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Rapid Assay for Detecting Enhanced Atrazine Degradation in Soil

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Atrazine is widely used to control broadleaf weeds and grasses in corn, sorghum, and sugarcane. Field persistence data published before 1995 showed that the average half-life of atrazine in soil was 66 d, and farmers expect to achieve weed control with a single application for the full season. However, reports of enhanced atrazine degradation in soil from fields that have a history of atrazine applications are increasing. A rapid laboratory assay was developed to screen soils for enhanced atrazine degradation. Soil (50 g) was placed in a 250 ml glass jar and treated with 7.5 ml of water containing atrazine (5 μg ai ml^{-1}) and capped with a Teflon-lined lid. The assay was conducted at room temperature (25 C). Soil subsamples (1.5 to 3 g) were removed at 0, 1, 2, 4, 8, and 16 d after treatment and extracted with an equal weight of water (wt/vol). The atrazine in the water extract was assayed with high-pressure liquid chromatography (HPLC). The half-life of atrazine in soils with a history of use was ≤ 1.5 d, whereas the half-life in soils with no history of atrazine use was > 8 d. The advantages of this assay are (1) the ease of set up; (2) the rapidity of extraction, and (3) the simplicity of the quantification of the atrazine.

Nomenclature: Atrazine; corn, *Zea mays* L.; sorghum, *Sorghum bicolor* (L.) Moench; sugarcane, *Saccharum officinarum* L.
Key words: Accelerated degradation, dissipation, half-life, microbial metabolism.

Atrazine is used in corn, grain sorghum, and sugarcane to control many broadleaf and certain grass weeds. In 2003, farmers applied an average rate of 1.1 kg ha^{-1} of atrazine on 68% of the corn in the United States (NASS 2004). Field persistence data published before 1995, the year when enhanced atrazine degradation was first reported in the literature, indicate the compound's average half-life in soil is 66 d, and farmers expected to achieve weed control with a single application for the full season (Bacci 1989; Frank et al. 1991; Gish et al. 1991, 1994; Khan et al. 1981; Ng et al. 1995; Sorenson et al. 1994; Winkelman and Klaine 1991; Workman et al. 1995) However, reports of enhanced atrazine degradation in soil from fields that have a history of atrazine applications are increasing (Barriuso and Houot 1996; Hang et al. 2003; Houot et al. 2000; Krutz et al. 2007; Ostrofsky et al. 1997; Popov et al. 2005; Pussemier et al. 1997; Shaner and Henry 2007; Vanderheyden et al. 1997; Yassir et al. 1999; Zablotowicz et al. 2006), and a loss of residual weed control has been reported by farmers and extension agents in Colorado and Mississippi (Krutz et al. 2007; Shaner and Henry 2007).

In the late 1990s a variety of atrazine-degrading bacteria, including members of the genera *Pseudomonas, Rhizobium, Acinetobacter, Agrobacterium,* and *Psuedoaminobacter,* were isolated from soil (Boundy-Mills et al. 1997; De Souza et al. 1995; Mandelbaum et al. 1995; Radosevich et al. 1995; Sadowsky et al. 1998). These bacteria rapidly mineralize atrazine to hydroxyatrazine by hydrolytic dechlorination followed by a series of reactions to produce cyanuric acid, which is further converted to urea via biuret. (Topp et al. 2000). It was subsequently discovered that the genes that encode for the enzymes that catabolize atrazine are carried on a large plasmid, and the plasmid has been found in a number of bacteria isolated from North America and Europe. (DeSouza et al. 1998; Smith et al. 2005; Topp et al. 2000).

Enhanced atrazine degradation is correlated with years of atrazine use and soil pH greater than 6.5 (Barriuso and Houot 1996; Hang et al. 2003; Harvey 1987; Houot et al. 2000; Krutz et al. 2007; Pussemier et al. 1997; Shaner and Henry 2007; Vanderheyden et al. 1997; Yassir et al. 1999; Zablotowicz et al. 2006). The half-life of atrazine ranges from 3.5 to 7 d in commercial fields in eastern Colorado, where atrazine had been used for 5 yr or more (Shaner and Henry 2007). In Mississippi, the half-life of atrazine in fields receiving continuous atrazine for 5 yr was 9 d compared with 17 d for an adjacent field that had not received any atrazine (Krutz et al. 2007). This increased rate of atrazine dissipation resulted in a 50% loss of residual activity of the herbicide (Krutz et al. 2007).

Soils can be screened in the laboratory for atrazine degradation in multiple ways. These usually include incubating soil with the herbicide, extracting the herbicide from the soil with an organic solvent at different time intervals, and analyzing the extract either by gas chromatography (GC) or by high-pressure liquid chromatography (HPLC) (Dean et al. 1996). Another commonly used method is to incubate soils with ^{14}C-atrazine and monitor the release of $^{14}CO_2$ from the soil (Krutz et al. 2007; Zablotowicz et al. 2006). Although these methods are relatively rapid, they have a number of limitations, including excessive handling of soil for extraction and analysis, availability of laboratories able to conduct experiments with radiolabeled materials, and disposal of radioactive and hazardous waste. These factors limit the number of samples that can be analyzed rapidly and efficiently.

Development of a rapid and accurate assay to determine the extent of enhanced atrazine degradation would be useful to advise farmers on whether or not they should modify their weed management practices. This assay should be simple and representative of the rate of dissipation of atrazine in the field. There are multiple ways to extract atrazine from soil but the simplest method is by aqueous extraction. This type of extraction more closely reflects the bioavailable fraction of atrazine in the soil (Barriuso et al. 2004; Johnson et al. 1999; Park et al. 2003) and has the advantages of not generating hazardous waste and being much less expensive that using organic solvents. Additionally, water should not extract many

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Table 1. Soil characteristics, crop rotation, initial water extraction, sorption coefficient (K_d), and half-life of atrazine in various soils.[a,b]

Location	No.	Soil series	Crop rotation	Crop	Triazine applied	Field	Initial water extract[c]	K_d	$T_{1/2}$
							ng ml^{-1}	L kg^{-1}	d
Colorado	CO1	Nunn clay loam	Continuous corn	Corn	+	CO 1	250	0.94 f	0.7 a
	CO2	Haxtun loamy sand	Continuous corn	Corn	+	CO 2	366	0.93 f	0.7 a
	CO3	Haxtun loamy sand	Continuous corn	Corn	+	CO 3	258	0.82 fg	0.7 a
	CO4	Weld silt loam	Continuous corn	Corn	+	CO 4	386	1.39 de	1.1 ab
	CO5	Nunn clay loam	Pasture–corn	Corn	+	CO 5	438	0.90 f	8.4 e
	CO6	Haxtun loamy sand	Wheat–fallow	Wheat	−	CO 6	282	0.48 g	10.2 e
	CO7	Weld silt loam	Wheat–fallow	Wheat	−	CO 7	339	0.87 fg	9.3 e
	CO8	Haxtun loamy sand	None	Grass waterway	−	CO 8	150	1.06 ef	7.5 e
	CO9	Weld silt loam	None	Grass waterway	−	CO 9	194	1.17 def	8.1 e
Illinois	IL1	Flannigan silt loam	Corn–soybean	Corn	+	IL 1	85	3.73 a	2.5 c
	IL2	Flannigan silt loam	Corn–soybean	Corn	+	IL 2	67	2.18 bc	2.6 c
	IL3	Lawson silt loam	Corn–soybean	Corn	+	IL 3	88	2.36 bc	2.7 c
	IL4	Lawson silt loam	Corn–soybean	Corn	+	IL 4	82	2.5 b	2.8 c
	IL5	Plano silt loam	Corn–soybean	Corn	+	IL 5	78	1.56 d	3.0 c
	IL6	Flannigan silt loam	Corn–soybean	Soybean	−	IL 6	106	3.52 a	5.0 d
	IL7	Flannigan silt loam	Corn–soybean	Soybean	−	IL 7	139	2.50 bc	5.7 d
	IL8	Flannigan silt loam	None	Grass ditchbank	−	IL 8	117	3.54 a	7.7 e
California	CA1	Hesperia fine sandy loam	Grapes	Grapes	+	CA 1	323	NA	0.7 a
	CA2	Hanford fine sandy loam	Grapes	Grapes	−	CA 2	352	NA	10.3 e
Mississippi	MS1	Dundee silt loam	Continuous corn	Corn	+	MS 1	189	NA	0.6 a
	MS2	Dundee silt loam	Corn–cotton	Corn	−	MS 2	194	NA	5.7 d

[a] Values followed by the same letter are not significantly different (P = 0.05).
[b] Abbreviations: K_d, binding constant for atrazine to soil; NA, not available.
[c] Initial water extract is measured in ng ml^{-1} of atrazine.

other compounds from the soil, which would make analysis simpler.

The objectives of this research were to (1) develop a rapid assay for screening soils for atrazine degradation that did not involve organic solvent extraction, and (2) determine the effectiveness of the screening method in detecting fields with enhanced atrazine degradation compared with standard protocols.

Materials and Methods

Soil Collection. Soils were collected from various locations in the United States. The location, herbicide, cropping history, and soil series are reported in Table 1. Soils were collected from the top 15 cm in each field, placed in a plastic bag, and stored at 4 C and were not allowed to dry until further analysis. Soils were passed through a 6.3-mm mesh sieve to remove large rocks and debris before use.

Laboratory Dissipation of Atrazine. *Incubation Procedure*. In all laboratory experiments with the soils described in Table 1, 50 g of moist soil was placed in 250-ml glass jars. The soil was treated with atrazine,[1] and the jars were sealed with Teflon-lined lids. These experimental units were incubated at 24 C and exposed to the lights in the laboratory (< 50 μM m^{-2} s^{-1}) for 8 to 10 h d^{-1}. Samples were taken from each soil at 0, 1, 2, 4, 8, 16, and 32 d after treatment (DAT) and extracted with water as described below. All treatments were replicated at least twice, and experiments were repeated at least twice.

Effect of Water Level, Atrazine Concentration, Mercuric Chloride, and Extraction Solvent on Degradation. Four preliminary experiments were conducted to determine the effect of soil moisture, herbicide concentration, microbiological processes, and extraction solvent on atrazine degradation. Two soils were used. CO2 was a Haxtun loamy sand and CO5 was a Nunn clay loam (Table 1). In the initial experiment, soil moisture content of CO5 was adjusted by adding 5, 6.5, 7.5, or 10 ml of water containing 5 μg ml^{-1} of atrazine to 50 g of soil (wet wt), which adjusted the water content between 19 and 29% (wt/wt). Subsamples were removed 1, 4, 6, 10, 14, and 21 DAT and extracted in water as described below. In the second experiment, 50 g of soils CO2 and CO5 were treated with 7.5 ml of water in which the amount of atrazine was varied to include 1, 2.5, and 5 μg ml^{-1}. Subsamples were removed 0, 1, 2, 3, 4, 7, 10, and 14 DAT and extracted in water as describe below. Because treating soil with $HgCl_2$ is an effective method to kill soil microorganisms (Wolfe et al. 1989), 5 mM $HgCl_2$[2] was added to the atrazine fortification solution to determine the role of microbiological processes on the dissipation of atrazine in a third experiment. Subsamples were collected 0, 1, 2, 3, 4, and 5 DAT and extracted in water as described below. In a fourth experiment, 50 g samples of CO2 and CO5 soils were treated with 7.5 ml of water containing atrazine (5 μg ml^{-1}). One set of subsamples was extracted with water and one set with toluene on 0, 1, 2, 4, 8, 16, and 32 DAT as described below.

Analysis of Atrazine. Water extraction and HPLC analysis of atrazine. Atrazine was extracted from the soils by weighing between 1.5 to 3 g of moist soil into a 15-ml conical tube[3] adding a volume of water (1 : 1 wt/vol), and vortexing the soil–water suspension twice for 10 s. The soil–water suspension (1.5 ml) was transferred to a 1.5 ml microfuge tube,[3] and the tube was centrifuged at 20,000 × *g* for 15 min. The supernatant (700 μl) was transferred to a 0.22-μm Teflon filter inserted in a microfuge tube[4] and filtered by centrifuging

at 4,000 × *g* for 5 min. The filtrate was transferred to a 1.5-ml HPLC vial and was analyzed with an HPLC instrument[5] equipped with a photodiode array detector.[6] Analytes were separated on a C18 column.[7] The mobile phase was acetonitrile : 5 mM ammonium acetate[8] adjusted to pH 4.5 (35 : 65 vol/vol) and was run isocratically at 40 C at a flow of 1 ml min^{-1}. The injection volume was 100 µl. Atrazine was detected at 223 nm, and the retention time was 8.7 min. The limit of detection was 10 ng ml^{-1} ($n = 8$).

Toluene extraction and gas chromatography–mass spectroscopy (GC/MS) analysis of atrazine. Atrazine was extracted and analyzed following the procedures of Shaner and Henry (2007). Five grams of soil were placed into a 50-ml glass centrifuge tube[3] with a Teflon-lined cap, and 5 ml of water and 5 ml of water-saturated toluene[8] was added. The tube was shaken horizontally for 2 h on a reciprocating shaker.[9] The samples were removed from the shaker and centrifuged for 20 min at 2,000 × *g*. One milliliter of the toluene phase was transferred to a 1-ml volumetric flask[3] to which 5 µl of a 0.1 mg ml^{-1} butylate[1] internal standard solution was added. The atrazine in the toluene phase was analyzed using a gas chromatograph equipped with a mass spectrometer[10] and monitoring the masses for butylate (mass-to-charge ratio [*m/z*] 146), and atrazine (*m/z* 200). A RTX-5 column[11] was used with a flow of helium[12] at 1 ml min^{-1}. The injection temperature was 280 C, and the detector temperature was 280 C. The program for detecting atrazine was as follows: initial oven temperature was 80 C (held 1 min), which was ramped at 30 C min^{-1} to 250 C and then held at 250 C for 1.5 min with a run time of 10 min. The retention times of butylate and atrazine were 6.4 and 7.8 min, respectively. The detection limit was 5 µg kg^{-1} of soil ($n = 10$). Quality control samples were included with each run and showed that the extraction efficiency for atrazine was 96 ± 3%.

The amount of water in each sample was determined by drying a sample at 105 C and determining the weight before and after drying. The amount of herbicide extracted from the soil was adjusted to the dry weight of soil.

Determination of atrazine binding to soil. Ten grams of air-dried soil was placed in a 50-ml centrifuge tube with a Teflon-lined cap. All samples were done with either three or four replicates per soil. Ten milliliters of a 0.02 M $CaCl_2$ solution with 0.5 mM $HgCl_2$ containing atrazine at a concentration of 1 µg ml^{-1} was added to each tube, and the tube was shaken for 24 h. Preliminary studies revealed that equilibrium had been reached after that time and that $HgCl_2$ did not affect the binding of atrazine to the soils (data not shown). $HgCl_2$ was added to the water phase to eliminate the possibility of atrazine being degraded during the equilibration period by bacteria in the soils. The samples were removed from the shaker and centrifuged for 20 min at 2,000 × *g*. Three milliliters of the equilibrium solution supernatant was transferred to a 10-ml glass test tube, which had a Teflon-lined cap, and 3 ml of water-saturated toluene was added. The tube was shaken for 1 h and then centrifuged for 5 min at 1,000 × *g* to separate the layers. The toluene phase was transferred to a 2-ml volumetric flask to which 10 µL of a 0.1 mg ml^{-1} butylate internal standard solution was added. The level of atrazine in the toluene phase was determined by GC/MS as described previously. The amount of herbicide sorbed by the soil was determined by the difference between the initial concentration of herbicide in the soil solution and the final concentration after equilibrating with the soil. The sorption coefficient (K_d) was calculated as shown in Equation 1:

$$K_d = [\textit{herbicide sorbed to soil } (\mu g/g)]/[\textit{herbicide in solution } (\mu g/ml)] \quad [1]$$

Field Dissipation of Atrazine in Colorado. *Field CO1*. This 10-ha field is located near Fort Collins, CO, and is a Nunn silt loam. This field was under conventional tillage and was irrigated with a linear sprinkler. Soil samples were taken from continuous corn plots treated with atrazine for 2 of the previous 5 yr. The field had not been treated with atrazine in 2005. The field was treated with a combination of atrazine, metolachlor, and mesotrione[14] at a rate of 0.84 kg ha^{-1} atrazine, 2.25 kg ha^{-1} metolachlor, and 0.225 kg ha^{-1} mesotrione on May 5, 2006. Three plots (3 m by 12 m) were established in the field, and samples were taken at 3, 10, 17, and 24 DAT. The herbicide was incorporated with 12.5 mm of irrigation water applied over 24 h after treatment. The field received supplemental irrigation of 150 mm of water during the time course of the experiment.

Field CO2. This 25-ha field, located near Haxtun, CO, is farmed using conventional tillage and center-pivot irrigation and contains both Iliff and Platner silt loam soils. The field had been in continuous corn for at least 5 yr with atrazine applied every year. The field received a commercial application of 0.84 kg ha^{-1} atrazine[15] on May 3, 2006. The field was retreated with atrazine at 0.67 kg ha^{-1} on May 31, 2006. The herbicide was incorporated both times by 25 mm of water applied over 2 d. The field received 40 mm of supplemental irrigation during the experiment. Three plots (3 m by 12 m) were established across the field, and samples were taken on 2, 9, 16, and 23 DAT after the second atrazine application. Soil samples were stored at −20 C until analyzed.

Field CO3. This 53-ha field is located near Yuma, CO, and soils in the field included Haxtun loamy sand, Manter loamy sand, and Julesburg loamy sand. This field was under strip-tillage with a 25-cm tilled strip over each row, which was on 76.2-cm centers and was irrigated by a center pivot. The field had been in continuous corn for at least 5 yr with atrazine applied every year. The field received 75 mm of supplemental irrigation during the period of sampling. A commercial application of 1.4 kg ha^{-1} of atrazine[16] was made on May 23, 2006, and the herbicide was incorporated with 25 mm of water applied over 2 d. Three plots (3 m by 12 m) were established across the field, and samples were taken 4, 7, 14, and 21 DAT. Soil samples were stored at −20 C until analyzed.

Statistical Analysis. Dissipation of atrazine was fitted to Equation 2 using SigmaPlot, Version 9[17]:

$$Y = Ae^{-kt} \quad [2]$$

Where, A is the mass of herbicide in soil at the first sampling time (mg kg^{-1}), k is the first-order rate constant (d^{-1}), and t

is time (d). Half-life ($T_{1/2}$) values for atrazine dissipation were calculated using Equation 3:

$$T_{1/2} = \ln(2)/k \quad [3]$$

The half-lives and K_d values of atrazine in the laboratory studies were subjected to ANOVA.[18] Treatment means were separated at the 5% level of significance using Fisher's Protected LSD test.

Results and Discussion

Effect of Water Level and Atrazine Concentration on Dissipation in Soil Assay. Atrazine degradation rates in soils are dependent on soil moisture content. Degradation is most rapid at 100% field capacity (Dinelli et al. 2000; Erickson and Lee 1989; Wang et al. 1995). The field capacity of CO5 is 23%. In this system there was no significant effect of soil moisture that ranged from below field capacity to above field capacity of CO5 (5, 6.5, 7.5, and 10 ml 50 g^{-1} soil, which corresponded to 19, 23, 26, and 29% soil moisture) on the atrazine half-life, which ranged from 4.9 to 6 d in soil CO5 (Figure 1). However if the soil was incubated in 50 ml of incubation solution per 50 g of soil, there was no detectable degradation of atrazine over 21 d (data not shown), indicating that too much water was detrimental in the assay. Atrazine degrades very slowly under anaerobic conditions (Accinelli et al. 2001; Seybold et al. 2001). Under the conditions of this assay, where the samples were not agitated, the system would probably become anaerobic, greatly reducing the rate of degradation. For convenience, the rest of the studies were conducted using 7.5 ml of incubation solution per 50 g of soil.

There was no significant effect of the concentration of atrazine in the incubation solution on the half-life of the herbicide in soils CO2 and CO5 (Figure 2). Gan et al. (1996) also found that the rate of atrazine degradation in two soils was unaffected by the concentration of the herbicide. However, it was more difficult to accurately measure the level of atrazine when the incubation solution contained 1 μg ml^{-1} compared with 5 μg ml^{-1}. The initial concentration of atrazine in the water extract was only 30 ± 3 ng ml^{-1} if the incubation solution contained 1 μg ml^{-1}, whereas it was 177 ± 10 ng ml^{-1} when the incubation solution had 5 μg ml^{-1}. For this reason, the remaining experiments were conducted using 5 μg ml^{-1} of atrazine in the incubation solution.

Effect of Mercury Poisoning on Atrazine Degradation. Atrazine degrades in the soil by chemical and biological processes (Wackett et al. 2002). There was no degradation of atrazine in the presence of 5 mM $HgCl_2$ after 6 d, whereas there was complete degradation of the herbicide in the nonpoisoned sample after 5 d in soil CO2 (Figure 3). Because treating soil with $HgCl_2$ is an effective method to kill soil microorganisms (Wolfe et al. 1989), these results indicate that the dissipation of atrazine in this assay system is primarily due to biological and not chemical processes.

Comparison of Water Extraction vs. Toluene Extraction. A comparison was made between water vs. toluene extraction of atrazine from two soils with different abilities to metabolize atrazine. In soil CO2, where atrazine dissipated rapidly, there was no significant difference in the calculated half-life between the two extraction methods. (Figure 4). In contrast, in soil CO5, the estimated half-life of atrazine was 8.4 d in the water extraction and 14.9 d in the toluene extraction (Figure 4). The difference between the two extractions is dependent on the ability of the solvent to remove atrazine from the soil. Toluene can extract 95 to 99% of atrazine from soil (Shaner and Henry 2007), whereas water was only able to extract 40 to 60% of the herbicide. In the highly metabolically active soil, there was no statistical difference between the calculated half-lives in the soils extracted with water or toluene. In the less-active soil, CO5, there was a statistical difference ($P = 0.05$), in the half-lives between the two extraction procedures, although both were relatively long. The difference between the two extraction procedures in CO5 is likely because of increased binding of atrazine to the soil with time, which results in the water not being able to extract the herbicide. Selim (2000) also reported time-dependent binding of atrazine. In spite of these differences, the water extraction method appears to be adequate to compare the relative rates of degradation of atrazine between soils and eliminates the need for organic solvent extraction.

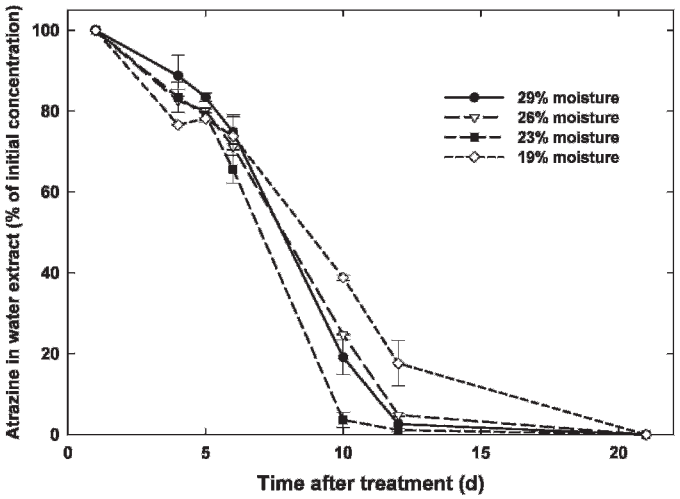

Figure 1. Effect of soil moisture on rate of dissipation of atrazine in soil water extract in CO5. Symbols are the means of three replications, and bars are the standard deviation.

Relationship Between Herbicide Binding to Soil and Water Extractability. All of the soils listed in Table 1 were treated with the same concentration of atrazine, and yet the amount of water-extractable atrazine varied over threefold (Table 1). The differences were because of the binding of atrazine to the soil. The concentration of atrazine in the water extract was negatively correlated to the K_d and the correlation was highly significant ($R = 0.7$, $P < 0.001$). These data indicate some of the limitations of this procedure: (1) There is a need to use significantly high initial concentration to detect atrazine in the water extract because of the loss of some material to sorption; the use of 5 μg ml^{-1} atrazine in the spike solutions should be enough in most cases, and (2) The baseline value for the screening method will be soil specific because differing K_d values. However, because there was not a significant effect of the initial concentration of atrazine on

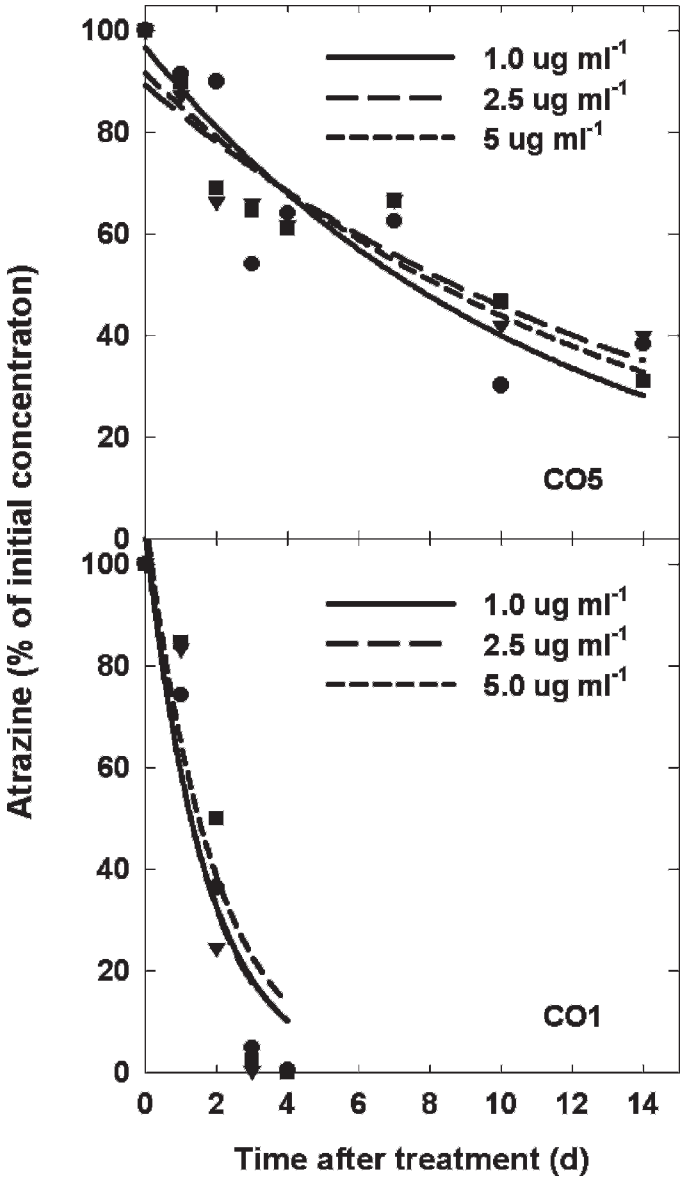

Figure 2. Effect of concentration of atrazine in incubation solution on rate of dissipation of atrazine in soil CO2 and CO5. Symbols represent the means of three replications. Bars are one standard deviation. The average $T_{1/2}$ was 1.4 d and 9 d for CO2 and CO5, respectively

the rate of degradation, the assay should work across a broad range of soil characteristics.

Relationship Between Herbicide History and Rate of Atrazine Degradation. The primary objective of this research was to develop a rapid assay that could be used to screen soils for degradation of atrazine. A wide number of soils were collected from a number of states from fields with varying histories of triazine herbicide use. In soil from fields that had a history of continuous triazine use (CO1 to 4, CA1, MS1), atrazine dissipated rapidly, with a half-life of less than 2 d (Table 1). Conversely, atrazine degraded slower in fields which did not have a history of atrazine use (CO5 to CO9, IL8, CA2, MS2) varying from 7.7 to 10.2 d (Table 1). Soils that had intermittent atrazine use (IL1 to IL7) had half-lives ranging from 2.5 to 5.7 d (Table 1). The half-lives of atrazine

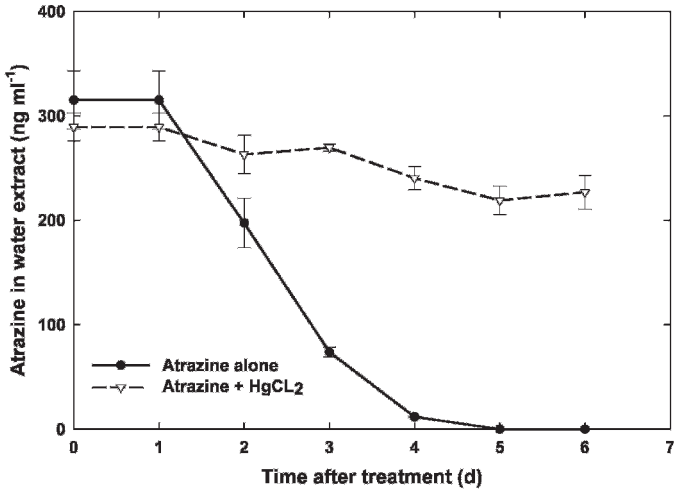

Figure 3. Effect of $HgCl_2$ on dissipation of atrazine in soil CO2. Symbols are means of three replications. Bars represent one standard deviation.

observed in this study are similar to those reported by Zablotowicz et al. (2006). They found that the half-life of atrazine in soils from Mississippi from fields with a history of atrazine use ranged from 2.3 to 3.1 d, whereas the half-life in a soil with no atrazine history was 17.3 d.

Interestingly, in the soils (CA1, CA2) collected from a California grape vineyard, one of which had been treated for many years with simazine, the half-life of atrazine was very

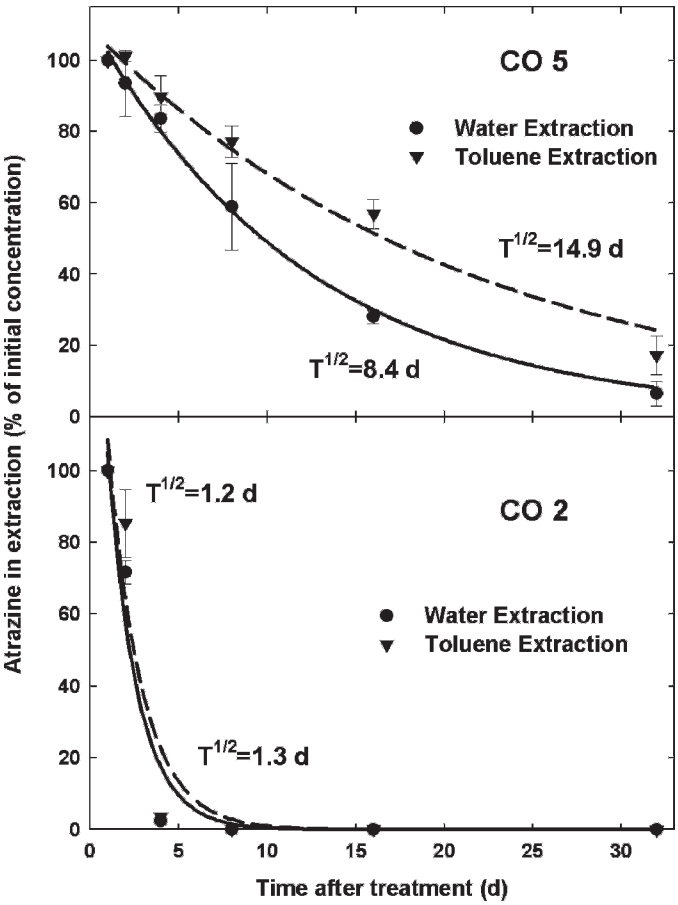

Figure 4. Comparison of atrazine dissipation in two soils (CO2 and CO5) with water vs. toluene extraction. Symbols are average of three replications, and bars are one standard deviation.

short in the soil with a history of simazine use compared with the rate of degradation in a soil with no triazine use history (0.7 vs. 10.3 d). The rate of simazine dissipation in these two soils was also determined using the same assay but substituting simazine for atrazine. In CA1, the half-life of simazine was 0.8 d compared with 11.5 d in the soil CA2 with no triazine history. Barriuso and Houot (1996) also observed rapid dissipation of simazine in soils exhibiting enhanced degradation of atrazine.

These results demonstrate the utility of this assay across a broad range of soils. The assay was done over a relatively short period, approximately 3 mo, by a single person on > 20 different soils.

Relationship Between Laboratory Assay and Field Dissipation. Soil from fields CO1, CO2, and CO3 rapidly metabolized atrazine in the laboratory assay. Atrazine was applied to these fields during the summer of 2006 and its rate of dissipation determined. The calculated half-lives of atrazine were 6.9 d, 3.0 d, and 3.0 d in CO1, CO2, and CO3, respectively (Figure 5). These are very short half-life values for atrazine, although they are in line with the values reported in other Colorado fields with a history of atrazine use (Shaner and Henry 2007). An attempt was made to measure the dissipation of atrazine in fields CO6 to CO9. The persistence of atrazine in these fields was longer compared with the corn fields, but we were unable to irrigate the fields, and extreme drought conditions confounded the results (data not shown). Although atrazine rapidly dissipated in the fields that also demonstrated rapid dissipation in the laboratory, further work needs to be conducted to fully establish the relationship between the laboratory degradation and persistence of atrazine in the field.

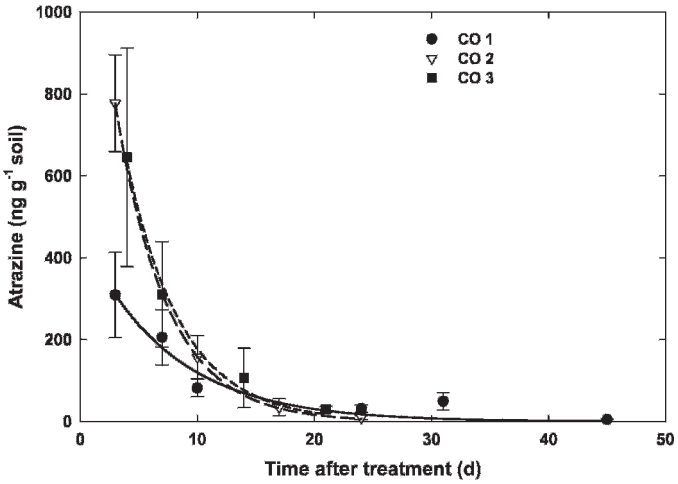

Figure 5. Dissipation of atrazine in Fields CO1, CO2, and CO3 in 2006. Symbols represent the means of three replications. Bars are one standard deviation. The $T_{1/2}$ for each curve is ■ 6.9 d; ▽ 3.0 d; ● 3.0 d.

Conclusion

The half-lives of atrazine in this assay, even in those soils with no history of atrazine use, were shorter than what has been reported in other studies (Barriuso and Houot 1996; Hang et al. 2003; Houot et al. 2000; Krutz et al. 2007; Ostrofsky et al. 1997; Popov et al. 2005; Pussemier et al. 1997; Shaner and Henry 2007; Vanderheyden et al. 1997; Yassir et al. 1999; Zablotowicz et al. 2006). The reason for this shorter half-life is likely because of the method of extraction. Water is a relatively poor solvent for atrazine, extracting only 40 to 60% of the available atrazine. However, water extractions are a better indicator of the bioavailable fraction of the herbicide compared with organic solvents because plants are only exposed to the herbicide that is in the soil water (Stalder and Pestemer 1980). This assay provided a rapid method for screening soils for enhanced atrazine degradation. In soils in which the herbicide dissipates quickly, the assay only requires 4 to 7 d for the atrazine in the water extract to go to nondetectable levels. The advantages of this assay are (1) the ease of set up, (2) the rapidity of extraction, and (3) the simplicity of the quantification of the atrazine. Additionally, atrazine bioavailable for microbial degradation would also be expected to be bioavailable as an herbicide for weed control. Water extracted very few other compounds from the soil that absorbed in the same wavelength as atrazine and the HPLC chromatogram was very clean, with few interfering peaks.

There is potential for this type of assay to be used at the field level by substituting an enzyme-linked immunosorbent assay (ELISA) detection system for the HPLC method used in this research. Ideally, an ELISA would be a 100-fold more sensitive than HPLC but can cost from $3 to $5 per sample analysis. There can also be interference with atrazine metabolites with ELISA, which could confound the results (Franek et al. 1995). Further work is being conducted to assess this possibility.

The results from this study suggest that if atrazine dissipates very rapidly in the laboratory assay ($T_{1/2} \leq 2$ d), then it is likely that the herbicide will also dissipate quickly in the field. More research needs to be done to confirm these findings and to develop a better relationship between the rate of degradation of atrazine in the laboratory and the field.

Sources of Materials

[1] Analytical grade atrazine and butylate, Aldrich Chemical Co., P.O. Box 355, Milwaukee, WI 53201.

[2] Mercuric chloride, Aldrich Chemical Co., P.O. Box 355, Milwaukee, WI 53201.

[3] Fisher Scientific, 4500 Turnberry Dr., Hanover Park, IL 60103.

[4] Ultrafree-MC, Millipore Corporation, 80 Ashby Road, Bedford, MA 01730.

[5] Shimadzu LC 10AT pump, Shimadzu Scientific Instruments, Inc., 7102 Riverwood Drive, Columbia, MD 21046.

[6] SPD M 10A detector, Shimadzu Scientific Instruments, Inc., 7102 Riverwood Drive, Columbia, MD 21046.

[7] Alltech Econosphere C_{18} column 5 µm and 150 by 4.6 mm, Alltech Associates, Inc, 2051 Waukegan Road, Deerfield, IL 60015.

[8] HPLC grade acetonitrile, toluene, and ammonium chloride, Aldrich Chemical Co., P.O. Box 355, Milwaukee, WI 53201.

[9] Eberbach two-speed reciprocating shaker, VWR International Inc., 17750 E 32nd Place, Suite 10, Aurora, CO 80011.

[10] Shimadzu GC-17A and GCMS QO 5050A, Shimadzu Scientific Instruments, Inc., 7102 Riverwood Drive, Columbia, MD 21046.

[11] RTX-5 (30 m by 0.25 mm) Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823.

[12] Ultrapure helium, 5.0, General Air Service and Supply, 1918 Heath Parkway, Fort Collins, CO 80524.

[13] JMC Soil Sampler, Clements Associates Inc., 1992 Hunter Avenue, Newton, IA 50208.

[14] Lumax, Syngenta Crop Protection, P.O. Box 18300, Greensboro, NC 27419.

[15] Aatrex 4L, Syngenta Crop Protection, P.O. Box 18300, Greensboro, NC 27419.

[16] Atrazine 90, UCPA, 1300 Corporate Center Curve Eagan, MN 55121

[17] SigmaPlot 9.0, Systat Software Inc. 501 Canal Blvd., Suite C, Point Richmonds, CA 94804.

[18] SyStat ver. 2.03, Systat Software Inc. 501 Canal Blvd., Suite C, Point Richmonds, CA 94804.

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