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ORIGINAL PAPER

Adult reproductive diapause in *Drosophila suzukii* females

Yifan Zhai[1] · Qingcai Lin[1] · Jinping Zhang[2] · Feng Zhang[2] ·
Li Zheng[1] · Yi Yu[1]

Abstract *Drosophila suzukii* (Diptera: Drosophilidae) is an emerging pest of soft fruits, but in this species diapause has not been thoroughly explored. We examined the effects of different temperatures and photoperiods on diapause induction and termination under laboratory conditions. There was variation in the ovarian development and oviposition rate under different photoperiods at 10 ± 1 °C, and the percentage of adults with immature ovaries was higher during the short photoperiod (8L:16D) than other photoperiods at 10 ± 1 °C. Adults were most sensitive to photoperiod within 3 days of eclosion. The optimal combination of photoperiod and temperature for diapause termination was a long photoperiod (16L:8D) at 25 ± 1 °C. The supercooling point was significantly reduced in reproductive diapause females, and trehalase, pyruvate kinase, sorbitol dehydrogenase, hexokinase and phosphofructokinase enzyme activities were significantly reduced (36.46, 57.85, 32.64, 54.68 and 24.59 %, respectively); glycogen and triglyceride levels were significantly increased (42.17 and 120.36 %). We conclude that *D. suzukii* is typical of short-day diapause species within a certain photoperiod range. This information might contribute to a more fundamental understanding of adult reproductive diapause for this important pest.

Keywords *Drosophila* · Reproductive diapause · Photoperiod · Diapause induction · Cold tolerance

Communicated by A. Biondi.

Special Issue: Spotted Wing Drosophila.

✉ Yifan Zhai
  zyifan@saas.ac.cn

✉ Yi Yu
  robertyuyi@163.com

1 Institute of Plant Protection, Shandong Academy of Agricultural Sciences, Jinan 250100, China

2 MoA-CABI Joint Laboratory for Bio-Safety, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

Key message

- We report adult reproductive diapause induction and termination in *D. suzukii*.
- *D. suzukii* was shown to be a typical of short-day diapause species.
- The 1–3-day period after eclosion is the most sensitive stage to photoperiod in *D. suzukii*.
- *D. suzukii* diapausing females showed higher cold tolerance than non-diapasonic ones.
- Diapausing females had lower enzymatic activities and higher energy reserves.

Introduction

Diapause is advantageous for avoiding adverse environmental conditions, enabling females to survive through severe winter conditions and then develop when temperatures and photoperiods increase (Denlinger 2002, 2008). In addition, diapause typically occurs at a specific developmental stage for each species, such as the embryo (e.g., *Bombyx mori*), larvae/nymph (e.g., *Loxostege sticticalis*), pupae (e.g., *Helicoverpa armigera*) or adult (e.g.,

Drosophila melanogaster) stages (Allen 2007; Jiang et al. 2010; Liu et al. 2010; Kobayashi et al. 2014). Adult reproductive diapause often occurs because many insect species overwinter as adults, in which the oogenesis and vitellogenesis processes usually stop (Baker and Russell 2009).

Drosophila suzukii (Diptera: Drosophilidae) has the evolutionary advantage over other *Drosophila* species, that is, a serrated ovipositor to break the skin of fresh or fruits and lay eggs inside. Now, it has become an economically important pest insect for berry and some stone fruits in the world, such as in the Americas, Europe and Eastern Asia (Chabert et al. 2012; Rota-Stabelli et al. 2013; Deprá et al. 2014; Zhai et al. 2014; Lin et al. 2014a, b; Cini et al. 2014), requiring the prompt development of sustainable control tools (Asplen et al. 2015; Daane et al. 2016; Haye et al. 2016; Murphy et al. 2016). In addition, *D. suzukii* adults survive for 3–12 weeks, and only adult flies can overwinter in various shelters (Kimura 2004; Dalton et al. 2011; Rossi-Stacconi et al. 2016). In the winter, adult *D. suzukii* is darker and has longer wings than in the summer (Kanzawa 1936). Several authors found that *D. suzukii* adults collected in autumn were reproductively immature, suggesting overwintering adult reproductive diapause (Mitsui et al. 2010; Rossi-Stacconi et al. 2016; Wang et al. 2016). Diapause has been studied for many Drosophilids, for example, *D. robusta*, *D. littoralis*, *D. montana*, *D. triauraria* and *D. melanogaster* (Carson and Stalker 1948; Lumme et al. 1973; Yamada and Yamamoto 2011; Salminen and Hoikkala 2013; Kubrak et al. 2014). However, to the best of our knowledge, there are no specific studies on the mechanisms affecting *D. suzukii* female reproductive diapause.

In the present study, *D. suzukii* diapause induction and termination were investigated under laboratory and field conditions. This work could thus provide the basis for further investigation on molecular regulation of reproductive diapause in this species.

Materials and methods

Study flies

We obtained the original *D. suzukii* colony from cherry orchards at Tai'an (35°67′N, 116°24′E) in Shandong Province, China, in 2012. The strain was reared in a continuous laboratory culture on artificial medium. The insects were maintained in the laboratory at 25 ± 1 °C under a 16L:8D daylight cycle and 70–80 % relative humidity.

Females were collected within 12 h after eclosion and reared on fresh artificial medium (50 g banana, 5 g yeast, 2 g flour, 3 g sugar, 2.5 g agar, 0.5 g nipagine and 150 ml H_2O) in glass jars (length: 400 mm; diameter: 200 mm) at

different photoperiods and temperatures for the experiments; see below.

Field experiments

A sugar-vinegar mixture was used to trap *D. suzukii* adults in a cherry orchard in Tai'an region, Shandong Province. Ten field plots in this orchard were selected. Three trees were then randomly selected from each plot, and three traps with the sugar-vinegar mixture were placed in each of the selected trees. The temperature and photoperiod in the orchard were recorded. *D. suzukii* adults were collected from these traps and delivered to the laboratory for dissection.

Diapause induction experiments

Newly emerged females were reared on artificial medium in phytotronic environments at different temperatures (5 ± 1, 10 ± 1, 15 ± 1, 20 ± 1 and 25 ± 1 °C) and photoperiods (4L:20D, 6L:18D, 8L:16D, 10L:14D, 12L:12D, 14L:10D and 16L:8D) for further observation (Williams et al. 2006). The females were dissected 15 days later, and the developmental status of the ovaries was assessed according to King (1970). The ovaries were dissected and classified into three categories: immature, developing and developed ovaries (Fig. 1). A female was scored as diapausing if the ovaries were without vitellogenin accumulation (immature ovaries); a female was scored as non-diapausing if vitellogenin was observed in the ovaries (developing and developed ovaries). The quadratic parabolic regression equation of the diapause critical photoperiod was fitted by R statistical software and used for the different photoperiod and diapause rate (arcsin square root transform) data. Approximately 40–50 individuals were observed in each of the three replicates for all treatments and placed onto fresh artificial medium in glass jars (Supplementary Table 1).

Determination of the sensitive stage to photoperiod

To determine the effect of developmental stages of *D. suzukii* on the diapause sensitivity to photoperiod, an experiment was conducted following Spieth (1995). Different developmental stages of *D. suzukii* from newly laid eggs to adults and the differently aged adults (from 1 to 15 days old at an interval of 3 days) were tested. All the insects were reared on a fresh artificial diet in glass jars initially under the standard condition (16L:8D, 25 ± 1 °C) and then transferred to photoperiods at 16L:8D and 8L:16D, respectively (see Tables 1, 2), a photoperiod at 10 ± 1 °C. The resulting females were dissected when they were 16 days old, and the number of eggs in the
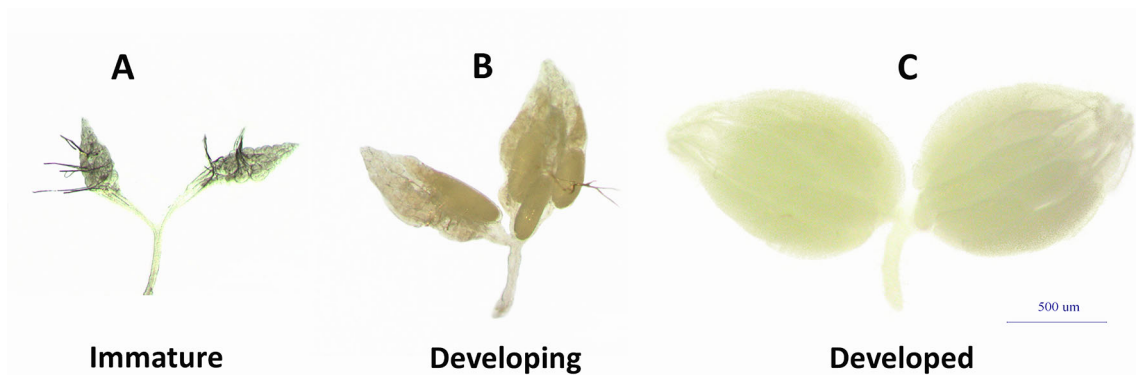
Fig. 1 Classification of ovaries into three developmental stages in *D. suzukii*. **a** Immature ovaries of a newly enclosed or diapausing female, **b** developing ovaries of incomplete vitellogenesis and **c** developed ovaries of a sexually mature female

Table 1 The diapause rate after photoperiods of 8L:16D and 16L:8D at different developmental stages in *D. suzukii* at 10 °C

Treatments no.	Different developmental stages						Total number of females	Oviposition rate (%)	Diapause rate (%)
	Egg	1st	2nd	3rd	Pupa	Adult			
1	S	L	L	L	L	L	135	100.0 ± 0.0a	0.0 ± 0.0f
2	S	S	L	L	L	L	159	85.5 ± 0.8b	15.4 ± 1.6e
3	S	S	S	L	L	L	144	68.3 ± 1.2c	25.2 ± 0.8d
4	S	S	S	S	L	L	156	65.5 ± 0.8c	23.8 ± 2.1d,e
5	S	S	S	S	S	L	165	66.8 ± 0.5c	28.2 ± 1.2d
6	S	S	S	S	S	S	144	0.0 ± 0.0e	100.0 ± 0.0a
7	L	S	S	S	S	S	165	0.0 ± 0.0e	100.0 ± 0.0a
8	L	L	S	S	S	S	144	0.0 ± 0.0e	100.0 ± 0.0a
9	L	L	L	S	S	S	126	8.5 ± 0.1d	88.2 ± 0.8c
10	L	L	L	L	S	S	150	7.8 ± 0.2d	92.3 ± 1.3b
11	L	L	L	L	L	S	138	12.8 ± 1.2d	83.5 ± 0.8c
12	L	L	L	L	L	L	135	100.0 ± 0.0a	0.0 ± 0.0f

L long photoperiod (16L:8D); *S* short photoperiod (8L:16D). The data represent the mean values ± SD, and the different letters denote a significant difference ($p < 0.05$, Tukey's post hoc test)

ovaries was counted and recorded. The oviposition and diapause rates were then calculated [oviposition rate = (number of females with oviposition/total number of females) × 100 % and the diapause rate = (number of females with immature ovaries/total number of females) × 100 %]. Approximately 40–60 individuals were tested in each treatment, and the experiment was replicated three times.

Diapause termination bioassay

Diapaused female individuals of *D. suzukii* were obtained after being treated for 15 days since the eclosion under the diapause-inducing photoperiod (8L:16D) at 10 ± 1 °C. The diapaused female adults (at the 16th day post-eclosion) were then treated respectively at four different temperatures (10 ± 1, 15 ± 1, 20 ± 1 and 25 ± 1 °C), each with three respective photoperiods (8L:16D, 12L:12D, 16L:8D)

for 15 days. The resulting adults were then dissected and the vitellogenin observed in the counted ovaries; 50–60 individuals were tested for each of the treatments, and the experiment was replicated three times (Supplementary Table 2).

Assessment of cold tolerance

Newly emerged females were reared under different photoperiods (8L:16D, 12L:12D, 16L:8D) and temperatures (10 ± 1, 25 ± 1 °C), and the supercooling point (SCP) and freezing point (FP) of the females (15th day post-eclosion) under different treatments were measured. The abdomen of each female was fixed with plastic tape to the tip of a thermocouple attached to a multichannel automatic recorder (TOPTEST TP9024, Top Electric Co., Shenzhen, China). The thermocouple with the female was placed inside an insulating tube in a cryogenic refrigerator to

Table 2 The diapause rate after photoperiods of 8L:16D and 16L:8D at different adult stages in *D. suzukii* at 10 °C

Treatments no.	Different adult stages					Total number of females	Oviposition rate (%)	Diapause rate (%)
	1–3 day	4–6 day	7–9 day	10–12 day	13–15 day			
1	S	L	L	L	L	165	29.2 ± 1.5d	53.2 ± 2.1b
2	S	S	L	L	L	129	33.5 ± 1.1d	50.1 ± 1.6b,c
3	S	S	S	L	L	174	32.7 ± 1.2d	57.4 ± 0.8b
4	S	S	S	S	L	117	27.1 ± 0.9d	63.8 ± 2.1a,b
5	S	S	S	S	S	144	13.2 ± 0.2e	80.2 ± 1.2a
6	L	S	S	S	S	135	55.5 ± 0.8c	38.3 ± 0.8c
7	L	L	S	S	S	135	88.5 ± 1.3b	10.8 ± 0.2d
8	L	L	L	S	S	126	100.0 ± 0.0a	0.0 ± 0.0e
9	L	L	L	L	S	165	100.0 ± 0.0a	0.0 ± 0.0e
10	L	L	L	L	L	144	100.0 ± 0.0a	0.0 ± 0.0e

L represents the long photoperiod (16L:8D), and *S* represents the short photoperiod (8L:16D). The data represent the mean values ± SD, and the different letters denote a significant difference ($p < 0.05$, Tukey's post hoc test)

ensure that the temperature of the adult decreased at a cooling rate of approximately 1 °C min^{-1}. The SCP and FP were identified as the lowest inflexion point and peak point in the temperature, respectively (Guo et al. 2006; Formby et al. 2013). We examined 40–50 individuals in each of three replicates for all treatments and placed them on fresh artificial medium in glass jars (Supplementary Table 3).

Assessment of metabolic enzyme activities and biochemical substances

To clarify the physiological adaptation of diapause (8L:16D) or non-diapause (16L:8D) females (15th day post-eclosion) of this pest at 10 ± 1 °C, some cold tolerance-related metabolic enzymes, such as trehalase (TRE), sorbitol dehydrogenase (SDH), pyruvate kinase (PK), alkaline phosphatase (ALP), hexokinase (HK) and phosphofructokinase (PFK), were quantified. The activities of metabolic enzymes were measured by commercial kits (Suzhou Comin Biotechnology Co., Ltd., Suzhou, China), and the absorbance of TRE, SDH, PK, HK and PFK were measured at 340 nm. ALP was measured at 510 nm. Glycogen (MAK016), triglyceride (TR0100) and protein (BCA1) were measured by commercial kits (Sigma-Aldrich Co. LLC., USA). Approximately 40–50 individuals were observed in each of three replicates for all treatments and were placed onto fresh artificial medium in glass jars (Supplementary Table 4).

Statistical analyses

The statistical analyses were performed using SPSS17.0 software. The effects of photoperiod and temperature and their interactions on the induction of diapause were tested using a general linear model (GLM). Differences between treatment levels were examined using ANOVA, followed by Tukey's analysis.

Results

Field investigation

To identify the population dynamics and female ovarian development stages of *D. suzukii*, we investigated cherry orchards at Tai'an from 2013 to 2014, and no adults were recorded to be trapped in winter (December–February). The first and last females with immature ovaries were trapped after and before winter, respectively. In addition, the proportions of females with developing and developed ovaries increased from April to June and then gradually decreased from July to November. During this time, the photoperiod and temperature of n-valley changed, and the photoperiod was gradually increased to more than 10 h. The mean temperature was gradually increased above 10 °C (Fig. 2).

Photoperiodic responses for reproductive diapause induction

Ovarian development stages of the females were measured in different photoperiod and temperature combinations, and there was variation of ovarian development stages under different temperatures and photoperiods (Fig. 3). The ovarian development was delayed with a lower temperature and short photoperiod, but development accelerateed with temperature rises and photoperiod increases. The ovarian development stage (Fig. 3a) and oviposition rate (Fig. 3b)

Fig. 2 The investigation of the population dynamics and female ovarian development stages of *D. suzukii* in the cherry orchards at Tai'an once a month. **a** The photoperiod and temperature were recorded on different survey dates under various field conditions. **b** Proportions of the females with immature ovaries are shown in the *slash* and those with developing and developed ovaries in *light* and *dark gray*, respectively
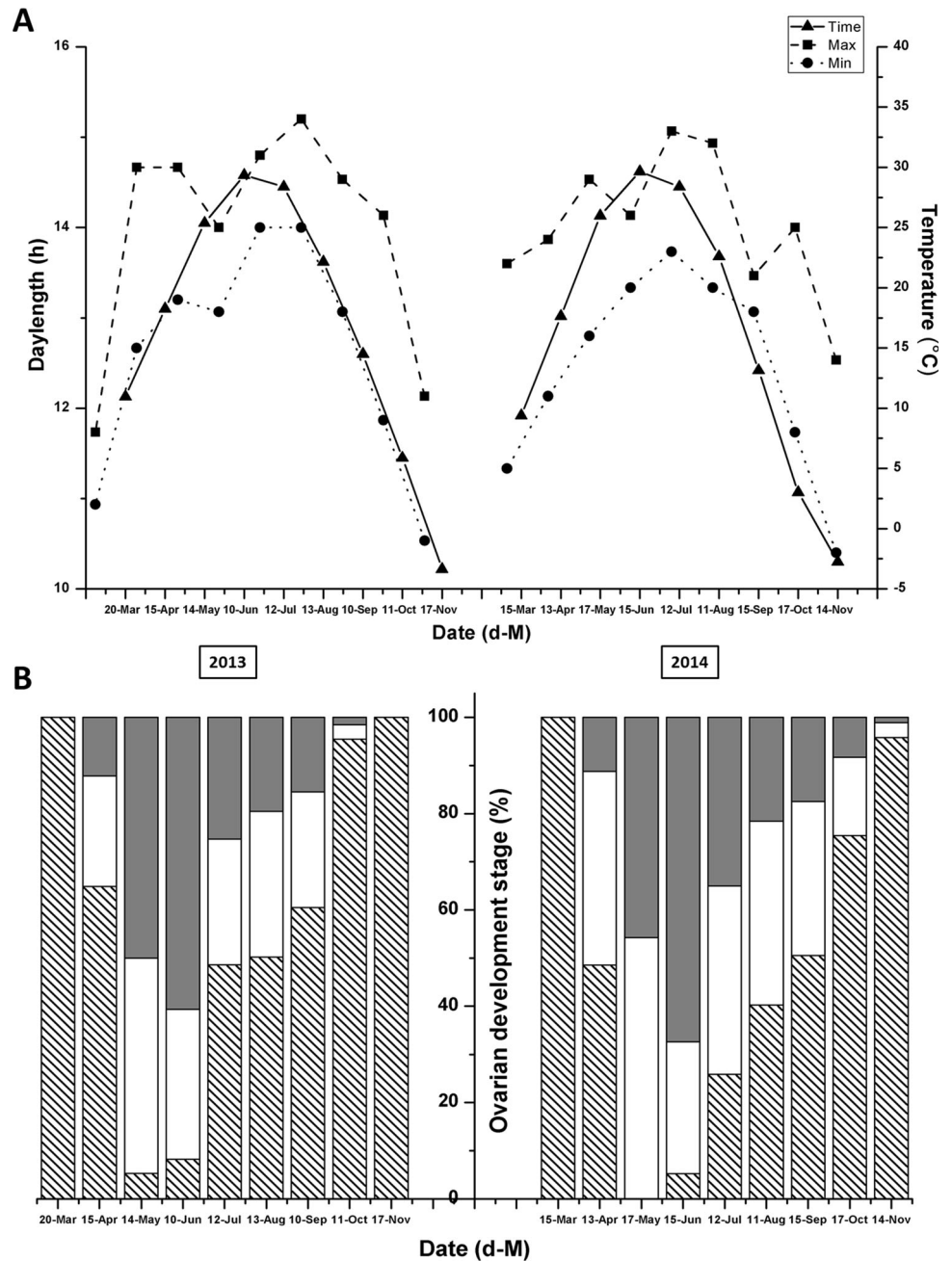
were different at 10 ± 1 °C, during the short photoperiod (8L:16D); all the proportions of the females had immature ovaries and no sign of oviposition. The ovaries developed more rapidly under a long photoperiod (16L:8D), all the proportions of the females had developing and developed ovaries, and the oviposition rate was 100 %. The photoperiod response showed *D. suzukii* was typical of long-day species, with a diapause critical photoperiod of 13.35 h at 10 °C, according to the regression equation

$$Y = -0.0281 X^2 + 0.4977 X - 0.851 \ (R^2 = 0.9325).$$

The most sensitive stage to the photoperiod

The diapause rate increased slowly with an increase in the numbers of short-day treatment (no. 1–5), and it suddenly reached 100 % at the adult stage during the short-day photoperiod (no. 6) (Table 1). The diapause rate remained at a high level for the adult stage during the short-day photoperiod (no. 7–11), and it suddenly decreased from 83.5 to 0 % when the adult was treated with the long-day photoperiod (no. 12) (Table 1). These results suggest that
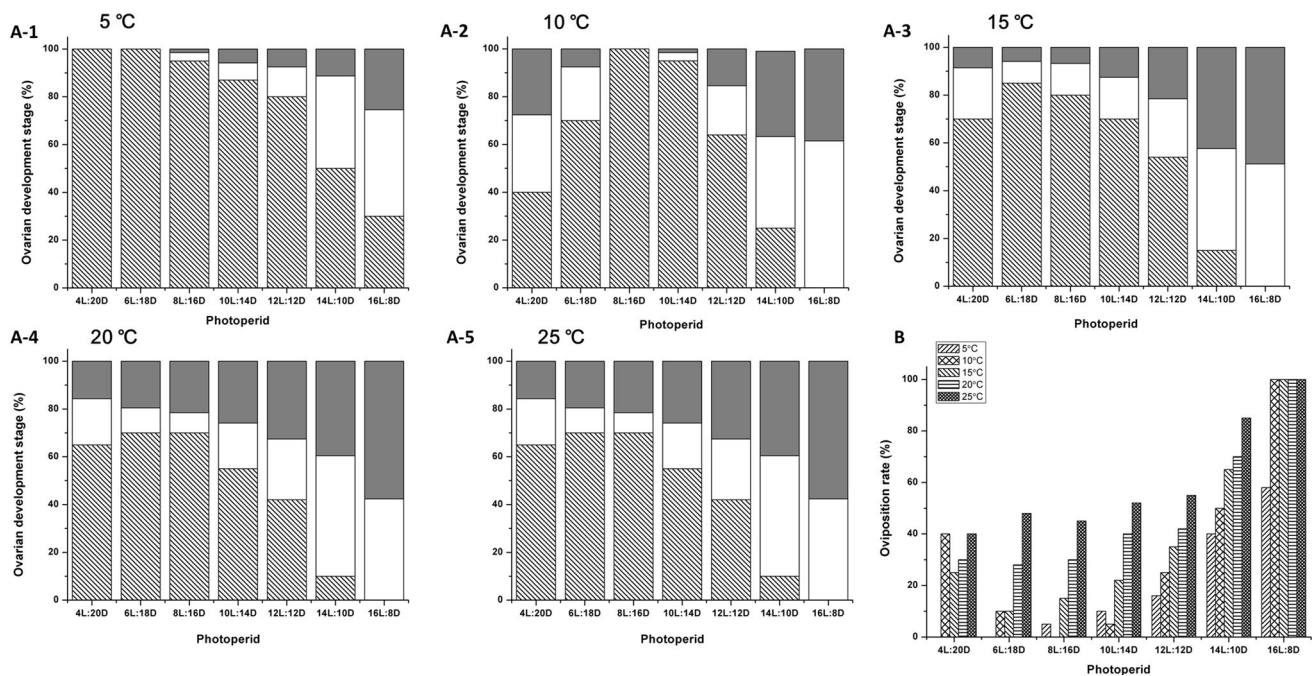
Fig. 3 Photoperiodic and temperature responses for diapause induction in *D. suzukii*. **a** Ovarian development stages and **b** oviposition rate of the females measured at different photoperiods (4L:20D, 6L:18D, 8L:16D, 10L:14D, 12L:12D, 14L:10D and 16L:8D) and temperatures (5 ± 1, 10 ± 1, 15 ± 1, 20 ± 1 and 25 ± 1 °C). **a** Proportions of the females with immature ovaries are shown in the *slash* and those with developing and developed ovaries in *light* and *dark gray*, respectively

the adult stage may be the most sensitive developmental stage to photoperiod in *D. suzukii*, while the egg may be the least sensitive stage (Table 1).

The diapause rate increased with a decreasing short-day photoperiod, suggesting that the short day photoperiod had a cumulative effect on diapause induction in *D. suzukii* (Table 2). No diapause was observed during the 1–15 days in the long-day photoperiod or the 10–15 days in the short-day photoperiod (Table 2). The diapause rate remained at 53.2 % when the adults were 1–3 days old in the short-day photoperiod (no. 1), and in contrast the diapause rate decreased from 80.2 to 38.3 % when the adults were 1–3 days old in the long-day photoperiod (no. 6). These results suggest that 1–3 days after eclosion may be the most sensitive stage to photoperiod in *D. suzukii*. The photosensitivity decreases as the adult age increased, until the 10th day after eclosion, and the short photoperiod has no effect on the reproductive diapause.

Effect of diapause-terminating photoperiod and temperature

The photoperiod significantly affected diapause termination at low temperatures (10 ± 1 °C), and the developing and developed ovaries increased with increasing photoperiod (Fig. 4). The low temperature and short photoperiod (8L:16D) maintain the reproductive diapause state, and long photoperiods can accelerate diapause development. In addition, temperature significantly influenced the diapause. The immature ovaries were significantly reduced under different photoperiods at high temperatures. This result indicates that the optimal combination of photoperiod and temperature for diapause termination was a long photoperiod (16L:8D) at 25 ± 1 °C.

Effect of photoperiod and temperature on cold tolerance

The SCP showed no significant differences between different photoperiods at high temperatures (25 ± 1 °C), while decreasing temperatures affected SCP or FP at different photoperiods (Fig. 5). According to previous diapause induction experiments, groups treated with different photoperiods at low temperatures were defined as diapausing or non-diapausing. A significant difference was observed between the diapausing (8L:16D) and non-diapausing (16L:8D) groups at low temperatures (10 ± 1 °C) in SCP and FP. This result suggests that the effect of photoperiod on SCP and FP decreases as the temperature increases, because the cold tolerance of diapause females was stronger compared with non-diapause females.

Physiological and biochemical changes

Diapause regulated several physiological and biochemical mechanisms, particularly modifying the activities of some

Fig. 4 Photoperiodic and temperature responses for diapause termination in *D. suzukii*. Ovarian development stages at different photoperiods (8L:16D, 12L:12D and 16L:8D) and temperatures (10 ± 1, 15 ± 1, 20 ± 1 and 25 ± 1 °C). Proportions of the females with immature ovaries are shown in the *grid* and those with developing and developed ovaries in *light* and *dark gray*, respectively
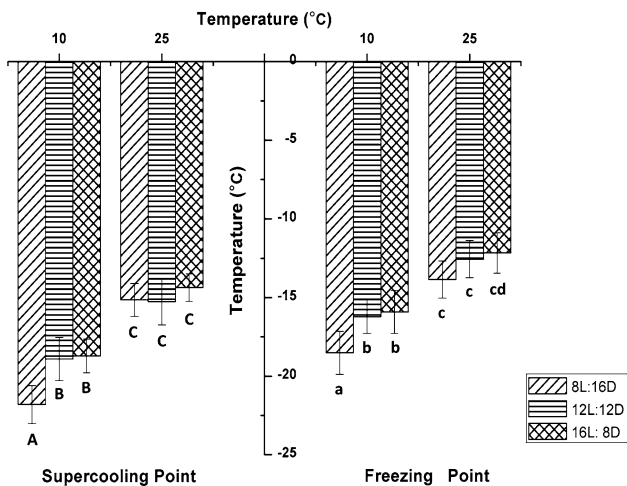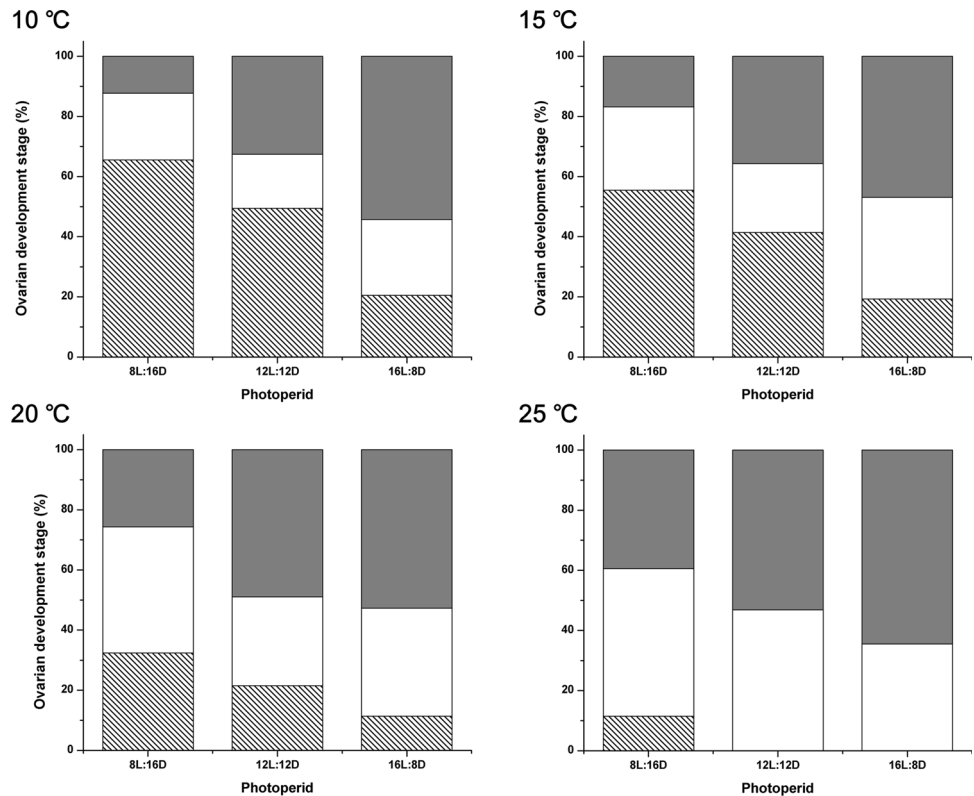

Fig. 5 Photoperiodic and temperature responses for SCP and FP. The SCP and FP of females at the 15th day after eclosion were measured at different photoperiods (8L:16D, 12L:12D and 16L:8D) and temperatures (10 ± 1, 25 ± 1 °C). The data represent the mean values ± SEM (*n* = 3), and the *values in the columns* followed by *different letters* denote a significant difference (*p* < 0.05, Tukey's post hoc test)

metabolic enzymes and energy reserve substances. The enzymatic activities of TRE, PK, SDH, HK and PFK significantly decreased (36.46, 57.85, 32.64, 54.68 and 24.59 %, respectively). On the contrary, the glycogen and triglyceride levels significantly increased (42.17 and

120.36 %) (Fig. 6). This result showed that the metabolic rate remained at lower levels through the modulation of enzymatic activity and accumulated cold tolerance substance content, thereby improving cold resistance during diapause.

Discussion

Adult reproductive diapause is a powerful overwintering strategy for many continental insect species including *Drosophila*. It enables females to survive several months through harsh winter conditions and then lay eggs when the temperature and photoperiod increase (Salminen and Hoikkala 2013). Worldwide, *Drosophila* has more than 1500 species, and most species are generally chill-susceptible and show both rapid cold-hardening and acclimation responses (Kelty and Lee 2001; MacMillan et al. 2009). *D. suzukii* is mainly found in warm-temperate regions (Kondo and Kimura 2008), and there is speculation that they enter a winter reproductive diapause (Walsh et al. 2010; Zerulla et al. 2015). Stephens et al. (2015) found that winter morphs of *D. suzukii* were produced by placing eggs at 10 °C with a photoperiod of 12L:12D, and they conjectured that these individuals might be in diapause. Although the overwintering site is not clear, it is generally thought that *D. suzukii* overwinters beneath leaf litter in
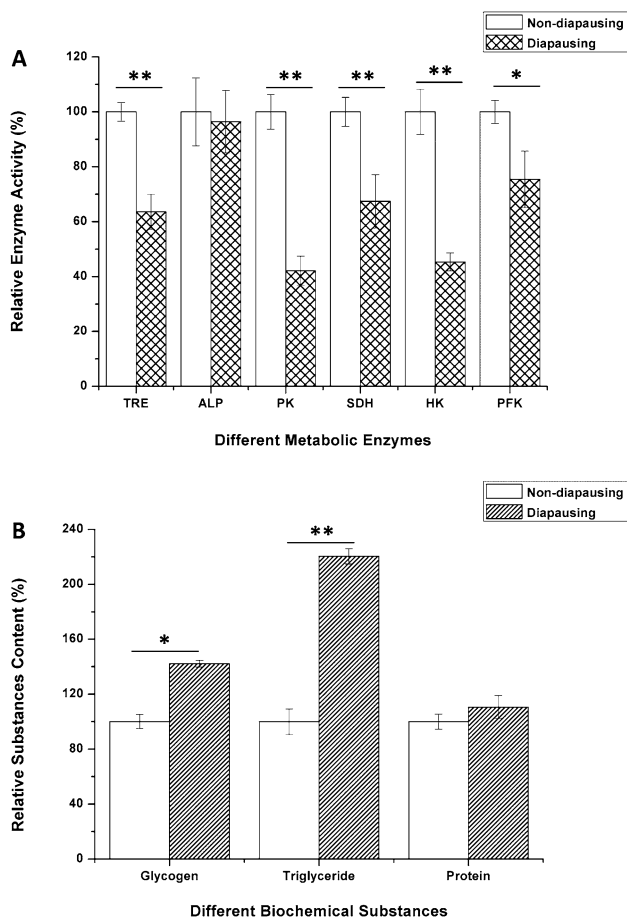
Fig. 6 Relative physiological and biochemical changes. **a** Relative enzyme activity and **b** relative substance content in diapausing and non-diapausing females. The data represent the mean values $\pm$ SEM ($n = 3$). *$p < 0.05$; **$p < 0.01$

built structures associated with an orchard or agricultural setting (Kanzawa 1939; Jakobs et al. 2015). We found almost all of the females trapped with immature ovaries in or around winter, and there was no record of adults trapped in winter (Fig. 2). According to the results obtained in the present study and other publications (Calabria et al. 2012; Jakobs et al. 2015; Rossi-Stacconi et al. 2016; Wang et al. 2016), we proposed that the diapause stage of overwintering was adult.

In warm-temperate regions, the photoperiod and temperature are the two key determinants of diapause-inducing environmental factors. Decades of research on insect diapause suggest that the relative importance of photoperiod and temperature in diapause regulation is widely variable and highly species-specific, and temperature often enhances or inhibits the induction of diapause through the photoperiod (Hodek and Hodkov 1988). In the present study, the ovarian development stages and oviposition rate were different at 10 ± 1 °C (Fig. 3). All of the females with immature ovaries at a short photoperiod (8L:16D) and

reproductive diapause phenomenon gradually weaken with increasing or decreasing temperatures. The ovaries developed quickly under a long photoperiod (16L:8D) at different temperatures for all of the females with developing and developed ovaries, and the oviposition rate was 100 % above 10 ± 1 °C. This showed that *D. suzukii* was typical of short-day diapause species within a certain photoperiod range. The reproductive diapause induction condition of a low temperature and short-day photoperiod agreed with the late autumn and early winter climatic conditions in warm-temperate regions, and this is consistent with other research on *Drosophila* species' reproductive diapause (Schmidt et al. 2008; Baker and Russell 2009; Kankare et al. 2010; Yamada and Yamamoto 2011; Rossi-Stacconi et al. 2016; Wang et al. 2016).

Different insect species exhibit different sensitivities to photoperiod, but the sensitive stage was not basically changed for a particular species (Beck 1980), such as the sensitive stage in maternity for most embryo diapause (Kobayashi et al. 2014), previous instars for most larvae/nymph diapause (Xiao et al. 2013), larvae stage for most pupae diapause (Liu et al. 2010) and preliminary stage of eclosion for most reproductive diapause (Xue et al. 2002). These results suggested that 1–3 days after eclosion is the most sensitive stage in *D. suzukii*, and the photosensitivity decreased with increasing days of adult stages (Table 1). A short photoperiod had a cumulative effect on diapause induction in *D. suzukii* (Table 2).

Insects' cold tolerance is influenced by various factors, and different insect species adjust their cold tolerance in different ways, such as the reduction of SCP, FP and water content, accumulation of cold tolerance substances, export of ice nucleating substances, regulation of metabolic enzyme activity, etc. Stephens et al. (2015) found that *D. suzukii* was a chill-intolerant insect, and winter-morph adults are the most cold-tolerant life stage. The lower lethal temperature of *D. suzukii* winter-morph adults was significantly colder than that of summer-morph adults, while the SCP of winter-morph adults was actually warmer than that of summer-morph adults and pupae. The SCP of *D. suzukii* was between −16 and −23 °C and was chill-susceptible (Jakobs et al. 2015). The SCP of adult *D. melanogaster* is approximately −20 °C and its lower lethal temperature above −10 °C (Chen and Walker 1994). Other *Drosophila* species may be tolerant to colder temperatures, such as when the lower lethal temperature is approximately −13 °C in *D. borealis* (Nyamukondiwa et al. 2011). The SCP was below −20 °C (Fig. 5), reflecting the fact that *D. suzukii* had some cold tolerance and could cope with the cold environment, thereby possibly explaining the *D. suzukii* outbreaks in recent years around the world.

The diapause stages accumulate cold tolerance substances, such as trehalose, sorbitol and glucose, glycerinum,

etc., to improve the cold tolerance and overcome severe winter environments (Rozsypal et al. 2013). TRE is a key enzyme in trehalose hydrolysis, and changes in the activity of this enzyme directly affect energy metabolism (Kamei et al. 2011). Sorbitol, through SDH, is converted to glycogen and subsequently utilized as energy for embryonic development (Rubio et al. 2011). PK, HK and PFK are the key enzymes in the glycolytic pathway, and PK mediates the conversion of phosphoenolpyruvic acid and ADP into pyruvic acid and ATP. HK catalyzes glucose from a steady state into an active state (Rider et al. 2011). In the present study, TRE, PK, SDH, HK and PFK enzyme activity levels significantly decreased, indicating that the diapause female improves cold tolerance through the accumulation of cold tolerance substances.

Diapause regulation has been shown as a pest control method, and several strong candidate genes/proteins can terminate diapause, causing them to fail to overwinter successfully. Zhou et al. (2015) found treatment with a recombinant TAT-caDH protein can affect larval development in H. armigera. Using RNAseq technology, several diapause candidate genes code for upstream regulators of a complex change of gene expression, which leads to a phenotypic switch from direct ontogeny to larval of the drosophilid fly Chymomyza costata (Poupardin et al. 2015). In a future study, several strong candidate genes/proteins of diapause will be investigated for functional studies, which may be useful for controlling the pest.

To our knowledge, this is the first study to ever report the adult reproductive diapause induction and termination in D. suzukii. Furthermore, our results emphasize the importance of understanding how D. suzukii is induced to initiate and terminate diapause under laboratory conditions of changing photoperiod and temperature, and we performed a preliminary examination of the physiological and biochemical mechanism. Follow-up studies will reveal the molecular mechanisms of reproductive diapause at the level of omics in D. suzukii females and provide instructions for pest control.

Author contribution

YZ and QL conceived and designed the research. YZ, QL and JZ conducted experiments. FZ and LZ analyzed the data. YZ and YY wrote the manuscript. All authors read and approved the manuscript.

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