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Environmental Fate of Spinosad. 1. Dissipation and Degradation in Aqueous Systems

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Spinosad is a bacterially derived insect control agent consisting of two active compounds, spinosyns A and D. The objective of this paper is to describe the environmental fate of spinosad in aquatic systems. To this end, several studies performed to meet regulatory requirements are used to study the fate and degradation in individual environmental media. Specifically, investigations of abiotic (hydrolysis and photolysis) and biotic (aerobic and anaerobic aquatic) processes are described. Understanding developed from the laboratory-based studies has been tested and augmented by an outdoor microcosm study. Understanding of aquatic fate is a building block for a complete environmental safety assessment of spinosad products (Cleveland, C. B.; Mayes, M. A.; Cryer, S. A. *Pest Manag. Sci.* **2001**, *58*, 70−84). From individual investigations, the following understanding of dissipation emerges: (1) Aqueous photolysis of spinosad is rapid (observed half-lives of <1 up to 2 days in summer sunlight) and will be the primary route of degradation in aquatic systems exposed to sunlight. (2) Biotic transformations contribute to spinosad's dissipation, but less so than photolysis; they will be of primary importance only in the absence of light. (3) Spinosad partitions rapidly (within a few days) from water to organic matter and soil/sediment in aquatic systems but not so rapidly as to replace sunlight as the primary route of dissipation. (4) Abiotic hydrolysis is relatively unimportant compared to other dissipation routes, except under highly basic (artificial) conditions and even then observed half-lives are ∼8 months. Degradation pathways are understood are follows: (1) Degradation primarily proceeds by loss of the forosamine sugar and reduction of the 13,14-bond on the macrolide ring under aqueous photolytic conditions. (2) Degradation to several other compounds occurs through biotic degradation. Degradation under anaerobic conditions primarily involves changes and substitutions in the rhamnose ring, eventually followed by complete loss of the rhamnose ring. Degradation under aerobic conditions was more extensive (to smaller compounds) with the loss of both the forosamine and rhamnose sugars to diketone spinosyn aglycon degradates. (3) Hydrolytic degradation involves loss of the forosamine sugar and water and reduction on the macrolide ring to a double bond at the 16,17-position.

KEYWORDS: Aerobic; anaerobic; aquatic; dissipation; hydrolysis; kinetics; microcosm; photodegradation; reduced risk; spinosad; spinosyn

INTRODUCTION

Spinosyns are fermentation products produced by the actinomycete *Saccharopolyspora spinosa*, a bacterial organism isolated from soil. Structurally, the spinosyns consist of a central macrolide ring system (aglycon) with a rhamnose sugar at the 9-position and a forosamine sugar at the 17-position. The term "spinosad" refers to the combination of two structurally similar active ingredients, spinosyns A and D (**Figure 1**), which differ by a methyl group at position 6 on the central macrolide ring. Spinosyns A and D have typically been isolated and commercially formulated from the fermentation system in a ratio of approximately 85:15 (as a natural product a wider range of ratios is possible). Experiments described within this paper have been conducted primarily with either isolated spinosyn A or D, but at times a mixture of the two was used as the test substance.

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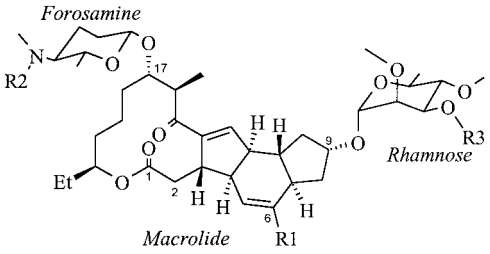

Spinosyn A: R1 = H, R2 = CH3, R3 = CH3
Spinosyn D: R1 = CH3, R2 = CH3, R3 = CH3
Spinosyn B: R1 = H, R2 = H, R3 = CH3
N-demethyl spinosyn D: R1 = CH3, R2 = H, R3 = CH3
Spinosyn J: R1 = H, R2 = CH3, R3 = H

Figure 1. Structure of spinosyns A, D, B, and J and N-demethylated spinosyn D.

Table 1. Key Physicochemical Properties of Spinosyns A and D

parameter	spinosyn A	spinosyn D
water solubility (ppm, w/v)		
pH 5	290	28.7
pH 7	235	0.332
pH 9	16	0.053
unbuffered	89.4	0.495
pK_a	8.10	7.87
adsorption partitioning	8.3 (sand)	similar results
coefficients (mL/g)	5.4 (loamy sand)	noted for
	25 (sandy loam)	spinosyn B
	323 (silt loam)	
	283 (clay loam)	
acetone solubility (g/100 mL)	16.8	1.01
vapor pressure (mmHg at 25 °C)	2.4E–10	1.6E–10
Henry's law (Pa m^3/mol at 20 °C)	1.89E–7	2.32E–5

There are many other structurally related spinosyns, but only spinosyns A and D have been commercially developed because they are the most active insecticide components. Our focus on spinosyns A and D was confirmed through the regulatory review process. On the basis of results of plant and animal metabolism and toxicity studies, the U.S. EPA concluded that spinosyns A and D constitute the toxic residues of concern for all matrices and that there is no need to address additional less active degradates/metabolites. Spinosyns A and D are the only compounds that comprise the residue to be monitored for tolerance enforcement in the United States (*2*).

Spinosad has a high level of activity and selectivity against certain insects that infest cotton, vegetables, tree crops, ornamentals, and turf (*3*, *4*). As of 2001, spinosad is registered in 37 countries for use in controlling pests in over 150 different crop commodities. Maximum use rates vary on crop and formulation; the highest single application rate in the United States is 0.16 lb/acre. Spinosad is classified as a reduced-risk compound by the U.S. EPA. Key properties are listed in **Table 1**.

Current approved uses of spinosad do not involve direct treatment of water, but as with all agrochemicals, it is important to understand the behavior of the compound in aquatic systems to adequately assess margins of safety. One of the greatest challenges of environmental fate research is the desire to predict behavior under real-world conditions, given there is large variation of potential conditions. To that end, it is desirable to understand isolated routes of dissipation in several prototype systems with a single test substance and then confirm that understanding under field conditions in which all of the possible routes of degradation are acting concurrently and with a formulated product. In Asia, spinosad is being evaluated for

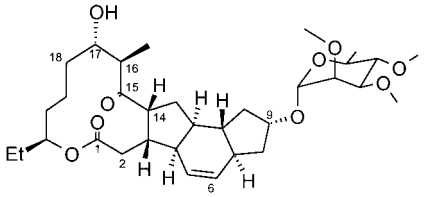

Figure 2. Structure of spinosyn A aqueous photodegradate A1, identified as 13,14-dihydrospinosyn A C-17 PsA.

efficacy for potential future use in rice, and therefore results from an aerobic flooded soil study are also available. To that end, this paper describes results of several investigations, which augment understanding from previous work (*5*, *6*):

- aqueous photolysis (sterile buffer, pH 7, natural sunlight with dark controls)
- anaerobic transformation (dark, water/sediment)
- aerobic degradation in flooded soil (laboratory simulation at high use rate, dark)
- hydrolysis (sterile, dark, pH 5, 7, and 9)
- outdoor aquatic microcosm (formulated product, natural water, sediment, natural sunlight).

The first four of the above investigations had two objectives: define the kinetics of dissipation of spinosad and identify the metabolite/degradation products for that system. The microcosm study was designed to provide information under real-world use conditions to augment understanding gained from the laboratory-based results.

MATERIALS AND METHODS

Standards and Reagents. For laboratory-based investigations (hydrolysis, photolysis, flooded soil, and anaerobic aquatic) both ^{14}C-labeled spinosyn A and ^{14}C-labeled spinosyn D were used in separate applications as test substances. The radiolabeled material was prepared by bacterial fermentation using [1,2-^{14}C]acetate as a carbon source (*7*). Radiolabeled carbon was incorporated into 21 positions of the central or macrolide ring system, the methyl at position 16, and the carbon of the ethyl function adjacent to position 21 on the macrolide ring system. For the various investigations, the radiochemical purity of the test substances ranged from 95 to 98% for spinosyn A and from 90 to 98.5% for spinosyn D. For the outdoor microcosm study, an experimental suspension concentrate formulation containing 42.9% spinosyns (non-radiolabeled, diluted to the normal range of spray dilutions) was applied.

Various analytical standards were used for quantitation of sample residues; the germane standards for verification or structural confirmation are discussed within the methods section of each individual study which follows. In general, the purities of ^{14}C-labeled spinosyn standards were spinosyn A, ~97%, spinosyn D, ~98%, spinosyn B, ~96%, and N-demethylated spinosyn D, ~98%. Several non-radiolabeled standards were available for retention time and spectral comparison purposes: spinosyn A, spinosyn B, spinosyn D, N-demethylated spinosyn D, spinosyn J (**Figure 1**); 13,14-dihydrospinosyn A C-17 PsA (**Figure 2**); the reverse pseudoaglycon and ketoreverse pseudoaglycon of spinosyn A (**Figure 3**); the 9,17-diketone spinosyn aglycon (**Figure 4**); and 3 dehydroxypseudoaglycon isomers of spinosyn A (**Figure 5**). An analytical standard for spinosad containing 76.1% spinosyn A and 11.9% spinosyn D was used for the immunoassay analysis in the microcosm study.

HPLC Analysis. Separations of spinosyns and other degradates were performed using either a YMC AQ-301 ODS, 10 cm × 4.6 mm i.d. (YMC Laboratories, Morris Plains, NJ), or a YMC AQ-303 ODS, 25 cm × 4.6 mm, column. Equipment and conditions are listed in **Table 2**. HPLC grade solvents were used. With molecular weights of >700, the spinosyns are insufficiently volatile for analysis by gas chromatography, so of the classical methods, HPLC-UV is the method of choice for routine chromatographic separations. However, the analytes are only moderately sensitive to UV detection. As a result, large sample sizes (followed by cleanup) may be needed.

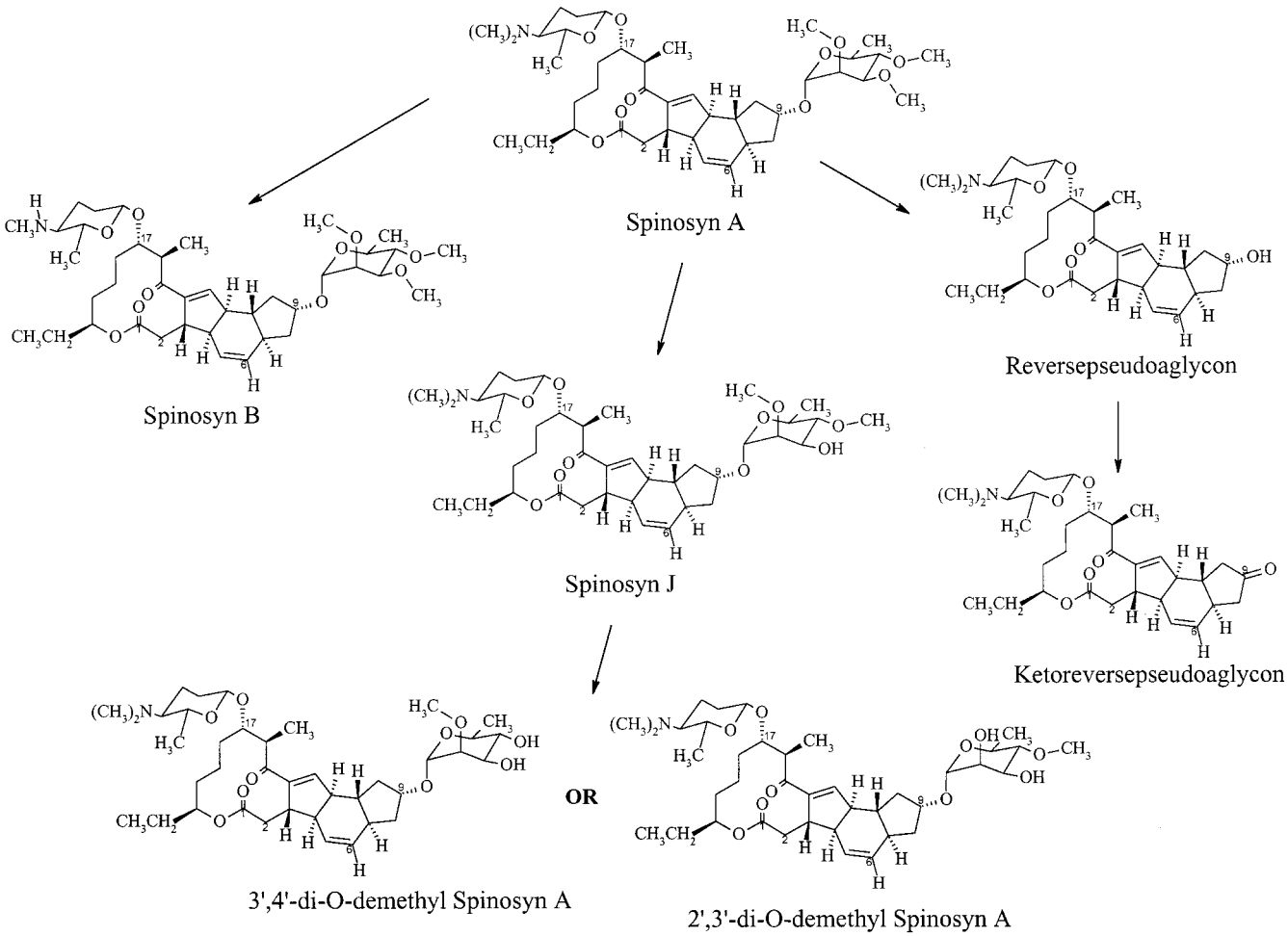

Figure 3. Proposed degradation pathway for spinosyn A under anaerobic aquatic conditions.

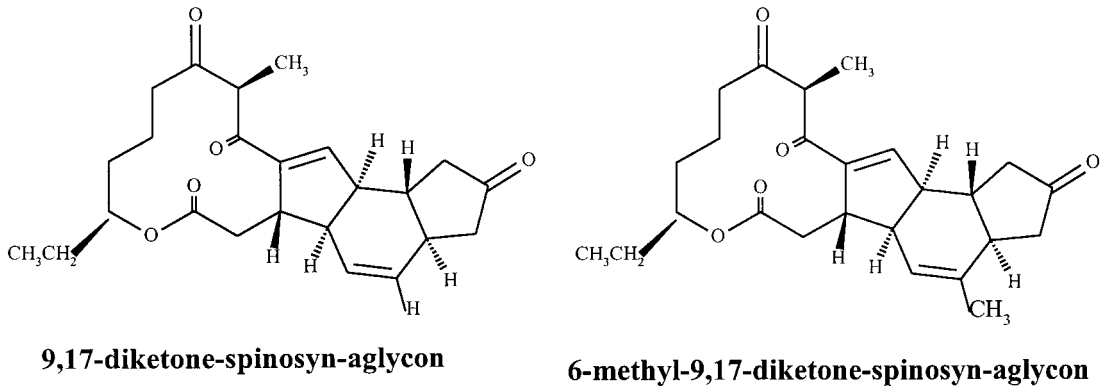

Figure 4. Degradates for spinosyns A (top) and D (bottom) under aerobic flooded soil conditions.

Immunoassay Analysis Method. A spinosad RaPID assay immunoassay (IA) kit (Strategic Diagnostics, Inc., Newark, DE) developed for analysis of spinosad-related compounds (*8*) was used in the outdoor microcosm study. The test kit employs spinosyn A for generation of the calibration curve. The method does not differentiate individual spinosyns, but instead measures total residues of spinosad and related degradates. Due to the sensitivity and specificity of the IA method, sample cleanup is minimized. For water, an aliquot (diluted with stabilizer if necessary) is mixed with enzyme-conjugated spinosad and paramagnetic particles coated with antibodies according to kit instructions. After incubation (30 min), the paramagnetic particles were retained within the tube using a magnetic field and the solution was decanted. After the particles had been washed, colorimetric reagents (3,3′,5,5′-tetramethylbenzidine as the reactant chromogen) are added and the sample is again incubated. Acid is added to stop the colorimetric reaction and the spinosad indirectly quantified by measuring light absorbance of the chromogen at 450 nm using an RPA-1 RaPID photometric analyzer (Strategic Diagnostics, Inc.). For IA analysis of sediment, samples are extracted using 65:27:8 (v/v/v) of methanol/5% aqueous NaCl/1 N NaOH. An aliquot of the extract is diluted, and the spinosad content is measured using the IA method described for water.

Degradation Analysis by MS. Mass spectral techniques were used for the identification of degradates in most of the experiments. The objective of the MS experiments is to identify unknowns from an isolated peak from subsamples. The MS methods are not applied to all samples. **Table 3** lists key aspects associated with each study.

Aqueous Photolysis. Sterile solutions (20 mL) of 0.01 M tris-(hydroxymethyl)aminomethane buffer (pH 7) in 25-mL Pyrex test tubes

Table 2. HPLC Conditions

sample analyzed	HPLC system[a]	mobile phase[b]	gradient conditions[c]
hydrolysis, aqueous photolysis dark controls	Hewlett-Packard 1050 or 1090 (250 nm)	42.5:42.5:15:0.1 (v/v/v/w) MeCN/MeOH/water/Amm Ac; 1.2 mL/min	none
aqueous photolysis	Hewlett-Packard 1050 or 1090, Beckman 171 RAM with solid cell; LSC of collected fractions	A: 10:10:80:0.1 (v/v/v/w) MeCN/MeOH/water/Amm Ac; B: 50:50:0.1 (v/v/w) MeCN/MeOH/Amm Ac; 1.8 mL/min	100% A to 100% B in 60 min; hold 100% B for 30 min
PNAP-PYR from aqueous photolysis	Hewlett-Packard 1050 or 1090 (288 nm)	65:35 (v/v) MeOH/water containing 0.0633 M pyridine; 1.0 mL/min	none
outdoor microcosm	Varian 9012 pump and 9050 detector (250 nm)	1:1:1 (v/v/v) MeCN/MeOH/2% (w/v) aq Amm Ac; 0.8 mL/min	none
aerobic flooded soil	Hewlett-Packard 1050 pump and detector (250 nm)	42:16:42 (v/v/v) MeCN/2% Amm Ac/MeOH; 1.5 mL/min	none
aerobic flooded soil	Hewlett-Packard 1050 pump and detector (250 nm)	A: 3:3:4 (v/v/v) MeCN/MeOH/2% (w/v) aq Amm Ac; B: 45:45:10 (v/v/v) MeCN/MeOH/2% (w/v) aq Amm Ac; 1.5 mL/min	100% A to 100% B in 25 min; hold B for 10 min
aerobic flooded soil	Hewlett-Packard 1050 pump and detector (250 nm)	A: 4:4:1 (v/v/v) MeCN/MeOH/2% (w/v) aq Amm Ac; B: 2% (w/v) aq Amm Ac; 1.5 mL/min	85% A and 15% B to 100% B in 30 min; hold B for 20 min
anaerobic water/sediment	Varian 9010 pump, Spectra-Physics 8773XR detector (250 nm), Berthold LB 506D RAM	A: 0.2% (w/v) aq Amm Ac; B: 0.2% (w/v) Amm Ac in 1:1 (v/v) MeCN/MeOH; 1.5 mL/min	60% B to 90% B in 25 min; hold 90% B for 15 min

[a] RAM = radioactivity monitor; LSC = liquid scintillation counter. [b] MeCN = acetonitrile, MeOH = methanol, Amm Ac = ammonium acetate. [c] All gradients are linear.

with Teflon perfluoropolyethylene-lined screw caps were fortified at a final concentration of 2.0 mg/L (2 ppm) using [^{14}C]spinosyns A or D in 0.1 mL of acetonitrile (cosolvent to increase solubility). Samples were maintained at 25 °C using a temperature-controlled bath and exposed to sunlight near Greenfield, IN, 39.8° N latitude. Spinosyn A samples were exposed in late June and spinosyn D samples in early July. Duplicate samples were removed and analyzed at time 0 and after 3, 21, 24, 42, and 48 h of exposure.

Sunlight intensity was monitored using a *p*-nitroacetophenone−pyridine (PNAP−PYR) chemical actinometer (*9*). The actinometer contained 5.0 mg/L PNAP in water with 0.0633 M PYR. Duplicate PNAP samples were prepared in 25-mL Pyrex test tubes and exposed to sunlight simultaneously with spinosad samples. Pyridine was redistilled from glass, and PNAP was recrystallized from methanol prior to use. The PNAP−PYR solutions were analyzed using a 25 cm × 4.6 mm i.d. Spherisorb ODS II column (Alltech, Avondale, PA).

Identification of aqueous photodegradates was performed by mass spectrometry (MS) using a Finnigan MAT 95Q. To prepare sufficient material for NMR of the one major degradates, 60 additional 20-mL samples of spinosyn A in pH 7 buffer were prepared and exposed to sunlight for 3 days. The degradate was extracted using dichloromethane and purified by reversed-phase TLC using $LKC_{18}F$ plates (Whatman, Clifton, NJ) developed with 47.5:47.5:5 (v/v/v) acetonitrile/methanol/water. The proton NMR spectrum of the isolated degradate was obtained using a Varian Gemini 300.

Anaerobic Water/Sediment. The sediment compartment of each biometer was prepared by adding 0.5 g of dried alfalfa, followed by ~104 g of wet sediment (equal to 50 g on a dry-weight basis), and 100 mL of surface water. Characteristics of the clay sediment and water

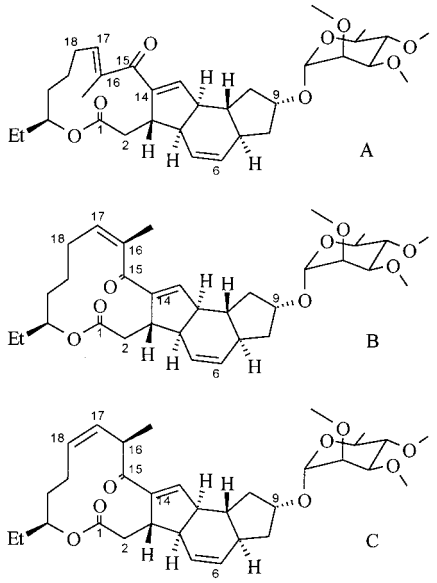

Figure 5. Spinosad degradate standards for the hydrolysis study: 3-dehydroxypseudoaglycon isomers of spinosyn.

Table 3. Key Aspects of MS Conditions for Degradation ID

study	instrument	ionization	sample introduction	acquisition rate
aqueous photolysis	Finnigan MAT 95Q (magnetic sector)	electron impact (EI), at 70 eV	direct insertion probe (DIP)	scan from *m/z* 50 to 800 in 1 s
anaerobic aquatic (A)	Finnigan MAT TSQ 70 (triple quadrupole)	particle beam with Vestec Universal Interface	HPLC	scan from *m/z* 100 to 900 in 1 s
anaerobic aquatic (B)	Finnigan MAT TSQ 70 (triple quadrupole)	electron impact (EI), at 70 eV	DIP	scan from *m/z* 100 to 900 in 1 s
nominal accurate mass	Finnigan MAT 95Q (magnetic sector)	electron impact (EI), at 70 eV	DIP	scan from *m/z* 100 to 800 in 1 s
hydrolysis	Finnigan MAT 95Q (magnetic sector)	electron impact (EI), at 70 eV	DIP	scan from *m/z* 35 to 800 in 1 s
aerobic flooded	Finnigan MAT Deca and Deca XP (ion trap)	atmospheric pressure chemical ionization (APCI) with alternating positive and negative polarity	HPLC via Agilent 1100 system	• scan range (LC-MS): *m/z* 100–1000 • scan range (LC-MS/MS): daughters of *m/z* 397.4, scan from *m/z* 105 to 500, with collision energy of 20%, 40% (for spinosyn A degradates) • scan range (LC-MS/MS): daughters of *m/z* 411.4, scan from *m/z* 105 to 500, with collision energy of 20%, 40% (for spinosyn D degradates)

Table 4. Characterization of Sediment and Water for Anaerobic Aquatic Study

water			sediment		
property/value	anaerobic	microcosm	property/value	anaerobic	microcosm
pH	7.7	7.6	pH	7.2	7.6
conductivity (μS)	1130	270	dry matter (%)	51.6	
hardness (mg/L)	495	110	total N (%)	0.18	
alkalinity (mg/L)	390	99	total P (%)	0.17	
total suspended solids (mg/L)		24	cation exchange capacity (mequiv/100 g)	24.1	15.8
N (mg/L)	1.5		org matter (%)	2.8	3.9
Zn (μg/L)	<50		extractable/reducible Mn (mg/L)	13/7	
Cu (μg/L)	<50		bulk density (g/cm^3)		1.13
Cd (μg/L)	<0.5		WHC (%) at 0.33 bar/15 bar		32.6/12.7
Pb (μg/L)	<5		sand %	23 (2 mm–63 μm)[b]	24
Ni (μg/L)	8.5		silt %	35 (63–2 μm)[b]	42.2
Cr (μg/L)	3.6		clay %	42 (<2 μm)[b]	33.8
turbidity	8.3 NTU[a]		texture	clay[c]	clay loam

[a] National turbidity units. [b] Based on ADAS classification. [c] Sediment is a clay under both ADAS and USDA classification systems.

taken from a pond near Thame, Oxon, U.K., are given in **Table 4**. After the compartment had been sealed with a greased stopper, 100 mL of 0.1 N sodium hydroxide was added to the other side to trap acidic volatiles, and a dip-tube was immersed in the NaOH. The top of the dip-tube was connected to an expansion bulb, and finally an in-line soda-lime trap was added to the end to prevent saturation of the NaOH hydroxide trap solution by absorption of CO_2 from the atmosphere. To promote anaerobic conditions, prepared biometers were purged for 2−3 min with nitrogen via the dip-tube while the greased stopper was released slightly to allow for gas flow. Prior to dosing with spinosad, biometers were incubated (25 ± 2 °C) in the dark for a minimum of 30 days to establish anaerobic conditions. Gas was released as needed by opening the dip-tube/side-flask joint slightly.

The biometers were dosed with ~87 μg of [^{14}C]spinosyn A or D in 0.10 mL of methanol, applied evenly over the surface of the water. Each biometer was purged with nitrogen, and the flasks were gently swirled to distribute the applied spinosyn in the surface water. Dosed biometers were incubated at 25 °C (dark), and individual spinosyn A and D biometer samples were withdrawn at 0, 3, 7, 14, 28, 56, 84, 170, and 365 days after treatment (DAT).

At each sampling point, the sodium hydroxide solution was removed and analyzed for radioactivity using liquid scintillation counting (LSC). The dissolved oxygen content of the water layer was determined prior to decanting from the sediment using a Griffin and George model 40 meter and a YRC/464-035V oxygen probe. The pH of each sample was determined and an aliquot withdrawn for radioassay.

Aliquots (75 mL) of the water layer were loaded on to a preconditioned C18 solid-phase extraction (SPE) cartridge and washed with 5−10 mL of additional water. Radiolabeled degradates were eluted with 10 mL of methanol. The methanol was removed under nitrogen at 40−50 °C. The residue was reconstituted in 0.5 mL of methanol and analyzed by HPLC.

Water (10 mL) was added to the sediment and the slurry transferred to a glass jar using 90 mL of 1:1 (v/v) acetone/methanol. The sediment samples were shaken for 20−30 min and centrifuged, and the supernatant was decanted. The procedure was repeated and the supernatant combined. A final extraction was performed with 100 mL of acetone, but the supernatant was retained separately. Extracted sediment was air-dried and homogenized, and aliquots of each were combusted to determine nonextractable radioactivity (NER). Analyses were conducted with a Harvey OX500 biological material oxidizer, with CO_2 recovered in a 1:1 (v/v) Carbosorb/Permafluor V (Canberra-Packard, Pangbourne, U.K.) mixture, which was analyzed by LSC.

Portions of the aqueous/methanol and acetone sediment sample extracts were combined, and the solvent was removed in a 40−50 °C water bath until only water remained in the samples. The samples were loaded on C18 SPE cartridges, eluted, concentrated, and reconstituted as described for the water samples in preparation for HPLC analysis using a flow-through radioactivity monitor (**Table 2**).

To assist in degradation identification, two biometer flasks for each spinosyn were prepared as described above except they were dosed at 10 and 3 times the rate above for spinosyns A and D, respectively. These four flasks were incubated for 126 days and prepared for analysis as above. Water and sediment extracts were analyzed by particle-beam liquid chromatography−mass spectrometry (PB-LC-MS) with a Vestec Universal Interface and a Finnigan TSQ70.

Table 5. Physiochemical Properties of the Aerobic Flooded Soils[a]

parameter	value	
soil series	volcanic	nonvolcanic
location	Ibaraki-ken, Japan	Fukuoka-ken, Japan
USDA textural class[a]	loam	loam
international textural class[b]	loam	clay loam
ADAS textural class[b]	sandy silt loam	sandy silt loam
cation exchange capacity (mequiv/100 g)	13.5	10.7
organic matter (%)	8.4	3.7
bulk density (g/cm^3)	0.72	0.92

[a] Analyses were conducted at AGVISE Laboratories, Northwood, ND. [b] By hydrometer method.

Aerobic Flooded Soil. Metabolism/degradation of spinosad was studied in biometer flasks with two Japanese soils, 1 cm depth, in the dark at 25 °C for 100 days. Given the variation in international classification systems, several soil properties and classifications are listed in **Table 5**. Test systems were attached to a low-pressure oxygen manifold to maintain aerobic conditions and were preincubated in an incubator for 11 days. The test system represented flooded rice paddy conditions (flooded with HPLC grade water at a 1:1 soil/water ratio). [^{14}C]Spinosyn A or D was applied at ca. 3.7 or 2.8 μg/mL to nonvolcanic and volcanic soils, respectively. This corresponds to an anticipated maximum application rate for spinosad in Japanese rice of 400 g/ha. Samples were analyzed at 0, 3, 7, 21, 35, 49, 71, 84, and 100 DAT.

The aqueous layer was extracted with 50:50 acetonitrile/methanol at 0 DAT and diluted with 50:50 acetonitrile/methanol at later time points. Soils were sequentially extracted with an extraction solution of 50:50 acetone/methanol followed by a second solution of 92:8 methanol/0.1 N NaOH. Water and soil extracts were analyzed by HPLC and TLC (20 × 20 cm EM Science silica gel 60 F254, 250 μm). For TLC, toluene/isopropyl alcohol/diethylamine (12:1:1) was used to develop plates viewed both with a UV lamp (254 nm) for reference standards and with a BioScan System 200 imaging scanner equipped with an Auto Changer 4000 for samples with ^{14}C. Cochromatography with analytical standards, NMR (Bruker DRX-600, Billerica, MA), LC-MS, and LC-MS/MS with atmospheric pressure chemical ionization on a Thermo Finnigan LCQ DECA XP (San Jose, CA) were used to identify degradates.

Hydrolysis. Sterile solutions of 0.01 M buffers prepared from sodium acetate (pH 5), tris(hydroxymethyl)aminomethane (pH 7), or sodium carbonate (pH 9) were placed in 20-mL ampules and fortified using 0.1 mL of a 400 mg/L acetonitrile solution of [^{14}C]spinosyn A or D for a final aqueous concentration of 2.0 mg/L of each spinosyn. Duplicate samples were analyzed by HPLC at time 0 and after 2, 7, 15, 22, and 30 days (dark, 25 °C).

Hydrolysis degradates at pH 9 were prepared for identification by incubation of additional samples of radiolabeled spinosyns A and D for 68 days. The degradates were analyzed using gradient HPLC and then further purified using LK6F silica gel TLC plates (Whatman, Clifton, NJ) developed with 99:1 (v/v) ethyl acetate/diethylamine. Degradates were isolated by TLC and their identities determined by electron impact mass spectrometry (EI-MS) on a Finnigan MAT 95Q (Finnigan, Bremen, Germany).

Outdoor Microcosm. Aquatic microcosms were constructed near Greenfield, IN, from insulated stainless steel tanks (1.7 m i.d. × 0.58 m) with ca. 50 cm of water and 5 cm of a clay loam sediment (**Table 4**) collected from a local pond. One control tank and three treated tanks were established 17 days prior to treatment. The microcosms were treated to simulate the direct overspray of a pond using a hand-held sprayer and the 480 g/L suspension concentrate (SC) formulation (42.9% spinosad) at a rate equivalent to a surface application of 100 g/ha.

Water samples down to the sediment were collected using disposable glass tubes. Aliquots collected from each quadrant of a tank were combined to form a sample. Samples were collected from each tank at time 0, 1, 2, 4, and 8 h after treatment, and 1, 2, 4, 8, and 15 DAT. Water samples were stored under refrigeration. For the HPLC analysis, the water samples were transferred to a separatory funnel with dilute NaOH and partitioned with MeOH and methylene chloride. The methylene chloride layer was brought to dryness, and then residues were dissolved in 1:1:1 MeOH/acetonitrile/2% ammonium acetate. For IA analysis, aliquots of water were used directly with the test kits as described for the IA method.

Sediment samples were collected by pressing an inverted wide-mouth (5 cm) jar into the sediment, covering the jar opening with a stainless steel plate, and removing the intact core. Care was taken to minimize surrounding sediment disturbance and cross-contamination by tilting the jar before the plate was inserted. Three sediment cores from each microcosm were combined into a single sample for analysis for each microcosm at each sampling event. Samples were collected at 1, 2, 4, 8, 15, and 35 DAT. Sediment samples were stored frozen and then homogenized in a hammer mill with dry ice. For HPLC analysis, a 20-g aliquot of sediment was extracted with 65:27:8 MeOH/5% NaCl/1 N NaOH by sonication and centrifuge, and samples were brought to known volume. An aliquot was acidified and transferred to a separatory funnel and hexane added. The sample was partitioned three times and the hexane layer collected and passed through sodium sulfate and then

Table 6. Photodegradation Results:[a] Percent of Radioactivity Applied

sample	exposed irradiation time (days)	actinometer corrected sunlight irradiation (days)	parent (%)	product 1 (%)	product 2 (%)	product 3 (%)	product 4 (%)
spinosyn A	0	0	97.95				
	0.125	0.25	83.05	3.3	4.8	3.7	
	0.875	0.45	67.65	7.45	7.0	4.7	
	1.0	0.59	59.3	**14.05**	4.1	7.5	
	1.75	1.01	45.1	**19.5**	5.1	*8.9*	
	2.0	1.32	37.05	***24.05***	*6.0*	8.25	
no acetonitrile[b]	2.0	1.32	30.5	**15.9**	4.65	7.6	
	0	dark control	93.75				
spinosyn D	0	0	94.9				
	0.125	0.23	76.1	5.8	4.1	2.65	2.9
	0.875	0.46	60.75	**12.25**	5.3	4.05	3.4
	1.0	0.79	46.9	**18.05**	*7.1*	5.65	*3.7*
	1.75	1.18	32.55	**16.45**	3.45	*5.95*	3.0
	2.0	1.66	23.7	***19.3***	3.05	5.15	3.4
no acetonitrile[b]	2.0	1.66	20.05	**15.55**	1.8	4.8	3.6
	0	dark control	94.15				

[a] Degradate percentages >10 are in boldface type; highest amount observed for each product is italicized. [b] No acetonitrile provided a check on the influence of solvent; no major differences were observed with or without solvent.

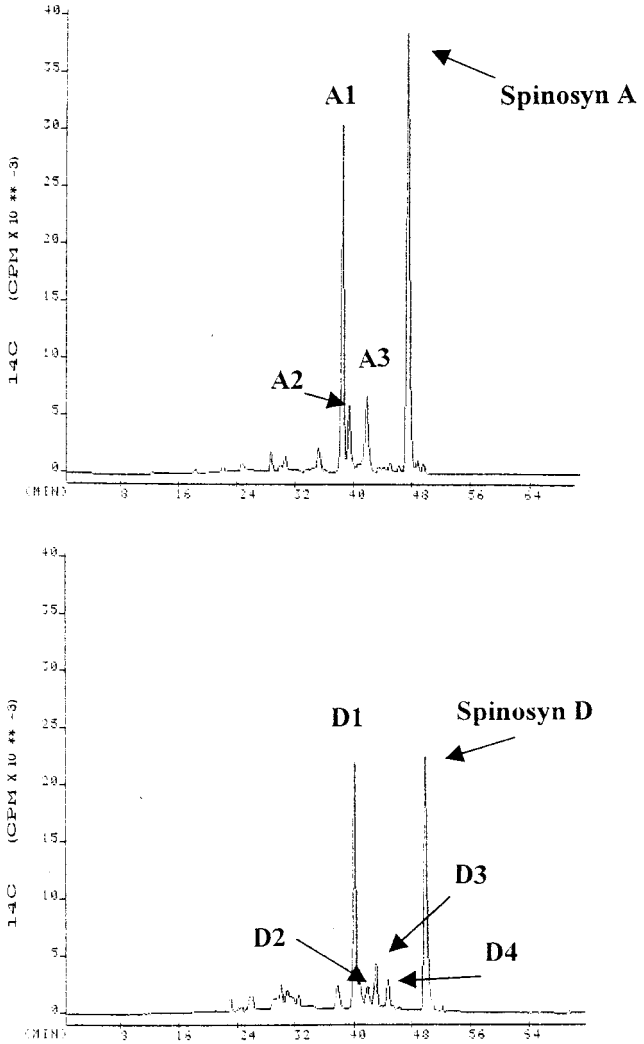

Figure 6. HPLC radiochromatograms of spinosyns A (top) and D (bottom) after exposure to summer sunlight for 48 h.

brought to dryness. The residues were loaded onto a silica solid-phase extraction (SPE) column with hexane, then eluted with solution of 3:1 MeOH/methylene chloride, and then brought to dryness and redissolved in 1:1:1 MeOH/acetonitrile/2% ammonium acetate. For IA analysis, 5-g aliquots of sediment were prepared as described for the IA method.

RESULTS AND DISCUSSION

Aqueous Photolysis. Degradation profiles based on percent of applied for spinosyns A and D are presented in **Table 6** as averages of duplicate samples. Photodegradation was rapid over the 2 days of sunlight exposure. Dark control samples indicated the spinosyns were essentially stable to hydrolysis during the experiment.

For photolysis to occur, a molecule must absorb in the range of light present at the earth's surface (>290 nm). Once a photon is absorbed, a bond must be broken (as opposed to reradiation by fluorescence or phosphorescence). The combination of labile bonds and an overlap of the absorption spectrum with the incoming solar radiation is responsible for photolability. Spinosyns A and D exhibit absorption maxima at ~340 nm that allow for photodegradation in sunlight.

The photodegradation rate of a chemical is dependent upon the photochemical quantum efficiency, sunlight intensity, and extinction coefficient according to (*10*)

$$k = \phi \sum_{\lambda} L_{\lambda} \epsilon_{\lambda}$$

where k is the first-order photodegradation rate (days^{-1}), ϕ is the quantum efficiency, L_{λ} is the light intensity (millieinsteins cm^{-2} days^{-1}), and ϵ_{λ} is the extinction coefficient (L cm^{-1} mol^{-1}).

Because natural sunlight was used as the photolytic source, variation in sample irradiation affected by time of day, cloud cover, and the geometry of sample containers needed to be assessed. Therefore, PNAP−PYR actinometers were used to normalize the observed degradation to standardized sunlight exposure conditions (summer, 40° N) based on the known photodegradation rate of PNAP at a given concentration of pyridine relative to the experimentally determined rate at each sampling point (*9*). **Table 6** lists actual exposure time and then normalized sunlight irradiation time. From these data, summer photodegradation first-order rate constants were 0.8 and 0.9 days^{-1} for spinosyns A and D, respectively, with half-lives of 0.9 and 0.8 days.

Using summer sunlight intensities (*11*) and experimentally derived extinction coefficients, $\Sigma L_{\lambda}\, \epsilon_{\lambda}$ values for spinosyns A and D were determined to be 38.3 and 38.7 days^{-1}, respectively, and photochemical quantum efficiencies were 0.019 and 0.021.

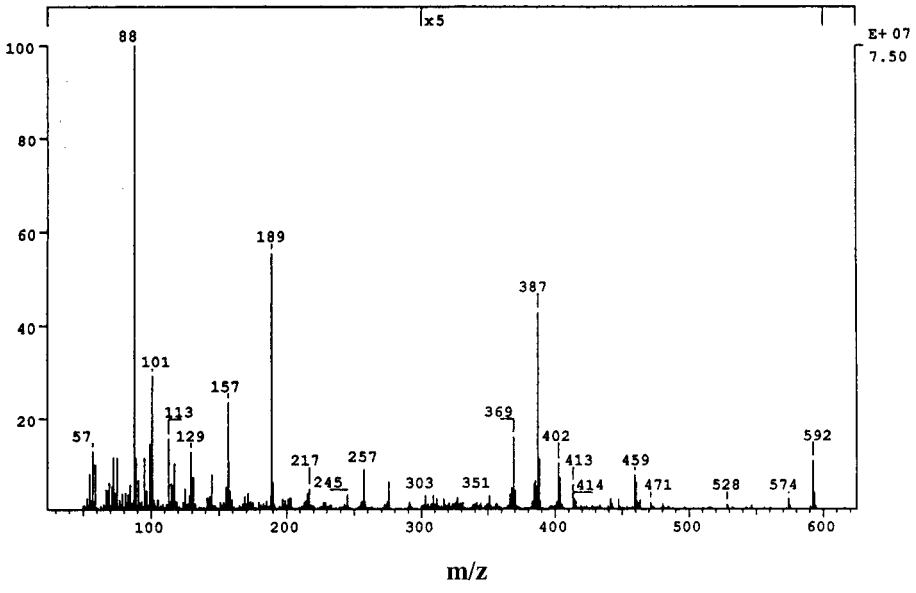

Figure 7. Probe-EI-MS spectrum of photolysis degradate A1, identified as 13,14-dihydrospinosyn A C-17 PsA.

Table 7. Degradation Profile (Percent of Applied) for Anaerobic Aquatic Study

sample matrix	RRT[a]	0 DAT	3 DAT	7 DAT	14 DAT	28 DAT	56 DAT	84 DAT	170 DAT	365 DAT
spinosyn A[a]										
NaOH trap (primarily $^{14}CO_2$)		0.1	0.0	0.1	0.1	0.1	0.2	0.3	0.2	0.3
water phase										
spinosyn A	1.0	***51.9***	**12.9**	7.4	3.5	2.5	1.5	1.3	0.8	0.6
spinosyn J[b]	0.86					0.4	*0.5*	0.4	0.4	0.3
spinosyn B	0.82	*6.1*	1.3	1.1	0.8	0.2	0.2		0.6	0.1
O-demethylated spinosyn J[b]	0.72					0.2	0.4	0.4	0.6	*0.8*
ketoreverse pseudoaglycon	0.64									*0.4*
sediment										
spinosyn A	1.0	**34.7**	***76.4***	**74.2**	**73.6**	**71.4**	**64.7**	**49.5**	**42.1**	**31.1**
spinosyn J	0.86			2.8	5.3	7.9	**10.4**	***11.8***	**11.0**	7.3
spinosyn B	0.82	3.0	4.2	3.0	4.6	1.9	3.2	2.2	*5.8*	1.6
O-demethylated spinosyn J[b]	0.72					3.3	6.5	9.5	**10.1**	***14.1***
ketoreverse pseudoaglycon	0.64						3.4	3.4	5.3	***14.0***
reverse pseudoaglycon isomer 1	0.62							*3.2*	2.5	2.8
reverse pseudoaglycon isomer 2	0.57							1.5	1.5	*3.7*
nonextractable residues		3.3	8.7	7.1	6.6	8.4	8.1	**10.3**	**13.3**	***17.4***
spinosyn D[c]										
NaOH trap (primarily $^{14}CO_2$)		0.1	0.8	0.9	1.6	1.7	1.9	1.9	0.7	0.9
water phase										
spinosyn D	1.0	***43.4***	**10.5**	4.7	3.6	2.8	1.6	2.2	0.9	0.7
spinosyn J of D	0.86	*4.6*	1.7				0.5		0.3	0.3
N-demethylated spinosyn D (B of D)	0.82	1.1	0.3	*1.3*	0.7	0.3	0.4		0.6	0.2
O-demethylated spinosyn J of D	0.77		0.1							*0.4*
unknown	0.74	*1.1*	0.5						0.04	
unknown	0.67		*0.2*						0.2	
unknown	0.62		*0.3*							0.3
sediment										
spinosyn D	1.0	**40.8**	**73.6**	**72.1**	**70.3**	**68.6**	**67.0**	**62.6**	**48.6**	**48.9**
spinosyn J of D	0.86		1.5	3.1	3.5	6.1	8.2	9.9	9.9	***11.6***
N-demethylated spinosyn D (B of D)	0.84	4.1	1.8	2.0	2.3	1.9	2.6	2.4	*6.5*	1.8
unknown (likely related to B of D)	0.82	1.9	*2.9*	2.2	3.5	1.1	1.3	1.0	2.0	
O-demethylated spinosyn J of D	0.76			1.2	2.5	2.9	3.5	4.1	5.6	*7.2*
unknown	0.71								1.3	*3.3*
reverse pseudoaglycon of D isomer 1	0.69								*2.3*	1.9
reverse pseudoaglycon of D isomer 2	0.66								*2.2*	1.2
nonextractable residues		5.0	11.2	8.4	7.2	**10.1**	9.0	**10.7**	**11.4**	**15.5**

[a] Unless noted, assignment verified against analytical standard. [b] Assignment based on accurate mass determination by EI-MS. [c] Assignments for spinosyn D pathway based on rise/decline profile of metabolites and comparisons of mass spectra with assigned spinosyn A degradates. [d] Compartments >10% of applied are in boldface type; the maximum observed percentages are italicized. [e] RRT = relative retention time.

Photodegradation of spinosyns A and D produced several degradates (**Figure 6**) and the three most prominent degradates of spinosyn A, designated A1 (later named 13,14-dihydrospinosyn A C-17 PsA), A2, and A3, reached maximum concentrations equivalent to 25, 7, and 9% of initial, respectively. Similarly, the spinosyn D degradates D1, D2, D3, and D4 reached maximum concentrations of 20, 8, 6, and 4% of initial, respectively.

The mass spectrum of A1 (**Figure 7**) suggested extensive degradation had taken place; the spectrum revealed a molecular ion at *m*/*z* 592 and the absence of the forosamine sugar ion, *m*/*z* 142. This suggested the loss of forosamine, leaving a hydroxyl at the 17-position and the addition of two protons to the macrolide. The presence of an *m*/*z* 189 ion indicated the rhamnose sugar was intact. The UV absorption spectrum of spinosyn A contains an absorption maxima at ~240 nm due to the 13,14-double bond conjugated with the carbonyl at position 15. This absorption was absent in A1, suggesting modification of this region.

NMR analysis of the A1 photodegradate indicated two protons were added to the 13,14-double bond and the 5,6-double bond was intact. The singlet for the proton at the 13-carbon of spinosyn A was absent in A1. In addition, the ^{13}C APT (attached proton test) (*12*) spectrum indicated two quaternary carbons with chemical shifts consistent with the ester carbon at position 1 and an isolated carbonyl at position 15.

A standard of the proposed structure 13,14-dihydrospinosyn A C-17 PsA for A1 (**Figure 2**) was prepared from spinosyn A by reduction of the 13,14-double bond and hydrolysis to remove the forosamine. Both isomers due to the asymmetric carbon at position 14 were isolated. The β isomer produced NMR and mass spectra identical to those obtained from A1, and therefore the structure of 13,14-dihydrospinosyn A C-17 PsA was confirmed.

MS analysis of A2 indicated a molecular ion at *m*/*z* 731, the same as spinosyn A, indicating rearrangement. Analysis of A3 produced a mass spectrum with a molecular ion at *m*/*z* 749, equivalent to spinosyn A with the addition of a water molecule. The exact positions of the rearrangement or addition of the water were not determined.

A similar degradation pattern for spinosyn D was observed by HPLC. The only degradate that was isolated in enough quantity for identification work was degradate D1. A molecular ion at *m*/*z* 606 was observed, which corresponds to the loss of the forosamine plus the addition of two protons and is congruent with the difference of 14 amu between spinosyns A and D.

Anaerobic Water/Sediment. At each sampling, the sediment layer was dark blue/gray and no dissolved oxygen was detectable in the water column, indicating that anaerobic conditions were maintained. The pH of the water in the flasks remained at ~7.5 throughout the experiment. Following application, applied radioactivity was observed to move from the water layer to the

Table 8. PB-LC-MS Identification of Spinosyn A Degradates from the Anaerobic Water/Sediment Study

relative retention	PB-LC-MS ions of interest	apparent MW	assigned structure
	Spinosyn A System		
1.00	189, 289, 449, 573, 731	731	spinosyn A
0.86	142, 189, 435, 559, 717	717	spinosyn J
0.82	128, 189, 449, 572, 717	717	spinosyn B
0.72	142, 189, 421, 544, 572, 703	703	O-demethylated spinosyn J
0.64	142, 259, 410, 496, 541	541	ketoreverse pseudoaglycon
0.62	142, 261, 435, 498, 543	543	reverse pseudoaglycon isomer
0.57	142, 261, 424, 498, 543	543	reverse pseudoaglycon
	Spinosyn D System		
1.00	142, 189, 463, 587, 701, 745	745	spinosyn D
0.86	142, 189, 355, 573, 686, 731	731	spinosyn J of D
0.82	128, 248, 404, 463, 731	731	N-demethylated spinosyn D (B of D)
0.76	142, 399, 559, 717	717	O-demethylated spinosyn J of D
0.71	142, 189, 273, 655, 727	727	unknown
0.69	142, 273, 512, 557	557	reverse pseudoaglycon of D isomer
0.66	142, 225, 363, 557	557	reverse pseudoaglycon of D

sediment layer (**Table 7**). By 14 DAT, <5% of the radioactivity remained associated with the water layer. No degradates >10% of applied were observed in the water compartment. Percentages of spinosyns A and D and degradates as measured by HPLC relative retention times (RRT) in the water and sediment layers are shown in **Table 7**. First-order half-lives of spinosyns A and D were calculated using the 0−170 DAT data and linear regression of the natural logarithm of the total amount of parent compound at each sampling time from a summation of the water layer and sediment extract HPLC results. The calculated half-lives for spinosyns A and D were 160 and 240 days, respectively, with R^2 values of 0.936 and 0.953.

Structural assignments for degradates listed in **Table 7** were made on the basis of the radiochemical chromatograms of the samples and mass spectral fragmentation patterns and comparison with analytical standards when available. The reconstructed ion chromatograms (RIC) were highly complex due to coextractants; RRTs from the HPLC radiochromatograms were used to locate peaks of interest. A summary of the fragment ions from mass spectra of the HPLC peaks and the corresponding degradate identification is presented in **Table 8**. The following assignments were based on verification with available analytical standards: spinosyn A, spinosyn B, spinosyn, D, ketoreverse psuedoaglycon of A, and reverse pseudoaglycon of A. Assignments of spinosyn J and O-methylated spinosyn J were made on the basis of accurate mass determination with EI-MS (Finnigan MAT 95Q, Bremen, Germany).

On the basis of their identity and the time course of their appearance, a proposed pathway for the degradation of spinosyn A is shown in **Figure 3**. In contrast to hydrolysis, degradation under anaerobic aquatic conditions primarily involves changes (spinosyn J) and substitutions (O-demethylation) in the rhamnose ring or complete loss of the rhamnose ring (reverse pseudoaglycons). The pathway for spinosyn D is believed to be similar; a number of degradates were seen in the water layer and sediment extracts with RRTs and elution profiles similar to those of spinosyn A degradates, suggesting that the observed degradates are the corresponding analogues of the spinosyn D degradates. Given the known 14 amu difference between spinosyns A and D, structural assignments for the spinosyn D degradates have been made on the basis of the correlation of fragmentation patterns of mass spectra between spinosyn A and spinosyn D degradates and RRT for individual degradates.

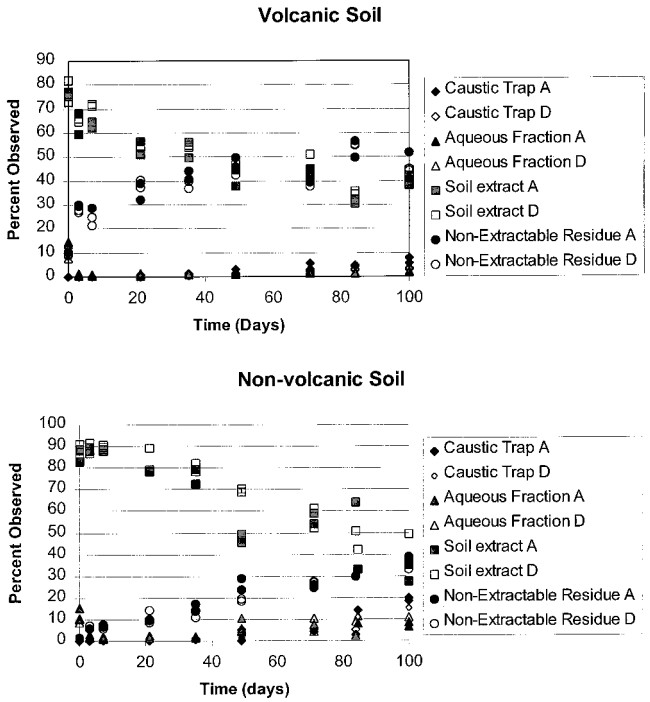

Figure 8. Kinetics of aerobic flooded soil partitioning.

Aerobic Flooded Soil. As seen in **Figure 8** spinosyns A and D degraded and partitioned in a similar fashion. There were slight differences in overall pattern between the two soils, but, in general, the spinosyns partitioned rapidly out of the aqueous phase between DAT 0 and DAT 3 as seen in the decline of the aqueous fraction. Over the course of the 100 days, there was a steady rise (up to ~20%) in the amount of ^{14}C observed in the caustic traps (confirmed as $^{14}CO_2$) and nonextractable soil residue (up to ~50%), and there was a corresponding decrease in the extractable soil residues. As metabolism/degradation increased at the later time points, multiple, low-level degradates were observed in the aqueous layer. Spinosyns A and D degraded with half-lives of 28 and 32 days in the nonvolcanic soil. Spinosyns A and D degraded with half-lives of 28 and 37 days in volcanic soil.

In the nonvolcanic soil (**Table 9**), spinosyn A was transformed into two major degradates (>10%): spinosyn B (confirmed by HPLC and TLC with standards) and 9,17-diketone spinosyn aglycon (**Figure 4**) as assigned by LC-MS

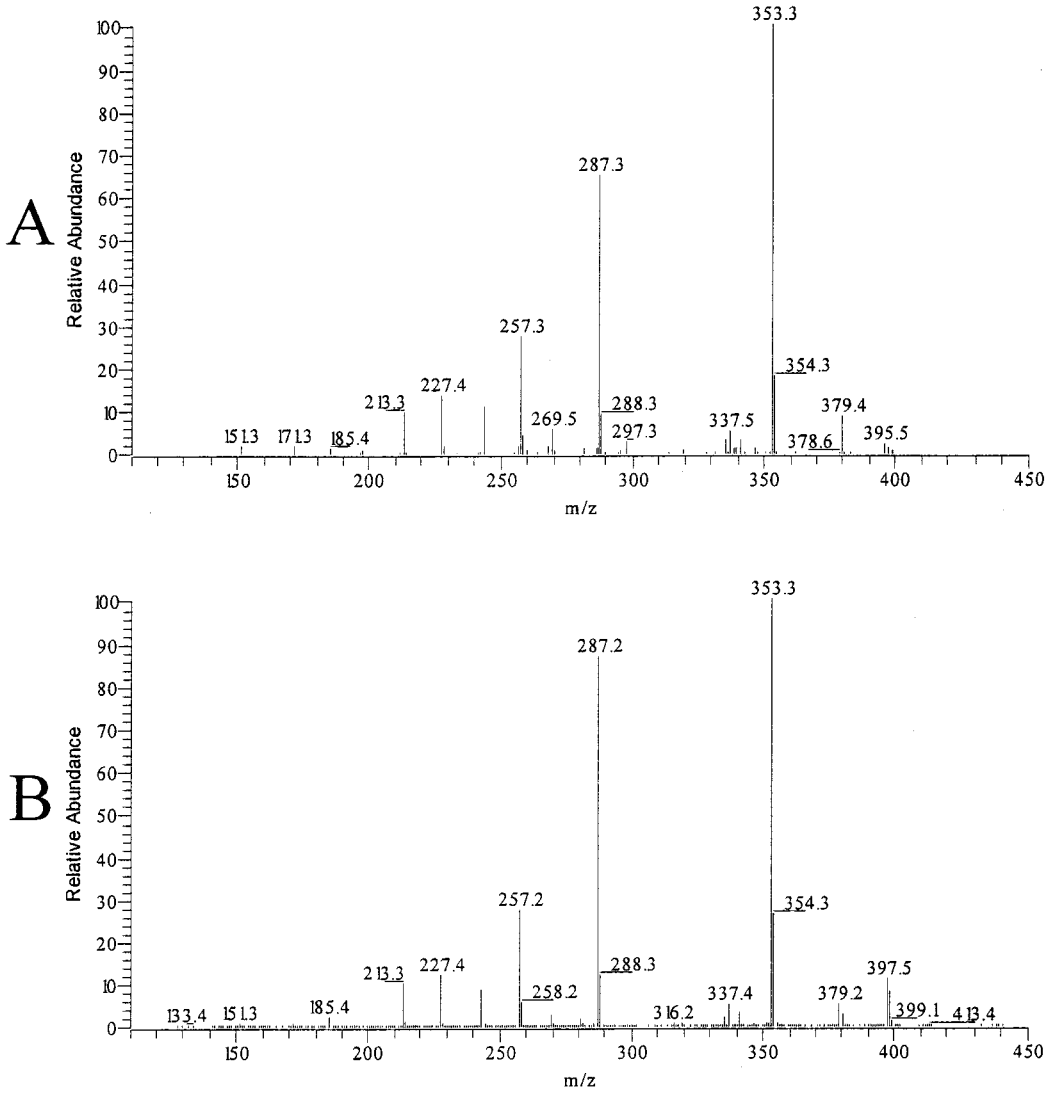

Figure 9. LC-MS/MS mass spectra of (A) aerobic flooded soil degradate and (B) 9,17-diketone spinosyn aglycon standard.

Table 9. Flooded Soil Experiment Results as Average Percent of Applied Activity

DAT	spinosyn A	spinosyn B	9,17-diketone spinosyn aglycon	spinosyn D	N-demethylated spinosyn D	6-methyl-9,17-diketone spinosyn aglycon
			Nonvolcanic			
0	80.8	9.4	0.0	82.5	5.7	0.0
3	78.9	7.4	0.0	68.7	12.1	0.0
7	74.2	8.7	0.0	77.4	7.5	0.0
21	59.0	12.8	1.1	49.1	16.4	2.9
35	32.5	26.5	4.5	44.7	17.5	3.7
49	12.3	9.9	15.8	27.5	18.4	7.3
71	14.5	14.6	13.2	11.3	9.0	10.5
84	13.1	15.7	6.2	12.8	7.3	7.3
100	7.5	5.4	7.5	12.3	5.5	6.7
			Volcanic			
0	72.8	7.4		75.1	3.7	
3	53.8	5.7		56.7	4.4	
7	41.4	12.3		59.6	6.3	
21	38.3	8.3		24.3	9.8	
35	20.5	15.6		23.9	11.2	
49	15.0	10.7		21.6	8.8	
71	8.5	10.9		15.0	9.9	
84	6.1	7.6		8.8	6.7	
100	6.4	8.8		12.9	7.7	

and NMR, confirmed by LC-MS/MS with standard. Several minor products were observed at or below 5% of applied. spinosyn D transformed into two major degradates, N-demethylated spinosyn D (confirmed by HPLC and TLC with standards) and 6-methyl-9,17-diketone spinosyn aglycon (**Figure 4**) as assigned by HPLC, LC-MS, and LC-MS/MS. In the volcanic soil, only spinosyn D and N-demethylated spinosyn D were observed as major degradates.

Assignment/confirmation of the degradates was facilitated by comparison of the chromatograms (on-line radioactivity monitor) with the ion chromatograms from LC-MS. The 9,17-diketone spinosyn aglycon eluted at 9.7 min and 6-methyl-9,17-diketone spinosyn aglycon eluted at 11.4 min from the nonvolcanic soil samples. Key information for identification of the 9,17-diketone spinosyn aglycon came from (1) LC-MS observation of an $[M - H]^-$ adduct at *m*/*z* 397.4, indicating the loss of both sugar rings; (2) NMR, the lack of a C-9 signal and the appearance of a quartet showed coupling to the methyl attributed to the H-16 methyl and no coupling to the H-17, indicating the assigned diketone structure; and (3) LC-MS/MS confirmation against a standard with key fragments at *m*/*z* 353.3, 288.3, 287.2, 257.2, 227.4, and 213.3 (**Figure 9**). The 6-methyl-9,17-diketonespinosyn aglycon has been assigned from the molecular weight from the LC-MS and LC-MS/MS fragmentation pattern and supported by the fact that all of the fragments are 14 mass units greater than those of the 9,17-diketone spinosyn aglycon.

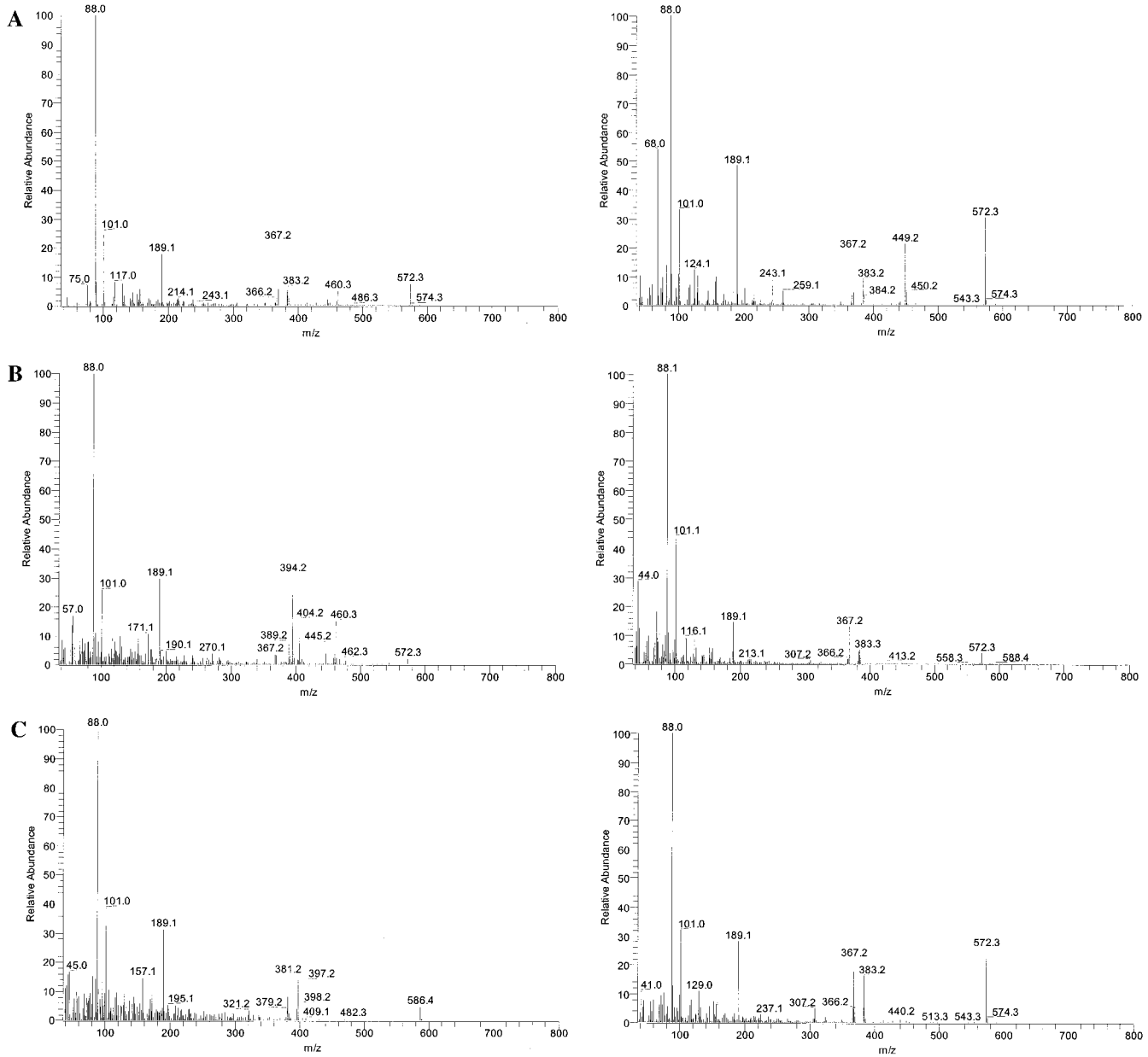

Figure 10. (A) Probe EI-MS spectra of spinosyn A hydrolysis degradate HA1 (left) and the associated dehydroxy-pseudoaglycon standard (right). (B) Probe EI-MS spectra of spinosyn A hydrolysis degradate HA2 (left) and the associated dehydroxypseudoaglycon standard (right). (C) Probe EI-MS spectra of spinosyn D hydrolysis degradate HD1 (left) and associated dehydroxypseudoaglycon standard (right).

Hydrolysis. At pH 5 and 7, essentially no degradation of spinosyn A or D was observed following incubation at 25 °C for up to 30 days; results are congruent with the dark controls of the photolysis experiment. At pH 9, both spinosyns degraded slowly, with degradation on the order of 10% over 30 days and calculated first-order half-lives of 200 and 259 days for spinosyns A and D, respectively.

Degradate identification employed electron impact positive ion mass spectrometry (EI-MS). EI mass spectra of spinosyn-related compounds generally contain an identifiable molecular ion. Spinosyn A exhibits a molecular ion at *m*/*z* 731, and the forosamine and rhamnose sugars are indicated by the fragment ions at *m*/*z* 142 and 189, respectively. HPLC chromatograms of the pH 9 spinosyn A and D hydrolysis samples were similar in that they both had degradation peaks (>10% applied radioactivity) which eluted prior to the parent peaks. Two hydrolysis products from spinosyn A and one from spinosyn D were isolated by TLC. The earliest eluting degradate peak for spinosyn A (HA1) had a mass spectrum that indicated a molecular ion at *m*/*z* 572 and the absence of the *m*/*z* 142 ion observed for spinosyn A (**Figure 10**). HA1 was assumed to form by the loss of the forosamine sugar and water and reduction on the macrolide ring to form a double bond between positions 16 and 17. This structure assignment was confirmed with a known standard, 3-dehydroxypseudoaglycon isomer A (**Figure 5**), by mass spectrometry, and by cochromatography (reversed-phase HPLC and silica gel TLC). The proposed assignment for HD1 is based on knowledge of HA1. The mass spectrum for HD1 was similar to that of HA1 (differing by 14 mass units, just as spinosyns A and D differ by 14 amu) and was marked by the absence of *m*/*z* 142 plus the presence of *m*/*z* 586, which represents the loss of the forosamine and water from the spinosyn D parent ion of *m*/*z* 746. The mass spectrum of HA2 (spinosyn A hydrolysis product) displays key spectral ions *m*/*z* 572, 189 (rhamnose sugar), 101, and 88 and no *m*/*z* 142 ion. It is likely to be structurally related to HA1 and analytical standard

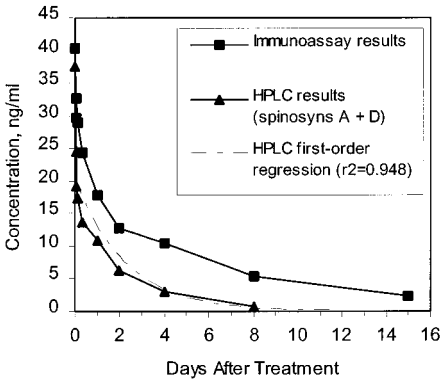

Figure 11. Analysis of microcosm water by immunoassay and HPLC.

Table 10. Results from Outdoor Microcosm Study

	mean (ppb)			
	in water		in sediment	
time (h)	[spinosyns A and D] by HPLC	[spinosad + degradates] by IA	[spinosyns A and D] by HPLC	[spinosad + degradates] by IA
0	37.6	40.4		
1	24.6	29.8		
2	19.3	32.7		
4	17.5	28.9		
8	13.6	24.4		
24	10.9	17.8	10.7	32.1[a]
48	6.2	12.7	13.3	39.2[a]
96	3.1	10.4	14.9	42.9[a]
192	1.7	5.4	14.0	56.0
360	<LOD	2.4	12.1	53.1
840			14.3	41.7[a]

[a] Between limit of detection of 20 ppb and limit of quantification of 50 ppb.

designated isomer B (**Figure 5**) based on cochromatography by reversed-phase HPLC and silica TLC.

Outdoor Microcosm. Microcosm water concentrations of spinosad, as determined by HPLC and IA, are shown in **Figure 11**. At time 0, the mean spinosad water concentration for the three replicate tanks was 38 ng/mL, as determined by HPLC. On the basis of the amount of material applied to the tanks, the initial concentration should have been ~20 ng/mL. The high initial concentrations were attributed to two factors. First, as material was sprayed on the water surface care was taken to avoid spraying near the edge of the tanks. Thus, the middle area treated and subsequently sampled received more material than the edges. Second, after application and prior to diffusion, the surface water would contain a high concentration of spinosad. Any surface water present on the outside glass surface of the sampling tube that dripped into the sample container during transfer of the sample in the tube to the sample bottle could significantly increase the concentration in the sample. At 2 h after application, mean water concentrations were approximately equal to the theoretical initial concentration.

As demonstrated in **Table 10**, spinosad declined rapidly in the water layer after application. By 8 DAT, the concentration in two of the three tanks had reached the HPLC method detection limit of 0.5 ng/mL. The HPLC degradation curve fit first-order kinetics with a correlation coefficient of 0.948 and yielded a half-life of 1.6 days. Exclusion of the samples up to 4 h after treatment from the data set increased the correlation coefficient to 0.997 and resulted in a half-life of 1.8 days for the sum of spinosyns A and D.

Table 11. Sensitivity of Spinosad Immunoassay Test Kit to Several Individual Spinosyns, Metabolites, and Degradates

high sensitivity (I_{50} < 2.0 ng/mL)	low sensitivity (I_{50} > 50 ng/mL)
spinosyn A	spinosyn H
spinosyn B	spinosyn J
spinosyn C	spinosyn L
spinosyn D	spinosyn A reverse pseudoaglycon
N-demethylated spinosyn D	spinosyn A aglycon
spinosyn E	
spinosyn F	
spinosyn K	
spinosyn A pseudoaglycon	

The focus of this outdoor investigation was to confirm the understanding gained in the laboratory studies under real-world conditions with a formulated product. Therefore, no radiolabel was available to track all degradates, but select degradates (closely related to spinosyn A or D) were monitored by HPLC and indirectly by IA. The measured mean water concentrations immediately after application were nearly identical between the HPLC and IA results, but the difference between the two methods increased with time after application. By design, the IA method also detects some degradates of spinosyns A and D. At 4 DAT, the IA method determined water concentration was 10 ng/mL (50% of applied), whereas HPLC indicated the water contained ~3 ng/mL spinosad (15%). Together information from the two methods indicates the presence of ~35% spinosad-related degradates (i.e., immunoreactive degradates) in the water. The HPLC method also measured spinosyn B and N-demethylated spinosyn D (**Figure 1**), but these degradates were found at mean concentrations of only 1.8 and 0.8 ng/mL, respectively, in the 4 DAT samples. **Table 11** indicates the relative sensitivity of various spinosyns to the IA method. The exposed samples are presumed to have contained the aqueous photodegradate 13,14-dihydrospinosyn A C-17 PsA, but this material is not detectable at the wavelength employed by the HPLC method, so confirmation was not possible.

The mean spinosad sediment concentration in the microcosm tanks increased after application to ~14% of applied at 4 DAT and then remained approximately constant through 35 DAT (**Table 10**). The agreement between the HPLC and IA method is tighter for the sediment analyses. This observation is congruent with results from the anaerobic sediment/water experiments, which indicates that once spinosad sorbs to the sediment, there will be slower degradation observed under anaerobic, dark conditions. The results show the majority of the applied spinosad degraded in the water column prior to partitioning to the sediment.

Conclusions. Taken together, these laboratory and outdoor studies provide fundamental understanding of the fate of spinosad in aquatic systems. The laboratory studies focused on individual mechanisms (hydrolysis, photolysis, and biotic degradation). In contrast, the intent of the outdoor microcosm study was not to further elucidate individual rates or fate but instead to confirm the general understanding derived from the laboratory studies, under conditions in which multiple processes were operative. Taken together, they indicate the spinosad will not be persistent and that photolysis is a rapid mechanism for degradation of spinosad in an aqueous system. This understanding is congruent with observations of spinosad's behavior in soil and on leaf surfaces; photolysis plays a key role in these compartments as well (*13*).

In the presence of sunlight, direct photodegradation of aqueous solutions of spinosyns A and D was rapid, with half-

lives of 1 day or less. Rapid photodegradation was confirmed in a natural water microcosm study where a half-life of 1−2 days was observed. Other degradation mechanisms have been observed as demonstrated in the anaerobic sediment and flooded soil systems and under basic aqueous conditions. Degradation primarily proceeds by loss of the forosamine sugar and reduction of the 13,14-bond on the macrolide ring under aqueous photolytic conditions, and degradation to several other compounds is possible through biotic and abiotic degradation.

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