Metabolic Pathways and Residue Levels of Thifensulfuron Methyl in Soybeans

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Radiolabeled thifensulfuron methyl [methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylate], the active ingredient in Pinnacle herbicide, was applied postemergence to greenhouse-grown soybean seedlings at 8 and 16 g of active ingredient/ha (2–4 times the labeled use rate), with and without surfactant. The soybeans were grown to maturity, and the metabolic pathways and total radiolabeled residue levels were determined. The concentration of thifensulfuron methyl in soybean foliage declined with half-lives ranging from 5 to 20 days to approximately 8 ppb 30 days after treatment. The identities and concentrations of surface residues and metabolites in soybean foliage were quantitated at 0, 7, and 30 days after treatment. Radiolabeled residues in mature soybean seeds produced from these elevated application rates were extremely low and ranged from 0.4 to 1.6 ppb.

INTRODUCTION

Crop protection chemicals play a crucial role in modern global food production, and they are very likely to remain important tools in agriculture well into the 21st century (Beyer, 1991). However, use of these tools raises legitimate concerns about their effects on nontarget organisms, water and air quality, and worker and food safety. One strategy to address these concerns is to develop effective agrichemicals that have minimal toxicity to nontarget organisms and can be applied at very low use rates. This paper quantitiates the extremely low residue levels in a raw agricultural commodity that derive from use of a sulfonylurea herbicide at very low application rates.

Thifensulfuron methyl [methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylate, formerly DPX-M6316] (Figure 1) is a sulfonylurea herbicide registered for postemergence broadleaf weed control in wheat and barley (Du Pont, 1985; Sionis et al., 1985), corn (Eberlein and Miller, 1989; Eberlein et al., 1989; Brewster et al., 1988), and soybeans (Du Pont, 1989). It is formulated as Pinnacle herbicide for use in soybeans with a postemergence application rate of 4.3 g of ai/ha (17.2 g of formulated product/ha). At this application rate, plus 0.125–0.25% v/v nonionic surfactant, thifensulfuron methyl controls or suppresses several broadleaf weeds including *Abutilon theophrasti* Medic, *Chenopodium album* L., *Amaranthus* spp., *Convolvulus arvensis* L., and *Xanthium pensylvanicum* Wallr. It is a short-residual herbicide due to its high susceptibility to microbial degradation in the soil, and treated fields may be replanted to rotational crops as soon as 45 days after treatment (Beyer et al., 1987a; Brown et al., 1987; Cambon and Bastide, 1992; Smith et al., 1990). Previous work has shown that tolerance of treated soybean seedings to thifensulfuron methyl results from its rapid metabolic inactivation in soybean foliage (Brown et al.,
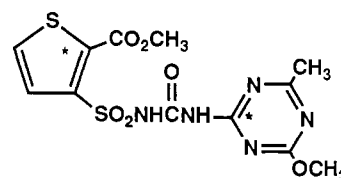

Figure 1. Chemical structure of thifensulfuron methyl. Asterisks show positions of ^{14}C radiolabels in separately labeled samples.

1990). The objectives of this study were to determine the metabolic pathways and residue levels of thifensulfuron methyl in soybean foliage during the first 30 days after treatment and the resultant residue levels in harvested pods and seeds (the raw agricultural commodity) in the crop grown to maturity.

MATERIALS AND METHODS

Chemicals. Separate samples of thifensulfuron methyl labeled with ^{14}C in the 2-position of the thiophene ring (23.3 μCi/mg; radiochemical purity >98%) and in the 2-position of the triazine ring (33.9 μCi/mg; radiochemical purity >99%) were synthesized at Du Pont NEN Products (formerly New England Nuclear), Boston, MA. All solvents used in the experiments were of HPLC grade; other chemicals were of reagent grade or better. Scinti-Verse 1 was used for all liquid scintillation counting (LSC) analyses. Metabolite standards were synthesized at E. I. du Pont de Nemours and Co. [see Levitt (1991) and Cuomo et al. (1991) for relevant general methods].

These experiments were conducted in compliance with those Good Laboratory Practice requirements specified in 40 CFR Part 160 and the residue chemistry guidelines defined in Federal Regulation 171-4(a)(2) applicable to plant metabolism studies.

Plant Growth and Treatment. Seeds of Miami soybean [*Glycine max* (L.) Merr.] (reg. no. 177) (Athow et al., 1984) were planted in 8-in. diameter plastic pots in unsterilized Sassafras sandy loam soil (sand, 65%; silt, 27.5%; clay, 7.5%; 1.2% organic matter; pH 6.8) and grown to maturity in a climate-controlled greenhouse equipped with supplemental lighting, located in Wilmington, DE. Seedlings were thinned as necessary to three to four vigorous plants per pot. The plants were watered either by hand or by an automatic drip irrigation system, and once a week a 20-20-20 NPK fertilizer at 3 g/L was included in the irrigation water.

Stock solutions of [*thiophene*-2-^{14}C]- and [*triazine*-2-^{14}C]thifensulfuron methyl were made up in water to produce the

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Residue Levels of Thifensulfuron Methyl in Soybeans

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Table I. Total Radioactive Residues Recovered from Treated Soybean Plants

treatment	days after application	tissue fresh wt,[a] g		concn of total radioactivity,[b] ppm	
		[*thiophene*-2-^{14}C]	[*triazine*-2-^{14}C]	[*thiophene*-2-^{14}C]	[*triazine*-2-^{14}C]
16 g/ha	0	48.8 (14)	46.7 (14)	0.860	0.582
	7	65.7 (11)	54.1 (12)	0.484	0.506
	30	335.0 (14)	332.3 (13)	0.098	0.066
	100 (seeds)	382.4 (24)	247.9 (19)	0.0016	0.0004
	100 (pods)	141.4 (24)	103.5 (19)	0.0089	0.0018
8 g/ha +0.25% X-77	0	43.6 (13)	37.7 (11)	0.648	0.629
	7	35.4 (11)	22.2 (6)	0.583	0.612
	30	151.2 (13)	116.3 (12)	0.141	0.174
	100 (seeds)	326.6 (20)	359.0 (20)	0.0015	0.0010
	100 (pods)	120.8 (20)	138.9 (20)	0.010	0.0042

[a] Number of plants shown in parentheses. [b] Calculated as thifensulfuron methyl equivalents.

Table II. Summary of Extractable and Bound Residues from Treated Soybean Plants

treatment	days after application	rinsed residues,[a] ppm		extractable residues,[a] ppm		bound residues,[a] ppm	
		[*thiophene*-2-^{14}C]	[*triazine*-2-^{14}C]	[*thiophene*-2-^{14}C]	[*triazine*-2-^{14}C]	[*thiophene*-2-^{14}C]	[*triazine*-2-^{14}C]
16 g/ha	0	0.747	0.498	0.123	0.088	0.002	0.003
	7	0.374	0.397	0.088	0.109	0.016	0.019
	30	0.053	0.040	0.032	0.024	0.008	0.005
8 g/ha + 0.25% X-77	0	0.222	0.295	0.348	0.376	0.004	0.016
	7	0.137	0.190	0.371	0.390	0.041	0.057
	30	0.024	0.031	0.088	0.136	0.018	0.016

[a] Concentration calculated as thifensulfuron methyl equivalents.

16 g of ai/ha without surfactant treatment. Radiochemical specific activities were not diluted with unlabeled thifensulfuron methyl.

Part of this stock solution was diluted 1:1 with water, agricultural nonionic surfactant X-77 (Ortho Products) was added to a final concentration of 0.25% (v/v), and the resulting solution was used for the 8 g/ha + 0.25% X-77 treatment. Subsamples of these solutions were analyzed by liquid scintillation counting, and their correct concentrations were confirmed (data not shown).

Individual pots of soybean seedlings (first to second trifoliate growth stage) were placed on a rotating turntable in a ventilated enclosure and were each sprayed with a 2 mL aliquot of the appropriate [14C]thifensulfuron methyl stock solution using a Preval hand-held sprayer to yield treatment rates of 16 g/ha without surfactant and 8 g/ha + 0.25% X-77. This spray volume per pot was equivalent to 65 gal/acre (approximately 600 L/ha) and provided thorough coverage of all foliage and soil surfaces.

Immediately after the spray solution had dried, a random sampling of plants was harvested (defined as day 0 samples) by excising the stem at soil level. The excised foliage was weighed and immediately rinsed in 80% ethanol (see below for details) to remove surface residues. The rinsate was reserved for subsequent analysis, and the rinsed foliage was frozen at -20 °C prior to extraction and analysis.

Additional plants were harvested at 7 and 30 days after treatment and were rinsed and frozen as described above. Fully mature soybeans and pods were harvested 100 days after treatment, weighed, and frozen (-20 °C) until analysis.

Analysis of Surface Residues. Freshly harvested soybean foliage was rinsed with 80% ethanol (EtOH) to remove surface residues for analysis, as follows: The foliage of each excised plant was fully immersed and swirled for 10–15 s in 350 mL of 80% EtOH and then rinsed with fresh 80% EtOH from a squeeze bottle. These rinsates were combined, and the total volume was determined. Aliquots were counted by LSC to determine total radiolabeled rinsed residues. The rinsed plants were blotted carefully and then were frozen at -20 °C prior to extraction and analysis. Day 0 and 7 excised plants were rinsed in their entirety; at 30 days, the plants were very large, and only the lower leaves (first through third trifoliates) that were actually sprayed were rinsed.

The rinsates were concentrated and analyzed as follows: 100 mL of each rinsate was rotary evaporated to dryness and the residue thoroughly resuspended in 5 mL of 5 mM potassium phosphate buffer, pH 7.5, by wrist-action shaking for 2 h. Three 200-μL aliquots were analyzed by LSC. Aliquots (200 μL) were

also analyzed by HPLC, and 1-min fractions were collected and analyzed by LSC (see below).

Extraction and Analysis of Soybean Foliage. Rinsed soybean plants were cut into small fragments with scissors and placed into a large mortar containing liquid nitrogen (LN₂). The frozen tissue was ground with a pestle into a fine powder, and the LN₂ was allowed to evaporate, leaving a thoroughly homogenized frozen powder. Total fresh weight of homogenized tissue was determined. A sample was removed, its fresh and dry weights were determined, and total radioactivity was determined by combustion analysis. The homogenized fresh tissue was extracted as follows: A 5- or 10-g aliquot of the homogenized tissue was added to 30 mL of 80% acetone in a 50-mL capped Corex centrifuge tube and vigorously shaken for 1 h at room temperature on a wrist-action shaker. The sample was then centrifuged for 10 min at 3000g, and the transparent, dark green supernatant fluid was reserved. The extraction was repeated twice with fresh 80% acetone, and the combined supernatant fluids from these three consecutive extractions were rotary evaporated to dryness (about 10 min at 40 °C using water aspiration). The residue was thoroughly resuspended in 20 mL of 80% acetone and transferred to a small vial. The acetone was evaporated under a stream of dry N₂, leaving a dark green aqueous extract. The sample volumes were carefully adjusted to 4.0 mL with distilled water, and three 100-μL aliquots were removed for LSC. The samples were then clarified by centrifugation, and 200-μL aliquots were analyzed by HPLC and TLC (see below).
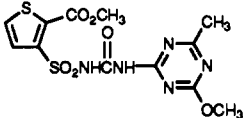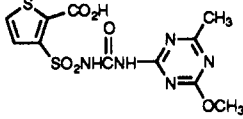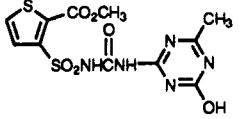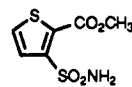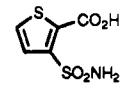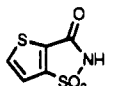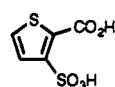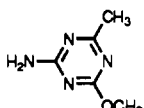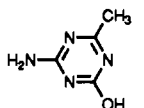
The thrice-extracted pellet was reserved, its dry weight determined, and unextracted radioactivity determined by combustion analysis.

Analysis of Mature Soybeans. Intact, filled mature soybean pods were harvested and weighed, the beans manually removed from the pods, and the beans and empty pods reweighed separately. Beans and empty pods were separately homogenized in a dry Waring blender to yield dry, coarse powders. Aliquots (0.4–0.6 g) of the homogenized dry powders were directly combusted to determine total radioactive residues.

Analytical Methods. Slightly different methods were employed for the separate analysis of samples treated with [*thiophene*-2-^{14}C]- or [*triazine*-2-^{14}C]thifensulfuron methyl.

[*Thiophene*-2-^{14}C] *Chromatography Conditions.* HPLC separations were performed on a Hewlett-Packard HP1090 liquid chromatograph equipped with a diode array detector set at 230 nm. The binary mobile phase was A = H₂O plus 0.1% formic acid and B = acetonitrile plus 0.1% formic acid. The stationary reversed-phase column was Du Pont Zorbax ODS (4.6 mm × 250

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Table III. Identity and Concentration of Thifensulfuron Methyl Metabolites Extracted from Soybean Foliage 30 Days after Treatment (DAT)[a]

structure	concn, ppm			standard HPLC RT, min	standard TLC R_f
	0 DAT	7 DAT	30 DAT		
thifensulfuron methyl	0.332[b] 0.216[c]	0.092[b] 0.087[c]	0.007[b] 0.008[c]	23.4[b] 29.1[c]	0.59 ± 0.02[b] 0.66 ± 0.02[c]
thifensulfuron acid	0.016[b] 0.026[c]	0.164[b] 0.118[c]	0.045[b] 0.049[c]	14.75[b] 15.64[c]	0.10 ± 0.015[b] 0.10 ± 0.015[c]
O-demethylthifensulfuron	ND[d] ND	ND ND	ND ND	14.14[b] 14.91[c]	0.35 ± 0.02[b] 0.20 ± 0.015[c]
thiophene-2-ester-3-sulfonamide	0.027	0.023	0.009	12.22	0.86 ± 0.02
thiophene-2-acid-3-sulfonamide	ND	0.011	ND	5.63	0.31 ± 0.07
thiophenesulfonimide	ND	ND	0.007	3.86	0.45 ± 0.02
thiophene-2-acid-3-sulfonate	ND	ND	0.007	2.80	
aminotriazine	0.053	0.077	0.023	7.30	0.83 ± 0.02
aminotriazinol	0.011	0.038	0.021	2.62	

[a] Application rate 8 g/ha + 0.25% X-77. [b] Methods and results for [thiophene-2-14C] label. [c] Methods and results for [triazine-2-14C] label. [d] Not detected.

mm) operated at 40 °C and a flow rate of 1.4 mL/min. One-minute fractions were collected and analyzed for radioactivity by LSC. The elution gradient was as follows: step 1, 5–20% B, 10-min linear gradient; step 2, 20–25% B, 5-min linear gradient; step 3, 25–35% B, 5-min linear gradient; step 4, 35–50% B, 5-min linear gradient; step 5, 50–70% B, 5-min linear gradient; step 6, 70–100% B, 5-min linear gradient.

[Triazine-2-14C] Chromatography Conditions. HPLC separations were performed on a Perkin-Elmer Series 4 liquid chromatograph equipped with a UV detector set at 245 nm. The binary mobile phase was A = H_2O plus 0.1% formic acid and B = acetonitrile plus 0.1% formic acid. The stationary reversed-phase column was Du Pont Zorbax ODS (4.6 mm × 250 mm) operated at 40 °C and a flow rate of 1.4 mL/min. One-minute fractions were collected and analyzed for radioactivity by LSC.

The elution gradient was as follows: step 1, 10–35% B, 30-min linear gradient; step 2, 35–75% B, 10-min linear gradient; step 3, 75–95% B, 10-min linear gradient.

[Thiophene-2-14C] Thin-Layer Chromatography (TLC). Sample aliquots (20 µL) were chromatographed using a stationary phase of Merck silica gel 60 F254, 250-µm thickness, and a mobile phase consisting of methylene chloride/methanol/concentrated ammonium hydroxide (70/30/3 v/v). Detection was by fluorescence quenching under UV light followed by autoradiography.

[Triazine-2-14C] Thin-Layer Chromatography (TLC). Sample aliquots (20 µL) were chromatographed using a stationary phase of Whatman LK6F silica gel plates, 250-µm thickness, and a mobile phase consisting of methylene chloride/methanol/concentrated ammonium hydroxide (145/50/5 v/v). Detection
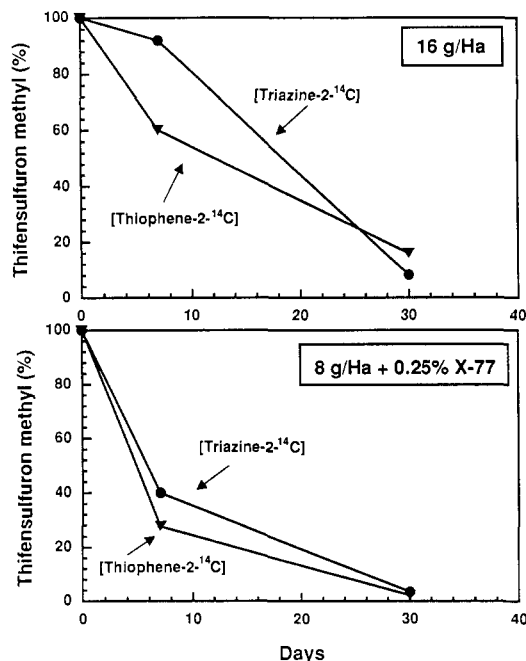
Figure 2. Concentrations of thifensulfuron methyl in soybean foliage, expressed as a percentage of the respective day 0 concentrations. Day 0 concentrations of [*triazine*-2-^{14}C]thifensulfuron methyl were 0.053 ppm at 16 g/ha and 0.216 ppm at 8 g/ha + 0.25% X-77. Day 0 concentrations of [*thiophene*-2-^{14}C]thifensulfuron methyl were 0.092 ppm at 16 g/ha and 0.332 ppm at 8 g/ha + 0.25% X-77.

was by fluorescence quenching under UV light followed by autoradiography.

Combustion analyses were performed using a Packard Instruments Tri-Carb 306 sample oxidizer. Samples were dried in a vacuum oven to constant dry weight prior to combustion.

RESULTS AND DISCUSSION

Thifensulfuron methyl is registered for postemergence broadleaf weed control in soybeans at an application rate of 4.3 g of ai/ha plus 0.125–0.25% v/v nonionic surfactant (Du Pont, 1989). In this study, separate samples of thifensulfuron methyl radiolabeled in the thiophene or triazine ring were applied at 8 g of ai/ha plus 0.25% Ortho X-77 surfactant and at 16 g of ai/ha without surfactant to greenhouse-grown soybean seedlings. These exaggerated application rates were chosen to simulate worst-case residue situations and were the highest rates that can be reasonably tolerated by the crop. The soybeans were grown to maturity, and resulting residue and metabolite patterns were followed for each of the four treatments (i.e., [*thiophene*-2-^{14}C] at 8 g of ai/ha plus 0.25% Ortho X-77 surfactant and 16 g of ai/ha without surfactant and [*triazine*-2-^{14}C] at 8 g of ai/ha plus 0.25% Ortho X-77 surfactant and 16 g of ai/ha without surfactant). Table I shows the number of harvested plants and the tissue fresh weights for foliage harvested at 0, 7, and 30 days after treatment and for mature seeds and pods harvested 100 days after treatment. The margin of soybean tolerance for thifensulfuron methyl can be narrow and is exacerbated by high rates of surfactant and hot, humid conditions (Du Pont, 1989). Some early crop injury was observed in this study, especially in the treatments with surfactant, where symptoms of leaf crinkling and stunting were observed shortly after treatment. Little or no visual injury was observed at 16 g of ai/ha without surfactant. Crop injury is reflected in Table I, where the fresh weights per plant at 8 g of ai/ha plus 0.25% X-77 at 30 days after treatment are about 40–50% those of the 16 g of ai/ha rate without
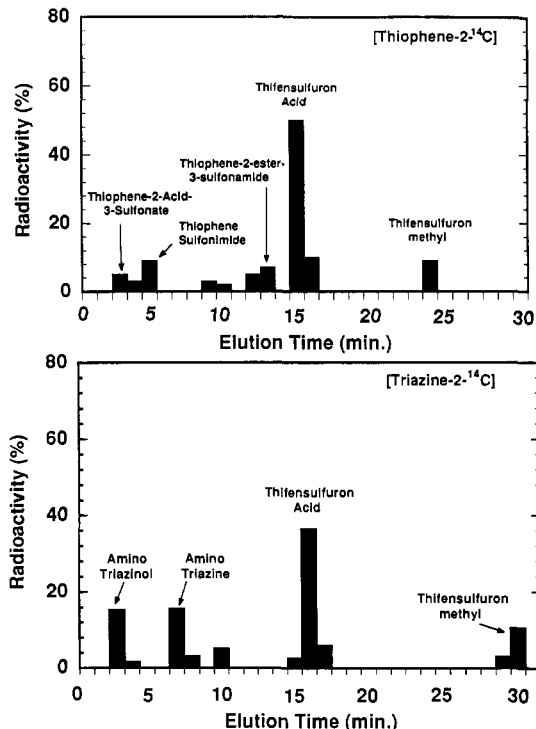

Figure 3. Reconstructed HPLC radiochromatograms of [^{14}C]thifensulfuron methyl and its metabolites extracted from soybean foliage 30 days after treatment with 8 g/ha radiolabeled compound + 0.25% X-77. One-minute fractions were collected and analyzed by LSC, and the data for each fraction are expressed as a percent of the total radioactivity in the chromatogram [2410 dpm ([*thiophene*-2-^{14}C]) and 4212 dpm ([*triazine*-2-^{14}C])]. The [*triazine*-2-^{14}C]- and [*thiophene*-2-^{14}C]thifensulfuron methyl-derived extracts were chromatographed under slightly different conditions, accounting for the difference in elution times of thifensulfuron methyl and thifensulfuron acid between the two chromatograms (see Materials and Methods).

surfactant. However, during the subsequent 70 days of growth, the remaining treated plants had recovered from this initial stunting, and the yields of seeds and pods per plant were approximately equal at both application rates (Table I).

The concentrations of total residues (surface plus tissue radioactivity) are also shown in Table I (expressed as thifensulfuron methyl equivalents). The data show an apparent effect of surfactant on spray retention; the concentrations of total foliage radioactive residues at day 0 are approximately 50–100% higher at 8 g of ai/ha plus surfactant than expected when compared to the residues retained at 16 g of ai/ha. These data are consistent with the increased foliage wetting and solution spreading observed during application of the sample containing surfactant. Table I shows that at these elevated application rates the concentrations of total radioactive residues at day 0 are each less than 1 ppm and fall to approximately 11–27% of the day 0 values by 30 days after application, primarily through growth dilution. Final radioactive residues at harvest were determined by combustion analysis and were very low in mature seeds, ranging from 0.0004 to 0.0016 ppm. Total radiolabeled residues in pods were slightly higher (0.001–0.010 ppm), reflecting the maternal origin of this tissue and its physical connection to the plant's translocation stream. These extremely low residues in mature seeds and pods made impractical their further characterization, but the metabolic pathways elucidated at 30 days after treatment (see below) plus the likelihood of reincorporation of ^{14}CO$_2$ into plant carbon metabolism suggest that these final residues are composed
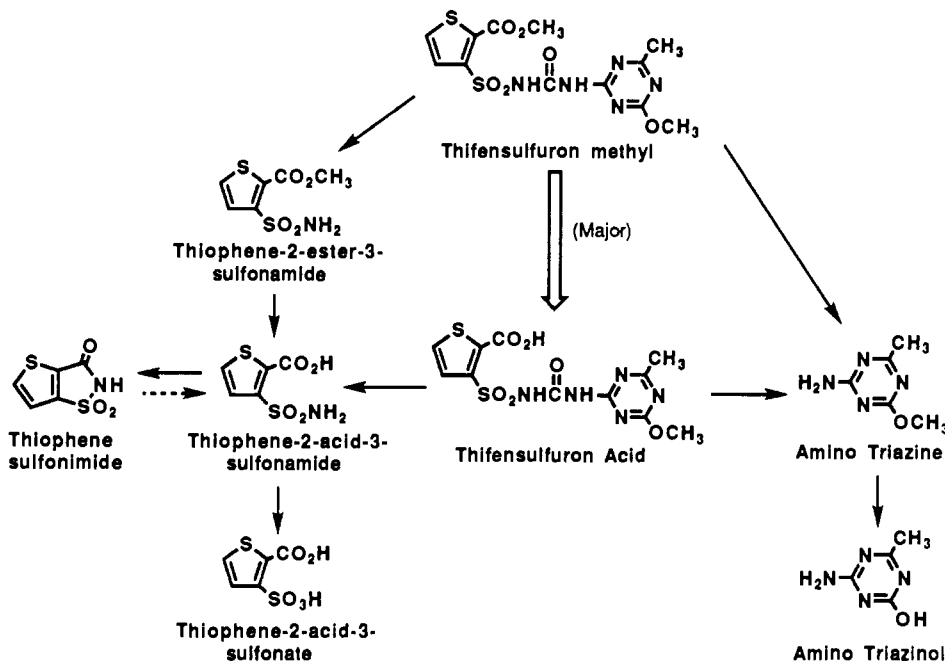
Figure 4. Proposed metabolic pathways for thifensulfuron methyl in soybean foliage.

of multiple components, each at extremely low absolute concentration.

The total foliage residues shown in Table I are composed of surface and penetrated (including extractable and bound) tissue residues. These residue fractions were separated and analyzed as described under Materials and Methods, and the results are shown in Table II. The data of Table II show that surfactant had the effect of increasing the tissue residue concentrations 3–4-fold over those obtained from the 16 g/ha treatment without surfactant. Thus, at 16 g of ai/ha without surfactant, between 14 and 22% of total retained residues penetrated into the foliage (defined as the sum of extractable and bound residues) between days 0 and 7, while thifensulfuron methyl penetrated much more effectively at 8 g of ai/ha plus X-77 surfactant, with between 54 and 64% of the retained residues having entered the plant tissues. The effects of surfactant on residues entering plant tissues likely result from a combination of spray retention, tissue penetration, and the indirect effect of reduced foliage fresh weight due to transient injury. While these data cannot distinguish among these mechanisms, they are consistent with the increased crop injury observed at 8 g of ai/ha plus 0.25% X-77 relative to the 16 g/ha without surfactant treatment (Table I).

As described under Materials and Methods, treated foliage was rinsed by repeated immersion in 80% ethanol to remove surface residues. The rinsed foliage was thoroughly extracted three times with 80% acetone, and residual radioactivity in the extracted pulp was determined by combustion analysis and defined as "bound" residues. The concentrations of these bound residues were highest at 7 days after treatment and declined to less than 20 ppb 30 days after treatment. The concentration of bound residues was higher in the 8 g of ai/ha plus X-77 surfactant, consistent with the greater concentration of extractable radiolabeled residues in this treatment.

While the surface, extractable, and bound residue concentrations in the 30-day foliage were quite low (Table I), they are higher than would be expected in field-grown soybeans due to the elevated application rates and because these greenhouse-grown plants did not experience any rain wash-off of surface residues. These plants were carefully

watered to avoid wash-off, and some of the applied radioactivity remained on the treated leaves throughout the 30-day period and beyond, potentially allowing continued cuticular uptake of these residues.

The rinsed and extractable residues from treated soybean foliage were analyzed by HPLC, as described under Materials and Methods. The rinsed (surface) residues were found to be >94% intact thifensulfuron methyl at 0, 7, and 30 days after application. Minor amounts of the thiophene-2-ester-3-sulfonamide and aminotriazine hydrolysis products (see Table III) composed the remainder of these surface residues (data not shown).

The extractable residues were analyzed to determine the metabolic pathway of thifensulfuron methyl in soybean tissue. Figure 2 shows the concentration of intact thifensulfuron methyl extracted from rinsed tissue at 0, 7, and 30 days after application. The data are expressed as percent of the respective day 0 concentrations, and data for both the thiophene-labeled and triazine-labeled treatments are shown. The concentration of intact thifensulfuron methyl in soybean foliage decreased with an approximate half-life of 12–20 days at 16 g of ai/ha without surfactant and 5–7 days at 8 g of ai/ha plus 0.25% X-77. Thus, thifensulfuron methyl appears to have been more rapidly metabolized in the plants that received the 8 g of ai/ha plus 0.25% X-77 treatment. Since the samples analyzed in Figure 2 were those residues remaining after the surface residues were removed by rinsing, these data suggest that the surfactant, in addition to increasing spray retention, also caused the thifensulfuron methyl to penetrate more readily into cells, where it could be more rapidly metabolized. In the treatments with 16 g of ai/ha without surfactant, the residues not removed by rinsing were apparently still not as thoroughly accessible to cellular metabolism, and perhaps a greater proportion remained in the apoplastic interstices between cells and only slowly diffused into the symplast. However, further studies are required to determine the basis for this observation.

The decline in thifensulfuron methyl concentration results from both metabolic degradation and general growth dilution, since these data are normalized from concentrations based on foliage fresh weight. Previous studies have shown that the metabolic half-life of thifen-

sulfuron methyl is 4–6 h when radiolabeled thifensulfuron methyl is taken up by soybean seedlings through the cut stem. As seen in Figure 2, the rate of metabolism is considerably slower in this study, where the herbicide was sprayed postemergence onto the foliage. This suggests that diffusion from cuticular and/or apoplastic compartments into the metabolically competent symplast is slow and probably rate-limiting to the degradation of thifensulfuron methyl compared to the relatively direct route of entry offered by cut-stem uptake used in prior studies [Brown et al., 1990; for reviews, see Brown (1990), Brown and Kearney (1991), and Brown et al. (1991a,b)].

Figure 3 shows reconstructed HPLC radiochromatograms of thiophene-labeled and triazine-labeled thifensulfuron methyl and metabolites extracted from soybean foliage 30 days after treatment with 8 g of ai/ha plus 0.25% X-77. The data for each 1-min fraction are expressed as a percent of the total radioactivity in the chromatogram; the analytical methods and equipment differed slightly in the analyses of the two labeled forms of thifensulfuron methyl, accounting for the different retention times in the chromatograms for thifensulfuron methyl and its deesterified free acid (see Materials and Methods). Figure 3 shows the extent of metabolism of thifensulfuron methyl in soybean foliage 30 days after postemergence treatment.

Metabolites were identified by cochromatography with authentic standards in two distinct systems (reversed-phase HPLC and normal-phase TLC). Previous studies have shown that the initial metabolite of thifensulfuron methyl in soybeans is its herbicidally inactive, deesterified free acid (Brown et al., 1990), formed with a half-life of 4–6 h when applied through the cut stem via the transpiration stream. Figure 3 shows that the deesterified thifensulfuron acid is also the major metabolite 30 days after postemergence treatment. Additional metabolites are indicated in Figure 3, and their HPLC and TLC retention times and concentrations are quantified in Table III. The thiophene-2-ester-3-sulfonamide, thiophene-2-acid-3-sulfonamide, and amino triazine metabolites result from hydrolysis of the sulfonylurea bridge, either through chemical or enzymatic mechanisms (Beyer et al., 1987b; Brown, 1990; Brown and Kearney, 1991). Additional metabolites 30 days after treatment include the thiophenesulfonimide and the thiophene-2-acid-3-sulfonate, likely derived from the thiophene-2-acid-3-sulfonamide, and the aminotriazinol, likely derived through oxidative O-demethylation of the aminotriazine [see Hutchison et al. (1984) and Cotterman and Saari (1989)]. The aminotriazinol is derived from the aminotriazine and not through bridge hydrolysis of a putative 4-hydroxy-6-methyl triazinyl thifensulfuron methyl (Table III). This compound is a known metabolite of thifensulfuron methyl in wheat (Cotterman and Saari, 1989), but the putative O-demethylthifensulfuron methyl was not detected in previous studies with soybeans (Brown et al., 1990); in this study it was detected neither by HPLC, where its standard is only closely resolved from the deesterified free acid, nor by silica gel TLC, where its standard is well-resolved from the deesterified free acid metabolite.

The metabolic pathways of thifensulfuron methyl during the first 30 days after postemergence application to soybean foliage are summarized in Figure 4.

CONCLUSIONS

The results of this study provide a quantitative example of the effect of low application rates on residue levels in treated crops. Postemergence applications of radiolabeled thifensulfuron methyl at 2 times (plus surfactant) and 4

times (without surfactant) the commercial application rate to soybean seedlings resulted in extremely low final residue levels in harvested soybeans. The initial metabolite of thifensulfuron methyl is its herbicidally inactive deesterified free acid, and subsequent degradation during the first 30 days after treatment follows established or reasonable metabolic routes. These results, combined with the likely further metabolism and growth dilution that should occur during the subsequent 70 days until harvest, suggest that this very low final residue burden is itself composed of multiple components (possibly including reincorporated $^{14}CO_2$), each present at levels far below 1 ppb. Therefore, commercialization of ultralow use rate crop protection chemicals represents a viable approach to addressing concerns about residue levels in agricultural commodities.

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