

Enhanced Degradation and Soil Depth Effects on the Fate of Atrazine and Major Metabolites in Colorado and Mississippi Soils

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The aim of this report is to inform modelers of the differences in atrazine fate between *s*-triazine–adapted and nonadapted soils as a function of depth in the profile and to recommend atrazine and metabolite input values for pesticide process submodules. The objectives of this study were to estimate the atrazine-mineralizing bacterial population, cumulative atrazine mineralization, atrazine persistence, and metabolite (desethylatrazine [DEA], deisopropylatrazine [DIA], and hydroxyatrazine [HA]) formation and degradation in Colorado and Mississippi *s*-triazine–adapted and nonadapted soils at three depths (0–5, 5–15, and 15–30 cm). Regardless of depth, the AMBP and cumulative atrazine mineralization was at least 3.8-fold higher in *s*-triazine–adapted than in nonadapted soils. Atrazine half-life ($T_{1/2}$) values pooled over nonadapted soils and depths approximated historic estimates ($T_{1/2}$ = 60 d). Atrazine persistence in all depths of *s*-triazine–adapted soils was at least fourfold lower than that of the nonadapted soil. Atrazine metabolite concentrations were lower in *s*-triazine–adapted than in nonadapted soil by 35 d after incubation regardless of depth. Results indicate that (i) reasonable fate and transport modeling of atrazine will require identifying if soils are adapted to *s*-triazine herbicides. For example, our data confirm the 60-d $T_{1/2}$ for atrazine in nonadapted soils, but a default input value of 6 d for atrazine is required for *s*-triazine adapted soils. (ii) Literature estimates for DEA, DIA, and HA $T_{1/2}$ values in nonadapted soils are 52, 36, and 60 d, respectively, whereas our analysis indicates that reasonable $T_{1/2}$ values for *s*-triazine–adapted soils are 10 d for DEA, 8 d for DIA, and 6 d for HA. (iii) An estimate for the relative distribution of DIA, DEA, and HA produced in nonadapted soils is 18, 72, and 10% of parent, respectively. In *s*-triazine–adapted soils, the values were 6, 23, and 71% for DIA, DEA, and HA, respectively. The effects of soil adaptation on metabolite distribution need to be confirmed in field experiments.

BACTERIA IN MANY AGRICULTURAL SOILS with an atrazine (6-chloro-*N*²-ethyl-*N*⁴-isopropyl-1,3,5-triazine-2,4-diamine) use history have acquired the novel genes *atzABC* and *trzN*, which code for enzymes that rapidly hydrolyze the herbicide (i.e., enhanced degradation) (Krutz et al., 2008b; Seffernick et al., 2000; Shapir et al., 2005). These genes are highly conserved, ubiquitous, and are widespread in soils of the United States where *s*-triazine herbicides are applied yearly for weed control in corn and sugarcane (de Souza et al., 1998; Krutz et al., 2008b). The mono-*n*-dealkylated metabolites of atrazine are also substrates for *atzA* and *trzN* gene products (Seffernick et al., 2000; Shapir et al., 2005). Atrazine, desethylatrazine (DEA), and deisopropylatrazine (DIA) persistence, therefore, is likely lower in soils with a bacterial population that is able to rapidly degrade *s*-triazine compounds (i.e., adapted soils).

Atrazine's average half-life ($T_{1/2}$) in nonadapted soil under aerobic laboratory conditions is 60 to 120 d (USEPA, 2006; Wauchope et al., 1992). Few persistence estimates for DEA ($T_{1/2}$ ranging from 33 to 72 d) or DIA ($T_{1/2}$ ranging from 32 to 40 d) are available (Bottoni et al., 1996; Kruger et al., 1993; Kruger et al., 1997), and, from a modeling standpoint, there is no consensus $T_{1/2}$ value for these metabolites in soil. In contrast, atrazine $T_{1/2}$ values in the Ap horizon of *s*-triazine–adapted soils under aerobic laboratory conditions range from 1 to 12 d (Krutz et al., 2008b). The persistence of DEA and DIA in *s*-triazine–adapted has not been reported.

The disparity in atrazine persistence between *s*-triazine–adapted and nonadapted soils could result in erroneous surface runoff predictions if default $T_{1/2}$ estimates are used for the former (Bakhsh et al., 2004; Chinkuyu et al., 2005; Ma et al., 2004; Muller et al., 2003; Neurath et al., 2007). Additionally, erroneous leaching predictions can occur if atrazine persistence in the subsurface horizons of *s*-triazine–adapted soils is lower than historic estimates (Di et al., 2001; Krutz et al., 2010; Leterme et al., 2007).

Atrazine, DEA, and DIA persistence in nonadapted soils increases with depth in the profile (Kruger et al., 1993; Kruger et al., 1997; Lavy et al., 1996; Miller et al., 1997), and prominent

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Abbreviations: AMBP atrazine-mineralizing bacterial population; DEA, desethylatrazine; DIA, deisopropylatrazine; HA, hydroxyatrazine; HPLC, high-performance liquid chromatography; LOQ, limit of quantitation; MPN, most probable number; RZWQM, Root Zone Water Quality Model.

fate and transport models can estimate this trend (Wauchope et al., 2004). Atrazine persistence in different horizons of *s*-triazine-adapted soils is relatively unknown, particularly in the United States. One Argentinean study indicates that atrazine persistence in *s*-triazine-adapted soils increases with depth in the profile and that atrazine $T_{1/2}$ values in subsurface horizons are at least twofold lower than in nonadapted soils (Hang et al., 2005). No comparative study exists for agricultural soils in the United States.

The objectives of this study were (i) to estimate the atrazine-mineralizing bacterial population (AMBP) in *s*-triazine-adapted and nonadapted soils and (ii) to measure cumulative atrazine mineralization, atrazine $T_{1/2}$ values, and metabolite (DEA, DIA, and hydroxyatrazine [HA]) formation and degradation in Colorado (CO) and Mississippi (MS) *s*-triazine-adapted and nonadapted soils collected from three depths (0–5, 5–15, and 15–30 cm). When sufficient data were available, persistence estimates for DEA and DIA were calculated for *s*-triazine-adapted soils. These values can be used for pesticide process submodules to estimate the differences in atrazine fate between *s*-triazine-adapted and nonadapted soils as a function of depth in the profile.

Materials and Methods

Site History and Soil Collection

s-Triazine-Adapted and Nonadapted Soil from Colorado

s-Triazine-adapted soil from Colorado was collected on 5 Apr. 2007 from an irrigated, strip-tilled, continuous corn production field that had been treated with atrazine since 2003. *s*-Triazine-adapted soil from Mississippi was collected on 12 Mar. 2007. The site history for the Mississippi *s*-triazine-adapted soil was previously described (Krutz et al., 2007; Zablotowicz et al., 2007). Briefly, the field had been under reduced tillage, planted to corn, and treated with atrazine since 2000. Nonadapted CO soil was collected on 5 Apr. 2007 from an irrigated, mixed cropping field that had not been treated with atrazine for the previous 10 yr. The previous crop had been sunflowers. The MS nonadapted soil was collected on 12 Mar. 2007 from a reduced tillage, continuous cotton field with no

prior atrazine exposure history for at least 12 yr. For the CO and MS soils, 20 cores were removed from the previous crop row using a zero-contamination plastic tube (2.3 cm diam. by 30 cm inner dimensions) (Clements Associates Inc., Newton, IA). The cores were divided into 0- to 5-cm, 5- to 15-cm, and 15- to 30-cm sections, and the soil was composited for each treatment. The soils were passed through a 2-mm sieve and stored at 5°C until study initiation. Herbicide and metabolite binding was determined as previously described (Shaner et al., 2009). Selected soil properties are presented in Table 1.

Atrazine Degradation and Metabolite Formation

Soil (100 g dry weight equivalent) weighed into screw-top flasks was fortified with technical-grade atrazine (99% purity) (Chemservice, Lancaster, PA) dissolved in high-performance liquid chromatography (HPLC)-grade acetonitrile resulting in an initial herbicide concentration of 1 $\mu\text{g g}^{-1}$. Soil moisture was adjusted to 70% field capacity by the addition of deionized water. Flasks were sealed with Teflon-lined caps and incubated in the dark at $20 \pm 2^\circ\text{C}$. At 1, 2, 4, 8, 16, 32, 56, 74, and 80 d after treatment, soil (5 g) was removed from the flasks and extracted for atrazine and metabolites. The weight of each flask was measured at each extraction. Water was added if needed to maintain desired soil moisture.

Herbicide and Metabolite Extraction

Soil (5 g) was weighed into a 50-mL plastic centrifuge tube and extracted with 15 mL 80:20 vol/vol MeOH/25 mmol L^{-1} ammonium acetate adjusted to pH 8.0. The suspension was agitated on a horizontal shaker for 30 min and centrifuged at $8000 \times g$ for 15 min, and the supernatant was transferred to 50-mL plastic centrifuge tubes. The extraction procedure was repeated, and supernatants were combined. The supernatant was then evaporated to <5 mL at 50°C with a Rapidvap (Labconco, Kansas City, MO), brought to 10 mL with deionized water, and concentrated on a C18 SPE column (Thermo Electron Corp., Logan, UT) preconditioned with 3 mL each of methanol, ethyl acetate, methanol, and distilled water. The column was dried under negative pressure for 90 min, and atrazine, DEA, and DIA were eluted with 2 mL ethyl acetate

Table 1. Selected soil properties of the *s*-triazine-adapted and nonadapted locations in Colorado and Mississippi soils.

Soil†	Depth	Sand	Silt	Clay	OM‡	pH
	cm	%			g C kg^{-1}	
CO, adapted	0–5	35.2	28.0	36.8	19.0	7.9
	5–15	35.2	28.0	36.8	19.0	8.0
	15–30	31.2	32.0	36.8	19.0	7.8
CO, nonadapted	0–5	28.8	47.2	24.0	15.0	8.1
	5–15	28.8	47.2	24.0	14.0	7.9
	15–30	28.8	47.2	24.0	13.0	7.9
MS, adapted	0–5	12.8	41.2	46.0	13.0	6.4
	5–15	16.8	53.2	30.0	13.0	6.3
	15–30	18.8	47.2	34.0	13.0	7.3
MS, nonadapted	0–5	16.8	27.2	16.8	18.0	6.8
	5–15	16.8	29.2	16.6	13.0	6.6
	15–30	18.8	33.2	16.7	8.0	6.7

† CO, Colorado; MS, Mississippi.

‡ OM, organic matter.

into 2-mL volumetric tubes. Samples were fortified with an internal standard of 10 μL of 0.1 mg mL^{-1} of butylate dissolved in acetonitrile, brought to volume with ethyl acetate, and analyzed with a gas chromatography/mass spectrometry. Subsequently, HA was eluted from the column with 2 mL 95:5 vol/vol methanol (MeOH)/0.1 mol L^{-1} HCl into 2-mL volumetric tube. Samples were brought to volume with MeOH and analyzed by HPLC.

Gas Chromatography/Mass Spectrometry Analysis

The parent compound and *N*-dealkylated metabolites were quantified by monitoring the masses of atrazine (M/Z 200), DEA (M/Z 172), DIA (M/Z 173), and butylate (M/Z 146) with a gas chromatograph equipped with a mass spectrometry detector (Shimadzu GC-17A and GC-MS QO 5050A; Shimadzu Scientific Instruments, Columbia, MD). Analyte separation was achieved on a 30-m by 0.25-mm RTZ-5 column (Restek, Bellefonte, PA) with a flow of helium at 1 mL min^{-1} . Injection and detector temperature were held at 280°C. Initial oven temperature was held at 80°C for 1 min, ramped to 250°C at 20°C min^{-1} , and held for 1.5 min. Total run time was 11 min. Under these conditions, the retention times of butylate, DIA, DEA, and atrazine were 6.51, 7.89, 7.96, and 8.44 min, respectively. Recovery of atrazine, DIA, and DEA from fortified soil samples was 95, 85, and 90%, respectively. Pesticide and metabolite concentrations in soil samples were adjusted based on these percent recoveries. The method limit of quantitation (LOQ) for atrazine, DIA, and DEA was 0.007 mg kg^{-1} .

High-Performance Liquid Chromatography Analysis

Hydroxyatrazine was identified and quantified by a Shimadzu 10AT HPLC separation module coupled with a Shimadzu SPP. M 10A detector. The HPLC was fitted with a 4.6-mm diameter by 150-mm length Alltech Econosphere C_{18} column. Mobile phase solvents were HPLC grade and consisted of 63:35 vol/vol 5 mmol L^{-1} ammonium acetate (pH 5.2)/acetonitrile. The injection volume was 100 μL , and separation was achieved in the isocratic mode with a flow rate of 1 mL min^{-1} . Under these conditions, HA was detected at 8.6 min at 236 nm. Hydroxyatrazine recovery from soil ranged from 75 to 85%, and the method LOQ was 0.007 mg kg^{-1} .

Mineralization of ^{14}C -ring-Labeled Atrazine

Mineralization of ^{14}C -ring-labeled atrazine was evaluated in biometer flasks as previously described (Krutz et al., 2007). Briefly, *s*-triazine-adapted and nonadapted soil (25 g dry weight equivalent) from CO and MS were fortified with a solution of technical grade atrazine (99% purity) (Chemservice, Lancaster, PA) and ^{14}C -atrazine (115 $\mu\text{Ci}/\text{mmol}$ specific activity; 94% radiological purity) (Sigma Chemical Company, St. Louis, MO) in deionized water. The initial herbicide concentration was 1 mg kg^{-1} , and the initial radioactivity was 86,580 Bq kg^{-1} . Final soil moisture contents were adjusted to 70% field capacity by adding deionized water, and biometers were incubated in the dark at $20 \pm 2^\circ\text{C}$. Evolved $^{14}\text{CO}_2$ was trapped in NaOH and quantified by liquid scintillation spectroscopy using Hionic-Fluor (PerkinElmer, Shelton, CT). To avoid saturation by CO_2 , NaOH was replaced after each sampling.

Most Probable Number of Atrazine-Mineralizing Bacterial Populations

The most probable number (MPN) of AMBPs was estimated for soils using ^{14}C -ring-labeled atrazine (0.05 mg L^{-1} and 2000 Bq L^{-1}) as previously reported (Zablotowicz et al., 2006), except that the carbon- and nitrogen-limited media were based on mineral salts from Vogel's media (Vogel, 1964). For each soil MPN, analysis was conducted on four replicate samples. Soils were serially diluted in phosphate buffer, and shell vials containing 800 μL of atrazine media were inoculated with 100 μL of soil dilution (1:10 to 1:10,000 dilution; five replicates per dilution). The shell vials were plugged with sterile polypropylene foam, placed in scintillation vials containing 1.0 mL of 1 mol L^{-1} NaOH , and capped with aluminum foil-lined caps. The MPN assemblies were incubated at 25°C for 28 d. Mineralization was determined by removing the inoculated shell vial, adding 15 mL of Hi-ionic cocktail, and counting by liquid scintillation spectroscopy. Estimates of AMBPs were calculated using MPN tables (Woomer, 1994).

Statistical Analysis

The experimental design was a split-split-plot arranged as a randomized complete block with three replications of each treatment. Soils were the main plot, depth was the subplot, and time was the sub-subplot. Analysis of variance and mean separation was performed using Proc Mixed (SAS version 9.1; SAS Institute Inc., Cary, NC). All results were considered significantly different at $p < 0.05$. Degradation data for atrazine and atrazine metabolites was fitted to Eq. [1] (SAS version 9.1; SAS Institute Inc., Cary, NC):

$$Y = Ae^{-kt} \quad [1]$$

where A (mg kg^{-1}) is the herbicide concentration in soil at the first sampling time [$t(\text{d}) = 0$], and k (d^{-1}) is the first-order rate constant. In some instances, insufficient data were available to model DEA, DIA, or HA kinetics; that is, compounds were only modeled if $<4\%$ of the parent compound remained and four or more data points were available to be fitted. Half-life values for all compounds were calculated from Eq. [2]:

$$T_{1/2} = 0.693/k \quad [2]$$

Results

Confirming that Dissipation Is Primarily Biologically Mediated

With the exception of HA, sorption coefficients for atrazine and the evaluated metabolites did not vary much among soils and depths (Tables 2–4). Within a given soil, K_d values for atrazine, DEA, DIA, and HA tended to decrease with depth in the soil profile, which was attributed primarily to declining levels of organic matter as depth in the profile increased. Moreover, for a given soil depth, K_d values generally were not different between *s*-triazine-adapted and nonadapted soils. A lack of significance in these tests indicates that differences in persistence estimates between *s*-triazine-adapted and nonadapted soils cannot be attributed to sorption and

Table 2. Atrazine and desethylatrazine sorption coefficients for Colorado and Mississippi *s*-triazine-adapted and nonadapted soil as a function of depth (0- to 5-, 5- to 15-, and 15- to 30-cm depth intervals) in the soil profile.

Soil†	Depth	L kg ⁻¹	
		Atrazine	Desethylatrazine
CO, adapted	cm		
	0–5	1.65 (0.059)‡	1.29 (0.023)
	5–15	1.56 (0.043)	1.24 (0.011)
CO, nonadapted	15–30	1.37 (0.042)	1.17 (0.015)
	0–5	1.50 (0.001)	1.24 (0.003)
	5–15	1.49 (0.007)	1.24 (0.016)
MS, adapted	15–30	1.45 (0.015)	1.23 (0.003)
	0–5	2.57 (0.239)	1.56 (0.074)
	5–15	2.04 (0.093)	1.40 (0.026)
MS, nonadapted	15–30	1.60 (0.025)	1.25 (0.015)
	0–5	2.78 (0.212)	1.74 (0.070)
	5–15	2.52 (0.169)	1.64 (0.070)
	15–30	2.16 (0.026)	1.45 (0.024)
	LSD _{0.05} (soil × depth)	0.2293	0.0865

† CO, Colorado; MS, Mississippi.

‡ Numbers in parentheses denote 1 SD from the mean.

limited bioavailability. Moreover, previous research from our laboratories indicated that enhanced atrazine mineralization or dissipation in *s*-triazine-adapted soils from Colorado and Mississippi was microbially mediated and could not be attributed to chemical hydrolysis (Shaner et al., 2007; Zablotowicz et al., 2007).

Atrazine-Mineralizing Bacterial Populations

Independent of depth, the AMBP in the CO and MS non-adapted soils was below the method limit of detection (i.e., 50 cells g⁻¹ soil) (Table 5). Conversely, an AMBP was detected at all depths in the CO and MS *s*-triazine-adapted soils. In the MS soils, the AMBP decreased with depth and was at least

sevenfold higher than that of the CO soils, with exception of the 15- to 30-cm depth.

Atrazine Mineralization

Cumulative atrazine mineralization was similar between CO and MS nonadapted soils (Fig. 1). Independent of depth, ≤2.3% of the herbicide was mineralized, and no statistical differences were observed among depths within or between nonadapted soils. Atrazine mineralization in MS *s*-triazine-adapted soil decreased with depth and, regardless of depth, was at least 49-fold higher than that of the MS nonadapted soil. Similarly, cumulative atrazine mineralization in the CO *s*-triazine-adapted soil decreased with depth and was at least 15.9-fold higher than that of the CO nonadapted soil, regardless of depth.

Atrazine Persistence

For all soils, atrazine T_{1/2} values decreased with depth. Atrazine T_{1/2} values pooled over nonadapted soils and depths approximated the historic estimate (60 d) (Table 5). For all depths, atrazine T_{1/2} values in *s*-triazine-adapted soils were at least fourfold higher than that of the non-adapted soils.

Metabolite Formation

Desethylatrazine

Desethylatrazine concentrations were markedly different between the *s*-triazine-adapted and nonadapted CO and MS soils (Fig. 2). In the CO soils, peak DEA concentrations occurred sooner and were of a lower magnitude in the 0- to 5-cm depth as compared with the 15- to 30-cm depth, regardless of herbicide use history. With exception of the 15- to 30-cm

Table 3. Main effects of soil and depth (0- to 5-, 5- to 15-, and 15- to 30-cm depth intervals) on the deisopropylatrazine partitioning coefficient.

Depth	CO, adapted†	CO, nonadapted	MS, adapted	MS, nonadapted	Depth mean
	L kg ⁻¹				
0–5 cm	1.74 (0.037)‡	1.32 (0.003)	1.80 (0.065)	2.07 (0.076)	1.65a
5–15 cm	1.41 (0.014)	1.33 (0.020)	1.70 (0.021)	1.99 (0.088)	1.60a
15–30 cm	1.38 (0.645)	1.31 (0.015)	1.53 (0.0330)	1.77 (0.049)	1.59a
Soil mean	1.51b§	1.32b	1.67ab	1.94a	

† CO, adapted: Colorado *s*-triazine adapted; CO, nonadapted: Colorado nonadapted; MS, adapted: Mississippi *s*-triazine adapted; MS, nonadapted: Mississippi nonadapted.

‡ Number in parentheses denotes 1 SD from the mean.

§ Main effect means within a column or row that are followed by the same letter are not significantly different at $P \leq 0.05$.

Table 4. Main effects of soil and depth (0- to 5-, 5- to 15-, and 15- to 30-cm depth intervals) on the hydroxyatrazine sorption partitioning coefficient.

Depth	CO, adapted†	CO, nonadapted	MS, adapted	MS, nonadapted	Depth mean
	L kg ⁻¹				
0–5 cm	1.68 (0.059)‡	1.31 (0.038)	10.49 (0.190)	9.58 (0.396)	5.76a
5–15 cm	1.52 (0.006)	1.39 (0.017)	10.24 (0.543)	9.98 (0.412)	5.78a
15–30cm	1.40 (0.003)	1.36 (0.023)	9.51 (0.156)	9.35 (0.227)	5.40b
Soil mean	1.53c§	1.35c	10.08a	9.63b	

† CO, adapted: Colorado *s*-triazine adapted; CO, nonadapted: Colorado nonadapted; MS, adapted: Mississippi *s*-triazine adapted; MS, nonadapted: Mississippi nonadapted.

‡ Number in parentheses denotes 1 SD from the mean.

§ Main effect means within a column or row that are followed by the same letter are not significantly different at $P \leq 0.05$.

Table 5. Most probable number for atrazine degraders, degradation rate constants, and half-life values for atrazine in Colorado and Mississippi *s*-triazine-adapted and non-*s*-triazine-adapted soil as a function of depth in the soil profile.

Soil†	Depth	MPN‡	<i>k</i>	Half-life	<i>r</i> ²
	cm	cells g ⁻¹ soil	d ⁻¹	d	
CO, adapted	0–5	311	0.2881 (0.2315–0.3446)§	2 (2.0–3.0)	0.96
	5–15	191	0.0684 (0.0581–0.0788)	10 (8.8–11.9)	0.99
	15–30	239	0.0277 (0.0246–0.0309)	25 (22.4–28.2)	0.98
CO, nonadapted	0–5	ND¶	0.0131 (0.0110–0.0151)	53 (45.9–63.0)	0.91
	5–15	ND	0.0078 (0.0063–0.0093)	89 (74.5–110.0)	0.90
	15–30	ND	0.0069 (0.0054–0.0084)	100 (82.5–128.3)	0.91
MS, adapted	0–5	3584	0.7262 (0.5560–0.8964)	<1 (0.8–1.2)	0.99
	5–15	2389	0.4810 (0.3762–0.5857)	1 (1.2–1.8)	0.98
	15–30	301	0.1529 (0.1236–0.1823)	5 (3.8–5.6)	0.99
MS, nonadapted	0–5	ND	0.0182 (0.0155–0.0210)	38 (33.0–44.7)	0.97
	5–15	ND	0.0196 (0.0167–0.0224)	35 (30.9–41.5)	0.97
	15–30	ND	0.0129 (0.0110–0.0148)	54 (46.8–63.0)	0.99
LSD _{0.05} (soil × depth)		532		11	

† CO, Colorado; MS, Mississippi.

‡ *k*, rate constant; MPN, most probable number.

§ Values in parentheses indicate 95% confidence intervals.

¶ ND, not determined.

depth, DEA concentrations were lower in CO *s*-triazine-adapted than in CO nonadapted soil from 16 d after inception until termination. A similar trend in DEA concentrations as a function of depth and herbicide use history was observed in the MS soils. The differences in DEA concentrations between MS *s*-triazine-adapted and nonadapted soils, however, were more apparent than in CO soils. Desethylatrazine *T*_{1/2} values were similar among *s*-triazine-adapted soils and averaged 10 d when pooled over depths and soils (Table 6).

Deisopropylatrazine

Generally, the temporal trend in DIA concentrations among depths between *s*-triazine-adapted and nonadapted soils was similar to DEA (Fig. 3). For instance, peak DIA concentrations occurred sooner and were of a lower magnitude in the 0- to 5-cm depth as compared with the 15- to 30-cm depth, regardless of herbicide use history. For all depths, excluding 15- to 30-cm, DIA concentrations were lower in CO *s*-triazine-adapted than nonadapted soil from 26 d after inception until termination. In MS *s*-triazine-adapted and nonadapted soils, there was no definitive trend in DIA concentrations as a function of depth. For all depths, however, DIA concentrations were lower in MS *s*-triazine-adapted than nonadapted soil from 16 d after inception until termination. Deisopropylatrazine *T*_{1/2} values were similar among *s*-triazine-adapted soils and averaged 8 d when pooled over depths and soils (Table 6).

Hydroxyatrazine

Two distinctive temporal trends in HA concentrations were observed (Fig. 4). First, HA concentrations in the 0- to 5-cm depth of CO *s*-triazine-adapted and the 0- to 15-cm depths of MS *s*-triazine-adapted increased rapidly within days of application relative to their respective non-

adapted soils, but HA concentrations in *s*-triazine-adapted soils returned to levels similar to their respective nonadapted counterparts by 8 d. Second, from 56 d after inception until termination, HA concentrations were higher in nonadapted soils relative to *s*-triazine-adapted soils, regardless of depth. From a regulatory perspective, it is important to note that HA concentrations in MS *s*-triazine-adapted soil exceeded 0.10 mg kg⁻¹ (i.e., approximately 10% of the parent compounds initial mass) by 56 d after inception, and the concentration was increasing at study termination, regardless of depth.

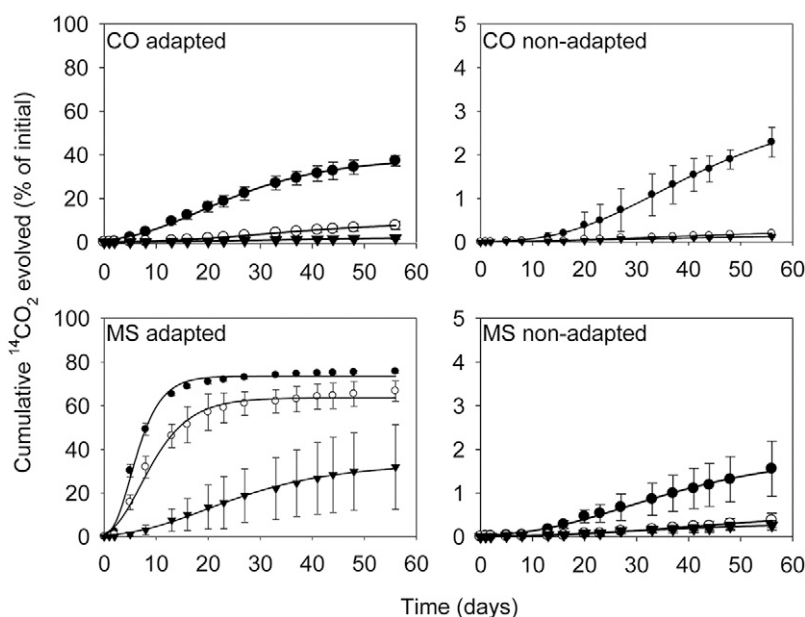


Fig. 1. Cumulative ¹⁴C₂ evolved reported as percent of total ¹⁴C-ring-labeled atrazine added in Colorado (CO) and Mississippi (MS) *s*-triazine-adapted and nonadapted soil as a function of depth in the soil profile. Symbols represent the mean of three replicates for each of the following treatments: 0- to 5-cm depth (closed circles), 5- to 15-cm depth (open circles), and 15- to 30-cm depth (closed triangles). Error bars indicate 1 SD and do not appear when smaller than the symbol for the mean. LSD_{0.05} (soil × depth × time) = 5.912. Note the differences in scale between *s*-triazine-adapted and non-adapted soils.

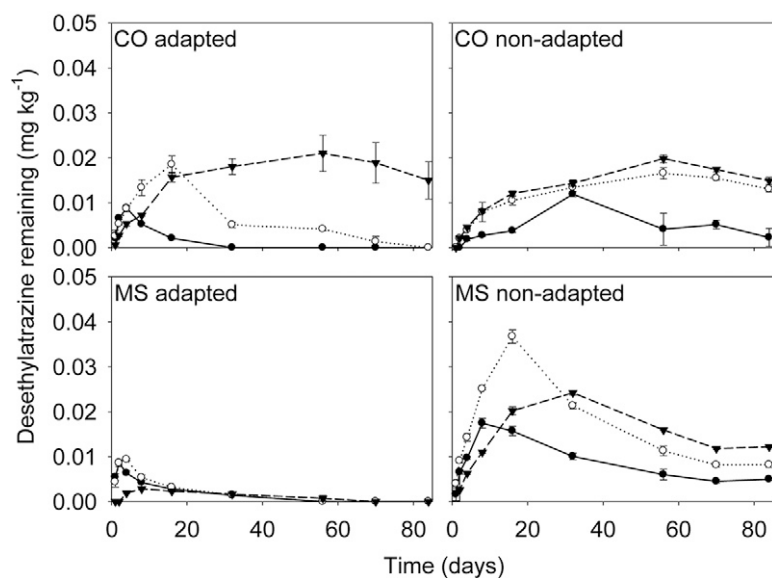


Fig. 2. Desethylatrazine (DEA) concentrations in Colorado (CO) and Mississippi (MS) *s*-triazine-adapted and nonadapted soil as a function of depth in the soil profile. Symbols represent the mean of three replicates for each of the following treatments: 0- to 5-cm depth (closed circles), 5- to 15-cm depth (open circles), and 15- to 30-cm depth (closed triangles). Error bars indicate 1 SD and do not appear when smaller than the symbol for the mean. $LSD_{0.05}(\text{soil} \times \text{depth} \times \text{time}) = 0.002$.

Discussion

A prerequisite for enhanced degradation is the selection of soil-borne bacteria with an adaptation that enables rapid pesticide degradation. In this study, an AMBP was detected only in soils with an atrazine use history, signifying that a previous atrazine application was likely the selective agent (Barriuso and Houot, 1996; Houot et al., 2000; Shaner and Henry, 2007; Shaner et al., 2007; Zablotowicz et al., 2006). Basic and applied studies indicate, however, that previous applications of *s*-triazine her-

bicides can select for bacteria able to rapidly degrade atrazine, a phenomenon known as cross-adaptation (Krutz et al., 2008a; Seffernick et al., 2000; Shapir et al., 2005). The adaptation in soil-borne bacteria from Colorado and Mississippi enabling rapid atrazine degradation was previously elucidated (i.e., the procurement of *atzABC* or *trzN* genes) (Krutz et al., 2008b).

Atrazine-mineralizing bacterial populations have been quantified in other soils with an atrazine exposure history, resulting in reduced herbicide persistence relative to historic estimates (Di et al., 2001; Zablotowicz et al., 2006). The AMBP data indicate the potential for reduced atrazine persistence in all depths of the *s*-triazine-adapted soil relative to the nonadapted soil. Additionally, the AMBP in MS *s*-triazine-adapted soil decreased with depth. This trend signifies that the selection of bacteria able to rapidly degrade atrazine is limited primarily to the upper root zone and that atrazine degradation in *s*-triazine-adapted soils decrease as depth in the profile increases. Exceptions are noted in that the AMBP was at least 27-fold higher in the 60- to 90-cm depth relative to the 0- to 30-cm depth of a New Zealand soil with a prior atrazine exposure history (Di et al., 2001; Sparling et al., 1998).

The cumulative mineralization and AMBP data were in agreement. For the nonadapted soils, no AMBP was detected, and cumulative mineralization approximated historic estimates of $\leq 2.5\%$, regardless of depth (Blume et al., 2004; Kruger et al., 1997). In MS *s*-triazine-adapted soil, cumulative mineralization decreased with depth, as was projected from the AMBP. An inverse relationship between cumulative atrazine mineralization and depth within *s*-triazine-adapted soils was previously noted (Hang et al., 2005; Hang et al., 2007). Cumulative

Table 6. Degradation rate constants and half-life values for desethylatrazine and deisopropylatrazine in Colorado and Mississippi *s*-triazine-adapted and nonadapted soils.

Soil†	Depth cm	$k\ddagger$ d^{-1}	Half-life d	r^2
Desethylatrazine				
CO, adapted	0–5	0.1355 (0.1193–0.1518)§	5 (4.6–5.8)	0.99
	5–15	0.0632 (0.0118–0.1147)	11 (6.0–58.7)	0.95
	15–30	–¶	–	–
MS, adapted	0–5	0.0918 (0.0511–0.1326)	8 (5.2–13.6)	0.98
	5–15	0.0838 (0.0385–0.1291)	8 (5.4–18.0)	0.98
	15–30	0.0345 (0.0154–0.0536)	20 (12.9–45)	0.95
Deisopropylatrazine				
CO, adapted	0–5	0.1019 (0.0914–0.1123)	7 (6.2–7.6)	0.99
	5–15	–	–	–
	15–30	–	–	–
MS, adapted	0–5	0.0735 (0.0203–0.1267)	9 (5.5–34.1)	0.91
	5–15	0.0916 (0.0484–0.1348)	8 (5.1–14.3)	0.96
	15–30	–	–	–

† CO, Colorado; MS, Mississippi.

‡ k , degradation rate constant.

§ Numbers in parentheses indicate 95% confidence intervals.

¶ Values were not calculated due to insufficient data.

mineralization in CO *s*-triazine-adapted soil also decreased with depth, but there was no relationship between cumulative mineralization and the AMBP.

The mineralization data reveal differences between *s*-triazine-adapted and nonadapted soils that have implications for the herbicides' fate and transport. First, atrazine catabolism is greater in *s*-triazine-adapted than in nonadapted soils, signifying lower total *s*-triazine residues in the former, regardless of depth. The potential transport for total *s*-triazine residue at all depths, therefore, is lower in *s*-triazine-adapted than in nonadapted soils (Krutz et al., 2008b). Second, for CO and MS *s*-triazine-adapted soils, cumulative mineralization decreased with depth. This trend indicates that atrazine residues leached into lower horizons of *s*-triazine-adapted soils will be more persistent and available for subsequent transport relative to residues in upper horizons, which is typical for nonadapted soils.

Atrazine $T_{1/2}$ values in nonadapted soils adhered to conventional thought. Atrazine persistence pooled over nonadapted soils and depths converged with the herbicides' historic persistence estimate of 60 d (Wauchope et al., 1992; USEPA, 2006), and $T_{1/2}$ values increased with depth (Blume et al., 2004; Kruger et al., 1993; Kruger et al., 1997; Miller et al., 1997). Conversely, atrazine persistence in all depths of the CO and MS *s*-triazine-adapted soils was at least twofold lower than historic estimates. Atrazine persistence in *s*-triazine-adapted soils increased as a function of depth in the soil profile, as was reported for an Argentina *s*-triazine-adapted soil (Hang et al., 2005).

The differences in atrazine persistence between *s*-triazine-adapted and nonadapted soils have a manifold impact on the modeling of atrazine fate and transport. First, precise model predictions are predicated on accurate pesticide persistence estimates (Di et al., 2001; Leterme et al., 2007). The default input value for atrazine persistence in commonly used fate and transport models is 60 to 120 d (USEPA, 2006; Wauchope et al., 1992). The discrepancy in $T_{1/2}$ values between *s*-triazine-adapted and nonadapted soils, regardless of depth, results in erroneous model predictions for *s*-triazine-adapted soils (Di et al., 2001; Leterme et al., 2007). Second, the Root Zone Water Quality Model (RZWQM) assumes (i) that the aerobic degradation half-life is constant from 0 to 25 cm depth, (ii) that $T_{1/2}$ values increase linearly from 25 to 75 cm depth, and (iii) that $T_{1/2}$ values are static past 100 cm depth (Wauchope et al., 2004). Our aerobic $T_{1/2}$ data demonstrate that atrazine persistence is not static over the 0- to 25-cm depth interval, regardless of herbicide use history. Moreover, the change in atrazine $T_{1/2}$ over the 0- to 25-cm depth interval is more dramatic for *s*-triazine-adapted than for nonadapted soils. These observations indicate that the maximum $T_{1/2}$ factor (i.e., the maximum multiple of the near-surface half-life that the half-life reaches at 100 cm [Wauchope et al., 2004]), will be greater for *s*-triazine-adapted than for nonadapted soils.

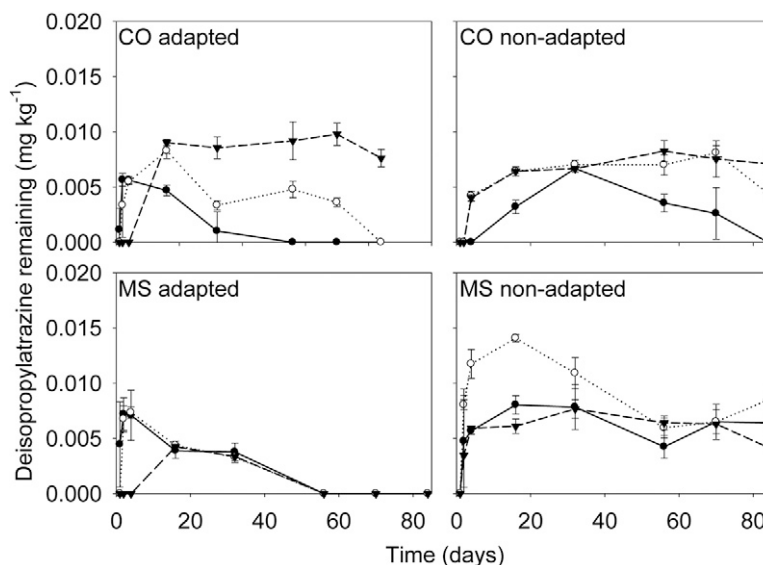


Fig. 3. Deisopropylatrazine (DIA) concentrations in Colorado (CO) and Mississippi (MS) *s*-triazine-adapted and nonadapted soil as a function of depth in the soil profile. Symbols represent the mean of three replicates for each of the following treatments: 0- to 5-cm depth (closed circles), 5- to 15-cm depth (open circles), and 15- to 30-cm depth (closed triangles). Error bars indicate 1 SD and do not appear when smaller than the symbol for the mean. $LSD_{0.05}(\text{soil} \times \text{depth} \times \text{time}) = 0.002$.

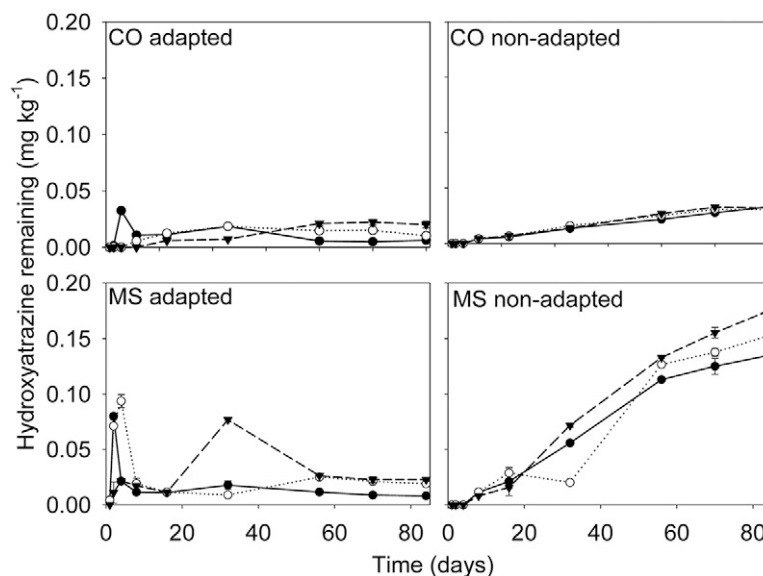


Fig. 4. Hydroxyatrazine (HA) concentrations in Colorado (CO) and Mississippi (MS) *s*-triazine-adapted and nonadapted soil as a function of depth in the soil profile. Symbols represent the mean of three replicates for each of the following treatments: 0- to 5-cm depth (closed circles), 5- to 15-cm depth (open circles), and 15- to 30-cm depth (closed triangles). Error bars indicate 1 SD and do not appear when smaller than the symbol for the mean. $LSD_{0.05}(\text{soil} \times \text{depth} \times \text{time}) = 0.003$.

There is a paucity of data for atrazine $T_{1/2}$ values at depths in excess of 30 cm in *s*-triazine-adapted soils. Atrazine persistence in an *s*-triazine-adapted soil from Argentina was at least twofold lower in the 0- to 18-cm depth compared with the 90-cm depth (Hang et al., 2005). A few studies report atrazine mineralization potential as a function of depth in *s*-triazine-adapted soils (Aislabie et al., 2004; Hang et al., 2005; Sparling et al., 1998), and results indicate that cumulative atrazine mineralization is zero- to twofold higher in surface soil as compared with that at the bottom of the root zone (i.e., at approximately 1 m

depth). There are no data on atrazine persistence in *s*-triazine-adapted soils below the root zone.

The concentration of *n*-dealkylated metabolites was generally lower in *s*-triazine-adapted than in nonadapted soils, regardless of depth. The explanation for this observation is multifaceted. First, soils exhibiting enhanced atrazine degradation are positive for *atzA* or *trzN* (Krutz et al., 2008b). These genes code for enzymes that rapidly convert atrazine to hydroxyatrazine (Seffernick et al., 2000; Shapir et al., 2005). Preferential conversion of atrazine to hydroxyatrazine, rather than to *n*-dealkylated *s*-triazine metabolites, may partially explain the lower concentration of *n*-dealkylated *s*-triazine metabolites in *s*-triazine-adapted soils (Krutz et al., 2008b). Recent data support this assumption in that a branched, serial, first-order decay model for atrazine in an *s*-triazine-adapted soil predicted that 71, 23, and 6% of the parent compound degraded to HA, DEA, and DIA, respectively (Webb et al., unpublished). These results are counterintuitive in that conventional atrazine degradation theory holds that HA formation arises primarily from chemical hydrolysis, that HA accounts for less than 10% of the parent compounds molar mass, and that microbial *n*-dealkylation governs the herbicide's fate in the soil environment. Second, *n*-dealkylated atrazine metabolites are substrates for the *atzA* and *trzN* gene products (Seffernick et al., 2000; Shapir et al., 2005). Lower concentrations of *n*-dealkylated atrazine metabolites in *s*-triazine-adapted soils may be due to the rapid conversion of *n*-dealkylated metabolites to hydroxyatrazine derivatives. Few persistence estimates for DEA and DIA in nonadapted soils are available, and no persistence estimates for atrazine's *n*-dealkylated metabolites are reported for *s*-triazine-adapted soils. The $T_{1/2}$ values for *n*-dealkylated metabolites in the surface horizon of nonadapted soil ranges from 32 to 72 d (Bottoni et al., 1996; Kruger et al., 1993; Kruger et al., 1997) and, in the case of DIA, is 173 d at a depth of 130 cm (Kruger et al., 1993). Our estimates for DEA and DIA persistence in *s*-triazine-adapted soils, particularly in the 0- to 5-cm depth, are at least threefold lower than the values reported in the literature. Accurate modeling of DEA and DIA transport in *s*-triazine-adapted soils will likely require lower persistence estimates than those currently reported.

Conclusions

The following recommendations regarding accurate atrazine fate and transport modeling are gleaned from the present work and our interpretation of previously published enhanced atrazine degradation data. Foremost, differences in atrazine persistence mandate a distinction between *s*-triazine-adapted and nonadapted soils. Two methods for identifying *s*-triazine-adapted soils exist (Shaner et al., 2007; Krutz et al., 2009). Regardless of the method used, a $T_{1/2}$ of 6 d is recommended for atrazine in *s*-triazine-adapted soils, whereas the historic estimate of 60 to 120 d is appropriate for nonadapted soils.

Second, the RZWQM assumes that herbicide degradation is static over the 0- to 25-cm depth range (Wauchope et al., 2004). Regardless of herbicide use history, our data indicate that atrazine degradation is not static over the 0- to 25-cm depth, particularly in *s*-triazine-adapted soils. This assump-

tion, therefore, may cause prediction errors in *s*-triazine-adapted and in nonadapted soils, with the potential for error exacerbated in the former. Field studies are required to elucidate prediction error associated with assuming static atrazine persistence in the 0- to 25-cm depth, particularly in the case of *s*-triazine-adapted soils.

Third, atrazine persistence, regardless of herbicide use history, increases with depth in the soil profile. The effect is greater in *s*-triazine-adapted than in nonadapted soils. The RZWQM allows the user to vary the maximum half-life factor (i.e., the multiple of the near-surface half-life that the $T_{1/2}$ reaches at 100 cm), and the default setting of 10 is often recommended (Wauchope et al., 2004). Based on the data presented by Wauchope et al. (2004), the default maximum $T_{1/2}$ factor is a logical estimate for nonadapted soils. The data presented herein indicate that the $T_{1/2}$ factor for *s*-triazine-adapted soils will be greater than 10. For example, the multiple of the near-surface $T_{1/2}$ that the CO *s*-triazine-adapted soil reached at the 15- to 30-cm depth was 12.5. Strong inference allows us to conclude that the $T_{1/2}$ factor will be greater than 12.5 at a depth of 100 cm because atrazine degrader numbers and activity decrease with depth in the profile of *s*-triazine-adapted soils.

Fourth, there is no consensus half-life value for DEA or DIA in the literature, and there is no indication as to the range of values that regulatory agencies use as input parameters. Based on previously published data, a default input value of 52 d for DEA and 36 d for DIA is recommended for nonadapted soils, whereas data from the present study indicate that $T_{1/2}$ values of 10 and 8 d are reasonable for DEA and DIA, respectively, in the 0- to 25-cm depth of *s*-triazine-adapted soils.

Fifth, HA or hydroxy-*s*-triazine derivatives are obligatory metabolites in *s*-triazine-adapted soils. However, there are no reported half-life values for HA in *s*-triazine-adapted or nonadapted soils. Clearly, HA persistence in soil requires further study. Until HA persistence data are available, HA persistence in nonadapted soils should be set at a value similar to that proposed by USEPA for atrazine (i.e., 90–120 d). In the case of *s*-triazine-adapted soils, previously published data indicate that HA persistence in *s*-triazine-adapted soils approaches that of atrazine (Krutz et al., 2006). Consequently, in *s*-triazine-adapted soils, a HA $T_{1/2}$ of 6 d is recommended.

Finally, the RZWQM allows the user to define the percentage of atrazine that is transformed to DEA, DIA, or HA, with a maximum of two metabolites modeled in concert with the parent compound. Reasonable estimates for the percent of atrazine transformed to these “daughter” species are nonexistent. Given that HA typically does not exceed 10% of the parent compounds molar mass (USEPA) and that a typical ratio for DEA to DIA concentrations in soil is 4:1, a reasonable ratio in nonadapted soils is 10% for HA, 72% for DEA, and 18% for DIA. For *s*-triazine-adapted soils, the ratios, as predicted by Webb et al. (unpublished), are recommended: 71% for HA, 23% for DEA, and 6% for DIA. Field-scale modeling studies are required to verify the effectiveness of these recommendations for decreasing the error associated with predicting atrazine fate and transport in *s*-triazine-adapted soils with default input values established for nonadapted soils.

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Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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