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Article

Effects of changing temperature on the physiological and biochemical properties of *Harmonia axyridis* larvae

Shasha Wang[1,], Xu Chen[2,**], Yan Li[1], Biying Pan[1], Shigui Wang[1], Huijie Dai[3], Su Wang[2,*], and Bin Tang[1,*]**

[1] College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou, Zhejiang, China
[2] Beijing Key Laboratory of Environment Friendly Management on Fruit Diseases and Pests in North China, Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing, China
[3] Weifang University of Science & Technology, Facility Horticulture Laboratory of Universities in Shandong, Weifang, China
* Corresponding authors: anthocoridae@163.com, tbzm611@yahoo.com
** These authors contributed equally to this study

With 5 figures and 3 tables

Abstract: *Harmonia axyridis* (Pallas) is an important natural enemy insect. Using fourth-instar larvae, we investigated whether changing temperature (5, 15 and 25°C) can enhance the cold tolerance of *H. axyridis* and determined optimal storage temperature conditions. Larvae were exposed to five altering temperature regimes:15/5 (15°C/12 h:5°C/24 h), 5/15/5 (5°C/12 h:15°C/12 h:5°C/24 h), 15/25/5 (15°C/12 h:25°C/12 h:5°C/24 h), 15/5/25/5 (15°C/12 h:5°C/12 h:25°C/12 h:5°C/24 h), and 5/25/15/5 (5°C/12 h:25°C/12 h:15°C/12 h:5°C/24 h). Compared with the larvae of control treatment (5°C/24 h), the larvae had lower supercooling points (SCPs) in the 15/25/5 and 5/25/15/5 groups, higher glycerol content in all treated groups (except for group 5/15/5), lower fat content in the 15/5/25/5 group, lower water content in the 15/25/5 and 15/5/25/5 groups, higher trehalose content in the 15/5, 15/25/5, and 15/5/25/5 groups, higher glucose content in the 15/25/5 group, and higher glycogen content in the 15/25/5, 15/5/25/5, and 5/25/15/5 groups. Compared with the control larvae, TRE1 activity of all treated larvae was significantly enhanced except for those in the 5/15/5 group. Also, the expression levels of three soluble trehalase genes (*TRE1-1, TRE1-2* and *TRE1-3*) and one membrane-bound trehalase gene (*TRE2*) increased after the changing temperature treatment, whereas *TRE1-4, TRE1-5*, and one membrane-bound like trehalase gene (*TRE2-like*) mRNA expression levels were very low. These results suggest that fourth-instar *H. axyridis* larvae exposed to a succession of different temperatures, including low temperatures, survived by reducing the SCP, accumulating carbohydrates and glycerol, and consuming fat. The temperature combination 15/25/5°C provided optimal storage conditions. These findings provide valuable insights for further elucidation of the cold resistance mechanism of ladybeetles and for obtaining an extended shelf life under low-temperature storage.

Keywords: Coccinellidae, trehalose metabolism, glycerol, RT-qPCR

1 Introduction

Aphids are major agricultural pests worldwide (van Emden et al. 2017; Hullé et al. 2020). They could induce notably extensive pesticide applications in crops (Johnson et al. 2009; Ragsdale et al. 2011; Heimpel et al. 2013), with multiple potential associated side effects on non-target arthropods (Lu et al. 2012; Jam & Saber 2018; Mohammed et al. 2018, and see Desneux et al. 2007 for a thorough review) as well as on targeted pests (e.g. hormesis effect or the selection of resistant populations, Guedes et al. 2016; Qu et al. 2015; Ullah et al. 2019a; 2019b), and this despite that natural enemies could be useful in the framework of biocontrol and Integrated Pest Management programs (Desneux et al. 2006; 2019; Ali et al. 2018; Jaworski et al. 2019). The ladybeetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is an important natural enemy that can be used to effectively control whiteflies, mites, aphids, and other pests (Koch 2003; Wang et al. 2017a; Chen et al. 2019), with effective limitation of aphid population growth in various crops (Koch 2003; Costamagna et al. 2008; Koch & Costamagna 2017). However, factors such as cannibalism, need of artificial diet, and low-temperature storage pose challenges for scaling up the commercial production of *H. axyridis* (Wu et al. 2016, Ovchinnikov et al. 2019).

Among insects that are resistant to freezing, the content of cryoprotective penetrants such as sorbitol, glycerol, trehalose, and proline has been found to be significantly increased

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