Acifluorfen Sorption and Sorption Kinetics in Soil

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Factors which influence kinetics of sorption for acifluorfen, a postemergence herbicide, in soil were evaluated. Twelve soils of varying characteristics were used in this study. Sorption kinetics experiments with 5.86 μM ^{14}C-labeled (19.6 Bq mL^{-1}) acifluorfen were conducted for equilibration times up to 96 h. Sorption was rapid for all soils, and most soils reached a pseudo-equilibrium after 24−48 h of equilibration. Apparent sorption increased at later times, possibly reflecting sorption of acifluorfen or metabolites, since acifluorfen is subject to biotransformation. Further investigation comparing sorption at 4 and 25 °C indicated that microbial metabolism was occurring for some soils at equilibration times longer than 24−48 h. Aminoacifluorfen was observed in methanol extracts of soils equilibrated 96 h. Soils were equilibrated for 24 h with ^{14}C-labeled acifluorfen (2.6−65.8 μM, 19.5 Bq mL^{-1}) to obtain sorption isotherms. Capacity for acifluorfen sorption in these soils generally increased with increasing soil organic carbon content, cation exchange capacity, and soil acidity.

INTRODUCTION

Acifluorfen (5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid) is used extensively as a postemergence herbicide in soybeans (*Glycine max* L.) (Johnson et al., 1978) and peanuts (*Arachis hypogaea*). Although postemergence herbicides are applied to foliage (Ritter and Coble, 1981a,b; Hook and Glenn, 1984; Willingham et al., 1989), some herbicide likely reaches the soil surface, depending on canopy density and washoff (Reddy et al., 1994).

Upon contact with soil, acifluorfen is subject to sorption processes. Mechanisms of herbicide sorption to organic components in soil include hydrogen bonding, electrostatic interaction, van der Waals forces, and hydrophobic partitioning (Hasset and Banwart, 1989; Stevenson, 1976). Acifluorfen sorption in soil has correlated positively with soil organic matter content (Pusino et al., 1993; Gennari et al., 1994b). Soil pH can also influence acifluorfen sorption. At soil pH above pK_a 3.5 (Roy et al., 1983), acifluorfen is predominantly negatively charged and would be expected to have little sorption in many soils. In soils with significant pH-dependent charge, increased sorption may result from increased anion exchange capacity. Lower water solubility of acifluorfen (solubility of acifluorfen acid = 120 mg L^{-1}, 25 °C) at reduced pH may also be a factor influencing retention. Protonation of the carboxyl group under acidic conditions could enhance hydrogen bonding (Pusino et al., 1993).

Acifluorfen sorption to two humic acids was inversely related to pH, but the magnitude of the pH effect varied between humic acids (Ruggiero et al., 1992). The work by Negre et al. (1995) studying effects of ethylene oxide sterilization on acifluorfen sorption in humic acid and whole soil substantiated these effects of pH and organic carbon on acifluorfen sorption. Gennari et al. (1994b) also observed a trend in increased acifluorfen sorption with decreasing pH when studying several soils of various physical and chemical properties.

Acifluorfen can form ligand exchange bonds with metal ions such as Cu(II) (Kozlowski, 1990), but the extent to which this may occur in soil is not well-known. Pusino et al. (1991) found that, when acifluorfen was equilibrated with homoionic montmorillonitic clays, divalent and trivalent cations were extracted from the clay interlayer and deposited as precipitates on the clay surface. Acifluorfen sorption in soil was positively correlated with cation exchange capacity (CEC) (Pusino et al., 1993). Saturating soil with Na$^+$ reduced acifluorfen sorption, while Ca^{2+} saturation increased sorption. This behavior may be due to the different solubilities of acifluorfen metal complexes: sodium acifluorfen is highly soluble, whereas calcium acifluorfen is less soluble ($K_{sp} \approx 1.5 \times 10^{-9}$). The likely binding site on the acifluorfen molecule for metal complexation is the carboxylate group (Kozlowski, 1990; Pusino et al., 1991), a contention supported by QSAR analysis of acifluorfen indicating that 70% of the negative molecular electrostatic potential is located in the vicinity of the NO$_2$-COOH region of the molecule (Nandihalli et al., 1992).

Existing research on acifluorfen sorption has ignored the time-dependent aspects of these reactions. Nevertheless, sorption kinetics may influence the fate and transport of chemical species in the soil environment and are important to assessing potential offsite transport and environmental impact. The primary objective of the present study was to characterize factors which influence sorption and sorption kinetics of acifluorfen in a wide variety of soils.

MATERIALS AND METHODS

Soils. Soils used in these studies included Dundee loam and silty clay loam (fine-silty, mixed, thermic Aeric Ochraqualf), Dundee silt loam from long-term no-tillage (NT) and conventional tillage (CT) soybeans, Weswood silt loam (fine-silty, mixed, thermic Fluventic Ustochrept), Mahan loamy fine sand and fine sandy loam (clayey, kaolinitic, thermic, Typic Hapludult), Miami silt loam (fine-silty, mixed, mesic, Typic Endoaquoll) from NT or CT soybeans, Ships clay (very-fine, mixed thermic Udic Chromustert), Sharkey clay (very-fine, montmorillonitic, nonacid, thermic Vertic Haplaquept), and Lafitte organic muck (euic, thermic Typic Medisaprist). Organic

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Table 1. Characteristics of the Soils

soil	pH, 1:1, CaCl$_2$	clay content (%)	CEC (cmol(+)kg^{-1})	organic carbon (g kg^{-1})	sorption K_d[a] (L kg^{-1})	sorption K_{oc}[b] (L kg^{-1})	Freundlich K_f[c]	Freundlich $1/N$[c]
Dundee loam (0–10 cm)	5.59	13.1	12.1	7.47	0.48	64.3	0.84(0.088)	0.85(0.028)
Dundee silty clay loam (0–10 cm)	5.08	35.2	19.5	7.48	0.56	74.9	0.89(0.062)	0.87(0.019)
Dundee silt loam (CT) (0–5 cm)	5.29	22.0	14.3	11.9	1.30	109.5	2.04(0.103)	0.82(0.015)
Dundee silt loam (NT) (0–5 cm)	5.13	22.0	16.7	22.4	3.15	140.5	5.22(0.252)	0.80(0.016)
Lafitte muck (0–10 cm)	4.10	20.0	78.0[d]	191.3	89.6	468.4	92.1(3.09)	0.90(0.035)
Mahan loamy fine sand (0–13 cm)	4.20	4.7	3.8	12.0	1.66	138.0	2.64(0.129)	0.80(0.015)
Mahan fine sandy loam (26–36)	4.40	14.8	4.1	0.10	0.86	8600	1.22(0.098)	0.86(0.023)
Miami silt loam CT (0–10 cm)	6.16	40.0	14.1	19.0	1.10	57.9	1.64(0.328)	0.80(0.055)
Miami silt loam NT (0–10 cm)	6.36	40.0	15.6	35.0	1.80	51.4	2.34(0.144)	0.87(0.018)
Sharkey clay (0–10 cm)	6.00	61.0	43.7	16.9	2.16	128.2	3.24(0.107)	0.82(0.010)
Ships clay (0–10 cm)	7.50	50.0	40.1	8.34	0.61	73.1	0.98(0.156)	0.82(0.043)
Weswood silt loam (0–10 cm)	7.70	21.0	16.4	3.11	0.30	95.2	0.47(0.064)	0.84(0.036)

[a] Linearized sorption $K_d = x/m/C$, where $x/m = \mu$mol kg^{-1}, $C = \mu$mol L^{-1}. [b] $K_{oc} = K_d/$(kg of organic C/kg of soil). [c] $K_f = [x/m]/C^{(1/N)}$; numbers in parentheses are the asymptotic standard errors. [d] Value was obtained from published values of representative Lafitte soils (Clark and White, 1978).

carbon, pH, CEC (Rhoades, 1982; Hendershot and Duquette, 1986), and clay content of soils are presented in Table 1. Soils were air-dried and sieved to <2 mm size particles.

Chemicals. Technical grade acifluorfen (98% purity) and aminoacifluorfen (98.9%) were purchased from Chem Service (West Chester, PA). CF^3-Ring-UL-^{14}C-labeled acifluorfen (99%, specific activity 667 MBq mmol^{-1}) was provided by BASF Corp. (Research Triangle Park, NC). Both [^{14}C]acifluorfen and technical-grade acifluorfen were used without further purification. Herbicide stock solutions were prepared in methanol and stored at 4 °C in the dark until use. Appropriate concentrations of ^{14}C and technical-grade working solutions were prepared in 0.01 M CaCl$_2$ or deionized H$_2$O.

Analysis. Measurement of radioactivity in all sample solutions was by scintillation counting (Packard Tri-Carb 4000, Packard Instrument Co., Downers Grove, IL) after mixing with Ecolume scintillation cocktail (ICN, Costa Mesa, CA).

Selected samples were analyzed by high-pressure liquid chromatography (HPLC) with a Waters (Waters, Milford, MA) HPLC system (Model 510 pump, 712 WISP autosampler, 490E UV detector, System Interface Module) equipped with an Alltima C-18 reversed-phase column (5 μm, 250 mm) (Alltech, Deerfield, IL). Mobile-phase conditions were isocratic acetonitrile:H$_2$0 (pH 3.2, H$_3$PO$_4$) (60:40) at 1 mL min^{-1} flow rate. Acifluorfen and metabolites were monitored using UV detection (230 and 296 nm), and ^{14}C-labeled analytes were monitored using a Beta-Ram detector (INUS Systems, Inc., Tampa, FL). Acifluorfen and aminoacifluorfen HPLC retention times (RTs) were 12.7 and 13.8 min, respectively.

In thin-layer chromatography (TLC) analysis, 50-μL sample aliquots were spotted on the pre-adsorbent area of silica gel TLC plates (20 × 20 cm, 250-μm silica gel, Whatman, Clifton, NJ). Plates were developed to 10 cm in a toluene:ethyl acetate: acetic acid:H$_2$O (50:50:1:0.5, v/v/v/v) solvent system. Chromatograms were analyzed with a Bioscan System 200 Imaging Scanner (Bioscan, Washington, DC). Acifluorfen and aminoacifluorfen corresponded to R_f values of 0.14 and 0.26, respectively.

Sorption Equilibrium and Kinetics. Batch techniques were used to characterize acifluorfen sorption. A soil:solution ratio of 1:4 was used with Lafitte due to high organic carbon content (Table 1), whereas a ratio of 1:2 was used with all other soils. Five grams of air-dry soil (2 g for Lafitte) were weighed into 25-mL Pyrex centrifuge tubes, 10 mL (8 mL for Lafitte) of acifluorfen solution was added, the tubes were sealed with Teflon-lined caps, and suspensions were equilibrated on a rotary shaker at 25 °C for prescribed periods of time. At the end of each shaking interval, samples were centrifuged 10 min (15g, 12 °C, Beckman J2-21, Beckman Instruments, Palo Alto, CA) and aliquots were counted for radioactivity. The difference between input solution concentration and supernatant concentration after shaking was attributed to sorption. Concurrent blanks indicated no herbicide sorption to the tubes and caps.

In the kinetics experiment, soils were treated with 5.86 μM ^{14}C-labeled (19.6 Bq mL^{-1}) acifluorfen. Reaction times on the shaker were 15 min or 1, 3, 6, 12, 24, 48, 72, or 96 h. In the equilibrium experiment, soils were treated with varying concentrations of ^{14}C-labeled acifluorfen (ranging from 2.6 to 65.8 μM, 19.5 Bq mL^{-1}) to establish isotherms. Approximate time to reach pseudo-equilibrium, as determined from the kinetics experiment, varied from 24 to 48 h depending on the soil; but for consistency, all soils in this experiment were equilibrated for 24 h. In both experiments, each treatment combination was replicated at least four times.

Microbial Population Dynamics and Biodegradation during Equilibration. The potential for microbial degradation of acifluorfen during equilibration was examined using Dundee silt loam (NT and CT) and Sharkey clay. Conditions of the kinetics experiment were duplicated, and serial dilutions of the resulting supernatants were spiral-plated on agar plates (Spiral Instruments, Bethesda, MD) to determine total and gram-negative bacteria in the supernatants (Reddy et al., 1995). Four replications were used.

A kinetics experiment was conducted at either 4 or 25 °C with Dundee silt loam (NT and CT) and Sharkey soils to ascertain the extent of apparent sorption possibly attributable to degradation. Other conditions were the same as in the previous 25 °C kinetics experiment. One solution with a concentration of 5.86 μM acifluorfen (39.3 Bq mL^{-1}) was used. Equilibration time periods were 1, 24, 48, 72, or 96 h, with four replications per soil and time period combination. At the end of each shaking interval, samples were centrifuged and aliquots were counted for radioactivity.

A more detailed study was conducted at 25 °C with Dundee silt loam CT soil to evaluate acifluorfen metabolism during incubation (incubation increments were 0, 24, 48, 96, and 144 h). Five grams (oven-dry equivalent weight) of field moist (16% w/w) soil were added to sterile 25-mL tubes, and the soil was treated with 8 mL of 30 μM acifluorfen (406 kBq L^{-1}) in 0.125 M, pH 7 potassium phospate buffer. Sufficient potassium phosphate buffer was added to bring the final volume to 10 mL. The samples were shaken for the previously specified intervals, and the aqueous phase was separated by centrifugation for analysis of acifluorfen and metabolites. The soils were then extracted with 10 mL of methanol followed by an extraction with 10 mL of methanol:0.5 N NaOH (95:5, v/v). Aliquots from each fraction were mixed with Ecolume scintillation cocktail and counted for total radioactivity. Extracts from each fraction were acidified with 1 N HCl, diluted with deionized water, filtered through a solid-phase C-18 extraction (SPE) column, and eluted with methanol. Acifluorfen and metabolites were analyzed using HPLC and TLC.

Effect of CaCl$_2$ as a Background Solution. Dundee silt loam CT and Sharkey clay soils were used to assess the effect of 0.01 M CaCl$_2$ background on acifluorfen sorption (Pusino et al., 1991, 1993). Soils were treated as described previously with 5.86 μM ^{14}C-labeled (0.959 kBq L^{-1}) acifluorfen dissolved in either 0.01 M CaCl$_2$ or deionized H$_2$O. Samples were equilibrated for 24 or 96 h at 25 °C, centrifuged, and sampled as described previously. The supernatant was analyzed with HPLC.

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After the supernatant was removed, soils were extracted twice with methanol:Tris buffer (0.05 M, pH 7) (80:20). The methanol extracting solution was buffered at pH 7 to minimize sorption due to protonation at low pH and to reduce extraction of humic material at basic pH. Solutions from the first methanol extraction were analyzed with HPLC with no further processing. Remaining methanol extracts were evaporated, diluted to 50 mL with acidified water (adjusted to pH 3 with H_3PO_4), filtered through C-18 SPE columns, and eluted with methanol. The eluted methanol samples were analyzed with TLC.

After the second extraction with methanol, soils were air-dried and ground with a mortar and pestle. Soil samples (0.3 g) were then combusted (Oxidizer 306, Packard Instrument Co.) to determine methanol-nonextractable ^{14}C.

Sorption of Aminoacifluorfen. Sorption of aminoacifluorfen was evaluated in the Dundee CT and Sharkey soils. Four grams of air-dry soil were treated with 8-mL aminoacifluorfen solutions at concentrations ranging from 3.02 to 30.1 μM in deionized H_2O. Samples were equilibrated on a shaker for 24 or 96 h at 25 °C and centrifuged for 10 min at 15g (12 °C), and supernatants were analyzed with HPLC methods described previously. Blanks containing no soil were run concurrently and indicated that sorption to Pyrex tubes and caps was minimal.

Statistical Analyses. A split plot design was used in all experiments. Analysis of variance statistical procedures were used to evaluate effects of variables such as soil and temperature. Standard error (s.e.) was used to indicate variability, and Fisher's least significant difference (LSD) test was used to separate means. Nonlinear regression was used to estimate model parameter coefficients.

Kinetic Models. The time-dependent increase in sorbed acifluorfen up to 48 h was described by a two-site equilibrium/kinetic model

$$S_1 = k_e C^{1/N} \tag{1}$$

$$dS_2/dt = k_f C^{1/N} - k_r S_2 \tag{2}$$

where S_i is sorbed concentration ($\mu mol\ kg^{-1}$), C is solution concentration (μM), k_e ($L\ kg^{-1}$) is the coefficient for sorption at sites 1 exhibiting instantaneous equilibrium, k_f ($L\ kg^{-1}\ h^{-1}$) and k_r (h^{-1}) are rate constants for sorption and desorption, respectively, from sites 2, $1/N$ accounts for sorption nonlinearity, and t (h) is time. Further sorption of ^{14}C beyond 48 h may reflect irreversible sorption of acifluorfen or degradation and sorption of metabolites as well as parent compound. Extension of the two-site model to include a third type of site may account for the former. Alternatively, acifluorfen degradation and time-dependent sorption of metabolites may be qualitatively described by assuming first-order kinetics:
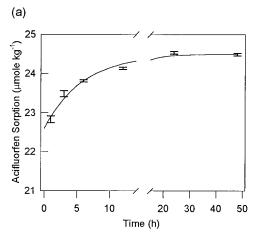
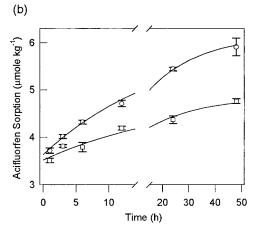$$dP/dt = k_d C - k_p P \tag{3}$$

$$dS_p/dt = (v/m) k_p P \tag{4}$$

where P is the solution concentration of all metabolic products (μM), S_P is concentration of sorbed metabolite ($\mu mol\ kg^{-1}$), k_d (h^{-1}) and k_p (h^{-1}) are first-order rate constants for substrate degradation and metabolite sorption, respectively, v is solution volume (L), and m is sorbent mass (kg).
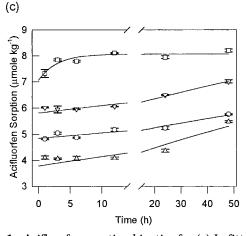
This two-site model (including extensions) was expressed in finite difference form and fitted to the data using nonlinear regression. To reduce the number of fitted parameters, the reaction order $1/N$ was taken from the sorption isotherm data (24-h reaction time). However, k_e, k_f, and k_r were not constrained such that $k_e + k_f/k_r = K_f$ (Freundlich coefficient at 24-h reaction time) because K_f may underestimate the equilibrium value.

RESULTS AND DISCUSSION

Factors Influencing Sorption. Acifluorfen sorption was initially rapid, with from 49% (Weswood) to

Figure 1. Acifluorfen sorption kinetics for (a) Lafitte muck soil; (b) Mahan soil sampled from the 0−13 cm (○) and 26−36 cm (□) depths; and (c) Dundee (CT, ○; NT, □) and Miami (CT, △; NT, ▽) soils. Bars associated with each symbol represent the standard error of each mean value. Curves represent best fits of data using eqs 1 and 2.

93% (Lafitte) of total ^{14}C sorption which occurred taking place within 1 h. In all soils, acifluorfen sorption up to 48-h reaction time was adequately described by the two-site model (eqs 1 and 2). Sorption kinetics up to 48 h for most of the soils are illustrated in Figures 1−3, and model parameters obtained by fitting the data are given in Table 2.

In light of apparent equilibrium conditions at 24−48 h and to minimize the potentially confounding effects of degradation, sorption capacity (Table 1; e.g., Figure 4) was evaluated after a 24-h shaking for all soils. The Freundlich sorption parameter (K_f) values ranged from
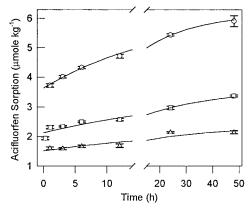
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Figure 2. Effect of soil pH on acifluorfen sorption kinetics in three surface soils: Weswood silt loam (△), Mahan loamy fine sand (○), and Dundee silty clay loam (□). Bars associated with each symbol represent the standard error of each mean value. Curves represent best fits of data using eqs 1 and 2.
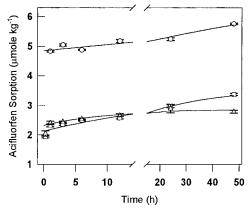

Figure 3. Comparison of acifluorfen sorption kinetics in Dundee loam (△), Dundee silt loam CT (○), and Dundee silty clay loam (□). Bars associated with each symbol represent the standard error of each mean value. Curves represent best fits of dat using eqs 1 and 2.

92.1 L kg^{-1} (Lafitte) to 0.47 L kg^{-1} (Weswood). All sorption isotherms were nonlinear and exponent (1/N) values less than 1 (Table 1), indicating decreasing fraction sorbed with increasing initial acifluorfen concentration. Variable acifluorfen sorption was attributed to several factors. Linear correlations indicated that organic carbon content ($r = 0.99***$), soil H$^+$ concentration ($r = 0.71**$), and CEC ($r = 0.78***$) were positively related to acifluorfen sorption (linearized K_d, Table 1). Multiple regression described these relationships as:

$$K_d = -1.05 + (0.102(\text{organic C}))** +$$
$$(12769(\text{CEC})[\text{H}])**, \quad R^2 = 0.99** \quad (5)$$

Sorptive capacity for the Lafitte muck was more than 1 order of magnitude higher than any other soil (Figure 1a and Table 1). Figure 1b compares sorption between two soil depths of Mahan having similar coarse texture and pH (Table 1) but greater organic carbon and acifluorfen sorption capacity in the surface soil. Higher acifluorfen sorption in the Dundee NT and Miami NT soils compared to their respective CT soils was also attributed to higher organic carbon (Figure 1c and Table 1). Higher organic carbon was also the probable reason for higher acifluorfen sorption in the Dundee CT and NT soils as compared to the other two Dundee soils (Table 1).

Soil pH can also influence acifluorfen sorption (Roy et al., 1983; Pusino et al., 1993; Ruggiero et al., 1992).

At pH levels above its pK_a, acifluorfen is substantially dissociated and subject to negative repulsion in most soils. Protonation of the carboxyl group under acid conditions generates the free acid, thus enhancing hydrogen bonding of acifluorfen to soil components. We compared three surface soils with relatively low organic carbon contents but varying pH: Weswood silt loam is derived from alluvial calcareous material, Dundee silty clay loam is moderately acidic, and Mahan loamy fine sand is acidic (Table 1). Acifluorfen sorption in these soils was inversely related to soil pH (Figure 2, Table 1). Also, some acid soils exhibit higher anion exchange capacity, which could increase sorption of anionic moieties of acifluorfen. This may have been a factor contributing to acifluorfen sorption in the acidic Mahan surface and subsoil, where Fe oxides are abundant and the dominant clay component is kaolinitic.

Cation exchange capacity has been cited as a factor in acifluorfen sorption (Pusino et al., 1993), and a significant positive relationship between CEC and acifluorfen sorption was observed in the present study (eq 5). Because the effect of CEC was interactive with pH and organic carbon, an increase in CEC did not always result in a direct increase in acifluorfen sorption (Table 1). Pusino et al. (1993) found that acifluorfen sorption correlated with organic matter content but not with clay content. Although the source of negative charge in soil is from both organic matter and clay, the negative charge from organic matter may therefore be more important to acifluorfen sorption than the charge derived from clay. Comparisons of acifluorfen sorption among Dundee loam, Dundee silt loam CT, and Dundee silty clay loam soils were used to illustrate this point (Table 1). The Dundee soil developed from Mississippi alluvial material and possesses montmorillonitic clay. Cation exchange capacity increased with increasing clay content for the three soils, but acifluorfen sorption did not increase in the same order (Table 1, Figure 3). Acifluorfen sorption was similar for the soils with the lowest and highest clay content (Dundee loam and Dundee silty clay loam, respectively) but the same organic carbon content. The highest acifluorfen sorption occurred in the soil with the highest organic carbon content but medium clay content (Dundee silt loam CT) (Table 1, Figure 3).
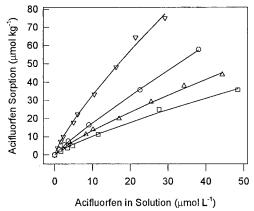
Since Ca−acifluorfen complexes may form in soil (Pusino et al., 1993), we examined the effect of 0.01 M CaCl$_2$ background on acifluorfen sorption. Acifluorfen sorption was greater in solutions with 0.01 M CaCl$_2$ than in H$_2$O for both soils (Table 3). Similarly, methanol-nonextractable ^{14}C increased with time for both CaCl$_2$ and H$_2$O solutions, but tended to be greater for the CaCl$_2$ solutions (Table 3).

Sorption experiments commonly use a salt solution like 0.01 M CaCl$_2$ to mask small differences in ion contents among soils. An important effect of using 0.01 M CaCl$_2$ as an equilibrating solution is increased displacement of acidic cations and increased acidity (Peech, 1965). Koskinen and Cheng (1983) found that increasing salt (CaCl$_2$) concentrations of equilibrating solutions increased sorption of weak acidic herbicides because of lower pH. We believe that potential explanations for enhanced acifluorfen sorption in the CaCl$_2$ solutions (Table 3) were a combination of an increase in the protonated moiety of acifluorfen at lower pH and reaction of the anionic moiety of acifluorfen with exchangeable Al or Fe. We discounted Ca−acifluorfen precipitation as a major mechanism of increased sorp-

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Table 2. Parameters for the Two-Site Equilibrium/Kinetic Model (Eqs 1 and 2) Used To Describe ^{14}C Sorption up to 48 h in Several Soils

soil	k_e (L/kg)	k_f (L/(kg h))	k_r (1/h)
Dundee loam (0−10 cm)	0.63 ± 0.02	0.01 ± 0.01	0.10 ± 0.04
Dundee silty clay loam (0−10 cm)	0.54 ± 0.06	0.02 ± 0.01	0.06 ± 0.06
Dundee CT silt loam, 4 °C (0−5 cm)[a]	1.84 ± 0.06	0.01 ± 0.01	0.01 ± 0.01
Dundee (CT) silt loam, 25 °C (0−5 cm)	1.75 ± 0.05	0.01 ± 0.01	0.01 ± 0.02
Dundee (NT) silt loam, 4 °C (0−5 cm)[a]	4.40 ± 0.06	0.06 ± 0.01	0.03 ± 0.01
Dundee (NT) silt loam, 25 °C (0−5 cm)	3.52 ± 0.38	0.38 ± 0.28	0.29 ± 0.17
Lafitte muck (0−10 cm)	33.5 ± 2.0	3.96 ± 0.65	0.06 ± 0.01
Mahan loamy fine sand (0−13 cm)	1.18 ± 0.02	0.06 ± 0.01	0.04 ± 0.01
Mahan fine sandy loam (26−36 cm)	1.05 ± 0.04	0.03 ± 0.01	0.04 ± 0.02
Miami (CT) silt loam (0−10 cm)	1.26 ± 0.13	0.02 ± 0.02	0.01 ± 0.03
Miami (NT) silt loam (0−10 cm)	2.24 ± 0.06	0.02 ± 0.01	0.01 ± 0.01
Sharkey clay, 4 °C (0−10 cm)[a]	1.97 ± 0.12	0.02 ± 0.01	0.01 ± 0.01
Sharkey clay, 25 °C (0−10 cm)	1.85 ± 0.05	0.10 ± 0.02	0.09 ± 0.02
Ships clay (0−10 cm)	0.68 ± 0.02	0.01 ± 0.01	0.04 ± 0.02
Weswood silt loam (0−10 cm)	0.39 ± 0.03	0.01 ± 0.01	0.04 ± 0.04

[a] Data to 96-h reaction time was fitted.

Figure 4. Freundlich sorption isotherms for acifluorfen showing the effects of tillage in Dundee silt loam (CT, △; NT, ▽) and Miami silt loam (CT, □; NT, ○) soils.

tion in our study for the following reasons. First, there was no evidence of precipitate formation observed in solutions with 0.01 M CaCl$_2$. Both water and CaCl$_2$ solutions were prepared from the same stock solution of [^{14}C]acifluorfen, and both final working solutions measured the same radioactivity. Had there been any significant precipitation, the radioactivity for the CaCl$_2$ solution would have been lower. The solutions in both H$_2$O and CaCl$_2$ were stable under refrigerated conditions and, after several weeks of storage, measured the same radioactivity as initial readings. Also, according to Pusino et al. (1993), the K_{sp} for Ca−acifluorfen is approximately 1.5×10^{-9}. For our conditions, it would have required an acifluorfen concentration of 387 μM to form Ca−acifluorfen precipitates, and our highest concentration (65.8 μM) was well below that point.

Sorption Kinetics. In several soils, sorption achieved a pseudo-plateau from about 24 to 48 h and then later increased. Representative data for acifluorfen sorption at 25 °C in Dundee CT, Dundee NT, and Sharkey soils are shown in Figure 5. Simple extensions of the two-site model to include nonequilibrium sorption at a third type of site did not adequately describe the continued increase in ^{14}C sorption. Since acifluorfen is subject to biotransformation, these ^{14}C sorption data probably include radiolabeled metabolites as well as the parent compound. Data for Dundee CT, Dundee NT, Miami CT, Miami NT, Sharkey, Ships, and Weswood soils were well described assuming acifluorfen degradation and metabolite sorption followed eqs 3 and 4.

Biodegradation. Little is published concerning acifluorfen degradation in soil. Gennari et al. (1994a)
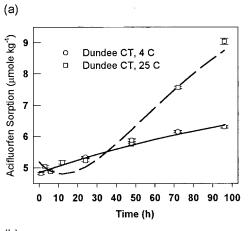
observed that, under anaerobic conditions, mixed enrichment cultures from soils with a history of acifluorfen exposure reduced acifluorfen to aminoacifluorfen when acifluorfen was the sole source of carbon. Inclusion of additional carbon sources such as sodium acetate promoted further degradation to other products. In our study, bacterial populations in soil suspensions of Sharkey and Dundee (NT and CT) soils indicated increased bacterial numbers, especially gram-negative, with time (Table 4). These bacteria may have transformed acifluorfen to a metabolite having a greater affinity for sorption to soil than acifluorfen. Although samples were well shaken, the rapid proliferation of bacteria may also have induced microaerophilic conditions which are potentially conducive to aromatic nitroreduction of acifluorfen.
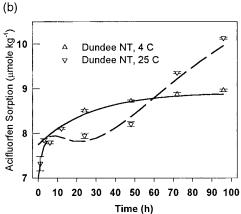
The carboxyl and amino functional groups and positions on the amino-substituted ring of aminoacifluorfen are identical with the functional groups of anthranilic acid (2-aminobenzoic acid). Gaston et al. (1996) have shown that anthranilic acid has a high affinity for a Dundee and Sharkey soil. If the carboxyl and amino groups are the active sorption sites for the anthranilic acid, they may also contribute to sorption of a metabolite such as aminoacifluorfen. Another diphenyl ether herbicide, bifenox [methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate], was rapidly hydrolyzed in soil to the acid form (carboxylic ester hydrolysis), and the nitro group was reduced to amino (Ohyama and Kuwatsuka, 1983). They hypothesized that, for the bifenox metabolites, the sorption associated with the carboxyl and hydroxl groups was related to hydrogen bonding and van der Waals forces, while hydrophobic bonding contributed to sorption at the amino group. Similar enhanced affinity for soil of amino derivatives of several diphenyl ether herbicides was observed by Niki and Kuwatsuka (1976). In our study, aminoacifluorfen sorption isotherms for Dundee CT and Sharkey soils indicated a high sorption affinity (Dundee CT: $K_f = 47.2$, $1/N = 0.41$; Sharkey: $K_f = 41.3$, $1/N = 0.72$). Average total recoveries of aminoacifluorfen (combined sorption solutions and methanol extracts) were only 9.9% (s.e. 1.23) and 17.8% (s.e. 0.89) for Dundee CT and Sharkey soils, respectively. Further metabolism of aminoacifluorfen during the 24-h equilibration was indicated by the presence of an unidentified peak in methanol extracts, especially in the Sharkey soil. Area of the unidentified peak increased with increasing concentration of added aminoacifluorfen, and the peak was not present in untreated soil extracts.
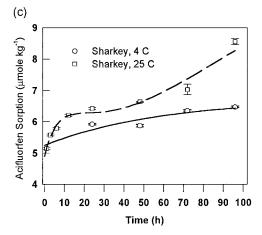
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Table 3. Effect of CaCl₂ on Acifluorfen Sorption, Methanol-Nonextractable ¹⁴C, and Solution pH after 24- or 96-h Equilibration with Dundee CT and Sharkey Soils

soil	shaking time (h)	acifluorfen sorption (μmol kg^{-1})[a]		methanol-nonextractable ¹⁴C (% of applied)[a]		supernatant pH[b]	
		0.01 M CaCl₂	water	0.01 M CaCl₂	water	0.01 M CaCl₂	water
Dundee CT	24	5.04a	2.81b	0.16a	0.03a	6.69	7.19
	96	7.99a	5.73b	20.8a	13.2b	6.60	7.24
Sharkey	24	5.65a	1.99b	7.91a	6.08b	6.63	7.53
	96	7.31a	4.58b	13.3a	10.1b	6.25	7.07
average		6.50a	3.78b	10.5a	7.35b		

[a] Within a row and for each parameter, means followed by the same letter are not significantly different. LSD α = 0.05. [b] pH was measured in supernatants pooled over replications.

(a)

(b)

(c)

Figure 5. Effect of equilibrating temperature on kinetics of acifluorfen sorption in (a) Dundee CT, (b) Dundee NT, and (c) Sharkey soils. Bars associated with each symbol represent the standard error of each mean value. Curves depicted as dashed lines (25 °C) represent best fits of data using eqs 1–4. Curves depicted as solid lines (4 °C) represent best fits of data using eqs 1 and 2.

Table 4. Changes in Bacterial Populations during 96-h Shaking

time (h)	total bacteria (log CFU mL^{-1})[a]	gram-negative (log CFU mL^{-1})[a]
	Sharkey	
1	6.46b	<4.20d
24	7.84a	5.05c
48	7.84a	6.31b
72	7.86a	6.51ab
96	7.89a	6.59a
	Dundee CT	
1	6.74c	4.70d
24	7.26b	5.78c
48	7.67a	6.34b
72	7.66a	6.54ab
96	7.61a	6.73a
	Dundee NT	
1	6.92c	4.88c
24	7.30b	5.82b
48	7.75a	6.60a
72	7.84a	6.67a
96	7.78a	6.65a

[a] Within a soil, means followed by the same letter are not significantly different. LSD α = 0.05.

Since sorption was based upon ¹⁴C remaining in solution after equilibration, other avenues of potential loss need to be addressed. One potential for loss of ¹⁴C from the mass balance equation is in the form of ¹⁴CO₂ (mineralized acifluorfen). However, upon the basis of results from Gaston and Locke (1996), it is doubtful that sufficient degradation occurred during this period to result in complete metabolization to ¹⁴CO₂. Gennari et al. (1994a) did not observe ether cleavage of acifluorfen in mixed enrichment cultures incubated for 56 d.

To further evaluate potential microbial degradation during sorption, Sharkey and Dundee NT and CT soils were equilibrated with acifluorfen solution for various time intervals at either 4 or 25 °C. Although factors related to sorption thermodynamics may be involved, reduced acifluorfen sorption at the lower temperature is consistent with what might be expected with lower microbial degradation (Figure 5).

Analysis of methanol extracts (after sorption) with the TLC toluene system for Dundee CT and Sharkey soils showed a major ¹⁴C peak, coeluting with acifluorfen (R_f 0.14), and a minor peak (R_f 0.26) that coeluted with aminoacifluorfen. The area represented by the minor peak increased with equilibration time (24–96 h), especially in the Dundee CT (Dundee CT, 0–14%; Sharkey, 0–3.9%). HPLC analysis of sorption supernatants and methanol extracts for both equilibration periods showed that most of the extractable ¹⁴C coeluted with acifluorfen. There were other minor peaks of radioactivity in some 96-h methanol extracts (4–13% of radioactivity), most consistently at a RT of 13.8 min, which coincided with an aminoacifluorfen standard.

Table 5. Degradation of Acifluorfen in Dundee CT Silt Loam Soil

	% of total [14]C Applied							
	aqueous extracts[a]			methanol extracts[a]				
(time (h)	acifluorfen	aminoacifluorfen	total	acifluorfen	aminoacifluorfen	other[b]	total	nonextract[a]
24	78.1(0.93)	<0.1(<0.001)	78.2	15.1(0.40)	<0.1(<0.01)	2.0(0.07)	17.2	1.3(0.11)
48	71.5(0.51)	<0.1(<0.001)	71.5	16.8(0.25)	0.7(0.04)	2.2(0.09)	19.7	4.0(0.14)
96	49.1(0.46)	4.2(0.39)	53.3	14.5(0.40)	10.4(0.32)	2.4(0.08)	27.2	9.8(0.23)
144	22.1(1.17)	12.7(0.24)	34.7	8.4(0.73)	19.2(1.14)	2.6(0.24)	29.7	17.1(0.57)

[a] Numbers in parentheses are standard errors. [b] Decarboxy acifluorfen and 4-(trifluoromethyl)-2-chlorophenol.

The study evaluating acifluorfen degradation in Dundee CT soil corroborated much that was observed previously. Acifluorfen accounted for 97−98% of extractable (aqueous and methanolic) ^{14}C after 24- and 48-h shakings, respectively (Table 5). Minor peaks in extracts coeluted with aminoacifluorfen, decarboxyacifluorfen, and 4-trifluoromethyl-2-chlorophenol. Aminoacifluorfen was the primary metabolite observed in extracts from the 96- and 144-h shakings. Nonextractable ^{14}C ranged from 1.3 to 17.1% during the 24−144-h shakings (Table 5). No significant quantity of ^{14}C was extracted using 5:95 NaOH:methanol until the 144-h shaking, when 4.3% was extracted (data not shown).

SUMMARY AND CONCLUSIONS

Several soils of diverse character were used to study factors which influence acifluorfen sorption. Sorption was rapid for all soils (percentage of total sorption ranged from 49% for Weswood to 93% for Lafitte within 1 h. Most soils reached a pseudo-equilibrium after 24−48 h of equilibration. Increases in apparent acifluorfen sorption at equilibration times longer than 24−48 h may reflect acifluorfen degradation and metabolite sorption. Further investigations comparing sorption at 4 and 25 °C indicated that microbial metabolism was occurring for some soils at equilibration times longer than 24−48 h. HPLC and TLC analyses of methanol extracts from soils after equilibration for 24 and 96 h showed that a metabolite, aminoacifluorfen, was present in the 96-h samples. Sorption studies with aminoacifluorfen indicated that it has a high affinity for soil, a factor which may help explain the irreversibility of sorption observed in these experiments. Capacity for acifluorfen sorption (24-h equilibration) in these soils in decreasing order was Lafitte ≫ Dundee NT > Sharkey > Miami NT > Mahan (0−13 cm) > Dundee CT > Miami CT > Mahan (26−26 cm) > Ships > Dundee scl = Dundee l > Weswood. Varying levels of acifluorfen sorption among soils were attributed to (a) organic carbon, (b) pH, and (c) CEC.

ACKNOWLEDGMENT

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