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Effects of cold storage on the biological characteristics of *Microplitis prodeniae* (Hymenoptera: Braconidae)

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Abstract

The endoparasitoid *Microplitis prodeniae* Rao and Chandry is an important potential augmentative biological control agent for lepidopteran pests of vegetables and tobacco. However, cold storage of pupae is required to ensure that sufficient parasitoids are available when they are needed in the field. In this study, pupae were maintained at 0, 4 or 10°C for 5–50 days after which the adults were evaluated for emergence, pre-emergence period, sex ratio, female longevity, oviposition period, and fecundity. Cold storage did not affect the pre-emergence period or proportion of females; however, there was a significant reduction in emergence, female longevity, oviposition period, and fecundity with increased exposure to cold. The pre-emergence period was approximately 5 days, and approximately 50% of the emergent parasitoids were females. A cold storage regime of 10 days at 10°C had no effect on the parasitoids and adult emergence was greater than 50% even after 20 days at 10°C. There was no carryover of the cold treatment from parental to F1 and F2 generations. Thus, *M. prodeniae* can be stockpiled for field release by exposing the pupae to a cold regime and subsequently holding them for adult emergence at 28°C.

Keywords: *Microplitis prodeniae*, cold storage, emergence rate, fecundity, biological control

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Introduction

The Oriental leafworm moth, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), infests vegetables in subtropical and tropical regions (Zhou *et al*., 2010; Dang *et al*., 2011), including Hanoi and its surrounding areas in Vietnam (Dang & Vu, 1999). *S. litura* is difficult to control due to its rapid development rate, adaptability to different host plants and strong dispersal capacity. Therefore, many different methods, especially insecticides, are used to suppress *S. litura* (Seth & Sharma, 2001; Bhatt *et al*., 2008; Liu *et al*., 2012). However, *S. litura* populations have developed resistance to some widely used insecticides (Brewer & Trumble, 1989; Kranthi *et al*., 2002). Given that pesticides can be harmful to humans and other non-targets as well as to the environment (Tong *et al*., 2013), research on parasitoids is important to find alternative controls for *S. litura* in field applications.

Parasitoids are important biological agents used in integrated pest management programmes. They have generated a great deal of interest because of their ability to suppress pest populations. *Microplitis prodeniae* Rao and Chandry (Hymenoptera: Braconidae) was first reported by Rao & Chandry (1950) and is a major parasitoid of *S. litura*. *M. prodeniae* is a solitary larval endoparasitoid that has three larval instars; the mature parasitoid larvae egress from the host and then spin cocoons (Z. Y., unpublished data). At 28°C, the total development time from egg to adult is approximately 13 days on *S. litura* (Dang & Ha, 1999); the egg stage of this parasitoid lasts for approximately 1 day and the larval and pupal periods are approximately 7 and 5 days, respectively (Z. Y., unpublished data). Adult longevity is

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3.5 and 4.0 days when fed with pure honey or a 50% sugar solution, respectively (Dang & Vu, 1999). Of the six instars of *S. litura*, *M. prodeniae* can parasitize the 1st through 4th instar larvae, however, the 2nd instar larval stage is the most preferred stage. The 2nd instar stage of *S. litura* has previously been observed to be the most suitable for parasitism (Z. Y., unpublished data). *M. prodeniae* females can discriminate between parasitized and unparasitized hosts. Generally, they lay just one egg per host; however, females will oviposit more than once in an attacked host when the exposure duration is long or the number of available hosts is small (Z. Y., unpublished data). The wasp has two population peaks, one of which occurs during the last 10 days of April and the other in the middle 10 days of June. These peaks are sufficient to control its host to some degree in tobacco, China (Chen *et al.*, 2003; Zhou *et al.*, 2010).

One of the main obstacles in biological control programmes is the failure to obtain large enough numbers of the natural enemies when they are required for release (Coudron *et al.*, 2007). Therefore, the development of cold storage techniques for biological control agents is considered extremely important because extended storage capability provides flexibility and efficiency for mass rearing (Greenberg *et al.*, 1996; Leopold, 1998; Tezze & Botto, 2004). Cold storage is also advantageous while shipping parasitoids and for stockpiling parasitoids when planning future releases (Ballal *et al.*, 1989). The effects of cold storage on the performance of parasitoids have received considerable interest (King, 1934; Hance *et al.*, 2007; Hawes & Bale, 2007; Leopold, 2007; Colinet & Hance, 2010; Alam *et al.*, 2016). These studies indicate that most parasitoids can be cold-stored for short periods with minimal reduction in fitness. Most often, survival, sex ratio, lifespan and fecundity have been considered the important indicators of the fitness of stored insect parasitoids, but intergenerational effects have also received some attention (Al-Tememi & Ashfaq, 2005; Colinet *et al.*, 2007; Colinet & Hance, 2009, 2010; Nadeem *et al.*, 2010; Chen *et al.*, 2011). Additionally, cold stress alters learning behaviour (van Baaren *et al.*, 2005), modify olfactory responses (Bourdais *et al.*, 2012), and affect the mating rate (Amice *et al.*, 2008). Cold stress also alters morphogenesis, acting on the morphology of wings and antennae (Sehnal, 1991; Bourdais *et al.*, 2006; Amice *et al.*, 2008; Colinet & Hance, 2009). The pupal stage is often considered to be the most suitable stage for short-term cold storage. Some experimental evidence has shown that pupae are more cold-tolerant than eggs, larvae or adults (van Lenteren & Tommasini, 2002).Although many studies have conducted research concerning the cold storage of braconid species (Hun *et al.*, 2005; Ismail *et al.*, 2010; Chen *et al.*, 2013; Silva *et al.*, 2013), to our knowledge, there are no reports concerning the cold storage of *M. prodeniae* to date. *M. prodeniae* has great potential as a candidate that may control *S. litura*. The estimated maximum numbers of the 1st, 2nd, and 3rd instar larvae that were parasitized by a single *M. prodeniae* female are 71.6, 78.4, and 41.5 larvae, respectively, over a 24-h period (Z. Y., unpublished data). It is meaningful to investigate the effects of cold storage on *M. prodeniae* because the ability to store *M. prodeniae* is directly related to using this parasitoid to control *S. litura*.

The objective of the present study was to evaluate the effect of storing cocoons at different low temperatures for varying periods on the emergence rates, pre-emergence period, female proportion, female longevity and fecundity of *M. prodeniae*. This demonstration of the suitability of *M. prodeniae* for short-term cold storage will improve the ability to transport and stockpile *M. prodeniae* used in biological control programmes of *S. litura* during the summer and autumn when such populations are most needed.

Materials and methods

Insect cultures

Hosts

Egg masses of *S. litura* were collected in 2012 from *Asparagus officinalis* leaves in Baodao Xincun, located in Danzhou City, Hainan Province, China (location: 19.52°N, 109.46°E). Thereafter, the host larvae were fed on a semi-synthetic diet (Ahmad *et al.*, 2007) in a transparent plastic container. The diet was changed daily to avoid bacterial contamination. The container was 20 cm in length, 13 cm in width, and 7 cm in height, covered with a screened and ventilated lid from hatching until pupation. Then, the *S. litura* pupae were transferred in groups to cages with 40-mesh nylon organza over aluminium frames for adult eclosion and egg laying. The cages were 60 cm in length, 40 cm in width, and 50 cm in height. We provided gauze as a substrate for egg deposition and 10% honey solution as food for the moths. The eggs were checked daily for larval hatching. The *S. litura* larvae were subsequently used to maintain the colony and for experiments.

Parasitoids

M. prodeniae was originally collected in 2013 from parasitized *S. litura* larvae on *A. officinalis* in a suburb of Sanya City, Hainan Province, China (location: 18.20°N, 109.50°E). The obtained population was reared in the laboratory using *S. litura* as hosts at 25 ± 1°C and 70 ± 10% RH with a 14 L:10 D photoperiod. The *M. prodeniae* adults were maintained in a clear transparent plastic box containing about forty 2nd or 3rd instar larvae. Three mated females were released into these containers for about 24 h. The wasps used in the experiments were 2 days old and had not previously been exposed to host larvae. Adult wasps were provided with a 10% sucrose-glucose-fructose mixture (1:1:1) as a dietary supplement.

The effect of cold storage on the emergence, development, and female longevity of M. prodeniae

Ten 24-h old cocoons of *M. prodeniae* were placed in a plastic tube with a diameter of 1.2 cm and height of 7.2 cm, plugged with cotton. Five tubes were randomly selected for each cold storage treatment; therefore, each treatment included 50 cocoons. Each tube was considered a replicate. The treatments consisted of combinations of three temperature levels (0, 4 and 10°C) with eight cold storage periods (5, 10, 15, 20, 25, 30, 40, and 50 days). The cold storage treatments consisted of storing the tubes in an incubator at 75 ± 5% RH in full darkness. The control group was maintained at the rearing conditions (28 ± 1°C, 75 ± 5% RH and a 14 L:10 D photoperiod). After each storage period was complete, the cocoons were transferred to another climatic chamber (28 ± 1°C, 75 ± 5% RH and a 14 L:10 D photoperiod) and observed daily. Adults were fed with a 10% aqueous sucrose-glucose-fructose mixture (1:1:1).

The effects of cold storage on the quality of *M. prodeniae* were evaluated by the following parameters: the emergence rates (no. of emerged adults/no. of cocoons × 100%); pre-emergence period after removal of cocoons from storage; female proportion of emerging adults (no. of emerged adult females/total individuals) and female longevity.

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The effect of cold storage on the fecundity of M. prodeniae

The effect of cold storage on fecundity was determined for the parental, Fl, and F2 generations. Pupae were stored at 10°C, 75 ± 5% RH in darkness for 10 and 20 days in an incubator. Each cold storage treatment was initiated with 400 24-h cocoons. The control group was kept at the rearing conditions (28 ± 1°C, 75 ± 5% RH and a 14 L:10 D photoperiod). After each storage period was completed, the cocoons were transferred to another climatic chamber (28 ± 1°C, 75 ± 5% RH and a 14 L:10 D photoperiod) and observed daily. Fifteen mating pairs (female–male) of newly emerged *M. prodeniae* were randomly selected. In a transparent plastic container as described above, 20 *S. litura* 2nd instar larvae were exposed to a single newly emerged mated female for 8 h. Female wasps were provided with a 10% sucrose-glucose-fructose mixture (1:1:1). The trial was repeated with new unparasitized 2nd instars each day until the female died to determine adult longevity. The exposed host larvae were dissected daily to determine whether an immature parasitoid was present and to record the number of immature parasitoids in all 20 host larvae. Then, the parasitoid's lifetime (no. of eggs laid by a female during her entire lifetime) and daily fecundity (no. of eggs laid by a female in a day) were calculated.

The female wasps of the Fl and F2 generations used in this study were progeny produced by a parental generation newly emerged from cocoons that had been maintained in cold storage at 10°C as described above. The fecundity values for the individual of the F1 and F2 generations were obtained using the same procedures as those described above for the individuals of the *M. prodeniae* parental generation.

Statistical analysis

All the data were analyzed with SAS software (SAS Institute, 1999). The effect of cold storage on the emergence, pre-emergence period, sex ratio, female longevity, and fecundity were analyzed with one-way analysis of variance, and multiple comparisons of means were carried out with Fisher's Protected Least Significant Difference (LSD) test (SAS Institute, 1999). Data were checked for normality and homoscedasticity before the comparison analysis. All significance levels were 5% unless otherwise noted.

Results

Effect of cold storage on the emergence, development and female longevity of M. prodeniae

The emergence of *M. prodeniae* at 28°C after removal of cocoons from cold storage declined as the storage duration increased compared with the control group, and the emergence rate decreased with colder storage temperatures. The emergence rates between treatments of 5 days and 10 days at 10°C were not significantly different from the emergence rate of the control group ($F = 44.5$; df = 10, 44; $P < 0.0001$; table 1). The emergence remained above 50% after both 5 days' storage at 4°C and after 5–20 days' storage at 10°C. However, adults did not emerge when the storage period of *M. prodeniae* cocoons exceeded 10 days at 0°C, 15 days at 4°C, or 50 days at 10°C.

The adult emergence times of all storage temperature treatments were not significantly different from that of the control group held at 28°C ($F = 1.5$; df = 10, 288; $P = 0.136$; table 2). The control group of *M. prodeniae* adults required an average of 5.1 days to emerge at 28°C.

Table 1. The effect of cold storage temperature and duration on the emergence rate of *M. prodeniae*.

Storage duration (days)	Adult emergence proportion (%)[1]		
	0°C	4°C	10°C
5	40 ± 3.16 e	52 ± 4.90 d	82 ± 6.63 ab
10	0	22 ± 2.00 f	80 ± 5.48 ab
15	0	0	78 ± 3.74 b
20	0	0	64 ± 2.45 c
25	0	0	40 ± 3.16 e
30	0	0	38 ± 3.74 e
40	0	0	12 ± 2.00 f
50	0	0	0
Control	90 ± 3.16 a	–	–

[1]All values are expressed as the means ± SE. Mean values with different letters are significantly different from one another (Fisher's LSD; $P < 0.05$).

Table 2. Effect of cold storage temperature and duration on the pre emergence period of *M. prodeniae* after removal of cocoons from storage.

Storage duration (days)	Adult emergence time after cold storage (days)[1]		
	0°C	4°C	10°C
5	4.73 ± 0.26 a	4.62 ± 0.16 a	4.59 ± 0.10 a
10	–	5.00 ± 0.20 a	4.63 ± 0.09 a
15	–	–	4.69 ± 0.11 a
20	–	–	4.75 ± 0.15 a
25	–	–	4.80 ± 0.13 a
30	–	–	4.84 ± 0.14 a
40	–	–	4.92 ± 0.08 a
Control	5.06 ± 0.12 a	–	–

[1]All values are expressed as the means ± SE. Mean values with different letters are significantly different from one another (Fisher's LSD; $P < 0.05$).

Cold storage did not significantly affect the proportion of females emerging from stored cocoons ($F = 0.5$; df = 10, 44; $P = 0.902$; table 3). The mean value was approximately 0.5.

Within each temperature treatment, female longevity decreased as the storage duration increased. In contrast, female longevity increased as the storage temperature increased within each treatment. Female longevity was not significantly affected after 5 days (longevity was approximately 12.3 days) and 10 days (longevity was approximately 12.2 days) storage at 10°C. However, female longevity under the other treatments was significantly lower than the value of 14.4 days for the control group ($F = 29.0$; df = 10, 139; $P < 0.0001$; table 4).

Moreover, emerging individuals were inactive and the emerged adults died or suffered from wing development problems after being stored for 30 days at 10°C. The number of individuals with wing development problems increased after being stored for 40 days at 10°C.

Effect of cold storage on the fecundity of M. prodeniae

There was no significant difference in the fecundity of the *M. prodeniae* parental generation between 10 days of storage at 10°C and the control group, but fecundity was significantly lower than the control group after 20 days of storage at 10°C

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Table 3. Effect of cold storage temperature and duration on the female proportion of *M. prodeniae* adults.

Storage duration (days)	Female proportion[1]		
	0°C	4°C	10°C
5	0.45 ± 0.03 a	0.47 ± 0.04 a	0.57 ± 0.06 a
10	–	0.40 ± 0.10 a	0.57 ± 0.03 a
15	–	–	0.45 ± 0.05 a
20	–	–	0.44 ± 0.06 a
25	–	–	0.51 ± 0.04 a
30	–	–	0.45 ± 0.05 a
40	–	–	0.40 ± 0.25 a
Control	0.54 ± 0.04 a	–	–

[1]All values are expressed as the means ± SE. Mean values with different letters are significantly different from one another (Fisher's LSD; $P < 0.05$).

Table 4. The effect of cold storage temperature and duration on the longevity of *M. prodeniae* females.

Storage duration (days)	Females longevity (days)[1]		
	0°C	4°C	10°C
5	4.22 ± 0.40 d	5.83 ± 0.46 d	12.33 ± 0.63 ab
10	–	5.25 ± 0.48 d	12.17 ± 0.59 ab
15	–	–	10.22 ± 0.58 bc
20	–	–	9.43 ± 0.75 c
25	–	–	8.30 ± 0.67 c
30	–	–	8.00 ± 0.61 c
40	–	–	4.83 ± 0.20 d
Control	14.38 ± 0.34 a	–	–

[1]All values are expressed as the means ± SE. Mean values with different letters are significantly different from one another (Fisher's LSD; $P < 0.05$).

($F = 72.1$; df = 2, 42; $P < 0.0001$; fig. 1). The fecundity of the *M. prodeniae* Fl and F2 generations after 10 and 20 days of storage at 10°C was not significantly different than the controls (Fl generation: $F = 2.1$; df = 2, 42; $P = 0.131$; F2 generation: $F = 2.3$; df = 2, 42; $P = 0.114$; fig. 1). In addition, no significant differences in fecundity among parents and their Fl and F2 offspring were found among control or the 10-day cold storage treatment (0 days: $F = 0.3$; df = 2, 42; $P = 0.778$; 10 days: $F = 0.2$; df = 2, 42; $P = 0.844$; fig. 1). The fecundity of F1 and F2 offspring was approximately twice that of their parents after the parent generation was cold-stored for 20 days ($F = 49.3$; df = 2, 42; $P < 0.0001$; fig. 1).

For parents, the ovipositional periods following storage for 0, 10, and 20 days were approximately 13, 13, and 9 days, respectively. Peak fecundity was reached after 2–4 days; however, peak daily fecundity declined as the cold storage duration increased. The daily fecundity of the 20-day cold storage group was reduced compared with the other groups. Females oviposited 27.78, 29.12, and 50.78% of the total eggs on days 1–3 under the 0-, 10- and 20-days storage treatments, respectively (fig. 2). After 20 days of cold storage, females laid the majority of their eggs in the first 3 days, while control females required more than 3 days to oviposit the majority of their eggs.

For the control group, the ovipositional patterns of the F1 and F2 generations were similar to that of the parental generation (figs 2–4). The ovipositional period after storage for the 0-, 10- and 20-day treatments were all approximately 13 days.

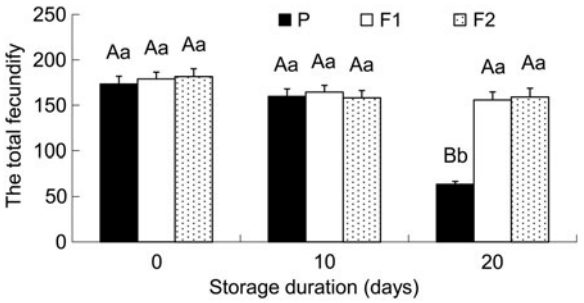

Fig. 1. Fecundity (mean ± SE) of *M. prodeniae* parents (P) and their Fl and F2 offspring at 28°C after cold storage. The uppercase letters indicate comparisons within different cold storage periods whereas the lowercase letters compare parents with their Fl and F2 offspring. Mean values with different letters are significantly different from one another (Fisher's LSD; $P < 0.05$).

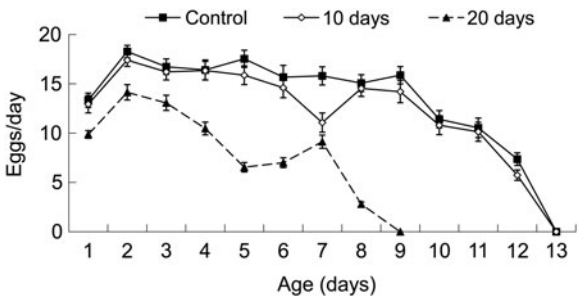

Fig. 2. Effect of the storage period at 10°C on the daily egg production of *M. prodeniae* parents.

Discussion

The effects of cold storage on the performance of parasitoids have received considerable attention from researchers. In addition to the obvious fitness indicators such as emergence, sex ratio, longevity and fecundity, many other important fitness traits might be affected by storage and therefore impact the effectiveness of biological controls. For instance, intergenerational effects and dispersal ability are important features that should be included in post storage quality assessment (Leopold, 2007; Colinet & Hance, 2010; Alam *et al.*, 2016). When planning cold storage of parasitoids, it is essential to determine the most appropriate storage temperatures and storage durations. Our results indicated that better emergence rates were obtained after 5 days of storage at 4°C and between 5 and 20 days of storage at 10°C. Developmental time can be extended by storing cocoons at cold temperatures, a characteristic that is valuable when stockpiling and transporting parasitoids. The sex ratio of the emerging adults was not significantly affected by cold storage. Adult longevity and fecundity were affected least by short-term storage at 10°C. Because cold storage longer than 10 days significantly affected the survival, adult longevity and fecundity of the parasitoid, short-term cold storage of cocoons for 10 days at 10°C may be better suited for *M. prodeniae*. Ballal *et al.* (1989) studied cold tolerance for cocoons of *Allorhogas pyralophagus* Marsh under laboratory conditions. With respect to survival and adult longevity, the most suitable storage temperature seemed to be 10°C. Silva *et al.* (2013) evaluated

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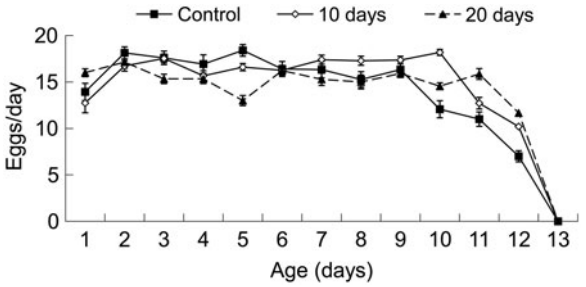

Fig. 3. Effect of the storage period at 10°C on the daily egg production of *M. prodeniae* Fl offspring.

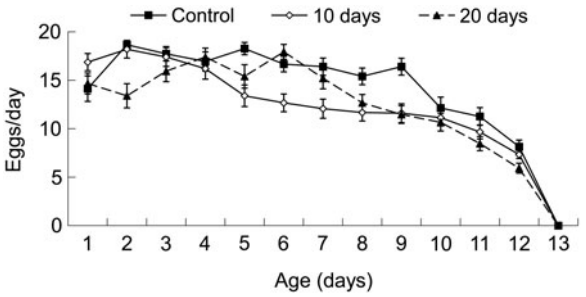

Fig. 4. Effect of the storage period at 10°C on the daily egg production of *M. prodeniae* F2 offspring.

the effects of constant low-temperature storage on *Diaeretiella rapae* (McIntosh), which can be stored for up to 24 days at 5° C. Our results are consistent with those of several authors that reported cocoons of parasitoid species of the family Braconidae were suitable for short-term cold storage (Ballal *et al.*, 1989; Silva *et al.*, 2013). Therefore, our results can form the basis for developing mass storage and shipping techniques.

Only 22% of the adults emerged when *M. prodeniae* cocoons were stored at 4°C for 10 days. Anonymous (1986) found that it is fatal to store *A. pyralophagus* cocoons beyond 4 days at 5°C. This differs from the results obtained by the present study and indicates that there may be a large interspecific variability in cold storage tolerance. We observed an increase in mortality following long-term cold storage of parasitoids, a result that is similar to previous reports (Hanna, 1935; Eisler & Pless, 1972) for *Euchalcidia carybori* Hanna and *Lysiphlebus testaceipes* Cresson, respectively. According to the results obtained in this research, the emergence percentage of *M. prodeniae* in the same period of storage (5 days) at a temperature of 10°C, 91.11%, at 4°C, 57.78%, and at 0°C, 44.44% was reduced when compared with that of the control group. This indicates that the emergence percentage of *M. prodeniae* after 5 days of storage decreases as the storage temperature decreases over the range of 10–0°C. Therefore, the most suitable cold storage condition for adult emergence was short-term storage at 10°C. To satisfy the production schedules of *M. prodeniae* it is important to know the time required for the wasps to emerge after cold storage, given that their developmental time is extended in cold storage. In addition, our observation that the adult emergence time is not altered by the cold storage of pupae is important for calculating accurate field release timing.

The sex ratio of emerging adults was not significantly affected by cold storage. This shows that differential pupal mortality did not occur based on sex due to the effects of cold storage. The number of released females is critical in parasitoids released in the field. Therefore, this result is important for biocontrol programmes.

The decrease in the longevity of parasitoids whose cocoons are exposed to prolonged low temperature observed in this study has also been reported for other parasitoid species of the family Braconidae such as *Bracon brevicornis* Wesmael (Jayanth & Nagarkatti, 1985) and *A. pyralophagus* (Ballal *et al.*, 1989). It is noteworthy that the longevity of parasitoids is closely related to their fat reserves (Ellers, 1995). We hypothesized that the low temperatures may decrease the amount of fat reserves, reducing the energy available to the adults. The physiological abnormalities following cold storage observed in this study were similar to those in a previous report (Hance *et al.*, 2007). We observed that adults emerged and died or had wing deformities after cold storage periods exceeding 30 days. The proportion of individuals with wing deformations increased after cold storage for 40 days at 10°C, showing that the exposure of pupae to prolonged low temperatures can result in an increased proportion of adults with developmental problems. It has been reported that lipids are a major source of energy used by parasitoids in maintaining vital functions (Adedokun & Denlinger, 1985; van Handel, 1993). We hypothesized that prolonged cold storage may decrease the lipid reserves of *M. prodeniae*. Although the parasitoids were able to complete their life cycles under these conditions, they probably had insufficient resources to emerge. Thus, when evaluating a cold storage technique, in addition to emergence rate, it is important to consider the effects of cold storage on the dispersion capability of *M. prodeniae*.

We observed a decrease in the fecundity of females obtained from cocoons stored for 20 days at low temperatures in this study. This result is consistent with other cold storage studies of parasitoid species of the family Braconidae (Archer & Eikenbary, 1973; Ballal *et al.*, 1989), in which the fecundity of females declined as the duration of the cold storage period of cocoons increased. This may be related to damage to the reproductive system of *M. prodeniae* due to exposure to cold. Generally, prolonged exposure to cold causes irreversible damage to the adult reproductive system (Rinehart *et al.*, 2000; Levie *et al.*, 2005; Hance *et al.*, 2007; Lacoume *et al.*, 2007; Colinet & Hance, 2009; Renault, 2011). However, the fecundity of *M. prodeniae* Fl and F2 offspring after the parental generation had undergone 20 days of storage at 10°C was not significantly different from that of the control group. This result indicates that the F1 and F2 offspring did not suffer any injury from their parents' cold storage. One potential reason is that cold storage may result in changes only at the physiological level and not at the genetic level. Furthermore, the ovipositional period of *M. prodeniae* after 20 days of storage at 10°C decreased. Hun *et al.* (2005) found that prolonged low temperature storage reduced the ovipositional period of *Microplitis mediator*, which agrees with our results. The decrease in the daily fecundity of females obtained from cocoons stored for 20 days at low temperatures observed in this study is similar to the results of Chen *et al.* (2011). Similarly, the fecundity of *M. prodeniae* during the first 3 days after cold storage treatment for 20 days was higher than that of the control group. This result is similar to that of *Anaphes ovijentatus*, reported by Jackson (1986) and probably occurs because cold storage affects the temporal oviposition pattern of *M. prodeniae*.

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Some studies have indicated that the exposure to fluctuating low temperatures has less harmful effects on braconid parasitoids than does maintenance at a constant low temperature (Colinet *et al*., 2006; Ismail *et al*., 2010). Additional research is needed to investigate whether this phenomenon also occurs with *M. prodeniae*. Moreover, Chen *et al*. (2013) found that diapausing parasitoids were more cold-tolerant than non-diapausing parasitoids. Therefore, we also need to examine a storage technique for inducing diapause in *M. prodeniae*.

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