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Effects of cold storage on life-history traits of *Aphidius matricariae*

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Abstract

Aphidius matricariae Haliday (Hymenoptera: Braconidae) is a polyphagous solitary endoparasitoid, attacking more than 40 species of aphids. This parasitoid is an important commercial product of many companies that produce biological control agents. Storage at low temperature increases the shelf life of many biocontrol agents, allowing companies to provide a steady and sufficient supply of insects for biocontrol programs. In the current study, the effects of cold storage of 1-day-old host mummies with *A. matricariae* for various time periods (5, 10, 15, 20, and 30 days) at 5 °C on the parasitoid's key life-history traits were investigated. Parameters assessed after storage included adult emergence rate, offspring sex ratio, adult longevity, oviposition period, fecundity, and life-table parameters (R_0, r, λ, T, and DT). Our results showed that the mummies of *A. matricariae* could be stored at 5 °C for 5 days without loss of quality and for 10–15 days with minimal reduction in quality (e.g., some reduction in adult longevity and R_0). If parasitoids were stored for >15 days, quality was more strongly affected. In conclusion, *A. matricariae* pupae could be stored at 5 °C for up to 15 days without significant negative post-storage effects on fitness of the parasitoid. These results could be used to improve the planning of mass rearing and mass release of *A. matricariae* in augmentative biological control programs.

Introduction

Mass rearing of biological control agents is an important tool for biological control programs, particularly for species used in augmentative releases (van Lenteren et al., 2003; Rezaei et al., 2020). Storage at low temperatures is an important method for increasing the shelf life of parasitoids and predators to provide a steady and sufficient supply of insects for biocontrol programs. Cold storage also allows the synchronized field release of control agents when needed (e.g., when an outbreak occurs). The development and optimization of efficient cold storage procedures for biocontrol agents can reduce the cost of biocontrol programs by extending the production period (Colinet & Boivin, 2011; Rathee & Ram, 2018).

Cold storage of biological control agents can affect various life-history parameters that are used as quality indicators, and subsequently reduce the performance of the stored parasitoids. Generally, the performance of stored individuals decreases with increasing exposure time and decreasing temperature (Colinet & Boivin, 2011). The tolerance to low temperature varies between biocontrol agents (Rathee & Ram, 2018). Cold storage may have negative effects on important biological parameters of insects, including immature survival, offspring sex ratio, adult longevity, flight capacity, and fertility (Archer et al., 1973; Colinet & Boivin, 2011). Generally, cold storage is done at temperatures above freezing, but near the lower developmental threshold of most insects (about 10 °C). For each species, it is critical to select the most tolerant life stage for

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cold storage (Archer et al., 1973; Hofsvang & Hagvar, 1977; Lins et al., 2013; Ismail et al., 2014; Kidane et al., 2015; Benelli et al., 2018). The 'mummy stage' (when the parasitoid is inside the mummified host aphid) of Aphidiinae parasitoids is considered to be the most suitable stage for storage (Levie et al., 2005; Colinet & Hance, 2010; Frere et al., 2011; Ismail et al., 2014).

Aphidius matricariae Haliday (Hymenoptera: Braconidae) is a polyphagous solitary endoparasitoid, of more than 40 species of aphids, in particular the green peach aphid, *Myzus persicae* (Sulzer), and the cotton aphid, *Aphis gossypii* Glover (both Hemiptera: Aphididae) (Rakhshani et al., 2005; Zamani et al., 2007; Hance et al., 2017; Rezaei et al., 2019). This parasitoid is one of the most important products of many companies producing biocontrol agents (Heimpel & Lundgren, 2000; Tahriri et al., 2007). It has a cosmopolitan distribution and occurs widely in Europe; however, it probably originated in northern India or Pakistan (Starý et al., 2000). Improving the mass rearing of *A. matricariae* will lead to more successful aphid control (Wei et al., 2003; Boivin et al., 2012). Hence, one of the essential components of any pest management program, including the release of *A. matricariae*, is the development of an effective method to store the parasitoid without altering its life-history traits (Colinet & Boivin, 2011; Rathee & Ram, 2018). Effective storage capability can ensure the production of large numbers of *A. matricariae* for release at the right time in crops (Archer et al., 1973; Benelli et al., 2018).

Studies on cold storage of biocontrol agents started about 90 years ago, particularly on Aphidiinae parasitoids (Colinet et al., 2006; Colinet & Hance, 2010; Frere et al., 2011; Silva et al., 2013; Mahi et al., 2014). Storage at low temperatures has been optimized for several species of Aphidiinae parasitoids, including *Lysiphlebus testaceipes* (Cresson) (Archer et al., 1973), *Aphidius colemani* Viereck, *Ephedrus cerasicola* Starý (Hofsvang & Hagvar, 1977), *Aphidius ervi* (Haliday) (Ismail et al., 2010), *Praon volucre* (Haliday) (Lins et al., 2013), and *Diaeretiella rapae* (McIntosh) (Silva et al., 2013). Moreover, Al-Antary & Abdel-Wali (2015) stated that the storage of *A. matricariae* at 4.5 °C for 1 or 2 weeks was better than for 3 weeks in terms of adult emergence, survival, and longevity, and they also reported that cold storage of *A. matricariae* failed without an acclimation period at 8 °C. In addition, Scopes et al. (1973) indicated that the mummies with *A. matricariae* could be stored successfully at 7 °C for 30 days with a normal level of adult emergence. In another study, Colinet & Hance (2010) determined that *A. matricariae* and *A. ervi* were more chill tolerant than *P. volucre*, *E. cerasicola*, or *A. colemani*. Colinet & Boivin (2011) and Rathee & Ram (2018) reviewed the effect of cold storage on the performance of natural enemies.

Life tables can be used as a tool for assessing natural enemy quality (Zamani et al., 2007; Tahriri et al., 2010). The net reproduction rate (R_0), intrinsic rate of increase (r), finite rate of increase (λ), mean generation time (T), and doubling time (DT) are the most important parameters that have been used for evaluation of natural enemies (Carey, 1993, 2001). The optimization of cold storage conditions based on demographic parameters has been done for *Cosmocomoidea* (formerly *Gonatocerus*) *ashmeadi* (Girault) (Chen et al., 2008) and *A. ervi* (Ismail et al., 2014). Moreover, the use of emergence rate, R_0, and cumulative fecundity curves of first-generation parasitoids was suggested for the evaluation of cold storage on parasitoids in biocontrol programs (Ismail et al., 2014). However, previous studies have not dealt with the effects of cold storage on life-table parameters of *A. matricariae*.

To optimize mass production and mass release programs of *A. matricariae*, we analyzed the effect of cold storage at constant 5 °C for various time periods (5, 10, 15, 20, and 30 days) on life-history traits of *A. matricariae*. We chose 5 °C because this temperature is above the development threshold of 3.5 °C determined by Zamani et al. (2007). Additional reasons for choosing 5 °C were: (1) many studies have reported that the suitable temperature for storage of Aphidiinae parasitoids ranges from 2 to 7 °C (Hofsvang & Hagvar, 1977; Colinet & Hance, 2010; Lins et al., 2013; Mahi et al., 2017); (2) storage temperature must be low enough to slow development especially for Aphidiinae parasitoids, which are harvested at the mummy stage (Colinet & Hance, 2010); (3) some development of parasitoids inside mummies can continue at 5 °C (Zamani et al., 2007; Ismail et al., 2014); and (4) 5 °C is easily available in refrigerators for storage of parasitoid mummies before release at target sites. We constructed the age-specific life table to comprehensively assess the quality of *A. matricariae* adults after exposure to the low temperature.

Materials and methods

Aphid and parasitoid rearing

The original population of *Myzus persicae* ssp. *nicotianae* Blackman (Hemiptera: Aphididae) was collected from tobacco fields of Khomeini Shahr county, Isfahan province, Iran (32°40'N, 51°33'E, 1 591 m above sea level) in September 2017. This subspecies is adapted to feed on tobacco, *Nicotiana tabacum* L. (Solanacae) (Blackman & Eastop, 2006; van Emden & Harrington, 2007). *Aphidius matricariae* inside host mummies were collected from a cucumber greenhouse in Varamin county (35°18N, 51°44'E, 969 m a.s.l.) in Tehran province, Iran. Tobacco plants, *N. tabacum* var. White Burley, were planted in the

glasshouse of Tarbiat Modares University, Tehran (35°44′N, 51°09′E, 1 273 m a.s.l.), at 25 ± 5 °C, 65 ± 5% r.h., and L16:D8 photoperiod. The plants were grown in plastic pots (10 cm diameter, 9 cm high) without any fertilizers or pesticides. The growth stage code of tobacco plants was 1105 (Papenfus & Billenkamp, 2019). These plants were used to rear the host aphids, *M. persicae* ssp. *nicotianae*. The host plant-aphid system was maintained in a ventilated cage (50 × 50 × 50 cm) in a growth chamber at 25 ± 1 °C, 70 ± 5% r.h., and L16:D8 photoperiod. Infested tobacco plants (3–4) were placed in each cage. To rear parasitoids, plants infested with third or fourth instar nymphs of *M. persicae* ssp. *nicotianae*, the host stages preferred by the parasitoid (Rezaei et al., 2019), were exposed to ca. 20 mated female adult parasitoids (<48 h old) in a ventilated cage (50 × 50 × 50 cm) for 24 h under the same environmental conditions used to rear the host aphids (Zamani et al., 2007). Then the aphids were removed from the cage and maintained on the host plants until mummies appeared. Newly formed mummies were used for the experiments.

A cotton ball soaked with a 30% honey in water solution was provided in the parasitoid oviposition cage and was renewed every 2 days (Wäckers, 2003). To obtain enough *A. matricariae* adults for our experiments, parasitoids were reared as needed under the standard environmental conditions, as described above. Parasitoids and host aphids were reared for five and seven generations, respectively, under the above-mentioned conditions before starting experiments.

Cold storage experiment

To determine the effect of cold storage on parasitoid quality, we used immature parasitoids of 1 day old (post-host mummy formation) (Colinet & Hance, 2010; Lins et al., 2013) which were then stored at a constant 5 °C for 5, 10, 15, 20, or 30 days, at 70 ± 10% r.h. in the dark (Colinet et al., 2006; Colinet & Hance, 2010; Lins et al., 2013). As the control, 1-day-old mummies of the parasitoid were maintained at 25 ± 1 °C and 70 ± 5% r.h. in the dark. For testing, mummies were placed in 5-cm-diameter Petri dishes. The experiment was arranged in a completely randomized design (subjects were randomly assigned to treatments). After the desired period of cold storage, all observations on parasitoid life history were made while insects were held in a growth chamber at 25 ± 1 °C, 70 ± 5% r.h., and L16:D8 photoperiod.

Adult emergence and offspring sex ratio

Aphid mummies were monitored daily for adult parasitoid emergence. Then, adults were sexed and the sex ratio (females/total) was calculated (Lins et al., 2013). This experiment was replicated 5× and each replication consisted of 30 host mummies.

Estimation of life-table parameters

To determine the demographic parameters of *A. matricariae*, tobacco leaf discs with petioles were placed in Petri dishes (12 cm diameter), each disc with 80 third-instar *M. persicae* ssp. *nicotianae* (i.e., the preferred host stage for the parasitoid), and the group of aphids was then exposed to one pair of 1-day-old *A. matricariae*. The petiole of each leaf disc was inserted between layers of wet tissue paper in order to maintain the humidity and the freshness of the leaf disc. Daily (between 10:00 and 11:00 hours), parasitoids were transferred to a new Petri dish with a fresh group of 80 third-instar aphids until the female died. The experiment was replicated 15× and each pair of parasitoids was considered a replicate. The potentially parasitized aphids, following their exposure to parasitoids, were maintained under the same environmental conditions as described above to determine the total number of mummified aphids. For each group, the number of emerging adults and their sex ratio were recorded. The tobacco leaf discs were replaced every 3 days to provide plant tissue for feeding of the parasitized aphids. A cotton ball with a 30% honey solution in water was placed in each Petri dish to feed *A. matricariae* adults and was replaced every 2 days. To evaluate life-table parameters, we randomly chose 150 immature stages of *A. matricariae* that were inside 1-day-old host mummies per treatment for the cold storage experiment.

Data obtained from all individual parasitoids were used to construct female age-specific life tables. Using their observed survival and fertility, the demographic parameters of *A. matricariae* females stored for different time periods were calculated, including net reproduction rate (R_0), intrinsic rate of increase (r), finite rate of increase (λ), mean generation time (T), DT, and life expectancy (e_x). According to Ismail et al. (2014), in the calculation of the demographic parameters, storage time was subtracted from the total time spent inside the host mummy. All equations and the life table construction follow Birch (1948) and Carey (1993, 2001). The jackknife method was used to estimate the pseudo-values of the life-history parameters r, R_0, λ, T, and DT (Maia et al., 2014).

Statistical analysis

Data were tested for normality with the Kolmogorov-Smirnov test before they were subjected to ANOVA. An arcsine transformation was used to transform percentage values for analysis (i.e., adult emergence and sex ratio). If significant differences were observed, means were compared with Tukey's honestly significant difference test

($\alpha = 0.05$). All statistical analyses were carried out using IBM-SPSS v.22.0 software (IBM, Armonk, NY, USA).

Results

Adult emergence and sex ratio

Adult emergence of *A. matricariae* was affected by cold storage time ($F_{5,24} = 125.52$, $P<0.001$; Figure 1). The percentage of adult emergence decreased with increasing storage period. Compared to the control, storage of mummies for 5 days at 5 °C showed no effect on the emergence rate of adults, and the lowest emergence was observed for the 30-day storage treatment (mean ± SE = 20 ± 1.1%).

The sex ratios of emerged adults of *A. matricariae* after cold storage did not differ among storage periods ($F_{5,24} = 0.39$, $P = 0.85$). The sex ratio of the emerged parasitoids varied from 0.51 ± 0.006 (control) to 0.57 ± 0.072 (30-day storage).

Age specific survival rate and life expectancy

The age-specific survival rates (l_x) of adult *A. matricariae* were 0.91 for the control and 0.85, 0.74, 0.63, 0.47, and 0.20, for the five increasing levels of storage, respectively (Figure 2). Likewise, life expectancy of the newly emerged *A. matricariae* adults was 19.08 days for the control and 17.35, 15.07, 12.83, 11.10, and 8.26 after 5, 10, 15, 20, and 30 days of cold storage, respectively (Figure 2). The first emerged control female started laying eggs after 11 days, compared to 11, 10, 9, 9, and 8 days for females stored for 5, 10, 15, 20, and 30 days, respectively (Figure 3).

Adult longevity, oviposition period, and fecundity

Both male ($F_{5,84} = 30.15$, $P<0.001$) and female ($F_{5,84} = 27.04$, $P<0.001$) adult longevity were affected by the duration of cold storage (Figure 4A). The longest

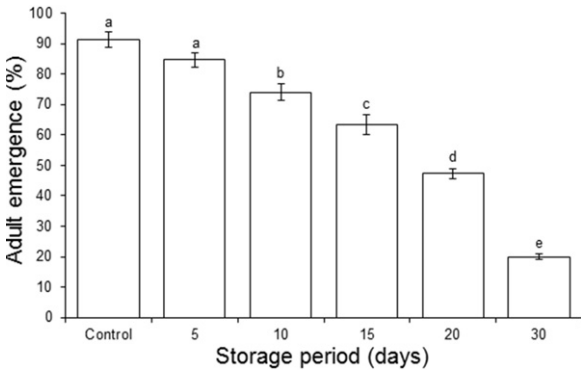

Figure 1 Mean (± SE; n = 5) adult emergence (%) of *Aphidius matricariae* for various storage periods (5–30 days) at 5 °C and the control treatment at 25 °C. Means capped with the same letter are not significantly different (Tukey's test: $P>0.05$).

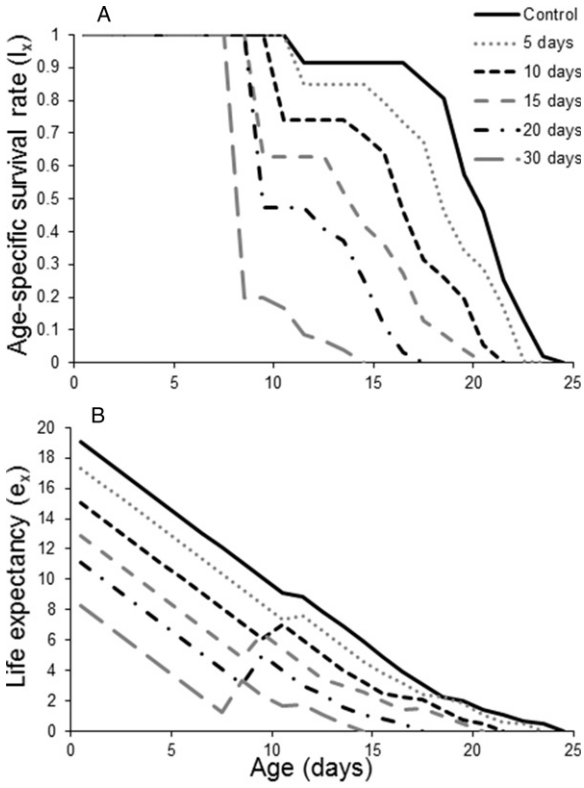

Figure 2 (A) Age-stage survival rate (l_x) and (B) life expectancy (e_x) of *Aphidius matricariae* stored for 5–30 days at 5 °C and the control treatment at 25 °C.

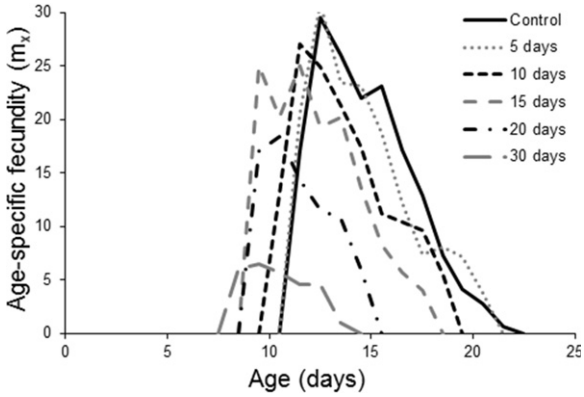

Figure 3 Age-specific fecundity (m_x) of *Aphidius matricariae* stored for 5–30 days at 5 °C and the control treatment at 25 °C.

(8.0 ± 0.38 days) female longevity was observed in the control, whereas the shortest longevity (2.5 ± 0.26 days) was in the 30-day cold storage treatments.

The pre-oviposition period of *A. matricariae* was not affected by cold exposure ($F_{5,56} = 0.23$, $P = 0.87$) (Figure 4B). However, the oviposition period itself differed

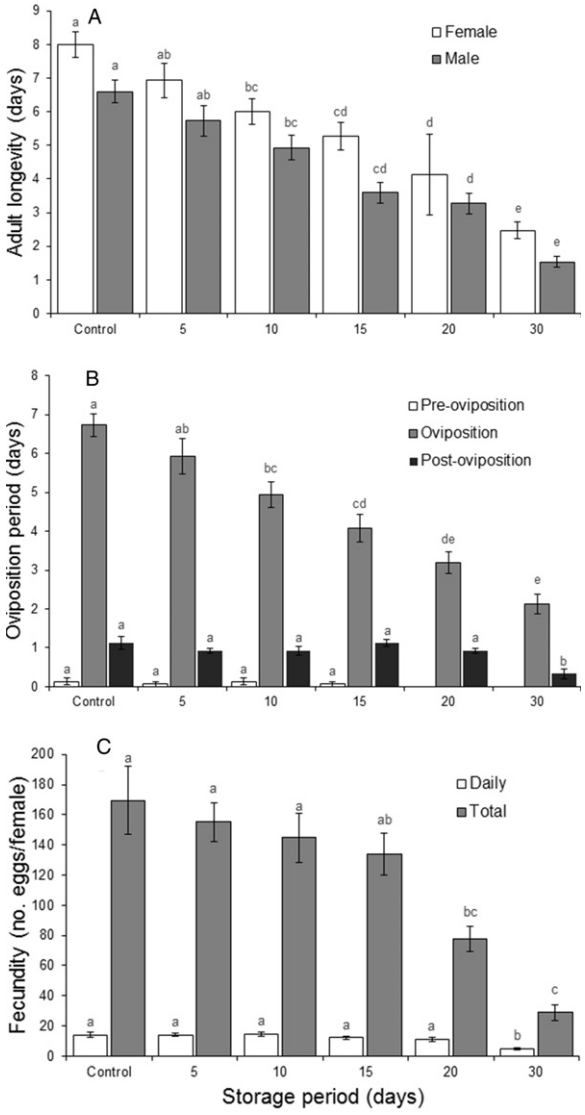

Figure 4 Mean (± SE; n = 15) (A) female and male adult longevity (days), (B) pre-, post-, and oviposition periods (days), and (C) daily and total fecundity (no. eggs per female) of *Aphidius matricariae* for 5–30 days at 5 °C and the control treatment at 25 °C. Means within a panel capped with the same letter are not significantly different (Tukey's test: P>0.05).

among cold storage treatments ($F_{5,84}$ = 25.76, P<0.001), with the longest (6.7 ± 0.30 days) oviposition period being in the control and the shortest (2.1 ± 0.26 days) in the 30-day cold storage. The post-oviposition period was also shorter in 30-day cold storage treatment compared to other treatments ($F_{5,84}$ = 7.00, P<0.001).

The total fecundity of *A. matricariae* was also affected by the duration of cold storage ($F_{5,84}$ = 14.18, P<0.001) (Figure 4C). The lifetime number of progeny per female was 169.4 ± 22.50 for the control treatment but only 28.8 ± 5.42 for the 30-day cold storage treatment. The daily rate of progeny production (number/female/day) was lower for the 30-day cold storage treatment (4.8 ± 0.90) than for the other treatments ($F_{5,84}$ = 7.11, P<0.001).

Population parameters

The R_0 values of *A. matricariae* differed among storage periods ($F_{5,90}$ = 8 149, P<0.001; Table 1). The mean values of R_0 ranged from 52.01 ± 0.38 female offspring/female for the control to only 1.51 ± 0.02 female offspring/female for the 30-day cold storage treatment. The intrinsic rate of increase (r) was also different ($F_{5,90}$ = 16 029, P<0.001), with the highest value (0.279 per day) in the 5-day storage treatment, and the lowest (0.039 per day) in the 30-day cold storage group. Similarly, the finite rate of increase (λ) was different between treatments ($F_{5,90}$ = 17 090, P<0.001). The mean values of λ ranged from 1.321 to 1.040 per day for the 5- and 30-day cold storage treatments, respectively. Also, the mean generation time (T) ($F_{5,90}$ = 7 228, P<0.001) and DT ($F_{5,90}$ = 336, P<0.001) were different between treatments. The control (14.81 ± 0.02 days) and 30-day storage (10.39 ± 0.02 days) treatments showed the highest and the lowest mean generation time, respectively. The longest DT was observed in 30-day storage treatment (18.02 ± 0.84 days).

Discussion

Mass rearing of biological control agents requires regular production followed by the ability to store the parasitoids produced, especially in the case of augmentative releases (van Lenteren et al., 2003; Wei et al., 2003; Rezaei et al., 2020). The present study showed that adult emergence, fecundity, adult longevity, oviposition period, and several population parameters of *A. matricariae* were all negatively affected by extended cold storage.

Adult emergence is a crucial parameter for the successful storage of any natural enemy (Colinet & Boivin, 2011). As reported in previous studies (Archer et al., 1973; Frere et al., 2011; Ismail et al., 2014; Mahi et al., 2014), increased duration of cold storage reduces adult emergence. For instance, Lins et al. (2013) reported that adult emergence of *P. volucre* in the control treatment was 93.1% whereas it was 63.5% after storage at 5 °C for 20 days. The emergence rate of *A. matricariae* significantly decreased when the storage period increased from 5 to 20 days at 2 °C (Colinet & Hance, 2010). A comparison of five Aphidiinae parasitoids showed that, in terms of adult emergence, *A. matricariae* and *A. ervi* are more chill tolerant than *A. colemani*, *E. cerasicola*, and *P. volucre* (Colinet & Hance, 2010).

Table 1 Mean (± SE; n = 15) demographic growth parameters in adults of *Aphidius matricariae* after storage of 1-day-old host mummies at 5 °C for various time periods

Time period (day)	R_0 (female offspring/female)	r (day^{-1})	λ (day^{-1})	T (days)	DT (days)
0 (Control)	52.01 ± 0.38a	0.267 ± 0.001c	1.306 ± 0.001c	14.81 ± 0.024a	2.60 ± 0.006b
5	50.41 ± 0.27b	0.279 ± 0.000a	1.321 ± 0.000a	14.07 ± 0.017b	2.49 ± 0.003b
10	37.36 ± 0.19c	0.271 ± 0.000b	1.312 ± 0.000b	13.33 ± 0.018c	2.55 ± 0.003b
15	28.33 ± 0.19d	0.270 ± 0.000bc	1.309 ± 0.001b	12.40 ± 0.019d	2.57 ± 0.004b
20	12.50 ± 0.11e	0.217 ± 0.001d	1.242 ± 0.001d	11.66 ± 0.014e	3.20 ± 0.011b
30	1.51 ± 0.02f	0.039 ± 0.001e	1.040 ± 0.001e	10.39 ± 0.021f	18.02 ± 0.837a

Means within a column followed by the same letters are not significantly different (Tukey's test: P>0.05).

However, no quality-control criteria for cold storage have been established for *A. matricariae*. Van Lenteren et al. (2003) reported that the acceptable adult emergence for *A. colemani* should be 45% or higher. Using this value, *A. matricariae* should not be stored for more than 20 days at 5 °C. Nevertheless, it is important to consider other quality-control parameters as well.

A female-biased sex ratio is also considered an important quality-control factor in augmentative biocontrol programs (Wei et al., 2003; Rezaei et al., 2020). Sex ratios may be distorted by cold storage and this distortion can arise from differential mortality between the sexes when immature stages are exposed to low temperatures (Rathee & Ram, 2018). In the present study, the sex ratio of *A. matricariae* was not significantly affected by exposure to low temperatures. This finding is consistent with Colinet & Hance (2010), Silva et al. (2013), and Mahi et al. (2014) who reported that both male and female Aphidiinae parasitoids show similar tolerance to low temperatures. Colinet et al. (2006) even reported better tolerance to low temperatures by females of some Aphidiinae parasitoids, including *A. colemani*, as did Hofsvang & Hagvar (1977) for *E. cerasicola*. According to the quality-control criteria reported by van Lenteren et al. (2003) for *A. colemani*, the sex ratio should be greater than or equal to 45% female, which was the case in our study for all cold storage treatments.

In the current study, the oviposition period and both male and female longevity changed with the duration of cold storage. At 5 °C, these parameters decreased with increasing storage time. The present results are in agreement with other studies conducted on *Exorista larvarum* (L.) (Benelli et al., 2018), *A. ervi* (Ismail et al., 2010), *Encarsia sophia* (Girault & Dodd) (Kidane et al., 2015), *P. volucre* (Lins et al., 2013), *Trichogramma carverae* Oatman & Pinto (Rundle et al., 2004), and *D. rapae* (Silva et al., 2013). One explanation for this effect may be the strong link between the amount of fat reserves and adult longevity (Silva et al., 2013; Kidane et al., 2015). The amount of fat reserves available for emerging adults of parasitoid declines linearly with the duration of cold exposure (Colinet et al., 2006; Ismail et al., 2010; Silva et al., 2013; Kidane et al., 2015). Furthermore, adult longevity can be influenced by the type and amount of food consumed in the adult stage (Heimpel et al., 1997; Munir et al., 2018).

Fecundity of *A. matricariae* ranged from 169.4 eggs per female (control) to 28.8 (30-day cold storage). When cold storage duration increased, total fecundity decreased. Cold storage may either cause retardation of egg maturation or malformation of reproductive organs in both males and females (Colinet & Boivin, 2011). In agreement with previous studies (Jalali & Singh, 1992; Silva et al., 2013; Ismail et al., 2014), our results demonstrated that the fecundity of *A. matricariae* decreased after cold storage. In addition, the nutritional status of parasitoids during their adult stage can affect fecundity (Heimpel et al., 1997; Dindo et al., 2019).

Demographic parameters of insects may depend on several factors, including rearing conditions (Carey, 1993; Haghani et al., 2006; Tahriri et al., 2010; Colinet & Boivin, 2011; Tazerouni et al., 2012). Few studies have assessed the demographic parameters of biocontrol agents after cold storage (Chen et al., 2008; Ismail et al., 2014). In the present study, all demographic parameters, including r, were significantly affected by increased cold storage. Typically, the decline in R_0 in storage treatments did not cause a proportional decrease in r values. This could be attributed to a decline in T with an increase in storage duration. In other words, the high value of r in the present study can be explained by higher R_0 and shorter T. This arises from the storage duration being subtracted from the total time spent in the mummified host stage. Ismail et al. (2014) compared the practical consequences of low-temperature effects on various demographic parameters and stated that, in order to evaluate the effects of the release of cold-stored parasitoids for biological control, it is better to use the emergence rate, R_0, and the cumulative fecundity curves of the first generation. One possible explanation for

this is that r is indicative of the population growth only if the population had reached a stable age structure (Carey, 1993; Ismail et al., 2014). However, R_0 for the 30-day storage was lower than that estimated for other treatments, which is probably due to reduced fertility and survival, as the reproductive system is particularly vulnerable to low-temperature effects.

As mentioned previously, it is important to consider the adult nutrition of *A. matricariae* (Godfray, 1994). Development continues at 5 °C, as T_0 for *A. matricariae* was estimated from linear regression equations to be 3.37 and 3.51 °C when the parasitoid was reared on *A. gossypii* and *M. persicae*, respectively (Zamani et al., 2007). The developmental rate of *A. matricariae* at low temperatures is not well known, and further studies are needed. Fluctuating thermal regimes may also have advantages, as documented in the biological characteristics of some parasitoids (Colinet et al., 2006; Ismail et al., 2014; Mahi et al., 2014; Rathee & Ram, 2018). Future studies on this for *A. matricariae* are therefore recommended.

An effective storage procedure is one that results in high survival and acceptable sex ratio and reproductive activity (Tauber et al., 1993; Chen et al., 2008; Ismail et al., 2014). The present study has shown that 1-day-old host mummies with *A. matricariae* immature stages can be stored at 5 °C for 5 days without loss of quality (except R_0) and for up to 15 days with some loss of quality (e.g., adult longevity and R_0). When the parasitoid is stored for longer than 15 days, quality was more strongly reduced. The results of this study can be used to improve the planning of mass rearing and mass release programs of *A. matricariae*.

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