Glutathione *S*-Transferases (GST) Activity of Quinclorac-Resistant Smooth Crabgrass (*Digitaria Ischaemum*)

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INTRODUCTION

Quinclorac controls crabgrass (*Digitaria* spp.) post-emergence in cool- and warm-season turfgrass species. Quinclorac-resistance has been confirmed in smooth crabgrass (*Digitaria ischaemum*) in Mississippi and California (Abdallah et al. 2006). Genetics and plant physiology play a significant role in herbicide resistance evolution among weedy species.

Two terminologies are often used to categorize the underlying mechanism of resistance: Target-site resistance (TSR) and non-target-site resistance (NTSR). Within the study of herbicide-resistant plants, the identification of target site-resistant populations has proven to be relatively straightforward, while the study and elucidation of resistance mechanisms involved in enhanced metabolism or uptake and translocation have been more poorly understood.

One of the well-established NTSR gene families is glutathione *S*-transferase (GST). In previous research, quinclorac-response GST genes were identified in quinclorac-resistant *Echinochloa crus-galli* (Li 0et al. 2013); GST activity in response to other herbicides also has been reported in the following grassy weeds: *Setaria faberi*, *Digitaria sanguinalis* and *Sorghum halepense* (Andrews et al. 1997), but little research has been conducted pertaining to NTSR mechanism on quinclorac-resistant smooth crabgrass.

MATERIALS AND METHODS

Plant Material and Growing Conditions

- Research was conducted as a completely randomized design (4 replications) repeated twice in time at Mississippi State University during summer of 2022.
- Quinclorac was sprayed to 3-leaf stage smooth crabgrass (Table 1) with an enclosed spray chamber, and plants were harvested aboveground 3 days after treatments (DAT) and stored at -80°C.

Statistical Analysis

ANOVA followed by pairwise-comparison test using PROC GLIMMIX in SAS version 9.4 was conducted to determine significant differences in GST specific activity among plant treatment rates for each variety. Results are reported as means +/- standard error.

Table 1. Quinclorac treatments were applied to 3-leaf stage smooth crabgrass at Mississippi State University near Starkville, MS, in 2022.

Active Ingredient	MSO[a] (% v/v)	Active Ingredient Rate		Trade name	Manufacturer	Formulation	Product rate
		lb. a.i. acre^{-1}	kg a.i. ha^{-1}			lb. gal^{-1}	fl. oz. acre^{-1}
Non-treated	-	-	-	-	-	-	-
Quinclorac	0.25	0.75	0.84	Drive XLR8	BASF	1.5	64
Quinclorac	0.25	6.75	7.6	Drive XLR8	BASF	1.5	576

[a]Methylated Seed Oil (Woznica et al. 2003)

Glutathione *S*-transferase Enzyme Assay

1. Tissue extracts were prepared by homogenizing leaf tissue (3-leaf-stage) in liquid nitrogen. Extraction was in 1 ml extraction buffer containing 0.2 M Tris-HCl (pH 7.5), 1 mM ethylenediaminetetraacetic (EDTA) and 10% polyvinylpolypyrrolidone (PVPP), then centrifuged for 20 minutes at 12,000 rpm then filtered.
2. A concentration of 10 µl from each filtered sample was placed in a 96-well plate. GST assay kit (Sigma Aldrich CS0410, St. Louis, MO) was used according to the manufacturer's instructions.
3. Enzyme activities were determined spectrophotometrically using a plate reader (Agilent BioTek Synergy LX Multi-Mode Reader, Fisher Scientific Company L.L.C., Pittsburgh, PA, USA) by measuring the conjugation of L-glutathione (GSH) to 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm over 6 min. Enzyme activity was calculated as GST specific activities.

OBJECTIVE

Identify the GST enzyme activity in resistant populations (MSU1 and MSU2) relative to a susceptible biotype (S).

HYPOTHESIS

GST activity is elevated in the MSU1 and MSU2 populations.

RESULTS

- GST activity was elevated in the MSU1 and MSU2 populations relative to the susceptible population. Activity was similar amongst two suspected resistant populations (Figure 1, Table 2).
- Pairwise comparisons test reveal differences in GST specific activity between the susceptible and the two resistant populations (Table 3).

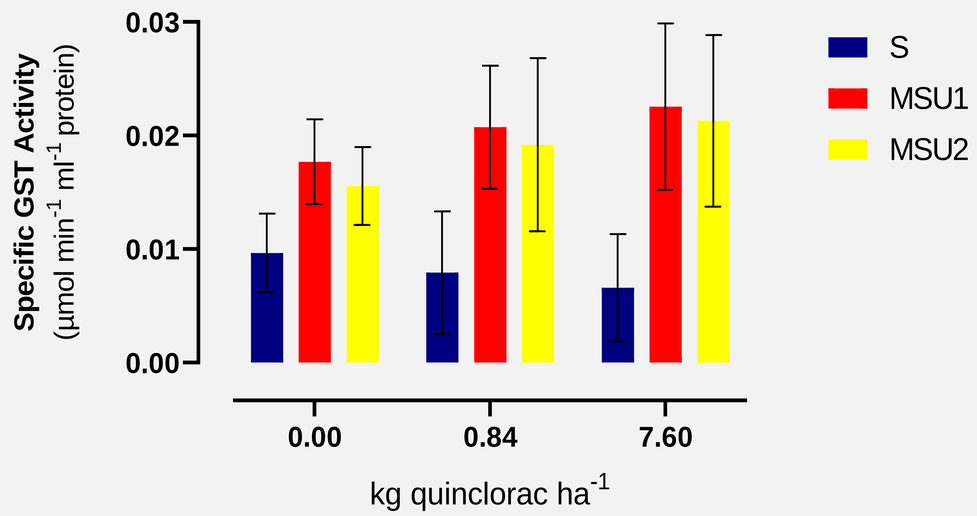

Figure 1. Specific glutathione S-transferase (GST) activity towards 1-chloro-2,4-dinitrobenzene (CDNB) in smooth crabgrass tissues with quinclorac treatment at 3 DAT. Values are presented as mean ± SE.

Table 2. Specific glutathione S-transferase (GST) activity towards 1-chloro-2,4-dinitrobenzene (CDNB) in smooth crabgrass with quinclorac treatment at 3 DAT. Values are presented as mean ± SE.

Population(s)	Specific GST (CDNB) Activity µmol min^{-1} ml^{-1} protein		
	Untreated Control	0.84 kg quinclorac ha^{-1}	7.6 kg quinclorac ha^{-1}
MSU1	0.01769363 ± 0.00199885	0.0207424 ± 0.00199885	0.02253623 ± 0.00199885
MSU2	0.01554487 ± 0.00199885	0.01918466 ± 0.00199885	0.02128945 ± 0.00199885
S	0.00966561 ± 0.00199885	0.00792594 ± 0.00199885	0.00659541 ± 0.00199885

Table 3. Pairwise F-test comparisons of specific GST activity on smooth crabgrass among quinclorac rates. Blue boxes represent statistical difference between population.

Population and Rate (kg quinclorac ha-1)	MSU1 (0)	MSU1 (0.84)	MSU1 (7.6)	MSU2 (0)	MSU2 (0.84)	MSU2 (7.6)	S (0)	S (0.84)	S (7.6)
MSU1 (0)		0.285	0.0917	0.4501	0.5998	0.2081	0.0061	0.001	0.0002
MSU1 (0.84)	0.285		0.528	0.0708	0.5836	0.8472	0.0002	<0.0001	<0.0001
MSU1 (7.6)	0.0917	0.528		0.0161	0.2403	0.6607	<0.0001	<0.0001	<0.0001
MSU2 (0)	0.4501	0.0708	0.0161		0.2027	0.0464	0.0417	0.009	0.0024
MSU2 (0.84)	0.5998	0.5836	0.2403	0.2027		0.4593	0.0013	0.0002	<0.0001
MSU2 (7.6)	0.2081	0.8472	0.6607	0.0464	0.4593		0.0001	<0.0001	<0.0001
S (0)	0.0061	0.0002	<0.0001	0.00417	0.0013	0.0001		0.5405	0.2816
S (0.84)	0.001	<0.0001	<0.0001	0.009	0.0002	<0.0001	0.5405		0.6395
S (7.6)	0.0002	<0.0001	<0.0001	0.0024	<0.0001	<0.0001	0.2816	0.6395	

CONCLUSION/DISCUSSION

- Quinclorac resistance in smooth crabgrass may be due to enhanced GST activity, which results in an enhanced capacity to detoxify the herbicide via glutathione conjugation.
- Elevated GST activity may be due to several confounding factors—principally the lack of tissue disruption experienced in susceptible populations due to excess accumulation of cyanide (Abdallah et al. 2006).
- Future research seeks to identify potential target-site mutations contributing to this phenomenon.

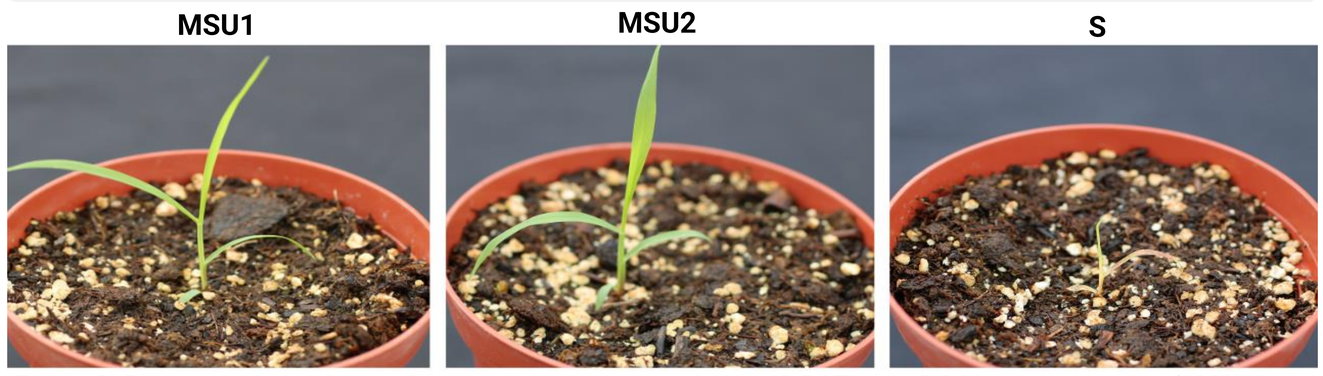

Figure 2. Photos depicting injury differences between populations 3 DAT after 0.84 kg quinclorac ha^{-1}. MSU1 and MSU2 are resistant populations. S is susceptible smooth crabgrass.

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