SPATIAL AND TEMPORAL CHANGES IN ATRAZINE DEGRADATION RATES IN SOIL

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ABSTRACT

Enhanced atrazine degradation has been documented in multiple fields in Colorado. A random survey of 70 fields in eastern Colorado showed that approximately 30% had enhanced atrazine degradation. However, the enhanced degradation is not necessarily long term. Twenty-five fields were tested in 2007 and then re-tested in 2009. In most of the fields the rate of atrazine degradation remained the same, but in other fields the rate either increased or decreased, suggesting that the populations of microorganisms responsible for degradation may not be stable. These results raise the question on how rapidly enhanced degradation can develop. A study was done to measure the spatial and temporal distribution of enhanced atrazine degradation. Four plots were established in a field that had shown low rates of atrazine degradation in 2007. The half life of atrazine in each of the plots was determined prior to atrazine application in August, 2009. There were major differences in the rates of atrazine degradation across the four plots, ranging between 4.3 and 11.7 days (d). Atrazine at 1.5 kg ha^{-1} was applied to whole field and soil samples from each plot were taken at 14, 28, 56 and 209 days after treatment (DAT). The rate of atrazine degradation increased with an average half life of 0.6 d at 56 DAT across all of the plots, although the differences among the plots remained. These data show the magnitude of the spatial and temporal differences in atrazine degradation and indicate that enhanced atrazine degradation can quickly arise in a soil soon after application of the herbicide.

Keywords: atrazine, herbicide, soil degradation

INTRODUCTION

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s triazine] is a widely used, soil-applied herbicide for controlling many broadleaf weeds in corn (Zea

mays L.), grain sorghum (*Sorghum bicolor* L.), and sugarcane (*Saccharum officinarum* L.). In 2005 66% of the corn in the U.S. was treated with atrazine at an average rate of 1.13 kg ha^{-1} (NASS, 2006). Farmers expect full season weed control from an atrazine application. The reported half-life of atrazine in the field is 60 days (Wauchope et al. 1992). However, farmers in eastern Colorado reported recently that atrazine was not giving the residual control expected (Shaner and Henry, 2007; Bridges et al., 2008). There could be several reasons for this lack of control including selection of tolerant or resistant weed biotypes leaching below the seed zone, or enhanced degradation. Most studies indicate the loss of atrazine efficacy is due to the selection of soil microbes that can rapidly degrade atrazine to CO_2 and urea (Krutz et al., 2010).

Variability in the rate of atrazine degradation in the field depends on atrazine-use history, soil pH, microbial communities, soil properties and landscape position (Vischetti et al., 1997; Liu et al., 2002; Muller et al., 2003; Charnay et al., 2005; Bending et al., 2006). A recent review paper by Krutz et al. (2010) described a multiple linear regression model for predicting atrazine persistence at the global scale. Their model identified soil pH and atrazine use history (number of consecutive years of atrazine application and whether or not atrazine had been applied at least one time in five years) as the significant input factors to predict atrazine half-life in soils from around the world.

What is not well understood is how rapidly enhanced atrazine degradation can develop or be lost after atrazine is no longer used? The objectives of this research were to determine 1) the stability of atrazine degradation rates in fields in eastern Colorado across two sampling years (2007 and 2009); 2) how rapidly enhanced atrazine degradation can develop within a single field; and 3) the spatial variability of atrazine degradation among plots within a single field.

MATERIALS AND METHODS

Sample Sites

Survey of Eastern Colorado. For objective 1, soils were collected from 21 different commercial farm fields across a broad geographical area of northeastern Colorado in November, 2007 and again in April, 2009 prior to herbicide applications. Soil samples were randomly collected across each of the fields by taking four to eight cores (5 cm diameter × 10 cm depth), which were composited into one sample per field. Samples were kept under cool conditions (~10 ^{o}C) for transport back to laboratory and immediately stored at 4°C.

Development of enhanced atrazine degradation. For objectives 2 and 3, soil was collected from a field near Greeley, CO. The soil is an Olney Fine Sandy Loam (Fine-loamy, mixed, superactive, mesic Ustic Haplargids). The field is under a 4 year rotation of dry beans-wheat-corn-sunflower. The soils were collected after the wheat was removed after the second year of the rotation. The soils had not been treated with atrazine for at least the previous three years but had been treated at earlier times. Four plots (9 m X 43 m) were established across the wheat area. These plots corresponded to the full irrigation treatment in 2008-2009 growing season. Soil cores were collected on 11 August, 31 August, 21

September, 19 October, 13 and 18 March 18, 2010. These dates corresponded to 7 days before herbicide application and 14, 28, 56, and 209 d after application, respectively. At each collection, four cores were removed in zero-contamination plastic tubes (2.3 cm dia X 30 cm inner dimensions) (Clements Associates Inc., Newton, IA). Collection sites were chosen at random across each plot. The soil cores were placed in a cooler and transported to the laboratory, where they were frozen at -80° C until analyzed. For analysis, each core was divided into three sections (0-5 cm; 5-15 cm; and 15-30 cm) and each section from the four columns of a plot were combined and mixed thoroughly.

Herbicide Treatment

Atrazine was applied uniformly across the field at 1.5 kg a.i. ha^{-1} with a tractor mounted sprayer set to deliver 200 l/ha at 207 kPa on 17 August 2009.

Atrazine degradation assay

Atrazine degradation of each of the soil samples was measured in the laboratory using the method of Shaner et al. (2007).

Dissipation calculation

Atrazine dissipation was fitted to Equation [1]:

$$C = C_0 e^{-kt} \qquad [1]$$

where C_0 is the concentration of atrazine in soil at time zero (mg kg^{-1}); k is the first-order rate constant (d^{-1}); and t is time (d). Half-life dissipation time (DT_{50}) values for atrazine in soil were calculated from Equation [2]:

$$T_{1/2} = \ln 2/k \qquad [2]$$

PCR Detection of 16Sr DNA and *atzC* Gene

The presence/absence of *atzC*, a gene encoding for atrazine degradation, was assessed to determine the potential of soils to degrade atrazine, DNA was extracted from 0.5 g of soil (n=45) using the FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH). DNA was visualized on 1% agarose gels containing 0.5 μg ml^{-1} ethidium bromide and quantified on a Nanodrop ND1000 microspectrometer (Nanodrop Technologies, Inc., Wilmington, DE). Amplification of *atzC* followed the protocol of Devers et al. (2007), and the protocol of Koike et al. (2007) was followed for amplification of 16S rDNA. PCR products were visualized on 2% agarose gels containing 0.5 μg ml^{-1} ethidium bromide. All soils yielded amplifiable DNA as verified by PCR amplification of 16S rDNA (not shown).

RESULTS

Change in atrazine degradation over time: field survey

A survey of fields in eastern Colorado was conducted in 2007 and the same fields were re-sampled in 2009. Twelve of the 21 fields sampled in 2007 showed enhanced atrazine degradation (DT_{50} <3 d) (Table 1) whereas only 2 of the fields showed slow degradation (DT_{50}>8 d). In 2009 there were some differences in the rate of atrazine degradation compared to 2007. In 14 of the fields, the rate of degradation was slower in 2009 compared to 2007; 2 of the fields showed more rapid degradation; and 5 of the fields remained the same. The differences between the years were correlated to changes in the herbicide use between 2007 and 2009. Where the rate of atrazine degradation decreased, atrazine had not been used the previous year, where atrazine had been used the previous year the fields showed more rapid degradation in 2009 vs. 2007. These results indicate that the ability of the soil microbes to degrade atrazine is not static or random, but is related to recent atrazine use history.

Table 1: Rate of atrazine degradation in soil collected from 21 fields in eastern Colorado in 2007 and 2009.

County	Number	DT_{50}[a]	
		2007	2009
		(d)	
Morgan	1	1.4	2.5
	2	1.8	2.7
	3	4.5	11.2
	4	5.2	5.3
Logan	5	3.6	4.0
	6	2.9	4.7
	7	18.4	3.0
	8	3.6	2.0
	9	2.5	2.8
	10	2.9	3.0
	11	3.5	15.5
	12	1.1	2.7
Phillips	13	1.1	3.8
	14	2.3	5.4
	15	8.5	6.1
	16	2.7	14.4
	17	2.4	4.5
	18	5.0	8.8
	19	4.6	7.4
	20	1.5	8.5
	21	2.0	6.7

[a] Dissipation time for 50% loss of herbicide.

Short Time Course of Development of Enhanced Atrazine Dissipation

A more detailed study on the dynamic changes in atrazine degradation was begun in 2007 in a field near Greeley, CO. Prior to the establishment of this test the field had been planted in a rotation of carrots, winter wheat, and corn. In 2006 the field where the test was conducted in 2009 had been planted in carrots. In 2007 the rotation changed to winter wheat, corn, sunflower, and dry beans. The soil from the winter wheat was sampled in 2007, prior to wheat planting. The soil was sampled again in 2008 after the wheat had emerged. Atrazine was applied to the wheat field on 13 May 2008. The rates of dissipation of atrazine in the treated field and in an adjacent non-treated field were evaluated in October, 2008 and again in May, 2009 (Figure 1).

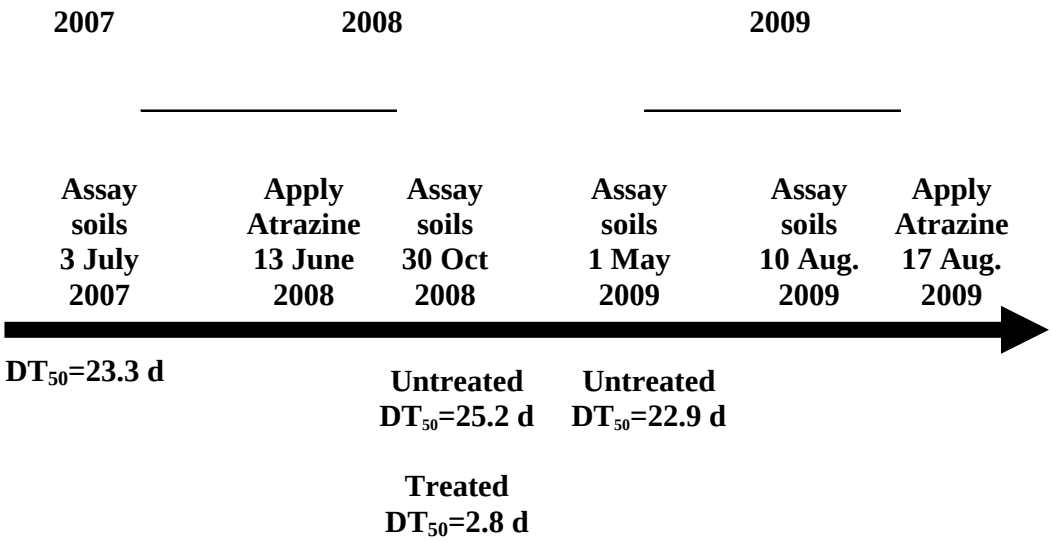

Figure 1: Time course of atrazine degradation rate in atrazine treated and untreated plots. DT_{50} is the time needed to dissipate 50% of the herbicide.

The DT_{50} of atrazine in the untreated soils from this field remained between 25.2 and 22.9 d across the three years. Surprisingly, the DT_{50} of atrazine in the treated field decreased from 25.2 d to 2.8 d. The soils treated with atrazine metabolized the herbicide almost 10 fold faster four months after application of the herbicide. These results indicate that the soil microbes that can metabolize atrazine were present in the soil prior to the 2008 application of the herbicide and their numbers and/or activity increased rapidly when atrazine was present.

The second study in the same area described above began in May, 2009. The land in the second study had been planted in winter wheat and had not received any applications of atrazine for at least the previous three years. When the half life of atrazine was assessed in May, 2009, the DT_{50} in this field was 22.9 d (Figure 1). Wheat was harvest in July, 2009 and 1.5 kg ha^{-1} was applied 17 August, 2009. Four plots were established across the field (Figure 2) and samples taken before and after atrazine application to determine how rapidly enhanced atrazine dissipation appeared. To determine the relationship between depth in the soil and dissipation, the soil samples were divided into three depths, 0-5 cm, 5-15 cm and 15-30 cm.

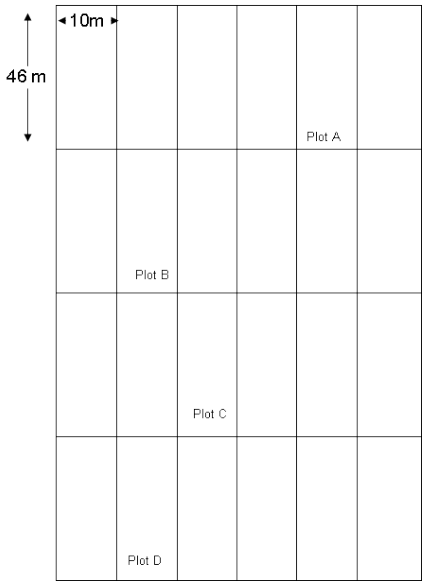

Figure 2. Layout of plots for time course of developing enhanced atrazine dissipation.

The DT_{50} of atrazine in the top 5 cm of soil varied across plots and over time (Figure 3). Plot A had the slowest DT_{50}, whereas Plot D had the most rapid DT_{50}. In all of the plots the rate of dissipation increased with time after atrazine application (Figure 3). By 56 days after treatment (DAT) the DT_{50} was the same across all of the plots (Figure 3). This rapid rate of dissipation was maintained over the winter, as shown by the DT_{50} at 209 DAT (Figure 3).

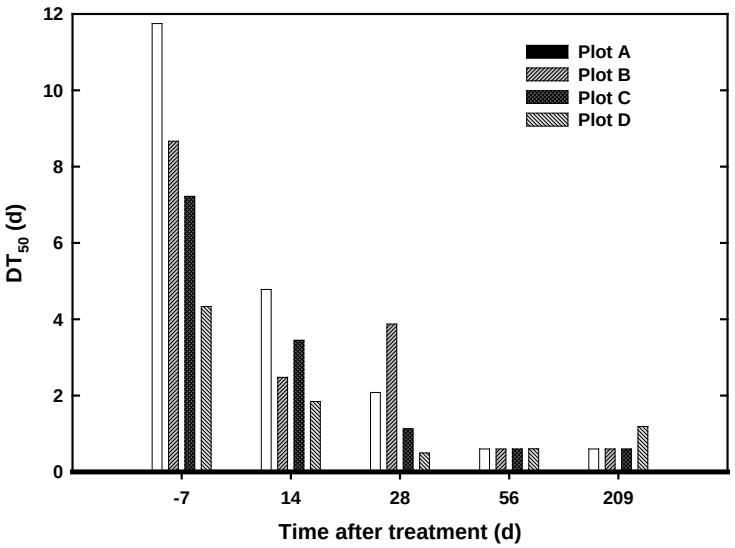

Figure 3: The change in the rate of DT50 over time for atrazine in the 0-5 cm soil depth of plots treated with atrazine on 17 August 2009.

There were major differences in the atrazine DT_{50} with depth over time in plot A (Figure 4). Prior to atrazine application, the DT_{50} in the 0-5 cm depth was 11.7 d. At the 5-15 cm and 15-30 cm depth the DT_{50} was 40.8 and 46.8 d, respectively, which is close to a four-fold slower rate of dissipation compared to the 0-5 cm depth (Figure 4). After application, the rate of atrazine dissipation increased over time at all depths, so that by 56 DAT the DT_{50} had dropped to 0.6, 4.0 and 5.6 d at 0-5, 5-15 and 15-30 cm depths, respectively. Thus, the soil was able to degrade atrazine approximately 10-fold more rapidly than before atrazine exposure. However, the rate of dissipation in the 0-5 cm depth always was faster than the other depths.

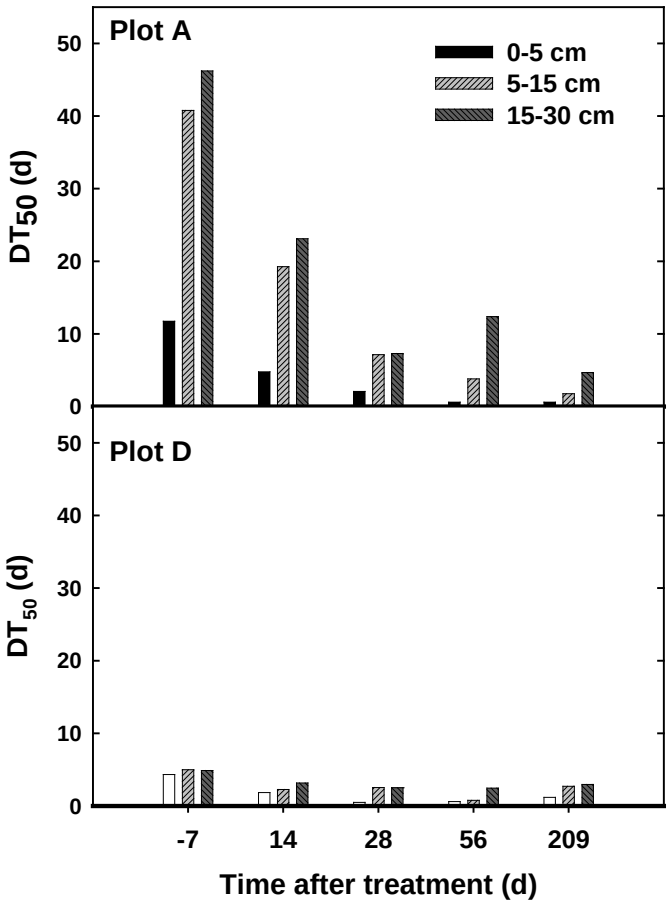

Figure 4: Effect of depth and time of atrazine degradation in Plots A and D after atrazine application.

The effect of depth on atrazine degradation in Plot D was different than what was observed in Plot A. Prior to atrazine application, there were no major differences in the DT_{50} across the depths (Figure 4), and atrazine dissipation rates were much faster than in Plot A. The DT_{50} were 4.3, 5.0 and 4.9 d for the 0-5, 5-15, and 15-30 cm depths, respectively. These rates are 3 to 8 fold faster than those in Plot A. The rate of dissipation of atrazine increased after application at all depths and the changes appeared to occur most rapidly in the 0-5 cm depth

compared to the other depths. (Figure 4). However, the soil from Plot D continued to metabolize atrazine more rapidly compared to Plot A. The data from Plots B and C were intermediate between Plots A and D (data not shown).

The other major differences between Plot A and Plot D, was the length of the lag period before degradation began in the lab assay. Soil from Plot A appeared to have a lag period of 21 to 35 d prior to atrazine application in the field (Figure 5) whereas the possible lag period in Plot D was 7 d. However, 28 d after atrazine was applied in the field, there did not appear to be any lag phase in the dissipation of atrazine in the soil from Plot D or in the upper 5 cm of soil from Plot A and the lag phase in the soil from the 5-15 and 15-30 cm depth dropped to 10 d (Figure 5.)

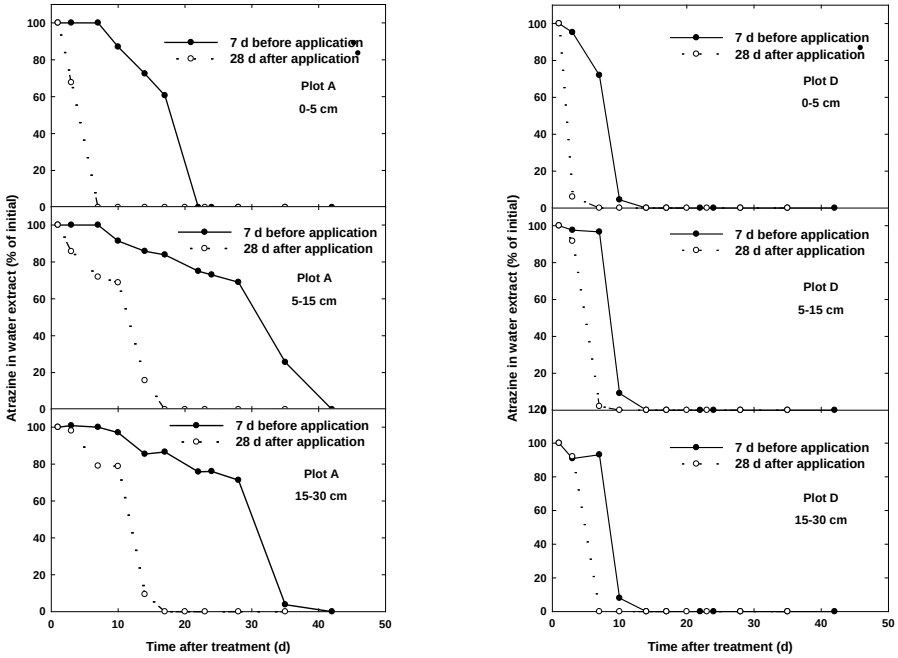

Figure 5: Dissipation of atrazine in laboratory assay in soil from different depths and time after application of atrazine in the field in Plot A (left) and Plot D (right).

The lag phase seen in these soils could indicate that the soil microbes capable of metabolizing atrazine were present but were not very abundant. Other researchers have observed similar changes in the lag phase in atrazine degradation after exposure to the herbicide (Krutz et al., 2010). The herbicide was probably not rapidly degraded until the level of the bacteria in the soil increased to some critical level. DNA was extracted from the soil and examined it for *atzC*, which encodes for a critical enzyme in the metabolism of atrazine in the soil (de Souza et al., 1998). We could detect *atzC* even in the 15-30 cm zone of the soil, although initially it was very faint. The level of *atzC* increased with time after exposure to atrazine in the field, particularly in the 0-5 cm depth (data not shown) indicating that the decrease in the lag phase was due to an increase in the level of atrazine metabolizing microbes in the soil after exposure to atrazine.

DISCUSSION

The phenomenon of enhanced atrazine dissipation in soils has been well documented across the world (Krutz et al., 2010). This rapid dissipation is due to the selection of soil microbes that can rapidly metabolized atrazine (Devers et al., 2007). Research on how rapidly the soil microbes adapt to the presence of atrazine has shown that this can occur within one growing season (Zablotowicz et al., 2006; Zablotowicz, 2007). However, there is little research on determining how soon after application this adaptation occurs. In this research we found that the soil microbes react very quickly to the presence of atrazine. We could measure more rapid degradation of atrazine in soil within 14 d after application and it reached its maximum level by 56 d after application. The initial detection of *atzC* in the soil showed that the genes responsible for atrazine degradation were present, although at relatively low levels. While the enzyme encoded by this particular gene does not initiate catabolism of atrazine, it is reported to occur along with *atzA*, which does encode the first enzyme in the degradation pathway, on the same plasmid, at least for *Pseudomonas* strain ADP (de Souza et al., 1998). Several studies have targeted *atzC* rather than *atzA* for detection and quantification of atrazine-degrading bacterial communities (e.g., Piutti et al., 2002; Martin-Laurent et al., 2003). Quantitative rather than qualitative assessment of *atzC* gene may have yielded better results in terms of predicting atrazine degradation activity. However, it has been found that the increased abundance of *atzC* in response to atrazine application was transient (Piutti et al., 2002; Martin-Laurent et al., 2003).

These results also show the variability in the ability of the soils to degrade atrazine across the field. The biggest differences occurred between Plot A and Plot D. Soil from Plot D was able to readily metabolize atrazine prior to application, although this rate increased after atrazine was applied whereas soil from Plot A did not metabolize atrazine well initially, but the rate increased over 10 fold by 56 d after application. One of the differences between these two areas of the field could be their nearness to an access road, which ran just outside Plot D. This road was treated with atrazine in the summer of 2009 to control weeds along its edges prior to treating the whole field. It is highly likely that there was some drift of atrazine into plot D, which could have resulted in inducing an increase in the population of soil microbes that could metabolize atrazine. Plot A was not close to this site of application.

The results from this study are important because they indicate that the ability of soils to rapidly degrade atrazine can be lost after the herbicide has not been used for at least 3 years. A similar observation was made by Shaner et al. (2009) who found that the rate of atrazine degradation in the field was dependent on how frequently a field had been treated with the herbicide. In soil from plots that had received yearly atrazine applications for four years, the herbicide dissipated very quickly in the laboratory, (DT_{50}=1.2 d) whereas in soil from a plot that had had only one application of atrazine in four years the DT_{50} was 3.8 d. A field that had no history of atrazine use had a DT_{50} of 9.3 d. Interestingly, in the field when atrazine was applied to the soil with 4 years of continuous atrazine use, the DT_{50} was 3.2 d, which was the same as the DT_{50} for the soil that had had only 1 application three years before (DT_{50}=3 d). The DT_{50} of atrazine in the soil that

had no previous history of herbicide use was 19.8 d. These results suggest that the same phenomenon seen in this study occurred in the previous study.

CONCLUSION

It has been shown that soils with enhanced degradation of atrazine lose residual weed control (Shaner and Henry, 2007; Krutz et al., 2007; Krutz et al., 2009). The results from this study indicate that once the soil has acquired this ability, it is not easily lost although initially the rate of degradation may decrease in the absence of the herbicide. However, once the herbicide is re-introduced to the soil, the microbes quickly respond and begin degrading the herbicide again.

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