



Research article

Artificial diets can manipulate the reproductive development status of predatory ladybird beetles and hold promise for their shelf-life management

Li Zhu ^{a,b,*}, Xin Chen ^c, Jia Lv ^{a,b}, Zhendong Huang ^{a,b,**}, Zhanxu Pu ^{a,b}, Shunmin Liu ^{a,b}

^a The Citrus Research Institute of Zhejiang Province, Taizhou, 318000, PR China

^b Key Laboratory of Fruit and Vegetable Function and Health Research of Taizhou, Taizhou, 318000, PR China

^c Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, 430070, PR China

ARTICLE INFO

Keywords:

Shelf-life management
Harmonia axyridis
Artificial diet
Nutrition accumulation

ABSTRACT

With the development of biological control methods, the predatory ladybird beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) has been widely used for pest control in agricultural production. Appropriate shelf-life management strategies could synchronize *H. axyridis* production with pest outbreaks, finally improving the effectiveness of biological control. Herein, we preliminarily explored whether an artificial diet could optimize the shelf-life management of *H. axyridis*. We compared the survival rate, nutrition accumulation, reproductive development, juvenile hormone (JH) related-gene expression levels, and stress resistance gene expression levels between aphid-fed and artificial diet-fed *H. axyridis* females. The results revealed that *H. axyridis* females maintained a high survival rate after being fed an artificial diet for 60 days, whereas the survival rate of aphid-fed females decreased. Continuous feeding of the artificial diet caused *H. axyridis* females to enter a diapause-like state, which was characterized by low JH levels, high triglycerides and trehalose accumulation, ovarian development inhibition, decreased Vgs expression levels, and increased stress resistance gene expression levels. This diapause-like state could be promptly recovered upon transferring to an aphid diet. These results indicate that the artificial diet could manipulate the reproductive development status of *H. axyridis* and lay the foundation for its shelf-life management.

1. Introduction

The multicolored Asian ladybeetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) is a beneficial predatory insect native to East Asia [1]. It exhibits outstanding effectiveness in controlling small arthropods such as aphids, coccids, and whiteflies [2]. In addition to laboratory bioassays, *H. axyridis* is extensively employed for pest management in orchards, greenhouses, crop fields, and other agricultural settings [2–6]. Furthermore, with increase in pest resistance following the use of chemical insecticides, integrated

* Corresponding author. The Citrus Research Institute of Zhejiang Province, Taizhou, 318000, PR China.

** Corresponding author. The Citrus Research Institute of Zhejiang Province, Taizhou, 318000, PR China.

E-mail addresses: zjuile0713@163.com (L. Zhu), hyhzd121505@163.com (Z. Huang).

<https://doi.org/10.1016/j.heliyon.2024.e29836>

Received 16 November 2023; Received in revised form 15 April 2024; Accepted 16 April 2024

Available online 17 April 2024

2405-8440/© 2024 The Citrus Research Institute of Zhejiang Province. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

pest management, which combines natural enemies with insecticides, is becoming increasingly important [7–10]. To satisfy the demands of pest management and make rational use of natural enemies, it is crucial to synchronize natural enemy production with pest outbreaks.

An appropriate shelf-life management strategy is a key approach to balance natural enemy production and pest control. Developing storage technologies based on insect development regulation, such as diapause induction and cold storage, is of great theoretical significance for the shelf-life management of natural enemies [11,12]. Cold storage constitutes the main method for prolonging the shelf-life of natural enemies, and it has been evaluated for parasitic wasps, predatory ladybirds and predatory mites [12–17]. The study indicated that 12 °C is suitable for the cold storage of the eggs and adults of *H. axyridis* and *C. septempunctata* as they maintained a high survival rate even after 30 days of cold storage [14]. However, the survival rate and fecundity of natural enemies decrease rapidly with increasing cold storage time [12–16,18,19]. Using cold acclimation, diapause induction, and interim nutrition supplementation can improve the survival rate of natural enemies during cold storage [13,14,18,20]. However, there remain numerous problems such as intricate operation, short storage time, and declining offspring fitness. The continuous optimization of natural enemy storage technology is urgently needed to address these issues as it can provide important technical support for the shelf-life management of natural enemies.

Developmental arrest caused by factors such as diapause and quiescence is gradually being applied to the shelf-life management of natural enemies [11,21]. The egg parasitoids could be stored for a full year after diapause induction, this enabling mass production and achieving better synchronization with the pest outbreaks [22]. The quiescence induced by low temperatures allows *Trichogramma evanescens* Westwood and *T. chilonis* Ishi to be stored for up to 30 days at 4 °C without significant loss [23]. In predacious mites, entering diapause ensures that predator synchronizes with the occurrence of its prey [24]. In addition, diapause lays the foundation for the storage, transportation, life prolongation, and population stability maintenance of predacious mites [21,25]. Reportedly, the reproductive diapause adults of *H. axyridis* can be cold stored at 10 °C for a long time, with the survival rate of the adults still exceeding 85 % after 120 days of cold storage [26]. Thus, using the diapause characteristics to manage the shelf-life of *H. axyridis* probably has certain commercial prospects [27].

Artificial diet serves as an important substitute food in the large-scale breeding of natural enemies. It includes a wide range of materials and facile preparation at low cost. Recently, an artificial diet was applied as a nutrition supplement before the cold storage of natural enemies. Reportedly, using an artificial diet as a nutrition supplement before storage can ensure that *C. septempunctata* adults can be stored for at least 60 days [28]. In *Podisus maculiventris*, eggs from artificial diet-fed adults exhibit higher hatching rates following cold storage compared with eggs from natural prey-fed insects [29]. Similarly, *H. axyridis* adults fed an artificial diet before cold storage exhibited a higher survival rate and lower weight loss during long-term cold storage than those fed natural prey [30]. In addition, continuously using artificial diet to feed predator adults often leads to pre-oviposition prolongation and low oviposition rates [31–33]. These features were considerably similar to those of reproduction diapause, indicating that artificial diet could potentially be used for manipulating reproductive development.

To explore whether artificial diet could be used to manipulate reproductive development in *H. axyridis*, this study detected some biological and biochemical characteristics after providing an artificial diet to newly emerged female beetles [27,34]. First, differences in nutrition accumulation, reproductive development, JH synthesis, JH responsive and stress resistance gene expression levels between artificial diet-fed and natural prey-fed beetles were compared. With the diet conversion experiment, the effects of continuous feeding of an artificial diet on the reproductive development of *H. axyridis* were further clarified. The relevant research results can provide an important theoretical basis for optimizing the storage technology of *H. axyridis*.

2. Methods

2.1. Insect rearing

The experimental population of *H. axyridis* was derived from Hubei Key Laboratory of Insect Resources Utilization and Sustainable Pest Management, Huazhong Agricultural University. The ladybirds were constantly fed on *Acyrthosiphon pisum* after introducing. Newly emerged *H. axyridis* female adults were collected daily and randomly divided into two groups, which were fed aphid (AP) and artificial diet (MF), respectively. All tested *H. axyridis* were reared at 25 ± 1 °C, 16 h light, 8 h dark, and relative humidity 60–70 %.

2.2. Artificial diet preparation

The artificial diet was prepared according to the formulation reported by Sun et al., 2018 (Table 1) [35]. First, the pork liver powder (Jiaxiang, Q/LJX0009S, China) and yeast extract (Oxoid, LP0021, England) were evenly mixed, and then the mixture was

Table 1
The formulation of the artificial diet of *Harmonia axyridis* [35].

Basic ingredients	Dosage	Additives	Dosage
Pork liver powder	10.0 g	Olive oil	1.1 ml
Yeast extract	1.0 g	Lin-seed oil	0.8 ml
Sucrose	2.0 g	Potassium sorbate solution (17 mg/ml)	1.0 ml
Honey	1.0 g	ddH ₂ O	15 ml

combined with a solution containing sucrose (Sangon Biotech, A502792-0500, China), honey, olive oil (Sangon Biotech, A502795-0100, China), linseed oil (Sanmark, 6948949300352, China) and potassium sorbate solution (Sangon Biotech, A504489-0500, China). After thorough mixing, the artificial diet was evenly divided and stored to -20°C for later use.

2.3. cDNA synthesis

The females from the AP, MF, and MF-AP treatments were quickly frozen in liquid nitrogen before total RNA extraction. RNase-free zirconia ceramic beads and 1 mL RNAiso Plus (Takara, 9109, Japan) were added to every 1.5 mL centrifuge tube. Then, a grinder (Jingxin, Shanghai, China) was used to grind for 2min at 65 HZ and 4°C . According to the instructions of RNAiso Plus, total RNA from each treated sample was gradually extracted using chloroform, isopropyl alcohol and anhydrous ethanol. After electrophoresis and total RNA concentration determination, 1 μg of total RNA was taken from each sample for cDNA synthesis (Takara, RR047A, Japan), and the obtained cDNA was diluted 20 times for subsequent real-time quantitative PCR (qRT-PCR) [34].

2.4. Survival rate

The daily survival rates of *H. axyridis* female adults after feeding on aphids and artificial diet were continuously observed for 10 days, each treatment contains three biological replicates ($n_{\text{AP}} = 26, 26, 26$; $n_{\text{MF}} = 27, 26, 28$). In addition, the survival of female adults in each treatment within 60 days was also analyzed, and the survival curves were drawn ($n_{\text{AP}} = 30$; $n_{\text{MF}} = 26$).

2.5. Changes in nutrition accumulation

To illustrate the differences in nutrition accumulation after feeding on different diets, this study evaluated individual fresh weight, total protein, triglyceride (TG), trehalose content and expression levels of key genes involved in the synthesis of fat and trehalose. The 10-day-old female adults from each treatment group were separately weighed on a one-thousandth balance (Sartorius, Germany) and their fresh weight was recorded ($n_{\text{AP}} = 50$; $n_{\text{MF}} = 52$). The total protein, TG and trehalose levels in 10-day-old female adults of each treatment group were determined using the respective content determination kits according to the manufacturers' instructions (Nanjing Jiancheng, total protein A045-4-2, TG A110-2-1, trehalose A149-1-1, China). The expression levels of lipogenesis gene (*fatty acid synthase*, *FAS*), trehalose synthesis gene (*trehalose-6-phosphate synthase*, *TPS*), and glycogen synthesis gene (*glycogen synthase*, *GS*) were detected via two-step qRT-PCR following the instructions of MonAmp™ ChemoHS qPCR Mix (Mona Bio, MQ00401, China) [34, 36]. *18S* and *28S* were considered as the reference genes [37]. All experiments included three biological replicates, each replicate contained three *H. axyridis* female adults.

2.6. Reproductive development and JH related-gene levels

The 10-day-old female adults were dissected in $1 \times$ PBS buffer and photographed using MD50 (Mingmei, Guangzhou, China). The lengths of the follicles were measured using Image J ($n_{\text{AP}} = 14$; $n_{\text{MF}} = 17$). The ovarian development degree classification and development index calculation were based on previous studies [27,38]. The expression levels of reproduction-related genes *vitellogenins* (*Vgs*), *vitellogenin receptor* (*VgR*) were detected using two-step qRT-PCR. Furthermore, the expression levels of the JH synthesis genes *farnesyl pyrophosphate synthases* (*FPPSs*), *farnesol dehydrogenase 1* (*FOLD1*), and *juvenile hormone acid methyltransferases* (*JHAMTs*), and the JH responsive gene *krüppel homolog1* (*Kr-h1*) were detected to assess the JH level in each treatment group.

2.7. Diet conversion experiment

The ingestion of a single artificial diet can induce reproductive inhibition in predators [32]. To investigate the effect of artificial diet on the reproductive inhibition of *H. axyridis*, a food conversion experiment was performed ($n_{\text{AP}} = 30$; $n_{\text{MF}} = 38$; $n_{\text{MF-AP}} = 26$). The artificial diet was supplied to the *H. axyridis* females since they emerged. After 10 days of feeding on the artificial diet, these females were then converted to consisting natural prey (recorded as MF-AP treatment). Females continuously fed an artificial diet or aphid served as controls. The expression levels of the reproduction-related genes (*Vgs* and *VgR*), JH synthesis genes (*FPPSs*, *FOLD1*, and *JHAMTs*), JH responsive genes (*Kr-h1*), and energy-related genes (*FAS*, *TPS*, and *GS*) in each treatment were also observed 7 days after food conversion.

2.8. Expression levels of heat shock protein

Heat shock protein (HSP) is a type of stress tolerance protein synthesized in insects and is involved in resisting adverse conditions such as cold, heat, and hypoxia [39]. In the diapause *H. axyridis* females, *HSP 21* and *HSP 68* exhibit high expression [34]. To compare the differences in the expression levels of HSPs after the consumption of artificial diet and aphids, the relative expression levels of *Hsp21* and *Hsp68* were detected in the cDNA from MF-7d, AP-7d, MF-10d and AP-10d treatments using two-step qRT-PCR. To further clarify the impact of diet conversion on HSPs expression, the relative expression levels of *HSP21* and *HSP68* were detected using two-step qRT-PCR at 7 days after food conversion.

2.9. Statistical analyses

The gene relative expression levels were calculated in Excel 2007. First, the CT values of 18S and 28S for all samples in the qRT-PCR reaction were calculated using geometric mean, and then the standardized values of these two reference genes were obtained [37,40]. Finally, $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression levels of target genes in each sample [41]. The statistically analysis of all data in this study was performed using IBM SPSS Statistic 19. Levene's test and independent sample *t*-test were respectively used for testing homogeneity of variance and significance among different treatments ($\alpha = 0.05$). Log-rank (Mantel-Cox) test was used to analyze the significance of differences between survival curves from AP and MF treatments. Additionally, the Kolmogorov-Smirnov test was used to assess the normal distribution of fresh weight and follicle length. Logarithmic transformation was applied to data that did not conform to normal distribution before conducting statistical analysis. All figures were drawn in GraphPad Prism 8.0.1, and the data in the figures were represented as mean \pm SD.

3. Results

3.1. *H. axyridis* females maintained high survival rates after consuming an artificial diet

Survival rate is one of the basic parameters used to evaluate the applicability of artificial diets in natural enemy production and shelf-life management. This study documented the survival of *H. axyridis* fed on aphid (AP) and artificial diet (MF) over 10 and 60 days since they emerged. It was observed that there was no significant difference between the survival rates of AP and MF within the initial 10 days after the beetles emerged (Fig. 1A). However, after continuously feeding for 60 days, the survival of AP significantly declined, with only 50 % of the individuals surviving on the 60th day (Fig. 1B). By contrast, the survival rate of MF was higher, with 84.6 % females surviving on the 60th day (Fig. 1B). And the survival curves of AP and MF treatments showed significant differences (Chi square = 7.346, $p = 0.0067$).

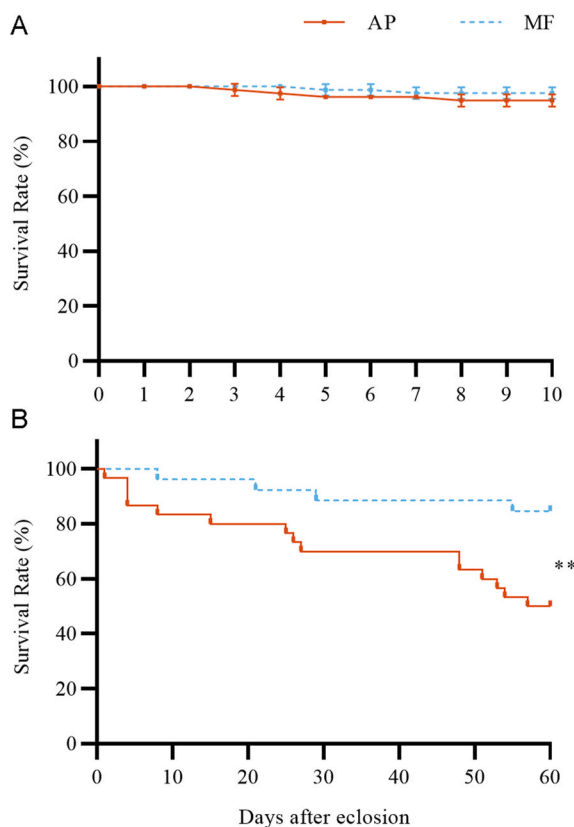


Fig. 1. The survival of *H. axyridis* females after feeding on different diets since their emergence. (A) Survival rate of the AP and MF females within 10 days. Both treatments contained three replications and were expressed as mean \pm SD ($n_{AP} = 26, 26, 26$; $n_{MF} = 27, 26, 28$), $\alpha = 0.05$. (B) Survival curves of the AP and MF females within 60 days, AP contained 30 females and MF contained 26 females.

3.2. Artificial diet stimulated the accumulation of TG and trehalose in *H. axyridis* females

To evaluate the nutrition accumulation of *H. axyridis* fed on different diets, the fresh weight and total protein, TG, and trehalose levels were detected in 10-day-old females from AP and MF treatment. The fresh weight did not show a significant difference between the AP and MF females ($p = 0.226$, Fig. 2A). The accumulation of TG and trehalose in MF females was significantly higher than that in AP females (Fig. 2B and D). However, the total protein content was slightly higher in the MF treatment group than in the AP treatment group ($p = 0.042$, Fig. 2C). The relative expression levels of *FAS*, *TPS*, and *GS* were further detected in the whole body of 10-day-old AP and MF females, and the results of qRT-PCR were consistent with those of nutrition accumulation determination, showing that the expression levels of *FAS* were higher when the *H. axyridis* was fed an artificial diet (Fig. 2E). Similarly, an artificial diet significantly stimulated the expression of *TPS* in 7-day-old *H. axyridis* (Fig. 2G). Conversely, *GS* was highly expressed in female beetles from AP treatment (Fig. 2F).

3.3. Artificial diet arrested the reproductive development of *H. axyridis* females

To understand the effects of different diets on ovarian development, the ovaries of 10-day-old females that fed on aphid and artificial diets were dissected (Fig. 3). The ovaries of *H. axyridis* females showed a large amount of vitellogenin deposition in the follicle after feeding on aphids for 10 days (Fig. 3A), and more than 50 % of the females exhibited mature oocytes (Fig. 3B). However, after feeding on the artificial diet, only 23.5 % of females had ovaries that developed into a grade II state of follicle growth (Fig. 3B). The ovary of 76.5 % females in the MF treatment still demonstrated an underdevelopment of grade I state of follicle (Fig. 3B). Their ovarian development index and follicle lengths were lower than those from AP treatment (Fig. 3A–D). The expression levels of *Vg1*, *Vg2*, and *VgR* also indicated that the ovary development of *H. axyridis* females was inhibited after the supply of an artificial diet (Fig. 3E–G). JH is an important upstream hormone that regulates reproductive development in insects [42]. The expression levels of *FPPS1*, *FPPS2*, *FOLD1*, *JHAMT1*, *JHAMT2*, and *Kr-h1* in the MF treatment were always significantly lower than those in the AP treatment (Fig. 3H–M).

3.4. Feeding on aphids could recover the reproductive inhibition caused by feeding on an artificial diet

To verify whether the reproductive inhibition caused by feeding on an artificial diet was irreversible or not, a food conversion experiment was performed using *H. axyridis* females that had been fed on an artificial diet for 10 days. The 10-day-old females from the MF treatment were converted to aphids feeding and kept for 7 days (recorded as MF-AP treatment); the females that continuously fed

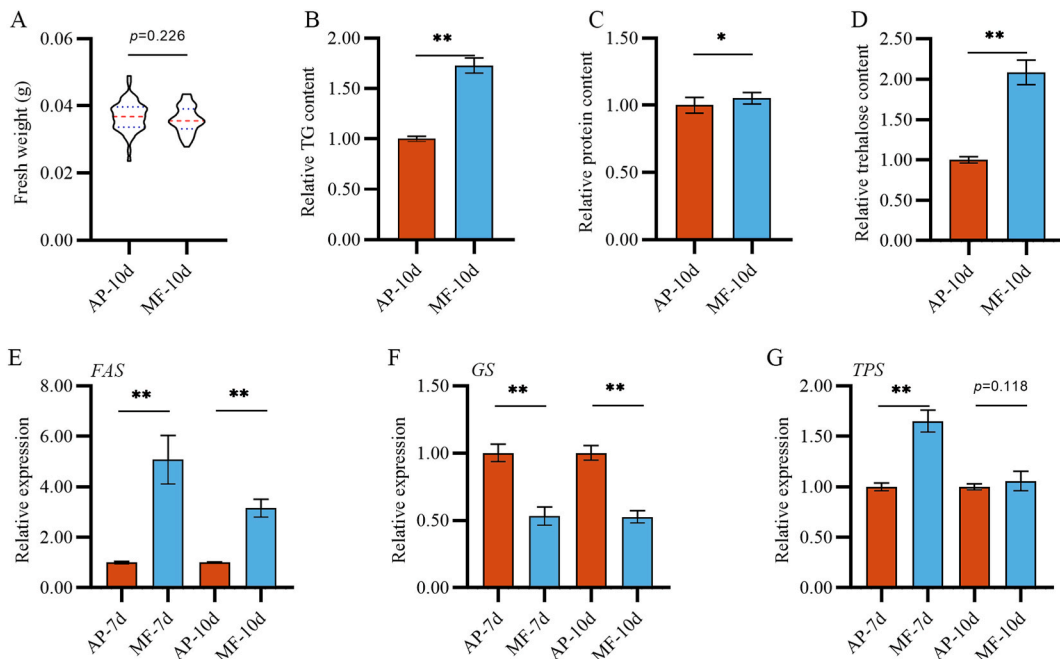


Fig. 2. Nutrition accumulation levels of *H. axyridis* females after feeding on different diets. Fresh weights (A) were respectively collected from 50 10-day-old females in the AP treatment group and 52 10-day-old females in the MF treatment group. The TG (B), total protein (C), and trehalose (D) levels were also detected in the 10-day-old females, and each treatment contained three replications. The relative expression levels of *FAS* (E), *GS* (F), and *TPS* (G) were detected in the whole body of the 7 and 10-day-old females in each treatment. Both treatments contained three replications. All the data were expressed as mean \pm SD. * represents $P < 0.05$, ** represents $P < 0.01$, p value represents no significant difference (independent sample t -test, $\alpha = 0.05$).

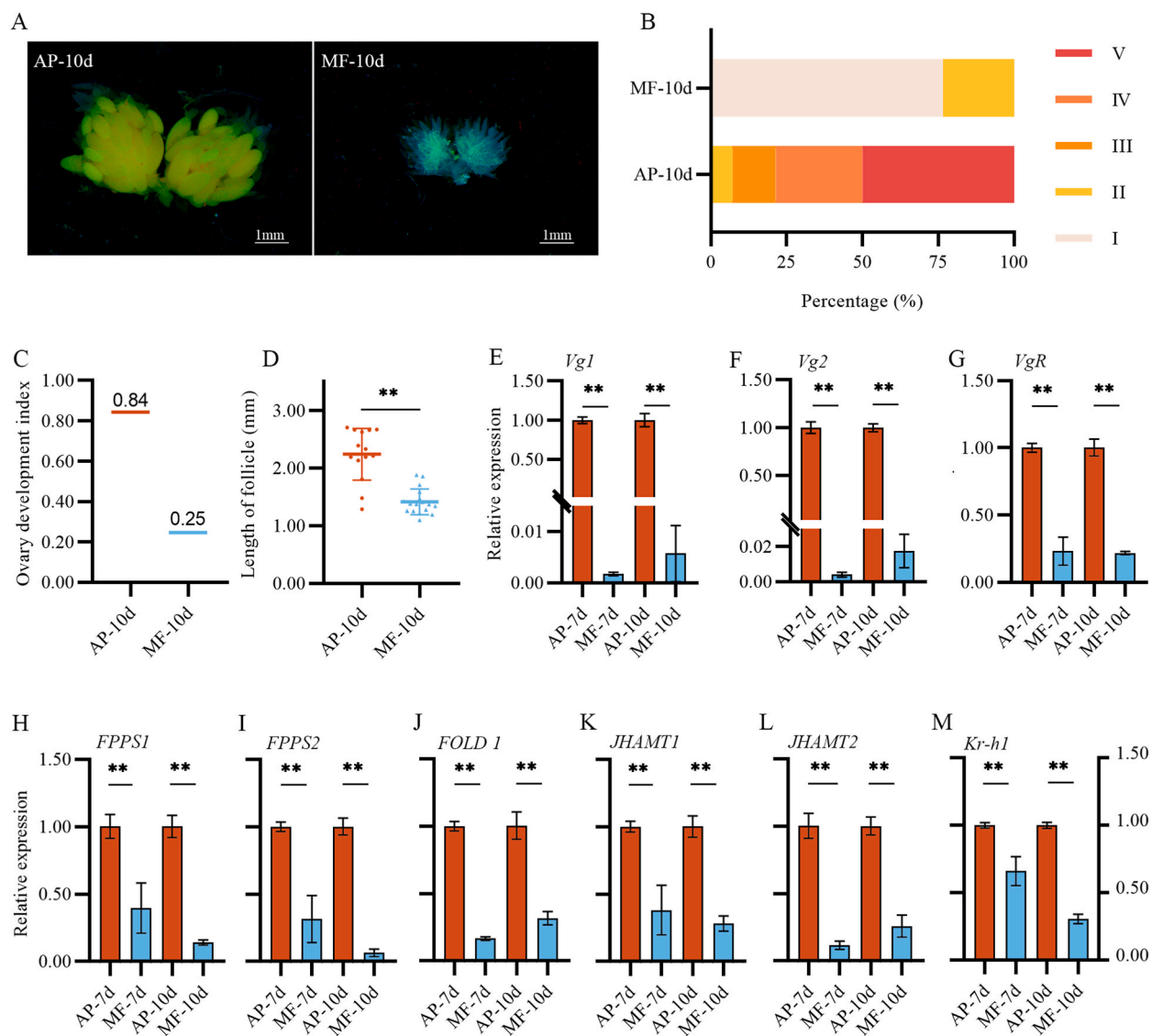


Fig. 3. Reproductive developments of *H. axyridis* females after feeding on different diets.

The ovarian development was assessed using ovarian yolk deposition (A), ovarian development degree (B), ovarian development index (C), and follicle length (D) of 10-day-old females from the AP and MF treatment groups. The mRNA expression levels of reproduction-related genes were also detected to evaluate reproduction development (E–G). The mRNA expression levels of JH synthesis genes (H–L) and responsive gene *Kr-h1* (M) were detected to evaluate JH levels in each treatment (H–M). The 7 and 10-day-old females from the AP and MF treatment groups were used for qRT-PCR, and every treatment contained three replications. The sample number of B–D: $n_{AP} = 14$, $n_{MF} = 17$. All the data were expressed as mean \pm SD. * represents $P < 0.05$, ** represents $P < 0.01$, p value represents no significant difference (independent sample t -test, $\alpha = 0.05$).

on an artificial diet or aphids served as controls. Through the dissection of ovaries, it was found that 100 % of the females in MF- AP treatment recovered ovarian development (Fig. 4A). Nearly 90 % of females had ovaries that reached grade V, 5 % reached grade IV, and 5 % reached grade III (Fig. 4C). The ovarian development index and the follicle length in the MF- AP treatment group were significantly increased, and there was no significant difference with the AP group (Fig. 4B and D). The expression levels of *Vg1*, *Vg2*, *VgR*, *FPSP1*, *FPSP2*, *FOLD1*, *JHAMT1*, *JHAMT2*, and *Kr-h1* were also significantly upregulated after conversion to aphids feeding, and were significantly higher than those of the females that continuously fed an artificial diet (Fig. 4E–M). The detection of nutrition accumulation-related genes revealed that the relative expression levels of *FAS* and *TPS* decreased significantly after conversion to aphids feeding, whereas the relative expression levels of *GS* increased in the MF- AP treatment group (Fig. 5A–C).

3.5. HSPs maintained high expression levels after feeding on an artificial diet

The expression levels of *HSP21* and *HSP68* in the MF treatment group were significantly higher than those in the AP treatment

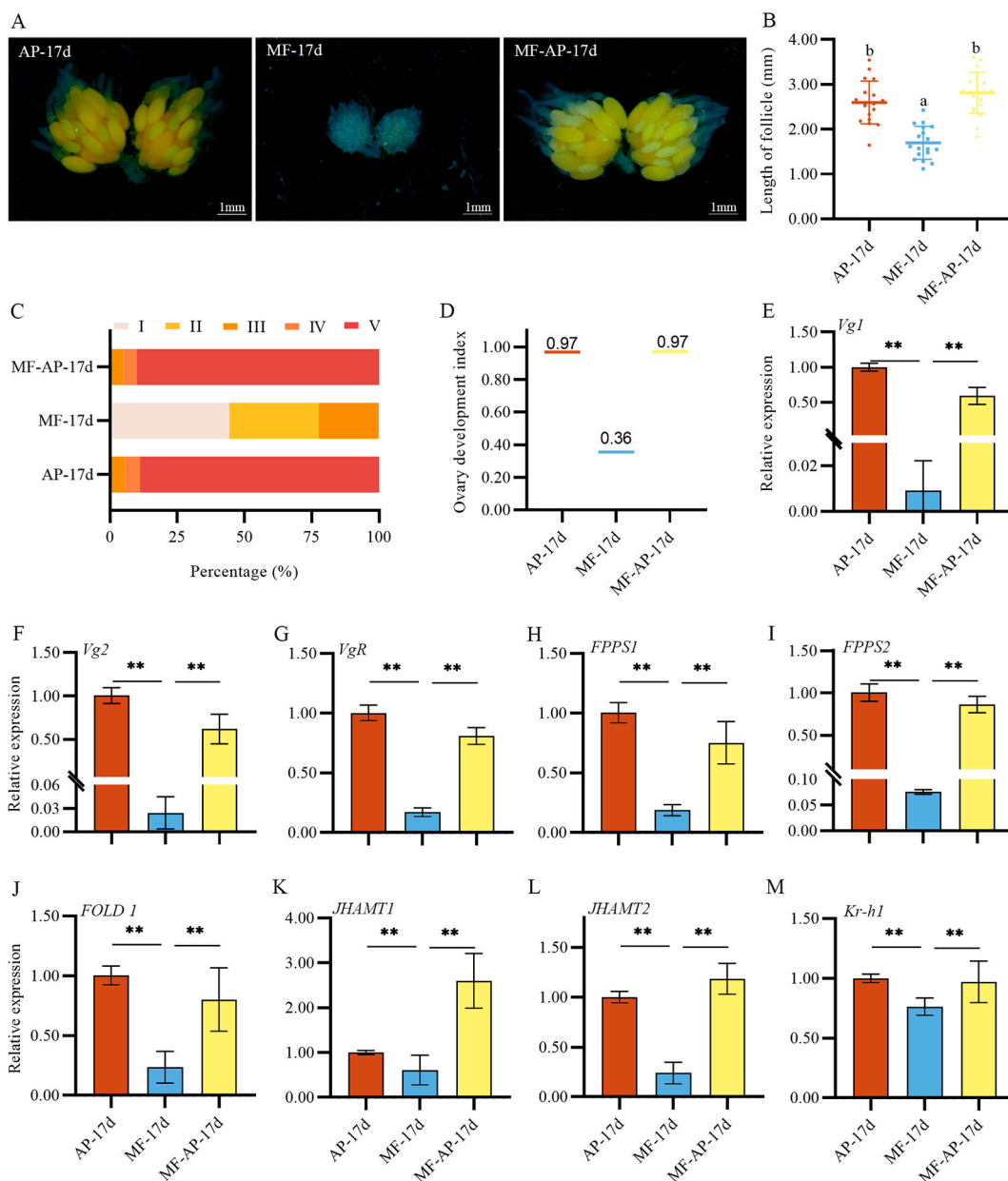


Fig. 4. Reproductive development of *H. axyridis* females after dietary conversion.

Ovarian yolk deposition (A), follicle length (B), ovarian development degree (C), and ovarian development index (D) were observed in 17-day-old females from the AP, MF, and MF- AP treatment groups. The mRNA expression levels of reproduction-related genes were also detected in the whole body of 17-day-old females (E– G). The mRNA expression levels of JH synthesis genes (H–L) and responsive gene *kr-h1* (M) were detected to evaluate JH levels after dietary conversion (H–M). The 17-day-old females from the AP, MF, and MF-AP treatment groups were used for qRT-PCR, and each treatment contained three replications. The sample number of B–D: $n_{AP} = 18$, $n_{MF} = 18$, $n_{MF-AP} = 20$. All the data were expressed as mean \pm SD. * represents $P < 0.05$, ** represents $P < 0.01$, p value represents no significant difference (independent sample t -test, $\alpha = 0.05$).

group (Fig. 6A and B). Furthermore, *HSP21* and *HSP68* still maintained high expression levels after converting to aphids feeding for 7 days, and there appeared to be no significant difference between the MF- AP and MF treatment groups (Fig. 6C and D).

4. Discussion

Using development regulation to optimize storage technology may be an important breakthrough in popularizing predators. To evaluate the application potential of artificial diet in the shelf-life management of *H. axyridis*, the survival rate, nutrition accumulation, reproductive development, and JH synthesis, responsive gene, and stress resistance gene expression levels were observed after feeding

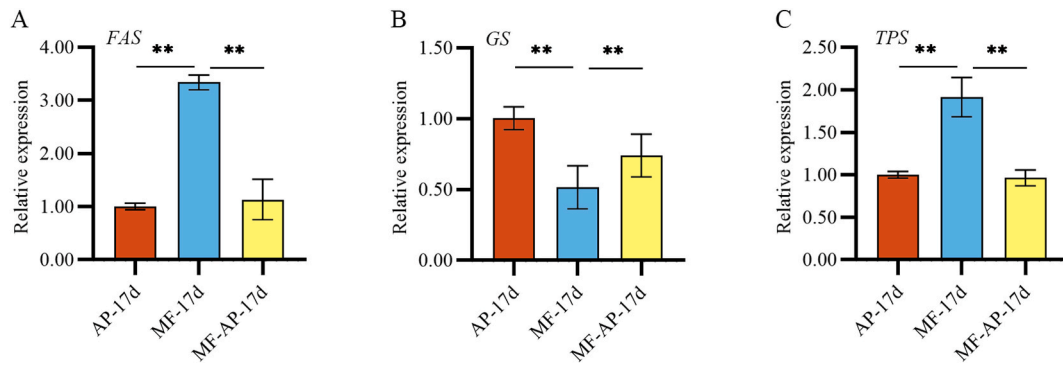


Fig. 5. Relative expression levels of *H. axyridis* nutrition accumulation genes after dietary conversion. The relative expression level of *FAS* (A), *GS* (B), and *TPS* (C) were detected in the whole body of 17-day-old females in each treatment group, and every treatment contained three replications. All the data were expressed as mean \pm SD. * represents $P < 0.05$, ** represents $P < 0.01$, p value represents no significant difference (independent sample t -test, $\alpha = 0.05$).

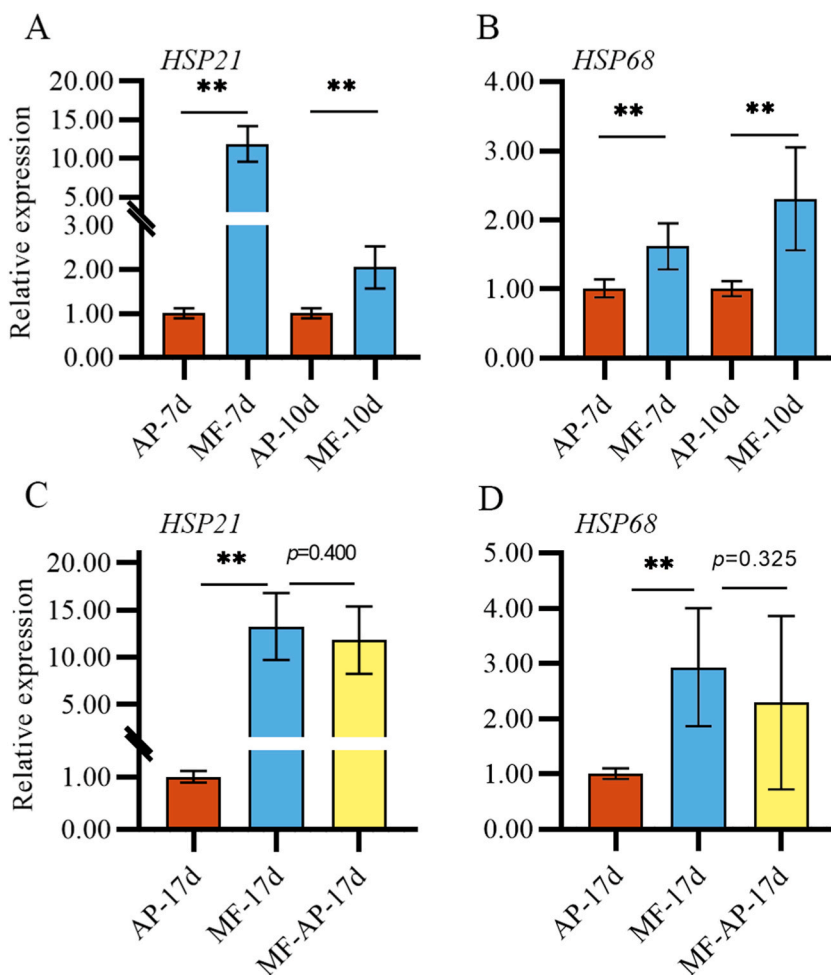


Fig. 6. Relative expression levels of *H. axyridis* female HSPs after feeding on different diets. The whole bodies of 7 and 10-day-old females from the AP and MF treatment groups were used for the qRT-PCR of *HSP21* (A) and *HSP68* (B). Furthermore, the expression levels of *HSP21* (C) and *HSP68* (D) after dietary conversion were detected in the whole body of 17-day-old females. Each treatment contained three replications. All the data were expressed as mean \pm SD. * represents $P < 0.05$, ** represents $P < 0.01$, p value represents no significant difference (independent sample t -test, $\alpha = 0.05$).

on artificial diet. The results indicated that providing an artificial diet to *H. axyridis* females had no significant negative effect on their survival rate, and the survival rate remained high within 60 days after emergence. After feeding on an artificial diet, *H. axyridis* females accumulated a large numbers of TGs and trehalose in their bodies, and the stress resistance gene expressions levels were also high. Continuous feeding on an artificial diet led to the reproductive inhibition of *H. axyridis* females, including ovarian development inhibition and *Vgs* expression level reduction. If these reproduction-arrested adults were transferred to aphid-feeding, they recovered from reproduction development immediately. These results suggest that artificial diets could be used to control the reproductive development status of *H. axyridis* and provide important technical tools for their shelf-life management.

Adequate nutrient accumulation may be an important guarantee for the shelf-life extension of natural enemies. Insufficient nutrition accumulation probably leads to reduced fecundity or high mortality after storage [43]. Herein, we found that the accumulation of TG and trehalose was significantly increased in the artificial diet-fed *H. axyridis* females. In fact, TG and trehalose are important energy reserves in insects [44]. They can enter the tricarboxylic acid cycle to provide energy for insects after a series of biochemical reactions. The findings provide an important theoretical basis and technical support for the shelf-life management of natural enemies.

In the research and development process of an artificial diet, it was found that simply feeding an artificial diet often caused the reproductive inhibition of predator adults, such as pre-oviposition prolongation and low oviposition [32,33]. Herein, the ovarian development of *H. axyridis* females was significantly inhibited after feeding on an artificial diet since they emerged, and the low expression levels of JH synthesis genes and *Kr-h1* also suggested a low level of JH titer in their bodies. In fact, the diapause *H. axyridis* females also presented with an arrested internal reproductive system, increased lipid and soluble sugar accumulation, low JH titer levels, and high *HSPs* expression levels [27,34]. In addition to the arrested ovarian development, large levels of TG and trehalose accumulated in the MF *H. axyridis* females, and the relative expression levels of *HSP21* and *HSP68* were significantly higher in the MF females than in the AP females. Therefore, *H. axyridis* probably entered a physiological state similar to reproductive diapause after being fed an artificial diet. This diapause-like state may have wide application prospects in the shelf-life management of *H. axyridis*.

Reportedly, a short day can induce low levels of JH in *H. axyridis* adults, and then trigger reproductive diapause [27,34,45]. Herein, the nutrition disparities between the artificial and aphids diet led to differences in the reproductive development in *H. axyridis* females. The reproductive arrest state caused by consuming artificial diet could be rapidly restored by switching to aphid-fed, which contrasts starkly with the reproductive diapause observed in ladybird beetles. In fact, nutritional quality is an important factor in ensuring the reproductive development and triggering endocrine signals in insects [46–48]. Various insects exhibit clear nutrient-limited oviposition. After feeding on flush shoots, the TOR signaling pathway is activated in *Diaphorina citri*, which subsequently affects JH levels and promotes oviposition [49]. Similarly, feeding on blood could activate JH synthesis, subsequently regulating oogenesis in mosquitoes [50]. Nutrient limitation in resting stage mosquitoes could increase the rate of follicular resorption [47]. Although the reproductive arrest caused by artificial diet feeding is not diapause, *H. axyridis* accumulated more TGs and trehalose and showed high expression levels of stress resistance genes, which would be beneficial for shelf-life management.

How to effectively control the occurrence and termination of diapause is the key technology for utilizing the diapause for the shelf-life management of natural enemies [21]. If the diapause maintenance period is too long or short, the supply of natural enemies will be out of sync with the pests, thus limiting the production and application of natural enemies [51]. A controllable diapause-like state might solve these problems effectively. In fact, reproductive inhibition caused by only feeding on an artificial diet has been reported in numerous natural enemies, and it can be easily recovered. After feeding on an artificial diet, *C. septempunctata* also enters a diapause-like state with inhibited reproductive development, which can be effectively alleviated following JH analog supplementation [52]. Herein, it was also found that *H. axyridis* females exhibited reproductive arrest, which is similar to diapause after feeding on an artificial diet, and the reproductive development could be quickly recovered after natural prey supplementation. This operation is simple and effective in solving the problem of long diapause maintenance. It should be noted that *H. axyridis* requires continuous provision of fresh artificial diet. However, this operation is somewhat simpler than mass-rearing aphids. In factories without suitable cold storage conditions, the artificial diet could be used for *H. axyridis* manipulating development and achieving shelf-life management.

Funding

This work was supported by the National Natural Science Foundation of China (Grant number 32202379) and Science and Technology Program of Zhejiang Province (Grant number 2023C04014).

Data availability statement

The original data of this study will be made available on request.

CRedit authorship contribution statement

Li Zhu: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Xin Chen:** Validation, Methodology, Data curation. **Jia Lv:** Data curation, Conceptualization. **Zhendong Huang:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Formal analysis. **Zhanxu Pu:** Software, Data curation. **Shunmin Liu:** Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] R. Koch, The multicolored Asian lady beetle, *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts, *J. Insect Sci.* 3 (2003) 32.
- [2] E.W. Riddick, Spotlight on the positive effects of the ladybird *Harmonia axyridis* on agriculture, *BioControl* 62 (3) (2017) 319–330.
- [3] P. Wu, J. He, Y. Ge, Z. Liu, R. Zhang, Comparison of predatory performance among three ladybird species, *Harmonia axyridis*, *Coccinella septempunctata* and *Hippodamia variegata*, feeding on goji berry psyllid, *Bactericera gobica*, *Insects* 15 (1) (2023) 19.
- [4] P. Wu, J. He, H. Dong, R. Zhang, Functional response and intraspecific competition of three ladybird species feeding on aphids on goji berry plants in laboratory and semi-field conditions, *Insects* 14 (11) (2023) 853.
- [5] T. Kuroda, K. Miura, Comparison of the effectiveness of two methods for releasing *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) against *Aphis gossypii* Glover (Homoptera: aphididae) on cucumbers in a greenhouse, *Appl. Entomol. Zool.* 38 (2) (2003) 271–274.
- [6] E.W. Riddick, Identification of conditions for successful aphid control by ladybirds in greenhouses, *Insects* 8 (2) (2017) 38.
- [7] I. Denholm, M.W. Rowland, Tactics for managing pesticide resistance in arthropods: theory and practice, *Annu. Rev. Entomol.* 37 (1992) 91–112.
- [8] S.E. Naranjo, P.C. Ellsworth, G.B. Frisvold, Economic value of biological control in integrated pest management of managed plant systems, *Annu. Rev. Entomol.* 60 (2015) 621–645.
- [9] K.L. Giles, B.P. McCornack, T.A. Royer, N.C. Elliott, Incorporating biological control into IPM decision making, *Curr. Opin. Insect Sci.* 20 (2017) 84–89.
- [10] S. Munir, A. Azeem, M. Sikandar Zaman, M. Zia Ul Haq, From field to table: ensuring food safety by reducing pesticide residues in food, *Sci. Total Environ.* 922 (2024) 171382.
- [11] W. Wang, L.S. Zhang, H.Y. Chen, Y.Y. Li, J. Zhang, Research progress in diapause of the lady beetles, *Plant Prot.* 37 (5) (2011) 27–33.
- [12] W.J. Li, J.H. Chen, G.A. Avila, M.Y. Ali, X.Y. Tian, Z.Y. Luo, et al., Performance of two egg parasitoids of brown marmorated stink bug before and after cold storage, *Front. Physiol.* 14 (2023) 1102216.
- [13] S.H. Sun, L.W. Zhao, J.Y. Qi, Z. Xu, L.L. Li, X.R. Xu, Supplementary nutrition of adult wasps and suitable stage for cold storage in mass rearing of *Choiuioia cunea* Yang (Hymenoptera: Eulophidae), *Chinese J. Biol. Control* 28 (3) (2012) 320–325.
- [14] J.C. Yang, Z.R. Shen, Effect of cold storage on survival of the ladybird beetles *Leis axyridis* and *Coccinella septempunctata*, *Acta Entomol. Sin.* 43 (Supplement) (2000) 211–214.
- [15] N.A. Ghazy, T. Suzuki, M. Shah, H. Amano, K. Ohyama, Effect of long-term cold storage of the predatory mite *Neoseiulus californicus* at high relative humidity on post-storage biological traits, *BioControl* 57 (5) (2012) 635–641.
- [16] U. Bernardo, L. Iodice, R. Sasso, P.A. Pedata, Effects of cold storage on *Thripobius javae* (=T. semiluteus) (Hymenoptera: Eulophidae), *Biocontrol Sci. Technol.* 18 (9) (2008) 921–933.
- [17] H. Colinet, G. Boivin, Insect parasitoids cold storage: a comprehensive review of factors of variability and consequences, *Biol. Control* 58 (2) (2011) 83–95.
- [18] S.B. Teng, Z.Q. Xu, The cold storage conditions of *Harmonia axyridis* eggs and adults in mass-rearing, *Chinese Bull. Entomol.* 42 (2) (2005) 180–183.
- [19] T.A. Coudron, H.J.R. Popham, M.R. Ellersieck, Influence of diet on cold storage of the predator *Perillus bioculatus* (F.), *BioControl* 54 (6) (2009) 773–783.
- [20] J. Zhao, Z.Z. Chen, F.Q. Zheng, F. Zhang, X.C. Yin, Y.Y. Xu, Effects of cold acclimation on developmental characteristics and fitness of *Harmonia axyridis* (Coleoptera: Coccinellidae) offsprings, *Acta Entomol. Sin.* 55 (7) (2012) 810–815.
- [21] D.L. Denlinger, Why study diapause? *Entomol. Res.* 38 (2008) 1–9.
- [22] L.A. Foerster, A.K. Doetzer, Cold storage of the egg parasitoids *Trissolcus basalis* (Wollaston) and *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae), *Biol. Control* 36 (2) (2006) 232–237.
- [23] M.M. Hasan, M.N. Parvin, C.G. Athanassiou, Effects of low temperatures on quiescence in *Trichogramma evanescens* Westwood and *T. chilonis* Ishii reared on *Plodia interpunctella* (Hubner): implications for mass rearing, *Sci. Rep.* 14 (1) (2024) 3220.
- [24] G. Broufas, Diapause induction and termination in the predatory mite *Euseius finlandicus* in peach orchards in northern Greece, *Exp. Appl. Acarol.* 25 (2002) 921–932.
- [25] X.M. Li, L. Li, C. Gao, Q.F. Tang, Evaluation of predation capacity in post-diapause predatory mite, *Neoseiulus barkeri*, *Chin. J. Biol. Control* 38 (5) (2022) 1149–1155.
- [26] Z. Liu, H.F. Xu, F.H. Kong, Z.B. Sun, B.Z. Zhu, Study on optimum cold storage conditions for adults of *Harmonia axyridis* Pallas, *Shandong Agri. Sci.* 6 (2009) 64–67.
- [27] Q. Gao, B.X. Wei, W. Liu, J.L. Wang, X.M. Zhou, X.P. Wang, Differences in the development of internal reproductive organs, feeding amount and nutrient storage between pre-diapause and pre-reproductive *Harmonia axyridis* adults, *Insects* 10 (8) (2019) 243.
- [28] Y.X. Sun, Y.N. Hao, M.L. Li, Effect of supplementation of artificial diet before storage on cold tolerance of *Coccinella septempunctata*, *Chin. J. Biol. Control* 36 (5) (2020) 708–713.
- [29] T.A. Coudron, M.R. Ellersieck, K.S. Shelby, Influence of diet on long-term cold storage of the predator *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae), *Biol. Control* 42 (2) (2007) 186–195.
- [30] Y.X. Sun, Y.N. Hao, C.Z. Liu, S.S. Wang, Artificial diet is fruitful pre-storage nutrition for long-term cold storage of laboratory-reared *Harmonia axyridis* (Pallas) adults, *Biol. Control* 139 (2019) 104075.
- [31] Z.C. Shen, C. Hu, H. Gong, A literature review on artificial diets for beetles, *Chin. Bull. Entomol.* 5 (1989) 313–316.
- [32] E.W. Riddick, Benefits and limitations of factitious prey and artificial diets on life parameters of predatory beetles, bugs, and lacewings: a mini-review, *BioControl* 54 (3) (2008) 325–339.
- [33] J. Xie, H. Wu, H. Pang, P. De Clercq, An artificial diet containing plant pollen for the mealybug predator *Cryptolaemus montrouzieri*, *Pest Manag. Sci.* 73 (3) (2017) 541–545.
- [34] Q. Gao, B. Li, Z. Tian, A. De Loof, J.L. Wang, X.P. Wang, et al., Key role of juvenile hormone in controlling reproductive diapause in females of the Asian lady beetle *Harmonia axyridis*, *Pest Manag. Sci.* 78 (1) (2022) 193–204.
- [35] Y.X. Sun, Y.N. Hao, T.X. Liu, A β -carotene-amended artificial diet increases larval survival and be applicable in mass rearing of *Harmonia axyridis*, *Biol. Control* 123 (2018) 105–110.
- [36] D.W. Zhang, Y. Li, M. Zhang, S.S. Wang, Z.J. Xiao, B.P. Zeng, et al., Expression and function of trehalase genes *TRE2-like* and *TRE2* during adult eclosion in *Harmonia axyridis* (Coleoptera: Coccinellidae), *Acta Entomol. Sin.* 62 (6) (2019) 663–671.
- [37] Q. Gao, B. Li, B.X. Wei, W. Liu, P. Wang, J.L. Wang, et al., Juvenile hormone regulates photoperiod-mediated male reproductive diapause via the methoprene-tolerant gene in the ladybeetle *Harmonia axyridis*, *Insect Sci.* 29 (1) (2021) 139–150.
- [38] H. Sakurai, T. Hirano, S. Takeda, Physiological distinction between aestivation and hibernation in the lady beetle, *Coccinella septempunctata bruckii* (Coleoptera: Coccinellidae), *Appl. Entomol. Zool.* 21 (3) (1986) 424–429.
- [39] A.M. King, T.H. MacRae, Insect heat shock proteins during stress and diapause, *Annu. Rev. Entomol.* 60 (2015) 59–75.
- [40] X. Yang, H. Pan, L. Yuan, X. Zhou, Reference gene selection for RT-qPCR analysis in *Harmonia axyridis*, a global invasive lady beetle, *Sci. Rep.* 8 (1) (2018) 2689.
- [41] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method, *Methods* 25 (4) (2001) 402–408.

- [42] V. Smykal, A.S. Raikhel, Nutritional control of insect reproduction, *Curr. Opin. Insect Sci.* 11 (2015) 31–38.
- [43] S.E. Flanders, The effect of cold storage on reproduction of parasitic Hymenoptera, *J. Econ. Entomol.* 31 (1938) 633–634.
- [44] D.A. Hahn, D.L. Denlinger, Energetics of insect diapause, *Annu. Rev. Entomol.* 56 (2011) 103–121.
- [45] H. Sakurai, T. Kawai, S. Takeda, Physiological changes related to diapause of the lady beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae), *Appl. Entomol. Zool.* 27 (4) (1992) 479–487.
- [46] R. Parthasarathy, S.R. Palli, Molecular analysis of nutritional and hormonal regulation of female reproduction in the red flour beetle, *Tribolium castaneum*, *Insect Biochem. Mol. Biol.* 41 (5) (2011) 294–305.
- [47] M.E. Clifton, F.G. Noriega, Nutrient limitation results in juvenile hormone-mediated resorption of previtellogenic ovarian follicles in mosquitoes, *J. Insect Physiol.* 57 (9) (2011) 1274–1281.
- [48] S. Roy, T.T. Saha, Z. Zou, A.S. Raikhel, Regulatory pathways controlling female insect reproduction, *Annu. Rev. Entomol.* 63 (2018) 489–511.
- [49] L. Jiang, P. Wu, L. Yang, C. Liu, P. Guo, H. Wang, et al., Transcriptomics and metabolomics reveal the induction of flavonoid biosynthesis pathway in the interaction of *Stylosanthes-Colletotrichum gloeosporioides*, *Genomics* 113 (4) (2021) 2702–2716.
- [50] F.G. Noriega, Nutritional regulation of JH synthesis: a mechanism to control reproductive maturation in mosquitoes? *Insect Biochem. Mol. Biol.* 34 (7) (2004) 687–693.
- [51] Y.Y. Li, L.S. Zhang, H.Y. Chen, Effect of biotic factors on diapause of parasitic wasps, *Chin. Bull. Entomol.* 47 (4) (2010) 638–645.
- [52] Z.H. Chen, J.D. Qin, X.M. Fan, X.L. Li, Effects of adding lipids and juvenoid into the artificial diet on feeding and reproduction of *Coccinella septempunctata* L., *Acta Entomol. Sin.* 27 (2) (1984) 136–145.