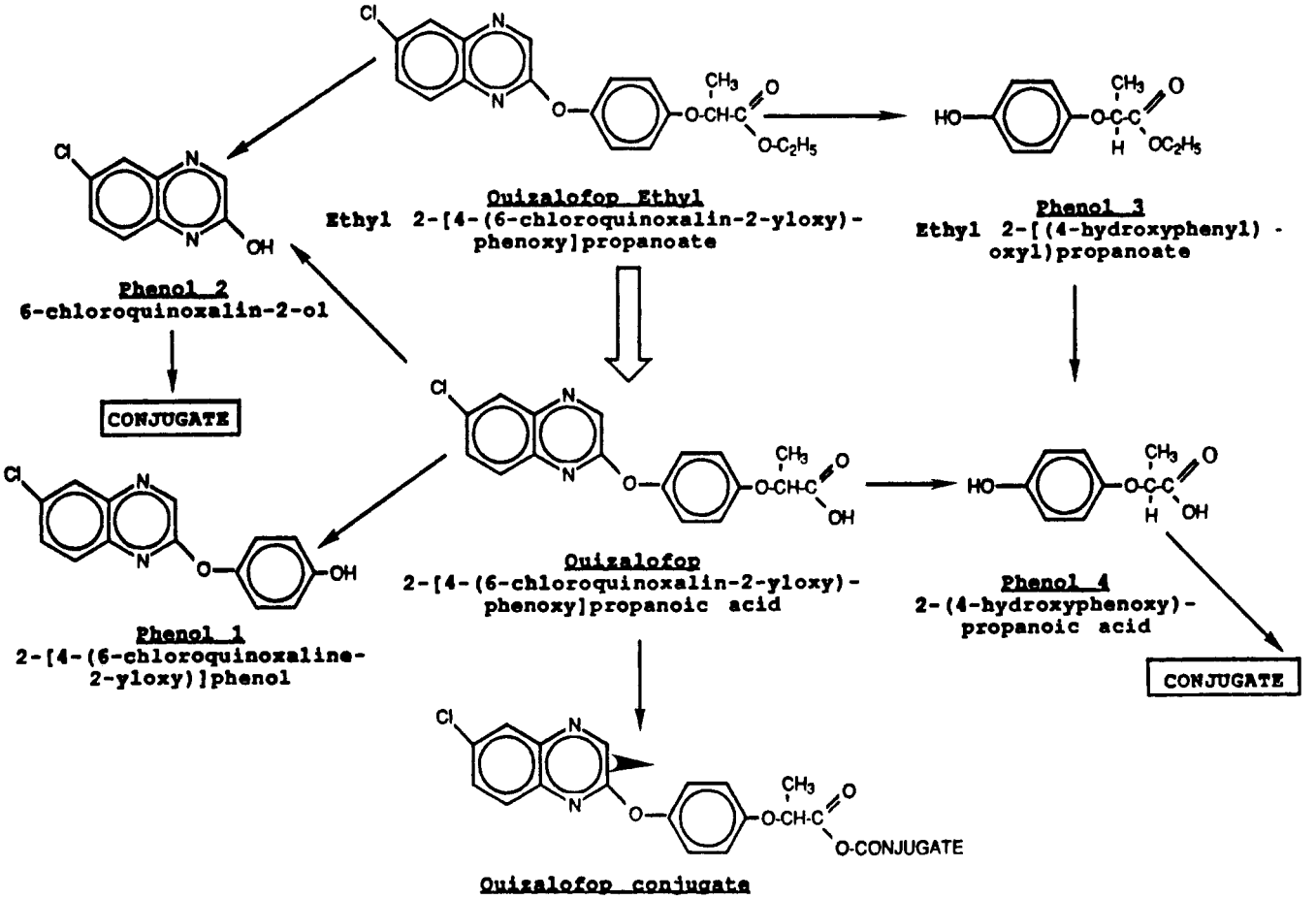

Figure 2. Proposed metabolic pathway of quizalofop-ethyl in soybean and cotton foliage.

In both the cotton and soybean studies, quizalofop-ethyl was rapidly hydrolyzed to quizalofop. In soybeans, this was followed by metabolism at one of the ether linkages producing phenol 1, phenol 2, phenol 3, and phenol 4. In addition, quizalofop, phenol 2, and phenol 4 undergo glucose conjugation. In cotton, metabolism at the ether linkage produced phenol 2 and phenol 4. Quizalofop, phenol 2, and phenol 4 also formed glucose conjugates. Glucose conjugation of 3-phenoxybenzoic acid (which is similar in structure to quizalofop) has been reported previously in cotton, tomato, cabbage, kidney bean, and cucumber plants (Mikami et al., 1984).

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1-[4-[(Trimethylsilyl)ethynyl]phenyl]-2,6,7-trioxabicyclo[2.2.2]octanes: A Novel Type of Selective Proinsecticide

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4-Alkyl-1-[4-[(trimethylsilyl)ethynyl]phenyl]-2,6,7-trioxabicyclo[2.2.2]octanes are potent insecticides with 24-h housefly topical LD_{50}s depending on the 4-substituent, i.e., 0.4–0.7 μg/g for *n*-propyl, *n*-butyl, and *tert*-butyl and 68 μg/g for cyclohexyl. The 4-*n*-propyl- and 4-*n*-butyl-1-[4-[(trimethylsilyl)ethynyl]phenyl] compounds have almost the same potency to houseflies as their 1-(4-ethynylphenyl) analogues, suggesting that the trimethylsilyl compounds may be proinsecticides. The microsomal cytochrome P_{450} inhibitor piperonyl butoxide antagonizes the toxicity of the [(trimethylsilyl)ethynyl]phenyl compounds by up to >714-fold and synergizes that of the ethynylphenyl compounds by 4- to 30-fold, indicating oxidative activation and detoxification, respectively. Mouse intraperitoneal LD_{50} values are 0.1–1.1 mg/kg for the ethynylphenyl compounds versus 3–>400 mg/kg for the [(trimethylsilyl)ethynyl]phenyl compounds. The 4-*n*-butyl-1-[4-[(trimethylsilyl)ethynyl]-phenyl] analogue is >930-fold more toxic to houseflies than to mice, establishing a remarkable level of selective toxicity among the trioxabicyclooctanes probably attributable to oxidative metabolic activation in houseflies but not in mice.

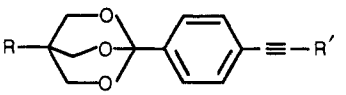
1,4-Disubstituted 2,6,7-trioxabicyclo[2.2.2]octanes are potent insecticides acting as $GABA_A$ receptor antagonists to inhibit GABAergic synaptic transmission (Palmer and Casida, 1985, 1987; Casida et al., 1988). 4-Alkyl-1-(4-ethynylphenyl)trioxabicyclooctanes are highly toxic to houseflies both with and without the synergist piperonyl butoxide (PB), achieving a level of potency comparable to that of the most effective established insecticides acting at other targets (Palmer and Casida, 1989). As with many other trioxabicyclooctanes (Casida et al., 1985), the 4-alkyl-1-(4-ethynylphenyl) analogues typically exhibit intraperitoneal LD_{50} values below 1 mg/kg to mice. Structural modification for selective toxicity therefore warrants special consideration.

This study focuses on possible proinsecticides that might more effectively undergo metabolic activation in insects than in mammals. Among the substituents examined, the [(trimethylsilyl)ethynyl]phenyl group proved to be of particular interest.

MATERIALS AND METHODS

Spectroscopy. Proton nuclear magnetic resonance (NMR) spectra were obtained at 300 MHz with a Bruker WM-300 spectrometer for samples dissolved in deuteriochloroform. Mass spectrometry (MS) utilized the Hewlett-Packard 5985 system with chemical ionization (230 eV with methane at 0.8 Torr).

Syntheses. The compounds examined are shown below:



	R			
R′	*n*-Pr	*n*-Bu	*t*-Bu	c-Hx
$SiMe_3$	1	2	3	4
H	5	6	7	8

Syntheses of **3**, **5**, and **7** have been described earlier (Palmer and Casida, 1989). Compounds **1**, **2**, **4**, **6**, and **8** were prepared by a similar route.

4-*n*-Butyl-1-[4-[(trimethylsilyl)ethynyl]phenyl]-2,6,7-trioxabicyclo[2.2.2]octane (**2**) was obtained as light brown needles: mp 124–126 °C: MS, $[M + 1]^+$ 345; NMR δ 7.55 and 7.40 (each 2 H, AA′BB′, aromatic), 4.10 (6 H, s, $CH_2O \times 3$), 1.35–1.15 (6 H, m, $(CH_2)_3$), 0.90 (3 H, t, CH_3CH_2), 0.20 (9 H, s, $(CH_3)_3Si$).

4-*n*-Propyl-1-[4-[(trimethylsilyl)ethynyl]phenyl]-2,6,7-trioxabicyclo[2.2.2]octane (**1**) was obtained as light tan flakes: mp 174–176 °C; MS, $[m + 1]^+$ 331; NMR δ 7.52 and 7.41 (each 2 H, AA′BB′, aromatic), 4.08 (6 H, s, $CH_2O \times 3$), 1.29–1.17 (4 H, m, CH_2CH_2), 0.91 (3 H, t, CH_3CH_2).

4-*n*-Butyl-1-(4-ethynylphenyl)-2, 6, 7-trioxabicyclo[2.2.2]octane (**6**) was obtained as pale yellow crystals: mp 85–87 °C; MS,

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