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EFFECTS OF THERMAL STRESS ON LIPID PEROXIDATION AND ANTIOXIDANT ENZYME ACTIVITIES OF ORIENTAL FRUIT FLY, *BACTROCERA DORSALIS* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

A change in temperature may be responsible for a variety of physiological stress responses in organisms, which are often associated with enhanced generation of reactive oxygen species (ROS) leading to oxidative damage. In the present study, the lipid peroxidation (LPO) levels of the oriental fruit fly, *Bactrocera dorsalis* (Hendel), were measured under thermal stress conditions of the relatively low (-5, -2.5, 0, and 5 °C) or high (35, 37.5, and 40 °C) temperatures. Subsequently, the time-related effect of thermal stress on activity of antioxidant enzymes including catalase (CAT), glutathione-S-transferases (GSTs), peroxidase (POX), superoxide dismutase (SOD), and total antioxidant capacity (T-AOC) were systematically determined. The results showed that LPO levels increased significantly in a time-dependent manner under thermal stress. The activities of CAT, GSTs, and SOD were significantly enhanced and likely provided a defense mechanism against oxidative damage due to the accumulation of ROS. POX and T-AOC levels were not significantly modified. These results suggest that thermal stress induces oxidative stress, and antioxidant enzymes likely play an important role in reducing oxidative damage in *B. dorsalis*.

Key Words: *Bactrocera dorsalis*, thermal stress, oxidative stress, antioxidant enzymes

RESUMEN

Un cambio en la temperatura puede ser responsable de una variedad de respuestas de estrés fisiológico en organismos, que son usualmente asociados al exceso en la generación de especies reactivas de oxígeno (ROS) llevando a un daño oxidativo. En el presente estudio, los niveles de peroxidación de lípidos (LPO) de la mosca de la fruta oriental, *Bactrocera dorsalis* (Hendel), fueron medidos bajo condiciones de estrés térmico con temperaturas relativamente bajas (-5, -2.5, 0, y 5 °C) o altas (35, 37.5, y 40 °C). Subsecuentemente, el efecto relacionado con el tiempo del estrés térmico sobre la actividad de enzimas antioxidantes incluyendo catalasa (CAT), glutathione-S-transferasa (GSTs), peroxidasa (POX), superóxido dismutasa (SOD), y capacidad antioxidante total (T-AOC) fueron determinadas sistemáticamente. Los resultados mostraron que los niveles de LPO aumentaron significativamente dependiendo del tiempo bajo estrés térmico. Las actividades de CAT, GSTs, y SOD aumentaron significativamente y posiblemente proporcionaron un mecanismo de defensa contra el daño oxidativo causado por la acumulación de ROS. Los niveles de POX y T-AOC no fueron modificados significativamente. Estos resultados sugieren que el estrés térmico induce estrés oxidativo, y que enzimas antioxidantes posiblemente juegan un papel importante reduciendo el daño oxidativo en *B. dorsalis*.

Temperature is one of the most important environmental factors that induces physiological changes in organisms. Previous studies suggested that thermal stress may diminish the antioxidant state and cause oxidative stress (An & Choi 2010), that such oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates, or to easily repair the resulting damage (Rahman et al. 2006). Heat stress signal transduction pathways and defense mechanisms are intimately associated with ROS (Pnueli et al. 2003), whereas low temperature stress induces H_2O_2 accumulation in cells (O'kane et al. 1996). Overproduction of ROS, including superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), and singlet oxygen ($^1O_2$), can lead to increased lipid peroxidation (LPO), protein oxidation and DNA damage (Nordberg & Arnér 2001), and may affect cell viability by causing membrane damage and enzyme inactivity (Livingstone 2001).

To maintain homeostasis and prevent oxidative stress as well as damage by ROS, organisms possess a complex defense system of non-enzymatic scavengers and a range of antioxidant en-

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zymes (Felton & Summers 1995). The antioxidant enzymes, including catalase (CAT), glutathione-S-transferases (GSTs), peroxidase (POX), and superoxide dismutase (SOD), play an important role in protecting cells and maintaining homeostasis by removing oxidative stress (Rudneva 1999). During the enzymatic reactions, SOD removes O_2^- through the process of dismutation to O_2 and H_2O_2 ($2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$), while CAT and POX break H_2O_2 into H_2O and O_2 (Kashiwagi et al. 1997). GSTs remove LPO or hydroperoxide products from cells (Dubovskiy et al. 2008). The process of LPO results in the formation of malondialdehyde (MDA), and its concentration can be an important indicator of oxidative damage (Lopez-Martinez et al. 2008); and LPO can be determined indirectly by measuring MDA concentration (Meng et al. 2009). In addition, total antioxidant capacity (T-AOC) is a measure of the ability of all antioxidants present in an organism to counteract oxidation (Ghiselli et al. 2000).

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is one of the most economically important fruit fly pests with larval stages being frugivorous on a wide range of fruit and vegetables (Clarke et al. 2005; Chen & Ye 2007). It is a major pest throughout Southeast Asia and further west through to Pakistan and north to southern China and Nepal; and it is currently expanding its range (Stephens et al. 2007). In China, the oriental fruit fly is mainly distributed in the southern and central areas, where the summer temperatures may reach or exceed 40 °C and the winter temperature decline to 0 °C or below. As a result of climate change, the potential global range for *B. dorsalis* is projected to extend further polewards as cold stress boundaries recede (Stephens et al. 2007). Environmental temperature is one of the most important factors to affect the distribution and population dynamics of *B. dorsalis*. However, excessive stress caused by changes in the environment beyond specific tolerance levels may damage cells, increased ROS production and activate antioxidant enzymes (Abele et al. 2002).

In this study, we describe changes of LPO levels and antioxidant enzyme activities under thermal stress in *B. dorsalis*. Our aim was to identify the oxidative stresses and the physiological responses of the oriental fruit flies to thermal stress for different treatment durations. We quantified changes in the concentration of MDA and in the activities of antioxidant enzymes (CAT, GST, POX and SOD), as well as the T-AOC.

Materials and Methods

Insects

Bactrocera dorsalis were originally collected from Fujian Province, China in 2009. The adults were reared in wire cages and fed an artificial diet consisting of yeast powder, honey, sucrose, vitamin C, and water. To obtain enough homogeneous individuals, 2 bananas were put into each cage to collect eggs every day. After hatching, larvae were reared on banana fruit in plastic basins with sand until pupation. Basins were kept in an incubator at 27 ± 1 °C, 70 ± 5% RH humidity and photoperiod cycle of 14:10 h L-D throughout development.

Thermal Stress

Thirty adult flies of 5-7-d-old were transferred into a 200-mL plastic cup for each treatment. Three cups were shocked at each target temperature, i.e., -5, -2.5, 0, 5, 35, 37.5, and 40 °C. The duration of each temperature treatment was 3, 6, and 9 h. Flies kept at a temperature of 27 °C served as a control. A programmable temperature controller (Sanyo Electric Co. Ltd., MIR-154, Japan) was used in all stress treatments. In order to check mortality, flies were allowed to recover at 27 °C for 30 min after shock. Then the surviving flies were frozen immediately in liquid nitrogen and stored at -80 °C until analysis. The treatments were replicated 3 times on 3 different days.

Protein Extraction

The protocols of protein extraction were carried out according to the commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). Treated fly samples were first homogenized in 0.9% N saline with a ratio of 1:9 (W_{flies}: $V_{normal\ saline}$). The resulting crude homogenates were centrifuged at 2,500 × rpm for 10 min at 4 °C and the supernatant was used for further analysis. Protein concentrations were determined according to the Bradford (1976) method with bovine serum albumin as the standard.

LPO Assay

Because the MDA concentration is an important indicator of oxidative damage, LPO levels were determined indirectly by measuring MDA concentration with commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) following the manufacturer's protocols with a slight modification. The absorbance was read by a Microplate Spectrophotometer (XMark™, BIO-RAD). The assay was conducted by measuring MDA formed by reacting with thiobarbituric acid to give a red species having a maximum at 532 nm. The MDA concentration was expressed as nmol of MDA produced per mg protein.

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Enzyme Activity Assay

The activities of CAT, POX, and SOD were determined with commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). Tests were conducted following the manufacturer's protocols and the absorbance was read in a Microplate Spectrophotometer (XMark™, BIO-RAD).

CAT activity was calculated by measuring the decrease due to H_2O_2 decomposition spectrophotometrically at 405 nm. One unit of CAT activity was defined as the amount that decomposes 1 µmol of H_2O_2 per s per mg protein. CAT activity was expressed as U mg^{-1} protein.

POX activity was determined at 420 nm by catalyzing the oxidation in the presence of H_2O_2 of a substrate. One unit of POX activity was defined as the amount that catalyses 1 µg substrate per min per mg protein.

SOD activity was determined at 550 nm by use of xanthine and xanthine oxidase systems of Crapo et al. (1978). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the xanthine oxidase system reaction in 1 mL enzyme extraction of 1 mg protein. SOD activity was expressed as U mg^{-1} protein.

GSTs activity was determined with 1-chloro-2, 4-dinitrobenzene (CDNB, Shanghai Chem. Ltd., Shanghai, China) and reduced GSH (Sigma) as substrates according to the procedure of Habig et al. (1974) with slight modifications. The 96-well microtiter plate with 100 µL CDNB (1% ethanol (V/V) included) and 100 µL GSH in normal saline in each well were held for 20 min at 37 °C. Subsequently 100 µL enzyme solutions were added per well, to give the final concentrations of 0.2 and 2.0 mM of CDNB and GSH, respectively. The change in absorbance was measured continuously for 5 min at 340 nm and 37 °C. Changes in absorbance per min were converted into nmol CDNB conjugated/min/mg protein from the extinction coefficient of the resulting 2, 4-dinitrophenyl-glutathione: $\varepsilon_{340\ nm}$ = 9.6 $mM^{-1}cm^{-1}$ (Habig et al., 1974).

T-AOC Assay

T-AOC was measured with commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). The absorbance was read at 520 nm. One unit of T-AOC was defined as the amount necessary to increase the absorbance by 0.01 per min per mg protein. T-AOC was expressed as U mg^{-1} protein.

Statistical Analysis

The three measurements of each assay for the control (27 °C) did not vary significantly (P < 0.05), so the data for the control were pooled in analysis. All the data from different treatments were subjected to One-Way or Two-Way Analysis Of Variance (ANOVA) (temperature and treatment duration) by the general linear model procedure of SPSS 12.0 for Windows (SPSS, Inc., Chicago, Illinois, USA), and mean differences were separated by the least significant difference (LSD) test when significant F-values were obtained (P < 0.05).

Results

No flies died at -2.5, 0, 5, 35, or 37.5 °C; however, none survived being stressed for 9 h at -5, and 40 °C. Dead flies were excluded from the assays.

Changes in LPO Levels

LPO levels (expressed as MDA) in response to thermal stress are shown in Table 1. MDA concentrations of *B. dorsalis* were significantly affected by the treatment temperatures ($F_{7, 44}$ = 777.94; P < 0.01) and durations ($F_{2, 44}$ = 56.38; P <

Table 1. Malondialdehyde (MDA) concentration (±SE) (nmol·mg^{-1}protein) of *B. dorsalis* exposed to thermal stresses.

Temperatures (°C)	MDA concentration (nmol·mg^{-1}protein)		
	3 h	6 h	9 h
-5	0.744 ± 0.017 b[1]	0.796 ± 0.024 b	—
-2.5	0.830 ± 0.023 b	0.541 ± 0.016 a	0.629 ± 0.005 b
0	1.000 ± 0.020 c	1.037 ± 0.025 c	1.567 ± 0.027 d
5	2.610 ± 0.051 e	1.914 ± 0.037 e	1.717 ± 0.006 e
27	0.540 ± 0.042 a	0.540 ± 0.042 a	0.540 ± 0.042 a
35	0.796 ± 0.001 b	1.052 ± 0.040 c	1.439 ± 0.046 c
37.5	1.008 ± 0.032 c	1.283 ± 0.024 d	0.707 ± 0. 011 b
40	1.674 ± 0.017 d	0.794 ± 0.089 b	—

[1]Means within a column followed by the different letters are significantly different (P < 0.05) in ANOVA (LSD).

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0.01), and the interactions between these treatment factors were significant ($F_{12, 44}$ = 130.88; P < 0.01), indicating stress temperature combined with prolonged duration significantly induced higher LPO levels.

The data in Table 1 show that the least concentration of MDA is formed at 27 °C, and as temperatures rise above or decline below 27 °C the concentrations of MDA increase. Thus as temperatures declined below 27 °C the concentrations of MDA increased to reach a maximum concentration at 5 °C, but as the temperature declined below 5 °C, the concentration of MDA progressively declined to reach only 0.744 at -5 °C or just 28% of the maximum at 5 °C. On the other hand as the temperature rose above 27 °C the concentration of MDA progressively increased with increasing temperature and duration to reach the highest levels at 35 °C after 9 h, at 37.5 °C after 6 h, and at 40 °C after 3h.

Changes in Activities of Antioxidant Enzymes

CAT activities of *B. dorsalis* were significantly different from the control when flies were exposed to treatment temperatures ($F_{7, 44}$ = 101.86; P < 0.01) and for the various durations ($F_{2, 44}$ = 36.63; P < 0.01), and there was a significant interaction between these two factors ($F_{12, 44}$ = 34.02; P < 0.01) (Fig. 1). When thermal stress lasted for 6 h, CAT activities peaked at -5, -2.5, 35, and 37.5 °C. When stressed for 3 h, CAT activity was highest at 40 °C (381.88 U·mg^{-1}protein). For 6 h and 9 h at -2.5 °C, CAT activity was 384.79 U·mg^{-1}protein and at 0 °C activity was 450.22 U·mg^{-1}protein.

GSTs activities were significantly affected by treatment temperatures ($F_{7, 44}$ = 58.74; P < 0.01) and durations ($F_{2, 44}$ = 25.81; P < 0.01), and there was a significant interaction between temperature and duration ($F_{12, 44}$ = 14.61; P < 0.01) (Fig. 2). GST activity first decreased after cold stress (3 h), then increased (6 h), while no obvious changes occurred at 5 °C. For heat stress, especially at 37.5 and 40 °C, GST activity increased significantly. For durations, the highest values were recorded as 52.21, 71.91 and 55.24 nmol·min^{-1}·mg^{-1}protein for 3 h at 40 °C, 6h and 9 h at 37.5 °C, respectively.

The effects of different amounts of thermal stress on POX activity of *B. dorsalis* are presented in Fig. 3. POX activities were significantly affected by treatment temperatures ($F_{7, 44}$ = 37.28; P < 0.01) and durations ($F_{2, 44}$ = 15.95; P < 0.01) and there was significant interaction between temperature and duration ($F_{12, 44}$ = 53.29; P < 0.01). However, unlike CAT or GST, POX activities all decreased both as the temperature rose above or fell below 27 °C, regardless of the duration.

SOD activities was significantly enhanced in *B. dorsalis* adults by exposure to treatment temper-

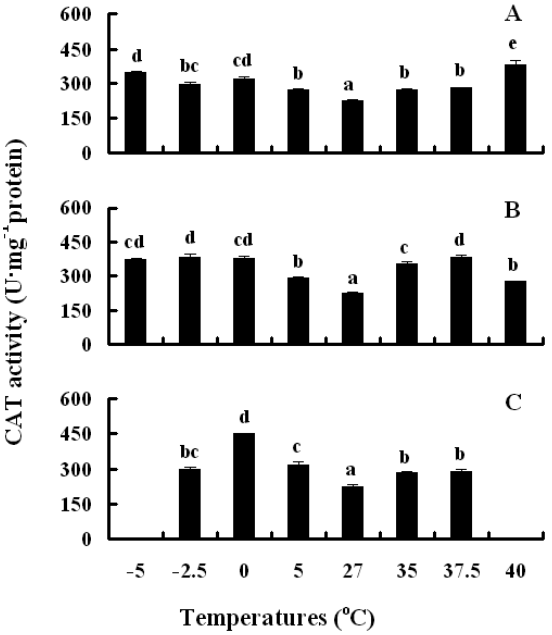


Fig. 1. Catalase (CAT) activity (U·mg^{-1}protein) of *B. dorsalis* exposed to thermal stresses for 3 h (A), 6 h (B) and 9 h (C). The temperature of 27 °C served as a control. Each value represents the mean (±SE) of 3 replications. Different letters above the bars indicate significant differences at P < 0.05 in ANOVA (LSD).

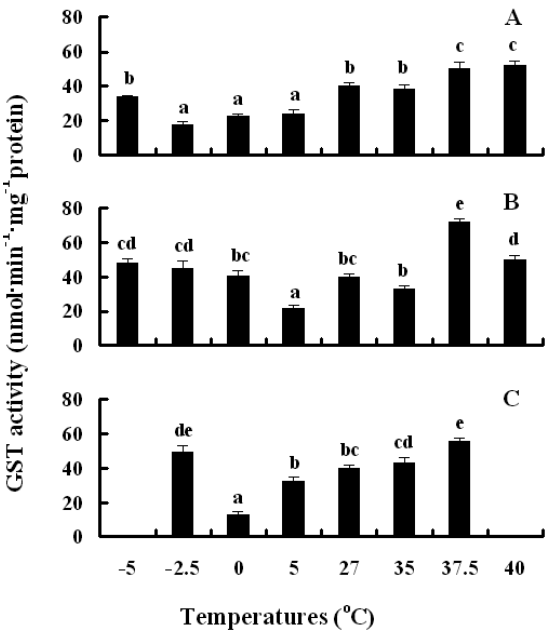


Fig. 2. Glutathione-S-transferases (GSTs) activity (nmol·min^{-1}·mg^{-1}protein) of *B. dorsalis* exposed to thermal stresses for 3 h (A), 6 h (B) and 9 h (C). The temperature of 27 °C served as a control. Each value represents the mean (±SE) of 3 replications. Different letters above the bars indicate significant differences at P < 0.05 in ANOVA (LSD).

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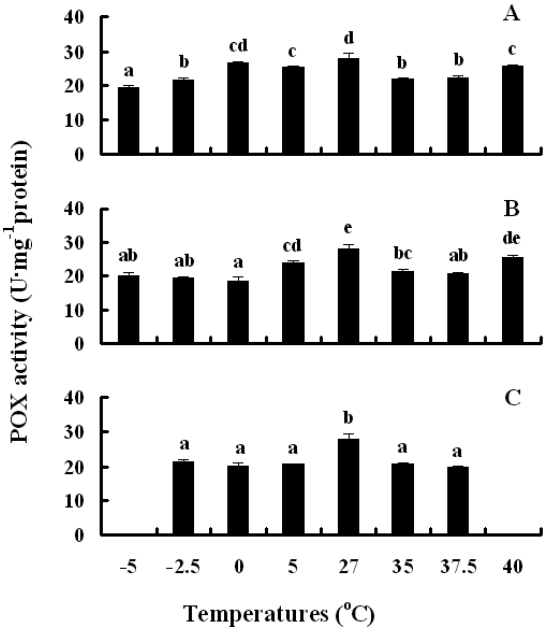


Fig. 3. Peroxidase (POX) activity (U·mg^{-1}protein) of *B. dorsalis* exposed to thermal stresses for 3 h (A), 6 h (B) and 9 h (C). The temperature of 27 °C served as a control. Each value represents the mean (±SE) of 3 replications. Different letters above the bars indicate significant differences at $P < 0.05$ in ANOVA (LSD).

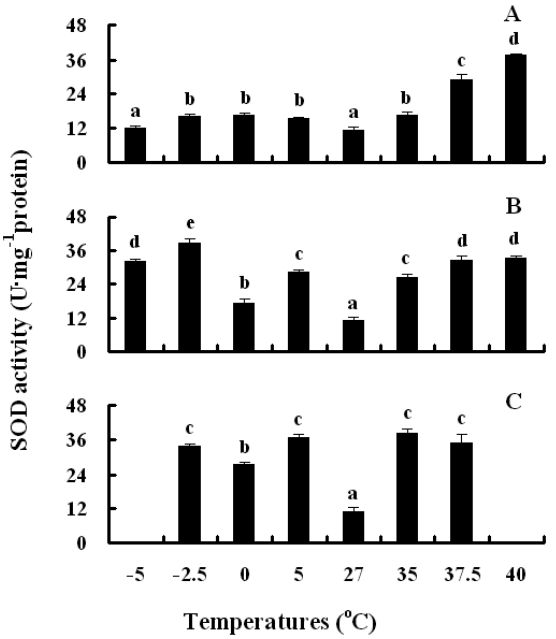


Fig. 4. Superoxide dismutase (SOD) activity (U·mg^{-1}protein) of *B. dorsalis* exposed to thermal stresses for 3 h (A), 6 h (B) and 9 h (C). The temperature of 27 °C served as a control. Each value represents the mean (±SE) of 3 replications. Different letters above the bars indicate significant differences at $P < 0.05$ in ANOVA (LSD).

atures ($F_{7,44} = 105.70$; $P < 0.01$) and for various durations ($F_{2,44} = 177.87$; $P < 0.01$), as presented in Fig. 4. Interaction between temperature and duration was significant ($F_{12,44} = 24.78$; $P < 0.01$). SOD activity increased in all treatments for 3 h when compared with control (27 °C), especially for heat stress. Under heat stress, SOD activity progressively increased with rising temperatures and peaked at 40 °C (37.73 U·mg^{-1}protein). Under cold stress, SOD activity was significantly enhanced after 6 h, compared to 3 h, and the highest value was attained at -2.5 °C (38.77 U·mg^{-1}protein). When treatments were extended to 9 h, all living flies had very high SOD activities and with the highest (38.47 U·mg^{-1}protein) recorded at 35 °C, but this amount was not significantly higher than at -2.5, 5 and 37.5 °C.

Changes in T-AOC

The total antioxidant capacity of *B. dorsalis* under different thermal stress is presented in Table 2. T-AOC was weakly but significantly affected by temperatures ($F_{7,44} = 5.11$; $P < 0.01$), but not by duration ($F_{2,44} = 1.74$; $P = 0.19$), and there was no significant interaction between temperature and duration ($F_{12,44} = 0.49$; $P = 0.91$). When stressed at 40 °C for 3 h, T-AOC significantly increased compared with the controls (27 °C).

Discussion

Temperature is one of the most important environmental variables that affect invertebrates (Bale et al. 2002). To understand the oxidative stress induced in *B. dorsalis* by environmental temperature changes, an index of oxidative stress (LPO), as well as the activity of antioxidant enzymes (CAT, GSTs, POX, and SOD) and T-AOC were measured.

A major oxidation product of peroxidized polyunsaturated fatty acids, MDA, has been used to determine the degree of LPO and as a biological marker of oxidative stress (Rio et al. 2005). MDA concentrations in *B. dorsalis* were significantly increased compared to the control when exposed either to heat or cold shock. Our results clearly demonstrated that in *B. dorsalis* thermal stress was accompanied by lipid peroxidation and other responses to oxidative stress, similar to other animals (An & Choi 2010; Yang et al. 2010). Thus we found that in response to heat stress, MDA concentration significantly increased both with temperatures ascending above 27 °C and temperature descending below 27 °C to reach its maximum value at 5 °C, indicating that this highly unfavorable condition causes much LPO product to be generated. Under much lower temperatures, e.g. 0 °C or -2.5 °C, the fly's metabolism may be di-

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TABLE 2. TOTAL ANTIOXIDANT ACTIVITY (T-AOC) (±SE) (U·MG^{-1}PROTEIN) OF *B. DORSALIS* EXPOSED TO THERMAL STRESSES.

Temperatures (°C)	T-AOC activity (U·mg^{-1}protein)		
	3 h	6 h	9 h
-5	14.130 ± 0.685 a[1]	14.709 ± 0.498 ab	—
-2.5	15.384 ± 0.734 a	14.366 ± 0.415 ab	14.887 ± 0.550 a
0	15.145 ± 0.451 a	14.199 ± 0.733 ab	14.336 ± 0.429 a
5	15.068 ± 0.457 a	14.120 ± 0.570 ab	14.961 ± 0.939 a
27	14.861 ± 0.796 a	14.861 ± 0.796 ab	14.861 ± 0.796 a
35	13.509 ± 0.465 a	13.546 ± 0.544 a	12.743 ± 0.581 a
37.5	15.341 ± 0.717 a	14.364 ± 0.537 ab	14.827 ± 1.112 a
40	17.754 ± 0.646 b	16.001 ± 0.428 b	—

[1]Means with a column followed by the different letters are significantly different ($P < 0.05$) in ANOVA (LSD).

verted along other pathways such as protein oxidation, or DNA damage (Nordberg & Arnér 2001) rather than only increased lipid peroxidation in response to extremely adverse conditions.

CAT, GSTs, POX, and SOD are important antioxidant defense enzymes, and they act in a coordinated manner to counteract oxidative stress generated by high concentrations of ROS inside the cell. Among these antioxidant enzymes in insects, CAT has been considered to be solely responsible for the scavenging H_2O_2, because insects are deficient in selenium-dependent glutathione peroxidase, which is a scavenger present in other organisms (Ahmad & Pardini 1990; Sohal et al. 1990). However, CAT removes H_2O_2 only at high cellular concentrations and is inefficient at removing H_2O_2 at low concentrations (Ahmad et al. 1991). Although CAT activity was too low to detect in citrus red mite exposed to thermal stress (Yang et al. 2010), it was significantly increased in the fat body of silkworm (*Bombyx mori* [L.]) 5th instars exposed to thermal stress (Nabizadeh & Kumar 2011) and in the cotton bollworm, *Helicoverpa armiger*a (Hübner), exposed to UV light irradiation (Meng et al. 2009). In this study, oriental fruit fly CAT activity increased significantly as temperatures deviated from the control of 27 °C. The over-expression of CAT under heat or cold shock resulted in the enzyme's enhanced removal of H_2O_2 and, hence, its prevention of damage by oxidative stress.

GSTs have been reported as a biomarker for assessing the environmental impact of organic xenobiotics that generate oxidative stress (Monteiro et al. 2006). However, when exposed to thermal stress for 3 h, oriental fruit fly GST activity increased significantly only at the highest temperatures (37.5 and 40 °C). With longer exposures GST activity also increased significantly at lower temperatures (-5 °C and -2.5 °C for 6 h) compared to the control (27 °C). This may suggest that GST is involved in the inactivation of accumulated toxic lipid peroxidation products and acute temperature-induced oxidative stress, such as occurs in the Asian citrus psyllid, *Diaphorina citri* Kuwayama, Hemiptera, Psyllidae (Marutani-Hert et al. 2010) and gold fish, *Carassius auratus* L., Cypriniformes: Cyprinidae (Lushchak & Bagnyukova 2006). It also had an obvious time-dependent effect, as in citrus red mite, *Panonychus citri* (McGregor), Acari, Tetranychidae (Yang et al. 2010).

Besides GST, insects have POX activities (Mathews et al. 1997). POX breaks down H_2O_2 (Lee et al. 2005). In this study, POX activities decreased significantly under thermal stress compared to the control. Fig. 3 shows that POX activity was highest at 27 °C, i.e., about 30 U·mg^{-1} protein, but the bar graphs also show that at no temperature or duration did POX decline below 20 U mg^{-1} protein. Thus despite declining by as much as one-third even under the most adverse conditions, POX maintains a substantial protectant against ROS under all conditions. However, in many animals, such as gold fish (Lushchak & Bagnyukova 2006) and honey bee, *Apis mellifera* L., Hymenoptera: Apidae (Corona & Robinson 2006), POX significantly increased under thermal stress. But POX of citrus red mite exposed to thermal stress did not change significantly (Yang et al. 2010).The functions of POX in response to thermal stress deserves further investigation.

SOD is an important constituent of cellular defense against oxidative stress and is among the most potent antioxidants known in nature (Bafana et al. 2011). It plays an important role in reducing the high level of superoxide radical induced by low or high temperatures (Celino et al. 2011). In this study, SOD activities under thermal stress significantly increased compared with control. This suggests that SOD was induced by temperature changes and thus protected the flies from thermal stress. Such a case was reported in 1969 (McCord & Fridovich 1969a, b). Both SOD and CAT directly scavenge ROS. SOD removes O_2^- through the process of dismutation to O_2 and

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H_2O_2, and then H_2O_2 is sequentially reduced to H_2O and O_2 by CAT (Kashiwagi et al. 1997). However, because in our results, CAT was present at higher levels than SOD, it would indicate that, under thermal stress, H_2O_2 was being produced directly by other processes.

As a tool to assess redox status, and as a representative measure of the total antioxidant capacity existing in the organism, the T-AOC assay has been widely used (Ghiselli et al. 2000; Meng et al. 2009; Sashidhara et al. 2011). T-AOC of *B. dorsalis* exposed to thermal stress did not change significantly compared to the control except when stressed at 40 °C for 3 h. This may suggest that T-AOC level was not significantly modified. Besides antioxidant enzymes, some non-enzymatic substances, such as trehalose (Mahmud et al. 2010) and α-tocopherol (Kaur et al. 2009), can play a role in antioxidant stress. Heat shock protein can cooperate with antioxidant enzymes to deal with ROS damage (Zhou et al. 2010). The result in this study may suggest that oriental fruit flies have evolved other defense mechanisms against thermal stress allowing the organisms to survive.

In conclusion, we have confirmed that thermal stress disturbs the redox balance in *B. dorsalis*, which leads to oxidative stress. This indicates that thermal stress damages oriental fruit flies, and is a major potential factor generating oxidative stress products. To counter this stress, antioxidant enzymes provide antioxidant defense and protection. POX activity and T-AOC play minor roles in scavenging deleterious LPO. Nevertheless, significantly enhanced CAT, GST and SOD activities in response to thermal stress are likely a defense mechanism against oxidative damage due to the accumulation of ROS. These processes may be mirrored in the fly's physiological adaptations. However, prolonged exposure to heat or cold shock resulted in decreased activities of CAT, GST and SOD, accompanied by impaired antioxidant capacity and high levels of oxidative stress.

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