Differential regulation of antioxidant enzymes in *Frankliniella occidentalis* (Thysanoptera: Thripidae) exposed to thermal stress

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Frankliniella occidentalis is an invasive insect pest that incites damage to ornamental and agronomic crops on a global scale. In this study, the effects of temperature on gene expression and enzyme activity were studied for superoxide dismutase (SOD), peroxidase (POD), and glutathione-S-transferase (GST) in *F. occidentalis*. SOD, POD and GST enzyme activity increased significantly at 35-37°C but declined as the temperature increased to 41°C. In a time course study at 35°C, SOD, POD and GST activities were significantly elevated at 0.5, 1 and 2 h in comparison to the control at 26°C. Expression patterns were evaluated for the three antioxidant genes under high and low temperature stress. In a time course study at -4°C, *SOD*, *POD* and *GST* expression peaked at 1 h and declined at 2 h of exposure. In contrast, when transcription was monitored at 35°C, expression was lowest at 1 h and increased at 2 h. The results provide data that will be useful in deciphering the role of antioxidant enzymes in the adaptation of *F. occidentalis* to climate change.

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Abstract

Frankliniella occidentalis is an invasive insect pest that incites damage to ornamental and agronomic crops on a global scale. In this study, the effects of temperature on gene expression and enzyme activity were studied for superoxide dismutase (SOD), peroxidase (POD), and glutathione-S-transferase (GST) in *F. occidentalis*. SOD, POD and GST enzyme activity increased significantly at 35-37°C but declined as the temperature increased to 41°C. In a time course study at 35°C, SOD, POD and GST activities were significantly elevated at 0.5, 1 and 2 h in comparison to the control at 26°C. Expression patterns were evaluated for the three antioxidant genes under high and low temperature stress. In a time course study at -4°C, *SOD*, *POD* and *GST* expression peaked at 1 h and declined at 2 h of exposure. In contrast, when transcription was monitored at 35°C, expression was lowest at 1 h and increased at 2 h. The results provide data that will be useful in deciphering the role of antioxidant enzymes in the adaptation of *F. occidentalis* to climate change.

Keywords *Frankliniella occidentalis*; thermal stress; oxidative defense; enzymatic activity; gene expression

Introduction

Temperature impacts the reproduction, development, and distribution of insects (Cossins & Bowle 1987; Worner 1998; Bale *et al*. 2002), and extreme temperatures are known elicitors of reactive oxygen species (ROS) in invertebrates. The excessive generation of ROS can damage cellular constituents, including lipids, proteins, and nucleic acids (Halliwell 1989; Kamata & Hirata 1999; Foyer & Noctor 2005; Lopez-Martinez *et al.* 2008). In order to survive, insects reduce or detoxify ROS through the action of antioxidants; these function as enzymatic and non-enzymatic scavengers that reduce lipid peroxidation and decrease damage to nucleic acids and proteins (Felton & Summers 1995; Lyakhovich *et al.* 2006; Krishnan *et al.* 2007). Peroxidase (POD), superoxide dismutase (SOD), and glutathione-S-transferase (GST) are antioxidant enzymes that defend cells from excessive levels of ROS (Felton & Summers 1995; Wang *et al.* 2001; Dubovskiy *et al.* 2008; Liu *et al.* 2020). SOD functions by degrading superoxide anions to hydrogen peroxide (H_2O_2) and oxygen, and H_2O_2 is subsequently converted to H_2O by POD (Kashiwagi *et al.* 1997; Wang & Li 2002; Liu & Ma 2007). GSTs function to detoxify compounds that are produced from lipid peroxidation (Ahmad *et al.* 1991; Kono & Shishido 1992; Dubovskiy *et al.* 2008).

The western flower thrips (WFT), *Frankliniella occidentalis*, damages both vegetables and ornamental plants on a global scale and is especially problematic in greenhouses (Morse & Hoddle 2006; Kirk & Terry 2015; Mouden et al. 2017). In addition to direct damage, WFT causes serious damage to plants by transmitting plant viruses such as the Tomato Spotted Wilt Virus (Pappu *et al.* 2009; Tomitaka 2019). WFT is endemic to the western region of North America and has spread

globally due to the transportation of agricultural products (Reitz 2009; Kirk & Terry 2015). According to the CABI Invasive Species Compendium, *F. occidentalis* has been discovered on all continents except Antarctica (https://www.cabi.org/isc/datasheet/24426). In mainland China, *F. occidentalis* was initially found in Beijing in 2003 (Zhang et al., 2003) and has since been discovered in at least ten provinces (Wu *et al.* 2017).

Previous studies indicated that temperature impacts development, sex ratios, reproduction, population growth, and mortality of *F. occidentalis* (Li *et al.* 2007; Li *et al.* 2011a; Zhang *et al.* 2012). During the hot summers in subtropical China, high temperatures may cause oxidative stress to *F. occidentalis*, particularly in greenhouses (Wang *et al.* 2014). Previous studies demonstrated that the expression of genes encoding catalase (CAT) and subsequent enzymatic activity were altered in *F. occidentalis* exposed to hot and cold stress (Shi *et al.* 2013; Qin *et al.* 2017). However, the impact of high and low temperatures on other antioxidant enzymes in *F. occidentalis* is unclear.

In this study, we investigated the effect of temperature stress on POD, SOD, and GST in *F. occidentalis*. The results provide important data on how antioxidant enzymes counteract oxidative damage in the WFT and provide a more comprehensive framework for understanding thermal tolerance in *F. occidentalis*.

Materials & Methods

Insects and temperature treatments

Frankliniella occidentalis populations were collected in Hangzhou, China, in 2008 and were reared with kidney bean, *Phaseolus vulgaris Linn*, at 25 ± 0.5°C and 70±5% relative humidity with a 16:8 h light:dark photoperiod as outlined by Li *et al.* (2011b). Newly emerged 2nd instar larvae were collected, and pools of 100 were exposed to high (31, 33, 35, 37, 39 or 41°C) or low (0, -2, -4, -6, -8 and -10°C) temperatures for 1 h in glass tubes as described (Chang *et al*., 2017). Through the results of the pre-experiment, 35°C and -4°C were decided as the model temperature on *F. occidentalis*，which was further explored by subjecting groups of individuals to 0, 0.5, 1, and 2 h of thermal stress; controls were maintained at 26°C (0 h time point). Following thermal stress, larvae were incubated at 26°C for 30 min and used a brush to touch it gently, thrips would be identified as surviving if it respond to the stimulus. Survivors were frozen in liquid nitrogen and stored at -80°C for future use. Four replicate pools were used for each temperature and time period.

Determination of enzyme activity

The assay kit used for protein extraction was from Nanjing Jiancheng Bioengineering Institute, Jiangsu, China. Treated samples were homogenized in 0.9% saline and then centrifuged at 2,500 × rpm for 10 min (Jia *et al*., 2011). Supernatants containing the enzyme fractions were collected, and protein content was determined using the Bradford (1976) method.

POD and SOD activities were assessed with commercially available kits (Qin *et al*., 2017). Absorbance values were obtained using the BioTek PowerWave HT Microplate

89 Spectrophotometer (Bio-Tek Instruments Inc., USA). GST activity was measured as a function of
90 reduced glutathione (GSH) using 10 mg of cytosolic protein and 1-chloro-2,4-dinitrobenzene
91 (CDNB; Shanghai Chem, Shanghai, China) as a substrate (Habig *et al.*, 1974; Attig *et al*., 2014).
92 GST activity was determined at A_{340} with a microplate spectrophotometer (Shanghai Xinmao
93 Instrument, Shanghai, China), and results are shown as μmol GSH-CDNB/min/mg protein.

94

95 **RNA isolation, partial cloning of *SOD*, *POD* and *GST*, and qRT-PCR**

96 The SV Total RNA Isolation System was used to isolate RNA from *F. occidentalis* as
97 recommended by the manufacturer (Promega, San Luis Obispo, CA, USA). RNA quality and
98 concentration were determined, and cDNA was generated from total RNA with the First Strand
99 cDNA Synthesis Kit (Clontech, Mountain View, CA, USA) as outlined previously (Zhang *et al.*,
100 2019).

101 "SOD", "POD" and "GST" were used as key words to search related gene fragments in the
102 transcriptome database of *F. occidentalis* after temperature treatments, respectively. According to
103 the obtained gene fragments, the corresponding primers (Table 1) were designed for fragment
104 verification. PCR products were cloned and sequenced as described (Zhang *et al.*, 2019).

105 Quantitative real-time reverse transcriptase PCR (qRT-PCR) was conducted using the
106 protocols described by Zhang *et al*. (2019). Specific primers (Table 1) were designed according to

the above verified fragments for qRT-PCR. Melting curve analysis was executed to analyze the specificity of PCR products. According to the evaluation results of Zheng *et al*. (2014) on the reliability of reference genes in *F. occidentalis*, Expression levels were normalized using reference genes *GAPDH* and *EF-1* for high and low temperature stress, respectively.

Statistical analyses

qRT-PCR data analyzing was conducted in Bio-Rad CFX Manager 3.1 software. The average Ct values of biological replicates were used to calculate the relative expression levels. The results of qPCR were analyzed with the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). Firstly, for all test samples and calibration samples, the Ct value of the housekeeping gene were used to normalize the Ct value of the target gene. Normalized results were ΔCt(test) and ΔCt(calibrator), respectively. And using ΔCt(calibrator) to normalize ΔCt(test) , ΔΔCt was obtained. The ratio of expression level was calculated by $2^{-\Delta\Delta Ct}$. Significant differences were detected by one-way analysis of variance (ANOVA) and Duncan's multiple comparisons test. Data were analyzed with SPSS v. 16.0 and considered significant at $P < 0.05$.

Results

Effect of high temperature stress on antioxidant activity

SOD activity increased with rising temperature from 31 to 37°C and was highest at 37°C. The activity of SOD activity began to decline at 39°C, and the level at 41°C was significantly lower than 37°C ($F_{6,19}$=4.245, P<0.05) (Fig. 1A). A similar pattern was observed with POD, where activity rose with increasing temperature, peaked at 35°C and was significantly lower at 41°C than 35°C ($F_{6,21}$=7.089, P<0.05) (Fig. 1B). GST activity was highest at 35°C (Fig. 1C) and began to decline with increasing temperature ($F_{6,21}$=8.312, P<0.05).

Temporal changes in antioxidant enzyme activity at 35°C

Antioxidant enzyme activity was significantly higher than the control (0 h, 26°C) when insects were exposed to 35°C for 0.5, 1 and 2 (SOD: $F_{3,10}$=10.005, P<0.05; POD: $F_{3,12}$=8.037, P<0.05; GSTs: $F_{3,10}$=5.815, P<0.05). No significant differences in antioxidant activity were detected between 0.5, 1 and 2 h of exposure (Fig. 2).

Expression of antioxidant genes in response to heat and cold stress

The expression of antioxidant genes was evaluated at 31, 33, 35, 37, 39 and 41°C; 26°C served as a control. *SOD* expression at 31°C was significantly lower than the control temperature of 26°C and showed further decreases in expression at 35°C to 37°C; however, expression peaked at 39°C and was comparable to the control (26°C) (Fig. 3A). With the exception of 39°C, *SOD* expression was inhibited by high temperatures, and the lowest expression level was observed at 35°C

($F_{6,18}$=71.329, P<0.05). In contrast, *POD* expression levels at 33°C and 39°C were significantly higher than the control at 26°C; however, expression levels at 31, 35, 37, and 41°C were significantly lower than the control and were inhibited by heat stress ($F_{6,17}$=1386.107, P<0.05) (Fig. 3B). *GST* expression was suppressed relative to the control at all elevated temperatures ($F_{6,20}$=652.115, P<0.05) (Fig. 3C). All three antioxidant genes shared an interesting increase in expression at 33°C and 39°C relative to the other elevated temperatures.

Expression of the three antioxidant genes was also evaluated in response to low temperature stress at 0, -2, -4, -6, -8 and -10°C. *SOD* expression showed a significant decline at 0 and -2°C relative to the control at 26°C; however, expression increased at -4°C and was comparable to the control at 26°C (Fig. 4A) ($F_{6,19}$=180.242, P<0.05). Interestingly, *SOD* expression declined and was significantly lower at -6, -8, and -10°C (Fig. 4A). *POD* expression was also significantly higher at -4°C than -0, -2, -6, -8, and -10°C (Fig. 4B). Similarly, *GST* expression was significantly higher at -4°C than -0, -2, -6, -8, and -10°C and was lowest at -6°C ($F_{6,20}$=183.310, P<0.05) (Fig. 4C).

Temporal changes in the expression of antioxidant genes

Compared to the control (0 h, 26°C), *SOD* and *GST* expression decreased significantly when 2nd instar larvae were exposed to 35°C for 0.5, 1 and 2 h (*SOD:* $F_{3,11}$=2189.970, P<0.05; *GST:* $F_{3,11}$=1709.476, P<0.05) and was lowest at the 1 h exposure period (Fig. 5A,C). *POD* expression was significantly upregulated at the 2 h time point and was higher than expression levels at 0

(control), 0.5 and 1 h ($F_{3,10}$=3425.185 P<0.05) (Fig. 5B).

After exposure to -4°C, the expression levels of the three antioxidant genes decreased significantly when compared to the control (*SOD:* $F_{3,10}$=814.378, P<0.05; *POD:* $F_{3,9}$=7339.947, P<0.05; *GST:* $F_{3,9}$=910.209, P<0.05). Interestingly, all three genes showed a peak in expression after a 1 h exposure to -4°C; however, it should be noted that expression at 1 h was lower than the control (Fig. 6).

Discussion

Insects are poikilotherms that are greatly impacted by temperature fluctuations (Cossins & Bowler 1987; Worner 1998; Bale *et al.* 2002). When exposed to thermal stress, insects sustain oxidative damage at the cellular level and respond with surplus levels of ROS (Lopez-Martinez *et al.* 2008; Cui *et al.* 2011; Li & Sattar 2019). ROS can cause direct damage to biological macromolecules and can also incite genetic mutations and cell death (Ryter *et al.* 2007). Antioxidant enzymes function to eliminate or reduce ROS levels in insects. Previous studies showed that SOD, POD and GST play important roles in the response of insects to ROS (Abele *et al.* 1998; An & Choi 2010; Celino *et al.* 2011; Liu *et al.* 2020). In this study, SOD, POD and GST activity increased significantly in response to high temperatures, which suggests that these enzymes function to remove excess ROS during thermal stress. Thus, our results are consistent with those reported for *Bactrocera dorsalis*, *Bombyx mori*, *Mononychellus mcgregori*, *Diaphorina citri* and *Neoseiulus cucumeris* (Lee *et al.* 2005; Jia *et al.* 2011; Marutani-Hert *et al.* 2010; Lu *et al.* 2014; Zhang *et al.*

183 2014). In a previous report, low temperature stress significantly altered SOD, POD, CAT and GST
184 activity in *F. occidentalis* (Shi *et al.* 2013). The increase in POD activity was likely the result of
185 elevated levels of SOD activity in response to H_2O_2. Although increased levels of antioxidant
186 enzymes suggests a defensive function of these enzymes in counteracting the negative effect of
187 ROS, there were no significant differences in SOD, POD or GST activity at 0.5, 1.0 and 2.0 h of
188 exposure to 35°C (Fig. 2). This might indicate that antioxidant enzyme activity is very sensitive to
189 high temperature stress and reached a threshold level at 0.5 h or earlier.
190 Many researchers have shown that temperature stress can lead to changes in antioxidant gene
191 expression in insects (Yang *et al.* 2019; Xia *et al.* 2019; Lu *et al.* 2017). Previous results showed
192 that temperature stress inhibited the transcription of *SOD*, *POD, GST* and related enzymes in
193 *Mythimna separate*, *Apis cerana cerana* and *Helicoverpa armigera* (Shen *et al.* 2016; Yang *et al.*
194 2019; Xia *et al.* 2019). These results reflect the diversity of molecular responses in organisms
195 exposed to external stress. In addition to recruiting antioxidant enzymes to remove ROS in
196 response to thermal stress, insects also respond by synthesizing osmoprotectants, altering
197 membrane lipid content, and expressing heat shock proteins (Chen & Kang 2005). A previous
198 study demonstrated that both high and low thermal stress induced *CAT* expression in *F.*
199 *occidentalis* (Qin *et al.* 2017); therefore, the down-regulation of *POD* in this study might be
200 attributed to increased expression of *CAT*. In the case of *SOD* and *GST*, thermal stress may induce
201 the synthesis of unknown substances that could inhibit transcription. Further research is needed to
202 validate or disprove these conjectures.

Differential regulation of antioxidant genes and enzymes has been reported in insects; for example, *POD*, *CAT* and *SOD* expression patterns were not necessarily correlated with enzyme activity during high temperature stress in *Mononychellus mcgregori* (Lu *et al.* 2017). In larvae of *Bombyx mori*, carboxylesterase activity was not correlated with gene expression (Liu *et al.* 2010). Elevated protein levels can be stressful for the organism, and the organism may inhibit gene transcription to maintain homeostasis. Conversely, if protein levels fall to a suboptimal level, the cell may respond by promoting transcription. Furthermore, transcription is often followed by post-transcriptional processing, degradation of transcription products, translation, post-translational processing and further modifications that impact protein levels. Further research is needed to understand the mechanisms that control the response of *F. occidentalis* to thermal stress.

Conclusions

This study reveals differential regulation of antioxidant gene expression and enzyme production in response to thermal stress. The results confirm the importance of antioxidant enzymes in modulating the response to thermal stress in *F. occidentalis*, and provide new avenues for further study of antioxidant mechanisms and physiological responses of *F. occidentalis*. The inconsistencies between gene expression and enzyme activity further illustrate the complexity of thermal adaptation in *F. occidentalis*. Future multidisciplinary research in genomics, transcriptomics, proteomics, and metabolomics will help explain the underlying mechanisms of thermal adaptation in *F. occidentalis*.

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Figure 1

Effect of high temperature stress on antioxidant enzyme activity in 2nd instar larvae of *F. occidentalis*.

Panels: (A) SOD, superoxide dismutase; (B) POD, peroxidase; (C) GST, glutathione-S-transferase. Larvae were exposed to 31, 33, 35, 37, 39, and 41°C for 1 h in glass tubes; 26°C was used as the control. Each value represents the mean (±SE) of four replications. Columns labeled with different letters indicate significance at $P < 0.05$ using ANOVA (Tukey's b(K) test).

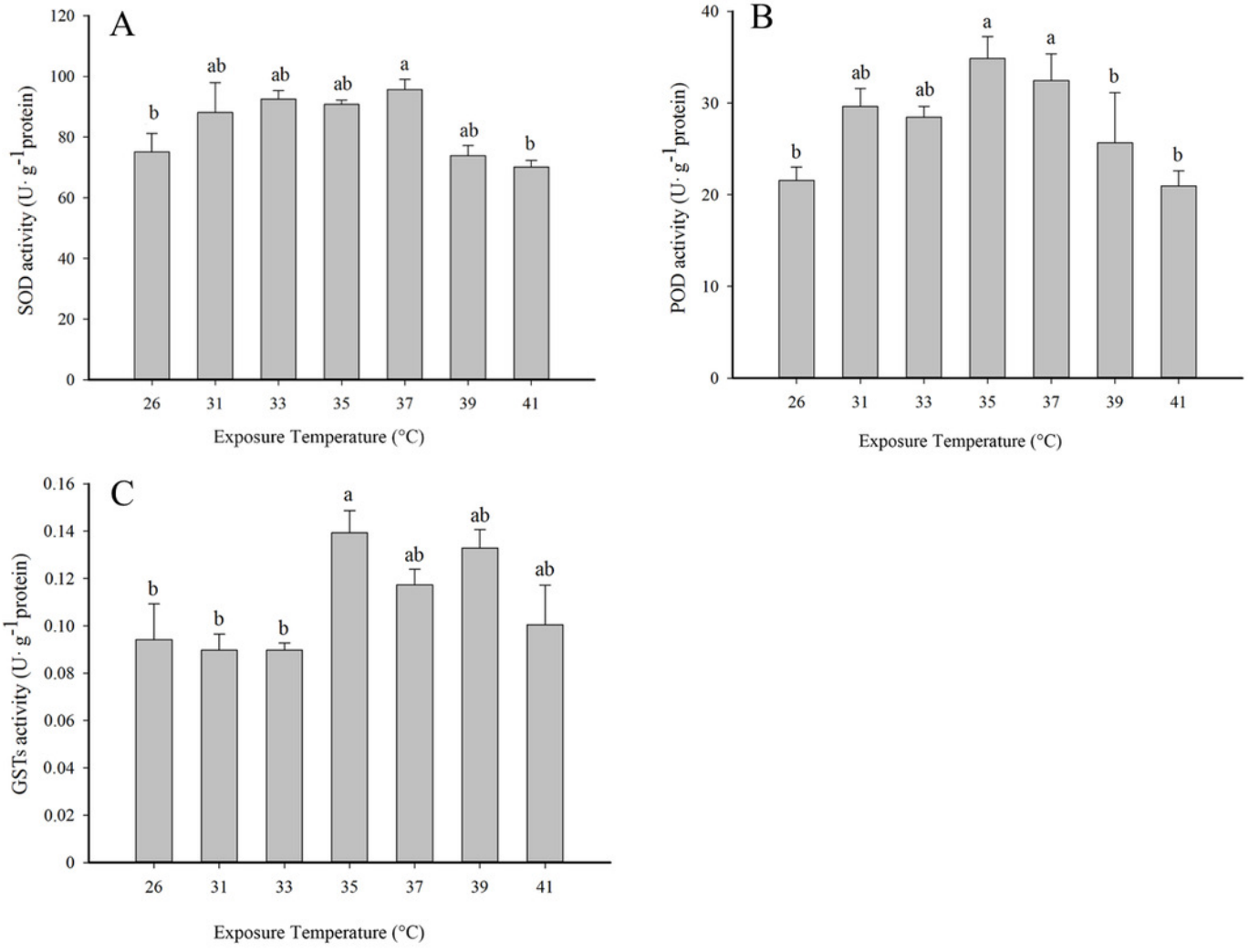


Figure 2

Temporal changes in antioxidant enzyme activity in 2nd instar larvae of *F. occidentalis* exposed to 35^{o}C.

Panels: (A) SOD, superoxide dismutase; (B) POD, peroxidase; (C) GST, glutathione-S-transferase. *F. occidentalis* was exposed to 35^{o}C for 0.5, 1, and 2 h and then analyzed for enzyme activity. The control group was maintained at 26°C (0 h time point). Columns show the mean (±SE) of four replications, and columns labeled with different letters indicate significance at $P< 0.05$ in ANOVA (Tukey's b(K) test).

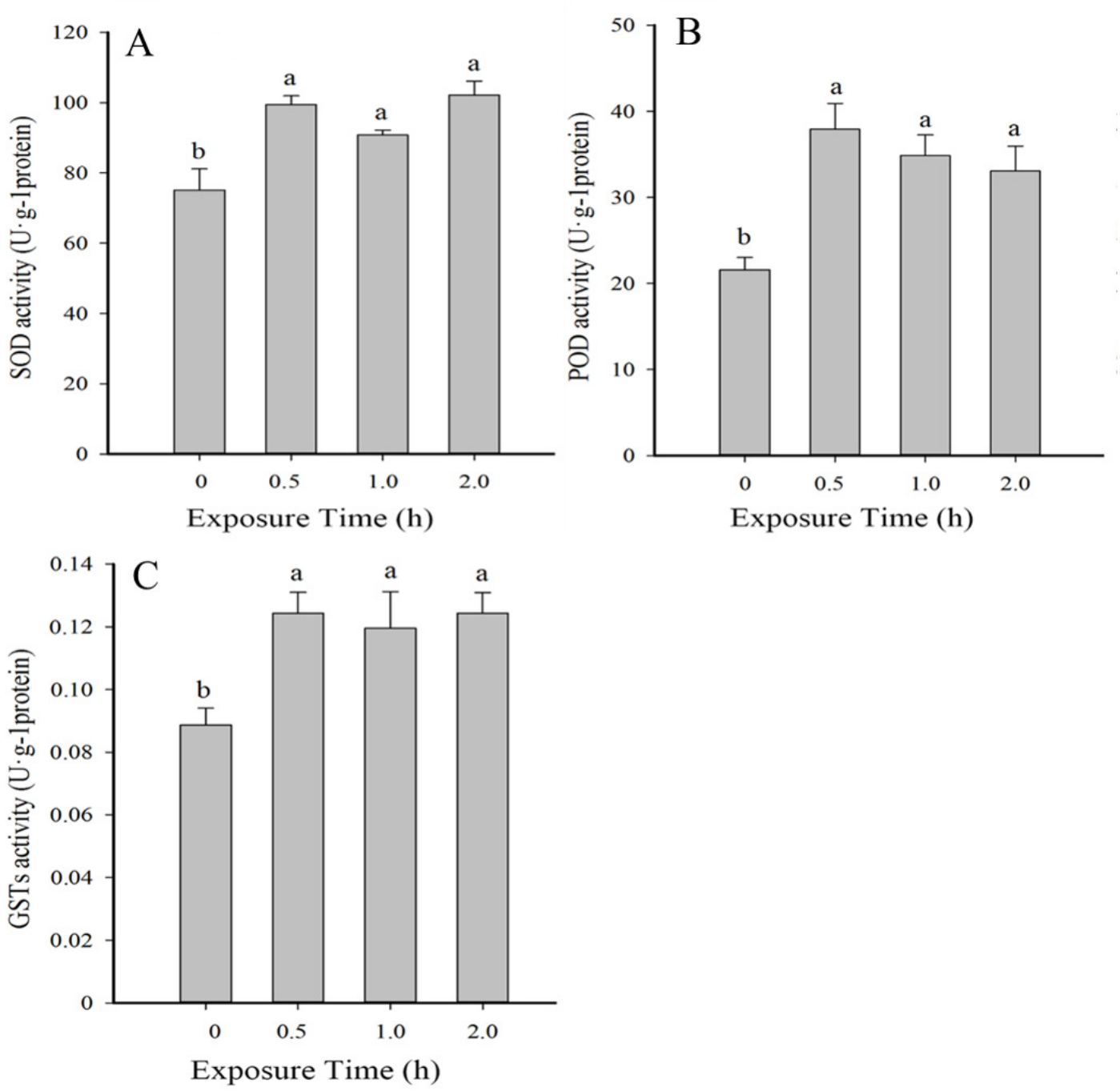
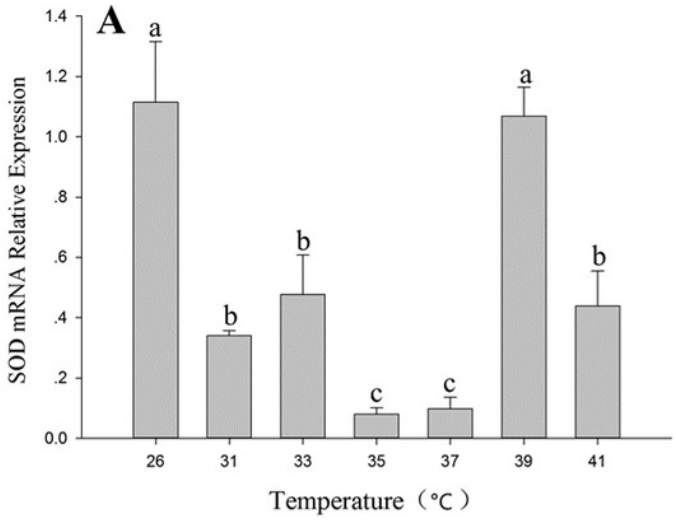
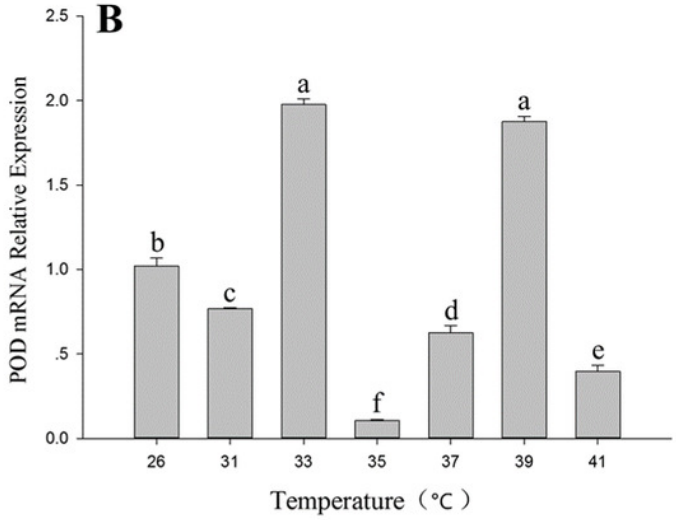

A
SOD activity (U·g-1protein)
120
100
80
60
40
20
0
b
a
a
a
0
0.5
1.0
2.0
Exposure Time (h)
B
POD activity (U·g-1protein)
50
40
30
20
10
0
b
a
a
a
0
0.5
1.0
2.0
Exposure Time (h)
C
GSTs activity (U·g-1protein)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
b
a
a
a
0
0.5
1.0
2.0
Exposure Time (h)

Figure 3

Effect of high temperature stress on expression of antioxidant genes in 2nd instar larvae of *F. occidentalis*.

Panels: (A) SOD, superoxide dismutase; (B) POD, peroxidase; (C) GST, glutathione-S-transferase. Larvae were exposed to 31, 33, 35, 37, 39, and 41^{o}C for 1 h in glass tubes; 26°C was used as the control. Expression levels were normalized with respect to *GAPDH*. Values represent the mean (±SE) of four replications, and columns labeled with different letters indicate significance at $P< 0.05$ in ANOVA (Tukey's b(K) test).


A
SOD mRNA Relative Expression
1.4
1.2
1.0
.8
.6
.4
.2
0.0
a
b
b
c
c
a
b
26
31
33
35
37
39
41
Temperature（℃）


B
POD mRNA Relative Expression
2.5
2.0
1.5
1.0
.5
0.0
b
c
a
f
d
a
e
26
31
33
35
37
39
41
Temperature（℃）

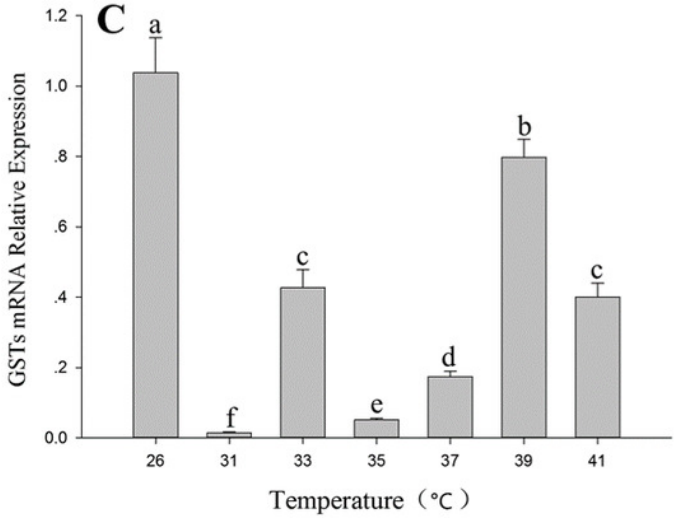

C
GSTs mRNA Relative Expression
1.2
1.0
.8
.6
.4
.2
0.0
a
f
c
e
d
b
c
26
31
33
35
37
39
41
Temperature（℃）

Table 1(on next page)

Primers used in the study

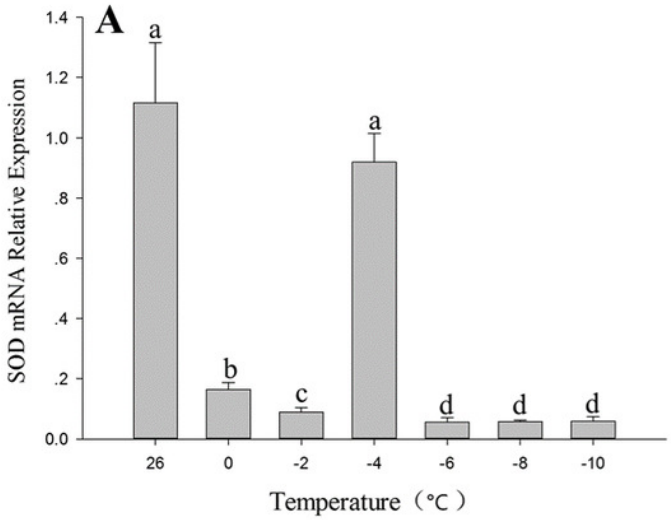
1

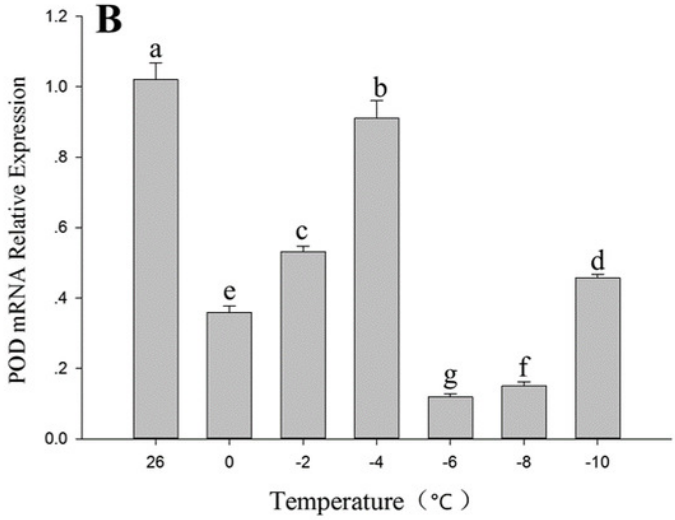
Primer name	Primer sequences	Tm (°C)	Length (bp)	E^c(%)
DP-*SOD*-F	AATGCTGCGTTCTCTGTTGTG	58.7	335	
DP-*SOD*-R	TCTGGTTTTGTTGTTTCAGGAGT	58.4		
DP-*POD*-F	CAACCCCGACCAGCCCTAC	62.3	600	
DP-*POD*-R	AAAAGGGGAAATCGGTGTCG	61.4		
DP-*GST*-F	TGACCGTGAACCAGACCGAG	61.3	431	
DP-*GST*-R	GATGCCGAAAATACTGAGTGTGG	61.4		
qPCR-*SOD*-F	GAAATAACTGGTTCCAAGGCACT	59.6	125	91.8
qPCR-*SOD*-R	AATGCTGCGTTCTCTGTTGTG	58.7		
qPCR-*POD*-F	CCGCACTGGGACGACGAGAC	65.8	235	96.4
qPCR-*POD*-R	CGATGAGCGAGTGGAAGTATCTGAA	64.8		
qPCR-*GST*-F	GCTGCTGCTGTGCTGGATTA	59.7	170	90.0
qPCR-*GST*-R	ACCGTGAACCAGACCGAGAC	59.4		
EF-1-F	TCAAGGAACTGCGTCGTGGAT	58.6	130	95.4
EF-1-R	ACAGGGGTGTAGCCGTTAGAG			
GAPDH-F	AAGGGTGCTCAGGTTGTTGCT	56.5	89	104.4
GAPDH-R	CGACCGTGGGTGGAGTCATAT			

Figure 4

Effect of low temperature stress on expression of antioxidant genes in 2nd instar larvae of *F. occidentalis*.

Panels: (A) SOD, superoxide dismutase; (B) POD, peroxidase; (C) GST, glutathione-S-transferase. Larvae were exposed to 0, -2, -4, -6, -8 and -10°C for 1 h in glass tubes; 26°C was used as the control. Expression levels were normalized with respect to *EF-1*. Values represents the mean (±SE) of four replications, and columns labeled with different letters indicate significance at $P< 0.05$ in ANOVA (Tukey's b(K) test).


A
SOD mRNA Relative Expression
1.4
1.2
1.0
.8
.6
.4
.2
0.0
a
b
c
a
d
d
d
26
0
-2
-4
-6
-8
-10
Temperature（℃）


B
POD mRNA Relative Expression
1.2
1.0
.8
.6
.4
.2
0.0
a
e
c
b
g
f
d
26
0
-2
-4
-6
-8
-10
Temperature（℃）

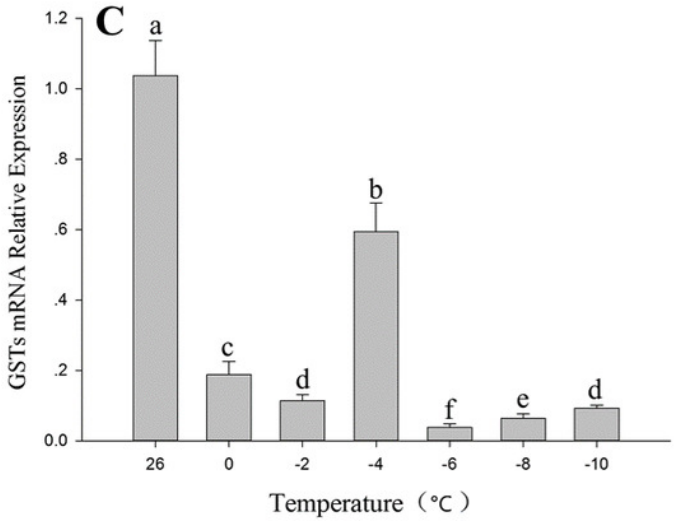
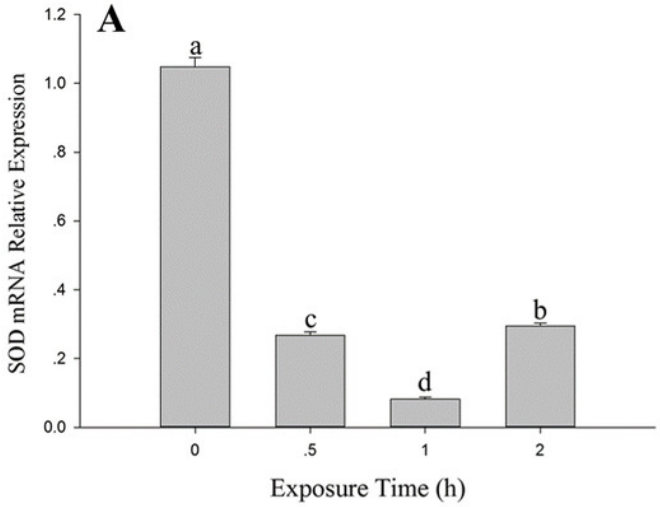
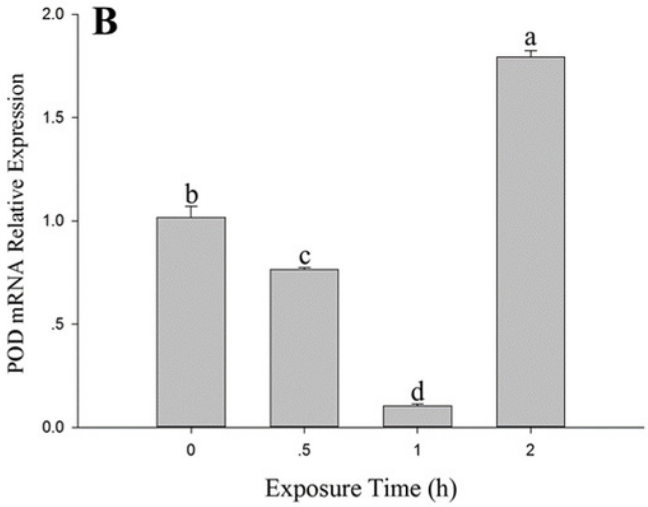

C
GSTs mRNA Relative Expression
1.2
1.0
.8
.6
.4
.2
0.0
a
c
d
b
f
e
d
26
0
-2
-4
-6
-8
-10
Temperature（℃）

Figure 5

Temporal changes in the expression of antioxidant genes in 2nd instar larvae of *F. occidentalis* exposed to 35^{o}C.

Panels: (A) SOD, superoxide dismutase; (B) POD, peroxidase; (C) GST, glutathione-S-transferase. *F. occidentalis* was exposed to 35^{o}C for 0.5, 1, and 2 h and then analyzed for gene expression; the control group was maintained at 26°C (0 h time point). Expression levels were normalized with respect to *GAPDH*. Columns show the mean (±SE) of four replications, and columns labeled with different letters indicate significance at $P< 0.05$ in ANOVA (Tukey's b(K) test).


A
SOD mRNA Relative Expression
1.2
1.0
.8
.6
.4
.2
0.0
a
c
d
b
0
.5
1
2
Exposure Time (h)


B
POD mRNA Relative Expression
2.0
1.5
1.0
.5
0.0
b
c
d
a
0
.5
1
2
Exposure Time (h)

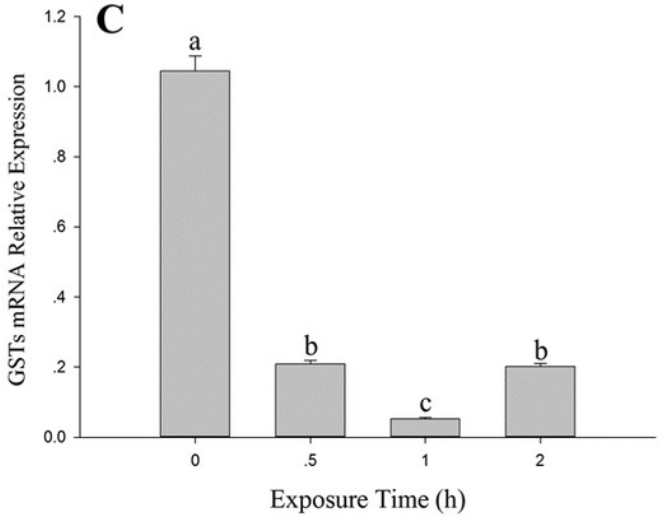
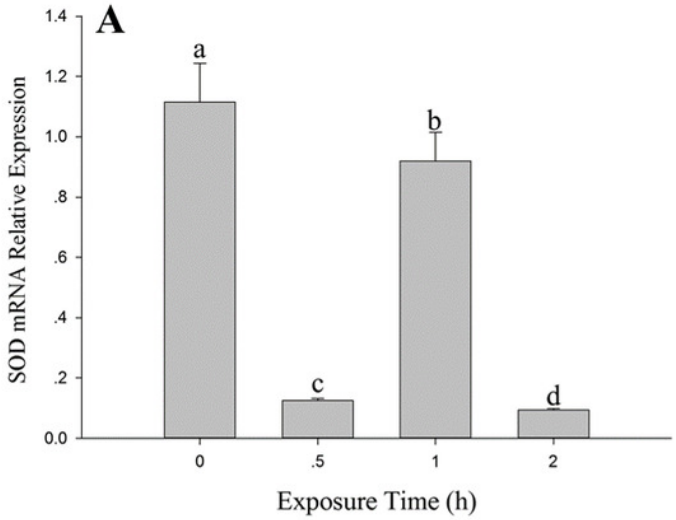
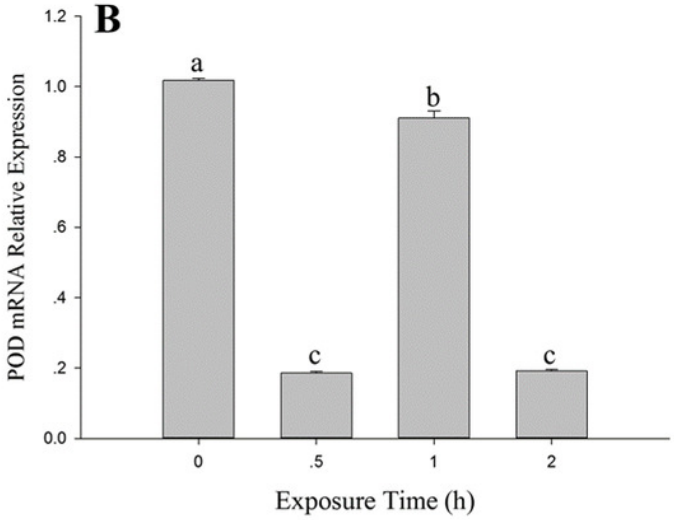

C
GSTs mRNA Relative Expression
1.2
1.0
.8
.6
.4
.2
0.0
a
b
c
b
0
.5
1
2
Exposure Time (h)

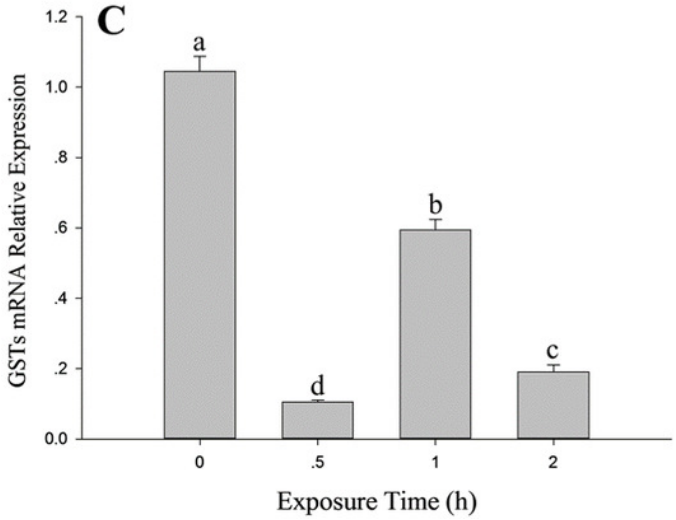
Figure 6

Temporal changes in the expression of antioxidant genes in 2nd instar larvae of *F. occidentalis* exposed to -4^{o}C.

Panels: (A) SOD, superoxide dismutase; (B) POD, peroxidase; (C) GST, glutathione-S-transferase. *F. occidentalis* was exposed to 4^{o}C for 0.5, 1, and 2 h and then analyzed for gene expression; the control group was maintained at 26°C (0 h time point). Expression levels were normalized with respect to *EF-1*. Columns show the mean (±SE) of four replications, and columns labeled with different letters indicate significance at $P< 0.05$ in ANOVA (Tukey's b(K) test).


A
SOD mRNA Relative Expression
1.4
1.2
1.0
.8
.6
.4
.2
0.0
a
b
c
d
0
.5
1
2
Exposure Time (h)


B
POD mRNA Relative Expression
1.2
1.0
.8
.6
.4
.2
0.0
a
b
c
c
0
.5
1
2
Exposure Time (h)


C
GSTs mRNA Relative Expression
1.2
1.0
.8
.6
.4
.2
0.0
a
d
b
c
0
.5
1
2
Exposure Time (h)