Evidence for cross-adaptation between *s*-triazine herbicides resulting in reduced efficacy under field conditions

L Jason Krutz,[1]* Ian C Burke,[2] Krishna N Reddy[1] and Robert M Zablotowicz[1]

[1]*United States Department of Agriculture, Agricultural Research Service, Southern Weed Science Research Unit, PO Box 350, Stoneville, MS 38776, USA*

[2]*Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164, USA*

Abstract

BACKGROUND: Enhanced atrazine degradation has been observed in agricultural soils from around the globe. Soils exhibiting enhanced atrazine degradation may be cross-adapted with other *s*-triazine herbicides, thereby reducing their control of sensitive weed species. The aims of this study were (1) to determine the field persistence of simazine in atrazine-adapted and non-adapted soils, (2) to compare mineralization of ring-labeled ^{14}C-simazine and ^{14}C-atrazine between atrazine-adapted and non-adapted soils and (3) to evaluate prickly sida control with simazine in atrazine-adapted and non-adapted soils.

RESULTS: Pooled over two pre-emergent (PRE) application dates, simazine field persistence was 1.4-fold lower in atrazine-adapted than in non-adapted soils. For both simazine and atrazine, the mineralization lag phase was 4.3-fold shorter and the mineralization rate constant was 3.5-fold higher in atrazine-adapted than in non-adapted soils. Collectively, the persistence and mineralization data confirm cross-adaptation between these *s*-triazine herbicides. In non-adapted soils, simazine PRE at the 15 March and 17 April planting dates reduced prickly sida density at least 5.4-fold compared with the no simazine PRE treatment. Conversely, in atrazine-adapted soils, prickly sida densities were not statistically different between simazine PRE and no simazine PRE at either planting date, thereby indicating reduced simazine efficacy in atrazine-adapted soils.

CONCLUSIONS: Results demonstrate the potential for cross-adaptation among *s*-triazine herbicides and the subsequent reduction in the control of otherwise sensitive weed species.
© 2008 Society of Chemical Industry

Keywords: enhanced degradation; cross-adaptation; weed control; atrazine; simazine

1 INTRODUCTION

Enhanced degradation is the phenomenon whereby a soil-applied pesticide is rapidly degraded by a population(s) of microorganisms that has developed the ability to use the compound as a carbon, energy and (or) nutrient source because of previous exposure to it or an analogue. Enhanced degradation has been reported for numerous agricultural products including nematicides, insecticides, fungicides and herbicides.[1–5] Agronomic problems associated with enhanced degradation, i.e. reduced pest control, intensify when structurally similar pesticides also degrade rapidly in the adapted soil, a phenomenon referred to as cross-adaptation.

Historically, enhanced degradation of highly substituted *s*-triazine herbicides was deemed unlikely because halogen and *n*-alkyl substituents were believed to retard microbial metabolism of the *s*-triazine ring. This assumption was supported by the inability of scientists to isolate bacteria able to catabolize highly substituted *s*-triazine herbicides, although attempts had been made since the introduction of the first s-triazine herbicide onto the market. However, in 1995, laboratories isolated bacteria able to catabolize atrazine, a highly substituted *s*-triazine herbicide.[6,7] Since this discovery, enhanced atrazine degradation has been reported for agricultural soils in Argentina, Belgium, Canada, France and the United States.[8–17] Moreover, a complete metabolic pathway describing the rapid catabolism of atrazine by bacteria has been elucidated (Fig. 1).

Assays conducted with purified atrazine chlorohydrolase and triazine hydrolase indicate potential for cross-adaptation among commercially available *s*-triazine herbicides (Fig. 1). Specifically, simazine is a substrate for purified atrazine chlorohydrolase, and most available *s*-triazine herbicides are substrates for purified triazine hydrolase.[32,33] However,

* Correspondence to: L Jason Krutz, United States Department of Agriculture, Agricultural Research Service, Southern Weed Science Research Unit, PO Box 350, Stoneville, MS 38776, USA
E-mail: jason.krutz@ars.usda.gov
(*Received 7 January 2008; revised version received 12 February 2008; accepted 18 February 2008*)
Published online 12 May 2008; DOI: 10.1002/ps.1601

© 2008 Society of Chemical Industry. *Pest Manag Sci* 1526–498X/2008/$30.00

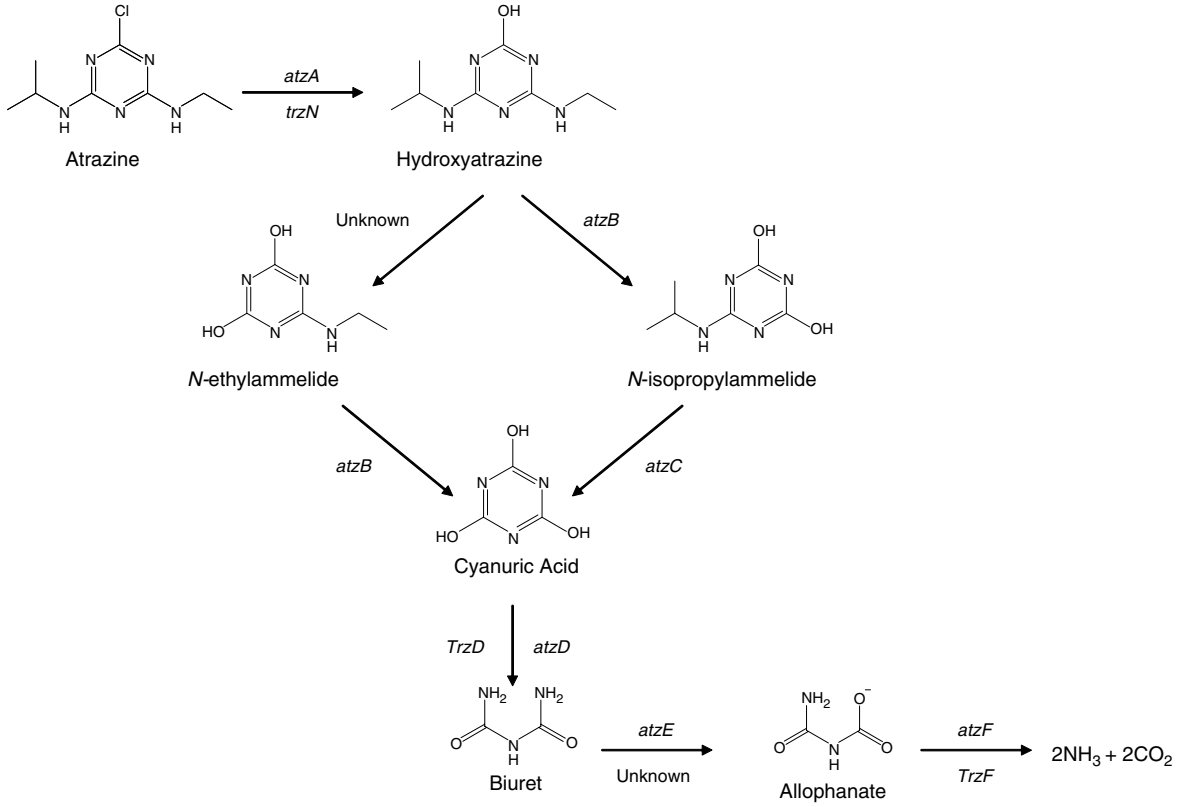

Figure 1. Proposed metabolic pathway(s) responsible for the rapid dissipation of atrazine and structurally similar triazine herbicides in soils exhibiting enhanced degradation. Abbreviations denote gene coding for the following enzymes: *atzA*, atrazine chlorohydrolase; *trzN*, triazine hydrolase; *atzB*, hydroxyatrazine ethylaminohydrolase; *atzC*, N-isopropylammelide isopropylaminohydrolase; *TrzD*, cyanuric acid amidohydrolase; *atzD*, cyanuric acid hydrolase; *atzE*, biuret hydrolase; *atzF*, allophanate hydrolase; *trzF*, allophanate hydrolase.[18–31].

cross-adaptation among *s*-triazine herbicides has not been confirmed under field conditions, nor has weed control been evaluated. The objectives of this study were (1) to determine field persistence of simazine in atrazine-adapted and non-adapted soils, (2) to compare mineralization of ring-labeled ^{14}C-atrazine and ^{14}C-simazine between adapted and non-adapted soils and (3) to compare prickly sida control with simazine in atrazine-adapted and non-adapted soils.

2 MATERIALS AND METHODS

2.1 Site history

The site history for the atrazine-adapted field has been described previously.[34] Briefly, the adapted soil was a Dundee silt loam (fine-silty, mixed, Typic Endoqualf), pH 6.7, 1.1% organic matter, CEC 15 $cmol_c$ kg^{-1}, and textural fractions of 26% sand, 55% silt and 19% clay. The field had been under reduced tillage, planted to corn (*Zea mays* L.) and treated with atrazine yearly since 2000. The soil has exhibited enhanced atrazine degradation since 2001, and bacteria isolated from the adapted soil were positive for *atzABC* and *trzN* genes.[35,36] The non-adapted soil was also a Dundee silt loam. The non-adapted field had been in cotton (*Gossypium hirsutum* L.)–soybean (*Glycine max* L.) rotation and has no known atrazine exposure history for at least 20 years.

2.2 Field preparation

Planting dates were 15 March and 17 April 2006 for both the atrazine-adapted and non-adapted field sites. Pre-emergence (PRE) options included (1) no PRE or (2) simazine at 1120 g AI ha^{-1}. Post-emergence (POST) options included (1) no POST or (2) glyphosate applied at 840 g AE ha^{-1} to V3–V4 stage corn. Corn, 'Garst 8347 RR', was planted in a conventional tillage seedbed at 7 seed m^{-1} of row, and tefluthrin [2,3,5,6-tetrafluoro-4-methylbenzyl (*Z*)-(1*RS*,3*RS*)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate; Force, Syngenta Crop Protection, Inc., Greensboro, NC] was applied in-furrow at 3700 g AI ha^{-1} at planting. Plots were 6.1 m in length by four rows wide, with rows spaced 102 cm apart. Simazine at 1120 g AI ha^{-1} was applied with a carbon dioxide powered backpack sprayer set to deliver 127 L ha^{-1} at 207 kPa through 11 002 flat-fan nozzles (Teejet 11 002 flat-fan nozzles; Spraying Systems Company, North Avenue, Wheaton, IL). Prickly sida (*Sida spinosa* L.) was seeded at 10 seed m^{-2} prior to planting using a shoulder-mounted rotary spreader. Prickly sida seed was mixed with sand (1 + 1 by volume) to facilitate even distribution from the spreader.

2.3 Field dissipation

The dissipation study was analyzed as a randomized complete block with twelve replications of each

treatment arranged in a split-split plot design. The whole plot was soil (adapted and non-adapted), the subplot was the simazine PRE application date (15 March and 17 April) and the subsubplot was time. From 0 to 42 days after simazine application, composite soil samples were collected from three locations in each plot to a depth of 5.0 cm. At each location, two samples were collected from in the plant row, and two samples were collected from the furrow between the beds. Composite samples were thoroughly mixed, and a subsample (50 g) was removed for herbicide analysis. Soil samples were stored at −5 °C until initiating extractions.

Simazine dissipation was fitted to the equation

$$C = C_0\ \mathrm{e}^{-kt} \quad (1)$$

where C_0 is the concentration of simazine in soil at time zero (mg kg^{-1}), k is the first-order rate constant (day^{-1}) and t is time (days). Half-life ($T_{1/2}$) values for simazine in soil were calculated from the equation

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

2.4 Mineralization assay

The mineralization study was analyzed as a randomized complete block with twelve replications of each treatment arranged in a split-split plot design. The whole plot was soil (adapted and non-adapted), the subplot was herbicide (simazine and atrazine) and the subsubplot was time. Mineralization of ^{14}C-ring-labeled simazine and atrazine was evaluated in biometer flasks as previously described.[34] For the simazine treatments, atrazine-adapted and non-adapted soils (25 g dry weight equivalent) collected from the 0–5 cm depth were fortified with a solution of technical-grade simazine (99% purity; Chemservice, Lancaster, PA) and ring-labeled ^{14}C-simazine (115 μCi mmol^{-1} specific activity, 94% radiological purity; Sigma Chemical Company, St Louis, MO) in deionized water. Similarly, for the atrazine treatments, adapted and non-adapted soil collected from the 0–5 cm depth were fortified with a solution of technical-grade atrazine (99% purity; Chemservice, Lancaster, PA) and ring-labeled ^{14}C-atrazine (115 μCi mmol^{-1} specific activity, 94% radiological purity; Sigma Chemical Company, St Louis, MO) in deionized water. The initial herbicide concentration for both triazine herbicides was 4 μg g^{-1}, and the initial radioactivity was 190 Bq g^{-1}. Biometers were incubated in the dark at 25 °C. Evolved ^{14}C-carbon dioxide was trapped in sodium hydroxide and quantified by liquid scintillation spectroscopy (LSS) using Hionic-Fluor (Perkin Elmer, Shelton, CT). Saturation of sodium hydroxide traps with carbon dioxide was avoided by replacing the trapping solution after each sampling. Soil was destructively sampled at 65 days. Air-dried soil was manually crushed into uniform particle size, and duplicate samples (0.30 g) were weighed onto Whatman 1 qualitative filter paper (Whatman Inc., Florham Park, NJ). Samples were combusted in a Packard model 306 oxidizer (Packard Instruments, Chicago, IL), and evolved ^{14}C-carbon dioxide was trapped in scintillation vials containing Carbo-Sorb and Permafluro (1 + 1 by volume, 20 mL; Packard Elmer, Meridian, CT). Radioactivity was determined by LSS. The amount of ^{14}C-carbon dioxide recovered from the combusted samples was added to the cumulative ^{14}C-carbon dioxide evolved to determine the mass balance of ^{14}C. Cumulative mineralization of ^{14}C-carbon dioxide was fitted to the Gompertz growth equation

$$y = a^* \exp(-\exp(-(t - t_0)/k))$$

where a is the plateau representing the maximum percent mineralization; t_0 is the abscissa of the inflection point representing the lag phase, k is the mineralization rate constant and t is time.

2.5 Herbicide analysis

2.5.1 Extraction and concentration

Soil (50 g) was agitated with methanol + water (8 + 2 by volume, 100 mL) for 24 h and centrifuged for 10 min at 6000 × *g*, and the supernatant was removed. The extract was concentrated by rotary evaporation, diluted in water (100 mL) and passed through a C_{18} solid-phase extraction column (Bakerbond; JT Baker, Phillipsburg, NJ) preconditioned with methanol (4 mL) followed by distilled water (4 mL) under negative pressure. Simazine was eluted with methanol (2 mL). Terbuthylazine was included as an internal standard.

2.5.2 Quantification

Simazine was identified and quantified with a Waters 2695 HPLC separations module (Waters, Milford, MA) equipped with a Waters 996 photodiode array detector (Waters, Milford, MA). The HPLC was fitted with a 2.1 mm diameter by 150 mm length Waters symmetry C_{18} column (Waters, Milford, MA). The mobile phase consisted of acetonitrile + water (4 + 6 by volume), and the flowrate was constant (0.3 mL min^{-1}). Recovery of simazine was 98.04%, and the instrument limit of detection was 0.11 μg mL^{-1} ($n = 8$).

2.6 Simazine efficacy in atrazine-adapted and non-adapted fields

To evaluate simazine efficacy, the field study was analyzed as a randomized complete block with a factorial arrangement of treatments. The factorial treatment arrangement consisted of planting date by PRE by POST treatment options, each replicated 3 times. Prickly sida density was evaluated 15, 30, 45 and 60 days after planting (DAP) from a single 1 m^2 plot randomly chosen from between the center two rows of each plot. Prickly sida emerges, develops symptoms and then dies in response to simazine before producing a true leaf; therefore, only weeds with one true leaf or more were counted, as these were considered to

have survived the herbicide treatment. All four rows of each plot were harvested using a combine modified for small plot research. Corn grain yield was adjusted to 15% moisture. Corn yields were higher at the 17 April planting date compared with the 15 March planting date; consequently, corn grain yields were standardized using the average yield of the weed-free treatment (simazine PRE fb glyphosate POST).

2.7 Statistics

Data variance was visually inspected by plotting residuals to confirm homogeneity of variance prior to statistical analysis. Data were subjected to analysis of variance using a mixed-model approach with replication considered as a random effect (SAS, 2006). Mineralization data were fitted to the Gompertz model using SAS NLIN, and 95% confidence intervals were calculated for a, t_0 and k (SAS, 2006).

3 RESULTS

3.1 Cross-adaptation

For simazine persistence, planting date ($P < 0.0001$) and soil ($P = 0.0010$) main effects were significant. Pooled over adapted and non-adapted soils, simazine half-life values were lower at the 17 April planting date ($T_{1/2} = 6.3$ d) than at the 15 March planting date ($T_{1/2} = 15.0$ days). Reduced simazine persistence at the 15 April planting date compared with the 15 March planting date was attributed primarily to higher average soil temperatures.[36] Pooled over planting date, simazine half-life values were lower in atrazine-adapted ($T_{1/2} = 7.6$ days) than in non-adapted soils ($T_{1/2} = 10.9$ days). Reduced simazine persistence in atrazine-adapted soils provides indirect evidence for cross-adaptation. Subsequent radiological assays were conducted to confirm this hypothesis.

For the radiological assay, a significant soil by time interaction was detected ($P < 0.0001$). From 3 days after study inception until study termination, cumulative mineralization pooled over simazine and atrazine was at least 3.5-fold greater in adapted than in non-adapted soil (Fig. 2). Maximum differences between soils occurred 5 days after inception, when cumulative mineralization of the evaluated *s*-triazine herbicides was 39.3-fold greater in atrazine-adapted than in non-adapted soils. Moreover, non-linear regression analysis indicated that the simazine and atrazine lag phase was 4.3-fold shorter and the mineralization rate constant was 3.5-fold higher in atrazine-adapted than in non-adapted soils (Fig. 2; Table 1). Similar mineralization kinetics between simazine and atrazine within atrazine-adapted soils is indicative of cross-adaptation.

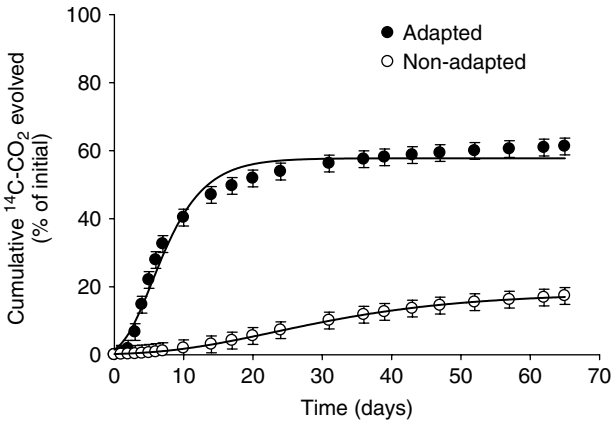

Figure 2. Cumulative [^{14}C]-carbon dioxide evolution pooled over atrazine and simazine in atrazine-adapted and non-adapted soils. Error bars indicate one standard deviation and do not appear when smaller than the symbol for the mean. Fitted values, indicated by the smooth line, are the best fit of the modified Gompertz growth equation $y = a^* \exp(-\exp(-(t - t_0)/k))$, where a is the plateau representing the maximum percentage mineralization, t_0 is the abscissa of the inflection point representing the lag phase, k is the mineralization rate constant and t is time.

3.2 Simazine efficacy

A differential effect of simazine on prickly sida density was observed between non-adapted and atrazine-adapted soils. At the 15 March planting date, prickly sida density in the non-adapted soil was 9.3-fold lower in simazine PRE compared with no simazine PRE ($P = 0.0052$; Table 2). Reduced prickly sida density in the simazine PRE treatment was expected, since the weed is typically controlled by the herbicide (York A, private communication). Conversely, in the atrazine-adapted soil, the contrast between no simazine PRE and simazine PRE for the 15 March planting date was not significant ($P = 0.2852$). A lack of significance in this contrast indicates that simazine had no statistical effect on prickly sida density in the atrazine-adapted soil. The respective trends within atrazine-adapted and non-adapted soils were also evident at the 17 April planting date; however, the contrast of no simazine PRE versus simazine PRE in the non-adapted soil was significant only at the $P = 0.0866$ level. These data indicate that simazine cross-adaptation decreases the herbicide residual control of prickly sida. However, under the conditions of this experiment, the economic significance of simazine cross-adaptation was minimal,

Table 1. Gompertz growth model estimates for atrazine and simazine in atrazine-adapted and non-adapted soils. Data for atrazine and simazine are pooled owing to a soil by time interaction ($P < 0.0001$)[a]

Soil	Maximum evolved (%)	Lag phase (days)	Mineralization rate (day^{-1})
Adapted	57.76 (56.42–59.09)	5.40 (5.02–5.79)	0.24 (0.20–0.28)
Non-adapted	18.14 (13.68–22.58)	23.06 (16.39–29.74)	0.07 (0.04–0.12)

[a] Numbers in parentheses indicate 95% confidence intervals as estimated by SAS NLIN.

Table 2. Interaction of planting date, soil and herbicide treatment on prickly sida density

Planting date	Field[a]	Simazine[b] PRE	Glyphosate[c] POST	Prickly sida density (plants m^{-2}) (± SE)
March 15	Adapted soil	−	−	4.5 (±2.0)
		−	+	0.3 (±0.2)
		+	−	1.0 (±0.7)
		+	+	0.3 (±0.2)
	Non-adapted soil	−	−	2.8 (±0.5)
		−	+	0.7 (±0.5)
		+	−	0.3 (±0.2)
		+	+	0.0 (±0.0)
April 17	Adapted soil	−	−	6.5 (±3.9)
		−	+	5.7 (±4.5)
		+	−	1.7 (±1.1)
		+	+	0.7 (±0.5)
	Non-adapted soil	−	−	2.7 (±1.4)
		−	+	1.0 (±0.6)
		+	−	0.5 (±0.5)
		+	+	0.3 (±0.3)

[a] The adapted soil had been treated with atrazine since 2000, has exhibited enhanced atrazine degradation since 2001 and was positive for the *atzABC* and *trzN* genes.[35,36] The non-adapted soil has no known atrazine exposure history for at least 20 years.
[b] Simazine pre-emergence (PRE) included no PRE (–) or simazine at 1120 g AI ha^{-1} (+).
[c] Glyphosate post-emergence (POST) included no POST (–) or glyphosate at 840 g AE ha^{-1} (+).

Table 3. Interaction of planting date, soil and herbicide treatment on yield and yield expressed as a percentage of the weed-free treatment

Planting date	Field[a]	Simazine[b] PRE	Glyphosate[c] POST	Yield (kg ha^{-1}) (± SD)	Yield (% weed free) (± SE)
March 15	Adapted soil	−	−	10 260 (±300)	99 (±0.03)
		−	+	10 590 (±290)	102 (±0.03)
		+	−	10 410 (±260)	100 (±0.03)
		+	+	10 230 (±260)	98 (±0.02)
	Non-adapted soil	−	−	8730 (±460)	90 (±0.05)
		−	+	9410 (±250)	97 (±0.03)
		+	−	9470 (±280)	98 (±0.03)
		+	+	9460 (±230)	98 (±0.02)
April 17	Adapted soil	−	−	10 970 (±700)	102 (±0.07)
		−	+	11 060 (±330)	102 (±0.03)
		+	−	10 690 (±470)	99 (±0.04)
		+	+	10 820 (±420)	100 (±0.04)
	Non-adapted soil	−	−	11 960 (±160)	106 (±0.01)
		−	+	11 720 (±350)	103 (±0.03)
		+	−	11 830 (±260)	104 (±0.02)
		+	+	11 750 (±210)	100 (±0.02)

[a] The adapted soil had been treated with atrazine since 2000, has exhibited enhanced atrazine degradation since 2001 and was positive for the *atzABC* and *trzN* genes.[35,36] The non-adapted soil has no known atrazine exposure history for at least 20 years.
[b] Simazine pre-emergence (PRE) included no PRE (–) or simazine at 1120 g AI ha^{-1} (+).
[c] Glyphosate post-emergence (POST) included no POST (–) or glyphosate at 840 g AE ha^{-1} (+).

as there was no observed corn grain yield loss in the atrazine-adapted fields at the 15 March or 17 April planting dates (Table 3).

4 DISCUSSION

The field persistence data coupled with the laboratory mineralization assay confirm cross-adaptation of simazine with atrazine-adapted soils. Previous reports confirmed that the atrazine-adapted soils evaluated in the present study were positive for the *atzABC* and *trzN* genes.[36] As simazine is a known substrate for the *atzA* and *trzN* gene products, the presence of these genes in the atrazine-adapted soils likely explains the biological mechanisms for cross-adaptation between the evaluated *s*-triazine herbicides.[32,33]

Furthermore, results from this study indicate potential for widespread cross-adaptation among commercially available *s*-triazine herbicides in agricultural soils positive for *atzA* and/or *trzN* genes. The severity of cross-adaptation will primarily depend on the enzymatic abilities of the bacteria responsible for enhanced *s*-triazine degradation. For example, the substrate specificity of the *atzA* gene product is restricted to *s*-triazine compounds containing Cl and F substituents.[32] Thus, for soils positive only for *atzA*, cross-adaptation among *s*-triazine herbicides will likely be limited to compounds with Cl and F substituents.

Pest Manag Sci **64**:1024–1030 (2008)
DOI: 10.1002/ps

Conversely, the substrate specificity of the *trzN* gene product encompasses *s*-triazine compounds with F, Cl, CN, N_3, SCH_3, SCH_2CH_3, $S(O)CH_3$ and OCH_3 substituents.[33] Consequently, cross-adaptation among most commercially available *s*-triazine herbicides is likely in agricultural soils positive for *trzN*. This assertion is startling, as enhanced atrazine degradation and the occurrence of *atzA* and/or *trzN* genes is suspected to be widespread across the western and southern corn-growing regions of the United States.[36]

Moreover, the prickly sida data confirm that reduced simazine persistence arising from cross-adaptation can reduce the efficacy of the compound. In view of the substrate range of the enzymes responsible for enhanced atrazine degradation, data indicate that cross-adaptation and a loss of residual weed control are probable among compounds in the *s*-triazine herbicide family, particularly if *trzN* is present. Consequently, the application of *s*-triazines PRE will likely be an ineffective stand-alone weed control program in adapted soils.

A viable weed control program for *s*-triazine-adapted and/or problem soils is presently in place throughout the United States. For example, in Mississippi most corn hectarage is planted to glyphosate-resistant hybrids, and glyphosate is applied either alone or in combination with herbicides possessing soil residual activity. Glyphosate is a non-selective broad-spectrum herbicide, and the pesticide is active on the same weed species as most *s*-triazine herbicides. Therefore, weeds surviving an *s*-triazine PRE treatment in an adapted soil can be managed with glyphosate POST, as was demonstrated in the present study (Table 3). However, glyphosate POST is not a viable option with non-glyphosate-resistant corn hybrids. Case in point, atrazine PRE failed to control weeds in non-glyphosate-resistant corn planted in an *s*-triazine adapted field (Shaner D, private communication). In summary, results from this study indicate potential for cross-adaptation among *s*-triazine herbicides resulting in reduced herbicidal efficacy.

REFERENCES

1 Singh BK, Walker A and Wright DJ, Cross-enhancement of accelerated biodegradation of organophosphorus compounds in soils: Dependence on structural similarity of compounds. *Soil Bio Biochem* **34**:1675–1682 (2005).

2 Racke KD and Coats JR, Enhanced degradation and the comparative fate of carbamate insecticides in soil. *J Agric Food Chem* **36**:1067–1072 (1988).

3 Walker A, Brown PA and Entwistle AR, Enhanced degradation of iprodione and vinclozolin in soil. *Pestic Sci* **17**:183–193 (1986).

4 Cotterill EG and Owen PG, Enhanced degradation in soil of tri-allate and other carbamate pesticides following application of tri-allate. *Weed Res* **29**:65–68 (1989).

5 Hole SJW and Powles SB, Reduced efficacy and enhanced degradation of carbetamide after repeated application in Australia. *Weed Res* **37**:165–170 (1997).

6 Mandelbaurm RT, Allan DL and Wackett LP, Isolation and characterization of a *Pseudomonas* sp. that mineralizes the *s*-triazine herbicide atrazine. *Appl Environ Microbiol* **61**:1451–1457 (1995).

7 Radosevich M, Traina SJ, Yue-Li H and Tuovinen OH, Degradation and mineralization of atrazine by a soil bacterial isolate. *Appl Environ Microbiol* **61**:297–302 (1995).

8 Barriuso E and Houot S, Rapid mineralization of the *s*-triazine ring of atrazine in soils in relation to soil management. *Soil Biol Biochem* **28**:1341–1348 (1996).

9 Hang S, Barriuso E and Houot S, Behavior of ^{14}C-atrazine in Argentinian topsoils under different cropping managements. *J Environ Qual* **32**:2216–2222 (2003).

10 Houot S, Topp E, Yassir A and Soulas G, Dependence of accelerated degradation of atrazine on soil pH in French and Canadian soils. *Soil Biol Biochem* **32**:615–625 (2000).

11 Ostrofsky EB, Traina SJ and Tuovinen OH, Variation in atrazine mineralization rates in relation to agricultural management practice. *J Environ Qual* **26**:647–657 (1997).

12 Pussemier L, Goux S, Banderheyden V, Debongnie P, Tresinie I and Foucart G, Rapid dissipation of atrazine in soils taken from various maize fields. *Weed Res* **37**:171–179 (1997).

13 Shaner DL and Henry HB, Field history and dissipation of atrazine and metolachlor in Colorado. *J Environ Qual* **36**:128–134 (2007).

14 Shaner DL, Henry WB, Krutz J and Hanson B, Rapid assay for detecting enhanced atrazine degradation in soil. *Weed Sci* **55**:528–535 (2007).

15 Vanderheyden V, Debongnie P and Pussemier L, Accelerated degradation and mineralization of atrazine in surface and subsurface soil materials. *Pestic Sci* **49**:237–242 (1997).

16 Yassir A, Lagacherie B, Houot S and Soulas G, Microbial aspects of atrazine biodegradation in relation to history of soil treatment. *Pestic Sci* **55**:799–809 (1999).

17 Zablotowicz RM, Weaver MA and Locke MA, Microbial adaptation for accelerated atrazine mineralization/degradation in Mississippi Delta soils. *Weed Sci* **54**:538–547 (2006).

18 Boundy-Mills K, De Souza ML, Mandelbaum RT, Wackett LP and Sadowsky MJ, The *atzB* gene of *Pseudomonas* sp. strain ADP encodes the second enzyme of a novel atrazine degradation pathway. *Appl Environ Micro* **63**:916–923 (1997).

19 Cheng C, Shapir N, Sadowsky NJ and Wackett LP, Allophanate hydrolase, not urease, functions in bacterial cyanuric acid metabolism. *Appl Environ Micro* **71**:4437–4445 (2005).

20 Shapir N, Cheng G, Sadowsky MJ and Wackett LP, Purification and characterization of TrzF: biuret hydrolysis by allophanate hydrolase supports growth. *Appl Environ Microbiol* **72**:2491–2495 (2006).

21 Shapir N, Sadowsky MJ and Wackett LP, Purification and characterization of allophanate hydrolase (*atzF*) from *Pseudomonas* sp. strain ADP. *J Bacteriol* **187**:3731–3738 (2005).

22 Sadowsky MJ, Tong Z, De Souza M and Wackett LP, *AtzC* is a new member of the amidohydrolase protein superfamily and is homologous to other atrazine-metabolizing enzymes. *J Bacteriol* **180**:152–158 (1998).

23 Mulbry WW, Zhu H, Nour SM and Topp E, The triazine hydrolase gene *trzN* from *Nocardioides* sp. strain C190: cloning and construction of gene-specific primers. *FEMS Micro Letters* **206**:75–79 (2002).

24 Fruchey I, Shapir N, Sadowsky MJ and Wackett LP, On the origins of cyanuric acid hydrolase: purification, substrates, and prevalence of *atzD* from *Pseudomonas* sp. strain ADP. *Appl Environ Micro* **69**:3653–3657 (2003).

25 de Souza ML, Seffernick J, Martinez B, Sadowsky M and Wackett LP, The atrazine catabolism genes *atzABC* are widespread and highly conserved. *J Bacteriol* **180**:1951–1954 (1998).

26 Smith D, Alvey S and Crowley DE, Cooperative catabolic pathways within an atrazine-degrading enrichment culture isolated from soil. *FEMS Microbiol Ecol* **53**:265–273 (2005).

27 Martinez B, Tomkins J, Wackett LP, Wing R and Sadowsky MJ, Complete nucleotide sequence and organization of the atrazine catabolic plasmid pADP-1 from *Pseudomonas* sp. strain ADP. *J Bacteriol* **183**:5684–5697 (2001).

28 Topp E, A comparison of three atrazine degradation bacteria for soil bioremediation. *Biol Fert Soils* **33**:529–534 (2001).

29 Shapir N, Osborne JP, Johnson G, Sadowsky MJ and Wackett LP, Purification, substrate range, and metal center of *atzC*: the *N*-isopropylammelide aminohydrolase involved in bacterial atrazine metabolism. *J Bacteriol* **184**:5376–5384 (2002).
30 Seffernick JL, McTavish H, Osborne JP, de Souza ML, Sadowsky MJ and Wackett LP, Atrazine chlorohydrolase from *Pseudomonas* sp. strain ADP is a metalloenzyme. *Biochemistry* **41**:14430–14437 (2002).
31 Wackett LP, Sadowsky MJ, Martinez B and Shapir N, Biodegradation of atrazine and related *s*-triazine compounds: from enzymes to field studies. *Appl Microbiol Biotechnol* **58**:39–45 (2002).
32 Seffernick JL, Gilbert J, Sadowsky MJ and Wackett LP, Substrate specificity of atrazine chlorohydrolase and atrazine-catabolizing bacteria. *Appl Environ Microbiol* **66**:4247–4252 (2000).
33 Shapir N, Rosendahl C, Johnson G, Andreina M, Sadowsky MJ and Wackett LP, Substrate specificity and colorimetric assay for recombinant TrzN derived from *Arthrobacter aurescens* TC1. *Appl Environ Microbiol* **71**:2214–2220 (2005).
34 Krutz LJ, Zablotowicz RM, Reddy KN, Koger CH III and Weaver MA, Enhanced degradation of atrazine under field conditions correlates with a loss of weed control in the glasshouse. *Pest Manag Sci* **63**:23–31 (2007).
35 Zablotowicz RM, Krutz LJ, Reddy KN, Weaver MA, Koger CH and Locke MA, Rapid development of enhanced atrazine degradation in a Dundee silt loam soil under continuous corn and in rotation with cotton. *J Agric Food Chem* **55**:852–859 (2007).
36 Krutz LJ, Shaner DL, Accienelli C, Zablotowicz RM and Henry WB, Atrazine dissipation in triazine-adapted and non-adapted soil from Colorado and Mississippi: implications of enhanced degradation on atrazine fate and transport parameters. *J Environ Qual* **37**:848–857.

Pest Manag Sci **64**:1024–1030 (2008)
DOI: 10.1002/ps