Journal of Insect Physiology 73 (2015) 47–52

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Antioxidant responses of *Propylaea japonica* (Coleoptera: Coccinellidae) exposed to high temperature stress

Shize Zhang [a], Wenyan Fu [a], Ning Li [a], Fan Zhang [b], Tong-Xian Liu [a,*]

[a] State Key Laboratory of Crop Stress Biology for Arid Areas and Key Laboratory of IPM of Crop Pests on the Northwest Loess Plateaus of Ministry of Agriculture, Northwest A&F University, Yangling, Shaanxi 712100, China
[b] Institute of Plant and Environmental Protection, Beijing Academy of Agricultural and Forestry Sciences, Banjingcun, Haidian, Beijing 100089, China

ARTICLE INFO

Article history:
Received 10 August 2014
Received in revised form 8 January 2015
Accepted 12 January 2015
Available online 20 January 2015

Keywords:
Propylaea japonica
Oxidative stress
Antioxidant response
High temperature stress

ABSTRACT

Temperature is one of the most important environmental factors, and is responsible for a variety of physiological stress responses in organisms. Induced thermal stress is associated with elevated reactive oxygen species (ROS) generation leading to oxidative damage. The ladybeetle, *Propylaea japonica* (Thunberg) (Coleoptera: Coccinellidae), is considered a successful natural enemy because of its tolerance to high temperatures in arid and semi-arid areas in China. In this study, we investigated the effect of high temperatures (35, 37, 39, 41 and 43 °C) on the survival and activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidases (POD), glutathione-S-transferases (GST), and total antioxidant capacity (TAC) as well as malondialdehyde (MDA) concentrations in *P. japonica* adults. The results indicated that *P. japonica* adults could not survive at 43 °C. CAT, GST and TAC were significantly increased when compared to the control (25 °C), and this played an important role in the process of antioxidant response to thermal stress. SOD and POD activity, as well as MDA, did not differ significantly at 35 and 37 °C compared to the control; however, there were increased levels of SOD, POD and MDA when the temperature was above 37 °C. These results suggest that thermal stress leads to oxidative stress and antioxidant enzymes play important roles in reducing oxidative damage in *P. japonica* adults. This study represents the first comprehensive report on the antioxidant defense system in predaceous coccinellids (the third trophic level). The findings provide useful information for predicting population dynamics and understanding the potential for *P. japonica* as a natural enemy to control pest insects under varied environmental conditions.

1. Introduction

Temperature is one of the most important environmental factors affecting growth, reproduction, distribution, abundance and phenology, but also induces physiological responses in organisms (Angilletta et al., 2002; Parmesan, 2006; Jia et al., 2011). Temperatures above the normal optimum are perceived as heat/thermal stress by all living organisms (Kotak et al., 2007; Nguyen et al., 2013). Surplus generation of reactive oxygen species (ROS) can cause oxidative damage under thermal stress. In general, there is a balance between the generation of ROS and the antioxidant processes. However, the balance is broken during periods of environmental stress, and surplus ROS was produced (Joanisse and Storey, 1996; Martinez et al., 2008; Lalouette et al., 2011). Excess ROS can result in lipid peroxidation that disrupts cell membrane fluidity and can lead to apoptosis as well as DNA damage in the form of mutations, base deletions, degradation and single-strand scission (Green and Reed, 1998; Monaghan et al., 2009). In insects these effects can occur in response to a variety of factors, including compensatory growth (Mangel and Munch, 2005), ingested plant photo-oxidants (Aucoin et al., 1995), and unfavorable environmental conditions (such as pollutants, temperature or hypoxic stress) (Zaman et al., 1995; Joanisse and Storey, 1998; Jing et al., 2005). In order to prevent damage by ROS, living organisms have evolved complex protective mechanisms for scavenging ROS, which include small molecular antioxidants and enzymatic components (Howe and Schilmiller, 2002). Antioxidative enzymes are the most important components in the scavenging system of ROS. Major antioxidative enzymes in insects include superoxide dismutase (SOD), catalase (CAT), peroxidases (POD) and glutathione-S-transferases (GST) (Dubovskiy et al., 2008; Felton and Summers, 1995; Wang et al., 2001). SOD is an important antioxidant defense, and its

* Corresponding author at: Key Laboratory of Applied Entomology, Northwest A&F University, Yangling, Shaanxi 712100, China. Tel.: +86 29 87092663.
E-mail address: txliu@nwsuaf.edu.cn (T.-X. Liu).

http://dx.doi.org/10.1016/j.jinsphys.2015.01.004
0022-1910/

enzymatic action results in the formation of hydrogen peroxide (H_2O_2) and oxygen (O_2). H_2O_2 is then converted by CAT and POD into water (H_2O) and oxygen. GST scavenges the products of lipid peroxidation or hydroperoxides from cells (Dubovskiy et al., 2008; Meng et al., 2009). Total antioxidant capacity (TAC) is a measure of the ability of all antioxidants present in an organism to counteract the oxidation of an indicator by an oxidant or to reduce an indicator substance (Ghiselli et al., 2000). Malondialdehyde (MDA) is a decomposition product of polyunsaturated fatty acid hydroperoxides, and its concentration is related to the degree of membrane lipid peroxidation (Meng et al., 2009). Therefore, lipid peroxidation can be determined indirectly by measuring MDA concentration.

The predaceous coccinellids are important natural enemies of numerous small phytophagous insects and acarines, and are therefore considered as good natural enemies (Obrycki and Kring, 1998). Predaceous coccinellids occur in most cropping systems and their impact on aphid populations is known to be important (Hodek and Honek, 1996; Obrycki et al., 2009). Predaceous coccinellids represent a third trophic level, which must cope with their own thermal stress. To our knowledge, however, little is known about the impact of heat stress on the physiological responses of predaceous coccinellids.

The ladybeetle, *Propylaea japonica* (Thunberg) (Coleoptera: Coccinellidae), is indigenous to many regions of Asia, including China, South Korea, Japan and India (Zhang et al., 2004). *P. japonica* preys on aphids, young larvae, eggs of many insect pests and whiteflies in the field and on greenhouse vegetables, and has 4–5 generations per year in northern China and 7–8 generations per year in southern China (Zhang et al., 2007, 2012). Furthermore, because of its tolerance to higher temperatures, *P. japonica* is considered as a very good natural enemy in the arid and semi-arid areas of China (Zhang et al., 2004).

In northern China, temperature is normally above 35 °C during the summer months, and the maximum daytime temperature exceed 40 °C (Climate Databases, Chinese Academy of Forestry). An interesting question concerning the temperature extremes is how they affect the physiological responses of the predaceous coccinellid, *P. japonica*. The present paper first describes the changes of the antioxidative enzyme activities and MDA of *P. japonica* under high temperature stress. The purpose of this study is to identify the oxidative stress and physiological responses of the ladybeetle to high temperature stress. The findings can provide useful information for predicting population dynamics and understanding the potential for *P. japonica* as an importantly natural enemy to control pest insects under in a variety of environments.

2. Materials and methods

2.1. Insect species

A *P. japonica* colony was initiated from approximately thirty pairs of adults, collected from a garden pea (*Pisum sativum* L.) field and a nearby corn field in the Experimental Farm of the Northwest A&F University (34°17′37.01″ N, 108°01′03.34″ E). The ladybeetles were reared in mesh covered cages (40 × 40 × 40 cm). Four potted broad bean seedlings with aphids (*Aphis craccivora*) were placed in each cage, and the seedlings were replaced with fresh ones with aphids when necessary. The ladybeetles were reared at 25 ± 1 °C, 50 ± 10% RH and 16L: 8D for at least 2 generations, and were then used in the experiments. An *A. craccivora* culture was initiated in the laboratory using aphids collected on garden pea in the same location as the ladybeetles. The culture was developed on potted broad bean plants in mesh-covered cages (60 × 60 × 60 cm).

2.2. Thermal stress

Ten *P. japonica* adults 8–10 d old were transferred into a 5 mL glass vial and exposed to heat stress for 1 h by immersion in a preheated water bath at 35, 37, 39, 41 and 43 °C respectively, after which the adults were allowed to recover at 25 °C for 2 h. The ladybeetles kept at a temperature of 25 °C served as a control. The living adults were frozen immediately in liquid nitrogen and stored at −80 °C until assay. Each treatment was repeated five times.

2.3. Sample preparation

The treated ladybeetles were homogenized in a cold mortar with a pestle in 0.05 M phosphate buffer solution (PBS) pH 7.8, containing 0.1 mM ethylenediamine tetraacetic acid (EDTA) and 1% (wt/vol) polyvinylpyrrolidone (PVP) under liquid nitrogen. The crude homogenates were centrifuged at 10,000*g* for 15 min at 4 °C. The supernatant was re-centrifuged at 10,000*g* for 15 min at 4 °C for determination of antioxidant enzyme activities. Protein concentrations were determined according to the Bradford (1976) method using bovine serum albumin as the standard.

2.4. Enzyme activity assay

The activities of SOD, CAT, POD and GST were determined using commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) following the manufacturer's protocols, with a slight modification as follows: the absorbance was read by using a Tecan Infinite M200 Microplate Reader (Tecan Group Ltd., Switzerland).

SOD activity was assayed by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) at 550 nm. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the NBT reduction caused by the superoxides generated from the reaction of photoreduced riboflavin and oxygen. The result was expressed as U mg^{-1} protein.

CAT activity was measured by following the consumption of H_2O_2 at 240 nm for 4 min. One unit of CAT activity was defined as the amount that reduced the level of H_2O_2 by 1 μmol per second per mg protein. The result was expressed as U mg^{-1} protein.

POD activity was assayed by following the change of absorption at 470 nm due to guaiacol oxidation. One unit of POD activity was defined as the amount that catalyzes 1 μg substrate per minute per mg protein. The result was expressed as U mg^{-1} protein.

GST activity was determined using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The change in absorbance was measured at 340 nm and the enzyme activity was expressed as nmol CDNB conjugate formed/min/mg protein using a molar extinction coefficient of 9.6 mM^{-1} cm^{-1}. The result was expressed as nmol min^{-1} mg^{-1} protein.

2.5. TAC assay

TAC was measured using a test kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) based on the generation of the Fe^{2+}-o-phenanthroline complex, as the overall reducing agents in sample supernatant reduced Fe^{3+} to Fe^{2+}, which reacted with the substrate o-phenanthroline. Stable color of Fe^{2+}-o-phenanthroline complex was measured at 520 nm by using the Microplate Reader (Tecan Group Ltd., Switzerland). One unit of TAC was defined as the amount necessary to increase the absorbance by 0.01 per minute per mg protein. The result was expressed as U mg^{-1} protein.