Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

Induction of Herbicide Detoxifying Enzyme in Maize by Chiral 3-Dichloroacetyl Oxazolidine

Li-Xia ZHAO[a], Qing-Rui WANG[a], Ying FU[a], Chun-Yan LI[b], Shuang GAO[a], Fei YE[a]

[a]*Northeast Agricultural University, College of Science, Harbin, 150030, P.R. CHINA*

[b]*Northeast Agricultural University, College of Resources and Environment, Harbin, 150030, P.R. CHINA*

ARTICLE INFO

Research Article DOI: 10.15832/ankutbd.456660
Corresponding Author: Fei YE, E-mail: yefei@neau.edu.cn, Tel: +86 (451) 551 915 07
Received: 13 February 2017, Received in Revised Form: 24 October 2017, Accepted: 30 October 2017

ABSTRACT

Safeners are important tools used to ensure to safe useof herbicide. The aim of this paper is to evaluate the protective effect of four safeners (R-28725, 3-dichloroacetyl oxazolidine and its two optical isomers) and investigate the mechanism of herbicide detoxication by safener. Laboratory studies were conducted to evaluate the effectiveness of safeners for protecting maize from the residues of preemergent herbicide fomesafen in Northeast Agricultural University, China. Physiological and biochemical tests were herein conducted under laboratory conditions, by using seed treatment with safeners and soil treatment with fomesafen, respectively. R-28725 provoked high glutathione level, glutathione-S-transferase activity and affinity of glutathione-S-transferase than other safeners, but R-isomer treatment resulted in complete reversal of injury caused by fomesafen.

Keywords: Herbicide safener; Herbicide detoxication; 3-Dichloroacetyl oxazolidine; Fomesafen; Glutathione-S-transferase

1. Introduction

Fomesafen [5-(2-chloro-α,α,α-trifluoro-p-tolyloxy)-N-mesyl-2-nitrobenzamide], a diphenyl-ether herbicide, controls broadleaf weeds by inhibiting protoporphyrinogen oxidase (PPOX), an important enzyme needed in chlorophyll biosynthesis. Fomesafen applied preemergence in soybean as a selective herbicide with both root and shoot activity (Peachey et al 2012). But, it has been reported that recommended dosage of fomesafen in soybeans may cause carryover injury to corn and the injury was varied significantly by plant variety and soil conditions (Rauch et al 2007; Cieslik et al 2014). When sweet corn was sown in high pH and low organic matter soils, injury of fomesafen was more serious compared with other soil types. For that reason, application of fomesafen in crops was restricted.

A key technology to increase herbicide selectivity is safeners. Herbicide safeners are synthetic compounds which can activate the tolerance of plants to herbicide without decrease herbicidal activity to weeds (Kraehmer et al 2014). The concept of safener was found by Otto Hoffman in the late 1940s. Research and development of new safeners proceeded in late 20th century, and products of safener were subsequently commercialized by agrochemical

companies. Although extensive work has been done, the mechanism of safener is not fully unveiled. Researchers have believed that structural similarity between herbicide and safener was essential for certain type of safeners (Bordas et al 2000). Research about safeners previously mainly focused on response of plants to safener. There is now a general consensus that some safeners enhanced the genes expression in plants related with exogenous compounds metabolism process such as glutathione-S-transferase (GST), cytochrome P450, and glutathione (GSH) (Matola & Jablonkai 2007; Del Buono & Ioli 2011). It has been reported that GSH-mediated detoxification was involved in the herbicide metabolism and detoxification response of plants (Riechers et al 2010). Skipsey et al (2011) found that a series of fenclorim derivatives induced GST and increased herbicide tolerance in rice. Da Silva et al (2014) also reported that fluxofenim induced GST and protect two sorghum hybrids from the injury of herbicide S-metolachlor. However, to our knowledge, there is no any report about safener for PPOX-inhibiting herbicides.

3-Dichloroacetyl-2,2-dimethyl-1,3-oxazolidine (R-28725) was proven effective in reducing herbicidal injury from ALS-inhibitor herbicides (Zhao et al 2014). Its analogue, 3-dichloroacetyl-2,2-dimethyl-4-ethyl-1,3-oxazolidine, has a chiral carbon atom that gives rise to two optical isomers. The aim of this research was to study the bioactivity of R-28725, two optical isomers and racemate of 3-dichloroacetyl-2,2-dimethyl-4-ethyl-1,3-oxazolidine as safeners for fomesafen. In addition, physiological and biochemical tests was conducted to assess the protective ability of these compounds and investigate the function of GSH, GST, PPOX in herbicide detoxification.

2. Material and Methods

The tested soil was *Mollisols-cryolls* clay loam type and collected from Horticulture Station, Northeast Agricultural University with a pH of 7.37 (Figure 1). The seedlings of maize cultivar, Dongnong 253 (*Zea mays* L.), was germinated and raised in a growth chamber at the Pesticide Chemistry Laboratory, Northeast Agricultural University. Fomesafen (99.5%, powder) was obtained from Aladdin Chemistry (Shanghai, China) to determine the GST activity *in vitro*. Fomesafen (250 g L^{-1}, liquid) was obtained from Dalian Songliao Chemical Industry Cmpany (Dalian, China) to use in other tests. R-28725, the racemate and two optical isomers of 3-dichloroacetyl-2,2-dimethyl-4-ethyl-1,3-oxazolidine were synthesized in our laboratories (99.0%) (Table 1).

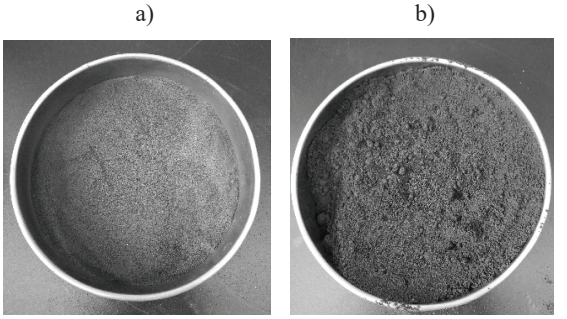

Figure 1- Tested soil (a) untreated soil (b) soil added with fomesafen

Table 1- Chemical name of safeners

Safener	*Chemical name*
R-28725	3-dichloroacetyl-2,2-dimethyl-1,3-oxazolidine
R-isomer	(R)-3-dichloroacetyl-2,2-dimethyl-4-ethyl-1,3-oxazolidine
S-isomer	(S)-3-dichloroacetyl-2,2-dimethyl-4-ethyl-1,3-oxazolidine
Racemate	(RS)-3-dichloroacetyl-2,2-dimethyl-4-ethyl-1,3-oxazolidine

Seedlings of maize were soaking for 12 hours in solution of safeners (0, 1, 5, 10, 25, 50, 100 mg kg^{-1}), the control was soaking in water (Figure 2). Then, the seeds were germinated in dishes in a growth chamber for 24 hours (Figure 3). The recommended field application dose of fomesafen was 3.75 mg kg^{-1} and an average half-life value of fomesafen was 50 d which means that the concentration of fomesafen in soil was 0.96 mg kg^{-1} after it had been applied 100 d (Rauch et al 2007; Wu et al 2014). So, in this study, 1 mg kg^{-1} was chosen as the concentration of fomesafen in soil. Sown these seeds in paper-cups (10 cm × 15 cm), 7 seeds per cup, containing

soil added with fomesafen with a depth of 13 cm, and incubated in a growth chamber with a 12/12 photoperiod, 26.5±1 ℃ temperature, 75% relative humidity. Each treatment was replicated three times.

Figure 2- *Zea mays* seeds soaking in water and solution of safeners

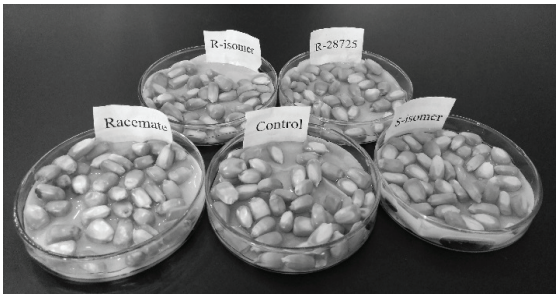

Figure 3- *Zea mays* seeds germinated in dishes

In order to calculate the recovery rate of maize, four parameters (plant height, root length, fresh weight of shoot, and fresh weight of root) of maize were measured 7 days after treatment. Recovery rate was calculated by Equation 1.

Where safener include the four safeners in this research and herbicide is fomesafen. The recovery rate of parameters of maize was calculated respectively.

The maize was washed and cutted to collect shoot and root tissues for biological assays. GSH level assay: GSH level was measured by UV-visible spectrophotomer as described previously (Ismaiel & Papenbrock 2014). To perform the determination, 5,5'-dithiobis (2-nitrobenzoic) acid (DTNB) was used in this study as chromogenic agent, absorbance data collected at 412 nm, and GSH level was calculated by comparing with standard working curve.

GST enzyme extraction and assay *in vivo*: The extraction and assay GST of was performed as described by Matola & Jablonkai (2007). The GST activity was obtained by measuring the level of conjugate composed from GSH and substrate. GST activity was expressed as level of conjugate per minute per mg of protein (μmol min^{-1} mg^{-1} protein).

GST activity assay *in vitro*: To determine the GST activity *in vitro* (against fomesafen in this study), the amount of fomesafen was determined by high performance liquid chromatography (HPLC) (Scarponi et al 2006; He et al 2010). GST enzyme was extracted from root of maize, and added with glutathione and fomesafen solution. After cultivated 2 hours, residue of fomesafen in this mixture was measured through HPLC. The GST activity *in vitro* was expressed as amount of fomesafen decreased per minute per mg of protein (nmol min^{-1} mg^{-1} protein).

$$\text{Recovery rate}(\%) = \frac{\text{Parameter of maize treated by safener and herbicide} - \text{Parameter of maize treated by herbicide}}{\text{Parameter of maize untreated} - \text{Parameter of maize treated by herbicide}} \quad (1)$$

Kinetic parameters of GST assay: The procedure described by Scarponi et al (2006) was followed to measure kinetic parameters of GST with modification. The GST activity was determined over a range of 1-chloro-2,4-dinitrobenzene (CDNB) concentration (0.13-4.14 mM) at a single GSH concentration of 5 mM.

PPOX enzyme extraction and assay: To investigate the effect of safener to target enzyme, PPOX activity was determined as described previously (Labbe et al 1985). PPOX activity was expressed as amount of protoporphyrin IX composed from protoporphyrinogen IX catalyzed by PPOX per hour per mg of enzyme (nmol h^{-1} mg^{-1} protein).

Statistical analysis: All data was performed by SPSS statistic software to determine statistical significance at 95% confidence level (P= 0.05) by Duncan's multiple-range test. All data reported were expressed as mean±standard deviation of three replicates.

3. Results and Discussion

3.1. Parameters of maize

The parameters of maize were significantly decreased by the treatment of fomesafen. When fomesafen applied at 1 mg kg^{-1} in soil, caused 34.42% to 39.36% decrease to root and shoot of maize, respectively.

The maize response to different concentrations of these four safeners was studied to get appropriate concentration of safeners offering maximum protection from fomesafen. The recovery rate of maize can be found in Figure 4. Significant differences were observed for recovery rate of maize to different concentration of safener in this study. Appropriate concentration of safener significantly decreased

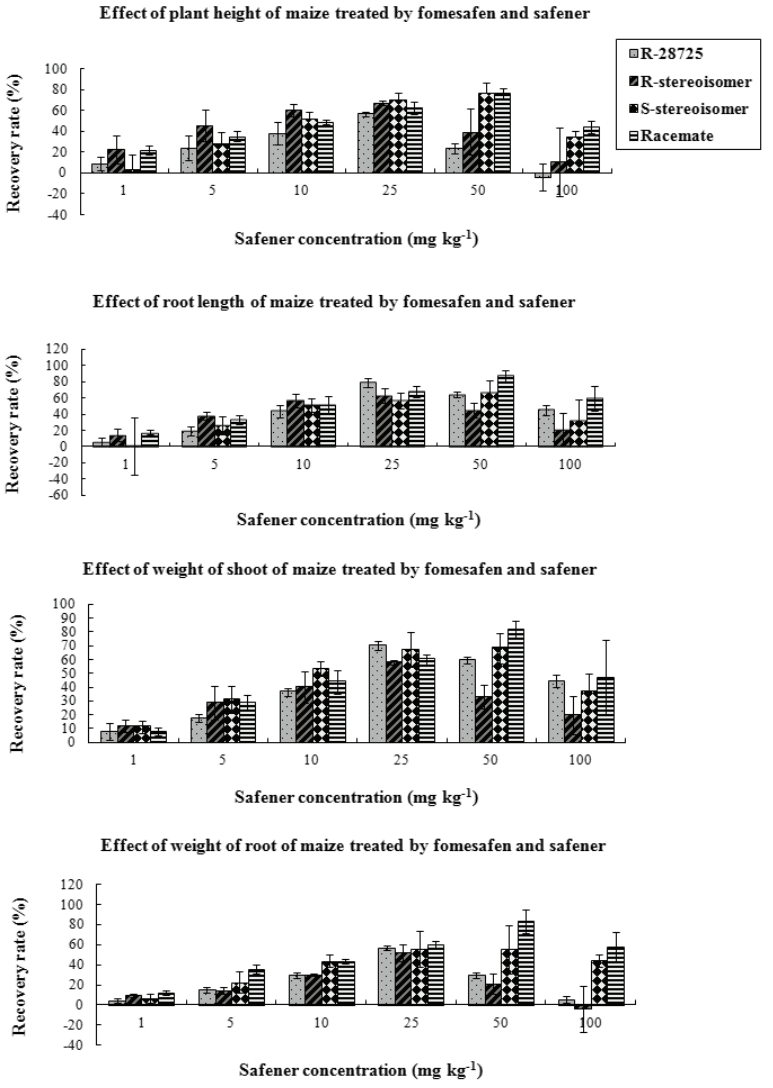

Figure 4- Recovery rate of parameters of maize effected by fomesafen and safener

herbicidal injury from fomesafen. The optimum concentration of safener for R-28725, and R-isomer were 25 mg kg^{-1}, and for S-isomer, and Racemate were 50 mg kg^{-1}. The results of recovery rate of maize soaked in the optimum concentration of safeners indicate that the order of protective ability of safeners was as follows: Racemate > R-28725 > S-isomer > R-isomer. Optimum concentration for each safener was then applied to maize for subsequent testing.

3.2. GSH level

Hatzios & Burgos (2004) reported that safeners could elevate the conjugation of herbicide with GSH through increasing the level of GSH in plants. Therefore, the level of GSH in plant was taken as an important index to check the protective ability of safener (Table 2). GSH levels in root and shoot of maize increased 43.4% and 30.1%, respectively, for fomesafen treatment compared with control. The level of GSH in root of maize treated by Racemate or R-28725 combined with fomesafen increased significantly by 84.0% and 101.2% compared with control, respectively, while the level of GSH in shoot of maize treated by Racemate combined with fomesafen increased 77.1% compared with control.

3.3. GST activity

Response of GST in maize treated by safener and fomesafen were investigated to discover the its role in detoxification process. The GST activity *in vivo* of maize treated by Racemate or R-28725 combined with fomesafen increased 88.6% and 85.0% compared with the control, respectively. The GST activity *in vitro* of maize treated by R-isomer or R-28725 increased 328.7% and 299.2% compared with the control, respectively (Table 3). The results of GST activity of maize indicate that R-28725 induced GST affinity for substrate significantly.

3.4. Kinetic parameters of GST

Further research was conducted for kinetic parameters of GST. The kinetic parameters V_{max} (the maximal reaction rate of detoxification reaction) and K_M (the concentration of substrate when the velocity of detoxification reaction is half of the maximum velocity) of GST were calculated by linear regression (Table 4). V_{max} value of GST for maize treated by R-28725 was raised to 2.02 times that of control and K_M value was decreased to 59.8% of control, indicating the strong inducement of GST caused by R-28725. It

Table 2- Effect of safeners and fomesafen on GSH level

Treatment	*GSH level in root ($\mu g\ g^{-1}$)*	*GSH level in shoot ($\mu g\ g^{-1}$)*
Control	3.268±0.2532 d	9.281±1.6320 c
Fomesafen	4.686±0.2233 c	12.071±0.5690 c
R-isomer+Fomesafen	4.732±0.1381 c	14.373±0.1657 b
S-isomer+Fomesafen	4.474±0.1797 c	13.231±0.2767 b
Racemate+Fomesafen	6.012±0.2349 b	16.491±0.2611 a
R-28725+Fomesafen	6.574±0.3296 a	14.262±0.2086 b

Mean ± standard deviation. Values sharing same letters differ non-significantly (P>0.05). The values correspond to averages of three replicates

Table 3- Effect of safeners and fomesafen on GST

Treatment	*GST activity in vivo ($\mu mol\ min^{-1}\ mg^{-1}$ protein)*	*Treatment*	*GST activity in vitro ($nmol\ min^{-1}\ mg^{-1}$ protein)*
Control	1.67±0.038 e	Control	0.254±0.0455 d
Fomesafen	2.26±0.064 d	Fomesafen	0.071±0.0158 e
R-isomer+Fomesafen	2.90±0.020 b	R-isomer	1.089±0.1075 a
S-isomer+Fomesafen	2.54±0.088 c	S-isomer	0.574±0.0294 c
Racemate+Fomesafen	3.15±0.075 a	Racemate	0.795±0.1000 b
R-28725+Fomesafen	3.09±0.120 a	R-28725	1.014±0.0340 a

Mean ± standard deviation. Values sharing same letters differ non-significantly (P>0.05). The values correspond to averages of three replicates

was consistent with the results of GST activity. Other safeners also induced the affinity of GST to substrate of conjugated reaction in some extent.

Table 4- Effect of safeners and fomesafen to kinetic parameters of GST

Treatment	V_{max} *(nmol min^{-1} mg^{-1} protein)*	K_m *(mmol L^{-1})*
Control	0.790±0.0300 e	1.950±0.0557 b
Fomesafen	0.567±0.0292 f	2.973±0.1429 a
R-isomer	1.437±0.0231 b	1.320±0.0100 d
S-isomer	0.913±0.0666 d	1.673±0.0569 c
Racemate	1.090±0.0346 c	1.570±0.0173 c
R-28725	1.597±0.0907 a	1.167±0.1443 d

Mean ± standard deviation. Values sharing same letters differ non-significantly (P>0.05). The values correspond to averages of three replicates

3.5. PPOX activity

Fomesafen harms plants via inhibition of PPOX, but safener protect plants by reducing the amounts of herbicide to reach to the targeted site in the plant (Rushing et al 2013). For that reason, PPOX activity is clearly critical for the resistant ability of plants. The effect of safeners and fomesafen on the PPOX activity was determined to investigate the protective effectiveness of safeners (Table 5). The results showed that fomesafen inhibited PPOX activity in maize significantly by 56.6% compared with the control and safeners could elevate PPOX activity of maize significantly. It is noteworthy that R-isomer could reverse the effects on maize caused by fomesafen totally.

Table 5- Effect of safeners and fomesafen on PPOX activity

Treatment	*PPOX Activity (nmol h^{-1} mg^{-1} protein)*
Control	0.505±0.0312 a
Fomesafen	0.219±0.0161 d
R-isomer+Fomesafen	0.519±0.0406 a
S-isomer+Fomesafen	0.237±0.0173 d
Racemate+Fomesafen	0.403±0.0196 b
R-28725+Fomesafen	0.332±0.0172 c

Mean ± standard deviation. Values sharing same letters differ non-significantly (P>0.05). The values correspond to averages of three replicates

While fomesafen contribute to improving crop yield, it can also pose a risk to those plants that are sensitive to them (Cieslik et al 2014). For that reason, effective safeners were developed to protect plants. But no safener has been developed to protect plant from diphenyl-ether herbicide. In order to develop safener for fomesafen, the protective effects of four safeners were studied in our laboratory. The results conclusively demonstrated that the maize injured by fomesafen was effectively protected by these safeners. The maize seeds that had been soaked in solution of safener were safe from fomesafen treatment. The results indicate that the application of these safeners produced high recovery rates of growth level of maize ranged from 51.80% to 87.21% with fomesafen applied at 1 mg kg^{-1} in soil.

For evaluation the enhancement of detoxification of maize, induced by safeners, the GSH level, GST, and PPOX activity of maize treated by fomesafen and safener were investigated. Our study has shown that these safeners caused enhancement of GSH level, GST, and PPOX activity of maize and affinity of GST enzyme to substrate. Enhancement of GSH level in root of maize, GST activity and affinity of GST to CDNB caused by R-28725 was greatest which caused 1.40-fold, 1.46-fold and 2.82-fold increase to GSH level, GST activity *in vivo* and V_{max} of GST, respectively. It is safe to say that these safeners induced the conjugation of herbicide with GSH catalyzed by GST to some extent. Consistent with previous studies, safeners significantly change the affinity of GST to substrate of conjugation reaction (Scarponi et al 2006). Fomesafen resulted in inhibition to plant by inhibiting PPOX activity. So, PPOX activity is an important index to maize treated by safener and fomesafen. R-isomer treatment resulted in complete reversal of injury caused by fomesafen. This might suggest that the protective ability of safener not only depends on the GSH and GST in maize (Jablonkai 2013).

4. Conclusions

From this study, it can be concluded that seed treatment with these four safeners present protective ability to injury caused by fomesafen. In addition, the excellent efficacy suggests that these safeners should be considered

for reducing of herbicide toxicity in maize. Therefore, these compounds can be a useful tool to protect maize from the injury of herbicide and improve selectivity between crop and weed. This study is the first one on the effect of chiral 3-dichloroacetyl oxazolidine and their interaction with fomesafen. However, in-depth studies are still needed to determine the exact mechanism of the enhancement of protective ability.

Acknowledgements

Supported by the National Nature Science Foundation of China (31401787, 31572042), the China Postdoctoral Science Foundation (2015M571384), Natural Science Foundation of Heilongjiang Province of China (C2015014), the Science and Technology Research Project of Heilongjiang Education Department (12541029, 12531027), the "Academic Backbone" Project of Northeast Agricultural University (16XG24) and the "Young Talents" Project of Northeast Agricultural University (14QC38).

References

Bordas B, Komives T, Szanto Z & Lopata A (2000). Comparative three-dimensional quantitative structure-activity relationship study of safeners and herbicides. *Journal of Agricultural and Food Chemistry* **48**: 926-931

Cieslik L F, Vidal R A & Trezzi M M (2014). Fomesafen toxicity to bean plants as a function of the time of application and herbicide dose. *Acta Scientiarum Agronomy* **36**: 329-334

Da Silva J R V, Martins C C, Da Silva Junior A C & Martins D (2014). Fluxofenim used as a safener on sorghum seed for S-metolachlor herbicide. *Bioscience Journal* **30**: 158-167

Del Buono D & Ioli G (2011). Glutathione S-transferases of Italian ryegrass (*Lolium multiflorum*): activity toward some chemicals, safener modulation and persistence of atrazine and fluorodifen in the shoots. *Journal of Agricultural and Food Chemistry* **59**: 1324-1329

Hatzios K K & Burgos N (2004). Metabolism-based herbicide resistance: regulation by safeners. *Weed Science* **52**: 454-467

He C Y, Li Y Q, Wang S J, Ouyang H X & Zheng B (2010). Determination of biphenyl ether herbicides in water using HPLC with cloud-point extraction. *Journal of Sichuan University* **41**: 148-152

Ismaiel A A & Papenbrock J (2014). The effects of patulin from *Penicillium vulpinum* on seedling growth, root tip ultrastructure and glutathione content of maize. *European Journal of Plant Pathology* **139**: 497-509

Jablonkai I (2013). Herbicide Safeners: Effective Tools to Improve Herbicide Selectivity. Herbicides-Current Research and Case Studies in Use. In: Dr. Andrew P (Ed.), InTech, DOI: 10.5772/55168. Available from: https://www.intechopen.com/books/herbicides-current-research-and-case-studies-in-use/herbicide-safeners-effective-tools-to-improve-herbicide-selectivity

Kraehmer H, Laber B, Rosinger C & Schulz A (2014). Herbicides as weed control agents: state of the art: I. weed control research and safener technology: The path to modern agriculture. *Plant Physiology* **166**: 1119-1131

Labbe P, Camadro J M & Chambon H (1985). Fluorometric assays for coproporphyrinogen oxidase and protoporphyrinogen oxidase. *Analytical Biochemistry* **149**: 248-260

Matola T & Jablonkai I (2007). Safening efficacy of halogenated acetals, ketals and amides and relationship between the structure and effect on glutathione and glutathione S-transferases in maize. *Crop Protection* **26**: 278-284

Peachey E, Doohan D & Koch T (2012). Selectivity of fomesafen based systems for preemergence weed control in cucurbit crops. *Crop Protection* **40**: 91-97

Rauch B J, Bellinder R R, Brainard D C, Lane M & Thies J E (2007). Dissipation of fomesafen in New York state soils and potential to cause carryover injury to sweet corn. *Weed Technology* **21**: 206-212

Riechers D E, Kreuz K & Zhang Q (2010). Detoxification without intoxication: Herbicide safeners activate plant defense gene expression. *Plant Physiology* **153**: 3-13

Rushing J B, Baldwin B S, Taylor A G, Owens V N, Fike J H & Moore K J (2013). Seed safening from herbicidal injury in switchgrass establishment. *Crop Science* **53**: 1650-1657

Scarponi L, Quagliarini E & Del Buono D (2006). Induction of wheat and maize glutathione S-transferase by some herbicide safeners and their effect on enzyme activity against butachlor and terbuthylazine. *Pest Management Science* **62**: 927-932

Skipsey M, Knight K M, Brazier-Hicks M, Dixon D P, Steel P G & Edwards R (2011). Xenobiotic responsiveness of Arabidopsis thaliana to a chemical series derived from a herbicide safener. *Journal of Biological Chemistry* **286**: 32268-32276

Wu X H, Xu J, Dong F S, Liu X G & Zheng Y Q (2014). Responses of soil microbial community to different concentration of fomesafen. *Journal of Hazardous Materials* **273**: 155-164

Zhao L X, Fu Y, Gao S, Xing Z Y, Wei L N & Ye F (2014). Protective responses induced by 3-dichloroacetyl oxazolidine safeners in maize (*Zea mays*). *International Journal of Agriculture and Biology* **16**: 1204-1208