SUGARCANE SOILS EXHIBIT ENHANCED ATRAZINE DEGRADATION AND CROSS ADAPTATION TO OTHER *s*-TRIAZINES

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ABSTRACT

Reports of reduced residual weed control with atrazine in Florida and Hawaii soils indicate that enhanced triazine degradation may be occurring across the entire United States sugarcane production region. A previously developed triazine degradation assay was used to determine if Florida and Hawaii soils were positive for enhanced atrazine degradation and if confirmed adapted soils were also cross-adapted with ametryn, and metribuzin. The objectives of this study were to 1) determine if soils collected from the United States sugarcane regions (i.e., Florida and Hawaii) exhibit enhanced atrazine degradation; and 2) determine if atrazine adapted soils also degrade other triazine herbicides used use in sugarcane production. Florida and Hawaii soils with a previous atrazine use history did exhibit enhanced atrazine degradation. Atrazine adapted soils were cross-adapted with ametryn, another s-triazine; however, the atrazine adapted soils were not cross-adapted with the non-symmetrical triazine herbicide, metribuzin. Results indicate that 1) enhanced atrazine degradation occurs across the full range of the United States sugarcane production region; and that 2) atrazine adapted sugarcane production soils will likely be cross-adapted with ametryn but not metribuzin. Consequently, metribuzin may be a viable alternative to for sugarcane production soils with reduced residual weed control arising from enhanced *s*-triazine degradation.

INTRODUCTION

The triazine herbicides have been a mainstay for control of grass and broadleaf weeds in many crops since the 1950s. Atrazine is the most widely used of the triazines with the primary uses in corn, sorghum and sugarcane. However, other triazines, particularly ametryn, simazine and metribuzin, also are critical for weed control in other crops such as sugarcane, tree and fruit crops and potatoes. In spite of the advent of new herbicide chemistries and the introduction of glyphosate- and glufosinate-resistant corn, atrazine is still the cornerstone for weed management in most of the corn grown in the U.S. (LeBaron et al. 2008). The triazines have become even more important as tools to manage weeds that have become resistant to other herbicides, such as the ALS-inhibitors and glyphosate. The availability of triazine herbicides is an important component in many integrated weed management programs (LeBaron et al. 2008).

One advantage of the triazines is the length of residual activity, which is from 8 to 10 weeks in most cropping situations. However, in the mid-1990s, two soil bacteria, *Pseudomonas sp. ADP* and *Nocardioides sp. C190*, were isolated and shown to rapidly degrade triazine herbicides (Mulbry et al. 2002; Wackett et al. 2002). These bacteria are widely distributed and bacterial isolates from four phyla originating from six continents have been documented (Krutz et al. 2010). Most of the isolates contain some or all of the gene homologs responsible for the enzymes that degrade atrazine on one or more self-transmittable plasmids (Wackett et al. 2002; Devers et al. 2005). The bacterial atrazine degradation pathway is initiated by one of two enzymes. Both enzymes dechlorinate atrazine to form hydroxyatrazine. However, one of the enzymes (*AtzA*) recognizes primarily the chlorotriazine herbicides, i.e. atrazine, simazine, propazine, terbuthylazine, mesoprazine, sebuthylazine, and associated mono-*n*-dealkylated metabolites, while the other enzyme (*TrzN*) metabolizes all commercially available *s*-triazine herbicides, in addition to some asymmetrical triazine compounds (Strong et al. 2002; Shapir et al. 2005). These findings suggest that selection of enhanced atrazine degradation in soil microbes could affect the dissipation of both symmetrical and asymmetrical triazine herbicides, which is known as cross adaptation.

Enhanced atrazine degradation has been documented in growers' fields in Colorado and Mississippi with a concomitant loss of weed control (Shaner and Henry, 2007; Krutz et al. 2008). We have conducted surveys on soils from multiple sites in the U. S. to determine the extent of enhanced atrazine degradation in different cropping systems (Shaner et al. 2007). These surveys utilized a relatively simple, rapid soil assay in which the soil is incubated with atrazine and changes in water extractable atrazine are measured over time (Shaner et al. 2007). This assay could be used as a method to survey soils, not only for atrazine degradation, but also other triazine herbicides.

Triazines are widely used for weed control in sugarcane. Atrazine is used on more than 70% of sugarcane production, and 94% of all ametryn sold is for sugarcane post-emergent weed control (Smith et al. 2008). Up to 8.9 kg ha^{-1} of atrazine can be applied to sugarcane per year, usually in two applications of approximately 4.45 kg ha^{-1} each (CDMS, 2009). Additionally, metribuzin is used on approximately 19% of the sugarcane hectarage (Smith et al. 2008). Growers depend on these triazines to control many broadleaf and grass weeds, particularly during the first eight to 10 weeks following cane emergence (Smith et al. 2008) and expect these herbicides to provide residual weed control throughout the establishment period. Atrazine is also widely used in a tank mix application at or after layby for postemergence control of vining weeds, such as annual morningglory, after canopy closure.

In the early 2000s sugarcane growers in Hawaii reported that atrazine residual activity was not providing the weed control expected (Michael Poteet, personal communication). Similarly, researchers reported that residual control of red morningglory (*Ipomoea coccinea* L.) by atrazine had decreased in recent years in Louisiana sugarcane fields (Griffin et al. 2000; Jones and Griffin, 2009.) Viator et al. (2000) determined that red morningglory had not become resistant to atrazine. In both

instances, the researchers suspected that poor atrazine performance was related to rapid degradation rather than herbicide resistance.

Hence, the objectives of this study were 1) determine the rate of atrazine degradation in soils collected from sugar cane fields in Florida and Hawaii; and 2) determine if the rapid assay can be used to screen for degradation of other triazine herbicides in these soils and other soils collected throughout the US.

MATERIALS AND METHODS

Soils were collected from fields in Florida and Hawaii in 2008. In addition, soils from other cropping systems were also collected in 2008. The upper 10 cm of soil were collected from multiple sites within each field. The soil was kept at field moisture levels and shipped and stored at 4 C until assayed. The soil series and recent triazine use history were recorded for each sample (Table 1).

Analytical grade triazines (Figure 1) were purchased from Sigma-Aldrich.

The rate of degradation of atrazine and the other triazines tested in the system was determined by the procedure described in Shaner et al. (2007). Briefly, 50 g of soil were placed in a 125 mL glass jar capped with a lined lid. The soils from Hawaii, California, Colorado and Mississippi were treated with 7.5 ml of water containing 5 ug l^{-1} of one of the analytical grade triazines being tested. The soils from Florida were treated with 20 ug l^{-1} of the triazines due to the high organic matter content of the soils. The soils were incubated at room temperature (25 C) in the dark. Soil samples (1.5-3 g) were removed at 1, 2, 4, 7, 14, 21 and 28 days after treatment (DAT). An equal amount of water (v/w) was added to the soil, the sample was mixed vigorously three times by vortexing and 1.5 ml of the slurry was transferred to a 1.5 mL microfuge tube. The microfuge tube was centrifuged at 15,000 x g for 10 minutes. Approximately 0.9 ml of the supernatant was transferred to a microfuge insert with a 0.45 um Teflon filter contained in a 1.5 ml microfuge tube. The sample was centrifuged at 10,000 x g for 10 minutes and the filtrate transferred to a 2 ml vial.

The amount of triazine in the filtrate was analyzed by high performance liquid chromatography (HPLC) as described by Shaner et al. (2007). Analytes were separated on a C_{18} column. The mobile phase was acetonitrile: 5mM ammonium acetate adjusted to pH 4.5 (35:65 v/v) and was run isocratically at 40 C at a flow of 1 ml minute^{-1}. The injection volume was 100 µl. The triazines were detected at 223 nm and the retention times were 5.6, 8.5, 13.8 and 15.7 minutes for metribuzin, atrazine, propazine and ametryn, respectively. The limit of detection was 5 ng ml^{-1} (n=8).

Triazine dissipation was fitted to Equation [1]:
$$C = C_0 e^{-kt} \qquad [1]$$

where C_0 is the concentration of the triazine in water extract at 1 DAT (mg l^{-1}); k is the first-order rate constant (d^{-1}); and t is time (d).

Half-life (DT_{50}) values for the triazine in the water extract were calculated from Equation [2]:

$$DT_{50} = \ln 2/k \qquad [2]$$

Correlation coefficients and statistical significance of the figures were calculated utilizing SigmaPlot 10.0.

RESULTS AND DISCUSSION

Atrazine dissipated rapidly in sugarcane soils from Hawaii and Florida that had been treated with a high rate of atrazine within 3 months of collecting the soil (Table 1). In Hawaii the soils with the most rapid degradation rate were also the ones where the growers were dissatisfied with the residual activity of atrazine (Michael Poteet, personal communication). These Hawaiian soils degraded atrazine approximately 10-fold faster than soils that did not have a history of atrazine use (Table 1.). However, not all of the soils recently treated with atrazine rapidly degraded the herbicide. There was one soil in Hawaii that had been recently treated with 3.3. kg ha^{-1} of atrazine that degraded atrazine relatively slowly ($DT_{50} = 6.8$ days) (Table 1). The reason for this slow rate of degradation could be due to the absence of the soil microorganisms that degrade atrazine. The fact that this soil did not rapidly degrade the other triazines (Table 1) supports the hypothesis.

In the four soils from Florida, the soil that had received two atrazine applications at 4.5 kg ha^{-1} each, degraded atrazine the most rapidly, followed by the soil that had only received one atrazine application. Both soils degraded atrazine 2- to 4-fold more rapidly than the soils that had no recent history of atrazine use (Table 1).

Soils were collected from other cropping systems to compare to the sugarcane soils. These soils also showed a strong relationship between history of atrazine (or simazine) use and dissipation rates. In soils collected from grape vineyards in CA, atrazine dissipated rapidly in soils that had a long history of simazine use but was approximately 4-fold slower in soil that had no history of simazine use. Likewise there was a two- to three-fold difference in the rate of atrazine dissipation in soils from CO and MS that had a history of triazine use compared to soils with no history (Table 1).

These results suggest that soil microbes that rapidly degrade atrazine have been selected or enriched in soils from numerous sites in the U.S and raise questions as to the rate of degradation of other triazines. To address this question, we used the rapid assay to evaluate the degradation rates of other triazine herbicides (Figure 1) in these soils. The triazines tested included propazine, although not used in sugarcane production is a very close analog to atrazine; and two triazines used in sugarcane production, ametryn, which differs from atrazine by having a thiomethyl substitution in place of the chlorine (Figure 1); and metribuzin, which is an asymmetrical triazine with a completely different substitution pattern on the triazine ring compared to atrazine (Figure 1). The results show a very strong correlation between the rate of degradation of atrazine and propazine (Table

1, Figure 2). This is not surprising since the first enzymes in the enhanced microbial degradation pathway appear to recognize all chlorotriazines. Similar results were observed when simazine was substituted for propazine (data not shown).

The ametryn degradation rate was also highly correlated to atrazine degradation (Table 1, Figure 2), although the correlation was not as high as with propazine. The primary reason for the lower correlation was that often, ametryn degraded more rapidly than atrazine. Across all locations, soil types and cropping histories, based on the DT_{50} analysis, there were no instances for which atrazine degraded more rapidly than ametryn (Table 1). These results suggest that in sugarcane production, if growers observe less than expected residual weed control with atrazine, switching to ametryn will not alleviate the problem of a lack of weed control.

Conversely, there was no correlation between metribuzin and atrazine dissipation rates (Table 1, Figure 2). This observation indicates that the enzyme(s) that degrade the *s*-triazine herbicides ametryn, atrazine, and propazine are not effective against the asymmetrical triazine herbicide metribuzin. Thus, metribuzin could be applied to soils exhibiting enhanced atrazine degradation without a reduction in residual weed control.

Results from this survey indicate that enhanced atrazine degradation occurs in various regions across the United States sugarcane production areas. Moreover, sugarcane production soils exhibiting enhanced atrazine degradation are cross-adapted with ametryn and propazine. Extensive use of these triazines for pre-emergence weed control in sugarcane-producing soils may lead to reduced yields via greater competition from certain weed species. Jones and Griffin (2008) reported a loss of control of morningglory in sugarcane fields in Louisiana, which they suspected was due to rapid dissipation of atrazine. These results support their conclusions.

Shaner and Henry, (2007) reported that there was approximately a 3- to 5-fold difference between the rates of degradation in the rapid assay compared to field dissipation. That is, if the half life of atrazine in the rapid assay was approximately 1 day, the rate of degradation in the field was between 3 and 5 days. Conversely, if the half life of atrazine in the rapid assay was 6 days, then rate of degradation in the field was 18 to 30 days. If these relationships hold for other triazines, these triazines would dissipate very rapidly in the field.

These data indicate, however, that the non-symmetrical triazine herbicide metribuzin is not cross-adapted with atrazine. Metribuzin, therefore, is a viable alternative for soils exhibiting enhanced atrazine degradation. Atrazine replacement costs in integrated weed management programs for sugarcane producers may be prohibitive, and further studies should identify other cost-effective solutions for areas experiencing enhanced degradation.

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Table 1: Soil characteristics, triazine use history, crop and DT$_{50}$ of triazines in soils collected from California, Colorado, Florida, Hawaii, and Mississippi.

State	Soil Series	Crop	Atrazine History	DT$_{50}$ (days)			
				Atrazine	Propazine	Ametryn	Metribuzin
Hawaii	Waiakoa silty clay loam	Sugarcane	4.5 kg ha^{-1} within 3 months	0.8 (0.1)[a]	2.1 (0.1)	1.1 (0.3)	11.7 (0.3)
	Paia silty clay	Sugarcane	4.5 kg ha^{-1} within 3 months	0.8 (0.1)	1.4 (0.1)	0.6 (0.1)	13.9 (0.5)
	Nonopahu clay	Sugarcane	4.5 kg ha^{-1} within 3 months	1.8 (0.1)	1.9 (0.1)	1.9 (0.1)	13.8 (0.3)
	Keahua silty clay loam	Sugarcane	None for 2 years	3.4 (0.1)	5.0 (0.2)	3.0 (0.1)	18.0 (1.4)
	Makaweli silty clay loam	Sugarcane	3.3 kg ha^{-1} within 3 months	6.8 (0.1)	9.1 (0.2)	7.1 (1.2)	15.6 (1.6)
	Makaweli silty clay loam	Sugarcane	None for 2 years	7.5 (0.1)	8.8 (0.1)	3.5 (0.2)	7.7 (0.5)
	Waiakoa silty clay loam	Pasture	None	11.9 (0.4)	16.5 (0.5)	3.8 (0.3)	4.0 (0.1)
	Waiakoa silty clay loam	Pasture	None	13.9 (0.7)	18.9 (1.1)	17.8 (0.2)	15.4 (0.2)
Florida	Pahokee muck	Sugarcane	9.0 kg ha^{-1} within 3 months	1.2 (0.1)	1.6 (0.1)	1.2 (0.1)	11.9 (4.8)
	Pahokee muck	Sugarcane	4.5 kg ha^{-1} within 3 months	3.1 (0.1)	3.4 (0.2)	1.2 (0.1)	10.2 (0.1)
	Pahokee muck	Fallow	None	6.9 (0.5)	7.2 (1.0)	2.3 (1.6)	11.0 (0.4)
	Pahokee muck	Non-cropland	None	6.1 (1.1)	7.3 (2.0)	--	6.6 (2.1)
California	Hesperia fine sandy loam	Grape	1.1 kg ha-1 simazine for >5 years	2.3 (0.1)	4.2 (0.1)	2.1 (0.1)	17.2 (0.5)
	Hanford fine sandy loam	Grape	None	13.1 (0.7)	15.9 (1.8)	5.3 (0.1)	7.9 (0.2)
Colorado	Fort Collins sandy clay loam	Corn	1.1 kg ha-1 for 3 years	4.8 (0.2)	4.8 (0.2)	5.0 (0.2)	15.0 (0.5)
	Fort Collins sandy clay loam	Sunflower	None	10.4 (0.6)	10.8 (0.6)	10.6 (0.3)	17.4 (0.1)
Mississippi	Leeper silty clay loam	Corn	1.1 kg ha-1 for >5 years	2.1 (0.1)	3.3 (0.1)	2.5 (0.1)	10.0 (0.1)
	Leeper silty clay loam	Cotton	None for 3 years	3.4 (0.1)	5.1 0.1	3.6 (0.2)	11.6 (0.3)
	Leeper silty clay loam	Blackberry	None	7.4 (0.1)	8.8 (0.1)	6.7 (0.1)	10.4 (0.1)

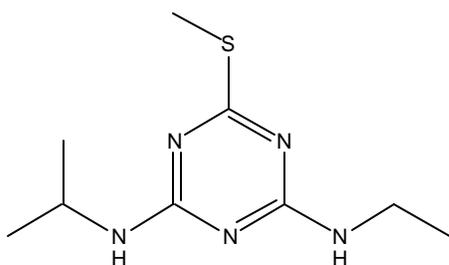[a]Standard error of the mean of four replicates.

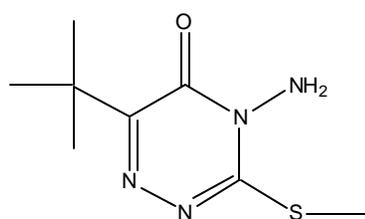**Figure 1: Structure of triazine herbicides**
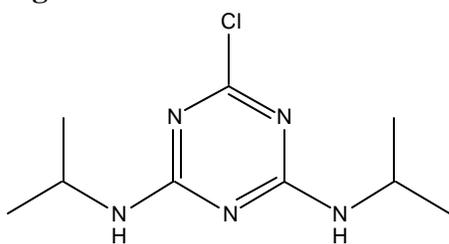
Atrazine
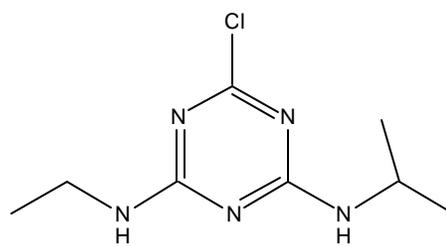
Propazine

Ametryn

Metribuzin

Figure 2: Relationship between DT_{50} of atrazine and other triazines in different soils. Correlations between propazine or ametryn DT_{50} and atrazine DT_{50} are significant at p<0.001. The correlation between metribuzin DT_{50} and atrazine DT_{50} is not statistically significant.