WALAILAK JOURNAL
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Effect of Cold Storage on Development of *Habrobracon hebetor* (Say) (Braconidae: Hymenoptera) Reared on *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae)

Atirach NOOSIDUM*, Weerachai SOMSRI and Angsumarn CHANDRAPATYA

Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand

(***Corresponding author's e-mail: fagrarn@ku.ac.th**)

Received: 23 March 2017, Revised: 1 March 2018, Accepted: 19 April 2018

Abstract

Habrobracon hebetor has been investigated as a successful biological control agent for larvae of pyralid moths in warehouses. Gaining new information on how to improve the storage procedure of *H. hebetor* under cold conditions could allow the development of approaches to maintain this parasitoid in pest management programs. This study investigated the effect of cold temperatures (10 and 15 °C) on the development of *H. hebetor* pupae reared on *Corcyra cephalonica*, which was kept for up to 3 weeks. Mortality of *H. hebetor* pupae during cold storage in all treatments was 4 - 19 %. Mortality of *H. hebetor* adults emerging during cold storage at 10 °C (28 %) and 15 °C (61 %) was significantly observed at 21 days of storage, while other treatments were less than 4 %. Numbers of emerging adults after cold storage from 7 - 14 days were 89 - 96 %, and the female percentage ranged from 13 - 39 %. The number of eggs laid per female from the emerging females after cold storage for 7 - 14 days was not significant as compared to the control treatment (43 - 53 eggs per female) after laying for 72 h. The sex ratios of emerging adults in all cold storages and the control treatment at 72 h after emerging were not significant.

Keywords: Insect parasitoid, rice moth, cold storage, sex ratio, biological control

Introduction

Biological control programs, a significant part of integrated pest management (IPM) programs, have been one of the environmentally-friendly methods used in controlling insect pests since the 19th century [1]. This control program has given an advantage mostly to users, consumers, and all non-target organisms, and it has been a promising alternative to pesticide application to control economically important insect pests [2]. The biological control method normally relies on predators, parasitoids, or pathogens which have efficiency in killing a specific pest during its entire life cycle. Several insect natural enemies, including predators and parasitoids, have been employed as bio-control agents, and various successful results have been reported all around the world [1-3]. Braconid wasps are parasitoid insects which normally cause insect mortality in natural fields, and many genera of Braconidae (*e.g., Cotesia*, *Apanteles*, and *Habrobracon*) are now playing important roles in biological control programs to manage various insect pests in several crops of worldwide organic farming [4]. Nowadays, *H. hebetor* (Say) is produced and sold commercially to control some stored-product moths [5].

Habrobracon hebetor (= *Bracon hebetor* Say) has long been known as an important biological control agent to inflict substantial mortality on various immature insects belonging to Order Lepidoptera [6,7]. This parasitoid is considered as one of the best potential biological control agents and a well-known polyphagous ectoparasitoid of many lepidopteran larvae infesting flour mills, warehouses, and food stores in storage facilities, especially the larvae of Family Pyralidae and Noctuidae [6,8-11]. Several researchers reported that the release of *H. hebetor* can deplete numbers of insect pests in both storage [6,8] and field

conditions [12], such as the Mediterranean flour moth (*Ephestia kuehniella* Zeller), Indian meal moth (*Plodia interpunctella* (Hübner)), rice moth (*Corcyra cephalonica* (Stainton)), greater wax moth (*Galleria mellonella* (L.)), and army worm (*Helicoverpa amigera* (Hübner)), because of its high reproductive and development rate, short generation time, and wide range of host species [6,7,10]. The female *H. hebetor* prefers to parasitize the larval stage of its host and then lays eggs on or near the outer surface of the paralyzed host [5]. Larvae of *H. hebetor* hatch within 2 days after egg laying and then the larva feeds on its host until it becomes an adult [13].

However, the success of biological control programs of *H. hebetor* depends on various factors, such as the number of the insect pest population and the number of the parasitoid population in the management area [14]. Therefore, a huge number of the parasitoid population must be used for implementation in the management area to suppress the insect pest population at an economical level [7]. Mass rearing and handling processes are important for the implementation of the biological control method of *H. hebetor*. Its fitness and performance, such as survival, development, size, longevity, fecundity, and sex ratio, should be modified during mass production [15,16]. Moreover, the effects of different factors on the behavioral ecology of *H. hebetor* are elated to parasitism succession in optimizing mass production of the parasitoid in biological control programs [13,17]. Host density and storage techniques are necessary to improve the flexibility and efficiency in mass production of *H. hebetor* [10,18].

C. cephalonica is normally used as one of the preferred diets for rearing *H. hebetor* in laboratory conditions for mass production of the parasitoid in many countries [7,19]. However, the rearing of *H. hebetor* on *C. cephalonica* larva has shown unsatisfying implementation results in biological control programs in Thailand. This wasp produces a lesser number of offspring in the next generation, and adults rapidly emerge from the pupal stage; thus, suitable host density and storage condition is still uncertain. The objective of this study was to evaluate the effect of cold temperatures on the storage of *H. hebetor* reared on *C. cephalonica* larva, in order to select suitable conditions for further development of mass rearing of this parasitoid.

Materials and methods

Insect rearing

Corcyra cephalonica

The target host used in this study, *C. cephalonica,* was originally obtained from the Bureau of Agricultural Commodities Promotion and Management, Department of Agricultural Extension (DOAE), Ministry of Agriculture and Cooperatives, Bangkok, Thailand, in 2015. The *C. cephalonica* colony was maintained on a diet of rice bran. Approximately 400 eggs were sprinkled into a rearing container (23 cm diameter; 10 cm height) containing 1200 grams of the diet, and the lid covered and sealed with glue tape. Eggs hatched after 4 - 5 days, with all larvae fed on an artificial diet for 25 - 30 days before turning into pupae and becoming adults. Adults (1 - 2 days old) were collected from the stock culture and held in a cage made of Polyethylene net (225 mesh) for oviposition. Environmental conditions were 28.0 ± 0.5 °C, 75 ± 5 % RH, and 12L: 12D photoperiod. Final instar larvae were used in all experiments.

Habrobracon hebetor

H. hebetor was obtained from the same place as *C. cephalonica*. This strain was previously used for a few years for the biological control of *Opisina arenosella* Walker, and was maintained in the laboratory on final instar larvae of *C. cephalonica* at the Department of Entomology, Kasetsart University, Thailand.

Adult parasitoids were introduced into a plastic container (7 cm diameter; 4.5 cm height) containing *C. cephalonica* final instar larvae and a paper dipped in 20 % honey, and the container then covered with a screen lid. Environmental conditions for rearing *H. hebetor* were the same as those used in rearing *C. cephalonica*. To obtain newly emerging parasitoids, adult female and male *H. hebetor* (3 couples) were released into a new rearing container with 5 *C. cephalonica* final instar larvae and removed after 24 h. Each parasitized larva was incubated separately, and the resulting adult parasitoids were used in the experiments.

Effect of cold storage on development of *H. hebetor*

Five last instar larvae of *C. cephalonica* were separately placed in a plastic cup (7 cm diameter; 4.5 cm height); then, 3 couples of *H. hebetor* adults were released into each cup. After 3 days, all *H. hebetor* were removed from the cups. All *C. cephalonica* cadavers, containing pupal stages of the ectoparasitoid from each cup, were transferred into a new cup and covered with aluminum foil to prevent water loss. Cups were kept under different conditions, consisting of 2 temperature levels (10 and 15 °C) with 3 cold storage periods (7, 14, and 21 days). Cold conditions were provided by temperature-controlled incubators. Five replicates were allocated to the cold storage treatments, and each treatment was repeated twice. The control treatment (without cold storage) was kept in the same rearing conditions. The effects of temperature and the cold storage period on the quality of parasitoids were evaluated by measuring the percentage of insect emerging and their sex ratio.

Egg laying and progeny production of diapause females after cold storage

One last instar larva of *C. cephalonica* was placed in a plastic cup (7 cm diameter; 4.5 cm height). One couple of *H. hebetor* obtained from the previous study in each treatment (2 temperature levels with 3 cold storage periods) was released into the cup. The couple of *H. hebetor* was transferred daily to a new cup containing a new last instar larva of *C. cephalonica* for 3 days (7, 14, and 21 days). Five replicates were performed for each treatment, and the experiment was repeated twice. The effects of temperature and cold storage period on daily parasitization was evaluated by measuring the percentage of insect progeny and their sex ratio.

Statistical analyses

Data in all experiments were analyzed by analysis of variance (one-way ANOVA) using a statistical program (R version 3.0.1.) [20], and Tukey's test (alpha = 0.05) was employed to compare mean values of adult emergence and sex ratio across the different cold storages of *H. hebetor.* Two-way ANOVA with temperature and storage period as the fixed effects were used to determine if the interaction between temperature and storage period significantly affected mortality of parasitoids, adult emergence, and the number of egg laid following storage.

Results and discussion

Effect of cold storage on development of *H. hebetor*

The effect of cold storage on the development of *H. hebetor* reared on *C. cephalonica* were observed by measuring (1) the mortality of *H. hebetor* adults emerging during cold storage, (2) the mortality of *H. hebetor* pupae during cold storage, and (3) the percentage of *H. hebetor* females after cold storage. Mortality of *H. hebetor* adults emerging during cold storage indicated that the interaction between period and temperature was found ($F = 11.59$; df = 2, 54; $P < 0.01$). Two temperature levels (10 and 15 °C) and 3 periods of cold storage (7, 14, and 21 days) had an effect on the mortality of *H. hebetor* adults emerging during cold storage ($F = 4.50$; df = 1, 54; $P = 0.03$ and $F = 75.87$; df = 2, 54; $P < 0.01$, respectively). Mortality of *H. hebetor* adults emerging during cold storage at 10 and 15 °C was significantly observed at 21 days of storage ($F = 37.61$; df = 6, 63; $P < 0.01$). Mortality of *H. hebetor* adults emerging during cold storage at 15 °C for 21 days was the highest (61.34 ± 3.90 %), followed by treatment of *H. hebetor* adults emerging during cold storage at 10 °C for 21 days (28.49 ± 8.86 %). Other treatments and the control treatment were not significant (**Table 1**).

Table 1 Mortality of pupae and emerging adults of *H. hebetor* during cold storage at 10 or 15 °C for 7, 14, and 21 days

Stage	Storage temperature (°C)	Storage length (day)	Mean±SE (%) [1/]
Pupa	control	-	5.14±2.10 a
	10	7	3.33±2.22 a
		14	5.96±2.24 a
		21	19.77±3.51 b
	15	7	5.75±1.97 a
		14	9.69±1.89 a
		21	5.64±2.81 a
Emerging adult	control	-	0.00±0.00 a
	10	7	0.00±0.00 a
		14	4.79±2.79 a
		21	28.49±8.86 b
	15	7	0.00±0.00 a
		14	0.00±0.00 a
		21	61.34±3.90 c

[1/] Means ± SE followed by the same letters in each stage are not significantly different, according to Tukey's test ($P > 0.05$)

The interaction between period and temperature affected the mortality of *H. hebetor* pupae during cold storage ($F = 7.91$; df = 2, 54; $P < 0.01$). Two temperature levels had no effect on the mortality of *H. hebetor* pupae during cold storage ($F = 0.45$; df = 1, 54; $P = 0.50$), while 3 periods of cold storage affected the mortality of *H. hebetor* pupae during cold storage ($F = 3.34$; df = 2, 54; $P = 0.04$). Mortality of *H. hebetor* pupae during cold storage in all treatments was less than 20 % (4.00 - 19.77 %), and significant difference among treatments was found ($F = 4.10$; df = 6, 63; $P < 0.01$). Mortality of *H. hebetor* pupae during cold storage at 10 °C for 21 days was the highest, while other treatments were not significant difference from the control (**Table 1**).

The interaction between period and temperature affected the number of *H. hebetor* adults emerging after cold storage ($F = 4.32$; df = 2, 54; $P = 0.02$). Two temperature levels and 3 periods of cold storage had an effect on the number of *H. hebetor* adults emerging after cold storage ($F = 5.20$; df = 1, 54; $P = 0.03$ and $F = 131.68$; df = 2, 54; $P < 0.01$, respectively). Adult males of *H. hebetor* emerging after cold storage in both temperatures at 21 days of storage were significantly different from the control and other treatments ($F = 28.04$; df = 6, 63; $P < 0.01$). Only 33.02 ± 3.72 % of emerging adults was found in a treatment at 15 °C for 21 days of storage, and 51.74 ± 6.68 % of emerging adults was also found in a treatment at 10 °C for 21 days of storage. The percentage of emerging adults of other treatments ranged from 89.25 - 96.67 %, and there was no significant difference from the control ($F = 55.40$; df = 6, 63; $P < 0.01$) (**Table 2**).

The interaction between period and temperature also affected the number of adult female *H. hebetor* emerging after cold storage ($F = 10.96$; df = 2, 54; $P < 0.01$). Two temperature levels had no effect on the number of adult female *H. hebetor* emerging after cold storage ($F = 0.01$; df = 1, 54; $P = 0.93$), while 3 periods of cold storage had an effect on the number of adult female *H. hebetor* emerging after cold storage ($F = 11.10$; df = 2, 54; $P < 0.01$). Numbers of adult female *H. hebetor* emerging after cold storage in all treatments (26.18 - 39.54 %) were not different from the control treatment (32.63 ± 4.19 %), except for female percentages in the treatment of 15 °C for 7 and 21 days of storage ($F = 7.85$; df = 6, 63; $P < 0.01$) (**Table 2**).

Table 2 Number of emerging adults of *H. hebetor* after cold storage at 10 or 15 °C for 7, 14, and 21 days

Sex	Storage temperature (°C)	Storage length (day)	Mean ± SE (%) [1/]
Total alive adults	control	-	94.86 ± 2.10 c
	10	7	96.67 ± 2.22 c
		14	89.25 ± 2.55 c
		21	51.74 ± 6.68 b
	15	7	94.25 ± 1.97 c
		14	90.31 ± 1.89 c
		21	33.02 ± 3.72 a
Female percentage of alive adults	control	-	32.63 ± 4.19 b
	10	7	39.54 ± 4.36 b
		14	27.92 ± 2.05 ab
		21	36.41 ± 4.51 b
	15	7	13.84 ± 3.37 a
		14	26.18 ± 4.25 ab
		21	62.62 ± 10.57 c

[1/] Means ± SE followed by the same letters in each stage are not significantly different, according to Tukey's test ($P > 0.05$)

Egg laying and progeny production of females after cold storage

Numbers of egg laid by females emerging from pupae after storage at 10 and 15 °C varied with the 3 periods of cold storage; the interaction between period and temperature was not found in all 3 times (24, 48 and 72 h) ($F = 0.21 - 1.41$; df = 5, 54; $P > 0.05$). The results showed that 2 temperature levels had no effect on the number of eggs ($F = 0.29 - 0.90$; df = 1, 54; $P > 0.05$), while 3 periods of cold storage affected the number of eggs ($F = 11.20 - 15.31$; df = 2, 54; $P < 0.01$). Those females which laid more eggs after removal from cold storage still varied with periods of cold storage of 24 and 48 h. Egg laying in all treatments of cold storage (9.00 - 14.10 eggs per female) were not significant as compared to the control treatment (13.50 ± 2.90 eggs per female) after laying for 24 h, except in the treatment of females stored at 10 and 15 °C for 21 days, with only 1.60 ± 1.39 and 3.70 ± 1.54 eggs per female found, respectively ($F = 5.7$; df = 6, 63; $P < 0.01$). After laying for 48 and 72 h, the number of eggs in the treatment of females stored at 10 °C and 15 °C for 21 days were significantly lower than the control treatment ($F = 5.7$; df = 6, 63; $P < 0.01$ and $F = 4.4$; df = 6, 63; $P < 0.01$, respectively). After 48 h, the number of eggs in all treatments ranged from 11.30 - 34.20 eggs per female. Females stored at 10 °C and 15 °C for 21 days at 72 h laid only 23.40 ± 4.90 and 23.10 ± 4.32 eggs per female, respectively, while in other treatments, females laid 43.20 - 45.50 eggs per female (**Table 3**).

Table 3 Number of eggs laid by *H. hebetor* females after cold storage at 10 or 15 °C for 7, 14, and 21 days

Time	Storage temperature (°C)	Storage length (day)	Mean ± SE (egg per female) [1/]
24	control	-	13.50 ± 2.90 c
	10	7	9.00 ± 2.01 bc
		14	13.70 ± 2.69 c
		21	1.60 ± 1.39 a
	15	7	11.80 ± 2.37 bc
		14	14.10 ± 3.05 c
		21	3.70 ± 1.54 b
48	control	-	29.00 ± 4.18 bc
	10	7	26.00 ± 3.36 bc
		14	34.20 ± 2.69 c
		21	11.30 ± 3.32 a
	15	7	29.40 ± 3.35 bc
		14	25.50 ± 4.57 bc
		21	13.70 ± 2.47 a
72	control	-	45.50 ± 5.71 b
	10	7	45.70 ± 3.84 b
		14	53.70 ± 5.22 b
		21	23.40 ± 4.90 a
	15	7	47.90 ± 6.09 b
		14	43.20 ± 7.96 b
		21	23.10 ± 4.32 a

[1/] Means ± SE followed by the same letters in each time are not significantly different, according to Tukey's test ($P > 0.05$).

The number of *H. hebetor* progeny developing from the previous eggs showed that the number of male progeny was 3 times higher than female progeny at 24, 48, and 72 h in all treatments (**Figures 1A - 1F**). At 24 h after emerging, the interaction between period and temperature affected the number of male progeny ($F = 3.19$; df = 2, 54; $P = 0.04$) (**Figure 1A**). Two temperature levels had no effect on the number of male progeny ($F = 9.92$; df = 1, 54; $P = 0.36$), while 3 periods of cold storage affected the number of male progeny ($F = 1.28$; df = 2, 54; $P = 0.26$). Significance in the number of male progeny was found in the control treatment (10.80 ± 2.48 individuals) compared with the treatments of 10 °C (0.30 individuals) and 15 °C (1.60 ± 0.95 individuals) for 21 days ($F = 6.1$; df = 6, 63; $P < 0.01$). The other 2 times (48 and 72 h) showed that the interaction between period and temperature had no effect on the number of male progeny ($F = 2.87$; df = 2, 54; $P = 0.06$ and $F = 3.06$; df = 2, 54; $P = 0.06$) (**Figures 1B - 1C**). The 2 temperature levels and 3 periods of cold storage had no effect on the number of male progeny ($F = 0.25$ - 2.80; df = 1, 54; $P > 0.05$ and $F = 2.96$ - 4.70; df = 2, 54; $P > 0.05$, respectively). Significance between the control treatment (21.40 ± 3.82 individuals) and other treatments (8.40 - 24.50 individuals) was not found at 48 h after emerging, but male progeny in the treatment of 10 °C for 14 days (24.50 ± 4.49 individuals) was significantly higher than male progeny at 10 °C (8.40 individuals) and 15 °C (9.90 ± 1.86 individuals) for 21 days ($F = 1.8$; df = 6, 63; $P < 0.01$). However, no significant difference was observed in the number of male progeny (20.20 - 35.10 individuals) at 72 h after emerging ($F = 1.3$; df = 6, 63; $P = 0.24$).

The number of female progeny showed that interaction between period and temperature for 3 times (24, 48 and 72 h) was not found ($F = 2.21$; df = 2, 54; $P = 0.11$; $F = 1.09$; df = 2, 54; $P = 0.34$ and $F = 1.83$; df = 2, 54; $P = 0.16$). Also, the 2 temperature levels and 3 periods of cold storage had no effect on

the number of female progeny ($P > 0.05$). Numbers of female progeny in all treatments at 24 h (0.30 - 2.70 individuals) were not significant ($F = 1.8$; df = 6, 63; $P = 0.09$). Significant differences between treatments were observed after 48 and 72 h ($F = 2.5$; df = 6, 63; $P = 0.03$ and $F = 1.8$; df = 6, 63; $P < 0.01$, respectively), but none of these were different from the control treatment. Especially, the number of female progeny from the treatment of 15 °C for 7 days (13.60 ± 3.36 individuals) was higher than the treatment of 10 °C for 21 days (**Figures 1D - 1F**).

The sex ratios between male and female progeny in all treatments were not significant ($P > 0.01$) in the control treatment at 3 times. The interaction between period and temperature for 3 times (24, 48, and 72 h) were not found ($F = 1.34$; df = 2, 54; $P = 0.26$; $F = 1.02$; df = 2, 54; $P = 0.36$ and $F = 0.74$; df = 2, 54; $P = 0.48$). The 2 temperature levels and the 3 periods of cold storage had no effect on the sex ratios after cold storage ($P > 0.05$). However, the proportions of males in all treatments at 24, 48, and 72 h ranged from 0.50 - 0.89, 0.59 - 0.92, and 0.62 - 0.91, respectively (**Figures 2A - 2C**).

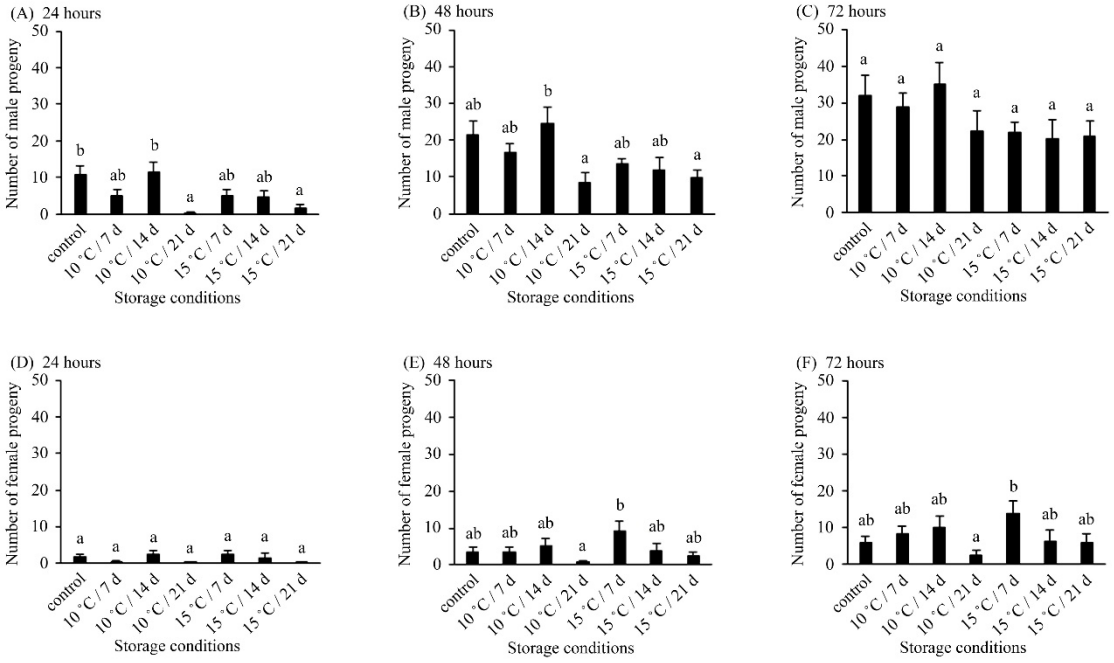

Figure 1 The effects of cold storage on male and female progeny of *H. hebetor* were tested for under various conditions: control (stored at room temperature), and stored at 10 or 15 °C for 7, 14, and 21 days; the average number of male progeny (1A - 1C) and female progeny (1D - 1F) of *H. hebetor* which emerged after cold storage for 24, 48, 72 h. The same letters above the bars indicate treatments without significant differences, according to Tukey's test ($P > 0.05$).

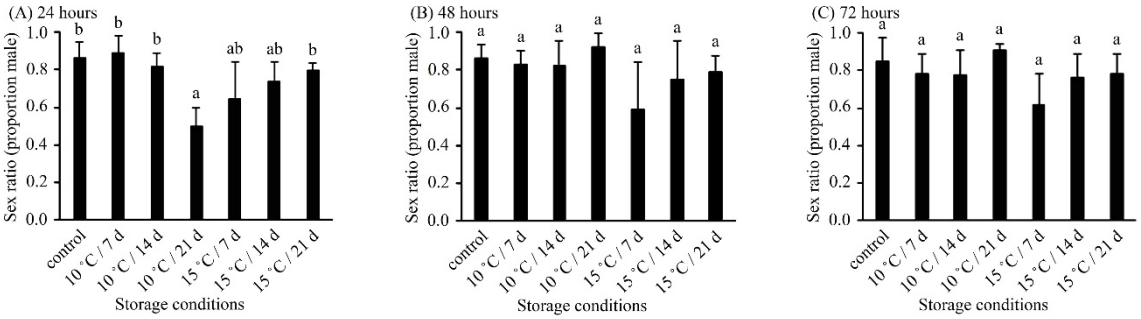

Figure 2 The effects of cold storage on sex ratio of *H. hebetor* were tested for under various conditions: control (stored at room temperature), and stored at 10 or 15 °C for 7, 14, and 21 days; the sex ratio of *H. hebetor* which emerged after cold storage for 24, 48, and 72 h (2A - 2C). The same letters above the bars indicate treatments without significant differences, according to Tukey's test ($P > 0.05$).

This study showed that *H. hebetor* can be effectively stored in cold conditions for up to 14 days, and can contribute to the improved use of *H. hebetor* as a biological control agent in pest management programs. Previous studies indicated that cold storage of *H. hebetor* for 14 - 21 days did not seem to affect its survival and reproductive capabilities, which are traits that need to be maintained after cold storage [9,10,21]. A few studies reported that cold storage provides positive effects on the performance of *H. hebetor* rearing on *P. interpunctella* [9,21] and *E. kuehniella* [10], and studies supported that the storage of *H. hebetor* in cold temperatures was considered as a valuable tool to provide more flexibility and efficiency in mass production [9,10,21,22].

The results of this study showed that most *H. hebetor* pupae that were stored at 10 and 15 °C for up to 3 weeks were still alive during testing. Mortality during cold storage, egg laying, number of progeny produced, and sex ratio after removal from cold storage were similar to the control. These results were similar to Chen *et al.* [9] who indicated that *H. hebetor* reared at 20 °C and stored for up to 8 weeks did not differ in most of their mortality, longevity, egg laying, and the number of progeny produced after removal from cold storage from culture parasitoids. Mortality of adult *H. hebetor* emerging during cold storage at 15 °C was significantly observed to be up to 61 % after 21 days of storage, and lower mortalities were found to be only 18 %. Mortality of *H. hebetor* pupae during cold storage in all treatments was 4 - 19 %. Similar to this result, the mortality of *H. hebetor* adults emerging during cold storage of parasitoids reared at 17.5 and 20 °C, and then stored at 5 °C for 4 weeks, were up to 24 - 42 %, depending on different photoperiods. Mortality of males reared at 20 °C exceeded 80 % after 8 weeks of storage, and no males survived after 16 weeks of cold storage [9]. The mortality might have been caused by the depletion of large resources during cold storage, resulting in reduced performance and accumulation of toxic metabolites that caused death or reduced fitness [9,23].

Egg laying after removal from cold storage of females reared at 10 and 15 °C varied with the period of cold storage. Egg laying of females emerging after cold storage was lower than that of cultured females in all treatments. Previous studies indicated that the number of eggs laid was always lower in stored insects than in cultured insects. This might have been because the parasitoids were recovering from chilling injuries after exposure to low temperatures [22,24], and eggs in the ovary might have been killed during storage [9]. In this study, egg laying cultures of *H. hebetor* averaged 23 - 53 eggs per female in *C. cephalonica* at 72 h, which was lower than the eggs obtained in some studies that found 253 eggs per female in *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) [25], 326 eggs per female in *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae) [26], 78 eggs per female in *Galleria mellonella* L. (Lepidoptera: Pyralidae) [27], 66 eggs per female in *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) [27], 400 eggs per female in *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) [21], and 311 eggs

per female in *P. interpunctella* [9]. Egg laying in all treatments of cold storage was each up to 14, 34, and 45 eggs per female after laying for 24, 48, and 72 h, respectively, and none of these were different compared to the control treatment. The differences in fecundity might be due to the different host species and, also, the different densities of parasitoids and larval hosts [9]. Fecundity in this study declined with increased cold storage period. Similarly, Chen *et al.* [21] reported that daily fecundity of *H. hebetor* declined when they were kept in cold storage longer than 30 days, and those female parents started to lay a few eggs on the second day. This phenomenon could be explained by the fact that parasitoids need to recover from chilling injuries after long exposure to low temperatures [22,24].

The percentages of female progeny produced by the females emerging after cold storage at 10 and 15 °C were 2 - 13 %, and they were always not different from the control (5 %). The percentage of female progeny for 21 days between cold storage treatment and culture treatment (13 vs. 30 %) after cold storage in this study was similar to Chen *et al.* [9], who revealed that the percentage of female emerging after cold storage at 5 °C was lower than cultured parasitoid (36 vs. 52 %). The sex ratios between males and females in all treatments were not significant in the control treatment at 3 periods, and ranged from 0.62 - 0.91 at 72 h. Close to this experiment, Yu *et al.* [28] found that the progeny sex ratio of *H. hebetor* reared on *P. interpuntella* remained at 0.5, while Rotary and Gerling [29] found 0.4 % of *H. hebetor* female when *A. kuehniella* was provided as a host. This result was similar to several previous researchers, who found that the sex ratio of *H. hebetor* offspring were approximately 1:1 by using *P. interpunctella* as a host [9,30-32]. The differences in sex ratios among different studies might be due to the use of different hosts, host or parasitoid densities, and temperature conditions [9,21]. Although male percentages of adult *H. hebetor* emerging after cold storage in both temperatures after 21 days of storage significantly decreased, the presence of a male after cold storage did not affect the sex ratio of progeny. Female percentages of *H. hebetor* adults emerging after cold storage in all treatments were not different.

Storage proteins were synthesized before the insects entered diapause, then released into the insect hemolymph and remained throughout diapause. The proteins disappeared quickly after diapause was terminated [9,33]. However, previous studies indicated that increasing the longer cold storage of *H. hebetor* females resulted in a decline in parasitism, longevity, and fecundity of parents and offspring [9,10,21,33]. For example, the ability of *H. hebetor* to parasitize *P. interpunctella* larvae declined with increasing cold storage period, and *H. hebetor* parents stored for 70 days and allowed to parasitize *P. interpunctella* larvae for 72 h caused only 44 % mortality, whereas control parents achieved 90 % mortality [21]. The offspring declined to increase in the cold storage period because living organisms had limited resources, which were spent on growth, reproduction maintenance [34], and maternal aging. Previous studies had suggested that *H. hebetor* might overwinter in the adult stage [9,10,21], while our data showed that *H. hebetor* adults could survive up to 21 days at 10 and 15 ºC during the pupal stage. Heat shock proteins (HSPs) were expressed in most organisms in response to a wide range of stressful environmental conditions [34]. HSPs increase when cells are exposed to abnormal temperatures or other stress conditions [34,35]. Because of this mechanism, oleic acid levels were increased, which helped cell membranes maintain their liquid crystalline state, and promoted cellular survival at low temperatures [36]. Another reason was an increasing number of fat bodies, which might help an insect to survive at low temperatures by serving as an organ for energy storage and utilization [37,38].

Conclusions

Our results showed that the pupal stage of *H. hebetor* can be stored at 10 and 15 ºC for up to 2 weeks without adversely affecting the performance of the parasitoid. Gaining new information on how to improve the performance of *H. hebetor* under cold conditions could lead to the development of approaches to keep and release this parasitoid in the agricultural field. This research proved that the use of *H. hebetor* as a biological control agent in pest management programs can be improved. Further research needs to be conducted on field performance of *H. hebetor* to improve performance after long-term cold storage.

Acknowledgements

This research was supported by the Kasetsart University Research and Development Institute (Grant# R-M 2.55). We also thank Dr. Watcharapong Chumchuen, Dr. Sheila Villamor, and Sirirut Mangtab for their valuable assistance.

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