

## UC Riverside

### UC Riverside Previously Published Works

**Title**

Acetaminophen detoxification in cucumber plants via induction of glutathione S-transferases.

**Permalink**

<https://escholarship.org/uc/item/39k4t8cq>

**Authors**

Sun, Chengliang  
Dudley, Stacia  
McGinnis, Michelle  
[et al.](#)

**Publication Date**

2019-02-01

**DOI**

10.1016/j.scitotenv.2018.08.346

Peer reviewed



# Acetaminophen detoxification in cucumber plants via induction of glutathione S-transferases

Chengliang Sun <sup>a,\*</sup>, Stacia Dudley <sup>a</sup>, Michelle McGinnis <sup>a</sup>, John Trumble <sup>b</sup>, Jay Gan <sup>a</sup>

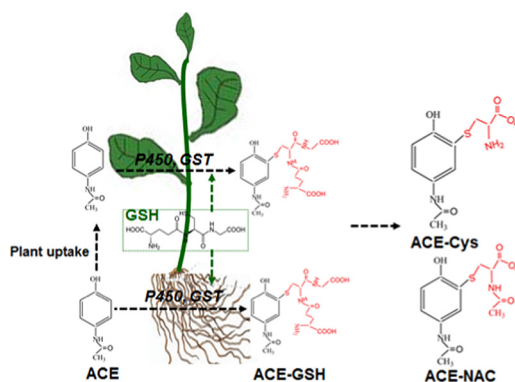
<sup>a</sup> Department of Environmental Sciences, University of California, Riverside, CA 92521, USA

<sup>b</sup> Department of Entomology, University of California, Riverside, CA 92521, USA

## HIGHLIGHTS

- Acetaminophen was taken up by plants and conjugated quickly with GSH;
- Activity of GST was significantly elevated after exposure to acetaminophen;
- The GSH conjugates were found to convert to cysteine and *N*-acetylcysteine conjugates;
- Enzymes involved in GSH regeneration were induced to maintain GSH homeostasis.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

### Article history:

Received 29 June 2018

Received in revised form 24 August 2018

Accepted 25 August 2018

Available online 27 August 2018

Editor: Yolanda Picó

### Keywords:

Acetaminophen

PPCPs

Glutathione S-transferases

Detoxification

Glutathione conjugation

## ABSTRACT

Many pharmaceutical and personal care products (PPCPs) enter agroecosystems during reuse of treated wastewater and biosolids, presenting potential impacts on plant development. Here, acetaminophen, one of the most-used pharmaceuticals, was used to explore roles of glutathione (GSH) conjugation in its biotransformation in crop plants. Acetaminophen was taken up by plants, and conjugated quickly with GSH. After exposure to 5 mg L<sup>-1</sup> acetaminophen for 144 h, GSH-acetaminophen conjugates were 15.2 ± 1.3 nmol g<sup>-1</sup> and 1.2 ± 0.1 nmol g<sup>-1</sup> in cucumber roots and leaves, respectively. Glutathione-acetaminophen was also observed in common bean, alfalfa, tomato, and wheat. Inhibition of cytochrome P450 decreased GSH conjugation. Moreover, the GSH conjugate was found to further convert to cysteine and *N*-acetylcysteine conjugates. Glutathione S-transferase activity was significantly elevated after exposure to acetaminophen, while levels of GSH decreased by 55.4% in roots after 48 h, followed by a gradual recovery thereafter. Enzymes involved in GSH synthesis, regeneration and transport were consistently induced to maintain the GSH homeostasis. Therefore, GST-mediated conjugation likely played a crucial role in minimizing phytotoxicity of acetaminophen and other PPCPs in plants.

© 2018 Elsevier B.V. All rights reserved.

## 1. Introduction

Climate change, continuing population growth, and urbanization are exerting an unprecedented pressure on fresh water supply, mandating the use of nontraditional water resources (Miller et al., 2016; Wu et al., 2014), such as treated municipal wastewater, for agricultural

\* Corresponding author.

E-mail address: [chengliang.sun@ucr.edu](mailto:chengliang.sun@ucr.edu) (C. Sun).

irrigation (Miller et al., 2016; Riemenschneider et al., 2017). Treated wastewater irrigation, however, poses potential risks because a multitude of trace contaminants, including numerous pharmaceutical and personal care products (PPCPs), are incompletely removed during wastewater treatment and may enter the soil-plant continuum (Lefevre et al., 2015; Malchi et al., 2014). Land application of biosolids and animal manure constitutes yet another route for such trace contaminants to enter agroecosystems (Bartrons and Peñuelas, 2017). Once in the agroecosystems, PPCPs may be translocated into edible plant parts and thus, enter the terrestrial food chains, including human diet. Consequently, plant accumulation of PPCPs is raising widespread concerns due to potentially deleterious effects on the environment and human health (Malchi et al., 2014; Nunes et al., 2014).

Studies over the past decade show that various PPCPs can be taken up from soil by plants (Carter et al., 2014). For instance, Wu et al. (2014) detected 16 PPCPs in edible tissues of eight common vegetables grown with treated wastewater irrigation. Additional studies showed that some PPCPs can pose significant phytotoxicity, leading to inhibition of plant growth (Bartrons and Peñuelas, 2017; Christou et al., 2016; Sun et al., 2018). For example, carbamazepine, an antiepileptic drug, displayed phytotoxic effects toward *Cucurbita pepo* at concentrations  $> 1 \text{ mg kg}^{-1}$  in soil (Carter et al., 2015). In contrast, other plant species appeared to be unaffected by PPCP exposure. For instance, no significant change in growth rate was noticed after *Phragmites australis* was exposed to  $60 \mu\text{g L}^{-1}$  ibuprofen (He et al., 2017). These findings imply that certain plants may have detoxification mechanisms contributing to their resilience to the stress of such xenobiotics. However, although an increasing amount of research has considered plant accumulation of PPCPs (Bartrons and Peñuelas, 2017; Malchi et al., 2014; Miller et al., 2016), little is known about the detoxification mechanisms of higher plants toward PPCPs.

A common pathway in most organisms for detoxifying xenobiotics is through glutathione (GSH) conjugation catalyzed by glutathione S-transferases (GSTs) (Bártíková et al., 2015; Coleman et al., 1997; Doty, 2008; Marrs, 1996). It has long been recognized that GSTs play an important role in normal cellular metabolism in plants (Marrs, 1996). In addition, GST mRNA responds quickly to exposure of xenobiotics (DeRidder et al., 2002), protecting the cell against oxidative stress (Marrs, 1996; Vontas et al., 2001) or chemical toxicity (Coleman et al., 1997). Previous research on xenobiotics such as herbicides shows that differences in GSH availability and in the portfolio of GST isoenzymes are associated with xenobiotic resistance (Coleman et al., 1997). Conjugation with various biomolecules was observed in plant metabolism of different PPCPs, including diclofenac (Huber et al., 2012; Fu et al., 2017a), ibuprofen (Marsik et al., 2017), triclosan (Macherius et al., 2012), benzotriazole (Lefevre et al., 2015) and acetaminophen (Bartha et al., 2010; Huber et al., 2009). However, GST-mediated conjugation may play a predominant role, but has been relatively understudied so far (Bartha et al., 2010). In particular, the entire process of GST-mediated detoxification, from GSH conjugation in the cytoplasm to vacuolar accumulation and processing of conjugates has yet to be elucidated in vivo.

Acetaminophen and structural analogs are phenolic compounds that are among ubiquitous environmental contaminants (Ashrap et al., 2017). Acetaminophen is one of the most used pain and fever relief medicines, and many billions of doses of acetaminophen are consumed each year (Crane et al., 2006; Huber et al., 2009). Acetaminophen was detected in the aquatic environment at  $0.01\text{--}0.3 \text{ mg L}^{-1}$  in the South Wales region of the UK (An et al., 2009; Kasprzyk-Hordern et al., 2008). Adverse effects of acetaminophen were also observed in fish and plants (Bartha et al., 2010; Kavitha et al., 2011). Cucumber is a crop plant, widely consumed, with high economic and ecological relevance, and recommended for use in phytotoxicity studies by U.S. Environmental Protection Agency (US EPA, 1996). In this study, we used acetaminophen to elucidate the mechanisms of GST-mediated detoxification in cucumber plants. Findings of this study may provide evidence

for PPCP detoxification via GSH conjugation, and will likely prompt further exploratory investigation on the role and value of this pathway for the numerous emerging contaminants.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Acetaminophen ( $\geq 99\%$ ) was purchased from Sigma-Aldrich (St. Louis, MO). Chemical characteristics of acetaminophen are shown in Table S1 in Supporting Information (SI). *d*<sub>4</sub>-Acetaminophen was obtained from C/D/N Isotopes (Pointe-Claire, Quebec, Canada). Standards of glutathione, cysteine and *N*-acetylcysteine conjugates of acetaminophen were acquired from Santa Cruz Biotechnology (Santa Cruz, CA). All other chemicals and reagents used in the enzyme activity measurement were of analytical grade or better (Text S1). Acetonitrile and methyl *tert*-butyl ether were of liquid chromatography grade (Fisher Scientific, Fair Lawn, NJ). Methanol and formic acid were Ultime grade (Fisher). Ultrapure water was prepared using a Milli-Q system (Millipore, Carrigtwohill, Cork, Ireland).

### 2.2. Plant culture and exposure experiments

Cucumber (*Cucumis sativus* L.) seeds were obtained from Fisher and transferred to a 72-hole plate filled with a mixture of vermiculite and perlite (3:1; v:v). Plants were cultivated under controlled conditions (12 h/25 °C day and 12 h/25 °C night regime; relative humidity of 75–80%; a light intensity of  $200 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) for 1 week and irrigated with half strength modified Hoagland nutrient solution, which is adequate for the seedlings' energy demands for cucumber growth and the low ionic strength nutrient solution permits a more precise computation of acetaminophen speciation than do more complete nutrient medium. Details on the solution composition are given in Table S2. Seedlings in the third-leaf stage were removed from the pots, rinsed with tap water, and placed in 500 mL glass jars (one plant per jar) containing aerated, half strength modified Hoagland nutrient solution. Each treatment contained three replicates, and each replicate was prepared from a single plant by separating into roots and shoots. The jars were covered with aluminum foil to prevent evaporation and exposure of roots to light. After 7 days of adaption, plants were exposed to acetaminophen by spiking 25  $\mu\text{L}$  stock solution ( $100 \text{ mg mL}^{-1}$  in methanol) to yield an initial concentration of  $5.0 \text{ mg L}^{-1}$ . The use of such a relatively high concentration was to facilitate metabolite identification. Simultaneously, treatment blanks (nutrient solution in jars without plants but with acetaminophen) and plant blanks (nutrient solution in jars with plants but without acetaminophen) were included in duplicate to determine abiotic losses of acetaminophen under the experimental conditions. All experimental materials were autoclaved, 121 °C for 30 min, before use.

A parallel group trial enrolled with acetaminophen at  $60 \mu\text{g L}^{-1}$  was included to simulate more realistic levels and to validate the high level treatment. The cultivation lasted for 20 days, with the nutrient solution renewed every other day (Fig. S1). Additionally, in order to determine if plants can detoxify acetaminophen via GSH conjugation in realistic field environment, cucumber seedlings were cultivated for 20 days in soil amended with 5% biosolids containing acetaminophen at an initial concentration of  $10 \text{ mg kg}^{-1}$  (Fig. S1). Cucumber roots were collected and freeze-dried for acetaminophen-GSH analysis.

A root exudate control was included to evaluate the effect of plant exudates on microbial degradation and GSH conjugation of acetaminophen in the hydroponic solution. Cucumber seedlings were cultivated in 500 mL glass jars containing aerated, half strength modified Hoagland nutrient solution, and the plants were removed after 7 days. The solution was brought back to 500 mL with half strength modified Hoagland nutrient solution, and then 25  $\mu\text{L}$  of acetaminophen stock solution was added to yield an initial concentration of  $5.0 \text{ mg L}^{-1}$ . The nutrient

solution samples were exposed to the same conditions, and were analyzed for acetaminophen and its conjugates.

To investigate whether GSH-acetaminophen can be transported from the root to the shoot, two-week-old cucumber plants were exposed to  $1 \text{ mg L}^{-1}$  GSH-acetaminophen for 2 days. Leaves were collected and extracts from the aerial parts were analyzed as described below.

To test whether cytochrome P450 was involved in GSH-conjugates formation, two P450 inhibitors, 1-aminobenzotriazole and piperonyl butoxide, were used to pretreat the plant on the basis of previous studies demonstrated specific inhibition effects (Kaspar et al., 2011; Chen et al., 2018). Cucumber seedlings were pretreated with 1-aminobenzotriazole (1 mM) or piperonyl butoxide (250  $\mu\text{M}$ ) for 12 h in 500 mL glass jars with aerated, half strength modified Hoagland nutrient solution, followed by spiking 25  $\mu\text{L}$  of acetaminophen stock solution to yield an initial concentration of  $5.0 \text{ mg L}^{-1}$ . After 96 h incubation, tissue samples were collected from both the treated and untreated plants. Extracts from the tissues were analyzed for GSH-conjugates. The specificity of these P450 inhibitors was evaluated by monitoring cytochrome P450 activity, GSH content and GST activity in cucumber tissues after the treatment. For better clarity, all the experiments performed was schematically represented Table S3.

Simultaneously, commercially available common bean (*Phaseolus vulgaris* L.), tomato (*Solanum lycopersicum* L.), alfalfa (*Medicago sativa* L.) and wheat (*Triticum aestivum* L.) seeds were germinated and transplanted under the same conditions as above. After 7 days of adaptation, plants were exposed to acetaminophen at  $5.0 \text{ mg L}^{-1}$ . After 96 h, plant roots were collected and analyzed for acetaminophen and its glutathione, cysteine and *N*-acetylcysteine conjugates.

#### Sampling, sample preparation and analysis

Cucumber seedlings treated with or without  $5 \text{ mg L}^{-1}$  acetaminophen were sacrificed at 0, 12, 24, 48, 72, 96, 120 and 144 h. Before sample preparation, roots were rinsed thoroughly with tap water. Harvested tissues were separated into roots and shoots and then frozen in liquid nitrogen. Enzyme activities of GST, glutathione reductase (GR),  $\gamma$ -glutamylcysteine synthetase ( $\gamma$ -ECS), and cytochrome P450, as well as contents of reduced glutathione in both roots and shoots, and lipid peroxidation were immediately analyzed after sampling, as described below. The remaining plant samples were freeze-dried and stored at  $-80 \text{ }^\circ\text{C}$  until chemical analysis.

At each sampling time, nutrient solution samples and the aqueous root exudate samples were collected and mixed with equal volumes of methanol. The mixture was added with  $d_4$ -acetaminophen as a surrogate, filtered through a PTFE syringe filter (0.2  $\mu\text{m}$ , Millipore, Carrigtwohill, Cork, Ireland) and stored at  $-20 \text{ }^\circ\text{C}$  before analysis.

To quantify acetaminophen and its glutathione, cysteine and *N*-acetylcysteine conjugates, the freeze-dried plant tissue samples were ground to a fine powder with a mortar and pestle, and a 0.5-g aliquot was used for solvent extraction. Each sample was spiked with  $d_4$ -acetaminophen as the recovery surrogate, and extracted, sequentially, with 20 mL methyl *tert*-butyl ether (MTBE), acetonitrile, and 0.5 M HCl solution in an ultrasonic water bath for 20 min for each extraction. The supernatant from MTBE and acetonitrile extraction was combined after centrifugation (15 min at 8000g) and reconstituted in 1.0 mL methanol after drying under nitrogen ( $\text{N}_2$ ). The extract was pooled with the supernatant from the HCl extraction. The pooled extract was loaded onto an Oasis<sup>TM</sup> HLB cartridge (150 mg, Waters, Milford, MA) that was preconditioned with 6 mL methanol and 12 mL deionized water, and eluted with 15 mL methanol under gravity. The eluate was evaporated to dryness under  $\text{N}_2$ , and the residue was recovered in 1.5 mL methanol:water mixture (1:1, v:v) and filtered (PTFE, 0.2  $\mu\text{m}$ ) before analysis.

The instrumental analysis was performed on a Waters ACQUITY ultra-performance liquid chromatography (UPLC) coupled to a Waters Micromass Triple Quadrupole mass spectrometer (QqQ) equipped with an electrospray ionization (ESI) interface (Waters, Milford, MA).

Acetaminophen, and its glutathione, cysteine and *N*-acetylcysteine conjugates were separated using ACQUITY UPLC BEH C18 column (2.1 mm  $\times$  100 mm, 1.7  $\mu\text{m}$  particle size, Waters) with water (containing 0.2% formic acid) and methanol as mobile phases. Electrospray ionization was operated in the positive mode. Detailed information on the instrumental analysis is given in Table S4.

### 2.3. Assays of enzyme activity, glutathione and lipid peroxidation

Fresh cucumber roots (0.5 g) were frozen in liquid nitrogen, and homogenized on ice with 5 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% polyvinylpyrrolidone. After centrifugation at 15,000 g at  $4 \text{ }^\circ\text{C}$  for 20 min, the supernatant was used for the assay of activities of glutathione S-transferase, glutathione reductase, and cytochrome P450 (Text S2).

Measurement of GST (EC 2.5.1.18) activity was carried out spectrophotometrically after the glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) adduct synthesis reaction (Gonzalez et al., 2010). Briefly, GST activity was measured in 2 mL of a reaction mixture containing 50 mM PBS (pH 7.0), 1 mM CDNB, 5 mM GSH and 100  $\mu\text{L}$  enzyme extract. The increase in absorbance as a result of GSH-CDNB synthesis was determined at 340 nm for 5 min on a Cary 50 UV-Visible spectrophotometer (Varian, Palo Alto, CA). The GST activity was calculated using the extinction coefficient of GSH-CDNB ( $\epsilon = 9.6 \text{ mM}^{-1} \text{ cm}^{-1}$ ).

To determine the activity of GR (EC 1.6.4.2), a 200  $\mu\text{L}$  aliquot of enzyme extract was added to the reaction mixture of 0.15 mM NADPH and 0.5 mM oxidized glutathione (GSSG) in 50 mM PBS (Sun et al., 2014). The absorbance decrease was monitored at 340 nm for 3 min after GSSG-dependent consumption of NADPH ( $\epsilon = 6.22 \text{ mM}^{-1} \text{ cm}^{-1}$ ).

For the assay of  $\gamma$ -ECS (EC 6.3.2.2), plant tissues (0.5 g) were homogenized and suspended in 5 mL of 0.1 M HCl. After centrifugation at 20,000 g for 10 min at  $4 \text{ }^\circ\text{C}$ , the supernatant was collected. The  $\gamma$ -ECS activity was measured according to Shan and Liang (Shan and Liang, 2010). An incubation solution was prepared with 800  $\mu\text{L}$  50 mM Tris-HCl (pH 7.6) containing 0.25 mM glutamate, 10 mM ATP, 1 mM dithioerythritol, 2 mM cysteine, and 400  $\mu\text{L}$  of the  $\gamma$ -ECS sample. The mixed solution was incubated for 1 h at  $25 \text{ }^\circ\text{C}$ , and then 800  $\mu\text{L}$  phosphorus agent (to detect cysteine-dependently generated  $\text{PO}_4^{3-}$  in the reaction; containing 3 mM  $\text{H}_2\text{SO}_4$ , distilled water, 2.5% ammonium molybdate and 10% ascorbic acid) was added and mixed. After incubation at  $45 \text{ }^\circ\text{C}$  for 25 min, the absorbance at 660 nm was measured on the UV-Visible spectrophotometer. One unit of  $\gamma$ -ECS activity was defined as 1 mmol cysteine-dependently generated  $\text{PO}_4^{3-}$  per minute.

The levels of proteins in the enzyme extracts were determined using Coomassie Brilliant Blue G-250 (Zhou et al., 2005). Briefly, 100  $\mu\text{L}$  of enzyme extract was added to 5 mL of 0.01% Coomassie Brilliant Blue G-250. After 5 min, the absorbance at 595 nm was measured on the UV-Visible spectrophotometer. The protein content of enzyme extracts was calculated by comparison with a standard curve using bovine serum albumin (BSA) as the standard. The protein concentrations in the extracts were estimated using the BSA standard curve.

The content of GSH was determined spectrophotometrically according to Jiang et al. (2012). Plant tissues were homogenized in 5 mL of cold 5% meta-phosphoric acid on ice and centrifuged at 12,000 g at  $4 \text{ }^\circ\text{C}$  for 15 min, and the supernatant was analyzed for GSH. To 0.5 mL of supernatant, 0.5 mL PBS (100 mM; pH 7.0) and 0.5 mL of 5'-dithiobis-2-nitrobenzoic acid (DTNB, dissolved in PBS) were added. After thorough mixing and incubation for 5 min, the absorbance at 412 nm was measured ( $\epsilon = 1.3 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ ).

Membrane lipid peroxidation, a typical indicator of stress phytotoxicity (Sun et al., 2014), was estimated by measuring the concentration of malondialdehyde, according to the reaction with thiobarbituric acid as described in Sun et al. (2018).

## 2.4. Quality control and quality assurance

All treatments were performed in triplicates. A procedural blank and a sample duplicate were included in every batch of 10 samples to monitor background contamination and reproducibility, and the calculated relative standard deviations were <10%. The  $d_4$ -Acetaminophen was used to account for any loss during sample preparation, matrix-induced ionization effects, and variations in instrumental response. Authentic standards were used to confirm the target analytes. A six-point calibration line was used for quantification with the  $r^2$  values of at least 0.99 for all analytes (Table S5). No acetaminophen or any of the conjugates was detected in the solvent or treatment blanks. Limits of detection (LOD) and quantification (LOQ) for individual acetaminophen and the conjugates were calculated as 3 and 10 times the signal-to-noise level from the low-level spiked samples ( $n = 3$ ; Table S6). The recoveries in all samples were within acceptable limits ranging from 75 to 110%, demonstrating good method accuracy and precision. Extraction efficiency was >91% for acetaminophen and its conjugates. The plant position was regularly rearranged every 2 days to avoid side-effects.

## 2.5. Statistical analysis

Data were statistically analyzed using the SPSS package (version 11.0; SPSS, Chicago, IL). The mean and standard deviation (SD) of each treatment were calculated. Statistical differences in enzymes activities and glutathione content between treated and untreated plants (at the same sampling points) were performed using  $t$ -test, and the differences in content of acetaminophen-GSH among different treatments were performed by the analysis of variance (one-way ANOVA) with Duncan post-hoc ( $P < 0.05$  and  $P < 0.01$ ).

## 3. Results and discussion

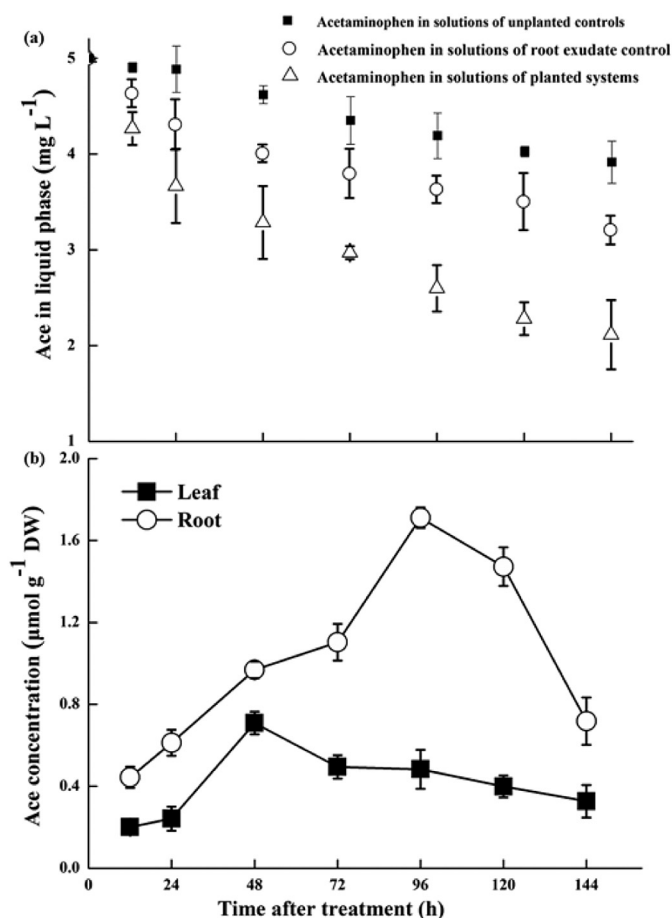
### 3.1. Phytotoxicity and uptake of acetaminophen by cucumber plant

#### 3.1.1. Concentration-dependent phytotoxicity of acetaminophen to cucumber plants

The biomass of both leaves and roots significantly decreased after 7 days when the concentration of acetaminophen was higher than  $10 \text{ mg L}^{-1}$  ( $P < 0.05$ ; Table S7). However, the lipid peroxidation increased by 30.6–92.3% in various plant tissues after exposure to  $5 \text{ mg L}^{-1}$  acetaminophen ( $P < 0.05$ ; Fig. S2); while no significant difference in biomass was observed between the treated and untreated plants at this exposure level. Our results were in agreement with that reported for other plant species (An et al., 2009; Bartha et al., 2010), suggesting that plants may be susceptible to phytotoxicity when exposed to acetaminophen at high levels.

#### 3.1.2. Dissipation of Acetaminophen in culture medium

The level of acetaminophen in the nutrient solution without plant, after the removal of plant (i.e., the root exudate control), or with cucumber plant, all decreased over time (Fig. 1a). Significant losses occurred in the unplanted control or the root exudate control ( $P < 0.05$ ). Considering that the cultivation system was not a sterile environment, both microbial and abiotic degradation may have contributed to the apparent acetaminophen dissipation in the solutions. This observation was consistent with Bartha et al. (2010), who also reported the occurrence of plant-independent, biotic processes that decreased the available acetaminophen in plant solution. Compared to the unplanted controls, however, acetaminophen in the growth media with plant disappeared significantly faster ( $P < 0.05$ ). For example, the average concentration of acetaminophen in the medium with plant decreased from  $5.0 \text{ mg L}^{-1}$  at the beginning to  $3.3 \pm 0.3 \text{ mg L}^{-1}$  at 48 h, and further to  $2.1 \pm 0.4 \text{ mg L}^{-1}$  at the end of cultivation (Fig. 1a). The significant differences ( $P < 0.05$ ) in acetaminophen concentration between the



**Fig. 1.** Time-dependent concentrations of acetaminophen in plant medium and tissues. (a) Solution with no plant control (filled square, ■), solution with root exudates (open circle, ○), and solution with cucumber plants (open triangle, △). (b) Concentrations of acetaminophen in cucumber leaves (filled square, ■) and roots (open circle, ○). The values shown are mean  $\pm$  SD ( $n = 3$ ). Ace, acetaminophen; DW, dry weight.

treatments suggested that acetaminophen was taken up from the nutrient solution into the plant.

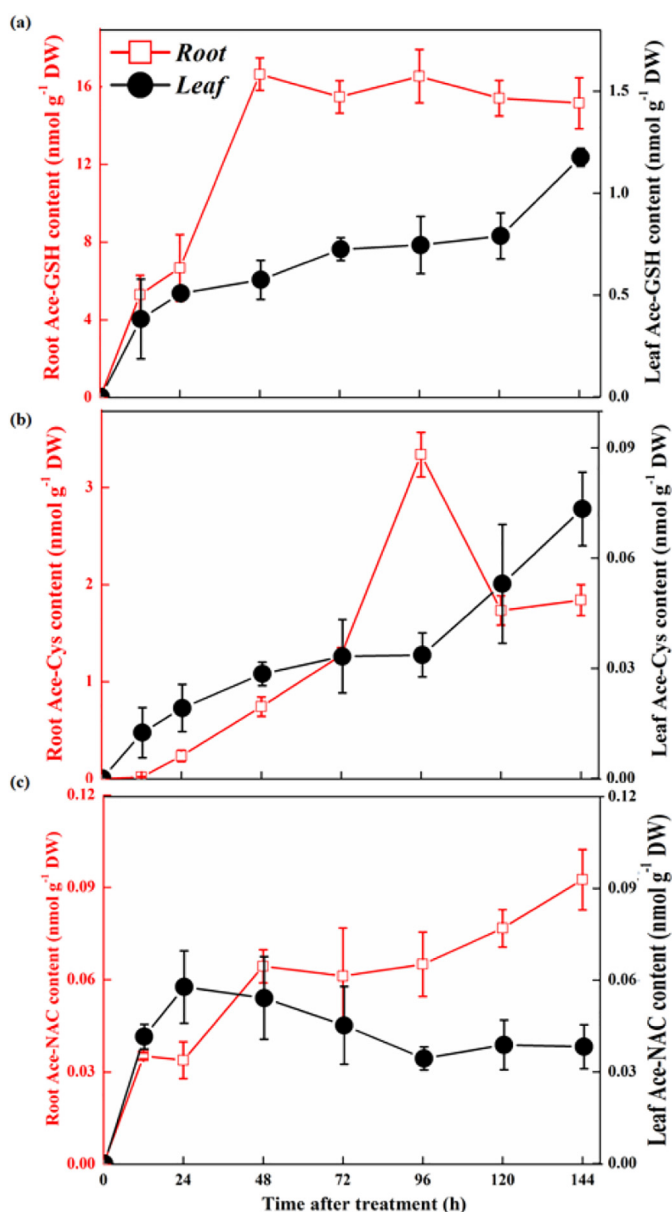
#### 3.1.3. Accumulation and translocation of acetaminophen in plants

Time-dependent concentrations of acetaminophen in cucumber roots and leaves are depicted in Fig. 1b. During the short growth period, acetaminophen was detected in both roots and leaves. The accumulation of acetaminophen in leaves suggested its acropetal translocation (from the root upward to aboveground) in the cucumber seedlings. However, acetaminophen concentrations in roots were 1.2–5.0 times higher than those in leaves. For example, after 96 h,  $1.7 \pm 0.1 \mu\text{mol g}^{-1}$  (dry weight) and  $0.5 \pm 0.1 \mu\text{mol g}^{-1}$  acetaminophen were detected in roots and leaves, respectively (Fig. 1b). The relatively lower levels of acetaminophen in leaves pointed to moderate transport to the aboveground and likely different transformation rates in these tissues. The level of acetaminophen reached the maxima at 96 h in roots and 48 h in the leaves, and a significant decrease was observed in both tissues thereafter ( $P < 0.05$ ), indicating active biotransformations in plant cells or release of parent and conjugated compounds from plant roots to the bathing solution. In plant tissues without exposure to acetaminophen, neither acetaminophen nor GSH conjugates were detected, indicating absence of cross contamination.

### 3.2. Formation of GSH-conjugated metabolites

In plants, GSH conjugates have been extensively studied because of their role in detoxification and selectivity of herbicides, such as

sulfonylureas (Brown and Neighbors, 1987), triazines (Guddewar and Dauterman, 1979) and chloroacetanilides (Field and Thurman, 1996). In comparison, only sporadic studies have considered GSH conjugation of other contaminants in plants. Detoxification via GSH conjugation was recently reported for the antibiotic chlortetracycline in plant cells (Farkas et al., 2007). In the present study, the concentration of GSH-acetaminophen conjugate in cucumber roots after 48 h was  $1.7 \pm 0.8 \text{ nmol g}^{-1}$ , and it remained relatively constant thereafter (Fig. 2a). In comparison, the level of GSH-acetaminophen in leaves continued to increase during the exposure and reached  $1.2 \pm 0.1 \text{ nmol g}^{-1}$  at the end of experiment (Fig. 2a). The relative differences between roots and leaves also suggested that roots were the main location for acetaminophen detoxification. This observation was in agreement with a previous study where extensive transformation of acetaminophen was also found in the root hairy cell culture of *Armoracia rusticana* (Huber et al., 2009).

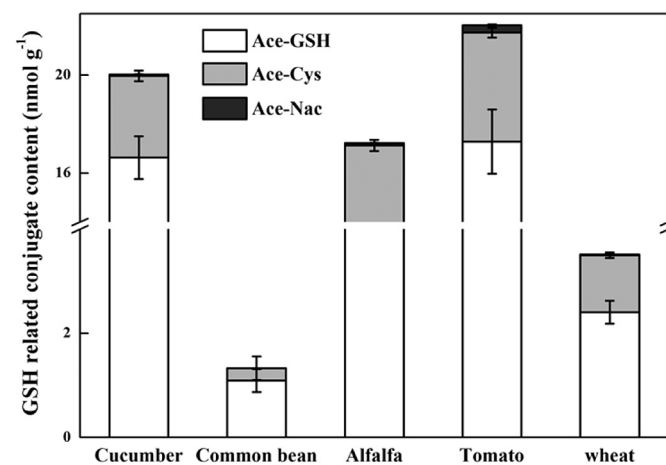


**Fig. 2.** Time-dependent concentrations of glutathione-related metabolites of acetaminophen in cucumber plants. (a) Acetaminophen-glutathione (Ace-GSH) in leaves (filled circle, ●) and roots (open square, □); (b) Acetaminophen-cysteine conjugate (Ace-Cys) in leaves (filled circle, ●) and roots (open square, □); and (c) Acetaminophen-*N*-acetylcysteine conjugate (Ace-NAC) in leaves (filled circle, ●) and roots (open square, □). The values shown are mean  $\pm$  SD ( $n = 3$ ). DW, dry weight.

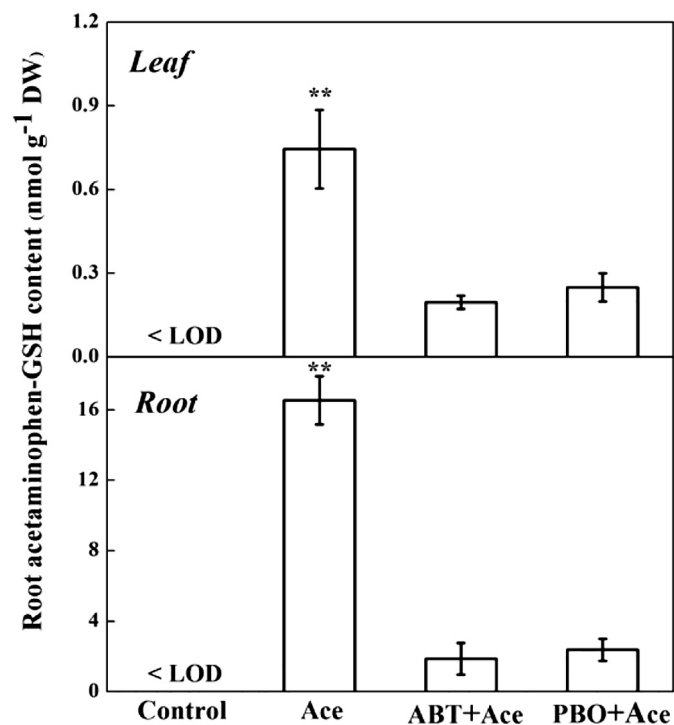
In the follow-up experiments, GSH-acetaminophen conjugate was also found in the roots of common bean, alfalfa, tomato, and wheat plants (Fig. 3). The ability to carry out GSH conjugation appeared to vary among plant species, with the overall level of GSH-conjugates following an order of tomato, cucumber, alfalfa > wheat > common bean ( $P < 0.05$ ). The differences in the conjugation of acetaminophen by GSH among plant species may be attributed to differences in GSH availability and the portfolio of GST isoenzymes, differences in Phase I reactions, or the involvement of other Phase II metabolism pathways. It must be noted that the commercial cultivars used as genotype may make a difference to the present results. Meanwhile, in all acetaminophen ( $5 \text{ mg L}^{-1}$ ) treatment crops, elevated lipid peroxidation was observed, with the damage being less pronounced in cucumber, tomato and alfalfa tissues (Fig. S2). Combining with the levels of GSH conjugates, this observation implied that the different tolerance to acetaminophen among different plant species may be due to their differences in GSH conjugation ability.

When cucumber plants were cultivated in nutrient solution containing acetaminophen at the lower level ( $60 \mu\text{g L}^{-1}$ ) or in soil amended with acetaminophen-containing biosolids, GSH-acetaminophen conjugate was also detected, even though the levels were generally lower (Fig. S1). This observation suggested that direct conjugation of acetaminophen with GSH in cucumber plants occurred under environmentally relevant conditions.

In mammalian systems, acetaminophen is oxidized by cytochrome P450 and peroxidases to a reactive quinone-like compound (*N*-acetyl-*para*-benzoquinone imine) (Potter and Hinson, 1987), which can then undergo GSH conjugation by GSTs. In the current study, it was noted that in cucumber leaves and roots, the total activity of cytochrome P450 ( $P < 0.05$ ; Fig. S3), but not GSH content or GST activity ( $P > 0.05$ ; SI Fig. S4), was significantly inhibited by 1-aminobenzotriazole or piperonyl butoxide, two specific cytochrome P450 inhibitors. With the pretreatment of 1-aminobenzotriazole or piperonyl butoxide, levels of GSH-acetaminophen decreased from  $0.7 \pm 0.1 \text{ nmol g}^{-1}$  to  $0.2 \pm 0.1$  or  $0.3 \pm 0.1 \text{ nmol g}^{-1}$ , and from  $16.5 \pm 1.4 \text{ nmol g}^{-1}$  to  $1.9 \pm 0.9$  and  $2.4 \pm 0.6 \text{ nmol g}^{-1}$  in cucumber leaves and roots, respectively (Fig. 4). These results clearly suggested that cytochrome P450 played a role prior to GSH-acetaminophen conjugation. However, there was only a slight difference in P450 activities between cucumber and common bean ( $P > 0.05$ ; Fig. S5); whereas cucumber displayed significantly higher GST activity than common bean ( $P < 0.01$ ; Fig. S5). It is therefore likely that different activities of GST also contributed to the different levels of acetaminophen-GSH conjugates. The results clearly showed that GSH-mediated detoxification of acetaminophen in plants differed from the known detoxification reactions of chlorinated herbicides,



**Fig. 3.** Concentrations of glutathione, cysteine and *N*-acetylcysteine conjugates of acetaminophen in roots of cucumber, common bean, alfalfa, tomato and wheat after 96 h exposure to  $5 \text{ mg L}^{-1}$  acetaminophen.



**Fig. 4.** Effects of cytochrome P450 inhibitors on glutathione conjugation of acetaminophen in cucumber roots. Cucumber seedlings were pretreated with 1-aminobenzotriazole (1 mM) or piperonyl butoxide (250  $\mu$ M) for 12 h, followed by exposure to 5.0 mg L<sup>-1</sup> acetaminophen for 96 h. The values shown are mean  $\pm$  SD (n = 3). \*\*Indicates significant difference between treatments at  $P < 0.01$ .

such as chlorimuron ethyl (Brown and Neighbors, 1987), alachlor (Field and Thurman, 1996), and metolachlor (Cottingham and Hatzios, 1992). For these chlorinated herbicides, conjugation occurs via a nucleophilic substitution of chlorine by the thiol residue of GSH, forming a polar GSH-conjugate. For acetaminophen, however, the potential site of GSH conjugation may involve alkyl groups (C=C) and result in the ring opening of acetaminophen. A recent study on ibuprofen metabolism in *Phragmites australis* also suggested that ibuprofen was not directly amenable to Phase II conjugation; rather, it needed to be first functionalized via Phase I activation (He et al., 2017). These findings together indicated that conjugation with GSH is a common and important detoxification pathway for xenobiotics of different structures in higher plants. For PPCPs such as acetaminophen, Phase I enzymes may be involved to create favorable reactive sites prior to GSH conjugation.

### 3.3. Fate of glutathione conjugates in plant tissues

Generally, conjugation to GSH occurs in the cytosol, but the accumulation of the conjugates in this compartment may also confer toxicological effects (Marrs, 1996). For example, the conjugate may block GST activity (i.e., product inhibition), leading to an accumulation of unconjugated electrophiles in plant cells, or they may encounter cytosolic enzymes (Coleman et al., 1997), which could result in conversion to toxic metabolites. It is generally assumed that the vacuole serves as a site for the deposition of xenobiotic conjugates in plants and fungi (Ghosh et al., 1999; Sandermann Jr, 1992; Tong et al., 2004; Verbruggen et al., 2009). Several studies have shown that GSH-conjugated arsenite was sequestered in the vacuole (Marrs, 1996; Tong et al., 2004). However, little is known about the biological mechanism underpinning GSH conjugates of organic contaminants across tonoplast. Although vacuole was not collected in this study, GSH-acetaminophen conjugate, however, was not detected in the xylem sap of cucumber exposed to acetaminophen (5.0 mg L<sup>-1</sup>). Furthermore, GSH-acetaminophen conjugates were also not detected in the

aboveground tissues of cucumber exposed to GSH-acetaminophen (1.0 mg L<sup>-1</sup>; Fig. S6). These results indicated that the parent compound was not metabolized in the xylem or GSH conjugate produced in the roots was not transported to the aboveground. However, all GSH-related conjugates were detected in the nutrient solution, ranging from 22.7  $\pm$  4.9 nmol L<sup>-1</sup> to 44.6  $\pm$  5.2 nmol L<sup>-1</sup> after 5 mg L<sup>-1</sup> acetaminophen exposure (Table S8), suggesting release of GSH-related conjugates from plant roots to the bathing solution. A similar phenomenon where plants released conjugated metabolites was previously observed for triclosan (Macherius et al., 2012), benzotriazole (Lefevre et al., 2015) and 2-mercaptobenzothiazole (Lefevre et al., 2015). Additionally, microorganisms in the growth medium could also convert acetaminophen to GSH conjugates, although this possibility was not explored in this study.

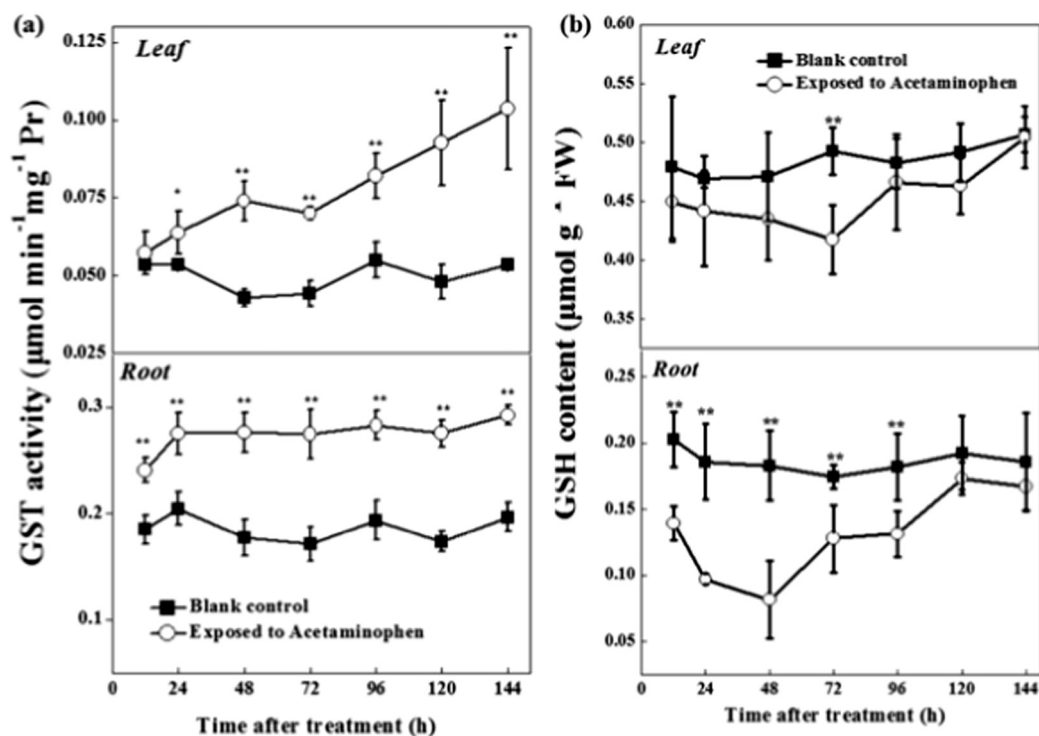
Glutathione conjugates produced in plant cells may undergo further transformations to yield cysteine conjugates, as previously shown for animals (Brüsewitz et al., 1997). Most herbicide-GSH conjugates in plants were rapidly metabolized by dipeptidases to remove the glycine residue of GSH to form cysteine conjugates, which could further yield N-acetylcysteine conjugates (Marrs, 1996). In this study, cysteine and N-acetylcysteine conjugates (chromatograms shown in Fig. S7) of acetaminophen were detected in both roots and leaves of cucumber, and the levels in roots were about 3.3  $\pm$  0.2 and 0.07  $\pm$  0.01 nmol g<sup>-1</sup>, respectively, after exposure to 5 mg L<sup>-1</sup> acetaminophen for 96 h (Fig. 2b&c). Glutathione-acetaminophen derived cysteine and N-acetylcysteine conjugates were also detected in roots of common bean, alfalfa, tomato, and wheat plants (Fig. 3), with levels ranging from 0.2  $\pm$  0.1 to 4.5  $\pm$  0.5 nmol g<sup>-1</sup> and from 0.02  $\pm$  0.01 to 0.3  $\pm$  0.09 nmol g<sup>-1</sup>, respectively. The amount of acetaminophen conjugates tended to decrease after 48 h and 96, in leaves and roots, respectively (Fig. S8), indicating that some conjugates were converted further or the conjugated metabolites were released back to the medium.

The content of acetaminophen-cysteine conjugate in leaves was consistently much smaller than that in roots throughout the experiment. Previous studies showed that the generated cysteine or N-acetylcysteine metabolites were exported to the apoplast, where they may be bound to lignin and cellulose (Marrs, 1996). Several metabolites of cysteine conjugates have been identified in plant cells (Fu et al., 2017b), although the precise fate and route of metabolism of cysteine conjugates is still uncertain. Further research is needed to understand the efflux of cysteine conjugates from the vacuole.

### 3.4. Acetaminophen-induced GST activity and changes in GSH homeostasis

Conjugation of xenobiotics with GSH is mediated by enzymes that belong to the superfamily of GSTs, which were previously shown to respond quickly to xenobiotics (Brüsewitz et al., 1997; He et al., 2017; Schröder and Collins, 2002). An evaluation of changes in GST activity was made to understand the mechanism by which acetaminophen was conjugated by GSH. Dynamic changes in GST activity in cucumber tissues during exposure are shown in Fig. 5a. The GST activity increased in both leaves and roots after exposure to acetaminophen ( $P < 0.05$ ). Compared to the blank control, GST activity was consistently elevated at each sampling point, amounting to 1.07–1.94 and 1.30–1.60 times that in the blank control in leaves and roots, respectively. A similar behavior was reported earlier in tomato leaves exposed to the fungicide chlorothalonil (McGonigle et al., 2000).

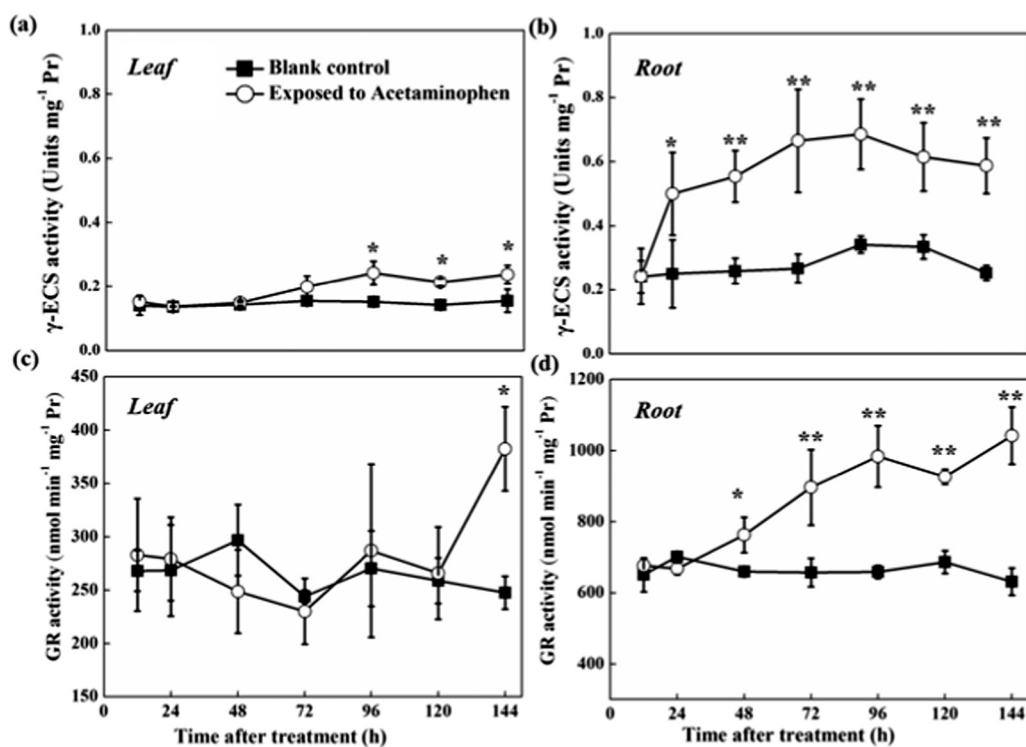
Although levels of PPCPs in wastewater effluents are usually below the levels used in this study (Guerra et al., 2014), it may be argued that continuous irrigation, active water evapotranspiration, and adsorption, may render the actual concentrations of PPCPs in the soil porewater considerably higher. Results from the biosolid amendment experiment further supported the conclusion that GSH detoxification may occur for acetaminophen under environmentally relevant conditions.



**Fig. 5.** Effect of acetaminophen on glutathione (a) *S*-transferase activity and (b) GSH content in cucumber leaves and roots. The values shown are mean  $\pm$  SD ( $n = 3$ ). Asterisks indicate significant difference between two treatments at each time point (\* $P < 0.05$  and \*\* $P < 0.01$ ). Pr, protein; FW, fresh weight.

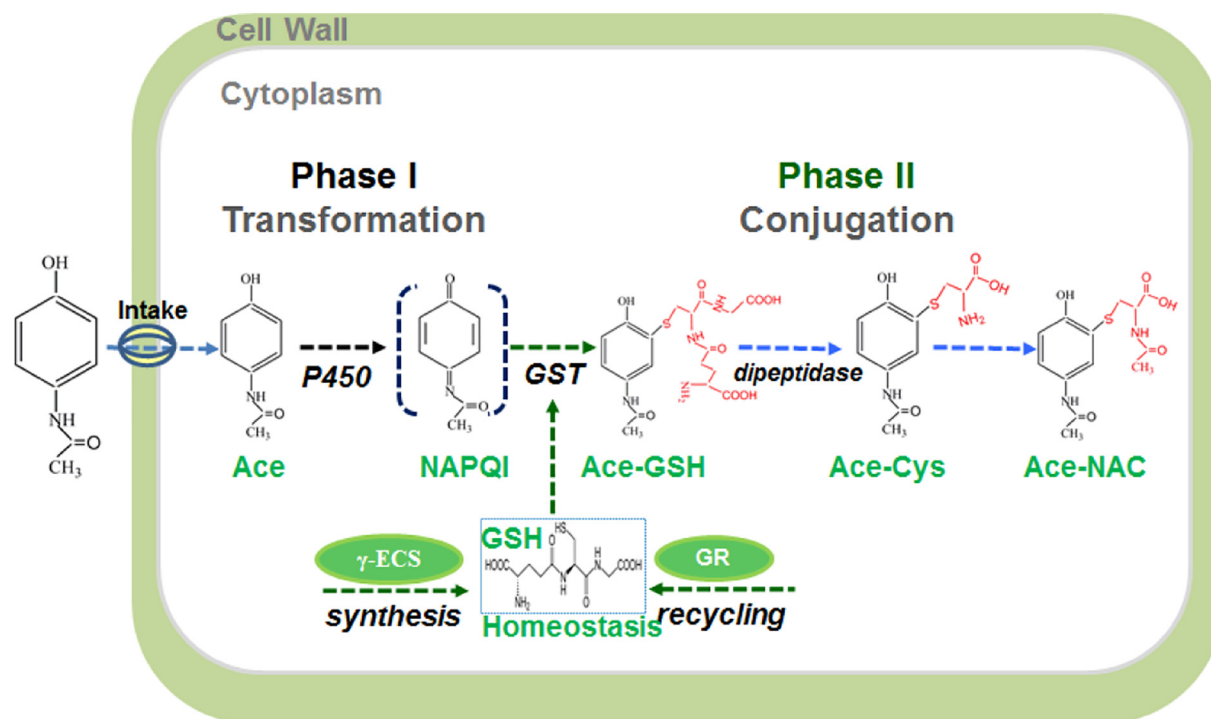
The GSH availability has been shown to correlate with the adaptation or response to xenobiotics in plants (Dixon et al., 2002), and is an important factor in determining cell sensitivity. For example, GSH availability has been used in understanding herbicide selectivity (Marrs, 1996). Any perturbation leading to a depletion of GSH can severely

impair a plant's defense against xenobiotics. Here, the concentration of GSH in cucumber roots decreased immediately after exposure to 5 mg L<sup>-1</sup> acetaminophen, although it gradually recovered to the normal level at 120 h; whereas GSH content only decreased at 72 h in cucumber leaves. Compared to the control, the level of GSH was reduced by 55.4%



**Fig. 6.** Effect of acetaminophen on activities of  $\gamma$ -glutamylcysteine synthetase ( $\gamma$ -ECS) and glutathione reductase (GR) in cucumber tissues. Activities of  $\gamma$ -ECS in (a) cucumber leaves and (b) roots; and activities of GR in (c) cucumber leaves and (d) roots. The values shown are mean  $\pm$  SD ( $n = 3$ ). Asterisks indicate significant difference between two treatments at each time point (\* $P < 0.05$  and \*\* $P < 0.01$ ). Pr, protein.





**Fig. 7.** A schematic representation of acetaminophen detoxification in plants via glutathione conjugation. Ace, acetaminophen; NAPQI, *N*-acetyl-para-benzoquinone imine; Ace-GSH, acetaminophen-glutathione; Ace-Cys, 3-cysteinyacetaminophen; Ace-NAC, 3-(*N*-acetyl-l-cystein-S-yl) acetaminophen; P450, cytochrome P450; GST, glutathione S-transferase;  $\gamma$ -ECS,  $\gamma$ -glutamylcysteine synthetase; GR, glutathione reductase.

in the root after 48 h and by 15.2% in the leaves after 72 h (Fig. 5b). These results were in agreement with the observation that roots were the tissues where more GSH conjugated metabolites were found. However, levels of GSH gradually recovered to a level similar to the control at the end of experiment (Fig. 5b), possibly due to rapid recycling via GR or synthesis of GSH by  $\gamma$ -ECS in plant cells (Wang et al., 2010).

In order to explore the roles of GR and  $\gamma$ -ECS in maintaining GSH homeostasis, their activities during the exposure were further monitored. In leaves, activities of GR and  $\gamma$ -ECS were elevated slightly, at about 1.53- and 1.54-fold after 144 h, respectively (Fig. 6). In contrast, treatment of acetaminophen significantly elevated  $\gamma$ -ECS activity in roots and showed a time-dependent response, increasing by 2.32-fold after 24 h ( $P < 0.05$ , Fig. 6b). A similar pattern was observed in GR activity in roots after exposure to acetaminophen (Fig. 6d); while significant increase in GR activity was only observed at 144 h in cucumber leaves (Fig. 6c). The finding indicated that activities of GR and  $\gamma$ -ECS were elevated to ensure sufficient GSH turnover in response to acetaminophen-induced consumption of GSH through GSH conjugation.

### 3.5. GSH detoxification pathway and environmental implications

Based on the results of this study, we propose a model to illustrate the operation of the GST detoxification pathway, which involves conjugation, accumulation and processing of these metabolites (Fig. 7). The present study provided strong evidence that transformation of acetaminophen, one of the most used antipyretic and analgesic drugs worldwide, was likely catalyzed first by cytochrome P450, and followed by GSH conjugation mediated by GSTs. The GSH conjugates were further transformed and released back to the medium. Meanwhile, enzymes involved in GSH synthesis, regeneration and transport appeared to work in concert to maintain GSH homeostasis during acetaminophen transformation and detoxification.

Detoxification by GSTs is known to play an important role in the biotransformations of a multitude of xenobiotics in plants (Bartha et al., 2014; Malan et al., 1990; Vontas et al., 2001). When emerging contaminants such as acetaminophen are introduced in agroecosystems, GST-

mediated detoxification may serve the purpose to minimize their potential phytotoxicity to susceptible plants. On the other hand, however, the conjugation may effectively conserve the parent compound and its biological activity, if deconjugation occurs, e.g., in the human digestive tract. Conjugates back transformation to other biologically active compounds has been reported for benzotriazole (Lefevre et al., 2015), triclosan (Macherius et al., 2012), and naproxen (Fu et al., 2017b). Thus, understanding the toxicological consequence of Phase II conjugates of such emerging contaminants in agricultural plants may improve risk assessment of reuse practices of treated wastewater and biosolids. Moreover, non-food plants capable of such detoxification may be used for removing such trace contaminants, in settings such as stormwater basins, wetlands and vegetative buffers.

### Acknowledgements

This work was financially supported by the U.S. Environmental Protection Agency STAR Program (No: R835829).

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.08.346>.

### References

- An, J., Zhou, Q., Sun, F., Zhang, L., 2009. Ecotoxicological effects of paracetamol on seed germination and seedling development of wheat (*Triticum aestivum* L.). *J. Hazard. Mater.* 169, 751–757.
- Ashrap, P., Zheng, G., Wan, Y., Li, T., Hu, W., Li, W., Zhang, H., Zhang, Z., Hu, J., 2017. Discovery of a widespread metabolic pathway within and among phenolic xenobiotics. *Proceedings of the National Academy of Sciences of the United States of America*, 201700558.
- Bartha, B., Huber, C., Harpaintner, R., Schröder, P., 2010. Effects of acetaminophen in *Brassica juncea* L. Czern.: investigation of uptake, translocation, detoxification, and the induced defense pathways. *Environ. Sci. Pollut. Res.* 17, 1553–1562.
- Bartha, B., Huber, C., Schröder, P., 2014. Uptake and metabolism of diclofenac in *Typha latifolia*-how plants cope with human pharmaceutical pollution. *Plant Sci.* 227, 12–20.

- Bártíková, H., Skálová, L., Stuchlíková, L., Vokřál, I., Vaněk, T., Podlípna, R., 2015. Xenobiotic-metabolizing enzymes in plants and their role in uptake and biotransformation of veterinary drugs in the environment. *Drug Metab. Rev.* 47, 374–387.
- Bartrons, M., Peñuelas, J., 2017. Pharmaceuticals and personal-care products in plants. *Trends Plant Sci.* 22, 194–203.
- Brown, H.M., Neighbors, S.M., 1987. Soybean metabolism of chlorimuron ethyl: physiological basis for soybean selectivity. *Pestic. Biochem. Physiol.* 29, 112–120.
- Brüsewitz, G., Cameron, B., Chasseaud, L., Görlner, K., Hawkins, D., Koch, H., Mennicke, W., 1997. The metabolism of benzyl isothiocyanate and its cysteine conjugate. *Biochem. J.* 162, 99–107.
- Carter, L.J., Harris, E., Williams, M., Ryan, J.J., Kookana, R.S., Boxall, A.B., 2014. Fate and uptake of pharmaceuticals in soil-plant systems. *J. Agric. Food Chem.* 62, 816–825.
- Carter, L.J., Williams, M., Böttcher, C., Kookana, R.S., 2015. Uptake of pharmaceuticals influences plant development and affects nutrient and hormone homeostases. *Environ. Sci. Technol.* 49, 12509–12518.
- Chen, G., Xu, H., Zhang, T., Bai, C., Dong, L., 2018. Fenoxaprop-P-ethyl resistance conferred by cytochrome P450s and target site mutation in *Alopecurus japonicus*. *Pest Manag. Sci.* 74, 1694–1703.
- Christou, A., Antoniou, C., Christodoulou, C., Hapeshi, E., Stavrou, I., Michael, C., Fatta-Kassinos, D., Fotopoulos, V., 2016. Stress-related phenomena and detoxification mechanisms induced by common pharmaceuticals in alfalfa (*Medicago sativa* L.) plants. *Sci. Total Environ.* 557, 652–664.
- Coleman, J., Blake-Kalff, M., Davies, E., 1997. Detoxification of xenobiotics by plants: chemical modification and vacuolar compartmentation. *Trends Plant Sci.* 2, 144–151.
- Cottingham, C.K., Hatzios, K.K., 1992. Basis of differential tolerance of two corn hybrids (*Zea mays*) to metolachlor. *Weed Sci.* 40, 359–363.
- Crane, M., Watts, C., Boucard, T., 2006. Chronic aquatic environmental risks from exposure to human pharmaceuticals. *Sci. Total Environ.* 367, 23–41.
- DeRidder, B.P., Dixon, D.P., Beussman, D.J., Edwards, R., Goldsbrough, P.B., 2002. Induction of glutathione S-transferases in *Arabidopsis* by herbicide safeners. *Plant Physiol.* 130, 1497–1505.
- Dixon, D.P., Laphorn, A., Edwards, R., 2002. Plant glutathione transferases. *Genome Biology* 3, reviews3004-1.
- Doty, S.L., 2008. Enhancing phytoremediation through the use of transgenics and endophytes. *New Phytol.* 179, 318–333.
- Farkas, M.H., Berry, J.O., Aga, D.S., 2007. Chlortetracycline detoxification in maize via induction of glutathione S-transferases after antibiotic exposure. *Environ. Sci. Technol.* 41, 1450–1456.
- Field, J.A., Thurman, E., 1996. Glutathione conjugation and contaminant transformation. *Environ. Sci. Technol.* 30, 1413–1418.
- Fu, Q., Ye, Q., Zhang, J., Richards, J., Borchardt, D., Gan, J., 2017a. Diclofenac in *Arabidopsis* cells: rapid formation of conjugates. *Environ. Pollut.* 222, 383–392.
- Fu, Q., Zhang, J., Borchardt, D., Schlenk, D., Gan, J., 2017b. Direct conjugation of emerging contaminants in *Arabidopsis*: indication for an overlooked risk in plants? *Environ. Sci. Technol.* 51, 6071–6081.
- Ghosh, M., Shen, J., Rosen, B.P., 1999. Pathways of As (III) detoxification in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U. S. A.* 96, 5001–5006.
- Gonzalez, A., Vera, J., Castro, J., Dennett, G., Mellado, M., Morales, B., Correa, J.A., Moenne, A., 2010. Co-occurring increases of calcium and organellar reactive oxygen species determine differential activation of antioxidant and defense enzymes in *Ulva compressa* (Chlorophyta) exposed to copper excess. *Plant Cell Environ.* 33, 1627–1640.
- Guddewar, M.B., Dauterman, W.C., 1979. Purification and properties of a glutathione-S-transferase from corn which conjugates s-triazine herbicides. *Phytochemistry* 18, 735–740.
- Guerra, P., Kim, M., Shah, A., Alae, M., Smyth, S., 2014. Occurrence and fate of antibiotic, analgesic/anti-inflammatory, and antifungal compounds in five wastewater treatment processes. *Sci. Total Environ.* 473, 235–243.
- He, Y., Langenhoff, A.A., Sutton, N.B., Rijnaarts, H.H., Blokland, M.H., Chen, F., Huber, C., Schröder, P., 2017. Metabolism of ibuprofen by *Phragmites australis*: uptake and phytodegradation. *Environ. Sci. Technol.* 51, 4576–4584.
- Huber, C., Bartha, B., Harpaintner, R., Schröder, P., 2009. Metabolism of acetaminophen (paracetamol) in plants—two independent pathways result in the formation of a glutathione and a glucose conjugate. *Environ. Sci. Pollut. Res.* 16, 206–213.
- Huber, C., Bartha, B., Schröder, P., 2012. Metabolism of diclofenac in plants—hydroxylation is followed by glucose conjugation. *J. Hazard. Mater.* 243, 250–256.
- Jiang, Y.P., Cheng, F., Zhou, Y.H., Xia, X.J., Mao, W.H., Shi, K., Chen, Z., Yu, J.Q., 2012. Cellular glutathione redox homeostasis plays an important role in the brassinosteroid-induced increase in CO<sub>2</sub> assimilation in *Cucumis sativus*. *New Phytol.* 194, 932–943.
- Kaspar, M., Grondona, M., Leon, A., Zambelli, A., 2011. Selection of a sunflower line with multiple herbicide tolerance that is reversed by the P450 inhibitor malathion. *Weed Sci.* 59, 232–237.
- Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. *Water Res.* 42, 3498–3518.
- Kavitha, P., Ramesh, R., Bupesh, G., Stalin, A., Subramanian, P., 2011. Hepatoprotective activity of *Tribulus terrestris* extract against acetaminophen-induced toxicity in a freshwater fish (*Oreochromis mossambicus*). *In Vitro Cellular & Developmental Biology* 47, 698–706.
- Lefevre, G.H., Müller, C.E., Li, R.J., Luthy, R.G., Sattely, E.S., 2015. Rapid phytotransformation of benzotriazole generates synthetic tryptophan and auxin analogs in *Arabidopsis*. *Environ. Sci. Technol.* 49, 10959–10968.
- Macherius, A., Eggen, T., Lorenz, W., Moeder, M., Ondruschka, J., Reemtsma, T., 2012. Metabolization of the bacteriostatic agent triclosan in edible plants and its consequences for plant uptake assessment. *Environ. Sci. Technol.* 46, 10797–10804.
- Malan, C., Greyling, M.M., Gressel, J., 1990. Correlation between CuZn superoxide dismutase and glutathione reductase, and environmental and xenobiotic stress tolerance in maize inbreds. *Plant Sci.* 69, 57–166.
- Malchi, T., Maor, Y., Tadmor, G., Shenker, M., Chefetz, B., 2014. Irrigation of root vegetables with treated wastewater: evaluating uptake of pharmaceuticals and the associated human health risks. *Environ. Sci. Technol.* 48, 9325–9333.
- Marrs, K.A., 1996. The functions and regulation of glutathione S-transferases in plants. *Annu. Rev. Plant Biol.* 47, 127–158.
- Marsik, P., Sisa, M., Lacina, O., Motkova, K., Langhansova, L., Rezek, J., Vanek, T., 2017. Metabolism of ibuprofen in higher plants: a model *Arabidopsis thaliana* cell suspension culture system. *Environ. Pollut.* 220, 383–392.
- McGonigle, B., Keeler, S.J., Lau, S.M.C., Koeppel, M.K., O'Keefe, D.P., 2000. A genomics approach to the comprehensive analysis of the glutathione S-transferase gene family in soybean and maize. *Plant Physiol.* 124, 1105–1120.
- Miller, E.L., Nason, S.L., Karthikeyan, K., Pedersen, J.A., 2016. Root uptake of pharmaceuticals and personal care product ingredients. *Environ. Sci. Technol.* 50, 525–541.
- Nunes, B., Antunes, S.C., Santos, J., Martins, L., Castro, B.B., 2014. Toxic potential of paracetamol to freshwater organisms: a headache to environmental regulators? *Ecotoxicol. Environ. Saf.* 107, 178–185.
- Potter, D.W., Hinson, J.A., 1987. Mechanisms of acetaminophen oxidation to N-acetyl-P-benzoquinone imine by horseradish peroxidase and cytochrome P450. *J. Biol. Chem.* 262, 966–973.
- Riemenschneider, C., Seiwert, B., Moeder, M., Schwarz, D., Reemtsma, T., 2017. Extensive transformation of the pharmaceutical carbamazepine following uptake into intact tomato plants. *Environ. Sci. Technol.* 51, 6100–6109.
- Sandermann Jr., H., 1992. Plant metabolism of xenobiotics. *Trends Biochem. Sci.* 17, 82–84.
- Schröder, P., Collins, C., 2002. Conjugating enzymes involved in xenobiotic metabolism of organic xenobiotics in plants. *International Journal of Phytoremediation* 4, 247–265.
- Shan, C., Liang, Z., 2010. Jasmonic acid regulates ascorbate and glutathione metabolism in *Agropyron cristatum* leaves under water stress. *Plant Sci.* 178, 130–139.
- Sun, C., Lu, L., Liu, L., Liu, W., Yu, Y., Liu, X., Hu, Y., Jin, C., Lin, X., 2014. Nitrate reductase-mediated early nitric oxide burst alleviates oxidative damage induced by aluminum through enhancement of antioxidant defenses in roots of wheat (*Triticum aestivum*). *New Phytol.* 201, 1240–1250.
- Sun, C., Dudley, S., Trumble, J., Gan, J., 2018. Pharmaceutical and personal care products-induced stress symptoms and detoxification mechanisms in cucumber plants. *Environ. Pollut.* 234, 39–47.
- Tong, Y.P., Kneer, R., Zhu, Y.G., 2004. Vacuolar compartmentalization: a second-generation approach to engineering plants for phytoremediation. *Trends Plant Sci.* 9, 7–9.
- U.S. Environmental Protection Agency, 1996. Ecological effects test guidelines (OPPTS 850.4200): Seed germination/root elongation toxicity test. EPA 712-C-96-154. Washington, DC.
- Verbruggen, N., Hermans, C., Schat, H., 2009. Mechanisms to cope with arsenic or cadmium excess in plants. *Curr. Opin. Plant Biol.* 12, 364–372.
- Vontas, J.G., Small, G.J., Hemingway, J., 2001. Glutathione S-transferases as antioxidant defence agents confer pyrethroid resistance in *Nilaparvata lugens*. *Biochem. J.* 357, 65–72.
- Wang, J., Jiang, Y., Chen, S., Xia, X., Shi, K., Zhou, Y., Yu, Y., 2010. The different responses of glutathione-dependent detoxification pathway to fungicide chlorothalonil and carbendazim in tomato leaves. *Chemosphere* 79, 958–965.
- Wu, X., Conkle, J.L., Ernst, F., Gan, J., 2014. Treated wastewater irrigation: uptake of pharmaceutical and personal care products by common vegetables under field conditions. *Environ. Sci. Technol.* 48, 11286–11293.
- Zhou, B., Guo, Z., Xing, J., Huang, B., 2005. Nitric oxide is involved in abscisic acid-induced antioxidant activities in *Stylosanthes guianensis*. *J. Exp. Bot.* 56, 3223–3228.