Differential regulation of antioxidant enzymes in *Frankliniella occidentalis* (Thysanoptera: Thripidae) exposed to thermal stress (#58282)

First submission

Guidance from your Editor

Please submit by **5 Apr 2021** for the benefit of the authors (and your $200 publishing discount) .

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Author notes
Have you read the author notes on the guidance page?

Raw data check
Review the raw data.

Image check
Check that figures and images have not been inappropriately manipulated.

Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.

Files
Download and review all files from the materials page.

6 Figure file(s)
1 Table file(s)
2 Raw data file(s)

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.
- Speculation is welcome, but should be identified as such.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

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

Jia-Wen Yuan [1], **Yutao Zheng** [1], **Ya-Wen Chang** [1], **Jing Bai** [1], **Jing Qin** [1], **Yu-Zhou Du** [Corresp. 1, 2]

[1] College of Horticulture and Plant Protection & Institute of Applied Entomology, Yangzhou University, Yangzhou, China

[2] Joint International Research Laboratory of Agriculture and Agri-Product Safety, Yangzhou University, Yangzhou, China

Corresponding Author: Yu-Zhou Du
Email address: yzdu@yzu.edu.cn

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.

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

Jia-Wen Yuan[1], Yu-Tao Zheng[1], Ya-Wen Chang[1], Jing Bai[1], Jing Qin[1], Yu-Zhou Du[1, 2]

[1] School of Horticulture and Plant Protection & Institute of Applied Entomology, Yangzhou University, Yangzhou 225009, P. R. China

[2] Joint International Research Laboratory of Agriculture and Agri-Product Safety, Yangzhou University, Yangzhou 225009, P. R. China

Corresponding author: Yu-Zhou Du, e-mail: yzdu@yzu.edu.cn

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

F. occidentalis populations were collected in Hangzhou, China, in 2008 and were reared at 25 ± 0.5°C with a 16:8 h light:dark photoperiod as outlined by Li *et al.* (2011b). Newly emerged 2nd

72 instar larvae were collected, and pools of 100 were exposed to high (31, 33, 35, 37, 39 or 41°C) or
73 low (0, -2, -4, -6, -8 and -10°C) temperatures for 1 h in glass tubes as described (Chang *et al*.,
74 2017). The temporal effect of 35°C and -4°C on *F. occidentalis* was further explored by subjecting
75 groups of individuals to 0, 0.5, 1, and 2 h of thermal stress; controls were maintained at 26°C (0 h
76 time point). Following thermal stress, larvae were incubated at 26°C for 30 min and mortality was
77 determined. Survivors were frozen in liquid nitrogen and stored at -80°C for future use. Four
78 replicate pools were used for each temperature and time period.
79
80 **Determination of enzyme activity**
81 Treated samples were homogenized in 0.9% saline and subjected to centrifugation as described
82 previously (Jia *et al*., 2011). Supernatants containing the enzyme fractions were collected, and
83 protein content was determined using the Bradford (1976) method.
84 POD and SOD activities were assessed with commercially available kits ~~as described~~ (Qin *et*
85 *al*., 2017). Absorbance values were obtained using the BioTek PowerWave HT Microplate
86 Spectrophotometer (Bio-Tek Instruments Inc., USA). GST activity was measured as a function of
87 reduced glutathione (GSH) using 10 mg of cytosolic protein and 1-chloro-2,4-dinitrobenzene
88 (CDNB; Shanghai Chem, Shanghai, China) as a substrate (Habig *et al.*, 1974; Attig *et al*., 2014).
89 GST activity was determined at A_{340} with a microplate spectrophotometer (Shanghai Xinmao
90 Instrument, Shanghai, China), and results are shown as μmol GSH-CDNB/min/mg protein.
91

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

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

98 Gene-specific primers (Table 1) were used to amplify cDNA from *F. occidentalis* based on
99 sequences obtained from the transcriptome (unpublished data). PCR products were cloned and
100 sequenced as described (Zhang *et al.*, 2019).

101 Quantitative real-time reverse transcriptase PCR (qRT-PCR) was conducted using the primers
102 in Table 1 and the protocols described by Zhang *et al*. (2019). Melting curve analysis was
103 executed to analyze the specificity of PCR products. Expression levels were normalized using
104 reference genes *GAPDH* and *EF-1* for high and low temperature stress, respectively.

105

106 **Statistical analyses**

107 The results of qPCR were analyzed with the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).
108 Significant differences were detected by one-way analysis of variance (ANOVA) and Duncan's
109 multiple comparisons test. Data were analyzed with SPSS v. 16.0 and considered significant at
110 $P < 0.05$.

111

112 Results

113 Effect of high temperature stress on antioxidant activity

114 SOD activity increased with rising temperature from 31 to 37°C and was highest at 37°C. The
115 activity of SOD activity began to decline at 39°C, and the level at 41°C was significantly lower
116 than 37°C ($F_{6,19}$=4.245, P<0.05) (Fig. 1A). A similar pattern was observed with POD, where
117 activity rose with increasing temperature, peaked at 35°C and was significantly lower at 41°C than
118 35°C ($F_{6,21}$=7.089, P<0.05) (Fig. 1B). GST activity was highest at 35°C (Fig. 1C) and began to
119 decline with increasing temperature ($F_{6,21}$=8.312, P<0.05).

120

121 Temporal changes in antioxidant enzyme activity at 35°C

122 Antioxidant enzyme activity was significantly higher than the control (0 h, 26°C) when insects
123 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;
124 GSTs: $F_{3,10}$=5.815, P<0.05). No significant differences in antioxidant activity were detected
125 between 0.5, 1 and 2 h of exposure (Fig. 2).

126

127 Expression of antioxidant genes in response to heat and cold stress

128 The expression of antioxidant genes was evaluated at 31, 33, 35, 37, 39 and 41°C; 26°C served as
129 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:*

150 $F_{3,11}$=1709.476, P<0.05) and was lowest at the 1 h exposure period (Fig. 5A,C). *POD* expression
151 was significantly upregulated at the 2 h time point and was higher than expression levels at 0
152 (control), 0.5 and 1 h ($F_{3,10}$=3425.185 P<0.05) (Fig. 5B).

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

158

159 ## Discussion

160 Insects are poikilotherms that are greatly impacted by temperature fluctuations (Cossins & Bowler
161 1987; Worner 1998; Bale *et al.* 2002). When exposed to thermal stress, insects sustain oxidative
162 damage at the cellular level and respond with surplus levels of ROS (Lopez-Martinez *et al.* 2008;
163 Cui *et al.* 2011; Li & Sattar 2019). ROS can cause direct damage to biological macromolecules
164 and can also incite genetic mutations and cell death (Ryter *et al.* 2007). Antioxidant enzymes
165 function to eliminate or reduce ROS levels in insects. Previous studies showed that SOD, POD
166 and GST play important roles in the response of insects to ROS (Abele *et al.* 1998; An & Choi
167 2010; Celino *et al.* 2011; Liu *et al.* 2020). In this study, SOD, POD and GST activity increased
168 significantly in response to high temperatures, which suggests that these enzymes function to
169 remove excess ROS during thermal stress. Thus, our results are consistent with those reported for
170 *Bactrocera dorsalis*, *Bombyx mori*, *Mononychellus mcgregori*, *Diaphorina citri* and *Neoseiulus*

171 *cucumeris* (Lee *et al.* 2005; Jia *et al.* 2011; Marutani-Hert *et al.* 2010; Lu *et al.* 2014; Zhang *et al.*
172 2014). In a previous report, low temperature stress significantly altered SOD, POD, CAT and GST
173 activity in *F. occidentalis* (Shi *et al.* 2013). The increase in POD activity was likely the result of
174 elevated levels of SOD activity in response to H_2O_2. Although increased levels of antioxidant
175 enzymes suggests a defensive function of these enzymes in counteracting the negative effect of
176 ROS, there were no significant differences in SOD, POD or GST activity at 0.5, 1.0 and 2.0 h of
177 exposure to 35^{o}C (Fig. 2). This might indicate that antioxidant enzyme activity is very sensitive to
178 high temperature stress and reached a threshold level at 0.5 h or earlier.
179 Many researchers have shown that temperature stress can lead to changes in antioxidant gene
180 expression in insects (Yang *et al.* 2019; Xia *et al.* 2019; Lu *et al.* 2017). Previous results showed
181 that temperature stress inhibited the transcription of *SOD*, *POD, GST* and related enzymes in
182 *Mythimna separate*, *Apis cerana cerana* and *Helicoverpa armigera* (Shen *et al.* 2016; Yang *et al.*
183 2019; Xia *et al.* 2019). These results reflect the diversity of molecular responses in organisms
184 exposed to external stress. In addition to recruiting antioxidant enzymes to remove ROS in
185 response to thermal stress, insects also respond by synthesizing osmoprotectants, altering
186 membrane lipid content, and expressing heat shock proteins (Chen & Kang 2005). A previous
187 study demonstrated that both high and low thermal stress induced *CAT* expression in *F.*
188 *occidentalis* (Qin *et al.* 2017); therefore, the down-regulation of *POD* in this study might be
189 attributed to increased expression of *CAT*. In the case of *SOD* and *GST*, thermal stress may induce
190 the synthesis of unknown substances that could inhibit transcription. Further research is needed to
191 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* during climate change. 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*.

Acknowledgments

We express our deep gratitude to the Testing Center of Yangzhou University. The authors acknowledge support from the Special Fund for Agro-Scientific Research in the Public Interest of China (201103026, 200803025).

References

Abele D, Burlando B, Viarengo A & Pörtnera H O. 1998. Exposure to elevated temperatures and hydrogen peroxide elicits oxidative stress and antioxidant response in the Antarctic intertidal limpet *Nacella concinna*. *Comparative Biochemistry and Physiology B: Biochemistry and Molecular Biology* **120**, 425-435.

Ahmad S, Duval D L, Weinhold L C & Pardini R S. 1991. Cabbage looper antioxidant enzymes: tissue specificity. *Insect Biochemistry* **21**, 563-572.

An M I & Choi C Y. 2010. Activity of antioxidant enzymes and physiological responses in ark shell, *Scapharca broughtonii*, exposed to thermal and osmotic stress: effects on hemolymph and biochemical parameters. *Comparative Biochemistry and Physiology B: Biochemistry and Molecular Biology* **155**, 34-42.

Bale J S, Masters G J, Hodkinson I D *et al*. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology* **8**, 1-16.

Bradford M M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248-254.

Celino F T, Yamaguchi S, Miura C *et al*. 2011. Tolerance of spermatogonia to oxidative stress is due to high levels of Zn and Cu/Zn superoxide dismutase. *PLoS ONE* **6**, e16938.

Chang Y-W, Chen J-Y, Lu M-X, Gao Y, Tian Z-H, Gong W-R, et al. 2017. Selection and validation of reference genes for quantitative real-time PCR analysis under different

238 experimental conditions in the leafminer *Liriomyza trifolii* (Diptera: Agromyzidae). *PLoS*
239 *ONE* **12(7)**, e0181862.
240 Chen B and Kang L. 2005. Adaptation of insects to environmental temperature stress and
241 population differentiation. *Progress in Natural Science*. **(03)**, 11-17.
242 Cossins A R, Bowler K. (Eds.) 1987. Temperature Biology of Animals. Chapman and Hall,
243 New York, pp. 125-157.
244 Cui Y D, Du Y Z, Lu M X & Qiang C K. 2011. Antioxidant responses of *Chilo suppressalis*
245 (Lepidoptera: Pyralidae) larvae exposed to thermal stress. *Journal of Thermal Biology* **36**,
246 292-297.
247 Dubovskiy I M, Martemyanov V V, Vorontsova Y L, Rantala M J, Gryzanova E V & Glupov
248 V V. 2008. Effect of bacterial infection on antioxidant activity and lipid peroxidation in the
249 midgut of *Galleria mellonella* L. larvae (Lepidoptera, Pyralidae). *Comparative Biochemistry*
250 *and Physiology Part C Toxicology and Pharmacology* **148**, 1-5.
251 Felton G W & Summers C B. 1995. Antioxidant systems in insects. *Archives of Insect*
252 *Biochemistry and Physiology* **29**, 187-197.
253 Foyer C H & Noctor G. 2005. Oxidant and antioxidant signalling in plant: a reevaluation of
254 the concept of oxidative stress in a physiological context. *Plant Cell and Environment* **28**,
255 1056-1071.
256 Habig W H, Pabst M J & Jakoby W B. 1974. Glutathione S-Transferases. *Journal of*
257 *Biological Chemistry* **249**, 7130-7139.
258 Hajer Attig, Naouel Kamel, Susanna Sforzini, Alessandro Dagnino, Jebali Jamel, Hamadi
259 Boussetta, Aldo Viarengo, Mohamed Banni. 2014. Effects of thermal stress and nickel
260 exposure on biomarkers responses in *Mytilus galloprovincialis* (Lam), *Marine Environmental*
261 *Research*, **94**, 65-71.
262 Halliwell B. 1989. Free-radicals, reactive oxygen species and human disease-acritical
263 evaluation with special reference to atherosclerosis. *British Journal of Experimental*
264 *Pathology* **70**, 737-757.

265 Jia F X, Dou W, Hu F & Wang J J. 2011. Effects of thermal stress on lipid peroxidation and
266 antioxidant enzyme activities of oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae).
267 *Florida Entomologist* **94**, 956-963.
268 Kamata H & Hirata H. 1999. Redox regulation of cellular signalling. *Cellular Signalling* **11**,
269 1-14
270 Kashiwagi A, Kashiwagi K, Takase M, Hanada H & Nakamura M. 1997. Comparison of
271 catalase in diploid and haploid *Rana rugosa* using heat and chemical inactivation techniques.
272 *Comparative Biochemistry and Physiology B: Biochemistry and Molecular Biology* **118**, 499-
273 503.
274 Kirk W D J & Terry L I. 2015. The spread of the western flower thrips *Frankliniella*
275 *occidentalis* (Pergande). *Agricultural and Forest Entomology* **5**, 301-310.
276 Kono Y & Shishido T. 1992. Distribution of glutathione S-transferase activity in insect
277 tissues. *Applied Entomology and Zoology* **27**, 391-397.
278 Krishnan N, Kodrík D, Turanli F & Sehnal F. 2007. Stage-specific distribution of oxidative
279 radicals and antioxidant enzymes in the midgut of *Leptinotarsa decemlineata*. *Journal of*
280 *Insect Physiology* **53**, 67-74.
281 Lee K S, Kim S R, Park N S *et al*. 2005. Characterization of a silkworm thioredoxin
282 peroxidase that is induced by external temperature stimulus and viral infection. Insect
283 Biochemistry and Molecular Biology **35**, 73-84.
284 Li H B, Shi L, Lu M X, Wang J J & Du Y Z. 2011a. Impact of temperature hardening on
285 thermal tolerance and reproduction in *Frankliniella occidentalis*. *Chinese Journal of Applied*
286 *Entomology* **36**, 437-442.
287 Li H B, Shi L, Lu M X, Wang J J & Du Y Z. 2011b. Thermal tolerance of *Frankliniella*
288 *occidentalis*: effects of temperature, exposure time and gender. *Journal of Thermal Biology*
289 **36**, 437-442.
290 Li J Z, Zhi J R, Yuan C M & Wang H. 2007. The effect of temperature on the development
291 of *Frankliniella occidentalis*. *Guizhou Agricultural Science* **5**, 1-5.

Li L J and Adili Sattar. 2019. Effect of temperature stress on the main antioxidant enzymes in the pupa of *Carpomya vesuviana* Costa. *Xinjiang Agricultural Sciences.* **56(11),** 2062-2071.

Liu C M & Ma J Q. 2007. Effects of different temperatures on cultivating and protection enzymes of *Polyrhachis dives*. *Journal of Xuzhou Normal University* **25**, 72-74.

Liu H T, Li B, Zhao G D, Zhang T, Gao R N, Wei Z G, Shen W D. 2010. Sexual differences in main detoxification enzymes and their gene expression in different instars of *Bombyx mori* larvae. *Acta Entomologica Sinica*. **53(5)**, 479－486.

Liu Li, Hou Xiao Lin, Yue Wen Bo, Xie Wen, Zhang Tao, Zhi Jun Rui. 2020. Response of Protective Enzymes in Western Flower Thrips (Thysanoptera: Thripidae) to Two Leguminous Plants. *Environmental entomology*. **49(5)**, 1191-1197.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods* **25(4),** 402–408

Lopez-Martinez G, Elnitsky M A, Benoit J B, Lee R E & Denlinger D L. 2008. High resistance to oxidative damage in the Antarctic midge *Belgica antarctica*, and developmentally linked expression of genes encoding superoxide dismutase, catalase, and heat shock proteins. *Insect Biochemistry and Molecular Biology* **38**, 796-804.

Lu F P, Chen Q, Chen Z H, Lu H, Xu X L & Jing F L. 2014. Effects of heat stress on development, reproduction and activities of protective enzymes in *Mononychellus mcgregori*. *Experimental and Applied Acarology* **63**, 267-284.

Lyakhovich V V, Vavilin V A, Zenkov N K & Menshchikova E B. 2006. Active defense under oxidative stress. The antioxidant responsive element. *Biochemistry Biokhimiia* **71**, 962-1183

Marutani-Hert M, Hunter W B & Hall D G. 2010. Gene response to stress in the Asian citrus psyllid (Hemiptera: Psyllidae). *Florida Entomologist* **93**, 519-525.

Morse J G & Hoddle M S. 2006. Invasion biology of thrips. *Annual Review of Entomology* **51**, 67-89.

Pappu H R, Jones R A & Jain R K. 2009. Global status of tospovirus epidemics in diverse cropping systems: successes achieved and challenges ahead. Virus Research **141**, 219-236.

Qin J, Lu M X, Zheng Y T & Du Y Z. 2017. Molecular cloning, characterization and functional analysis of catalase in *Frankliniella occidentalis*. *Annals of the Entomological Society of America* **110**, 212-220.

Reitz S R. 2009. Biology and ecology of the western flower thrips (Thysanoptera: Thripidae): The making of a pest. Florida Entomologist **92**, 7-13.

Ryter S W, Kim H P, Hoetzel A *et al*. 2007. Mechanism of cell death in oxidative stress. *Antioxid Redox Signal* **9**, 49-89.

Shen S, Wang M, Li X, Li S, van Oers MM, Vlak JM, Braakman I, Hu Z, Deng F, Wang H. 2016. Mutational and functional analysis of N-linked glycosylation of envelope fusion protein F of *Helicoverpa armigera* nucleopolyhedrovirus. *J Gen Virol*. **97(4)**, 988-999.

Shi L, Li H B, Kim H J., Wang J J & Du Y Z. 2013. Effect of low temperature stress on antioxidase activity of western flower thrips，*Frankliniella occidentalis. Chinese Journal of Applied Entomology* **50**, 1062-1067.

Tomitaka, Y. 2019. Studies on the interaction between tomato spotted wilt tospovirus and thrips. *J. Gen. Plant Pathol*. **85**, 465–467.

Wang J C, Zhang B, Li H G, Wang J P & Zheng C Y. 2014. Effects of Exposure to High Temperature on *Frankliniella occidentalis* (Thysanoptera: Thripidae), under Arrhenotoky and Sexual Reproduction Conditions. *Florida Entomologist* **97**, 504-510.

Wang M & Li Z Z. 2002. Studies on the activities of enzymes of protective system during diapause of sawfly *Chinolyda flagellicorni*. *Scientia Silvae Sinicae* **38**, 100-104.

Wang Y, Oberley L W & Murhammer D W. 2001. Evidence of oxidative stress following the viral infection of two lepidopteran insect cell lines. *Free Radical Biology and Medicine* **30**, 1254-1262.

Worner S P. 1998. Ecoclimatic assessment of potential establishment of exotic pests. *Journal of Economic Entomology* **81**, 973-983.

Wu SY, Tang L D, Zhang X R, Xing Z L, Lei Z R & Gao Y L. 2017. A decade of a thrips invasion in China: lessons learned. *Ecotoxicology*. **27(7)**, 1032-1038.

Xia Z Y, Qin M, Wang H F, Liu Z G, Wang Y, Zhang W X, Xu B H. 2019. Effect of low temperature stress on antioxidant indexs and cold tolerance gene expression in *Apis cerana cerana* during overwintering period. *Chinese Journal of Animal Nutrition*. **31(3)**, 1250-1258.

Yang Hang, Wang Xiaoyun, Pei Haiying, Fan Dong. 2019. Cloning a Peroxidase cDNA Sequence from the Oriental Armyworm, *Mythimna separate* Walker and Its Induction to Different Temperature Stress. *Chinese Journal of Biological Control.* **35(01)**, 44-52.

Zhang G H, Liu H, Wang J J & Wang Z Y. 2014. Effects of thermal stress on lipid peroxidation and antioxidant enzyme activities of the predatory mite, *Neoseiulus cucumeris* (Acari: Phytoseiidae). *Enperimental and Applied Acarology*. **64**, 3-85.

Zhang X, Qin J, Yuan J, Lu M, Du Y. 2019. Cloning of a new HSP70 gene from western flowerthrips, *Frankliniella occidentalis*, and expression patterns during thermal stress. *PeerJ* . **7**, e7687

Zhang Y J, Wu Q J, Xu B Y & Zhu G R. 2003. The occurrence and damage of *Frankliniella occidentalis* (Thysanoptera: Thripidae): a dangerous alien invasive pest in Beijing. *Plant Protection* **4**, 58-59.

Zhang Z J, Zhang Y J, Xu B Y, Zhu G R, Wu Q J. 2012. Effects of temperature on development，reproduction and population growth of the western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Acta Entomologica Sinica.* **55(10)**, 1168-1177.

Table 1(on next page)

Primers used in this study

1

Primer name	Primer sequences	Tm (°C)	Length (bp)
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
qPCR-*SOD*-R	AATGCTGCGTTCTCTGTTGTG	58.7	
qPCR-*POD*-F	CCGCACTGGGACGACGAGAC	65.8	235
qPCR-*POD*-R	CGATGAGCGAGTGGAAGTATCTGAA	64.8	
qPCR-*GST*-F	GCTGCTGCTGTGCTGGATTA	59.7	170
qPCR-*GST*-R	ACCGTGAACCAGACCGAGAC	59.4	
EF-1-F	TCAAGGAACTGCGTCGTGGAT	58.6	130
EF-1-R	ACAGGGGTGTAGCCGTTAGAG		
GAPDH-F	AAGGGTGCTCAGGTTGTTGCT	56.5	89
GAPDH-R	CGACCGTGGGTGGAGTCATAT		

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).

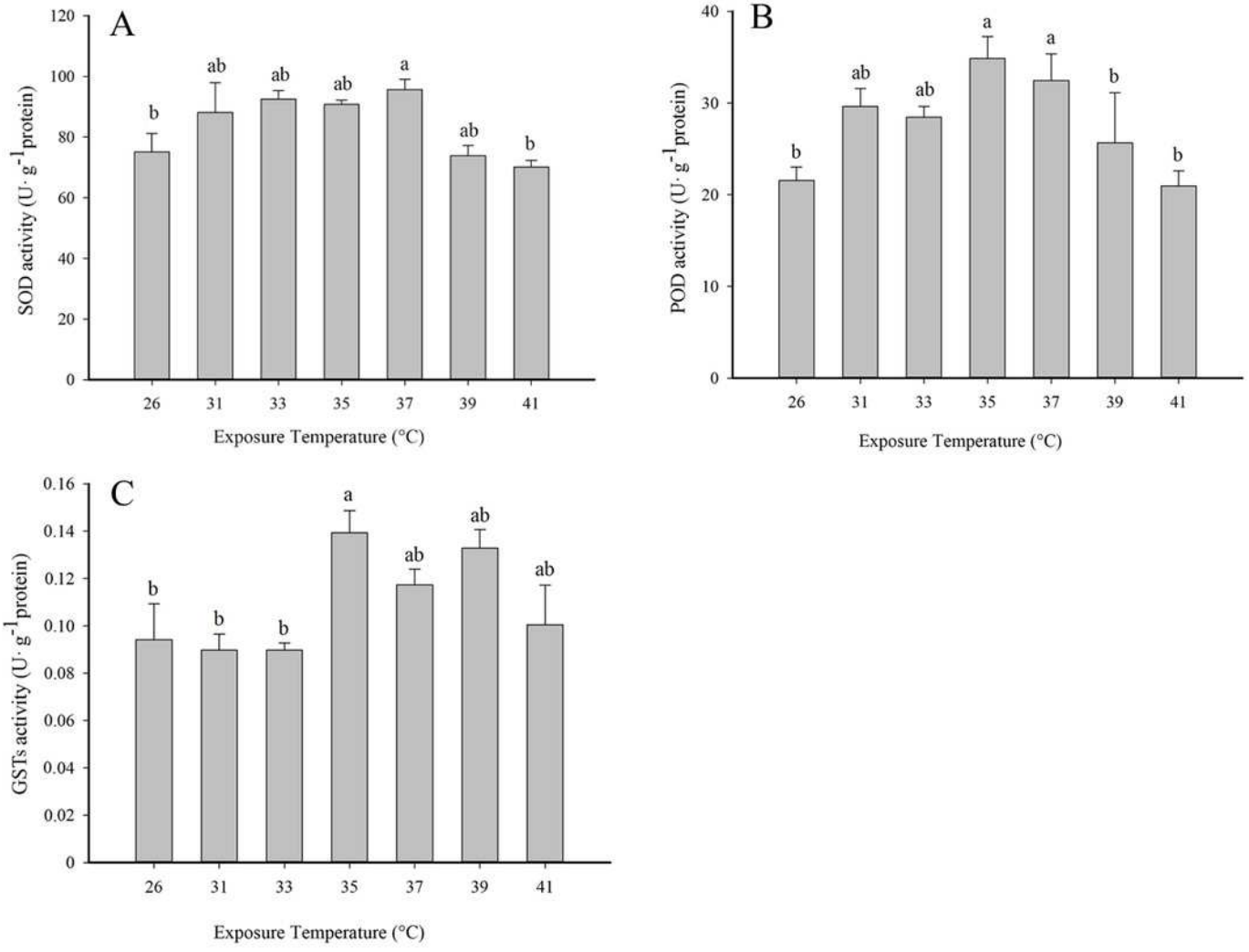


Figure 2

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).

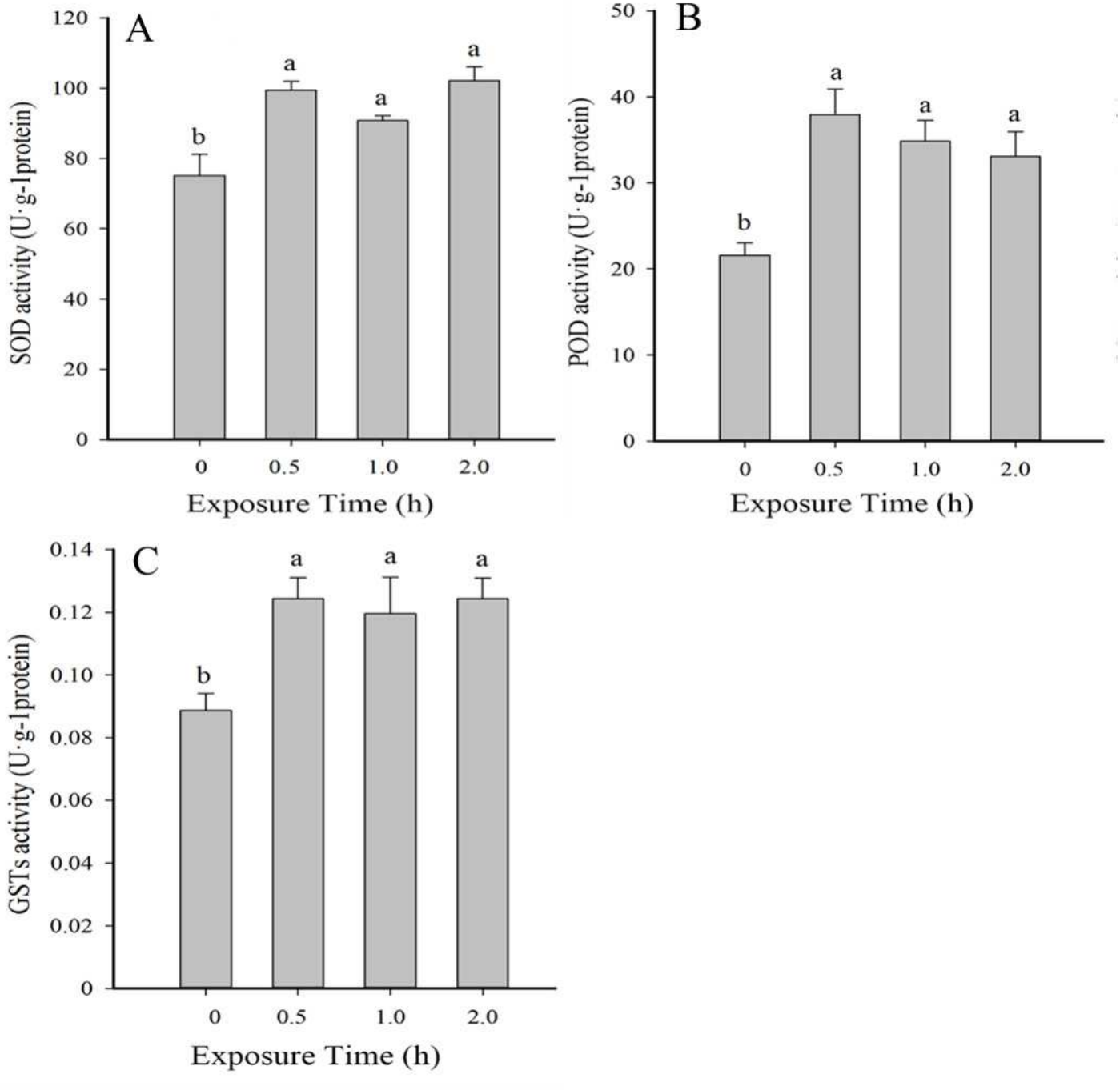
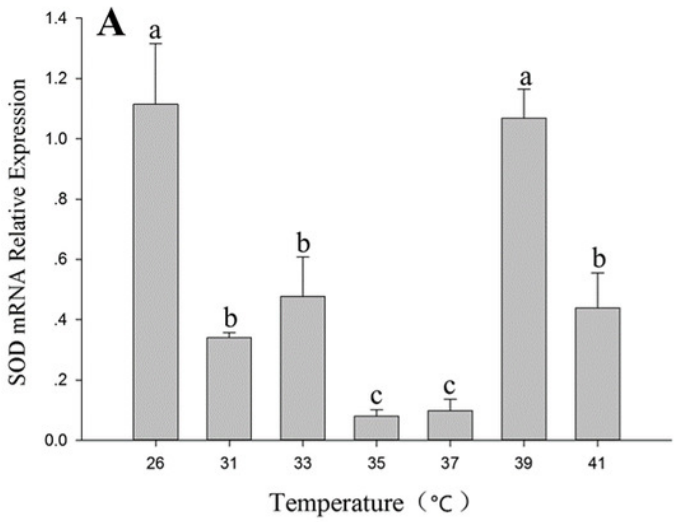
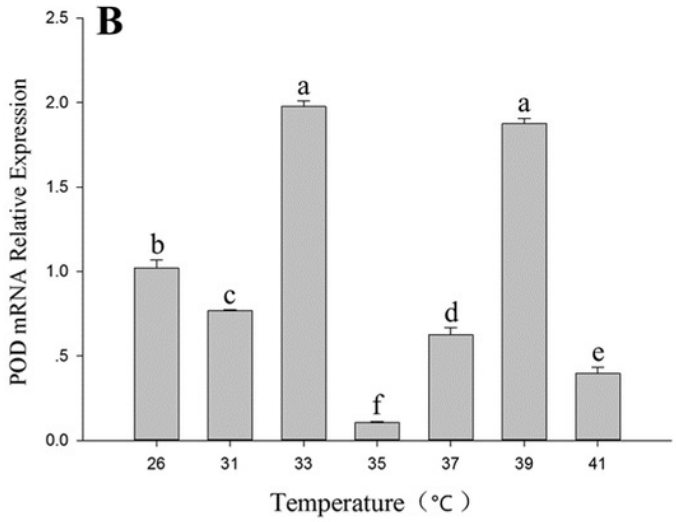

A
SOD activity (U·g-1protein)
120
100
80
60
40
20
0
b
a
a
a
0
0.5
1.0
2.0
Exposure Time (h)
B
POD activity (U·g-1protein)
50
40
30
20
10
0
b
a
a
a
0
0.5
1.0
2.0
Exposure Time (h)
C
GSTs activity (U·g-1protein)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
b
a
a
a
0
0.5
1.0
2.0
Exposure Time (h)

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°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（℃）

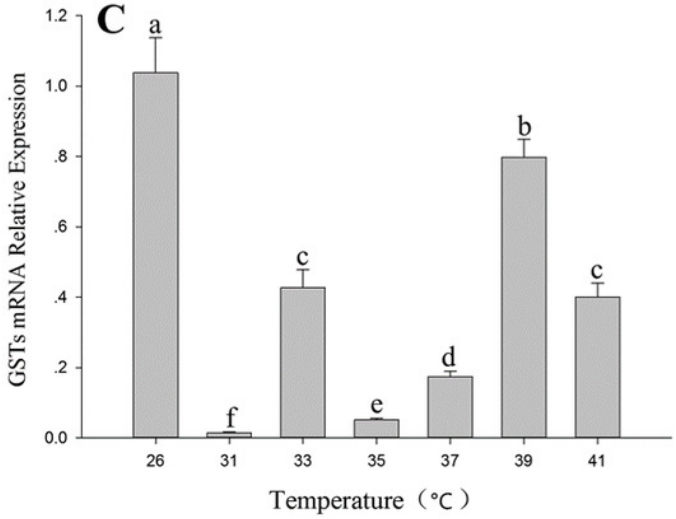
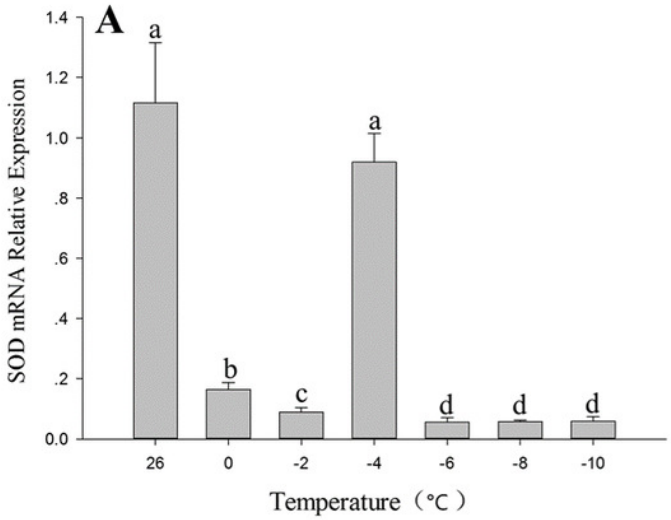
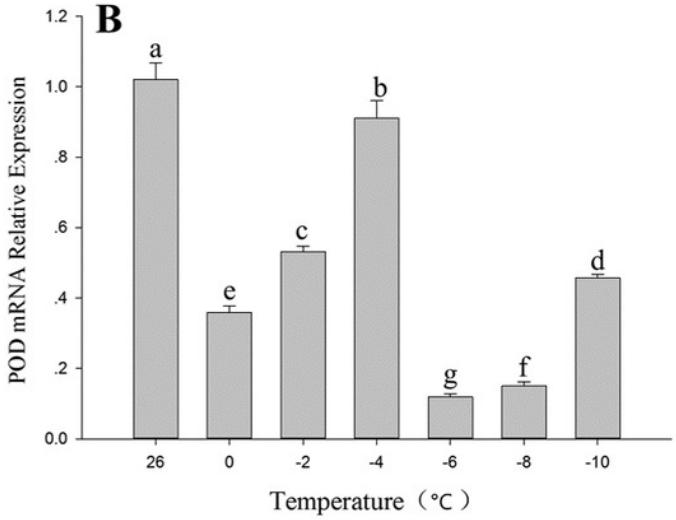

C
GSTs mRNA Relative Expression
1.2
1.0
.8
.6
.4
.2
0.0
a
f
c
e
d
b
c
26
31
33
35
37
39
41
Temperature（℃）

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（℃）

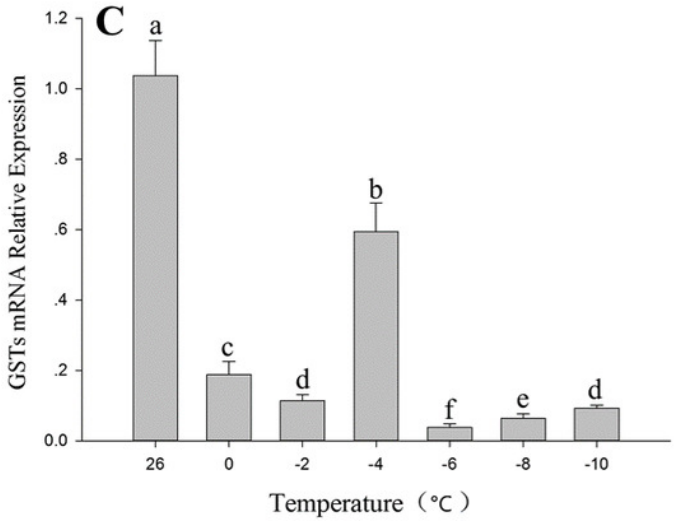
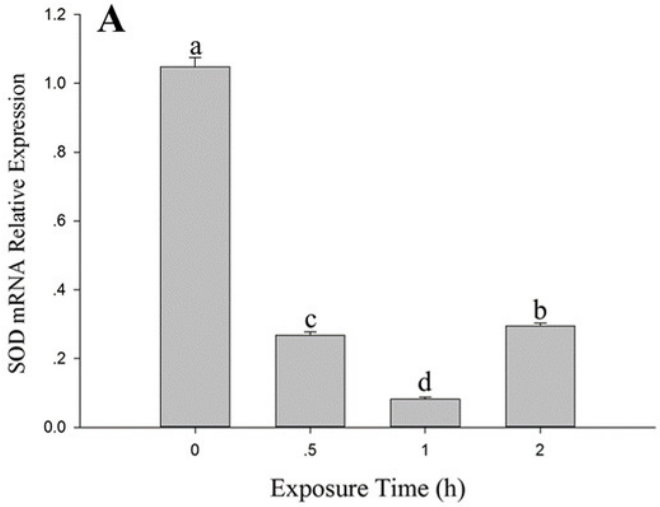
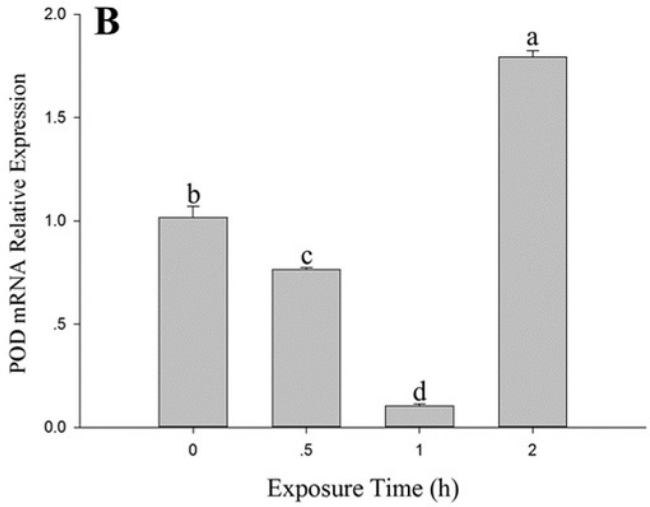

C
GSTs mRNA Relative Expression
1.2
1.0
.8
.6
.4
.2
0.0
a
c
d
b
f
e
d
26
0
-2
-4
-6
-8
-10
Temperature（℃）

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)

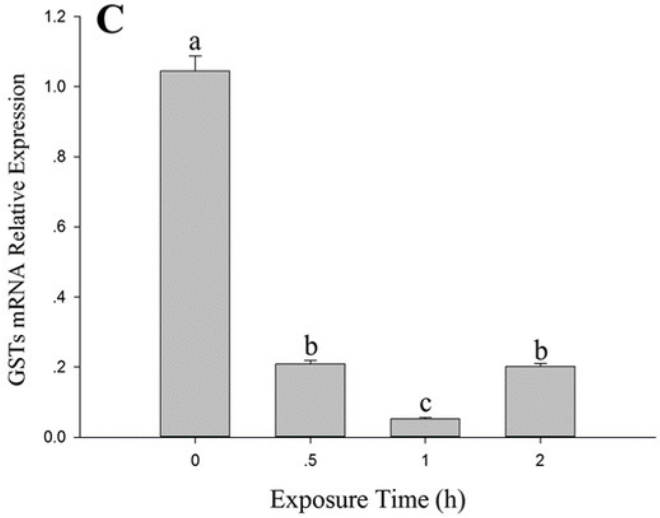
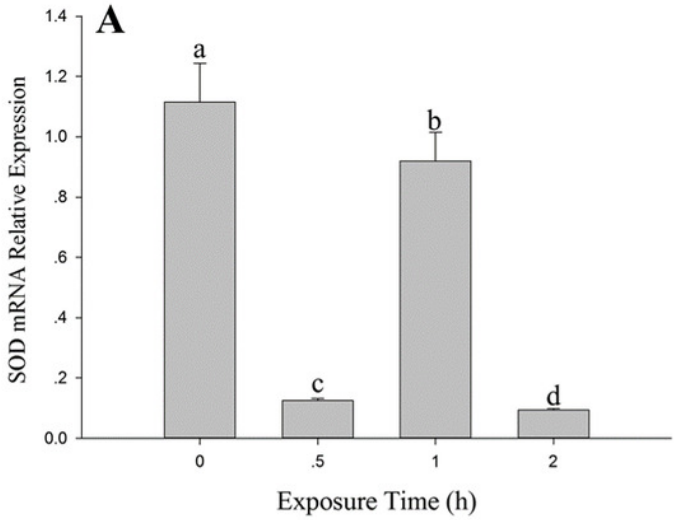
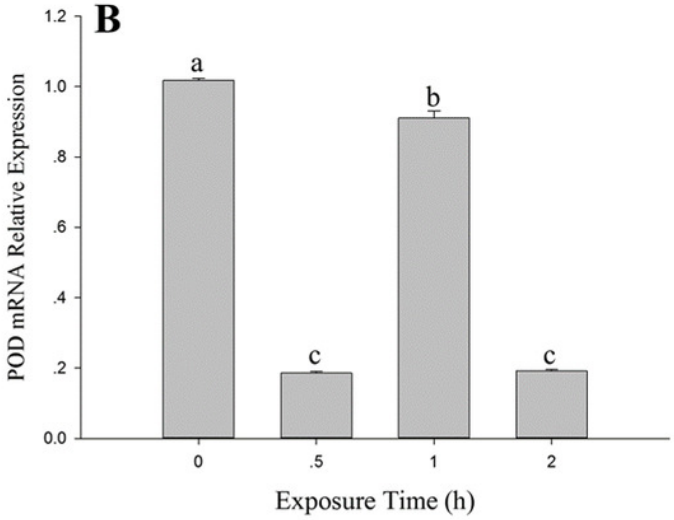

C
GSTs mRNA Relative Expression
1.2
1.0
.8
.6
.4
.2
0.0
a
b
c
b
0
.5
1
2
Exposure Time (h)

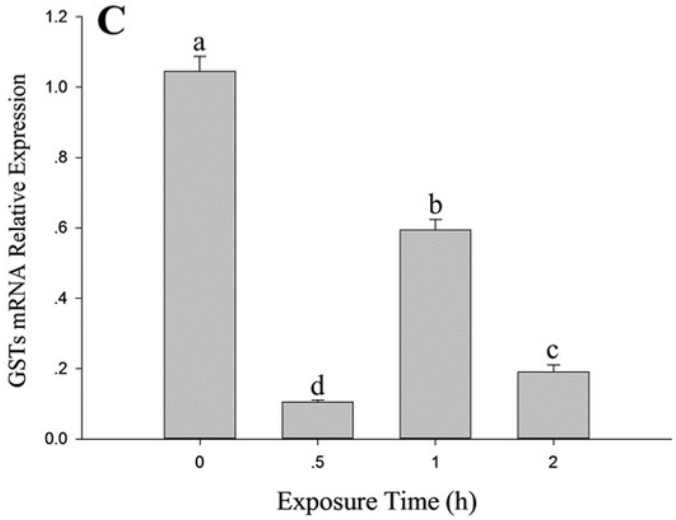
Figure 6

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)