

Microbial adaptation for accelerated atrazine mineralization/degradation in Mississippi Delta soils

Robert M. Zablotowicz

USDA-Agricultural Research Service, Southern
Weed Science Research Unit, P.O. Box 350,
Stoneville, MS 38776; rzablotowicz@ars.usda.gov

Mark A. Weaver

USDA-Agricultural Research Service, Southern
Weed Science Research Unit, P.O. Box 350,
Stoneville, MS 38776

Martin A. Locke

USDA-Agricultural Research Service, National
Sedimentation Laboratory, Water Quality and
Ecology Research Unit, Oxford, MS 38655

Most well-drained Mississippi Delta soils have been used for cotton production, but corn has recently become a desirable alternative crop, and subsequently, atrazine use has increased. Between 2000 and 2001, 21 surface soils (0 to 5 cm depth) with known management histories were collected from various sites in Leflore, Sunflower, and Washington counties of Mississippi. Atrazine degradation was assessed in 30-d laboratory studies using ^{14}C -ring-labeled herbicide. Mineralization was extensive in all soils with a history of one to three atrazine applications with cumulative mineralization over 30 d ranging from 45 to 72%. In contrast, cumulative mineralization of atrazine from three soils with no atrazine history was only 5 to 10%. However, one soil with no history of atrazine application mineralized 54 and 29% of the atrazine in soils collected in 2000 and 2001, respectively. Methanol extracted 15 to 23% of the ^{14}C -atrazine 7 d after treatment in soils having two applications within the past 6 yr, whereas 65 to 70% was extracted from no-history soils. First-order kinetic models indicated soil with 2 yr of atrazine exposure exhibited a half-life of less than 6 d. Most probable number (MPN) estimates of atrazine-ring mineralizing-microorganisms ranged from 450 to 7,200 propagules g^{-1} in atrazine-exposed soils, and none were detected in soils with no history of atrazine use. Although most soils exhibited rapid atrazine mineralization, analysis of DNA isolated from these soils by direct or nested polymerase chain reaction (PCR) failed to amplify DNA sequences with primers for the *atzA* atrazine chlorohydrolase gene. These results indicate that microbial populations capable of accelerated atrazine degradation have developed in Mississippi Delta soils. This may reduce the weed control efficacy of atrazine but also reduce the potential for off-site movement. Studies are continuing to identify the genetic basis of atrazine degradation in these soils.

Nomenclature: Atrazine; cyanazine; DEA, de-ethyl atrazine; DIA, de-isopropyl atrazine; corn, *Zea mays* L.; cotton, *Gossypium hirsutum* L.

Key words: Accelerated degradation, atrazine, microbial metabolism, mineralization.

Atrazine is one of the most widely used herbicides for broadleaf weed control in North American corn, sorghum [*Sorghum bicolor* (L.) Moench], and sugarcane (*Saccharum officinarum* L.) production. Consequently, atrazine is one of the most frequently detected herbicide contaminants in ground- and surface water (Burkhart and Kolpin 1993; Scribner et al. 2000; Thurman et al. 1991) and is of concern for toxicity to both humans and the environment (Solomon et al. 1996). Most well-drained soils of the Mississippi Delta have been historically cropped to cotton. However, corn has recently become a more desirable alternative crop. Atrazine is the most widely used herbicide for corn production. However, most cotton grown in the Mississippi Delta was treated with the related triazine, cyanazine. Cyanazine was removed from the market in 2000. With the change in Mississippi Delta cropping systems to increased corn production, relatively high levels ($> 10 \mu\text{g } \mu\text{l}^{-1}$) of atrazine have been detected in Mississippi Delta lake waters (Zablotowicz et al. 2004) with concentrations similar to those observed in mid-western U.S. streams (Thurman et al. 1991).

Atrazine is considered to be moderately persistent in soil with a half-life ranging from 30 d to greater than 1 yr (Blumhorst and Weber 1994; Houot et al. 1998; Sparling et al. 1998). In recent years, soils showing the potential for rapid mineralization of atrazine have been identified

throughout the world (Barriuso and Houot 1996; Yassir et al. 1999). Historically, the major route of biological atrazine transformation observed in soil was *N*-dealkylation to the products de-ethyl atrazine (6-amino-2-chloro-4-isopropyl-1,3,4-triazine, DEA) and de-isopropyl atrazine (6-amino-2-chloro-4-ethyl-1,3,4-triazine, DIA) (Esser et al. 1975). Atrazine dechlorination to hydroxyatrazine was initially considered to be due primarily to abiotic chemical hydrolysis (Blumhorst and Weber 1994; Esser et al. 1975). In the past decade, bacterial strains possessing atrazine chlorohydrolase, *atzA* (DeSouza et al. 1995, 1998), and related amidohydrolase enzymes, e.g., *trzN* (Mulbry et al. 2002; Topp et al. 2000), have been reported as common mechanisms for enhanced atrazine degradation in soil. Once atrazine is dechlorinated, further enzymatic hydrolytic transformations result in atrazine-ring cleavage, mineralization, and subsequent release of ammonium (Martinez et al. 2001). Nitrogen released from atrazine metabolism serves as a nitrogen source for atrazine-degrading bacteria (Bichat et al. 1999).

The objectives of this study were to characterize patterns of atrazine degradation (mineralization, metabolite formation, and kinetics) in Mississippi Delta soils and to ascertain the role of cropping history, herbicide history, and management practices on atrazine persistence.

TABLE 1. Cropping history, chemical, and biological soil characteristics of soils used in the studies.^a

Soil	Corn/atrazine history	Tillage	Organic matter ^b	pH	Sand	Silt	Clay	Total bacteria	Total fungi
			(%)		(%)	(%)	(%)	— log 10 cfu g ⁻¹ soil —	
2000									
Beasley 1	1998, 1999	CT	1.1 (0.2)	5.7 (0.3)	31 (1)	36 (8)	33 (3)	7.8 (0.1)	5.8 (0.1)
Beasley 2	1998	CT	1.9 (0.3)	6.6 (0.3)	21 (4)	35 (10)	40 (5)	7.7 (0.1)	5.4 (0.1)
Beasley 3	None	CT	1.1 (0.2)	6.2 (0.3)	22 (2)	57 (2)	21 (1)	7.3 (0.2)	5.0 (0.2)
Indian Mound	1997, 1998, 1999	CT	1.2 (0.3)	7.0 (0.4)	7 (3)	75 (3)	18 (2)	7.7 (0.1)	5.4 (0.1)
GW-Mon	1997	CT	0.8 (0.1)	5.8 (0.1)	27 (5)	57 (4)	16 (1)	7.4 (0.1)	5.4 (0.1)
Walker 1A	1998	NT	1.9 (0.1)	6.8 (0.2)	38 (5)	41 (4)	19 (5)	8.1 (0.1)	5.1 (0.1)
Walker 1B	1998	NT	0.9 (0.1)	5.6 (0.2)	43 (5)	33 (6)	24 (2)	7.7 (0.2)	4.8 (0.1)
Walker 2	1998, 1999	CT	1.5 (0.2)	6.2 (0.5)	30 (2)	53 (1)	17 (1)	7.3 (0.2)	4.9 (0.1)
Walker 3	1999	NT	4.2 (0.2)	7.3 (0.2)	26 (3)	54 (2)	20 (1)	8.6 (0.3)	6.2 (0.2)
Stoneville 1	None	CT	1.0 (0.1)	6.7 (0.3)	25 (2)	58 (1)	17 (2)	7.0 (0.1)	5.2 (0.1)
Stoneville 2	None	NT	1.8 (0.1)	6.8 (0.2)	24 (6)	56 (1)	17 (1)	7.4 (0.1)	5.5 (0.1)
LSD 0.05			0.3	0.5	7.0	7.0	4.0	0.1	0.2
2001									
Beasley 3	None	CT	1.5 (0.2)	6.4 (0.2)	22 (2)	57 (2)	21 (1)	7.7 (0.1)	5.1 (0.1)
Elizabeth	1997, 2000	CT	1.3 (0.2)	6.0 (0.2)	25 (3)	61 (2)	14 (2)	7.4 (0.1)	5.1 (0.1)
GW-Leland	1999, 2000	CT	1.0 (0.2)	5.9 (0.2)	21 (2)	59 (2)	20 (2)	8.1 (0.1)	5.1 (0.1)
GW-Mon	1996, 2000	CT	1.1 (0.2)	5.8 (0.1)	27 (5)	57 (4)	16 (1)	7.6 (0.1)	4.9 (0.1)
Thighman-1	1997, 1998, 2000	NT	3.0 (0.6)	5.9 (0.1)	29 (10)	41 (5)	30 (7)	7.9 (0.1)	5.4 (0.1)
Thighman-2	1999, 2000	NT	1.9 (0.2)	6.2 (0.1)	17 (5)	45 (3)	37 (5)	8.0 (0.1)	4.8 (0.1)
Thighman-3	None	CT	1.9 (0.2)	6.5 (0.2)	13 (3)	41 (2)	46 (1)	8.0 (0.1)	5.3 (0.2)
Thighman-4	2000	CT	2.1 (0.4)	5.8 (0.2)	15 (5)	58 (3)	27 (7)	8.0 (0.1)	5.6 (0.2)
Walker-1A	1998, 2000	NT	2.1 (0.2)	7.0 (0.2)	38 (5)	41 (4)	21 (3)	8.1 (0.1)	5.0 (0.1)
Walker-1B	1998, 2000	NT	1.1 (0.2)	6.0 (0.2)	43 (6)	33 (6)	24 (2)	7.1 (0.1)	4.9 (0.1)
LSD 0.05			0.3	0.5	7	5	6	0.1	0.1

^a Abbreviations: cfu, colony forming units; CT, conventional tillage; NT, no tillage.

^b Mean and standard deviation of four replicates.

Materials and Methods

Soils

Surface soils were collected from experimental sites and grower farms in Leflore, Washington, and Sunflower counties of Mississippi, in 2001 and 2002 (Table 1). In this survey, only 4 of the 17 individual soils (Beasley 3, GW-Mon, Walker 1a, and Walker 1b) were assessed in both years of this study. Based on soil survey data (Soil Survey Staff 1959a, 1959b, 1959c), most soils were a Dundee (fine-silty, mixed, thermic Typic Endoaqualfs) and also included Forrestdale (fine, smectitic, thermic Typic Endoaqualfs), Dowling (very-fine smectitic, Thermic Vertic Epiaquepts), and Alligator (very-fine, smectitic Thermic Alic Dytraquepts) soil series.

The soils were collected in early March, before planting and application of herbicide and fertilizer. Sampling points were georeferenced, with global positioning system and coordinates identified using a Trimble Pathfinder ProXR¹ to enable precise resampling. Cropping and herbicide history for at least the past 6 to 10 yr before sample collection were obtained from farm managers. Soils were collected from both conventional tillage (CT) and no-tillage (NT) systems. At each site, four replicate samples were collected about 100 m apart, in a rectangular grid using a surface sterilized sampling probe (5 cm in diameter). Replicate samples from a field were within a similar soil type and landscape position. Each replicate soil sample consisted of a composite of 10 subsamples collected to a depth of 0 to 5 cm. Soils were maintained at 4 C until processed. Enumeration of micro-

bial propagules was conducted within 2 d of collection, whereas atrazine degradation studies were conducted within 2 to 4 wk after collection. Soils were analyzed for organic matter and nitrate content by the University of Arkansas Soil Analysis Laboratory. Electrical conductivity and pH was determined in an aqueous soil suspension (2:1). Soil textural analysis was determined by the hydrometer method (Gee and Bauder 1986). Total bacterial and fungal propagules were determined by serial-dilution plating and spiral plating on 10% tryptic soy agar and rose Bengal potato dextrose agar, respectively (Reddy et al. 2003).

Degradation Studies

For each soil, 30 g of soil (air-dried weight equivalents) were added to three 250-ml polypropylene centrifuge bottles and one biometer flask (Bartha and Pramer 1965). Soils were treated with a mixture of ¹⁴C-ring-labeled² and -unlabeled atrazine³ to attain a concentration of 1.25 µg g⁻¹ and 149 Bq g⁻¹ (a total of ~ 252,000 dpm flask⁻¹). Following treatment of soils with atrazine, soils were adjusted to 30% moisture content (wt/wt) by adding additional distilled water, and soils were incubated at 28 C. Soils treated in centrifuge bottles were extracted 0, 7, and 13 d after treatment (DAT), and biometer flasks were extracted 30 DAT. For 0 d analysis, soils were extracted within 2 h of treatment.

To monitor atrazine mineralization, the side-arm traps of the biometer flasks were filled with 10 ml of 1 N sodium hydroxide, which was periodically removed and replaced

with fresh solution. Trapped $^{14}\text{CO}_2$ in the sodium hydroxide was determined on duplicate 1-ml aliquots by liquid scintillation spectroscopy⁴ (LSS) using Hi-Ionic scintillation fluid⁵, at 15 ml sample⁻¹. To recover atrazine and metabolites, treated soils were extracted with 100 ml of aqueous methanol (80 : 20 v/v methanol : water) for 20 h and centrifuged (10 min at $6,000 \times g$), and the supernatant was removed. Soil was reextracted with another 100 ml of aqueous methanol. Methanol extracts were combined and weighed, and radioactivity recovered was determined by LSS using Ecolume scintillation fluid⁶. Soils were further extracted with 0.5 M dibasic potassium phosphate (pH 7.5) and acetonitrile (3 : 1) for 24 h according to Lerch et al. (1997), and the radioactivity recovered in the supernatant was determined following centrifugation. The extracts were concentrated by rotary evaporation, diluted in 150 ml of water, acidified with 100 μl 1.0 N HCl, and further concentrated on 3-ml C₁₈-solid-phase extraction (SPE) columns⁷. The herbicide and metabolites were eluted from the SPE column in 4 ml of methanol and concentrated to 1.5 ml under nitrogen (N₂) gas. Most (> 95%) of the radioactivity was retained and eluted from the SPE column using this method. Herbicides and metabolites recovered in the methanol extracts were determined using thin-layer chromatography (TLC) and linear imaging scanning⁸. Aliquots (100 μl) were spotted on silica gel plates (250 μm thick). TLC plates were developed to 10 cm using toluene : ethyl acetate solvent (50 : 50 v/v), and retention factor (R_f) values for triazine standards were atrazine = 0.67, DEA = 0.40, DIA = 0.23, and hydroxyatrazine = 0.00 (Blumhorst and Weber 1994). Nonextractable radioactivity remaining in soils following the three extractions (bound fraction) was determined by oxidation as described by Locke et al. (1996), combusting soils with a biological oxidizer⁹ using a mixture of Carbo-Sorb E¹⁰ and Permafluor E¹¹ cocktail and counting radioactivity by LSS. Data for recovery of atrazine, mineralization and nonextractable ^{14}C were subjected to ANOVA using the general linear model procedure in SAS (SAS 2001). Means were separated using Fisher's protected LSD test at $P = 0.05$. Atrazine mineralization was modeled to fit second-order degradation kinetics using the three-parameter, Gompertz growth model ($y = ae^{-e[-k(t-t_0)]} + ct$) using Sigma Plot version 7.0¹² as used by others (Martin-Laurent et al. 2004; Piutti et al. 2002a, 2002b). Parameters determined include a , the plateau representing maximum percentage of mineralization; t_0 , the abscissa of the inflection point; k , the mineralization rate constant; and c , the rate of ^{14}C cometabolic mineralization; and t , time. The atrazine degradation data were fit to first-order kinetics using PCSAS NLIN procedure (SAS 2001). The first-order degradation rate constant (k) and initial herbicide concentration (C_0), standard deviation, and the 95% confidence interval were calculated using the NLIN procedure, and the half-life ($T_{1/2}$) was calculated from k ($T_{1/2} = 0.693/k$). Pearson correlations between atrazine-degrading parameters and soil characteristics were assessed using SAS, the Proc Corr procedure (SAS 2001).

Assessment of Atrazine-Degrading Populations

The MPN of atrazine-mineralizing populations were estimated for soils collected in 2000, using ^{14}C -ring-labeled atrazine (0.05 $\mu\text{g ml}^{-1}$ and 20 Bq ml^{-1}) in nitrogen-limited media (Jayachandran et al. 1998). Soils were serially diluted

in phosphate buffer, and shell vials containing 1 ml of atrazine media were inoculated with 100 μl of soil dilution (1 : 10 to 1 : 10,000 dilution, five replicates per dilution) and incubated at 28 C for 30 d. Mineralization was determined by removal of the inoculated shell vial, addition of 15 ml of Hi-ionic cocktail, and counting by LSS. Estimates of atrazine-mineralizers were calculated using MPN tables (Woomer 1994).

DNA was extracted from soils collected in 2001, and soils were exposed to 2.5 $\mu\text{g g}^{-1}$ atrazine for 30 d. At least two separate DNA extractions and subsequent polymerase chain reaction (PCR) were conducted for the four replicates of the 10 soils. About 300 mg of moist soil was extracted using an Ultra Clean soil DNA kit¹³. DNA was further purified using Promega Wizard DNA clean-up kits¹⁴. The presence of atrazine chlorohydrolase *atzA* genes was assessed by traditional PCR using forward and reverse primers 5'CCATGTGAACCAGATCCT3' and 5'GGTAATGTGGACGCTTCA3' (DeSouza et al. 1995), respectively. PCR protocols used a 2-min denaturation step at 94 C, followed by 25 cycles of 1 min at 94 C, 1 min at 55 C, and 1 min at 72 C, with a final extension step of 7 min at 72 C. Plasmid DNA containing the *atzA* gene (DeSouza et al. 1995) was used as a positive control. Nested PCR was according to Shapir et al. (2000) and used the *AtzA* internal primers, 5'GCACGGGCGTCAATTCTA3' and 5'GACAGTTGAAGGAATGCG3'. All reactions were with Finnzyme DyNAzyme polymerase¹⁵, using manufacturer's supplied buffer, without additional magnesium chloride.

Results and Discussion

Soil Characterization

A partial characterization of selected chemical, physical, and biological properties of the soils studied is summarized in Table 2. Data are not shown for all parameters, e.g., electrical conductivity and nutritional status (extractable nitrate, phosphate, and potassium). Of the 17 soils evaluated, four were evaluated for atrazine degradation in both years of the study. A wide range in organic matter contents was observed (0.7 to 4.3%). For example, two of the higher organic matter contents measured were in Thighman 1, no-tillage soil, with a balansa clover (*Trifolium michelianum* Savi) cover crop, and Walker 3, no-tillage soil, amended with cotton gin waste. Most soils were a silt loam to silty clay loam texture (mean silt ~ 50%), with one soil (Thighman 3), a silty clay. Soil pH ranged from moderately acidic to slightly above neutral (5.2 to 7.5). Soils with higher organic matter content contained the greatest number of bacterial and fungal propagules.

Atrazine Mineralization

Atrazine was rapidly mineralized in 15 soils that had a history of one to four atrazine applications (Table 2), with cumulative mineralization representing 45 to 73% of the ^{14}C -atrazine applied. Representative mineralization for five soils assayed in 2000 and 2001 are presented in Figure 1. In three soils with no atrazine history (Stoneville CT, Stoneville NT, and Thighman 3), only 5 to 10% of applied atrazine was mineralized during the 30-d incubation. However, one soil, with no atrazine history (Beasley 3), miner-

TABLE 2. Atrazine mineralization in Mississippi Delta soils collected in 2000, cumulative mineralization observed at select times, and parameters of atrazine mineralization kinetics after fitting to the Gompertz growth model.

Soil	Atrazine history	Observed mineralization ^a			Modeled mineralization parameters		
		7 DAT ^b	13 DAT	30 DAT	<i>A</i>	<i>ti</i>	<i>k</i>
—————% atrazine applied—————							
2000							
Beasley 1	2	36 (15)	50 (12)	60 (7)	58.2 (1.0)	4.7 (0.2)	0.26
Beasley 2	1	27 (5)	54 (3)	72 (2)	71.2 (0.5)	7.0 (0.1)	0.21
Beasley 3	0	12 (6)	32 (17)	54 (25)	55.2 (0.3)	9.6 (0.1)	0.17
GW-Mon	1	24 (3)	51 (5)	66 (1)	65.8 (0.4)	7.2 (0.1)	0.14
Indian Mound	3	40 (3)	62 (7)	71 (6)	68.6 (0.8)	4.6 (0.1)	0.3
Walker 1A	1	10 (8)	28 (11)	53 (2)	53.7 (0.6)	10.5 (0.1)	0.18
Walker 1B	1	8 (3)	29 (24)	46 (25)	45.4 (1.2)	9.2 (0.4)	0.18
Walker 2	2	37 (10)	56 (12)	67 (7)	64.4 (0.9)	5.2 (0.2)	0.29
Walker 3	1	11 (3)	19 (6)	52 (7)	58.1 (1.3)	13.7 (0.2)	0.14
Stoneville 1	0	1 (1)	2 (1)	8 (5)	31 ^c	38	0.04
Stoneville 2	0	1 (1)	3 (1)	10 (1)	43 ^c	39.5	0.04
LSD 0.05		11	17	18			
Mean 2000							0.18
2001							
Beasley 3	0	3 (1)	11 (5)	28 (15)	32.5 (0.6)	13.3 (0.2)	0.13
Elizabeth	2	42 (8)	54 (6)	58 (5)	55.9 (0.7)	3.5 (0.1)	0.37
GW-Leland	2	47 (2)	54 (2)	59 (2)	56.3 (0.6)	3.2 (0.1)	0.50
GW-Mon	2	46 (6)	54 (2)	59 (6)	56.1 (0.7)	3.2 (0.1)	0.42
Thighman 1	3	50 (4)	57 (5)	62 (5)	59.0 (0.7)	3.2 (0.1)	0.48
Thighman 2	2	35 (10)	51 (5)	57 (5)	56.5 (0.6)	4.9 (0.1)	0.33
Thighman 3	0	2 (1)	3 (1)	5 (1)	6.4 (0.5)	10.7 (1.0)	0.09
Thighman 4	1	37 (10)	48 (5)	56 (3)	54.2 (0.6)	4.5 (0.1)	0.33
Walker 1A	2	48 (2)	62 (2)	67 (1)	64.4 (0.6)	3.4 (0.1)	0.46
Walker 1B	2	51 (1)	60 (1)	64 (2)	61.8 (0.5)	3.4 (0.1)	0.46
LSD 0.05		9	6	8			

^a Mean and standard deviation in parentheses.

^b Abbreviation: DAT, days after treatment.

^c Predicted mineralization kinetics parameters, parameters exceed study duration.

alized 54 and 29% of the applied atrazine in soils collected in 2000 and 2001 respectively, with a relatively wide variance among the four replicate samples.

Atrazine mineralization kinetics were fit to a Gompertz three-parameter growth model (Piutti et al. 2002a, 2002b) for most soils with an $r^2 > 0.999$ (Table 2). Except for the

two Stoneville soils with low mineralization potential, the values for the plateau of percentage of mineralization predicted (*a*) was in good agreement with observed maximum mineralization at 30 DAT. The modeled lag phase before rapid mineralization (*ti*) ranged from 3 DAT to greater than 38 DAT. Of the four soils evaluated both years, the *ti* values were lower in three of the soils collected in 2001 when compared with those collected in 2000. The exception was Beasley 3 soil with no history of corn or atrazine use, where *ti* was greater in soil collected in 2001. The mineralization rates (*k*) of rapidly degrading soils ranged from greater than 0.21 to 0.46 (arbitrary units), whereas soils having a limited mineralization were generally less than 0.10. The *k* values for the three soils with atrazine history increased in 2001 compared with 2000 because of another year of atrazine exposure, whereas the *k* value for Beasley 3 decreased from 2000 to 2001. The mineralization kinetic parameters *a* and *ti* observed for degrading soils were similar to that reported elsewhere (Krutz et al. 2006; Martin-Laurent et al. 2004; Piutti et al. 2002b). Although extensive mineralization (40 to 50% of atrazine added) was observed in certain soils following 1 yr of atrazine exposure (Walker 1A, 1B, and 3), a more consistent mineralization was observed following the second year of atrazine application. This is especially evident for NT soils as illustrated by samples collected from Walker 1A, and Walker 1B in 2000 compared with the same soils

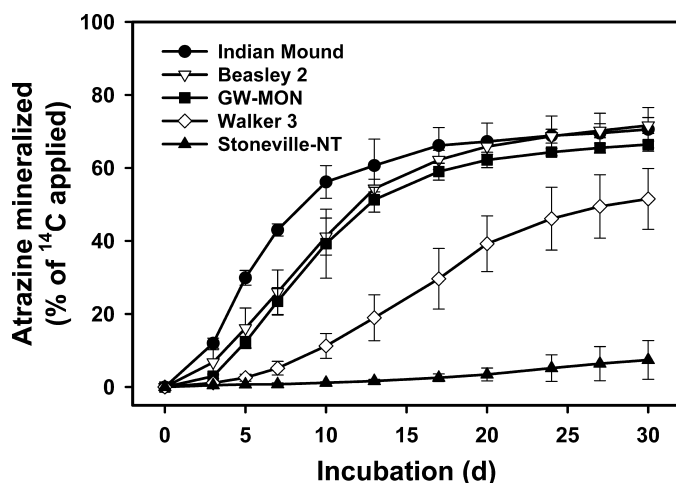


FIGURE 1. Atrazine mineralization in five soils (Indian Mound, Beasley 2, GW-MON, Walker 3, Stoneville 2) collected in 2000; mean and standard deviation of four replicates.

TABLE 3. Summary of atrazine degradation characteristics of Mississippi Delta soils collected in 2000, and most probable number (MPN) estimate of atrazine degraders.

Soil	Atrazine history	Methanol-extractable atrazine ^a			Nonextract-able ¹⁴ C 30 DAT	Degradation constant (<i>k</i>)	Half life (d)	MPN atrazine degraders propagules
		7 DAT ^b	13 DAT	30 DAT				
		% applied						
Indian Mound	3	13 (5)	5 (2)	3 (1)	4 (1)	0.269 (.016)	2.6	3.31 b
Walker 2	2	15 (3)	5 (1)	3 (2)	6 (2)	0.256 (.013)	2.7	3.09 bc
Beasley 1	2	23 (10)	13 (5)	7 (2)	18 (8)	0.161 (.016)	4.3	3.86 a
Beasley 2	1	37 (7)	14 (3)	4 (2)	12 (2)	0.122 (.011)	5.7	3.81 a
GW-Mon	1	40 (3)	16 (1)	6 (1)	15 (3)	0.114 (.005)	6.1	3.11 bc
Walker 1B	1	50 (13)	24 (14)	6 (1)	8 (2)	0.086 (.031)	8.0	3.05 cd
Walker 3	1	57 (3)	36 (10)	8 (4)	25 (2)	0.080 (.005)	8.7	2.66 d
Beasley 3	0	58 (5)	26 (18)	12 (12)	15 (3)	0.079 (.012)	8.8	0.00 e
Walker 1A	1	63 (3)	36 (10)	6 (4)	10 (1)	0.068 (.007)	10.2	3.08 bc
Stoneville 1	0	70 (3)	60 (6)	40 (1)	19 (2)	0.032 (.008)	21.7	0.00 e
Stoneville 2	0	72 (6)	63 (3)	49 (5)	19 (3)	0.015 (.003)	46.2	0.00 e
LSD 0.05		9	12	4	5			0.30

^a Mean and standard deviation (in parentheses) of four replicates.

^b Abbreviation: DAT, days after treatment.

in 2001 (Tables 2 and 3), where *ti* values were reduced by two-thirds, and *k* values nearly tripled. These accelerated rates of atrazine mineralization observed in most of the Mississippi soils are similar to those reported for certain soils worldwide, e.g., Australia (Sparling et al. 1998), France (Barriuso and Houot 1996; Martin-Laurent et al. 2004; Yassir et al. 1999), Israel (Shapir et al. 2000), and the United States (Ostrofsky et al. 1997). However, most of the soils examined in other studies had a more extensive history of atrazine exposure (> 10 yr). All the Mississippi soils evaluated in the current study had several years of exposure to cyanazine, between 1990 and 2000, associated with cotton production. However, this apparently did not translate into higher mineralization for atrazine in the “no-atrazine history” soils.

Atrazine Dissipation

A rapid dissipation of methanol-extractable atrazine was observed in soils that exhibited extensive atrazine mineralization (Tables 3 and 4; Figure 2). Methanol recovered 15

to 25% of the ¹⁴C-atrazine applied 7 d after treatment in most soils with a history of two atrazine applications. In three of the soils with no history of atrazine application, about 58 to 72% of atrazine was recovered by methanol extraction at 7 d. At 30 DAT, all soils with at least 1 yr of atrazine treatment had less than 10% of atrazine applied recovered by methanol extraction, whereas greater than 40% was recovered in three of the soils with no history of atrazine application. The exception was soil sampled from Beasley 3 with 12% of the atrazine applied recovered at 30 DAT in soils collected in 2000; however, for soils collected in 2001, 34% of the atrazine applied was recovered at 30 DAT. Evaluation of methanol-extractable atrazine using first-order kinetics indicated that atrazine half-lives for soils having at least 1 yr of exposure to atrazine ranged from 2.5 to 8.8 d. Soils with no atrazine history exhibited atrazine half-lives of greater than 17 d, except for soils from the Beasley 3 site collected in 2000. Although the Beasley 3 site had no record of atrazine application for at least 10 yr preceding this survey, it is possible that there may have been movement of soil from other regions of this farm from tillage equipment

TABLE 4. Summary of atrazine-degradation characteristics of Mississippi Delta soils collected in 2001.

Soil	Atrazine history	Methanol-extractable atrazine ^a			Nonextractable ¹⁴ C 30 DAT	Degradation constant (<i>k</i>)	Half life (d)
		7 DAT ^b	13 DAT	30 DAT			
		% applied					
Thighman 1	3	11 (2)	6 (1)	2 (1)	10 (2)	0.275 (.022)	2.5
Thighman 2	2	20 (4)	10 (1)	4 (2)	13 (1)	0.197 (.014)	3.5
Thighman 4	1	18 (11)	9 (3)	4 (1)	12 (1)	0.211 (.023)	3.3
GW-Leland	2	13 (3)	10 (3)	4 (2)	9 (1)	0.252 (.026)	2.8
GW-Mon	2	14 (4)	11 (2)	3 (1)	18 (1)	0.225 (.017)	3.1
Walker 1A	2	12 (4)	8 (2)	3 (1)	9 (1)	0.254 (.022)	2.7
Walker 1B	2	8 (1)	6 (1)	2 (1)	8 (1)	0.305 (.029)	2.3
Elizabeth	2	16 (8)	9 (1)	5 (1)	11 (2)	0.225 (.002)	3.1
Beasley 3	0	61 (2)	55 (9)	34 (6)	18 (4)	0.040 (.005)	17.3
Thighman 3	0	66 (3)	60 (2)	46 (4)	24 (1)	0.017 (.002)	40.8
LSD 0.05		8	4	3	3		

^a Mean and standard deviation (in parentheses) of four replicates.

^b Abbreviation: DAT, days after treatment.

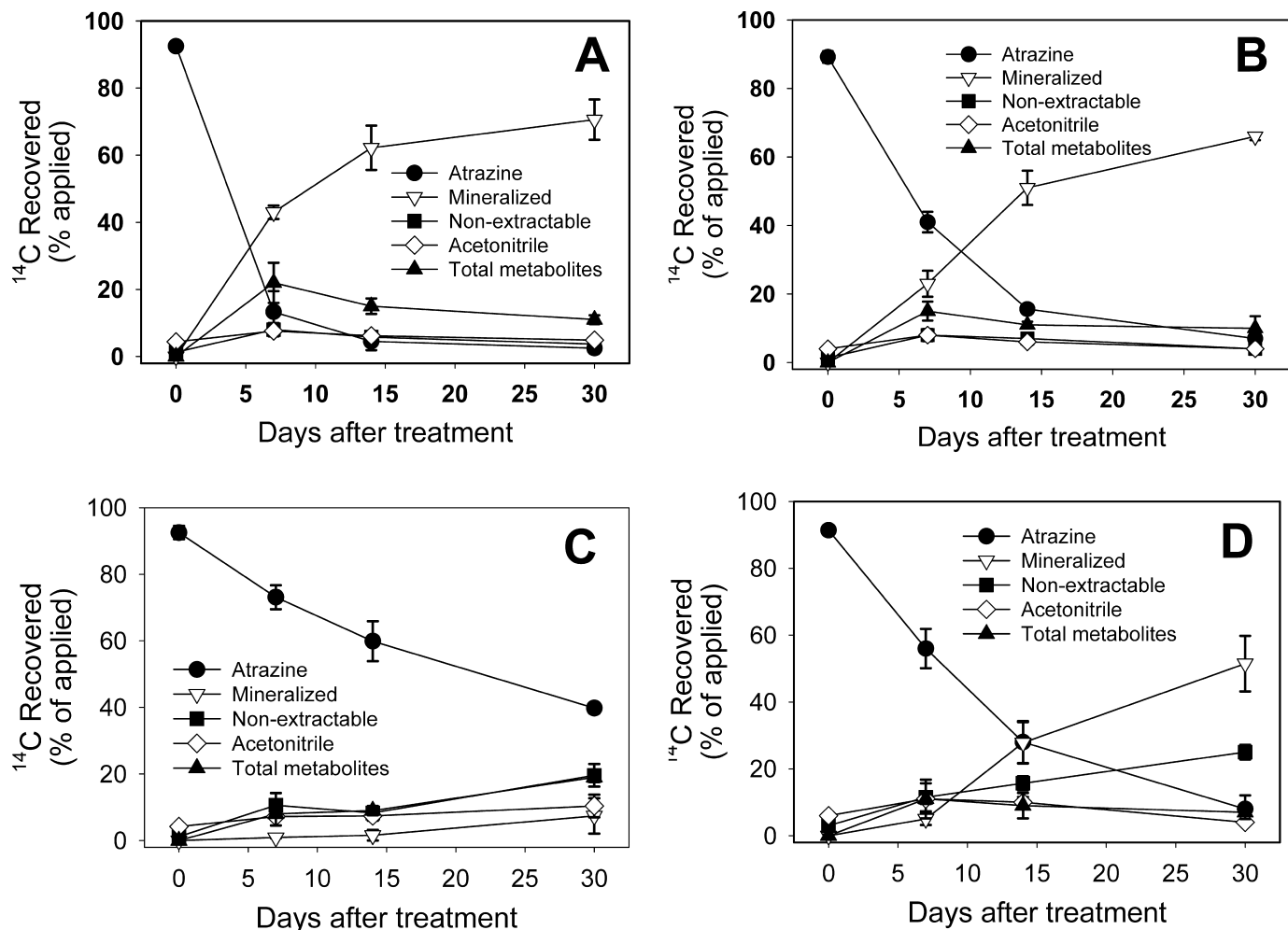


FIGURE 2. Atrazine-degradation parameters measured in four soils collected in 2000: (A) Indian Mound, (B) GW-Mon, (C) Stoneville 2, and (D) Walker 3. Mean and standard deviations of methanol-extractable ^{14}C -atrazine, $^{14}\text{CO}_2$ mineralized, nonextractable ^{14}C , acetonitrile : potassium phosphate-extractable ^{14}C , and total methanol-extractable ^{14}C metabolites. If not shown, the standard deviations of certain parameters are less than the size of the symbol.

used on other fields in this farm where corn was planted in 1998. Alabama field trials have indicated an average half-life of atrazine of less than 20 d in warm Southern U.S. soils (Buchanan and Hiltbold 1973).

MPN estimates of atrazine-mineralizers ranged from 450 to 7,200 propagules g^{-1} in soils exposed to atrazine, but none were detected in unexposed soils (Table 3). In certain soils, e.g., Beasley 1 and 2, a wide variance of atrazine-degrading populations were enumerated. Estimates of atrazine-ring degraders observed in Mississippi soils with a history of atrazine exposure were typically more numerous than those observed elsewhere (Jayachandran et al. 1998; Sparling et al. 1998). Analysis of DNA extracted from 2001 soils by either direct or nested PCR failed to amplify DNA sequences for *atzA* atrazine chlorohydrolase. However, the discovery of other atrazine degraders having unique chlorohydrolases, e.g., the *trzN* gene from *Nocardioides* (Mulbry et al. 2002), with extended substrate ranges for triazines indicates that other genera of bacteria may be responsible for atrazine degradation in certain soils. Regardless, the potential for accelerated atrazine degradation developed rapidly in Mississippi soils. Cyanazine degrades very rapidly in Mississippi Delta soils (Staddon et al. 2004), and all soils evaluated have had extensive exposure to that herbicide (at least three applica-

tions in the past 6 yr). It is possible that bacteria originally adapted for cyanazine have broadened their substrate use and are capable of metabolizing atrazine.

A mass-balance accounting for at least 88% of the ^{14}C added was achieved for all soils at 30 DAT (data not shown). Recovery of the ^{14}C label as mineralized CO_2 , methanol-extractable atrazine and DEA, acetonitrile mixed-mode extractable, and nonextractable for representative soils collected in 2000 are summarized in Figure 2. Compared with many herbicides, e.g., alachlor (Locke et al. 1996), cyanazine (Staddon et al. 2004), and fluometuron (Zablotowicz et al. 2000), relatively little of the ^{14}C -atrazine applied remained in the nonextractable fraction (less than 25%, Tables 2 and 3). The highest level of ^{14}C -label recovered in the nonextractable fraction was observed in soils with high organic matter content and relatively lower mineralization, e.g., Walker 3 (Table 2) that was NT and also amended with cotton waste. This is consistent with observations by Houot et al. (2000). Comparing the two Stoneville soils having no exposure to atrazine (Table 2), a greater amount of the ^{14}C -label was incorporated into the nonextractable fraction of NT compared to CT soils, as demonstrated for alachlor (Locke et al. 1996) and fluometuron (Zablotowicz et al. 2000).

TABLE 5. Methanol-extractable atrazine metabolites 30 days after treatment, 2000 and 2001 soils.

Soil	Total metabolites ^a	De-ethyl atrazine	De-Isopropyl atrazine	Polar metabolites
———— % of ¹⁴ C applied recovered ————				
2000				
Beasley 1	4.7 (2.0)	3.4 (0.5)	0.9 (1.0)	0.4 (0.6)
Beasley 2	11.0 (6.0)	5.3 (0.8)	3.9 (5.1)	1.9 (1.0)
Beasley 3	7.8 (2.6)	5.8 (1.7)	1.8 (1.0)	0.2 (0.4)
Indian Mound	10.7 (1.3)	7.1 (2.8)	1.2 (0.9)	2.4 (1.9)
GW-Mon	9.9 (3.5)	7.3 (2.7)	1.5 (0.3)	2.4 (1.9)
Stoneville 1	11.5 (5.4)	10.6 (5.4)	0.4 (0.8)	0.5 (1.1)
Stoneville 2	18.7 (3.3)	12.6 (3.4)	2.2 (0.8)	3.8 (0.6)
Walker 1A	13.8 (3.3)	9.2 (2.8)	2.6 (1.0)	2.2 (1.1)
Walker 1B	9.3 (2.6)	7.7 (1.8)	1.2 (0.8)	0.4 (0.8)
Walker 2	12.7 (2.4)	8.1 (2.7)	2.2 (0.6)	2.6 (0.7)
Walker 3	6.7 (2.0)	4.1 (1.9)	1.0 (0.3)	1.6 (0.7)
LSD 0.05	4.2	3.5	2.0	1.0
Mean 2000	10.6 (3.8)	7.4 (2.7)	1.7 (1.0)	1.7 (1.2)
2001				
Beasley 3	9.7 (3.2)	5.2 (1.4)	0.3 (0.5)	4.2 (1.7)
Elizabeth	7.4 (1.1)	2.3 (1.1)	0.7 (0.7)	4.3 (1.1)
GW-Leland	8.0 (2.2)	2.7 (0.7)	< 0.1	5.3 (1.9)
GW-Mon	9.4 (1.3)	3.7 (0.9)	0.6 (0.4)	5.1 (1.5)
Thighman 1	7.8 (1.9)	2.3 (0.8)	< 0.1	5.5 (2.0)
Thighman 2	8.2 (0.9)	2.0 (0.8)	0.1 (0.2)	6.0 (0.9)
Thighman 3	8.5 (2.4)	4.2 (1.8)	1.0 (0.6)	3.3 (0.8)
Thighman 4	8.5 (2.8)	2.2 (0.4)	0.7 (0.4)	5.6 (2.2)
Walker 1A	10.0 (1.4)	3.5 (0.5)	0.4 (0.5)	6.1 (1.3)
Walker 1B	9.1 (1.6)	4.1 (1.6)	< 0.1	4.9 (1.8)
LSD 0.05	NS	1.4	0.6	2.3
Mean 2001	8.7 (0.9)	3.2 (1.1)	0.4 (0.3)	5.0 (0.9)

^a Mean and standard deviation of four replicates.

In 2000, DEA was the major metabolite observed in methanol extracts (Table 5), with between 3.4 to 10.6% of the applied atrazine recovered as DEA at 30 DAT (61 to 92% of total metabolites), depending on the soil. DEA was less frequently detected, with the amounts of DIA accumulated typically less than 20% of total metabolites in 2000 and less than 10% in 2001. In soils collected in 2001, polar compounds (recovered at the origin in TLC) were the major metabolites recovered in methanol extracts (Table 4), with between 3.3 and 6.1% of the applied atrazine recovered as hydroxy-atrazine derivatives at 30 DAT (38 to 73% of total metabolites). Using an antibiotic-inhibition assay, Levanon (1993) demonstrated that the mineralization of the alkyl side-chains of atrazine was attributed to fungal metabolism, whereas the mineralization of the triazine ring was due to bacterial metabolism. *N*-dealkylation reactions are fairly commonplace in many soil fungi; however, the ability to mineralize the atrazine ring is limited to select bacteria possessing a series of hydrolytic enzymes. The sequential extraction of soil with the mixed-mode acetonitrile-potassium phosphate buffer system following aqueous methanol extraction, recovered an additional 4 to 6% of the initial radioactivity at 0 DAT and between 4 and 10% at 30 DAT. This extraction system was designed for enhanced recovery of the polar hydroxyl atrazine derivatives (Lerch et al. 1997, 1999). A limited increase in mixed-mode extractable radioactivity over time verifies the TLC analysis that only limited amounts of polar hydroxylated-atrazine metabolites accu-

mulate in these soils (average of 1.7% in 2000 and 5% in 2001).

Relationship Between Soil Characteristics and Atrazine Degradation Parameters

Relationships between atrazine degradation parameters and soil characteristics were assessed using Pearson's correlations, with both years data pooled in the analysis. Atrazine degradation parameters considered for this assessment included atrazine mineralized (7 and 30 DAT), total atrazine recovered (7 and 30 DAT), nonextractable ¹⁴C-residues (30 DAT), and extractable DEA (30 DAT) (Table 6).

Among the six atrazine degradation parameters, the most consistent relationship was with atrazine history. Atrazine mineralization was positively correlated with atrazine history, whereas atrazine and DEA recovery and nonextractable ¹⁴C were negatively correlated with atrazine history. The correlation of more rapid atrazine mineralization in atrazine history soils is consistent with observations of others (Barriuso and Houot 1996; Martin-Laurent et al. 2004; Ostrofsky et al. 1997; Yassir et al. 1999). It would be expected that if degradation was slower in history soils, less atrazine and DEA would be available for recovery at 30 d. Similar arguments could be made for lower levels of nonextractable ¹⁴C at 30 d in history soils. A similar relationship was observed between the MPN estimates of atrazine degraders and all atrazine degradation parameters except for nonextractable ¹⁴C-residues (2000 samples only).

The bioavailability of most herbicides for microbial biodegradation is limited by the sorption to organic matter or clay minerals (Alexander 1994; Skow and Johnson 1997). In addition, organic carbon substrates may affect microbial community structure and the potential for degradation of herbicides such as atrazine (Rhine et al. 2003). In regard to mineralization and atrazine recovery, there was no consistent correlation between organic matter or clay content. However, nonextractable ¹⁴C-residues were positively correlated with both organic matter and clay content. Other researchers have observed that carbon addition had no significant effect on atrazine degradation in adapted soils, but atrazine degradation via *N*-dealkylation was stimulated by carbon in nonadapted soils (Abdelhafid et al. 2000). DEA recovered at 30 DAT was also positively correlated with silt content, negatively correlated with clay content, and weakly correlated with organic matter content. Soil pH was a greater contributor to atrazine fate in soils because there was a positive correlation between soil pH and atrazine recovered at both dates and a negative correlation between atrazine mineralized at 7 DAT. DEA recovered at 30 DAT was also positively correlated with pH. Studies of Canadian and French soils (Houot et al. 2000) indicated that accelerated mineralization of atrazine was only observed in soils with a pH greater than 6.5; however, all of the Mississippi Delta soils in this study with a history of atrazine exposure and a pH of less than 6.0 (Beasley 1, GW-Mon, Walker 1B) mineralized greater than 46% of the applied atrazine during 30 d. In several studies (Abdelhafid et al. 2000; Rhine et al. 2003), atrazine mineralization was reduced by inorganic nitrogen forms. There was no correlation between nitrate content and atrazine mineralization or atrazine recovered; however, nonextractable atrazine residues were positively corre-

TABLE 6. Pearson correlations observed among characteristics of soils collected in 2000 and 2001 and several atrazine degradation parameters: atrazine mineralized (at 7 and 30 d), total atrazine recovered (at 7 and 30 d), nonextractable ¹⁴C (at 30 d), and de-ethyl atrazine (at 30 d).

Soil characteristic	Atrazine mineralized ^a		Atrazine recovered		¹⁴ C-nonextractable 30 DAT	De-ethyl atrazine 30 DAT
	7 DAT	30 DAT	7 DAT	30 DAT		
Atrazine history	0.774 (< 0.001)	0.691 (< 0.001)	-0.784 (< 0.01)	-0.735 (< 0.001)	-0.426 (< 0.001)	-0.461 (< 0.001)
MPN atrazine degraders	0.638 (< 0.001)	0.687 (< 0.001)	-0.702 (< 0.001)	-0.792 (< 0.001)	-0.278 (0.068)	-0.683 (< 0.001)
Organic matter	-0.117 (0.289)	-0.016 (0.886)	0.113 (0.304)	-0.562 (0.611)	0.411 (< 0.001)	-0.209 (0.058)
pH	-0.348 (0.001)	-0.169 (0.123)	0.372 (< 0.001)	0.267 (0.014)	0.160 (0.147)	0.305 (0.005)
Electrical conductivity	-0.217 (0.047)	0.132 (0.231)	0.188 (0.084)	-0.155 (0.158)	0.182 (0.098)	0.018 (-0.970)
Nitrate	-0.172 (0.117)	0.115 (0.298)	0.115 (0.298)	-0.136 (0.216)	0.256 (0.019)	-0.075 (0.495)
Phosphate	-0.205 (0.062)	0.028 (0.795)	0.197 (0.072)	-0.145 (0.186)	0.249 (0.022)	-0.104 (0.349)
Potassium	-0.400 (< 0.001)	-0.147 (0.181)	0.384 (< 0.001)	0.131 (0.233)	0.467 (< 0.001)	0.131 (0.223)
Sand	0.089 (0.422)	0.119 (0.282)	-0.027 (0.801)	-0.221 (0.043)	-0.202 (0.066)	-0.023 (0.798)
Silt	0.050 (0.651)	-0.056 (0.612)	0.003 (0.979)	0.118 (0.287)	-0.073 (0.510)	0.270 (0.013)
Clay	-0.021 (0.847)	-0.056 (0.616)	0.003 (0.997)	0.087 (0.429)	0.320 (0.003)	-0.331 (0.002)
Bacteria	-0.073 (0.505)	-0.069 (0.530)	0.076 (0.491)	-0.053 (0.628)	0.386 (0.003)	-0.312 (0.004)
Fungi	-0.190 (0.084)	0.025 (0.815)	0.131 (0.245)	0.019 (0.863)	0.309 (0.004)	0.038 (0.733)

^a The number in parentheses under the correlation coefficient is the probability $> r$. Parameters that are significantly correlated ($P > 0.05$ level) are highlighted in bold.
^b Abbreviations: DAT, days after treatment; MPN, most-probable number.

lated with nitrate concentration. No significant correlation was observed between either bacterial or fungal propagule density on atrazine mineralization or recovery. However, both parameters were positively correlated with nonextractable atrazine, and bacterial propagules were negatively correlated with DEA recovered at 30 DAT. Other studies have observed no significant relationship between microbial biomass and atrazine mineralization (Yassir et al. 1999), although those researchers observed that bacteria were more active in atrazine degradation compared with fungi.

Conclusion

In conclusion, accelerated degradation of atrazine (50 to 70% of the triazine ring mineralized) was observed in Mississippi Delta soils that had a short history of exposure to atrazine of one to two applications. This suggests that development and enhancement of specific microbial populations capable of mineralizing the atrazine ring developed in Mississippi Delta soils in a relatively short period after exposure to atrazine. Relatively high populations of atrazine degraders ($> 1,000$ degraders g^{-1} soil) were associated with soils that exhibited enhanced atrazine degradation. Half-lives of atrazine in soils exhibiting accelerated mineralization were typically less than 10 d. Accelerated degradation of atrazine may reduce the weed control efficiency of this herbicide and may also reduce potential for off-site movement. Red morningglory (*Ipomoea coccinea* L.) control was reduced in Louisiana sugarcane fields with extensive history of atrazine use (> 10 yr), and biotypes of this species collected at these sites did not exhibit atrazine resistance (Viator et al. 2002). Future studies need to consider atrazine dissipation in the field and the efficacy of weed control attained with atrazine under Mississippi conditions.

Sources of Materials

- ¹ Pathfinder ProXR, Trimble Navigation, LTD, 935 Stewart Drive, Sunnyvale, CA 94085.
- ² Uniformly labeled ring ¹⁴C-atrazine was a generous gift of Novartis, now Syngenta Crop Protection, 1800 Concord Pike, Wilmington, DE 19850.
- ³ Technical-grade atrazine, de-ethyl atrazine, deisopropyl atrazine, and hydroxyl atrazine were purchased from ChemService, Inc., 660 Tower Lane, West Chester, PA 19381.
- ⁴ Packard TriCarb 4000 series, Packard Instruments Co., 800 Research Parkway, Meriden, CT 06450.
- ⁵ Hi-Ionic scintillation cocktail, Packard Instruments Co., 800 Research Parkway, Meriden, CT 06450.
- ⁶ Ecolume scintillation cocktail, ICN, 3300 Hyland Avenue, Costa Mesa, CA 92626.
- ⁷ J. T. Baker, 222 Red School Lane, Phillipsburg, NJ 08895.
- ⁸ Bioscan Imaging System 200, Bioscan Inc., 4590 Macarthur Boulevard, Washington, D.C. 20007.
- ⁹ Packard TriCarb Oxidizer 306, Packard Instruments Co., 800 Research Parkway, Meriden, CT 06450.
- ¹⁰ Carbo-Sorb E. Packard Instrument Co., 800 Research Parkway, Meriden, CT 06450.
- ¹¹ Permafluor E+. Packard Instrument Co., 800 Research Parkway, Meriden, CT 06450.
- ¹² Jandel Scientific Software, P.O. Box 7005, San Rafael, CA 94912-7005.
- ¹³ MO BIO Laboratories, P.O. Box 606, Solana Beach, CA 92075.

¹⁴ Promega. 2800 Woods Hollow Road, Madison, WI 53711-5399.

¹⁵ Finnzyme DyNAzyme polymerase, New England Biolabs, Ipswich, MA 01938-2723.

Acknowledgments

We thank Earl Gordon and Melanie Patterson for technical assistance in conducting this research, and Syngenta for supplying radiolabeled atrazine. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Literature Cited

- Abdelhafid, R., S. Houot, and E. Barriuso. 2000. Dependence of atrazine degradation on C and N availability in adapted and non-adapted soils. *Soil Biol. Biochem.* 32:389–401.
- Alexander, M. 1994. *Biodegradation and Bioremediation*. San Diego: Academic.
- Bartha, R. and D. Pramer. 1965. Features of a flask and method for measuring the persistence and biological effect of pesticides in soil. *Soil Sci.* 100:68–70.
- Barriuso, E. and S. Houot. 1996. Rapid mineralization of the s-triazine ring of atrazine in relation to soil management. *Soil Biol. Biochem.* 28:1341–1348.
- Bichat, F., G. K. Sims, and R. L. Mulvaney. 1999. Microbial utilization of heterocyclic nitrogen from atrazine. *Soil Sci. Soc. Am. J.* 63:100–110.
- Blumhorst, M. R. and J. B. Weber. 1994. Chemical versus microbial degradation of cyanazine and atrazine in soils. *Pestic. Sci.* 42:79–84.
- Buchanan, G. A., and A. E. Hiltbold. 1973. Performance and persistence of atrazine. *Weed Sci.* 21:413–416.
- Burkart, M. R., and D. W. Kolpin. 1993. Hydrologic and land-use factors associated with herbicides and nitrate in near-surface aquifers. *J. Environ. Qual.* 22:646–656.
- De Souza, M. L., L. P. Wackett, K. L. Boundy-Mills, R. T. Mandelbaum, and M. J. Sadowsky. 1995. Cloning, characterization, and expression of a gene region from *Pseudomonas* sp. strain ADP involved in the dechlorination of atrazine. *Appl. Environ. Microbiol.* 61:3373–3378.
- DeSouza, M. L., J. Seffernick, B. Martinez, M. J. Sadowsky, and L. P. Wackett. 1998. The atrazine catabolism genes *atzABC* are widespread and conserved. *J. Bacteriol.* 180:1951–1954.
- Esser, H. O., G. Dupuis, E. Ebert, G. Marco, and C. Vogel. 1975. s-Triazines. Pages 129–208 in P. C. Kearney and D. D. Kaufman, eds. *Herbicides: Chemistry, Degradation, and Mode of Action*. Volume 1. New York: Marcel Dekker.
- Gee, G. W., and J. W. Bauder. 1986. Particle size analysis. Pages 383–411 in A. Klute, ed. *Method of Soil Analysis*, Part 1. Agron. Monogr. 9, Madison, WI: ASA and SSSA.
- Houot, S., E. Barriuso, and V. Bergheaud. 1998. Modifications to atrazine degradation pathways in a loamy soil after addition of organic amendments. *Soil Biol. Biochem.* 30:2147–2157.
- Houot, S., E. Topp, A. Yassir, and G. Soulas. 2000. Dependence of accelerated degradation of atrazine on soil pH in French and Canadian soils. *Soil Biol. Biochem.* 32:615–625.
- Jayachandran, K., N. B. Stolpe, T. B. Moorman, and P. J. Shea. 1998. Application of ¹⁴C-most probable-number technique to enumerate atrazine-degrading microorganisms in soil. *Soil Biol. Biochem.* 30:523–529.
- Krutz, L. J., T. J. Gentry, S. A. Senseman, I. L. Pepper, and D. P. Tierney. 2006. Mineralization of atrazine, metolachlor, and their respective metabolites in vegetated filter strip and cultivated soil. *Pest Manag. Sci.* In press.
- Lerch, R. N., E. M. Thurman, and P. E. Blanchard. 1999. Hydroxyatrazine in soils and sediments. *Environ. Toxicol. Chem.* 18:2161–2168.
- Lerch, R. N., E. M. Thurman, and E. L. Kruger. 1997. Mixed-mode sorption of hydroxylated atrazine degradation products in soil: a mechanism for bound residues. *Environ. Sci. Technol.* 31:1639–1645.
- Levanon, D. 1993. Roles of fungi and bacteria on the mineralization of atrazine, alachlor, malathion, and carbofuran in soil. *Soil Biol. Biochem.* 25:2097–2205.
- Locke, M. A., L. A. Gaston, and R. M. Zablotowicz. 1996. Alachlor biotransformations and sorption in soil from two soybean tillage systems. *J. Agric. Food Chem.* 44:1128–1134.
- Martin-Laurent, F., L. Cornet, L. Ranjard, J. C. Lopez-Gutierrez, L. Philippot, C. Schwartz, R. Chaussod, G. Cartoux, and G. Soulas. 2004. Estimation of atrazine-degrading genetic potential and activity in three French agricultural soils. *FEMS Microbiol. Ecol.* 48:425–435.
- Martinez, B., J. Tomkins, L. P. Wackett, R. Wing, and M. J. Sadowsky. 2001. Complete nucleotide sequence and organization of atrazine catabolic plasmid pADP-1 from *Pseudomonas* sp. strain ADP. *J. Bacteriol.* 183:5684–5697.
- Mulbry, W. M., H. Zhu, S. N. Nour, and E. Topp. 2002. The triazine hydrolase gene *trzN* from *Nocardiodex* sp. Strain C190: Cloning and construction of gene-specific primers. *FEMS Microbiol. Lett.* 206:75–79.
- Ostrofsky, E. B., S. J. Traina, and O. H. Tuovinen. 1997. Variation in atrazine mineralization rates in relation to agricultural management practices. *J. Environ. Qual.* 26:647–657.
- Piutti, S., S. Hallet, S. Rousseaux, L. Philippot, G. Soulas, and F. Martin-Laurent. 2002a. Accelerated mineralization of atrazine in maize rhizosphere soil. *Biol. Fert. Soils.* 36:434–441.
- Piutti, S., A. L. Marchand, B. Lagacherie, F. Martin-Laurent, and G. Soulas. 2002b. Effect of cropping cycles and repeated herbicide applications on the degradation of diclofop-methyl, bentazone, diuron, isoproturon, and pendimethalin in soil. *Pest Manag. Sci.* 58:303–312.
- Reddy, K. N., R. M. Zablotowicz, M. A. Locke, and C. Koger. 2003. Cover crop, tillage, and herbicide effects on weeds, soil properties, microbial populations, and soybean yield in the lower Mississippi Delta. *Weed Sci.* 51:987–994.
- Rhine, E. D., J. J. Fuhrmann, and M. Radosevich. 2003. Microbial community response to atrazine exposure and nutrient availability: linking degradation capacity to community structure. *Microbial Ecol.* 46:145–160.
- [SAS] Statistical Analysis Systems. 2001. *SAS User's Guide*. Version 8.1. Cary, NC: SAS Institute.
- Scribner, E. M., W. A. Battaglin, D. A. Goolsby, and E. M. Thurman. 2000. Changes in herbicide concentrations in Midwestern streams in relation to changes in use, 1989–1998. *Sci. Total Environ.* 248:255–263.
- Shapir, N., S. Goux, R. T. Manelbaum, and L. Pussemier. 2000. The potential of soil microorganisms to mineralize atrazine as predicted by MCH-PCR followed by nested PCR. *Can. J. Microbiol.* 46:425–432.
- Skow, K. M. and C. R. Johnson. 1997. Effect of sorption on biodegradation of soil pollutants. *Adv. Agron.* 58:1–56.
- Soil Survey Staff. 1959a. Washington County, Mississippi. USDA Soil Conservation Service. Washington, D.C.: U.S. Government Printing Office.
- Soil Survey Staff. 1959b. Leflore County, Mississippi. USDA Soil Conservation Service. Washington, D.C.: U.S. Government Printing Office.
- Soil Survey Staff. 1959c. Sunflower County, Mississippi. USDA Soil Conservation Service. Washington, D.C.: U.S. Government Printing Office.
- Solomon, K. R., D. B. Baker, R. P. Richards, K. R. Dixon, S. J. Klaine, T. W. La Pointe, R. J. Kendall, C. P. Weiskopf, J. M. Giddings, and J. P. Giesey. 1996. Ecological risk assessment of atrazine in North American surface waters. *Environ. Toxicol. Chem.* 15:31–76.
- Sparling, G., R. Dragten, J. Aislabie, and R. Fraser. 1998. Atrazine mineralization in New Zealand topsoils and subsoils: influence of edaphic factors and numbers of atrazine-degrading microbes. *Aust. J. Soil Res.* 36:557–70.
- Staddon, W. J., M. A. Locke, and R. M. Zablotowicz. 2004. Spatial variability of cyanazine dissipation in soil from a conservation-managed field. Pages 179–193 in M. T. Nett, M. A. Locke, and D. A. Pennington, eds. *Water Quality Assessments in the Mississippi Delta: Regional Solutions*, National Scope. ACS Symp. Ser. 877.
- Thurman, E. M., D. A. Goolsby, M. Meyer, and D. W. Kolpin. 1991. Herbicides in surface waters of the Midwestern United States—the effect of spring flush. *Environ. Sci. Technol.* 25:1794–1796.
- Topp, E., W. M. Mulbry, H. Zhu, S. M. Nour, and D. Cupels. 2000. Characterization of s-triazine herbicide metabolism by a *Nocardiodex* sp. isolated from agricultural soils. *Appl. Environ. Microbiol.* 66:3134–3141.
- Viator, B. J., J. L. Griffin, and E. P. Richard, Jr. 2002. Evaluation of red morningglory (*Ipomoea coccinea*) for potential atrazine resistance. *Weed Technol.* 16:96–101.
- Woomer, P. L. 1994. Most probable number counts. Pages 59–80 in S. H. Mickelson and J. M. Bingham, eds. *Methods of Soil Analysis*, Part 2:

- Microbiological and Biochemical Properties. Book Series No. 5, Madison WI: Soil Science Society of America.
- Yassir, A., B. Lagacherie, S. Houot, and G. Soulas. 1999. Microbial aspects of atrazine biodegradation in relation to history of soil treatment. *Pestic. Sci.* 55:799–809.
- Zablotowicz, R. M., M. A. Locke, L. A. Gaston, and C. T. Bryson. 2000. Interactions of tillage and soil depth on fluometuron degradation in a Dundee silt loam. *Soil Tillage Res.* 57:61–68.
- Zablotowicz, R. M., M. A. Locke, R. N. Lerch, and S. S. Knight. 2004. Dynamics of herbicide concentrations in Mississippi Delta oxbow lakes and the role of planktonic microorganisms in herbicide metabolism. Pages 134–149 in M. T. Nett, M. A. Locke, and D. A. Pennington, eds. *Water Quality Assessments in the Mississippi Delta: Regional Solutions, National Scope*. ACS Symp. Ser. 877.

Received October 19, 2004, and approved January 13, 2006.