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Residues of Reduced Herbicides Terbuthylazine, Ametryn, and Atrazine and Toxicology to Maize and the Environment through Salicylic Acid

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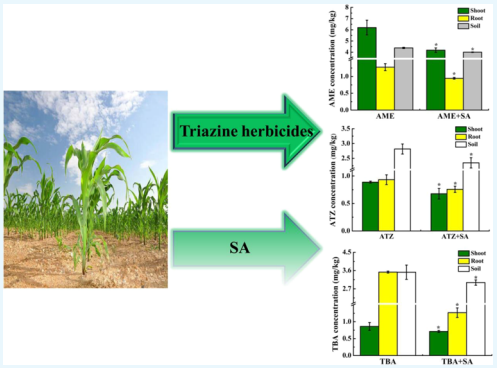
ABSTRACT: Terbuthylazine (TBA), ametryn (AME), and atrazine (ATZ) are triazine family herbicides. They are dominantly used in the field of cereal crops like wheat and maize for prevention of upland from annual gramineous and broad-leaved weeds, with attributes of weed efficiency broad spectrum and good market performance. Salicylic acid (SA) is a kind of natural plant growth regulator existing widely in the plant kingdom and participating in many physiological and defense processes. In this study, the effects of SA on the detoxification and degradation of herbicides TBA, AME, and ATZ in maize were investigated. When maize plants were exposed to 6 mg kg^{-1} of the triazine herbicides, the growth and chlorophyll concentration were reduced, while the membrane permeability increased. After maize was sprayed with 5 mg kg^{-1} SA, the herbicide-induced phytotoxicity was significantly assuaged, with the increased content of chlorophyll and decreased cellular damage in plants. Activities of several biomarker enzymes such as SOD, POD, and GST were repressed in the presence of SA. The concentration of the triazine herbicides in maize and the soil determined by high-performance liquid chromatography was drastically reduced by spraying SA. Using LC/Q-TOF-MS/MS, six metabolites and nine conjugates of AME in maize and soil were characterized. The relative contents of AME metabolites and conjugates in maize with SA were higher than those without SA. These results suggest that SA is able to promote the detoxification and decay of these triazine herbicides in maize and soil.



INTRODUCTION

Agricultural herbicides (pesticides) are synthetic chemicals widely used for field weeding to promote crop productivity. However, the reality is that not all applied herbicides are utilized by their targets; rather, in most cases, only a small proportion of them are absorbed by the targets, whereas most part of the herbicides reside inside the soil. Therefore, the accumulation of pesticides in the environment has become a serious problem in protecting the ecosystem and poses a threat to crop production, food safety, and human health.[1,2]

Triazine herbicides rank first among the world herbicide markets due to their broad spectrum and low cost. Triazine herbicides belong to a typical class of photosynthetic inhibitors that target photosynthetic system II and interfere with the electron transport by blocking the activity of Di protein.[3] Triazine herbicides are dominantly applied to the maize cropping fields. Due to their environmental persistence with a long half-life period, they are often found in soil, surface water, and groundwater environments.[4,5] Accumulation of the pesticides in humans through food chains triggers many ecotoxicological effects that come up with the carcinogenic and mutagenic diseases and dysfunction in human hormone homeostasis.[6,7] Therefore, it is imperative to understand how these nontarget plants or crops deal with the accumulation and toxicity of the pesticides and the mechanism for the metabolism and degradation of the pesticides in these plants.

Most of crops readily absorb triazine pesticides like atrazine from environments.[8,9] When plants are exposed to excessive atrazine, the reactive oxygen species (ROS) are massively generated.[9,10] The enhanced accumulation of atrazine has been reported to modify the activity of antioxidant enzymes and gene expression in cereal crops.[11,12] Peroxidase (POD) is an active antioxidant enzyme in plants, catalyzing the removal of hydrogen peroxide by oxidizing target substances such as phenols or other metabolites and thereby eliminating ROS-triggered damage to the cells.[13] POD is considered as a biomarker of plant resistance to multiple environmental stresses.[14] Glutathione *S*-transferase (GST) is one of the most important metabolic enzymes for antioxidation, and some of them are responsible for transformation of pesticides.[14] Under environmental stress, excess ROS not only activates the

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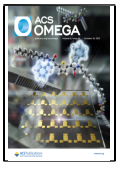

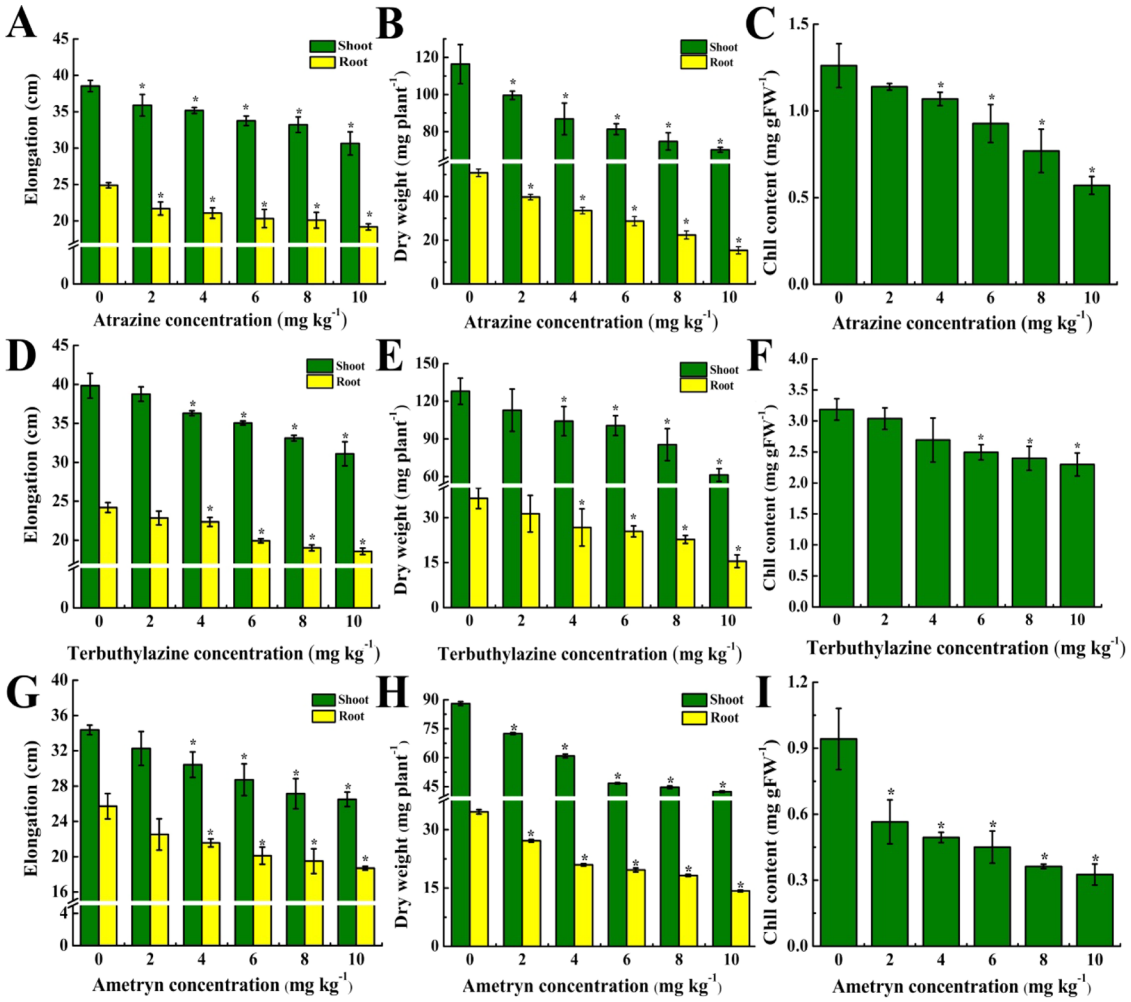

Figure 1. Effect of atrazine (A−C), terbuthylazine (D−F), and ametryn (G−I) on the growth of maize. Seedlings grew respectively in the soil with three herbicides at 0, 2, 4, 6, 8, and 10 mg/kg for 7 days. (A, D, G) Elongation of maize. (B, E, H) Dry mass of maize. (D, F, I) Chlorophyll content of maize. Values are means ± standard deviations (n = 3). Asterisks indicate the significant difference between the treatments with control and treatment ($p < 0.05$).

antioxidant capacity but also activates multiple signaling molecules. Salicylic acid, one of the signaling molecules, is a phenolic regulator of plant growth that is known for its role in mediating the defense of plants against pathogens.[15] Recent studies have shown that SA can also promote the degradation of pesticides in wheat, rice plants, and rhizosphere.[16−20] An understanding of how SA changes the activity of POD, GST, and ROS in crops under pesticide stress can help us figure out the mechanism of SA in alleviating the stress.

Maize containing a high nutritional value is the most important crop in the world. The phytotoxicity and residues of terbuthylazine (TBA), ametryn (AME), and atrazine (ATZ) in maize and soil directly affect seriously the crop production and food safety. Thus, the purpose of the study is to help understand how SA reduces the toxicity and damage of triazine to maize plants and promotes the degradation of triazine in maize and soil.

RESULTS AND DISCUSSION

Toxicological Response of Maize to Three Triazine Herbicides. Maize seedlings were grown in the soil treated with ATZ, TBA, and AME with gradient concentrations at 0, 2, 4, 6, 8, and 10 mg kg^{-1}. The biomass of maize was monitored. We found that all three herbicides could inhibit the growth of maize seedlings, and the effect was more obvious with the increase in herbicide concentrations (Figure 1). The chlorophyll content of maize leaves decreased significantly with the increase in herbicide concentrations (Figure 1C,F,I) because triazine herbicides are a class of photosynthesis inhibitors that can block electron transport in photosynthesis.[3] When the concentration of ATZ in the soil reached 6 mg kg^{-1}, the shoot length of maize was 12.4% lower than that of the control group, and the root length decreased by 18.4% (Figure 1A). This is consistent with the dry weight of the maize shoots and roots treated with ATZ (Figure 1B). The dry weight of maize decreased with the increase in herbicide concentrations (Figure 1B,E,H). These results indicated that the elevated concentration of the herbicides caused the severe phytotoxicity to the maize growth (Figure 1).

Salicylic Acid Mitigated the Toxicity of Triazine Herbicides to Maize. To test whether SA plays a role in reducing the toxicity of triazine herbicides to maize, 6 mg kg^{-1} in the soil was selected as the treatment concentration of three triazine herbicides. In the presence of herbicides, the elongation, chlorophyll content, and dry weight of maize with SA spray were higher than those without SA. The

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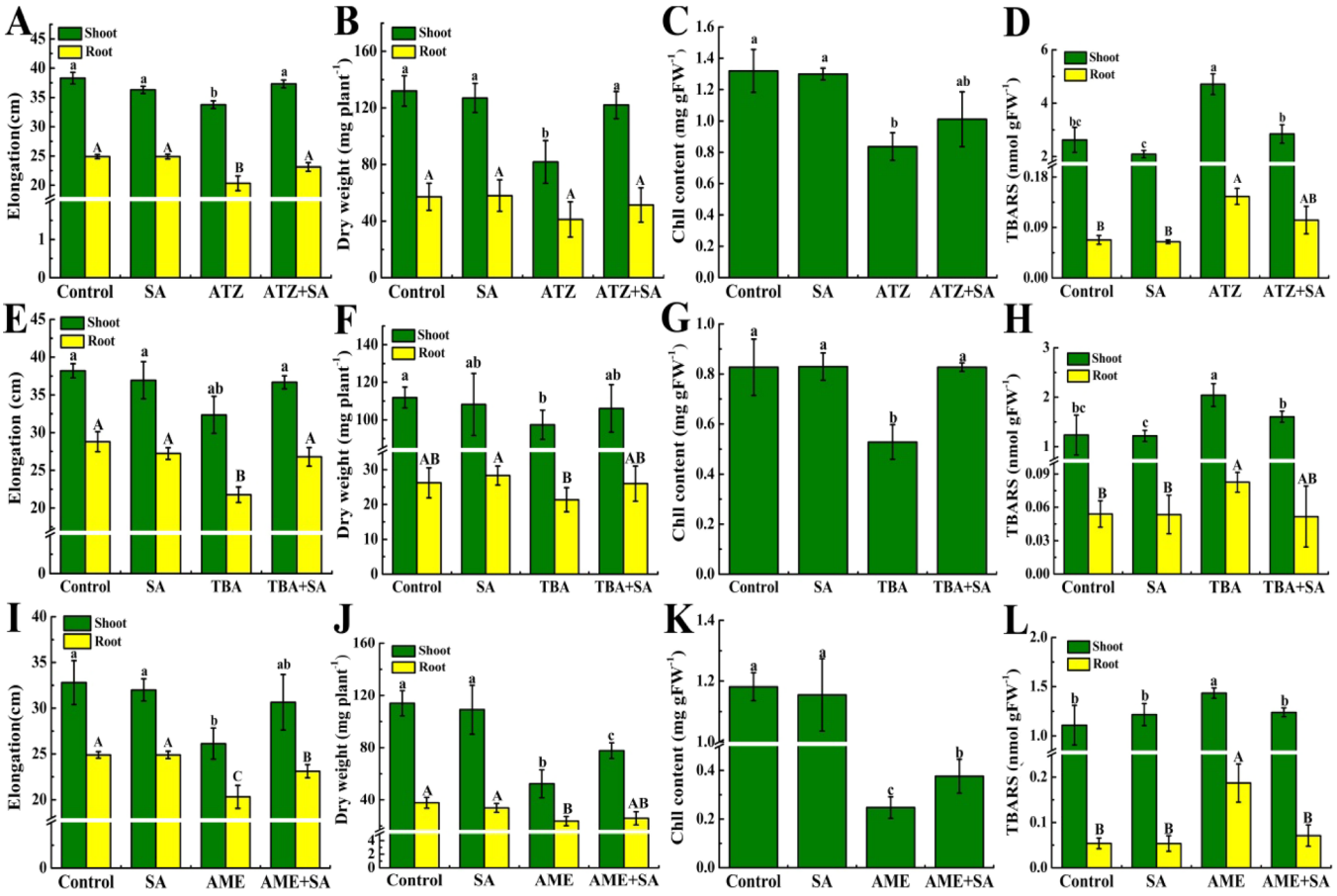

Figure 2. Effect of salicylic acid (SA) on the growth of maize under atrazine (ATZ) (A–D), terbuthylazine (TBA) (E–H), and ametryn (AME) (I–L) exposure. Seedlings grew respectively in the soil with three herbicides (6 mg/kg) for 4 days. After that, the leaves were sprayed with 5 mg/L SA every day for 6 days. (A, E, I) Elongation of maize. (B, F, J) Dry mass of maize. (D, G, K) Chlorophyll content of maize. (D, H, L) TBARS content of maize. Values are means ± standard deviations (n = 3). Different letters indicate the significant difference between the treatments (p < 0.05).

concentration of thiobarbituric acid reactive substance (TBARS) representative of the damage of a cellular membrane in maize with SA was lower than that without SA (Figure 2), suggesting that the growth of maize was improved, and the toxicological effect was decreased after spaying SA on maize growing in the soil. In Figure 2I, the shoot and root elongation values of maize under AME stress were significantly lower than those of the control, which were reduced by 20.3 and 18.4%, respectively. But when maize was under AME + SA, the shoot and root elongation values were 17.3 and 13.8% higher than those of the control under AME alone, respectively. The dry weights of the shoot and root under AME + SA treatment were 1.48- and 1.09-fold over those of the control (Figure 2J). The chlorophyll content of maize with AME was only one-fifth of that of the control, while with AME + SA, it increased by 10% (Figure 2K). These results point out that herbicide AME severely impeded the growth of maize, but the inhibitory effect could be restored partially by the supply of salicylic acid. The effect of SA on the growth of maize under ATZ and TBA treatment was consistent with AME + SA treatment. When plants are exposed to herbicides, the dynamic balance of the production and removal of reactive oxygen species or free radicals in plants is sabotaged.[21] The pesticide-induced peroxidation can boost production of additional free radicals, thus damaging the membrane lipids and producing a large amount of TBARS.[21] As shown in Figure 2L, the TBARS content of maize at 6 mg kg^{-1} AME in the soil was the highest in four treatments, suggesting that the maize seedlings were seriously injured after AME stress. In the AME + SA group, the TBARS content in the shoot of maize was lower than that in the AME group but not significantly different from that in the control group, indicating that the damage of AME to maize was significantly repaired after SA spray.

SA-Regulated Activities of Some Enzymes. Research has indicated that most of the abiotic stress is caused by the accumulation of superoxide radicals ($O_2^{\cdot-}$) and H_2O_2 in plants.[12,22] The oxidative stress in plants dysfunctions many critical macromolecular molecules (such as enzymes and proteins) and other important substances (*e.g.*, lipids) in the cells.[23] Superoxide dismutase (SOD) is the first line of enzymes in the active oxygen scavenging system, catalyzing the $O_2^{\cdot-}$ disproportionation reaction to produce H_2O_2 and O_2.[24] Peroxidase (POD) is another important antioxidant for attenuating oxidative stress by transformation of H_2O_2 into water and oxygen.[25] Studies show that the stress degree of plants is positively correlated with the activities of SOD and POD in plants within a certain range.[25] So, the enzyme activity of SOD and POD in maize reflects the stress level of three herbicides to maize. Glutathione *S*-transferase (GST) has the ability to promote the binding of reducing glutathione to harmful substances in plants.[26] Glutathione (GSH) with a reactive sulfhydryl group (−SH) plays an important role in the

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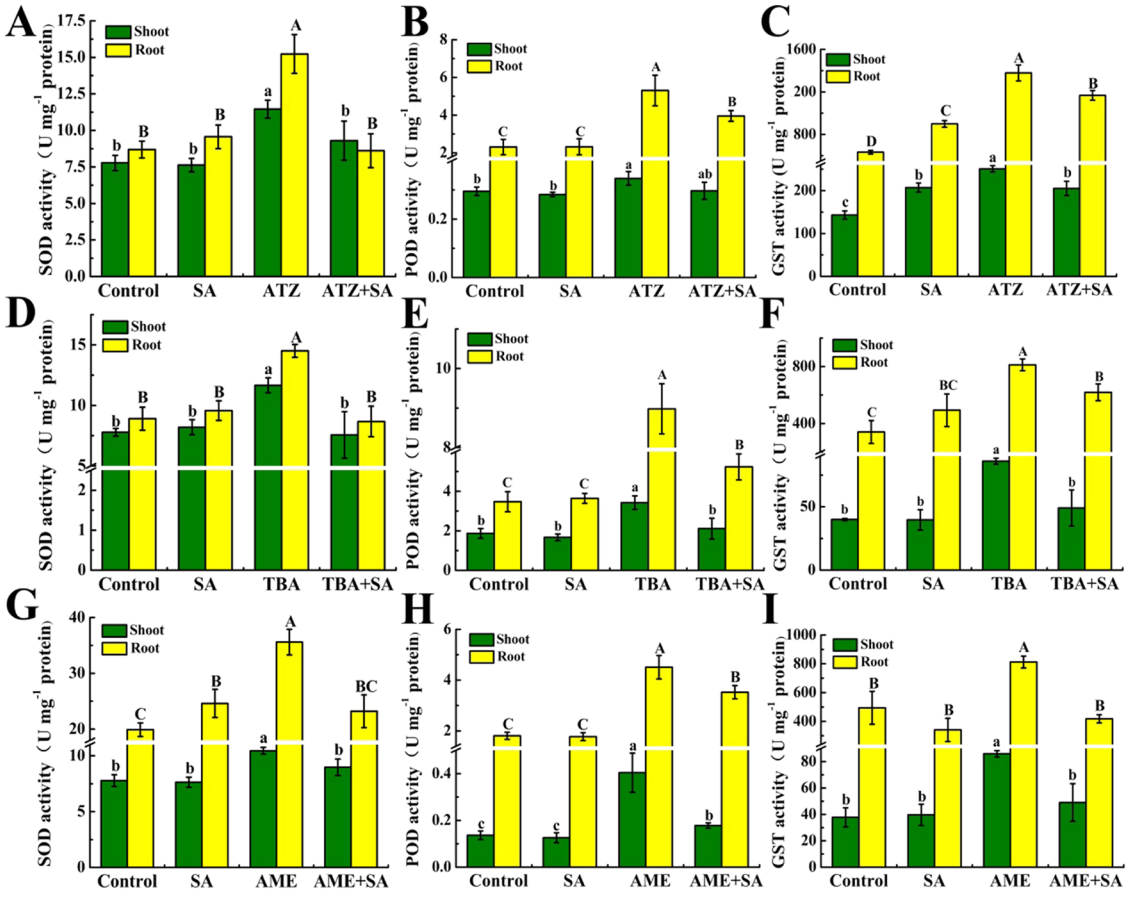

Figure 3. Effect of salicylic acid (SA) on the activities of SOD (A, D, G), POD (B, E, H), and GST (C, F, I) in maize under ATZ (A−C), TBA (D−F), and AME (G−I) exposure. Seedlings grew respectively in the soil with three herbicides (6 mg/kg) for 4 days. After that, the leaves were sprayed with 5 mg/L SA every day for 6 days. Values are means ± standard deviations (n = 3). Different letters indicate the significant difference between the treatments (p < 0.05).

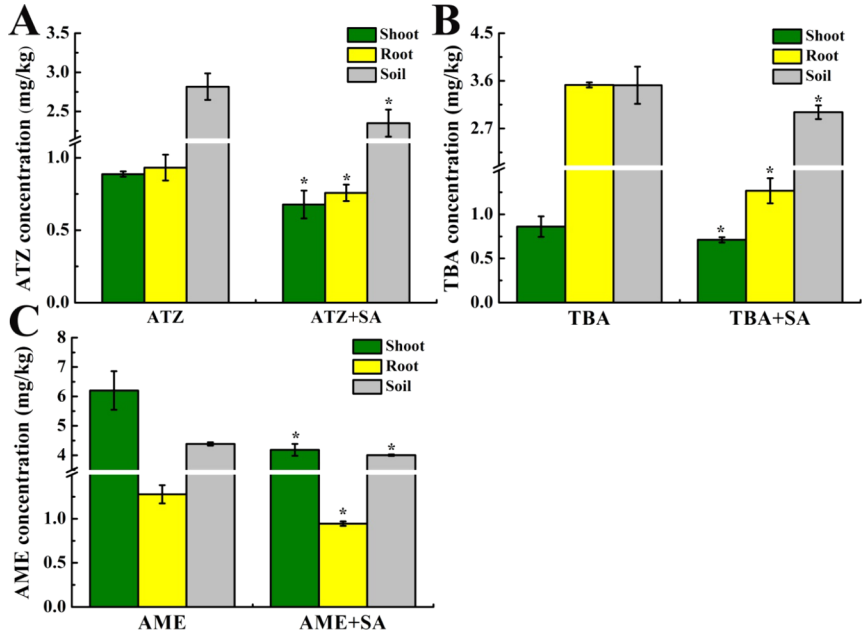

Figure 4. Effect of salicylic acid (SA) on the accumulation of ATZ (A), TBA (B), and AME (C) in maize and residues of three herbicides in the soil. Seedlings grew respectively in the soil with three herbicides (6 mg/kg) for 4 days. After that, the leaves were sprayed with 5 mg/L SA every day for 6 days. Values are means ± standard deviations (n = 3). Different letters indicate the significant difference between the treatments (p < 0.05).

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degradation of pesticides.[27] Therefore, GST can be also used to evaluate the accumulation of herbicides in maize and promotion of SA on the degradation process. As shown in Figure 3, there were the highest activities of SOD, POD, and GST in maize under exposure to three herbicides. The SOD activities in the shoot and root treated with AME were 14.1 and 34.8% higher than those treated with AME + SA, respectively (Figure 3G). The POD activities in the shoot and root treated with AME were 55.9 and 21.8% higher than those treated with AME + SA, respectively (Figure 3H). Under the stress of AME, the activity of GST in maize was greatly increased, and the activities of GST in the shoot and root were 2.27 and 1.64 times those of control, respectively (Figure 3I). These results indicate that spraying SA can mitigate the oxidative stress of herbicides to maize and increase the resistance of maize to the herbicides. Previous studies from our lab and others show that application of SA can promote the detoxification of pesticides such as isoproturon and propazine in Arabidopsis, rice, wheat, rapeseed, and other plant species.[19,20] The current study identified detoxification of three different pesticides in maize crops as affected by SA. Considering the different materials used, there is no evidence that allows us to infer the difference between the mechanisms for detoxification. Further research on identifying SA-regulated enzymes or proteins that specifically mediate the metabolism and degradation of the three different pesticides in maize will help figure out whether these studies with different pesticides share the same or different mechanisms.

SA Reduced the Accumulation of Triazine Herbicides in Maize and Soil. To investigate the reason of SA mitigation of the toxicity of triazine herbicides to maize, accumulation of three herbicides in maize with or without SA application was evaluated. Compared to the treatment without SA, the accumulation of ATZ in the shoot, root, and soil under SA treatment was reduced by 23.7, 18.7, and 16.6%, respectively (Figure 4A). The accumulation of TBA in the shoot and root under SA treatment was reduced by 17.4 and 64.0%, and the residue of TBA in the soil was reduced by 14.5% (Figure 4B). The same trend was observed under AME + SA treatment, with the concentration of AME being reduced by 32.5, 26.0, and 8.7% in the shoot, root, and soil, respectively (Figure 4C). The data indicated that SA can promote the degradation of three herbicides in maize and soil. Our previous studies have shown that disruption of the SA synthetic intermediate Ospal (phenylalanine ammonia lyase) results in drastically higher levels of the pesticide isoproturon accumulated in rice, while spraying exogenous SA can reverse the process; identification of genomic loci of isoproturon-exposed *Ospal* rice revealed that SA-mediated lower accumulation of the pesticide is associated with several degradation enzymes including cytochromes, glycosyltransferases, and ATP-binding cassettes, suggesting that SA functions in resist isoproturon phytotoxicity and degradation through activation of the phase I−III reaction pathway.[19,20] Although this study tested the maize with a group of different pesticides, it may be speculated that the regulatory mechanism works in a similar way. Meanwhile, the bioconcentration factor (BCF) was analyzed by calculating the percentage of herbicide concentrations that were allocated between the root and shoot tissues in maize.[23] The BCFs of the maize shoot and root under treatment of the herbicides and SA were significantly lower than those under only treatment of the herbicides (Table 1), confirming that the SA supply can decrease the accumulation of the three herbicides in maize tissues.

Table 1. Bioconcentration Factors (BCFs) for ATZ, TBA, and AME in the Shoot and Root of Maize[a,b]

		BCF	
herbicide	treatment	shoot	root
ATZ	ATZ	0.317 ± 0.036	0.334 ± 0.073
	ATZ + SA	0.293 ± 0.088	0.326 ± 0.068
TBA	TBA	0.251 ± 0.082	1.013 ± 0.162
	TBA + SA	0.237 ± 0.029	0.424 ± 0.093
AME	AME	1.416 ± 0.236	0.292 ± 0.038
	AME + SA	1.045 ± 0.081	0.236 ± 0.011

[a]Concentrations of three herbicides were determined in the root, shoot, and soil after maize exposure to herbicides at 6 mg/kg for 4 days. [b]BCF: herbicide concentrations in maize tissues over those in the soil.

Effects of SA on Degradation of Ametryn in Maize and Soil. To analyze the proposed metabolism and degradation pathway of triazine herbicides in maize and soil with or without SA, AME was taken as an example to characterize the metabolism and degradation products using a high-resolution liquid chromatography AB SCIEX Triple TOF 5600 mass spectrometer (LC-Triple TOF 5600 MS). No AME or SA treatment alone was used as a control. The mass spectrometry data (MS^2) of AME derivatives in maize and soil are summarized in Table 2. The structures of six metabolites and nine conjugates of AME were characterized by analyzing ion chromatograms and the MS^2 data generated by the collision-induced dissociation (CID) fragmentation mode (Figures S1−S3). It was found that there were five metabolites (Figure 5A) and eight conjugates in the maize shoot, wherein the conjugates m/z 388 and m/z 378 were detected only in the shoot (Figure 5B). There are five metabolites in the root (Figure 5C) and seven conjugates, of which m/z 415 was found only in the root (Figure 5D). Six metabolites were detected in the soil, where m/z 186 was identified only in the soil (Figure 5E).

After exogenous salicylic acid treatment, the residual concentrations of AME in the shoot, root, and soil were significantly reduced, while most of the metabolites and conjugates were significantly increased compared to the control group. In the shoot, the relative contents of AME metabolites m/z 212, m/z 200, and m/z 242 (Figure 5A) and six conjugates m/z 388, m/z 517, m/z 430, m/z 301, m/z 378, and m/z 406 with SA (Figure 5B) were significantly higher than those without SA (control). The relative content of AME metabolites and conjugates in the maize root had a similar tendency. Similarly, the relative content of three metabolites and five conjugates in the root of maize treated with salicylic acid was significantly higher than that of the control group (Figure 5C,D). Under AME exposure, the relative content of the six metabolites in the soil planted with maize with SA was higher than that in the control (soil planted with maize without SA) (Figure 5E). These results infer that the application of SA could mitigate the toxicity of AME to maize by facilitating the metabolism of AME in maize including the decrease in the AME concentration and promotion of the degradation of AME. On the basis of the identified metabolites and conjugates, the metabolism and degradation pathways of AME in maize and soil were proposed. AME was degraded

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Table 2. Summary of MS^2 Data for Ametryn (AME), Metabolites, and Conjugates Identified in Maize and Soil[a]

no.	acronym	chemical formula	t_R (min)	theor m/z, $[M + H]^+$	exptl m/z, $[M + H]^+$	delta (ppm)
1	IPU (isoproturon)	$C_{12}H_{18}N_2O$	7.26	207.1419	207.1117	0.5
2	AME (ametryn)	$C_9H_{17}N_5S$	4.39	228.1277	228.1275	−1
no.	metabolites	chemical formula	t_R (min)	theor m/z, $[M + H]^+$	exptl m/z, $[M + H]^+$	delta (ppm)
1	6-hydroxymethyl-AME	$C_9H_{17}N_5O$	3.67	212.1506	212.1504	−0.8
2	6-monodemethyl-AME	$C_8H_{15}N_5S$	3.37	214.1121	214.1120	−0.2
3	deisopropyl-AME	$C_6H_{11}N_5S$	3.54	186.0808	186.0807	−0.6
4	4,6-monodemethyl-AME	$C_7H_{13}N_5S$	3.54	200.0964	200.0962	−1.3
5	2-methyl-AME	$C_{10}H_{19}N_5S$	4.45	242.1433	242.1432	−0.7
6	4-OH-AME	$C_9H_{17}N_5OS$	4.11	244.1226	244.1225	−0.4
no.	conjugates	chemical formula	t_R (min)	theor m/z, $[M + H]^+$	exptl m/z, $[M + H]^+$	delta (ppm)
1	Cys/Ser *S*-monodemethyl-AME	$C_{14}H_{25}N_7O_4S$	3.17	388.1761	388.1760	−0.4
2	hmGSH *S*-hydroxymethyl-2-didesmethyl-AME	$C_{19}H_{32}N_8O_7S$	3.36	517.2187	517.2191	0.7
3	Cys/Glu-*S*-monodemethyl-AME	$C_{16}H_{27}N_7O_5S$	3.68	430.1867	430.1866	−0.1
4	GSH *S*-didesmethyl-AME	$C_{18}H_{30}N_8O_6S$	3.40	487.2081	487.2078	−0.8
5	Cys/Ser *S*-monodemethyl-deisopropyl-didesmethyl-AME	$C_9H_{15}N_7O_4S$	3.98	318.0979	318.0976	−0.8
6	Cys *S*-monodemethyl-AME	$C_{11}H_{20}N_6O_2S$	3.72	301.1441	301.1442	0.4
7	Cys/Asn *S*-monodemethyl-AME	$C_{15}H_{26}N_8O_4S$	10.88	415.1870	415.1872	0.4
8	3-OH-didesmethyl-AME-*O*-glucoside	$C_{13}H_{23}N_5O_6S$	2.97	378.1441	378.1438	−0.8
9	3-OH-AME-*O*-glucoside	$C_{15}H_{27}N_5O_6S$	3.35	406.1754	406.1751	−0.9

[a]Isoproturon, internal standard; t_R, retention time; theor m/z, theoretical m/z; exptl m/z, experimental m/z.

mainly through hydrolysis (−OH), dealkylation (−R), and demethylation ($-SCH_3$) in maize and soil. Meanwhile, the metabolites of AME were combined with glucose and amino acid to get their transformation and possibly make them easier to be degraded (Figure 6).

CONCLUSIONS

Three herbicides (TBA, AME, and ATZ) hindered the growth of maize, and the phenomenon was more obvious with the increased concentrations of herbicides. When maize was treated with 5 mg kg^{-1} exogenous SA in the presence of the herbicides, the plants grew better than those without SA treatment. The accumulation of three herbicides in the maize and maize-planted soil was reduced after SA spay. Furthermore, treatment with SA promoted the accumulation of the AME derivatives in maize and soil. These results allowed us to conclude that SA is able to accelerate the metabolic degradation of the herbicides in maize and soil.

EXPERIMENTAL SECTION

Materials and Treatments. ATZ (98% pure) was provided by Syngenta (Nantong China); TBA (98.2% pure) and AME (98.5% pure) were provided by Academy of Agricultural Sciences in Jiangsu, Nanjing, China. Salicylic acid is of analytical grade. Since very low concentrations of SA were used in the study, the SA treatment solution was prepared by directly dissolving the solid chemical in water. The pesticide-free soil sampled from the 0−20 cm surface layer at the experimental station of Nanjing Agricultural University was manually crumbled, air-dried, ground, and sieved through a 1−3 mm sieve prior to use. The major chemical properties of soil were as follows: organic carbon, 2.13%; total N, 1.26 g kg^{-1}; available P, 34.3 mg kg^{-1}; available K, 91.5 mg kg^{-1}; pH 7.6. The maize seeds (Jiangnan Huanuo) were sterilized with a 3% solution of H_2O_2, rinsed, and germinated on moist filter paper for 24 h. After germination, seedlings (8 per pot) were transferred to a plastic pot (1 L) with 1000 g of dried soils with 6 mg kg^{-1} of three triazine herbicides, respectively. When the third true leaf (3 days) of maize was well developed, the aerial parts of the plant were sprayed with 5 mg kg^{-1} SA once a day for 6 days. Meanwhile, plants sprayed with water were used as a control. Seedlings were grown in a chamber under controlled conditions (temperature, 30/25 °C; light/dark cycle, 14/10 h; light intensity, 300 μmol photons m^{-2} s^{-1}) and watered each day to retain 70% soil moisture. When harvested, shoots and roots of maize were individually sampled and immediately analyzed.

Measurement of Growth and Physiological Parameters. Elongation of roots and shoots was measured with a ruler. To determine the dry mass of maize, plant tissues were oven-dried at 70 °C for 72 h and weighed. The chlorophyll content in tissues was quantified according to the method of Song et al.[12] Fresh leaves (0.3 g) of maize were extracted with 5 mL of 80% acetone (80:20, v/v) for 48 h in the dark. The chlorophyll content was measured by reading the absorbance of the supernatant at 649 and 665 nm. The chlorophyll content was calculated according to the following formula: chlorophyll concentration (mg gFW^{-1}) = $[(6.10A_{665} + 20.04A_{649}) \times$ extract volume$]$/fresh weight × 1000.

The thiobarbituric acid reactive substance was measured.[12] Fresh tissues (roots or shoots) of maize (0.3 g) were extracted with 3 mL of 0.67% (w/v) trichloroacetic acid solution in an ice bath. The extracting solution was centrifuged at 12,000*g* for 30 min. The supernatant (2 mL) was mixed with 2 mL of a solution of 0.5% thiobarbituric acid and 20% trichloroacetic acid. The mixture was boiled for 30 min in a water bath, quickly cooled to room temperature, and centrifuged at 12,000*g* for 5 min. The absorbance of the supernatant was measured at 450, 532, and 600 nm. TBARS was calculated according to the following formulas:

$$\text{TBARS content } (\mu\text{mol L}^{-1}) = 6.45(A_{532} - A_{600}) - 0.56A_{450}$$

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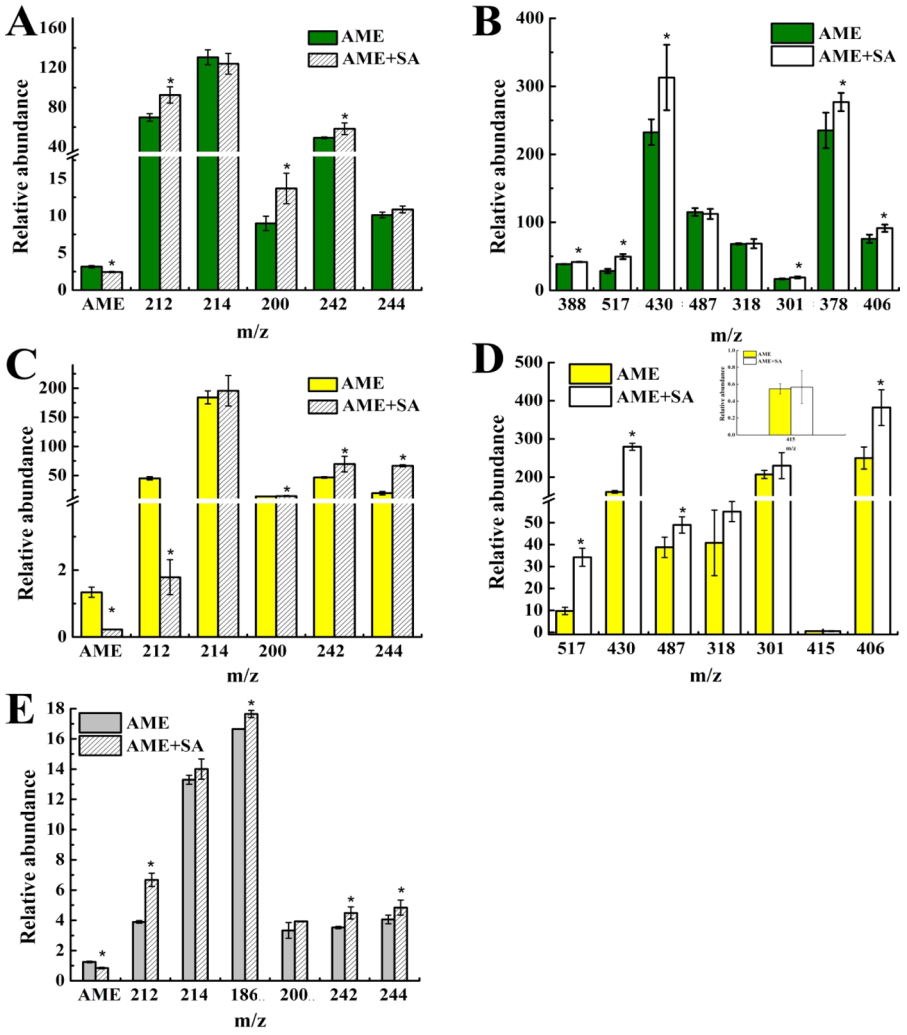

Figure 5. Relative content of AME derivatives in maize. AME-derived metabolites (A) and conjugates (B) in the shoot, metabolites (C) and conjugates (D) in the root, and AME metabolites (E) in the soil. Seedlings grew in the soil with AME (6 mg/kg) for 4 days. After that, the leaves were sprayed with 5 mg/L SA every day for 6 days. Values are means ± standard deviations (n = 3). Asterisks indicate the significant difference between the treatments (p < 0.05).

$$\text{TBARS content (nmol gFW}^{-1}) = (\text{MDA content (}\mu\text{mol L}^{-1}) \times 4\text{ mL})/\text{mass}$$

Assay of Enzyme Activities. The roots or shoots of fresh maize (0.3 g) were homogenized in 3 mL of precooled extraction buffer containing 50 mM Tris−HCl (pH 7.8), 1 mM ethylenediaminetetraacetic acid (EDTA), and 1.0% (w/w) polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12,000g at 4 °C for 20 min. The supernatant was used as a crude enzyme for the assay of enzyme activities.

The enzyme activity of SOD was measured using the method of nitrotetrazolium (NBT) photochemical reduction.[23] The amount of enzyme, which usually causes a 50% photochemical reduction inhibition rate, is defined as an enzyme activity unit (U). The mixture reaction system of 3 mL contained 30 μL of enzyme extract, 50 mM phosphate buffer (pH 7.8), 10 mM methionine, 1.17 mM riboflavin, and 56 mM NBT. The absorbance of mixture reaction solution was measured at 560 nm.

The mixture reaction solution (3 mL) contained 100 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 10 mM H_2O_2, and 50 μL of crude extract. The enzyme activity of POD in mixture reaction solution was determined at 25 °C for 5 min by the change in absorbance at 470 nm due to guaiacol oxidation.[28]

The enzyme activity of GST was assayed by the change in absorbance at 340 nm at 25 °C for 5 min.[12] The absorbance per minute was changed to 0.1 as the unit of enzyme activity. The mixture reaction system (3 mL) consisted of 2.85 mL of 100 mM PBS (pH 7.4), 45 μL of 3.3 mM reduced glutathione (GSH), 100 μL of crude enzyme solution, and 5 μL of 30 mM 1-chloro-2,4-dinitrobenzene (CDNB, soluble in ethanol). The reaction was started by the addition of CDNB. The protein concentration in the extracts of plants was determined by the dye-binding method according to Liang et al.[16]

Analysis of the Content of Three Herbicides in Maize and Soil. The plant tissues (2 g) and soil (5 g) were mixed with liquid nitrogen and extracted respectively with 10 mL of acetone/water solution (3:1, v/v) for 30 min under ultrasonic waves and centrifuged at 4000g for 8 min. The extraction process was repeated in triplicate. The supernatant was concentrated to remove acetone at 40 °C in a vacuum rotary evaporator. The residual water was loaded onto an LC-C18 solid phase extraction (SPE) column. The eluent was

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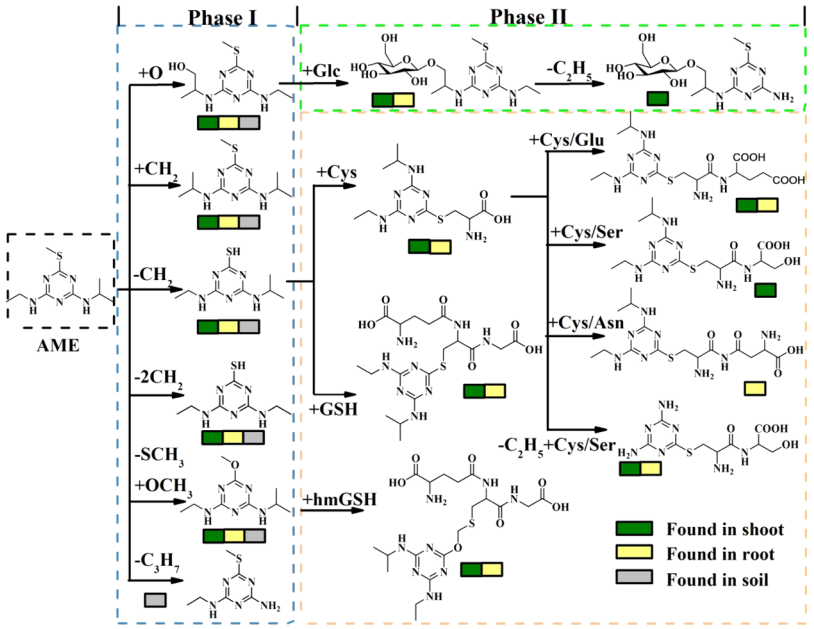

Figure 6. Proposed pathways of AME degradation in maize and soil.

discarded. The column was washed with 2 mL of methanol.[29] The washing solution was collected for high-performance liquid chromatography (HPLC) analysis (Waters 515, Waters Technologies Co. Ltd.). The extraction method of three herbicides from maize and soil was performed in the same way.

The content of three herbicides was measured respectively by HPLC under the following conditions: Hypersil reverse phase C18 column (Thermo, 250 mm × 4.6 mm); mobile phase: methanol/water (65:35, v/v); UV detector: 235 nm (ATZ), 225 nm (TBA), 225 nm (AME); flow rate, 0.6 mL/min; injection volume, 20 μL. The spiked recoveries of three triazine herbicides from soil and maize are displayed in Table S1.

Characterization of Degradation Products of Ametryn. The AME metabolites and conjugates in maize soil were determined by the Shimadzu LC 20ADXR LC system performed with an accelerator TOF analyzer equipped with an AB SCIEX Triple TOF 5600 mass spectrometer (LC-TOF-MS/MS). The autosampler temperature was set to 40 °C. The injection volume was 20 μL. Separation was performed on a Poroshell 120 EC-C18 column (50 mm × 2.1 mm, 2.7 μm, Thermo Fisher Scientific Co.). The mobile phase consisted of 0.1% aqueous formic acid (solvent A) and 0.1% formic acid in acetonitrile (solvent B) at a flow rate of 0.3 mL/min. The elution steps were set as follows: (1) 5% B for 1 min, 5 to 50% B for 1 to 3 min, 50 to 80% B for 3 to 8 min, 80 to 90% B for 8 to 13 min, 90 to 100% B for 13 up to 20 min, and 100% B for 10 min, (2) return to initial conditions, and (3) balance for 1 min before the next sample injection. The TOF-MS^2 parameters were as follows: an electrospray positive ion source (ESI^+) was used to monitor positive ions ($[M + H]^+$) of AME and its products; scanning range: m/z 100−800; atomization temperature: 300 °C; atomizing gas flow rate: 500 L h^{-1}; capillary voltage: 4000 V; cone voltage: 30 V.

Statistical Analysis. Each result shown in the figures and tables is the mean of three biological replicates. The values are expressed as means ± standard deviations. The data between different treatment groups were compared statistically by analysis of variance (ANOVA) followed by the least significant difference (LSD) test if the ANOVA result is significant at the $p < 0.05$ level. All data were calculated using the model procedure in SPSS 20.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c04315.

Supplemental Table S1: spiked recoveries of ametryn (AME), atrazine (ATZ), and terbuthylazine (TBA) in the soil, shoot, and root; Supplemental Figure S1: extracted ion chromatograms of metabolites and conjugates of ametryn (AME) in maize; Supplemental Figure S2: MS^2 spectra of AME metabolites; Supplemental Figure S3: MS^2 spectra of AME conjugates (PDF)

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Notes

The authors declare no competing financial interest.

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