

Dissipation of Fomesafen, Saflufenacil, Sulfentrazone, and Flumioxazin from a Tennessee Soil under Field Conditions

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Fomesafen, saflufenacil, and sulfentrazone had similar parameters for their mass spectrometry analysis, all being analyzed in negative mode with similar ionization energies. Flumioxazin was analyzed in positive mode using different ionization temperatures and voltage energies, and a larger injection volume (10 µl compared with 2-5 µl) due to lower liquid chromatography-mass spectrometry (LC-MS) detector response. Quantitative limits of detection in soil were < 5 parts per billion by weight for all herbicides. The field study was conducted three times (2010, 2011, 2012) with four blocks of each treatment each year. Herbicide concentrations over time were based on field samples that were later extracted and quantified using the described LC-MS procedures. Data were examined using a simple first-order (SFO) equation with each year-by-herbicide treatment combination regressed using SigmaPlot version 12.5 to determine regression parameters. The SFO rate constant was used to determine a half life, or DT₅₀ (in days) for each curve. All data were analyzed using a GLMMix ANOVA procedure using SAS version 9.3 and contrast statements were used to directly compare each herbicide comparison. Slopes for each herbicide use the SFO curve and were estimated using SAS. The order from shortest to longest DT_{50} was flumioxazin (21.1 d) = saflufenacil (21.4 d) < formesafen (45.6 d) < sulfentrazone (70.8 d). These results concur with the labeled recrop recommendations after application for flumioxazin and saflufenacil, which have shorter cotton plant-back restrictions compared with sulfentrazone and fomesafen. In these studies, none of the herbicides was highly persistent (all half-lives < 100 d), so none would be expected to be persistent pollutants in the environment, although further research is needed in this area.

Nomenclature: Soybean, *Glycine max* (L.) Merr.

Key words: Degradation, herbicide, LC-MS, persistence, soils.

The herbicides examined in this report are in different chemical families, but all inhibit protoporphyrinogen oxidase (protox, PPO), an enzyme of chlorophyll and heme biosynthesis catalyzing the oxidation of protoporphyrinogen IX to protoporphyrin IX. This protox inhibition leads to accumulation of protoporphyrin IX, the first light-absorbing chlorophyll precursor. In a cascading physiological series, free radicals are formed, resulting in degradation of lipids and various membranes, which allows cells to disintegrate rapidly (Duke et al.1991).

Flumioxazin is a dicarboxamide herbicide developed by Valent (Senseman 2007a). Reported halflives in soil are 12 to 18 d under laboratory conditions (Ferrell and Vencill 2003) and 10 to 32 d under field conditions (Alister et al. 2008). In aqueous systems flumioxazin degradation was affected by the presence of light and increased pH (Kwon et al. 2004; Shibata et al. 2011). Flumioxazin

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available in soil solution with an increase in soil water content (Ferrell et al. 2005). Flumioxazin water solubility is 1.79 mg L^{-1} and there is no apparent effect of pH of water solubility (Table 1). Flumioxazin is used in a wide range of crops including soybean, cotton, and several others (Anonymous b 2013). Fomesafen is a diphenylether herbicide developed by Syngenta Crop Protection (Senseman 2007b).

adsorption to soil was most highly correlated with organic matter, although it can become readily

by Syngenta Crop Protection (Senseman 2007b). Water solubility is greater at higher pHs, and is ~ 50 mg L⁻¹ (Table 1). Reported half-lives range from 28 to 60 d (Rauch et al. 2007) and 37 d (Cobucci et al. 1998). Mobility and bioavailability of fomesafen in soils is expected to be lower at low pH than at high or neutral pH (Guo et al. 2003; Weber 1993; Weber et al. 1993). The primary pathway of fomesafen degradation in soils is by microbial activity (Feng et al. 2012). Some data have suggested a possible risk of runoff from treated areas, depending on various environmental factors (Potter et al. 2011). Renewed interest in fomesafen use has been caused by its utility to manage glyphosate-resistant weeds in major row crops (Knezevic et al. 2009).

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Table 1. Attributes of various herbicides and details relevant to experimental conductance.

Herbicide	Water solubility	Effect of pH on water solubility	Molecular mass	Field application dosage	Field application dosage ratio to lowest
	mg L^{-1}		Apparent mass units	g ha $^{-1}$	Ratio
Flumioxazin	1.79	None	354.34	210	1.4
Fomesafen (acid)	~ 50	Greater at higher pH	438.76	280	1.87
Saflufenacil	210 @ pH7	Greater at higher pH	500.0	150	1.00
Sulfentrazone	110	Greater at higher pH	387.19	210	1.4

Saflufenacil is a pyrimidinedione herbicide developed by BASF (Grossman et al. 2010). Its primary use is preplant burndown application (Grossman et al. 2011) and it is labeled in several crops including corn, soybean, and cotton (Anonymous d 2013). Reported half-lives of saflufenacil were 13 d in surface soils and 32 d in subsurface soils (Papiernik et al. 2012). Observed low sorption to soil and rapid dissipation suggested that saflufenacil would be readily available for degradation or plant uptake in the plant root zone. However, saflufenacil injured several rotational crops when applied at higher rates in Canadian soils (Robinson and McNaughton 2012). Water solubility is directly related to pH and is 210 mg L^{-1} at pH 7 (Table 1). Saflufenacil has become a potential herbicide alternative for the effective control of glyphosate-resistant horseweed [Conyza canadensis (L.) Cronq.] in cotton (Waggoner et al. 2011).

Sulfentrazone is a phenyl triazolinone herbicide developed by FMC (Senseman 2007c). The published literature showed several estimates of field half-lives: 14 to 30 d in Colorado (Shaner 2012), 16 d in Georgia (Grey et al. 2007), and 24 to 113 d in Tennessee (Ohmes et al. 2000). Sulfentrazone half-lives under controlled conditions range from 21 to 111 d, with half-lives being correlated to soil pH and soil organic carbon content (Szmigielski et al. 2012). Sulfentrazone sorption was reported to be greater at lower soil pHs when the molecule is more likely to leach in soils (Grey et al. 1997, Ohmes and Mueller 2007, Reddy and Locke 1998, Shaner 2012). Field studies have consistently shown the possibility of sulfentrazone injury to rotational crops depending on environmental conditions (Main et al. 2004, Ohmes et al. 2000, Pekarek et al. 2010). Sulfentrazone has potential to effectively control problematic glyphosate-resistant weeds in soybean (Knezevic et al. 2009).

The herbicides examined in this research all have potential utility to manage glyphosate-resistant

weeds. Other commonly used modes of action have widespread resistance and may provide incomplete control. The use of PPO-inhibiting herbicides has become foundational to effective weed control in many producers' fields. The relative duration of control from the various herbicides remains in question. This research provides a direct comparison of the dissipation of these four chemicals under field conditions. This report also provides details about chemical analysis and optimizing mass spectrometry for the respective chemicals. Improvements in the chemical analysis of herbicides, especially by LC-MS, have been made in recent years, but few details concerning the analysis of these specific chemicals in soil extracts are available in the published literature.

Materials and Methods

Field Site Establishment. Field studies were conducted over a 3-yr period (2010 to 2012) in a loam soil near Knoxville, TN (35.972695N, 83.855008W). A different plot area with no previous residual herbicide application was used each year. The soil series was a Sequatchie loam (fine-loamy, siliceous, thermic, Typic Paleudult) with 36% sand, 44% silt, 20% clay, 1.9% organic matter, and pH of 6.0. The studies were arranged in a randomized complete block design with four replications. Each plot was four rows wide (3 m) by 10 m in length. Soybean (Allen variety, the glyphosate isoline of 5601T, CV441-PI 630984) was planted using field equipment at a depth of 1 cm in early May of each year. Herbicides were applied immediately after soybean planting in 190 L ha⁻¹ of water carrier at 275 kPa using 8002 flat-fan nozzles. The field study was maintained weed-free by applying POST glyphosate as needed. Rainfall data were collected by an on-site weather station, which was located ~ 150 m from the field site.

Soil samples from 0- to 8-cm depth were collected using a hand-held, 10-cm-diam golf-type cup cutter. This sampler collected a large volume of

Table 2. Chemical analysis settings in methods used to determine herbicide concentrations.

Parameter	Units	Flumioxazin	Fomesafen	Saflufenacil	Sulfentrazone
Mobile phase acetontrile : water	v/v	70:30	80:20	70:30	60:40
Injection volume	μl	10	2	2	5
Drying gas	$L min^{-1}$	5.0	10	11	6.0
Nebulizer pressure	Bar	60	60	60	30
Drying gas temperature	С	30	250	250	250
Vaporizer temperature	С	30	250	200	175
Mode		Positive	Negative	Negative	Negative
Capillary voltage	Volt	1,800	3,000	3,000	600
Corona current	μΑ	1.0	0	0	20
Charging voltage	Volt	1,500	1,000	1,000	1,000
Single ion monitored	Apparent mass units	355.10	437.00	499.00	385.00
Limit of detection	Ppbw	5	1	0.5	1
Retention time	Min	5.72	4.86	5.69	5.64

soil (each sample ~ 1,000 g) and performed well under a variety of soil conditions. Two soil samples from each plot were placed into a plastic bag that was closed and immediately placed in a cooler and then quickly (< 30 min) into a freezer at ~ -10 C. Care was taken not to allow samples inside the bags to remain in the sun and possibly degrade the herbicides. Soil samples were collected the day of application (day after treatment [DAT] 0) and at approximately 7, 14, 21, 28, 42, 56, and 70 DAT in 2010 and 2011, and also at ~100 and 200 DAT in 2012.

Soil Sample Processing. The chemical analysis was based upon previous work with a similar soil (Mueller and Steckel 2011). For sample processing, the soil sample in each bag was allowed to thaw for approximately 30 min and thoroughly homogenized by hand while still inside the plastic bag; then a 40 \pm 0.5 g subsample was placed into a 250-ml low-density polyethylene Nalgene bottle (www. Fishersci.com). These subsamples were refrozen until extraction. Herbicide was extracted from the subsample by adding 2 ml of methanol per gram of soil and shaking for 14 h on a reciprocating shaker. Extracts were passed through a 0.45-µm filter (FisherBrand 25-mm, 0.45-µm polytetrafluoroethylene, nonsterile, cat. no. 09-730-21 from www. fishersci.com) before liquid chromatography, and 2 to 10 µl were injected directly without concentration. The separation was accomplished using a C18 column (150 mm by 4.6 mm with 3-µm packing, Phenomenex Luna column, part no. 00F4251-E0 from www.phenomenex.com). Column temperature was 25 C. All mobile-phase mixtures utilized acetonitrile and water (all solvents LC-MS grade), both fortified with 0.1% formic acid (Fluka chemicals, from www.SigmaAldrich.com). Retention

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times for the various herbicides were from 4.8 to 5.7 min (Table 2). Recoveries from fortified untreated soil samples indicated that recovery was 85 to 94% for each herbicide (data not shown). After extractions, all recoveries were corrected for antecedent soil moisture in each sample. The limit of detection for each herbicide was approximately 3 parts per billion soil basis.

Analytical Method Development. An Agilent LC-MS system (www.Agilent.com) was used to analyze the samples. The analytical system consisted of a quaternary pump with low-pressure mixing, an automated sample injector capable of 1- to 100-µl injections, a thermally controlled column compartment, a diode array detector, and a single quadrapole mass spectrometer. The general method development outline involved making ~ 10 ppmw stock solutions of each herbicide in methanol and utilizing the diode array detector to verify retention times. The mobile phase for all herbicides consisted of acetonitrile : water in various ratios and both mobile-phase constituents were fortified with 0.1% formic acid to maintain constant ion strength. The mobile-phase ratios were optimized so that the retention time of the parent herbicide was from 5 to 7 min, thus having a capacity factor in our system of approximately 2.2. An aliquot of the stock solution was injected and the resulting chromatogram scanned at the appropriate retention time for that specific herbicide using MS to determine the appropriate ion for later use. The goal of method development was to find the ionized parent molecule for each herbicide, searching in both negative and positive ionization modes. Once the parent ion's apparent mass unit size was discovered, subsequent method development utilized the fast injection analysis feature of the Agilent software to

Table 3. Regression descriptors for herbicide dissipation curves.

Year	Chemical	a ^a	b ^a	r^{2a}	Half-life
		ppbw			d
2010	Flumioxazin	112.2	0.0757	0.84	9.2
2010	Fomesafen	124	0.0290	0.58	23.9
2010	Saflufenacil	122.4	0.0626	0.94	11.1
2010	Sulfentrazone	89.4	0.0158	0.87	43.9
2011	Flumioxazin	63.5	0.0315	0.93	22.0
2011	Fomesafen	197.9	0.0220	0.92	31.5
2011	Saflufenacil	68.4	0.0391	0.78	17.7
2011	Sulfentrazone	126.3	0.0192	0.89	36.1
2012	Flumioxazin	199.2	0.0455	0.92	15.2
2012	Fomesafen	112.8	0.0094	0.65	73.7
2012	Saflufenacil	88.8	0.1179	0.95	5.9
2012	Sulfentrazone	138.6	0.0100	0.82	69.3
Average ^b	Flumioxazin	124.9	0.03285		21.1
Average ^b	Fomesafen	144.9	0.01521		45.6
Average ^b	Saflufenacil	93.2	0.03238		21.4
Average ^b	Sulfentrazone	118.1	0.00979		70.8

^a Parameter estimates and r^2 for year-by-herbicide curves were based on regressing means of each day after treatment using Sigmaplot 12.5 version software.

^b Simple First Order regression analysis across years based on SAS regression of log-ppbw using mixed-model analysis. Parameter estimates for initial concentration based on numerical average of parameter *a* estimates from other analyses.

optimize the signal response for that particular herbicide's apparent ion. Once the MS parameters were optimized, the final method for each herbicide examined the sample extracts using single ion monitoring (SIM) for the apparent molecular ion to maximize sensitivity and minimize baseline interferences. Optimized MS analytical conditions are listed in Table 2.

Regression analysis is an important component in examining herbicide dissipation in soils. Many modelers prefer to have a SFO rate constant for use of their models, and those values were calculated using regression functions embedded within Sigma-Plot version 12.5. All data were analyzed using a mixed-model ANOVA procedure using SAS version 9.3 (Cary, NC) and contrast statements were used to directly compare each herbicide comparison. Slopes for each herbicide use the SFO curve and were estimated using SAS version 9.3 to compare the different herbicides.

Results and Discussion

Manufacturers of mass spectrometers have not standardized their nomenclature with respect to operating conditions for different brands of instruments. The instrument used in our research was manufactured by Agilent Technologies. The settings for analysis will provide direct utility or at least partial insight into analytical methods for these herbicides (Table 2). Fomesafen, saflufenacil, and sulfentrazone had similar parameters for their MS analysis, all being in negative mode and similar ionization energies. Injection volumes were lower (2) to 5 µl) due to greater detector response. Flumioxazin was analyzed in positive mode using less energetic ionization energies and a larger injection volume (10 μ l), which was due to a lower detector response. Flumioxazin lacks a functional pK_a , so it behaves differently from the other herbicides with respect to pH effects (Table 1). All four herbicides would be considered to be moderately water soluble (Table 1). All have molecular masses > 350, which allows for greater sensitivity in the chemical analysis due to fewer interfering ions when the MS is operated in SIM mode.

The application dosage for the herbicides varied from 150 to 280 g ha⁻¹ (Table 1). These are all within the label application rates for these chemicals and no soybean injury was observed from any treatments in the field (data not shown). The soybeans were present to provide the actual soil environment under which the herbicides would be dissipating. Previous research has indicated that the presence of a crop can affect herbicide dissipation under field conditions (Gallaher and Mueller 1996).

The first-order regression analysis provided several parameters for use in discussing the data. The parameter "a" is a measure of herbicide concentration in parts per billion at the time of application, presented for each individual curve and

Table 4. GLMMix ANOVA for log ppbw showing contrast statements of each possible pair-wise comparison of herbicides.

Herbicides compared	F-value	Prob. $> F$
Fomesafen vs. saflufenacil	49.98	< 0.0001
Fomesafen vs. sulfentrazone	5.02	0.0255
Fomesafen vs. flumioxazin	53.08	< 0.0001
Saflufenacil vs. sulfentrazone	86.56	< 0.0001
Saflufenacil vs. flumioxazin	0.04	0.8467
Sulfentrazone vs. flumioxazin	90.75	< 0.0001

as an average of 3 yr for each herbicide (Table 3). Thus the average initial flumioxazin concentration based on the regression analysis was 124.9 ppb. The theoretical ratio of the four herbicides normalized to the lowest initial application rate, saflufenacil at 150 g ai ha^{-1} , for the four herbicides in alphabetical order are 1.4 : 1.87 : 1.0 : 1.4 (Table 2). The same ratios calculated using parameter a are 1.34 : 1.55 : 1.00 : 1.26, which is in close agreement with the targeted application dosage from a relative perspective. Initial concentrations were somewhat lower than anticipated, but this may have been due to initial sampling being somewhat deeper (and thus possibly diluting the soil sample) or due to the regression analysis for parameter a crossing the y-intercept at a value below the 0 DAT concentration. The initial herbicide application appears to have been conducted properly on the basis of this analysis. Contrast statements indicate clear dissipation rate differences (Table 4). All direct comparisons are different (P < 0.05), except saflufenacil compared with flumioxazin. Data from all 3 yr indicated consistent results in herbicide dissipation (Table 3).

Rainfall has a clear direct and indirect effect on herbicide dissipation under field conditions, with possible leaching below the sampling zone and microbial degradation of the herbicides. Rainfall patterns for the 3 yr were similar in the total amount rainfall during the sampling interval (Figure 1). In 2010 there were somewhat drier periods from 3 to 8 wk after herbicide application, and this might have slowed herbicide dissipation. Rainfall during the week before herbicide application of greater than 50 mm provided for adequate soil moisture for soybean growth and possible herbicide degradation in 2010. The early part of the growing season of 2011 was dry, with less than 20 mm of rain from 0 to 4 wk after herbicide application (Figure 1). Rainfall after 5 wk was abundant and would have promoted herbicide dissipation. In 2012 there was abundant rainfall from 0 to 5 wk after herbicide application, and

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Figure 1. Rainfall at field location in Knoxville, TN in 2010 to 2012, normalized to the day of planting for each year.

there was a dry interval from 6 to 8 wk after planting (Figure 1). In 2012 some later sampling points were added to the design, although many were close to the previous estimates of herbicide concentration.

Herbicide dissipation in 2010 followed firstorder kinetics, with r^2 from 0.58 to 0.94 (Table 3). Half-lives varied from 11.1 to 43.9 d, with saflufenacil and flumioxazin having shorter halflives than fomesafen, which was shorter than sulfentrazone. In general, the largest standard errors around each sampling point were found early in the sampling interval, especially on 0 DAT (Figure 2).

Herbicide dissipation in 2011 fit first-order kinetics (Figure 3), with $r^2 > 0.78$ (Table 3). Standard errors for fomesafen and sulfentrazone



Figure 2. Herbicide dissipation in a field study in Knoxville, TN in 2010. Data points represent mean \pm standard error of four replications. Solid line is first-order regression curve. See Table 3 and text for parameter values.



Figure 3. Herbicide dissipation in a field study in Knoxville, TN in 2011. Data points represent mean \pm standard error of four replications. Solid line is first-order regression curve. See Table 3 for parameter values.

data appear to be greater than for the other two chemicals in 2011.

Herbicide dissipation fit first-order kinetics in 2012 (Figure 4, Table 3). The 0-DAT concentration for flumioxazin was substantially higher in 2012, and this concentration was also subject to a greater amount of error (Figure 4). The addition of the data point at greater than 200 DAT provided no



Figure 4. Herbicide dissipation in a field study in Knoxville, TN in 2012. Data points represent mean \pm standard error of four replications. Solid line is first-order regression curve. See Table 3 and text for parameter values.

change in the error from the line previously established for the other data points. The standard errors associated with the sulfentrazone measurements tended to be greater than for the other three herbicides in 2012, for reasons the authors do not know.

Averaged over the 3 yr the herbicides grouped into two types of dissipation, with saflufenacil and

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flumioxazin having relatively short half-lives (21.1 and 21.4 d, respectively), and fomesafen and sulfentrazone having somewhat longer half-lives (46 and 71 d respectively) (SAS analysis). This differential persistence is consistent with the amount of residual control of the flumioxazin and the saflufenacil products, in that they provide a short period of residual weed control of sensitive species. Fomesafen and sulfentrazone tend to provide more control into the growing season, and this is consistent with the half-lives reported here. These results would concur with the labeled recrop after application for flumioxazin and saflufenacil, which have less designated time needed before cotton may be planted compared with sulfentrazone and fomesafen (Anonymous 2013a,b,c,d; Waggoner et al. 2011).

Environmental parameters can greatly affect herbicide persistence in soils. In each year there was adequate rainfall to move the herbicide into the soil and to allow for herbicide degradation during the growing season. In the absence of rainfall or irrigation, herbicide persistence is greatly reduced because of a lack of movement into the soil and thus a greater potential for rapid photodegradation or volatilization losses. With no rainfall soon after PRE herbicide application the weeds may still germinate and grow through any herbicide residue present. The emphasis in this study was on the dissipation of these herbicides, and no efficacy data were recorded. The presence or absence of high populations of weeds would have compromised and confounded the dissipation data.

The PPO-inhibiting herbicides are currently essential components of herbicide use strategies to manage glyphosate-resistant weeds. This study documented that all four materials should provide some residual control and that, if rainfall is adequate during the early part of the growing season, the chances for rotational crop injury are minimal. Higher application doses, more frequent applications, applications later in the growing season, environmental conditions that disfavor herbicide degradation, or the planting of sensitive rotational crops could all result in possible crop injury.

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