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Performance of diapausing parasitoid wasps, *Habrobracon hebetor*, after cold storage

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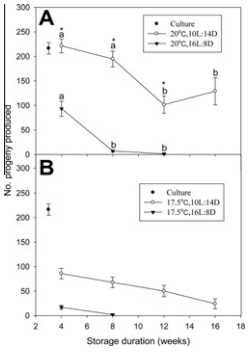
HIGHLIGHTS

- Diapause female survival is greater than for nondiapause females during cold storage.
- Diapause females produce more progeny than nondiapause females after cold storage.
- Female percentage of F1 offspring is lower than in the culture after cold storage.
- Diapause females can be stored at 5 °C for up to 8 weeks.

GRAPHICAL ABSTRACT



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ABSTRACT

The ectoparasitoid *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) is an important potential biological control agent for lepidopterous pests of stored products. We investigated the effects of long-term cold storage of diapausing and nondiapausing *H. hebetor* on their performance after cold storage. Mortality during storage increased with increasing storage duration, and the mortality of diapausing females was lower than that of nondiapausing females after 8, 12, and 16 weeks of storage. Longevity, egg laying, number of progeny produced, and time to 50% egg laying were all reduced, as compared with the culture females when parasitoids were reared at conditions that do not induce diapause. But, for females reared at 20 °C at conditions that induce diapause, all of these quality parameters did not differ from those of culture insects when the storage duration was 8 weeks or less. The percentage of female F1 offspring was always lower for cold stored insects than for the culture insects. Presence of a male after cold storage did not impact any of the quality parameters measured. Thus, rearing parasitoids at 20 °C and 10L:14D and then storing them for up to 8 weeks at 5 °C would produce parasitoids that are similar to culture parasitoids, except that the percentage of females is lower than that in the cultures (36% vs. 52%).

1. Introduction

The ectoparasitoid *Habrobracon hebetor* (Say) (=*Bracon hebetor*) (Hymenoptera: Braconidae) is considered to be one of the most important biological control agents of several pyralid moth pests in warehouses due to its rapid population growth (Press et al., 1982; Balevski, 1984; Huang, 1986; Keever et al., 1986; Brower and Press, 1990). It has been used to suppress moth populations in stored products (Press et al., 1982; Balevski, 1984; Huang, 1986; Brower and Press, 1990; Cline and Press, 1990; Garba and

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Gaoh, 2008; Zhang, 2009) and in field crops (Uwais et al., 2006; Imam et al., 2007).

There are two problems which reduce the desirability of *H. hebetor* as a potential biological control agent. One problem is obtaining sufficient numbers for release (Coudron et al., 2007), and another problem is a shortage of host larvae for mass production of *H. hebetor* when the demand is high. When demand is low, overproduction of host larvae can be wasteful. Cold storage has been considered as an option to overcome these problems for other parasitoid species (Foerster and Nakama, 2002; Pitcher et al., 2002; Bradley et al., 2004; Rundle et al., 2004; Tezze and Botto, 2004; Levie et al., 2005; López and Botto, 2005; Chen et al., 2008). Cold storage provides flexibility and efficiency in mass production (Greenberg et al., 1996; Leopold et al., 1998; Tezze and Botto, 2004), and minimizes the costs of maintaining a colony during periods when releases are not required (Coudron et al., 2007). Cold storage also improves genetic stability because fewer generations are reared.

A concern with storing parasitoids is possible loss of vitality during storage. Storing diapausing insects is one possible method for maintaining vitality during cold storage. Diapause is one of the major strategies used as a means to survive unfavorable environmental conditions, and it is well documented that insect chilling resistance is enhanced during diapause (Foerster and Doetzer, 2006; Denlinger, 2008). However, diapause in *H. hebetor* has not been well studied (Adashkevich and Saidova, 1985). Our previous study showed that adult *H. hebetor* can enter reproductive diapause when reared at low temperature and short photoperiod (Chen et al., 2012). However, we don't know whether reproductive diapause prolongs the length of time for which *H. hebetor* can be stored at low temperature without loss of vitality.

In this study, we evaluate the quality of *H. hebetor* adults after cold storage. Adults were reared at conditions that appear to induce reproductive diapause, and then emerged adults were stored at cold temperatures for varying periods of time. Several quality parameters were measured to determine the effects of different lengths of cold storage on reproduction and development.

2. Materials and methods

2.1. Insect rearing

2.1.1. Hosts

The Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), was reared in 3.8-l glass jars on an artificial diet consisting of cracked wheat (1000 g), wheat shorts (1000 g), wheat germ (100 g), brewer's yeast (80 g), glycerine (240 ml), honey (240 ml), and 120 ml of water (McGaughey and Beeman, 1988). Pupae were collected from the stock culture by placing corrugated cardboard (2-cm high by 5.7-cm diameter) in the glass jar as a pupation site (larvae crawl into the corrugations to pupate), and then putting the pupae in another glass jar for adults to emerge and lay eggs. About 50 mg of eggs (ca. 2000 eggs) were then transferred to a 500-ml glass jar filled about one-third with artificial diet. The culture was maintained at 30.0 ± 0.5 °C, 65 ± 5% relative humidity (RH), and 16L:8D photoperiod. Last instar larvae were used as hosts for parasitoids in the experiments.

2.1.2. Parasitoids

A field strain of *H. hebetor* used in the experiments was collected in Parlier, CA, in October 2009, and the studies described in this manuscript were conducted with this strain during the next year. Adult parasitoids were introduced into 15-dram plastic vials (3.2-cm diameter by 8.3-cm high) containing 30 last instar *P. interpunctella*. Vials were covered with lids that had a 0.5-cm diameter hole covered with fine mesh screen. Parasitoids were reared at 27.5 ± 0.5 °C, 65 ± 5% RH, and 16L:8D photoperiod.

2.2. Obtaining diapause and nondiapause H. hebetor for cold storage

To obtain *H. hebetor* eggs for experiments, 10 pairs of adults from the culture were allowed to oviposit for 24 h on 30 last instar *P. interpunctella*. Females are differentiated based on the presence of an ovipositor. The larvae with *H. hebetor* eggs were placed in Percival I-36VL chambers set at either 17.5 or 20 °C, 65% RH, and either 10L:14D (diapause-inducing conditions) or 16L:8D (conditions that do not induce diapause) (Chen et al., 2012). Adult emergence was checked every 3.5 days. After emergence, females and males were left together at their treatment conditions for 3.5 days to mate. After the 3.5–7.0-day post-emergence period, 450 females and 450 males from each condition were stored with the sexes separated at 5.0 °C. We only stored mated females because they can produce both male and female progeny without additional mating, which would be desirable in a mass rearing program. There were 30 adults per container. Tests at 17.5 and 20 °C were conducted sequentially.

2.3. Quality parameters measured after cold storage

After 4, 8, 12, 16, and 20 weeks of storage, three replicates of 30 females and 30 males from each storage condition were removed from cold storage. Mortality during cold storage was recorded.

We determined longevity and fecundity of females after cold storage in two ways. First, we determined the ability of females to mate and lay eggs after cold storage by pairing females with a 0- to 3-day-old male from the cultures in a 15-dram vial containing 9 *P. interpunctella* last instars. The vials were placed at 27.5 °C, 65 ± 5% RH, and 16L:8D photoperiod, and the adults were moved to a new vial containing 9 new *P. interpunctella* last instars every 3.5 days until the female died. After removal of the parasitoids, numbers of *H. hebetor* eggs on larvae were counted. Sex ratio of adults emerging from these eggs was determined. There were 15 pairs of adults set up for this test at each condition. We also determined whether sperm in the female's spermatheca were still active after cold storage using exactly the same experimental techniques, except that the females were placed in vials without males.

We determined longevity and fertility of males after cold storage by pairing one male that had been removed from cold storage with one 0- to 3-day-old unmated female from the cultures. Procedures used were the same as for females above. Unmated females were obtained by placing individual pupae from the culture in a small petri dish.

To determine whether longevity and fecundity differed from that of culture insects, 15 pairs of 0- to 24-h-old adults from the culture were treated as above.

2.4. Statistical analysis

Mortality during cold storage, longevity, fecundity, time of 50% egg laying, total number of progeny produced, and F1 offspring female percentage were analyzed with a two- or three-way ANOVA. When interactions were significant, we then used one- or two-way ANOVA's to examine effects of one factor within each level of the other factor. Tukey's-*b* test ($P = 0.05$) was used for mean comparisons (SPSS Inc., 2007). We used Dunnett's-*t* test to determine whether treatment means differed from those for the culture insects. Only replicates in which there were more than 50 progeny adults were used to calculate the sex ratio. Biological parameters were compared separately among different photoperiods at each temperature.