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

32 Temperature impacts the reproduction, development, and distribution of insects (Cossins & Bowle

33 1987; Worner 1998; Bale *et al.* 2002), and extreme temperatures are known elicitors of reactive

34 oxygen species (ROS) in invertebrates. The excessive generation of ROS can damage cellular

35 constituents, including lipids, proteins, and nucleic acids (Halliwell 1989; Kamata & Hirata 1999;

36 Foyer & Noctor 2005; Lopez-Martinez *et al.* 2008). In order to survive, insects reduce or detoxify

37 ROS through the action of antioxidants; these function as enzymatic and non-enzymatic

38 scavengers that reduce lipid peroxidation and decrease damage to nucleic acids and proteins

39 (Felton & Summers 1995; Lyakhovich *et al.* 2006; Krishnan *et al.* 2007). Peroxidase (POD),

40 superoxide dismutase (SOD), and glutathione-S-transferase (GST) are antioxidant enzymes that

41 defend cells from excessive levels of ROS (Felton & Summers 1995; Wang *et al.* 2001; Dubovskiy

42 *et al.* 2008; Liu *et al.* 2020). SOD functions by degrading superoxide anions to hydrogen peroxide

43 (H_2O_2) and oxygen, and H_2O_2 is subsequently converted to H_2O by POD (Kashiwagi *et al.* 1997;

44 Wang & Li 2002; Liu & Ma 2007). GSTs function to detoxify compounds that are produced from

45 lipid peroxidation (Ahmad *et al.* 1991; Kono & Shishido 1992; Dubovskiy *et al.* 2008).

46 The western flower thrips (WFT), *Frankliniella occidentalis*, damages both vegetables and

47 ornamental plants on a global scale and is especially problematic in greenhouses (Morse & Hoddle

48 2006; Kirk & Terry 2015; Mouden *et al.* 2017). In addition to direct damage, WFT causes serious

49 damage to plants by transmitting plant viruses such as the Tomato Spotted Wilt Virus (Pappu *et*

50 *al.* 2009; Tomitaka 2019). WFT is endemic to the western region of North America and has spread

51 globally due to the transportation of agricultural products (Reitz 2009; Kirk & Terry 2015).

52 According to the CABI Invasive Species Compendium, *F. occidentalis* has been discovered on all

53 continents except Antarctica (https://www.cabi.org/isc/datasheet/24426). In mainland China, *F.*

54 *occidentalis* was initially found in Beijing in 2003 (Zhang et al., 2003) and has since been

55 discovered in at least ten provinces (Wu *et al.* 2017).

56 Previous studies indicated that temperature impacts development, sex ratios, reproduction,

57 population growth, and mortality of *F. occidentalis* (Li *et al.* 2007; Li *et al.* 2011a; Zhang *et al.*

58 2012). During the hot summers in subtropical China, high temperatures may cause oxidative stress

59 to *F. occidentalis*, particularly in greenhouses (Wang *et al.* 2014). Previous studies demonstrated

60 that the expression of genes encoding catalase (CAT) and subsequent enzymatic activity were

61 altered in *F. occidentalis* exposed to hot and cold stress (Shi *et al.* 2013; Qin *et al.* 2017). However,

62 the impact of high and low temperatures on other antioxidant enzymes in *F. occidentalis* is unclear.

63 In this study, we investigated the effect of temperature stress on POD, SOD, and GST in *F.*

64 *occidentalis*. The results provide important data on how antioxidant enzymes counteract oxidative

65 damage in the WFT and provide a more comprehensive framework for understanding thermal

66 tolerance in *F. occidentalis*.

67

68 **Materials & Methods**

69 **Insects and temperature treatments**

70   *Frankliniella occidentalis* populations were collected in Hangzhou, China, in 2008 and were

71   reared with kidney bean, *Phaseolus vulgaris Linn*, at 25 ± 0.5°C and 70±5% relative humidity with

72   a 16:8 h light:dark photoperiod as outlined by Li *et al.* (2011b). Newly emerged 2nd instar larvae

73   were collected, and pools of 100 were exposed to high (31, 33, 35, 37, 39 or 41°C) or low (0, -2,

74   -4, -6, -8 and -10°C) temperatures for 1 h in glass tubes as described (Chang *et al.*, 2017). Through

75   the results of the pre-experiment, 35°C and -4°C were decided as the model temperature on *F.*

76   *occidentalis*,which was further explored by subjecting groups of individuals to 0, 0.5, 1, and 2 h

77   of thermal stress; controls were maintained at 26°C (0 h time point). Following thermal stress,

78   larvae were incubated at 26°C for 30 min and used a brush to touch it gently, thrips would be

79   identified as surviving if it respond to the stimulus. Survivors were frozen in liquid nitrogen and

80   stored at -80°C for future use. Four replicate pools were used for each temperature and time period.

81

82   **Determination of enzyme activity**

83   The assay kit used for protein extraction was from Nanjing Jiancheng Bioengineering Institute,

84   Jiangsu, China. Treated samples were homogenized in 0.9% saline and then centrifuged at 2,500

85   × rpm for 10 min (Jia *et al.*, 2011). Supernatants containing the enzyme fractions were collected,

86   and protein content was determined using the Bradford (1976) method.

87   POD and SOD activities were assessed with commercially available kits (Qin *et al.*, 2017).

88   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 *GST1*, 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 Transcriptome sequencing was performed on *F. occidentalis* exposed to low temperature (-

102 13° C), high temperature (40° C) and normal temperature control (26° C) at the Shanghai

103 Biotechnology corporation by Illumina sequencing platform. The RNA-seq data were deposited

104 with the Sequence Read Archives PRJNA73493 at NCBI. To remove adapter contamination,

105 low‐quality bases and bases artificially introduced during library construction, we trimmed all

106 raw   reads   using   Trimmomatic   0.32   (http://www.usadellab.org/

107 cms/index.php?page=trimmomatic) before transcript assembly, while the unpaired reads were

108   discarded (A.M. Bolger *et al.*, 2014). The clean reads were mapped to the sequences in the rRNA

109   database of all published insects downloaded from NCBI to discard rRNAs using SOAP. Only the

110   clean reads with the standard of Q30 > 85% and processed with Trimmomatic 0.32 and SOAP

111   were used for further analysis (X.Y. Gu *et al.*, 2019). Clean data were assembled with Trinity to

112   obtain a high-quality unigene library (M.G. Grabherr *et al.*, 2011). The unigene library of three

113   species were first assembled to obtain individual UniGene databases; the general UniGene library

114   was obtained by clustering the three individual databases through CD-Hit to facilitate comparison

115   of expression patterns (L. Fu *et al.*, 2012). The transcripts selected in the clustering united as

116   unigenes using the De Bruijn graph algorithm; CD-Hit was used to reduce sequence redundancy

117   and improve the performance of other sequence analyses (L. Fu *et al.*, 2012; Y. Yang *et al.*, 2013).

118   For functional annotation, we obtained information on unigenes using BLAST (cut-off e-value of

119   10-5) with protein databases such as NR (NCBI nonredundant database), Swiss-Prot, GO (Gene

120   Ontology), COG (Clusters of Orthologous Groups), KOG (euKaryotic Orthologous Groups),

121   eggNOG, Pfam (Protein family) and KEGG (Kyoto Encyclopedia of Genes and Genomes). "

122   Superoxide Dismutase ", " Peroxidase " and " glutathione-s-transferase " were used as key words

123   to search related gene fragments in the transcriptome database of *F. occidentalis*, respectively.

124   The fragment gene cDNA of the *SOD*, *GST1* and *POD* was

125   submitted to GenBank (accession no. MZ364120, MZ364118 and MZ364119, respectively).

126   According to the obtained gene fragments, the corresponding primers (Table 1) were designed for

127   fragment verification. PCR products were cloned and sequenced as described (Zhang *et al.*, 2019).

128   Quantitative real-time reverse transcriptase PCR (qRT-PCR) was conducted using the

129   protocols described by Zhang *et al*. (2019). Specific primers (Table 1) were designed according to

130   the above verified fragments for qRT-PCR. Melting curve analysis was executed to analyze the

131   specificity of PCR products. According to the evaluation results of Zheng *et al*. (2014) on the

132   reliability of reference genes in *F. occidentalis*, expression levels were normalized using reference

133   genes *GAPDH, RPL32* and *EF-1, 18S* for high and low temperature stress, respectively.

134

135   **Statistical analyses**

136   qRT-PCR data analyzing was conducted in Bio-Rad CFX Manager 3.1 software. The average Ct

137   values of biological replicates were used to calculate the relative expression levels. The results of

138   qPCR were analyzed with the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). Firstly, for all test

139   samples and calibration samples, the Ct value of the housekeeping gene were used to normalize

140   the Ct value of the target gene. Normalized results were ΔCt(test) and ΔCt(calibrator),

141   respectively. And using ΔCt(calibrator) to normalize ΔCt(test) , $\Delta\Delta$Ct was obtained. The ratio of

142   expression level was calculated by $2^{-\Delta\Delta Ct}$. The lg(X) method was used to transform the expression

143   level data for normality and homogeneity of variance. Significant differences were detected by

144   one-way analysis of variance (ANOVA) and Duncan's multiple comparisons test.   Data were

145   analyzed with SPSS v. 16.0 and considered significant at $P < 0.05$.

146

Results

Effect of high temperature stress on antioxidant activity

149 SOD activity increased with rising temperature from 31 to 37°C and was highest at 37°C. The

150 activity of SOD activity began to decline at 39°C, and the level at 41°C was significantly lower

151 than 37°C ($F_{6,19}$=4.245, $P<0.05$) (Fig. 1A). A similar pattern was observed with POD, where

152 activity rose with increasing temperature, peaked at 35°C and was significantly lower at 41°C than

153 35°C ($F_{6,21}$=7.089, $P<0.05$) (Fig. 1B). GST activity was highest at 35°C (Fig. 1C) and began to

154 decline with increasing temperature ($F_{6,21}$=8.312, $P<0.05$).

155

Temporal changes in antioxidant enzyme activity at 35°C

157 Antioxidant enzyme activity was significantly higher than the control (0 h, 26°C) when insects

158 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, $P<0.05$;

159 GSTs: $F_{3,10}$=5.815, $P<0.05$). No significant differences in antioxidant activity were detected

160 between 0.5, 1 and 2 h of exposure (Fig. 2).

161

Expression of antioxidant genes in response to heat and cold stress

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

164   a control. *SOD* expression showed significant decreases in expression at 35°C to 37°C; however,

165   expression peaked at 39°C and was comparable to the control (26°C) (Fig. 3A). With the exception

166   of 35°C to 37°C, *SOD* expression was not significantly changed by high temperatures

167   ($F_{6,18}$=29.203, *P*<0.05). In contrast, *POD* expression levels at 33°C, 37°C and 39°C were

168   significantly higher than the control at 26°C; however, except that the expression level was

169   significantly decreased at 35°C, there was no significant difference in the expression level of 31°C

170   compared with the control ($F_{6,18}$=51.745, *P*<0.05) (Fig. 3B). *GST1* expression was suppressed or

171   unaffected relative to the control at all elevated temperatures ($F_{6,17}$=32.682, *P*<0.05) (Fig. 3C). All

172   three antioxidant genes shared a common insensitivity in response to high temperature. Even under

173   some temperatures, the expression level was higher than that of the control, but the relative

174   expression level was not very high. .

175   Expression of the three antioxidant genes was also evaluated in response to low temperature

176   stress at 0, -2, -4, -6, -8 and -10°C. *SOD* expression showed a significant decline at all temperatures

177   relative to the control at 26°C; although the expression of -4 °C was higher than other temperatures,

178   it was also inhibited by low temperature (Fig. 4A) ($F_{6,20}$=243.607, *P*<0.05). *POD* expression was

179   also strongly inhibited , with the lowest expression appeared at -6 °C. ($F_{6,18}$=51.909, *P*<0.05) (Fig.

180   4B). Like *SOD* and *POD*, the *GST1* expression were decreased compared with the control relative

181   with low temperature. ($F_{6,17}$=32.682, *P*<0.05) (Fig. 4C).

182

183 **Temporal changes in the expression of antioxidant genes**

184 Compared to the control (0 h, 26ºC), *SOD* expression decreased significantly when 2nd instar larvae

185 were exposed to 35°C for 0.5, 1 and 2 h (*SOD: $F_{3,12}$*=31.689, *P*<0.05) and was lowest at the 1 h

186 exposure period (Fig. 5A). *POD* expression was significantly upregulated at 0.5 h and 2 h and was

187 higher than expression levels at 0 (control) and 1 h, with the peak appeared at 2 h. ($F_{3,12}$=72.243,

188 *P*<0.05) (Fig. 5B). *GST1* expression pattern was similar to POD (Fig. 5C). Although there was no

189 significant difference at 0.5 h compared to the control (*GST1: $F_{3,11}$*=1709.476, *P*<0.05).

190   After exposure to -4°C, the expression levels of the three antioxidant genes decreased

191 significantly when compared to the control (*SOD: $F_{3,10}$*=201.898, *P*<0.05; *POD: $F_{3,11}$*=204.420,

192 *P*<0.05; *GST1: $F_{3,10}$*=72.835, *P*<0.05). Interestingly, all three genes showed a peak in expression

193 after a 1 h exposure to -4°C; however, it should be noted that expression at 1 h was lower than the

194 control (Fig. 6).

195

196 **Discussion**

197 Insects are poikilotherms that are greatly impacted by temperature fluctuations (Cossins & Bowler

198 1987; Worner 1998; Bale *et al.* 2002). When exposed to thermal stress, insects sustain oxidative

199 damage at the cellular level and respond with surplus levels of ROS (Lopez-Martinez *et al.* 2008;

200 Cui *et al.* 2011; Li & Sattar 2019). ROS can cause direct damage to biological macromolecules

201 and can also incite genetic mutations and cell death (Ryter *et al.* 2007). Antioxidant enzymes

202   function to eliminate or reduce ROS levels in insects. Previous studies showed that SOD, POD

203   and GST play important roles in the response of insects to ROS (Abele *et al.* 1998; An & Choi

204   2010; Celino *et al.* 2011; Liu *et al.* 2020). In this study, SOD, POD and GST activity increased

205   significantly in response to high temperatures, which suggests that these enzymes function to

206   remove excess ROS during thermal stress. Thus, our results are consistent with those reported for

207   *Bactrocera dorsalis*, *Bombyx mori*, *Mononychellus mcgregori*, *Diaphorina citri* and *Neoseiulus*

208   *cucumeris* (Lee *et al.* 2005; Jia *et al.* 2011; Marutani-Hert *et al.* 2010; Lu *et al.* 2014; Zhang *et al.*

209   2014). In a previous report, low temperature stress significantly altered SOD, POD, CAT and GST

210   activity in *F. occidentalis* (Shi *et al.* 2013). The increase in POD activity was likely the result of

211   elevated levels of SOD activity in response to H_2O_2.   Although increased levels of antioxidant

212   enzymes suggests a defensive function of these enzymes in counteracting the negative effect of

213   ROS, there were no significant differences in SOD, POD or GST activity at 0.5, 1.0 and 2.0 h of

214   exposure to 35°C (Fig. 2). This might indicate that antioxidant enzyme activity is very sensitive to

215   high temperature stress and reached a threshold level at 0.5 h or earlier.

216   Many researchers have shown that temperature stress can lead to changes in antioxidant gene

217   expression in insects (Yang *et al.* 2019; Xia *et al.* 2019; Lu *et al.* 2017). Previous results showed

218   that temperature stress inhibited the transcription of *SOD*, *POD, GST1* and related enzymes in

219   *Mythimna separate*, *Apis cerana cerana* and *Helicoverpa armigera* (Shen *et al.* 2016; Yang *et al.*

220   2019; Xia *et al.* 2019). These results reflect the diversity of molecular responses in organisms

221   exposed to external stress. In addition to recruiting antioxidant enzymes to remove ROS in

222   response to thermal stress, insects also respond by synthesizing osmoprotectants, altering

223   membrane lipid content, and expressing heat shock proteins (Chen & Kang 2005). A previous

224   study demonstrated that both high and low thermal stress induced *CAT* expression in *F.*

225   *occidentalis* (Qin *et al.* 2017); therefore, the down-regulation of *POD* in this study might be

226   attributed to increased expression of *CAT*. In the case of *SOD* and *GST1*, thermal stress may induce

227   the synthesis of unknown substances that could inhibit transcription. Further research is needed to

228   validate or disprove these conjectures.

229   Differential regulation of antioxidant genes and enzymes has been reported in insects; for

230   example, *POD*, *CAT* and *SOD* expression patterns were not necessarily correlated with enzyme

231   activity during high temperature stress in *Mononychellus mcgregori* (Lu *et al.* 2017). In larvae of

232   *Bombyx mori*, carboxylesterase activity was not correlated with gene expression (Liu *et al.* 2010).

233   Elevated protein levels can be stressful for the organism, and the organism may inhibit gene

234   transcription to maintain homeostasis. Conversely, if protein levels fall to a suboptimal level, the

235   cell may respond by promoting transcription. Furthermore, transcription is often followed by post-

236   transcriptional processing, degradation of transcription products, translation, post-translational

237   processing and further modifications that impact protein levels. Further research is needed to

238   understand the mechanisms that control the response of *F. occidentalis* to thermal stress.

239

240   **Conclusions**

241   This study reveals differential regulation of antioxidant gene expression and enzyme production

242 in response to thermal stress. The results confirm the importance of antioxidant enzymes in

243 modulating the response to thermal stress in *F. occidentalis*, and provide new avenues for further

244 study of antioxidant mechanisms and physiological responses of *F. occidentalis*. The

245 inconsistencies between gene expression and enzyme activity further illustrate the complexity of

246 thermal adaptation in *F. occidentalis*. Future multidisciplinary research in genomics,

247 transcriptomics, proteomics, and metabolomics will help explain the underlying mechanisms of

248 thermal adaptation in *F. occidentalis*.

249

250 **Acknowledgments**

251 We express our deep gratitude to the Testing Center of Yangzhou University. The authors

252 acknowledge support from the Special Fund for Agro-Scientific Research in the Public Interest of

253 China (201103026, 200803025).

254 **References**

255 Abele D, Burlando B, Viarengo A & Pörtnera H O. 1998. Exposure to elevated temperatures

256 and hydrogen peroxide elicits oxidative stress and antioxidant response in the Antarctic

257 intertidal limpet *Nacella concinna*. *Comparative Biochemistry and Physiology B:*

258 *Biochemistry and Molecular Biology* **120**, 425-435.

259 Ahmad S, Duval D L, Weinhold L C & Pardini R S. 1991. Cabbage looper antioxidant

260 enzymes: tissue specificity. *Insect Biochemistry* **21**, 563-572.

261 A.M. Bolger, M. Lohse, B. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina

262 sequence data, *Bioinformatics* **30**, 2114-2120.

263 An M I & Choi C Y. 2010. Activity of antioxidant enzymes and physiological responses in

264   ark shell, *Scapharca broughtonii*, exposed to thermal and osmotic stress: effects on

265   hemolymph and biochemical parameters. *Comparative Biochemistry and Physiology B:*

266   *Biochemistry and Molecular Biology* **155**, 34-42.

267   Bale J S, Masters G J, Hodkinson I D, Awmack C, Bezemer TM, Brown VK, Butterfield J,

268   Buse A, Coulson JC, Farrar J, Good JEG, Symrnioudis I, Watt AD, Whittaker JB . 2002.

269   Herbivory in global climate change research: direct effects of rising temperature on insect

270   herbivores. *Global Change Biology* **8**, 1-16.

271   Bradford M M. 1976. A rapid and sensitive method for the quantitation of microgram

272   quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*

273   **72**, 248-254.

274   Celino F T, Yamaguchi S, Miura C, Ohta T, Tozawa Y, Iwai T, Miura T. 2011. Tolerance of

275   spermatogonia to oxidative stress is due to high levels of Zn and Cu/Zn superoxide dismutase.

276   *PLoS ONE* **6**, e16938.

277   Chang Y-W, Chen J-Y, Lu M-X, Gao Y, Tian Z-H, Gong W-R, et al. 2017. Selection and

278   validation of reference genes for quantitative real-time PCR analysis under different

279   experimental conditions in the leafminer *Liriomyza trifolii* (Diptera: Agromyzidae). *PLoS*

280   *ONE* **12(7)**, e0181862.

281   Chen B and Kang L. 2005. Adaptation of insects to environmental temperature stress and

282   population differentiation. *Progress in Natural Science*. **(03)**, 11-17.

283   Cossins A R, Bowler K. (Eds.) 1987. Temperature Biology of Animals. Chapman and Hall,

284   New York, pp. 125-157.

285   Cui Y D, Du Y Z, Lu M X & Qiang C K. 2011. Antioxidant responses of *Chilo suppressalis*

286   (Lepidoptera: Pyralidae) larvae exposed to thermal stress. *Journal of Thermal Biology* **36**,

287   292-297.

288   Dubovskiy I M, Martemyanov V V, Vorontsova Y L, Rantala M J, Gryzanova E V & Glupov

289   V V. 2008. Effect of bacterial infection on antioxidant activity and lipid peroxidation in the

290   midgut of *Galleria mellonella* L. larvae (Lepidoptera, Pyralidae). *Comparative Biochemistry*

291   *and Physiology Part C Toxicology and Pharmacology* **148**, 1-5.

292   Felton G W & Summers C B. 1995. Antioxidant systems in insects. *Archives of Insect*

293   *Biochemistry and Physiology* **29**, 187-197.

294   Foyer C H & Noctor G. 2005. Oxidant and antioxidant signalling in plant: a reevaluation of

295   the concept of oxidative stress in a physiological context. *Plant Cell and Environment* **28**,

296   1056-1071.

297   Habig W H, Pabst M J & Jakoby W B. 1974. Glutathione S-Transferases. *Journal of*

298   *Biological Chemistry* **249**, 7130-7139.

299   Hajer Attig, Naouel Kamel, Susanna Sforzini, Alessandro Dagnino, Jebali Jamel, Hamadi

300   Boussetta, Aldo Viarengo, Mohamed Banni. 2014. Effects of thermal stress and nickel

301   exposure on biomarkers responses in *Mytilus galloprovincialis* (Lam), *Marine Environmental*

302   *Research*, **94**, 65-71.

303   Halliwell B. 1989. Free-radicals, reactive oxygen species and human disease-acritical

304   evaluation with special reference to atherosclerosis. *British Journal of Experimental*

305   *Pathology* **70**, 737-757.

306   Jia F X, Dou W, Hu F & Wang J J. 2011. Effects of thermal stress on lipid peroxidation and

307   antioxidant enzyme activities of oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae).

308   *Florida Entomologist* **94**, 956-963.

309   Kamata H & Hirata H. 1999. Redox regulation of cellular signalling. *Cellular Signalling* **11**,

310   1-14

311   Kashiwagi A, Kashiwagi K, Takase M, Hanada H & Nakamura M. 1997. Comparison of

312   catalase in diploid and haploid *Rana rugosa* using heat and chemical inactivation techniques.

313   *Comparative Biochemistry and Physiology B: Biochemistry and Molecular Biology* **118**, 499-

314   503.

315   Kirk W D J & Terry L I. 2015. The spread of the western flower thrips *Frankliniella*

316   *occidentalis* (Pergande). *Agricultural and Forest Entomology* **5**, 301-310.

317   Kono Y & Shishido T. 1992. Distribution of glutathione S-transferase activity in insect

318   tissues. *Applied Entomology and Zoology* **27**, 391-397.

319   Krishnan N, Kodrík D, Turanli F & Sehnal F. 2007. Stage-specific distribution of oxidative

320   radicals and antioxidant enzymes in the midgut of *Leptinotarsa decemlineata*. *Journal of*

321   *Insect Physiology* **53**, 67-74.

322   Lee KS, Kim SR, Park NS, Kim I, Kang PD, Sohn BH, Choi KH, Kang SW, Je YH, Lee SM,

323   Sohn HD, Jin BR. 2005. Characterization of a silkworm thioredoxin peroxidase that is

324   induced by external temperature stimulus and viral infection. Insect Biochemistry and

325   Molecular Biology **35**, 73-84.

326   L. Fu, B. Niu, Z. Zhu, S. Wu, W. Li. 2012. CD-HIT: accelerated for clustering the next-

327   generation sequencing data, *Bioinformatics* **28**, 3150-3152.

328   Li H B, Shi L, Lu M X, Wang J J & Du Y Z. 2011a. Impact of temperature hardening on

329   thermal tolerance and reproduction in *Frankliniella occidentalis*. *Chinese Journal of Applied*

330   *Entomology* **36**, 437-442.

331   Li H B, Shi L, Lu M X, Wang J J & Du Y Z. 2011b. Thermal tolerance of *Frankliniella*

332   *occidentalis*: effects of temperature, exposure time and gender. *Journal of Thermal Biology*

333   **36**, 437-442.

334   Li J Z, Zhi J R, Yuan C M & Wang H. 2007. The effect of temperature on the development

335   of *Frankliniella occidentalis*. *Guizhou Agricultural Science* **5**, 1-5.

336   Li L J and Adili Sattar. 2019. Effect of temperature stress on the main antioxidant enzymes

337   in the pupa of *Carpomya vesuviana* Costa. *Xinjiang Agricultural Sciences.* **56(11),** 2062-

338   2071.

339   Liu C M & Ma J Q. 2007. Effects of different temperatures on cultivating and protection

340   enzymes of *Polyrhachis dives*. *Journal of Xuzhou Normal University* **25**, 72-74.

341   Liu H T, Li B, Zhao G D, Zhang T, Gao R N, Wei Z G, Shen W D. 2010. Sexual differences

342   in main detoxification enzymes and their gene expression in different instars of *Bombyx mori*

343   larvae. *Acta Entomologica Sinica*. **53(5)**, 479－486.

344   Liu Li, Hou Xiao Lin, Yue Wen Bo, Xie Wen, Zhang Tao, Zhi Jun Rui. 2020. Response of

345   Protective Enzymes in Western Flower Thrips (Thysanoptera: Thripidae) to Two

346   Leguminous Plants. *Environmental entomology*. **49(5)**, 1191-1197.

347   Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time

348   quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods* **25(4),** 402–408

349   Lopez-Martinez G, Elnitsky M A, Benoit J B, Lee R E & Denlinger D L. 2008. High

350   resistance to oxidative damage in the Antarctic midge *Belgica antarctica*, and

351   developmentally linked expression of genes encoding superoxide dismutase, catalase, and

352   heat shock proteins. *Insect Biochemistry and Molecular Biology* **38**, 796-804.

353   Lu F P, Chen Q, Chen Z H, Lu H, Xu X L & Jing F L. 2014. Effects of heat stress on

354   development, reproduction and activities of protective enzymes in *Mononychellus mcgregori*.

355   *Experimental and Applied Acarology* **63**, 267-284.

356   Lyakhovich V V, Vavilin V A, Zenkov N K & Menshchikova E B. 2006. Active defense

357   under oxidative stress. The antioxidant responsive element. *Biochemistry Biokhimiia* **71**, 962-

358   1183

359   Marutani-Hert M, Hunter W B & Hall D G. 2010. Gene response to stress in the Asian citrus

360   psyllid (Hemiptera: Psyllidae). *Florida Entomologist* **93**, 519-525.

361   M.G. Grabherr, B.J. Haas, M. Yassour, J.S. Levin, D.A. Thompson, I. Amit, et al. 2011. Full-

362   length transcriptome assembly from RNA-Seq data without a reference genome, *Nat.*

363   *Biotechnol*. 29, 644-652.

364   Morse J G & Hoddle M S. 2006. Invasion biology of thrips. *Annual Review of Entomology*

365   **51**, 67-89.

366   Pappu H R, Jones R A & Jain R K. 2009. Global status of tospovirus epidemics in diverse

367   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, Park JW,Choi AMK . 2007. Mechanisms 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

394   invasion in China: lessons learned. *Ecotoxicology*. **27(7)**, 1032-1038.

395   Xia Z Y, Qin M, Wang H F, Liu Z G, Wang Y, Zhang W X, Xu B H. 2019. Effect of low

396   temperature stress on antioxidant indexs and cold tolerance gene expression in *Apis cerana*

397   *cerana* during overwintering period. *Chinese Journal of Animal Nutrition*. **31(3)**, 1250-1258.

398   X.Y. Gu, Y. Zhao, Y. Su, J.J. Wu, Z.Y. Wang, J.T. Hu, L.J. Liu, Z.H. Zhao, A.A. Hoffmann,

399   B. Chen, Z.H. Li. 2019. A transcriptional and functional analysis of heat hardening in two

400   invasive fruit fly species, *Bactrocera dorsalis* and *Bactrocera correcta*, Evol. Appl **12**, 1147-

401   1163

402   Yang Hang, Wang Xiaoyun, Pei Haiying, Fan Dong. 2019. Cloning a Peroxidase cDNA

403   Sequence from the Oriental Armyworm, *Mythimna separate* Walker and Its Induction to

404   Different Temperature Stress. *Chinese Journal of Biological Control.* **35(01)**, 44-52.

405   Y. Yang, S.A. Smith. 2013. Optimizing de novo assembly of short-read RNA-seq data for

406   phylogenomics, *BMC Genomic* **14**, 328.

407   Zhang G H, Liu H, Wang J J & Wang Z Y. 2014. Effects of thermal stress on lipid

408   peroxidation and antioxidant enzyme activities of the predatory mite, *Neoseiulus cucumeris*

409   (Acari: Phytoseiidae). *Enperimental and Applied Acarology*. **64**, 3-85.

410   Zhang X, Qin J, Yuan J, Lu M, Du Y. 2019. Cloning of a new HSP70 gene from western

411   flowerthrips, *Frankliniella occidentalis*, and expression patterns during thermal

412   stress. *PeerJ* . **7**, e7687

413   Zhang Y J, Wu Q J, Xu B Y & Zhu G R. 2003. The occurrence and damage of *Frankliniella*

414   *occidentalis* (Thysanoptera: Thripidae): a dangerous alien invasive pest in Beijing. *Plant*

415   *Protection* **4**, 58-59.

416   Zhang Z J, Zhang Y J, Xu B Y, Zhu G R, Wu Q J. 2012. Effects of temperature on

417   development,reproduction and population growth of the western flower thrips,

418   *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Acta Entomologica Sinica.* **55(10)**,

419   1168-1177.

420 Zheng Y T, Li H B, Lu M X, Du Y Z, 2014. Evaluation and validation of reference genes for

421 qRT-PCR normalization in *Frankliniella occidentalis* (Thysanoptera: Thripidae). PLos ONE,

422 **9(10)**, e111369.

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 (Ducan 's test).

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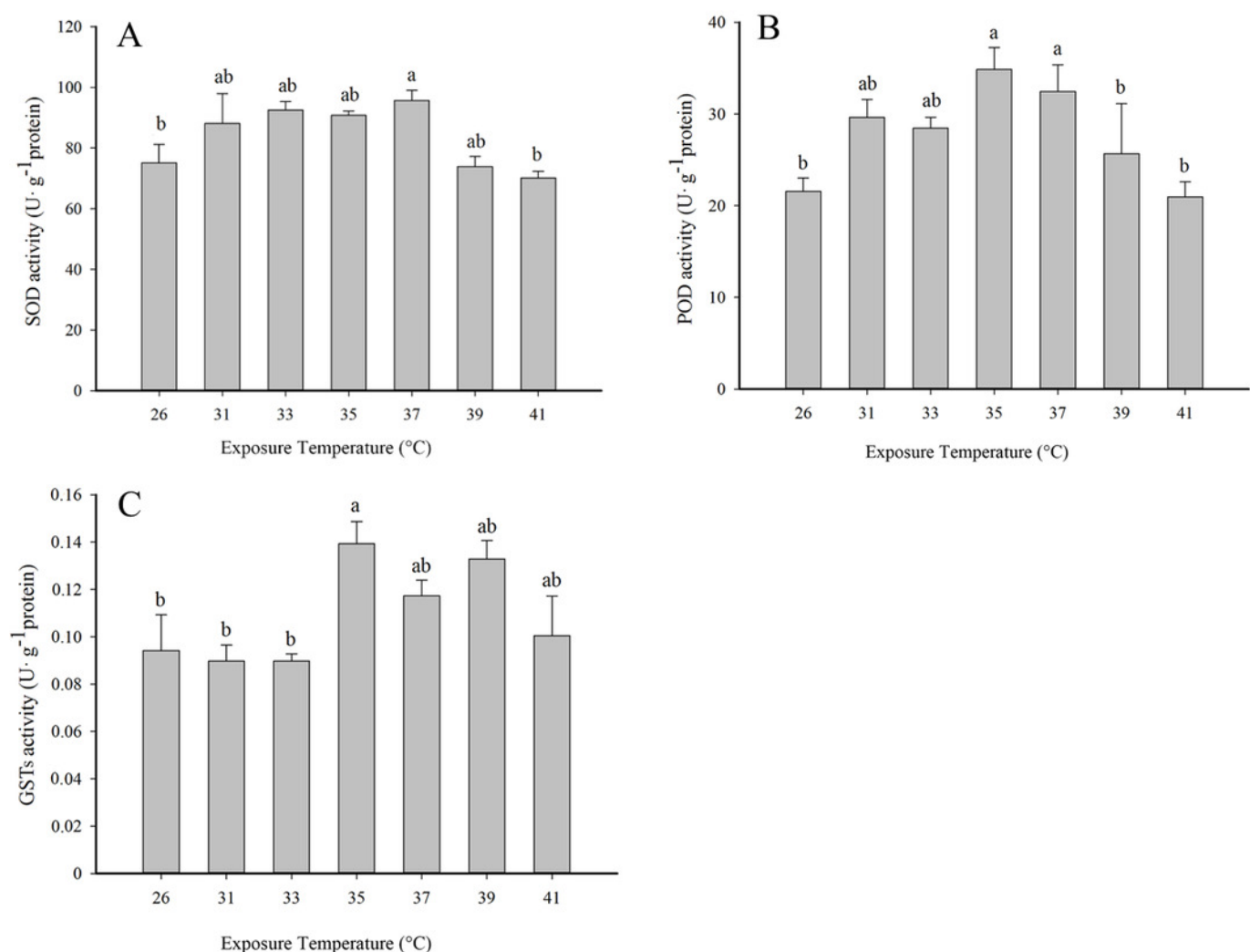


Figure 2

Temporal changes in antioxidant enzyme activity in 2nd instar larvae of *F. occidentalis* exposed to 35°C.

Panels: (A) SOD, superoxide dismutase; (B) POD, peroxidase; (C) GST, glutathione-S-

transferase. *F. occidentalis* was exposed to 35°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 (Ducan 's test).

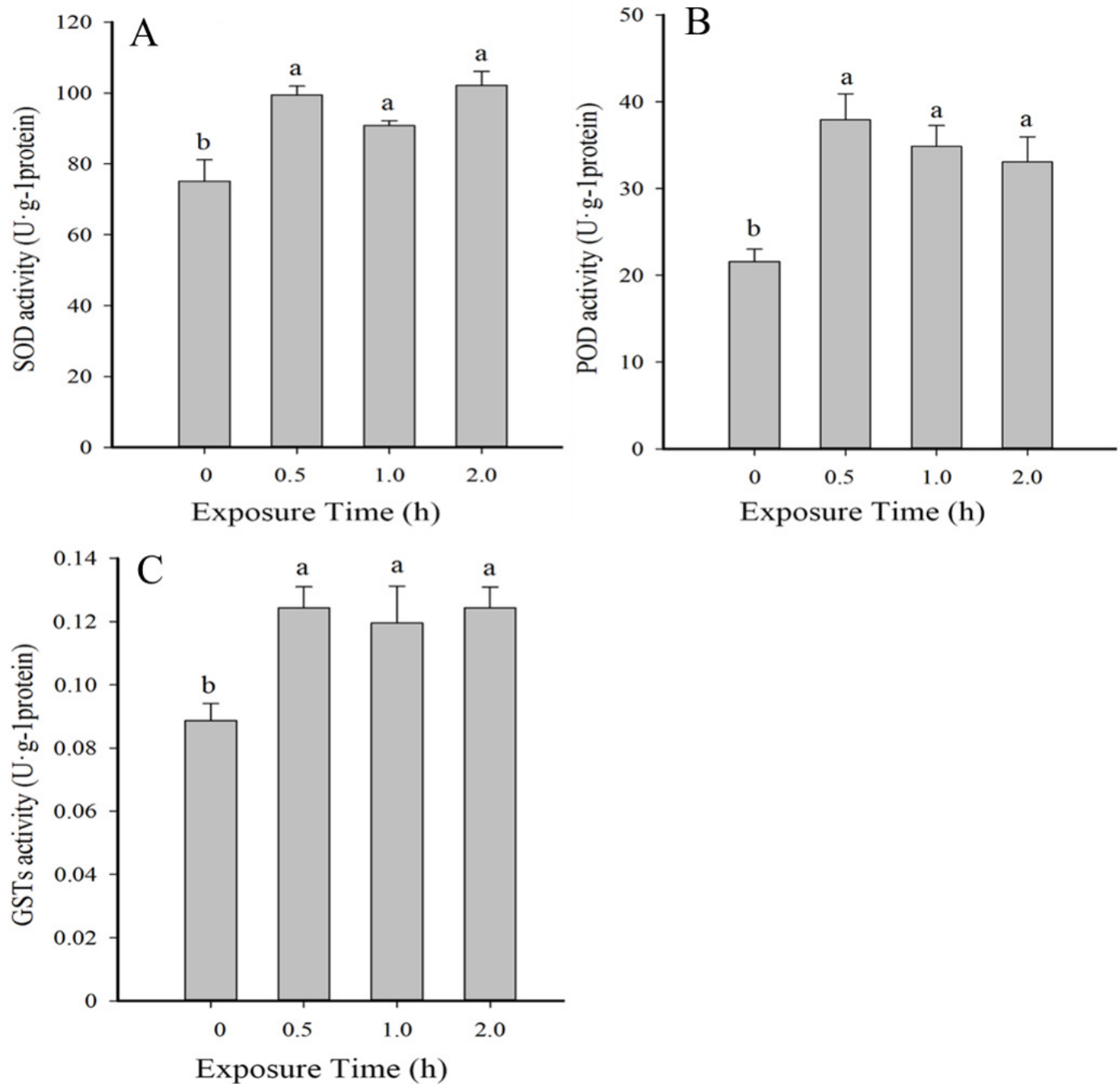



Figure 3

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 (Ducan 's test).

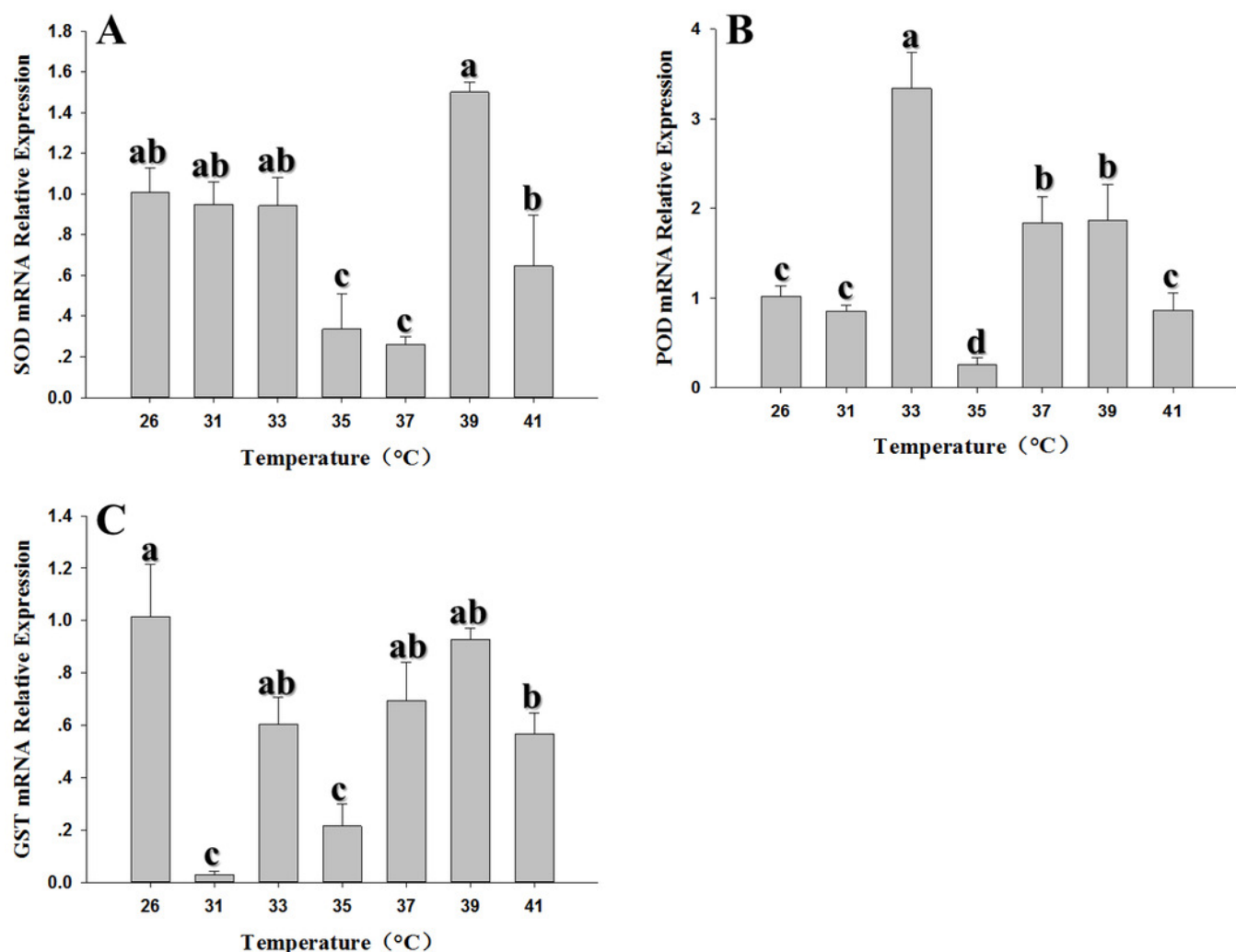



Figure 4

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 (Ducan's test).

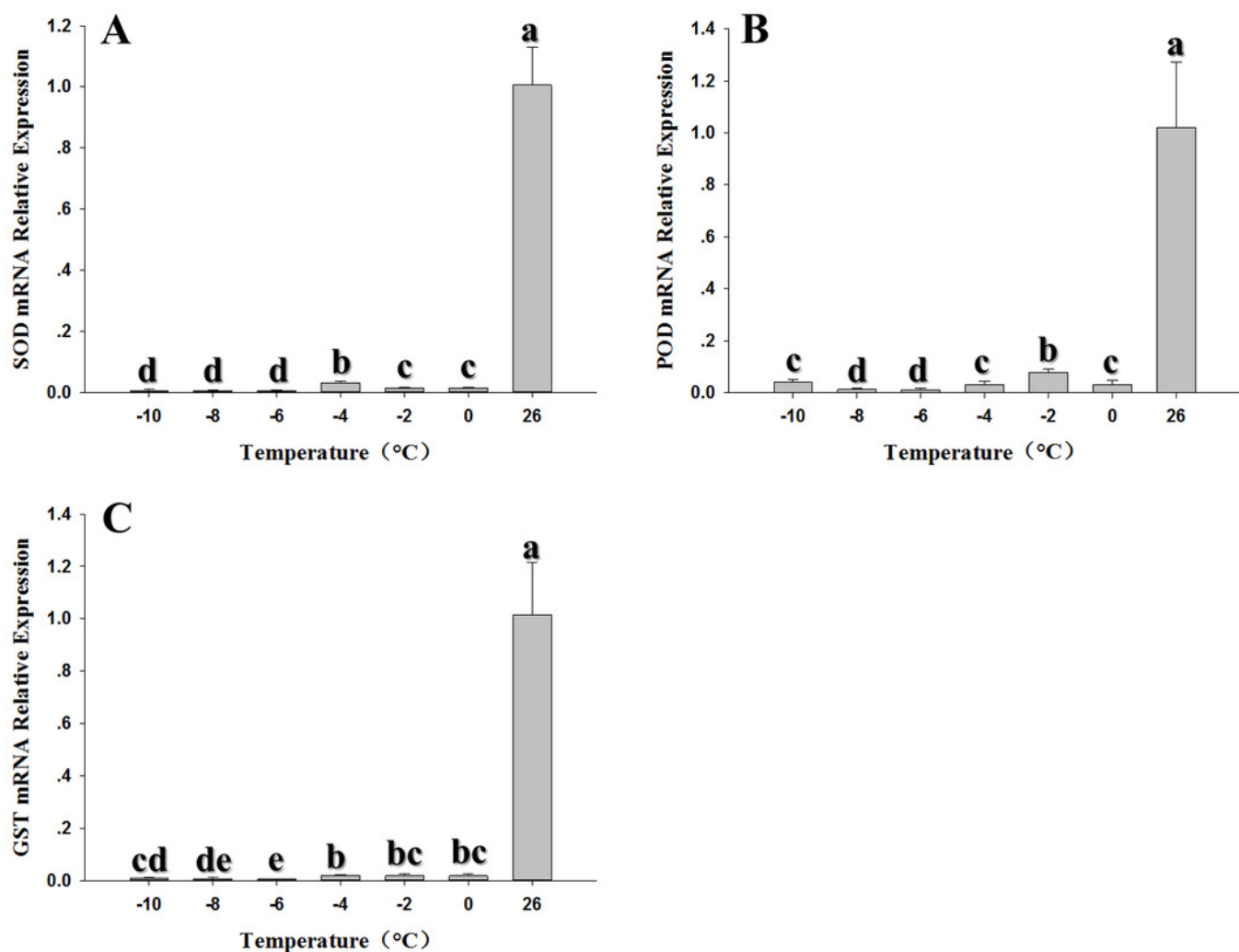



Figure 5

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

Panels: (A) SOD, superoxide dismutase; (B) POD, peroxidase; (C) GST, glutathione-S-transferase. *F. occidentalis* was exposed to 35°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 (Ducan 's test).

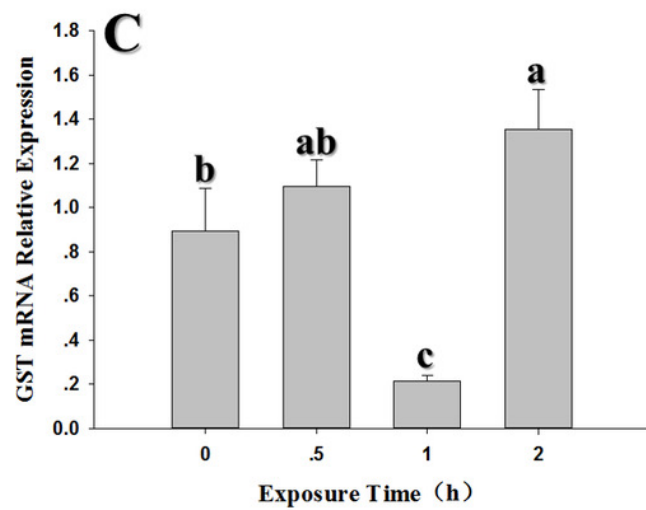
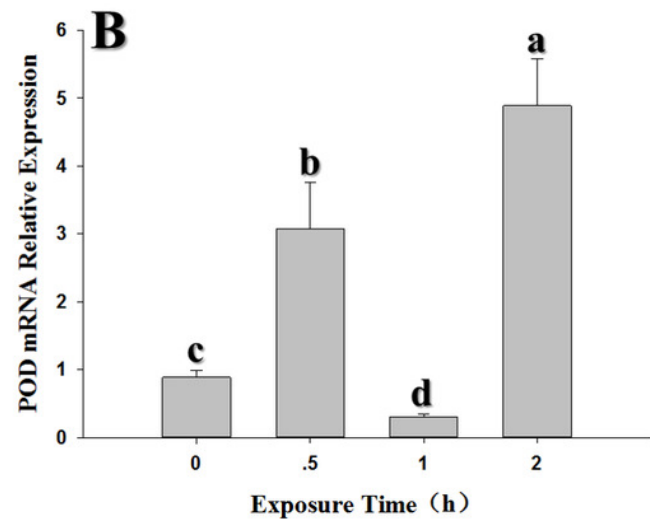
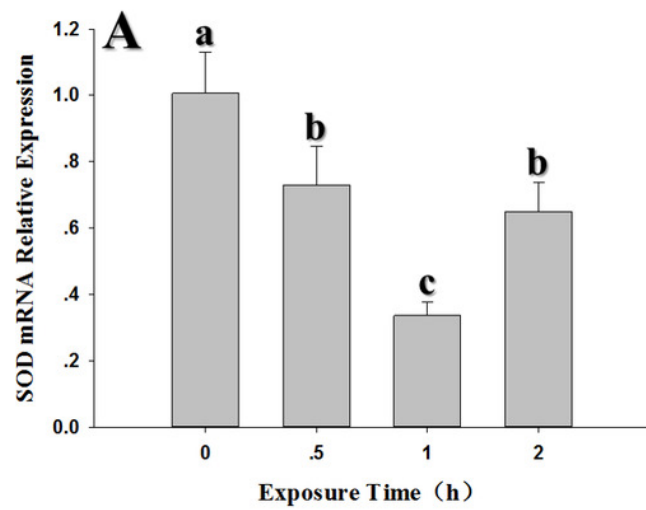
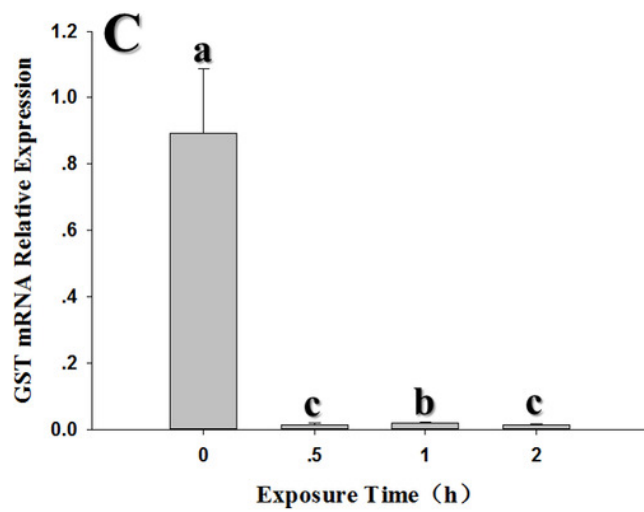
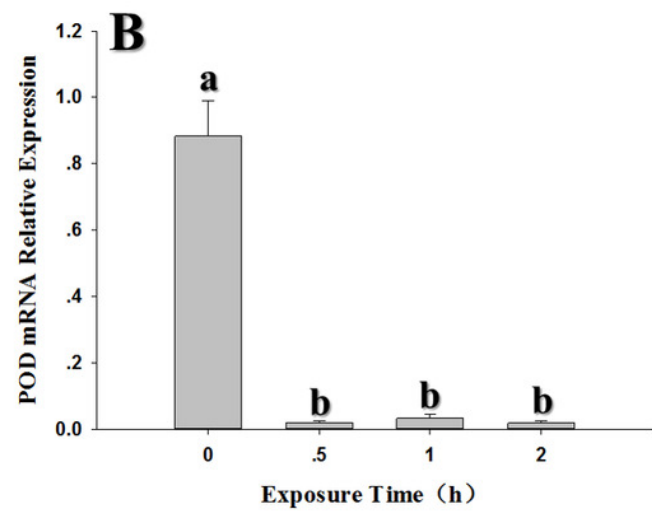
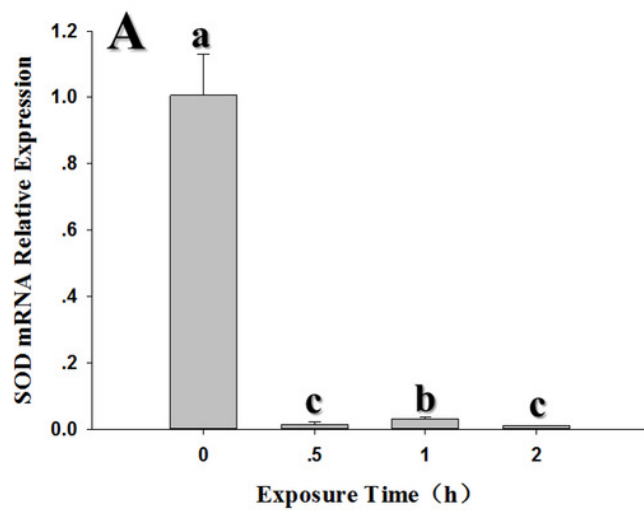



Figure 6

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

Panels: (A) SOD, superoxide dismutase; (B) POD, peroxidase; (C) GST, glutathione-S-transferase. *F. occidentalis* was exposed to 4°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 (Ducan's test).

Primers used in the study

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			
18S-F	AACACGGGAAACCTCACCA	55.4	116	108.9
18S-R	CAGACAAATCGCTCCACCAA			
RPL32-F	CAACATCGGTTATGGAAGCA	55.0	141	100.1
RPL32-R	ACAGCGTGGGCAATTTCAGC			
GAPDH-F	AAGGGTGCTCAGGTTGTTGCT	56.5	89	104.4
GAPDH-R	CGACCGTGGGTGGAGTCATAT			