INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
12–196/2013/15–1–101–106
http://www.fspublishers.org

Full Length Article

Carbohydrate Metabolism and Antioxidant Defense during Diapause Development in Larvae of Oriental Fruit Moth (*Grapholita Molesta*) at Low Temperature

Chao He[1], Quan-Ke Meng[2], Xiang-Bin Yang[1] and Lei Hua[1*]
[1]*College of Plant Protection, Northwest A and F University, Yangling, Shaanxi 712100, China*
[2]*Department of Biology and Chemical Engineering, Sanmenxia Polytechnic, Sanmenxia, Henan 472000, China*
[*]For correspondence: zhibao1022@yahoo.com

Abstract

Oriental fruit moth, *Grapholita molesta* (Lepidoptera: Tortricidae), is a major pest of tree fruits worldwide, overwinters in mature larva diapause. However, little is known about the physiological explanation for diapause development in this pest. In this study, we analyzed the dynamic metabolic processes of carbohydrate, protein and antioxidant enzymes in diapausing larvae, which were maintained at low temperatures of 4 and 10°C under laboratory conditions, and 24°C as control for up to 4 months. After 100 days of maintenance, diapause could be terminated at low temperatures, as indicated by the pupation rate, the larvae were more conducive to complete diapause development at 10°C compared to 4°C. During diapause, total sugar and glycogen content decreased and trehalose content increased at low temperatures; glycerol content significantly increased at 4°C, but not at 10°C; protein content did not significantly changed at low temperature, but slightly decreased during the middle phase of diapause. Additionally, the activities of SOD and CAT increased and POD activities decreased during the middle phase of diapause at low temperatures. Our results indicate that temperature and diapause intensity significantly affected the carbohydrate content and antioxidant enzymes activities of the diapausing larvae, low temperature plays a predominant role in diapause termination, and antioxidant enzymes have a similar role in the regulation of energy metabolism as carbohydrate. © 2013 Friends Science Publishers

Keywords: *Grapholita molesta*; Diapause development; Low temperature; Carbohydrate metabolism; Antioxidant enzyme

Introduction

In insects, diapause is an important survival strategy to escape adverse environmental conditions when metabolic rate depresses significantly and morphogenesis ceases or significantly slows down (Koštál, 2006). Diapause is controlled by more than one environmental condition (Denlinger, 2002); of these importance of temperature on the induction, maintenance and termination of diapause has been well documented (Hondelmann & Poehling, 2007; Yamamura *et al.*, 2008; Terao *et al.*, 2012), especially low temperature can directly affect the occurrence, distribution and reproduction of some insects (Tauber *et al.*, 1986).

In the long-term course of evolution, the insects already have been selected long ago to adapt to low temperature and can successfully survive in extreme environmental conditions. Diapause is a complex an adaptive response that involves many-defined physiological, biochemical and endocrinological adjustments in insects (Bale, 2002). Insects during the period of diapause development may have distinct response to low temperature (Denlinger, 1991). Many studies have documented that low molecular weight sugars and polyols are important energy substance and intermediate metabolites in many insect species during diapause (Storey & Storey, 1991). Metabolic adjustment of these compounds can ensure effective resource utilization, keeping a dynamic balance of nutrition, enhance the level of cold hardiness of diapausing insect and thus increasing the chances of winter survival (Ring & Danks, 1994; Khani *et a1*., 2007). Accordingly, studies of the expression and activity of antioxidant enzymes in insects suggest that the regulation of prooxidant/antioxidant equilibrium affects numerous physiological processes and may impair survival growth, development, fecundity, fertility and adult life span (Felton & Summers, 1995; Pardini, 1995; Šešlija *et al.*, 1999; Jovanović-Galović *et al.*, 2007). Some studies suggest that biochemical mechanisms of cold hardiness and freezing injury may be connected with antioxidative defense (Grubor-Lajsic *et al.*, 1997; Joanisse & Storey, 1998; Stanic *et al.*, 2004; Zhao & Shi, 2010).

Oriental fruit moth, *Grapholita molesta* (Busck), is a key pest of stone fruit worldwide. This moth passes the winter as mature diapausing larvae for about 5 months under natural conditions in Northwest China. Whether the diapausing larvae can successfully overwinter, affects the population dynamics of this species in the following year.

To cite this paper: He, C., Q.K. Meng, X.B. Yang and L. Hua, 2013. Carbohydrate metabolism and antioxidant defense during diapause development in larvae of oriental fruit moth (*Grapholita Molesta*) at low temperature. *Int. J. Agric. Biol*., 15: 101–106

Studies on the diapause ecology of oriental fruit moth effect of photoperiod and temperature (Dickson & Sanders, 1945; Dickson, 1949) and humidity (Chaudhry, 1956) on diapause have been well documented. However, to our knowledge, very little work has been devoted to investigate the physiological and biochemical mechanisms of diapause in this species. The dynamic biochemical processes that govern diapause of oriental fruit moth can help us understand the physiological adaptation mechanisms of diapause of this pest and provide novel targets for pest control.

The objectives of our study were to (i) assay the changes of carbohydrate content and the activities of antioxidant enzymes and (ii) obtain a dynamic overview of the biochemical metabolic of oriental fruit moth.

Materials and Methods

Insects

A colony of oriental fruit moth was maintained at 24±1°C, 16 h light period, and 60±10% RH under laboratory conditions. Founder individuals for this colony were collected from the local abandoned pear orchards in August 2009. Larvae maintained on green thinning apples with a method similar to the one described by Pree (1985). After three generations, their progeny were used for the next experiments.

Newly hatched larvae were reared under a 12 h light period at 24°C, 98-100% mature larvae entered diapause (Dickson, 1949). In our study, 14 days after cessation of feeding, the larvae that had not pupariated were regarded as being in diapause and completed prediapause development (day zero), and then used for the experiments. The diapause larvae were maintained at the following temperature regimes: (1) at 24°C, 12 h light period to retain the diapause state as controls and (2) exposed to 4 and 10°C for up to 4 months (30 days as a month) in darkness, respectively. During this stage, the larvae were subsampled every 20 days, part of them were placed under 16 h light period at 24°C and observed the rate of pupation to determine diapause termination, another part of them were stored at –20°C until used for biochemical measurements.

Estimation of Total Sugar, Glycogen and Protein

Larvae were weighed and dried at 100°C for 1 h. After cooling, added 100 μL of saturated Na_2SO_4 and 200 μL of methanol and crushed with a glass rod. The rod was rinsed with 100 μL of water and then with 3 mL of methanol: chloroform (1:1 v/v). If two layers formed, 300 μL of methanol were added and centrifuged, the precipitate was extracted twice with 1 mL, 500 μL of 66% respectively of ethanol saturated with Na_2SO_4 and centrifuged. The pooled supernatant was taken for measurement of total sugars. The precipitate was kept at 55°C for 5 min and dissolved by adding 500 μL of 30% KOH, then the mixture boiled for 20 min. After cooling, extracted twice in 1 mL of 95% ethanol, 0.5 mL of water and 1 mL of 95% ethanol, respectively, and then centrifuged. The pooled supernatant was taken for measurement of protein, the precipitate was dissolved in 2 mL water, taken for measurement of glycogen (Zhou *et al.*, 2004).

Total sugar assay: A 300 μL sample of total sugar was diluted to 500 μL with water were mixed with 2 mL of 0.2% anthrone solution, then boiled for 15 min. After cooling, absorbance of the reaction mixture was taken at 620 nm and compared with the calibration curve obtained by analysis of glucose standards.

Glycogen assay: A 500 μL sample of glycogen was mixed with 2 mL of 0.2% anthrone solution and then boiled for 15 min. After cooling, absorbance of the reaction mixture was taken at 620 nm and compared with the calibration curve obtained by analysis of glucose standards (Moghadam *et al.*, 2011).

Trehalose assay: A 500 μL sample of total sugar added 1 mL 0.15 M H_2SO4 and boiled for 10 min. After cooling, added 1 mL 30% KOH and boiled for 10 min. After cooling, the mixture was mixed with 2 mL of 0.2% anthrone solution and boiled for 15 min. After cooling, absorbance of 620 nm was read and compared with the calibration curve obtained by analysis of glucose standard (Wu *et al.*, 2004).

Protein assay: A 50 μL sample of protein was diluted to 1 mL with water, added 5 mL of Coomassie brilliant blue G-250 solution. After 2 min, absorbance was read at 595 nm and compared with the calibration curve obtained by analysis of bovine serum albumin standard (Bradford, 1976).

Glycerol assay: Larvae were weighed and homogenized with 500 μL water and centrifuged for 10 min at 5,000 g, the precipitate was dissolved in 500 μL water and centrifuged again. The pooled supernatant was used for measurement of glycerol. A 300 μL sample of glycerol was diluted to 1 mL, added oxidant (325 mg sodium periodate dissolved in 125 mL of water, added with 20 g of ammonium acetate & 15 mL of glacial acetic acid followed by dilution of mixture was diluted to 250 mL with water) 2 mL and chromogenic reagent (1 mL acetylacetone diluted to 250 mL with isopropyl alcohol) 2 mL at 60°C for 15 min. After cooling, absorbance was taken at 420 nm and glycerol content was calculated from the standard (Wu & Yuan, 2004).

Antioxidant enzyme assay: Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities were determined using assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Larvae were weighed and homogenized in 10 volumes of ice-cold 50 mM phosphate buffer (pH 7.0), then centrifuged for 10 min at 12,000 g at 4°C, the supernatant was taken as the enzyme source.

SOD activity was measured by inhibition of superoxide radical production in a xanthine-xanthine oxidase reaction. One unit of SOD activity was defined as the amount of enzyme required to inhibit the rate of SOD by

50% per mg protein in 1 mL reaction mixture at 37°C. POD activity was measured by measuring change in absorbance at 420 nm, according to the principle of POD catalyzed H_2O_2. One unit of POD activity was defined as the amount of enzyme that converted 1 μg of substrate per mg protein per min at 37°C. CAT activity was determined by measuring the disappearance of H_2O_2 at 240 nm. One unit of CAT activity was defined as the amount of enzyme required to degrad H_2O_2 in the reagent at the absorbance of 0.5–0.55 per g protein per second at 25°C. The enzyme protein concentrations were measured with the method of Bradford (1976) with bovine serum albumin as a protein standard.

Statistical Analysis

All experiments were replicated three times for each treatment with two larvae. The values were expressed as the mean ± SEM. All data were analyzed by one-way analysis of variance (ANOVA) with Tukey's multiple comparison test, using SPSS version 19.0 for Windows. $P < 0.05$ was considered significant.

Results

Effect of Cold Exposure on Diapause Termination

The pupation rate of diapause larvae was significant differences at 4 and 10°C, 10°C acted to terminate diapause more quickly ($P < 0.01$). Diapause larvae could not pupate at 24°C until day 120 (Fig. 1).

Total Sugar

Total sugar content was significantly lower at low temperatures than that of at 24°C ($P < 0.01$). At 4°C, total sugar content continuously decreased until day 60, reached the lowest level of 7.58 μg/mg (decreased by 44.47%). At 10°C, total sugar content significantly increased on day 20 and at day 40, reached the lowest level of 7.65 μg/mg (decreased by 43.96%). At 24°C, total sugar content was significantly increased on day 20, then decreased gradually from days 20 to 100, reached the lowest level of 12.90 μg/mg and decreased by 5.49% (Fig. 2a).

Glycogen

Glycogen content was significantly lower at 4 and 10°C compared to 24°C ($P < 0.01$). The changes of glycogen were fairly similar by day 100 at 4 and 10°C, initially continuously decreased and then increased. The lowest value of glycogen 1.76 μg/mg was on day 60, decreased by 76.34% at 4°C and 3.18 μg/mg on day 40, decreased by 57.26% at 10°C. At 24°C, glycogen increased significantly until day 40, decreased greatly on day 60, reached the lowest value of 5.79 μg/mg and decreased by 22.18% (Fig. 2b).

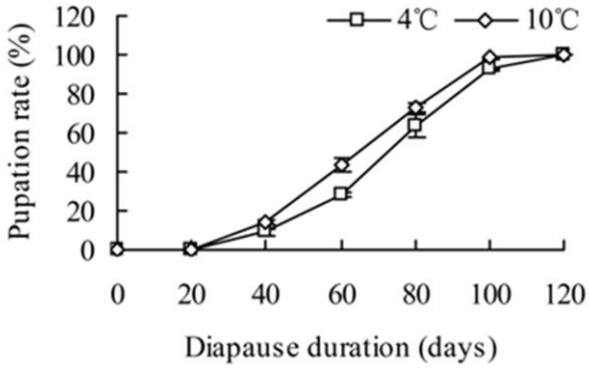

Fig. 1: Effects of exposure to low temperature on pupation rate in diapause larvae of oriental fruit moth at 24°C. The number of larvae analyzed for each transfer day raged between 60 and 100

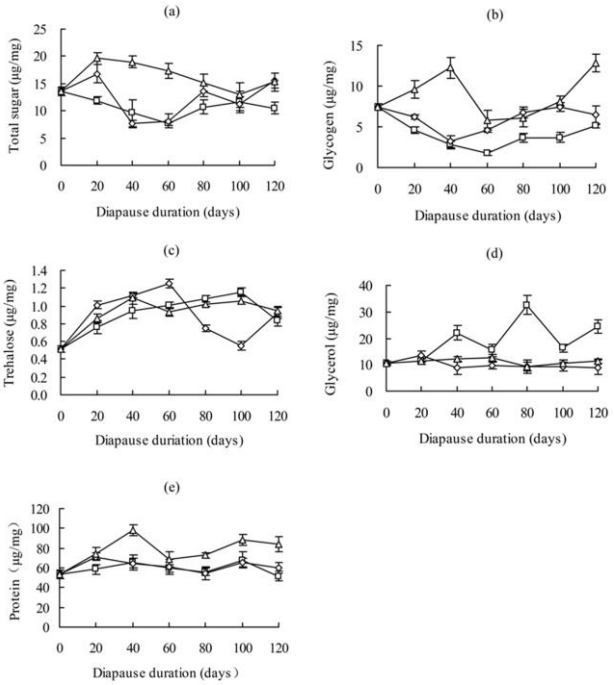

Fig. 2: Total sugar (a), glycogen (b), trehalose (c), glycerol (d) and protein (e) contents of diapause larvae of oriental fruit moth during diapause development at three different temperatures 4°C (-□-), 10°C (-◇-) and 24°C (-△-) for up to 120 days

Trehalose

Trehalose content increased significantly during diapause development at 4 and 10°C, as well as at 24°C ($P < 0.01$). At 4°C, trehalose content consistent increased on day 100, reaching a peak of 1.16 μg/mg and increased 2.23-folds. At 10°C, trehalose content significantly increased by day 60, reaching a peak of 1.25 μg/mg and increased 2.40-folds. At 24°C, trehalose content significantly increased at the first 40

days, then decreased slightly on day 60 and finally remained relatively stable level until day 120 (Fig. 2c).

Glycerol

Glycerol content was significantly affected by low temperature ($P < 0.01$). At 4°C, glycerol content significantly increased to a peak of 22.10 μg/mg, 32.57 μg/mg, and 24.54 μg/mg on day 40, 80, and 120, respectively, and increased more than two-folds. At 10°C, glycerol content increased on day 20, then decreased greatly on day 40, and finally remained constant level until day 120. At 24°C, Glycerol content slightly increased at the first 60 days, then decreased greatly on day 80 (Fig. 2d).

Protein

Protein content was significantly lower at cold temperature compared to 24°C ($P < 0.01$). The changes of protein was fairly similar at 4 and 10°C, initially increased, by day 40 at 4°C and day 20 at 10°C, then decreased until day 80. At 24°C, protein content increased significantly by day 40, and decreased greatly on day 60, then gradually increased until day 100 (Fig. 2e).

SOD

At 4°C, SOD activity decreased on day 20, then continuously increased to the highest level of 11.55 U/mg proteins on day 80 and increased by 45.28%. At 10°C, SOD activity changed followed the same trend as were observed at 4°C, increased to the highest level of 10.73 U/mg protein on day 100, and increased by 34.97%. At 24°C, SOD activity significantly decreased by day 20, then consistent increased to the highest level of 13.41 U/mg protein until day 120 (Fig. 3a).

POD

At 4°C, POD activity changed insignificantly by day 100, and slightly increased on day 120 ($P < 0.05$). At 10°C, POD activity consistent decreased at a very low rate by day 60, then increased until day 100, and significantly decreased on day 120 ($P < 0.05$). At 24°C, POD activity consistently decreased by day 60, then significantly increased to the highest level on day 120 (Fig. 3b).

CAT

At 4°C, initially CAT activity decreased on day 20, then rose sharply and reached a peak on day 80. At 10°C, CAT activity decreased greatly on day 40, then significantly increased and reached a peak on day 80 ($P < 0.01$). At 24°C, CAT activity consistent decreased by day 60, then rose sharply on day 80, and significantly decreased on day 100 ($P < 0.01$) (Fig. 3c).

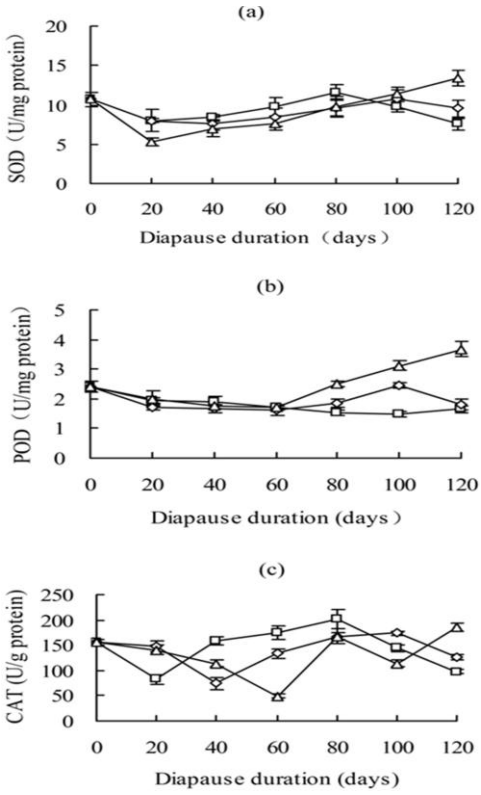

Fig. 3: The activities of SOD (a), POD (b) and CAT (c) in diapause larvae of oriental fruit moth during diapause development at three different temperatures 4°C (-□-), 10°C (-◇-) and 24°C (-△-) for up to 120 days

Discussion

In this study, diapause larvae of oriental fruit moth could be terminated by exposing the larvae to low temperature for a sufficiently long times which responded to 4 and 10°C as an environmental stimulus for the termination of diapause. It seems to be more conducive to complete diapause development at 10°C compared to 4°C. The result indicated that diapause intensity of diapausing larvae at 4°C was higher than that of at 10°C.

During diapause development, the initiation phase of diapause was considered to span a period of approximately 20 days. During this period, large amounts of energy sources (trehalose, glycerol & protein) were stored, and antioxidant enzymes (SOD & CAT) activities decreased simultaneously. During the maintenance phase (>20 days), high levels of cryoprotectants (glycerol) were accumulated in diapause larvae, whilst SOD and CAT activities gradually increased. These phenomena showed that antioxidant enzymes have a similar role in the regulation of energy metabolism as carbohydrate. During diapause, glycogen and protein were converted into other substances, while the activity of SOD and CAT were increased, which enhanced the protective

ability to regulate cold resistance of diapause larvae.

Studies have showed glycogen is used as an energy source material often gradually reduce when insect is overwintering (Goto *et al.*, 1997; Koštál *et al.*, 2004; Bemani *et al.*, 2012); our results also supported this point. At 4 and 10°C, in general, the levels of glycogen had gone through two phases. It degraded gradually in the early stage and continuously increased in the late stage. A lowest value of glycogen appearing at different times indicated that the metabolic rate was different in diapause larvae at different low temperatures. The utilization rate of glycogen at 4°C was significantly higher than that of at 10°C. A total sugar level was also decreased at low temperature with a decrease of glycogen in diapausing larvae. Trehalose, the major insect blood sugar, has previously been considered a cryoprotectant in some insect species (Storey & Storey, 1991; Goto *et al.*, 2001; Koštál *et al.*, 2001). We found, not only at 4 and 10°C but also at 24°C, trehalose content significantly increased and maintained at high level, but the concentrations of trehalose of oriental fruit moth was much lower than the levels reported in other cold hardy insects (Storey & Storey, 1991; Goto *et al.*, 2001; Koštál *et al.*, 2001). This indicated that trehalose may have no real role as cryoprotectant and not closely related to cold hardiness. This phenomenon has also been observed in several other insects (Li *et al.*, 2001; Khani *et al.*, 2007). Glycerol is the most common low molecular weight cryoprotectant found in overwintering insects (Lee, 1991), this is also true in diapause larvae of oriental fruit moth. Glycerol level was significantly increased at 4°C and could stop synthesizing when accumulate to a certain concentration, while at 10 and 24°C, glycerol content did not significantly accumulate. The results show that between glycerol and low temperature have certain relationship, only reach at determinate low temperature, the synthesis of glycerol can be induced. Protein level did not significantly chang at low temperature, initially increased, then slightly decline during diapause development. This showed that protein as a structural component and biological function of macromolecules in insect have associated with diapause development (Lee & Denlinger, 1996), and can transform into other substances to enhance themselves protection.

Studies have showed that there are some antioxidant enzymes in physiological and biochemical metabolic of insect, and play an important role in scavenging oxygen free radicals, protect the insect avoiding damage (Trofimov, 1975; Felton & Summers, 1995; Pardini, 1995; Wang *et al.*, 2012). In diapause larvae of oriental fruit moth, SOD and CAT activities were dropped in early diapause, and then gradually increased with the extension of the time of diapause. SOD and CAT activities were significant higher at low temperature compared to 24°C, the results show that temperature and diapause intensity have significant effect on the activity of POD, SOD and CAT, the low temperature can promote the activity while high temperature can decrease it. However, it seems that different temperatures had no significant effect on the activity of POD by day 60. Diapause larvae in the early stage of diapause, the activities of SOD and CAT decreased, it can be related to metabolic intensity in insects. When insects entered diapause, its metabolic rate declined (Koštál *et al.*, 2004), inducing the ability to synthesis of SOD and CAT was also decline. As other organisms, it has a coordinating role in the activities change of SOD, POD and CAT of diapause larvae, also is a evolved mechanisms of long-term adaptation.

In conclusion, there are different patterns of biochemical changes in diapause development of oriental fruit moth exposed to 4 and 10°C. Accumulation and utilization of carbohydrate were significantly affected by the temperature and diapause intensity, and some biochemical substances could only occur under determinate temperature. Antioxidant enzymes have a similar role in the regulation of energy metabolism as carbohydrate.

Acknowledgement

We thank some undergraduate students for their help in rearing the oriental fruit moth larvae in our laboratory. This study is supported by the “948” project (2006-G54) of China’s Ministry of Agriculture.

References

Bale, J.S., 2002. Insects at low temperatures: from molecular biology to distributions and abundance. *Phil. Trans. Royal Soc. B*, 357: 849–862

Bemani, M., H. Izadi, K. Mahdian, A. Khani and M.A. Samih, 2012. Study on the physiology of diapause, cold hardiness and supercooling point of overwintering pupae of the pistachio fruit hull borer, *Arimania comaroffi*. *J. Insect Physiol*., 58: 897–902

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

Chaudhry, G.U., 1956. The development and fecundity of the oriental fruit moth, *Grapholitha* (*Cydia*) *molesta* (Busck) under controlled temperatures and humidities. *B. Entomol. Res.*, 46: 869–898

Dickson, R.C., 1949. Factors governing the induction of diapause in the oriental fruit moth. *Ann. Entomol. Soc. Am*., 42: 511–537

Dickson, R.C. and E. Sanders, 1945. Factors inducing diapause in the oriental fruit moth. *J. Econ. Entomol*., 38: 605–606

Denlinger, D.L., 1991. Relationship between cold hardiness and diapause. *In*: Lee, R.E. and D.L. Denlinger (eds.), *Insects at Low Temperature*, pp: 174–198. Chapman and Hall, New York, USA

Denlinger, D.L., 2002. Regulation of diapause. *Annu. Rev. Entomol.*, 47: 93–122

Felton, G.W. and C.B. Summers, 1995. Antioxidant systems in insects. *Arch. Insect Biochem.*, 29: 187–197

Grubor-Lajsic, G., W. Block, M. Telesmanic, A. Jovanovic, D. Stevanovic and F. Baca, 1997. Effect of cold acclimation on the antioxidant defense system of two larval Lepidoptera (Noctuidae). *Arch. Insect Biochem.*, 36: 1–10

Goto, M., M. Fujii, K. Suzuki and M. Sakai, 1997. Factors affecting carbohydrate and free amino acid content in overwintering larvae of *Enosima leucoteniella*. *J. Insect Physiol.*, 44: 87–94

Goto, M., Y. Sekine, H. Outa, M. Hujikura and K. Suzuki, 2001. Relationships between cold hardiness and diapause, and between glycerol and free amino acid contents in overwintering larvae of the oriental corn borer, *Ostrinia furnacalis*. *J. Insect Physiol.*, 47: 157–165

Hondelmann, P. and H.-M. Poehling, 2007. Diapause and overwintering of the hoverfly *Episyrphus balteatus*. *Entomol. Exp. Appl.*, 124: 189–200

Jovanović-Galović, A., D.P. Blagojević, G. Grubor-Lajšić, M.R. Worland and M.B. Spasić, 2007. Antioxidant defense in mitochondria during diapause and postdiapause development of European corn borer (*Ostrinia nubilalis*, Hubn.). *Arch. Insect Biochem.*, 64: 111–119

Joanisse, D.R. and K.B. Storey, 1998. Oxidative stress and anti-oxidants in stress and recovery of cold-hardy insects. *Insect Biochem. Mol. Biol.,* 28: 23–30

Khani, A., S. Moharramipour and M. Barzegar, 2007. Cold tolerance and trehalose accumulation in overwintering larvae of the codling moth, *Cydia pomonella* (Lepidoptera, Tortricidae). *Eur. J. Entomol.*, 104: 385–392

Koštál, V., M. Šlachta and P. Šimek, 2001. Cryoprotective role of polyols independent of the increase in supercooling capacity in diapausing adults of *Pyrrhocoris apterus* (Heteroptera: Insecta). *Comp. Biochem. Physiol. B*, 130: 365–374

Koštál, V., M. Tollarová and J. Šula, 2004. Adjustments of the enzymatic complement for polyol biosynthesis and accumulation in diapausing cold-acclimated adults of *Pyrrhocoris apterus*. *J. Insect Physiol.*, 50: 303–313

Koštál, V., 2006. Eco-physiological phases of insect diapause. *J. Insect Physiol.*, 52: 113–127

Lee, R.E., 1991. Principles of insect cold hardiness. *In*: Lee, R.E. and D.L. Denlinger (eds.), *Insects at Low temperature*, pp: 17–46. Chapman and Hall, New York, USA

Lee, K.Y. and D.L. Denlinger, 1996. Diapause-regulated proteins in the gut of pharate first instar larvae of the gypsy moth, *Lymantria dispar*, and the effect of KK-42 and neck ligation on expression. *J. Insect Physiol.*, 42: 423–431

Li, Y.P., M. Goto, S. Ito, Y. Sato, K. Sasaki and N. Goto, 2001. Physiology of diapause and cold hardiness in the overwintering pupae of the fall webworm *Hyphantria cunea* (Lepidoptera: Arctiidae) in Japan. *J. Insect Physiol.*, 47: 1181–1187

Moghadam, E.B., H. Izadi, M.A. Samih, S. Moharramipour and K. Mahdian, 2011. Effect of insect growth regulators, temperature and overwintering on larvae of pistachio leaf white borer (*Ocneria terebinthina*). *Int. J. Agric. Biol.*, 13: 375–380

Pardini, R.S., 1995. Toxicity of oxygen from naturally occurring redox-active pro-oxidants. *Arch. Insect Biochem.*, 29: 101–118

Pree, D.J., 1985. Grapholita molesta. *In*: Singh P. and R.F. Moore (eds.). *Handbook of Insect Rearing*, Vol. *2*, pp: 307–311. Elsevier, Amsterdam, The Netherlands

Ring, R.A. and H.V. Danks, 1994. Desiccation and cryoprotection: overlapping adaptations. *CryoLetters*, 15: 181–190

Šešlija, D., D. Blagojević, M. Spasić and N. Tucić, 1999. Activity of superoxide dismutase and catalase in the bean weevil (*Acanthoscelides obtectus*) selected for postponed senescence. *Exp. Gerontol.*, 34: 185–195

Stanic, B., A. Jovanovic-Galovic, D.P. Blagojevic, G. Grubor-Lajsic, R. Worland and M.B. Spasic, 2004. Cold hardiness in *Ostrinia nubilalis* (Lepidoptera: Pyralidae): Glycerol content, hexose monophosphate shunt activity and antioxidative defense system. *Eur. J. Entomol.*, 101: 459–466

Storey, K.B. and J.M. Storey, 1991. Biochemistry of cryoprotectants. *In*: Lee, R.E. and D.L. Denlinger (eds.), *Insects at Low Temperature*, pp: 64–93. Chapman and Hall, New York, USA

Tauber, M.J., C.A. Tauber and S. Masaki, 1986. *Seasonal Adaptations of Insects.* Oxford University Press, Oxford, UK

Terao, M., Y. Hirose and Y. Shintani, 2012. Effects of temperature and photoperiod on termination of pseudopupal diapause in the bean blister beetle, *Epicauta gorhami*. *J. Insect Physiol.*, 58: 5737–5742

Trofimov, V.B., 1975. Some unusual physiological characteristics of diapause in *Neodiprion sertifer*. *Ekologiya*, 5: 85–88

Wang, Y., L.J. Wang, Z.H. Zhu, W.H. Ma and C.L. Lei, 2012. The molecular characterization of antioxidant enzyme genes in *Helicoverpa armigera* adults and their involvement in response to ultraviolet-A stress. *J. Insect Physiol.,* 58: 1250–1258

Wu, J.X., F. Yuan and L. Su, 2004. Changes of carbohydrate content in larvae of the wheat midge, *Sitodiplosis mosellana* (Gehin) during mature and diapauses stage. *Acta Entomol. Sin.*, 47: 178–183

Wu, J.X. and F. Yuan, 2004. Changes of glycerol content in diapause larvae of the orange wheat blossom midge, *Sitodiplosis mosellana* (Gehin) in various seasons. *Entomol. Sin.*, 11: 27–35

Yamamura, S., M. Ikarashi and M. Sasaki, 2008. Dual photoperiodic regulation to enable univoltine life cycle in alpine silver-Y moth, *Syngrapha ottolenguii* (Noctuidae: Plusiinae) without obligatory diapause. *Appl. Entomol. Zool.*, 43: 1105–1112

Zhao, L.C. and L.G. Shi, 2010. Metabolism of hydrogen peroxide between diapause and non-diapause eggs of the silkworm, *Bombyx mori* during chilling at 5°C. *Arch. Insect Biochem. Physiol.,* 74: 127–134

Zhou, G., M. Flowers, K. Friedrich, J. Horton, J. Pennington and M.A. Wells, 2004. Metabolic fate of [^{14}C]-labeled meal protein amino acids in *Aedes aegypti* mosquitoes. *J. Insect Physiol.*, 50: 337–349

(Received 27 April 2012; Accepted 24 September 2012)