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Abstract

Cold storage is a one means of preserving parasitoids prior to release in augmentation biological control programs. This study examined the feasibility of storing larval and pupal stages of a sexual population of *Lysiphlebus fabarum* Marshall (Braconidae: Aphidiinae) at 6.0 and 8.0 °C (± 1.0 °C), 50–60% RH, and 14L:10D photoperiod. These life stages were stored for periods of 1, 2 and 3 weeks under fluctuating thermal regimes (2.0 h daily at 21.0 ± 1.0 °C). Generally, pupae gave better results than larvae, 6.0 °C was better than 8.0 °C, and were better than constant, considering wasp survival, wasp size (tibial and antennal lengths), egg load and egg size. The best results were obtained with pupae stored for two weeks under a fluctuating temperature regime at 6.0 °C. Females emerging from this treatment did not differ from controls (developing directly at 21.0 °C) in body size, egg size, or progeny sex ratio and suffered less than 20% mortality. Egg loads were reduced in these wasps, but the reductions were substantially less than occurred in other two week storage treatments. Wasps stored in this manner successfully parasitized similar numbers of aphids as controls and produced similar progeny sex ratios. These results reveal a suitable set of low temperature conditions that can be used to delay the development of *L. fabarum* for two weeks with minimal impacts on wasp fitness.

Key words: body size, egg load, egg size, fitness, fluctuating temperatures

Introduction

Temperature is one of the most important physical factors affecting all aspects of insect life, from its direct effects on physiology and development, to more subtle effects on behavior and fitness (Ratte, 1985; Lee, 1991). Since low temperatures slow insect physiology, cold storage can be a valuable tool for the preservation and transport of ben-

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eficial insects prior to their release in biological control programs (Bourdais et al., 2012). However, given species-specific responses to low temperature conditions, cold-storage regimes require optimization for individual species to minimize any negative effects on parasitoid fitness (Leopold et al., 1998; Colinet & Hance, 2009).

The storage of parasitoids at low temperatures for biological control purposes has been studied for many years (Schread & Garman, 1934; Hanna, 1935). The many practical advantages include increased shelf-life (Leopold, 1998), convenience for transport to market (Colinet & Boivin, 2011), longer pre-release periods for consumers (Leopold, 1998), and better synchronization of desired developmental stages for release (Hofsvang & Hagvar, 1977; Chen et al., 2008). Nevertheless, cold storage can exact physiological costs that result in high rates of mortality (Levie et al., 2005), and negative effects on life history and behaviour (Colinet & Boivin, 2011). The traits potentially impacted include morphological symmetry (Bourdais et al., 2006), longevity (Amice et al., 2008), mobility (Tezze & Botto, 2004), fecundity, fertility and sex ratio (Levie et al., 2005), mating success (Amice et al., 2008; Colinet & Hance, 2009; Bourdais et al., 2012), offspring fitness (Chen et al., 2008), learning (van Baaren et al., 2005) and responsiveness to kairomones (Herard et al., 1988).

The temperature chosen for cold storage usually involves the determination of the lower threshold temperature for development and selecting a storage temperature slightly below it (Leopold, 2007; Ismail et al., 2013). Duration of storage is an important factor influencing insect survival under cold conditions because it is analogous to dosage (Kostal et al., 2004, 2006). Generally, increasing cold dosage causes progressively more refrigeration damage, eventually resulting in death (Bale, 1996; Kostal et al., 2006). Thus, duration of storage must be considered as a primary variable when calibrating conditions to minimize mortality and fitness reduction for any particular life stage (Amice et al., 2008).

Periodic transfer to a warmer temperature for short periods, i.e., a fluctuating temperature regime, has been shown to significantly increase survival of cold-stored insects relative to storage at a constant cold temperature (Renault et al., 2004), and especially for Aphidiine parasitoids (Ismail et al., 2010, 2013). For example, a fluctating

temperature regime significantly reduced mortality of *Aphidius colemani* Viereck compared to constant cold (Colinet et al., 2006, 2007). Short warm periods appear to reduce the accumulation of cold injury and provide opportunities for physiological repair mechanisms to operate, thus reducing mortality and fitness impacts. The rate of temperature change experienced by the insect can also affect its fitness and survival. Slower cooling provides greater opportunity for acclimation and activation of cold resistance mechanisms (Chown & Nicolson, 2004). For example, a transition period of intermediate temperature increased the survival of *Aphidius rhopalosiphi* (De Stefani-Peres) mummies compared to direct transfer to storage conditions (Levie et al., 2005). Parasitoid larvae and mummies are the aphidiine life stages most commonly stored (Archer et al., 1973; Hofsvang & Hagvar, 1977), and survival may also vary with age within a life stage (Colinet & Boivin, 2011).

Negative effects of cold storage on fitness may be observed immediately after storage or in the next generation (Colinet & Boivin, 2011). Various combinations of biological and behavorial parameters are typically quantified in parasitoids post-storage to assess these impacts, most commonly body size, which can have important effects on many components of parasitoid fitness. Larger individuals may live longer, have better mating success, higher fecundity, a larger proportion of female progeny, and even better dispersal ability (Godfray, 1994), any or all of which may correlate with their field performance (van Lenteren et al., 2003). For example, female body size has been shown to correlate with egg load and egg size in both sexual (AR, unpublished) and asexual (Ameri et al., 2013a, b) strains of *L. fabarum*.

Aphidiine wasps are solitary, koinobiont parasitoids of aphids that can be useful in their biological control (Stary, 1970). *Lysiphlebus fabarum* Marshall is a common aphidiine parasitoid in central Europe where it attacks more than 70 species of aphids in agricultural and horticultural crops (Stary, 1986; Volkl, 1992). Although both sexual (arrhenotokous) (Rakhshani et al., 2005) and uniparental (thelytokous) (Rasekh et al., 2010) populations of this species occur in Iran, the former appear to be more widely distributed. Currently, aphid control in Iran relies heavily on pesticides in both field and greenhouse settings, whereas any improved availability of biological control agents such as *L.*

fabarum would likely aid in reducing pesticide use. Cold storage is also an important part of successful mass rearing. In the present study, we compared the impact of different storage periods at two storage temperatures (6.0 or 8.0 °C) on *L. fabarum* survival, body size, sex ratio, egg load, egg size, and post-storage developmental period when the parasitoids were stored as either late instar larvae (within aphids) or pupae (within mummies). Furthermore, we compared the adult performance of wasps from the most suitable storage temperature with that of wasps developing directly. The objective was to identify storage conditions that would delay the emergence of wasps destined for distribution in augmentation programs with minimal impacts on their fitness.

Methods

Insect rearing

A stock colony of black bean aphid, *Aphis fabae* Scopoli, was established from material collected in bean fields in Khozestan province, Iran in spring, 2012, and mummies of the parasitoid *L. fabarum* were obtained from the same samples. The stock colony of *A. fabae* was maintained on potted broad bean, *Vicia fabae* L., grown in pots filled with fertilized sawdust. All insects and experiments were held in climate-controlled growth chambers at 21.0 ± 1.0 °C, 65–75% RH, and a 16L: 8D photoperiod.

For experiments, emerging parasitoids were held in mixed sex (1:1 SR) groups of 12 in ventilated plastic cylinders (20.0 x 8.0 cm) and supplied with diluted honey (30%) and water on cotton rolls, refreshed twice daily. All experimental wasps were obtained from a single generation. To obtain a large, synchronous cohort of aphids for parasitism, excised broad bean shoots were each infested with 100 adult aphids from the stock colony. The cut stem of the bean shoot was immersed in a small vial of fertilized water (N:P:K = 20:20:20) to maintain turgor and placed in a cylinder (as above) under the same physical conditions of the stock colony. The adult aphids were removed after 12 h and the nymphs left in situ to develop to the second nymphal instar (54 ± 6 h). Based on previous work (Ameri et al., 2013b), second instar nymphs are the most suitable stage for parasitism by *L. fabarum*. Emerging wasps were held in mixed sex groups (as above) to

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permit mating. In order to produce synchronous wasp cohorts, excised bean shoots bearing ca. 200 second instar aphids were each exposed to 30 two d-old mated females in a plastic cylinder (as above). After 10 hours, wasps were removed, the parasitized aphids left in situ to complete development, and the fertilizer solution refreshed as needed to sustain the shoot.

Insects for experiments were reared the same as the control treatment, with the exception that infested bean shoots were transferred to individual cold treatments once they reached the appropriate developmental stage (late instar larvae = 120 ± 6 h post-parasitism; newly-formed pupae in mummies = 144 ± 6 h post-parasitism).

Cold storage experiments

Parasitoids were transferred to cooling conditions on the bean shoots so as to prevent any damage associated with their removal from plant tissues. Because a stepwise reduction in temperature before cold storage improves survival (Singh & Srivastava, 1988), temperature transitions (growth chamber to cold storage and back) were performed stepwise with a two degree change every two hours until the target temperature was reached (6.0 or 8.0 °C). At each storage temperature, parasitoids were subjected to one of two further treatments, either a constant temperature regime, or a daily exposure to 21 ± 1 ºC for two hours (hereafter, the fluctuating temperature regime). Finally, the experiment was replicated with three different cold storage periods (1, 2 or 3 weeks) with a control (direct development at 21.0 ± 1 ºC). While in storage, all insects were held in a 10:14 L:D photoperiod to correspond to late-season conditions that would be consistent with cold weather.

After completion of each storage treatment, insects were transferred back to 21.0 ºC and observed twice daily until emergence of adult wasps, whereupon the survival rate and the sex ratio (percent females) were determined. The newly emerged wasps (6-10 h old) were then killed by a two-minute exposure to alcohol vapor. The hind tibia and antennae of each individual female were then photographed using a digital camera (Nikon Coolpix S10, Nikon Corporation, Tokyo, Japan) attached to a binocular microscope at

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100x magnification and measured with a precision of 0.01 mm. To test for the possibility of asymmetry, the number of antennomeres was counted on both antennae. Subsequently, the ovaries of each female were dissected on a glass slide in saline solution (7.5g NaCl l^{-1}) and photographed using the same equipment. The resolution of egg images was enhanced digitally (Adobe Photoshop CS5, Adobe Corporation, San Jose, CA, USA) so that eggs could be accurately counted. Then, one photograph was taken from a randomly selected group of 15-20 mature eggs at 240x magnification to estimate egg size. The 2D area of each egg image was determined to an accuracy of 1 μm^2 using ImageJ software (National institutes of Health, Bethesda, Maryland, USA) and egg size for each female tallied as the mean area of 15 images.

Parasitoid performance post-storage

Based on the results obtained in the previous experiment, the storage of pupae at 6.0 ºC for two weeks yielded wasps with body sizes and egg sizes not different from untreated controls, with a minimal reduction in egg load. Therefore, another cohort of wasps was produced under these conditions and their performance compared to that of control wasps that developed directly at 21.0 ºC. Following treatment, mummies were isolated in 1.5 ml plastic Eppendorf tubes and, following adult emergence, pairs were established in ventilated plastic cylinders (as above) with a 30% honey solution and water provided on cotton rolls. Each pair (8.0 ± 2.0 h old) in each treatment (n = 20 per treatment) was then introduced into an excised bean shoot bearing 35 second instar aphids in a ventilated plastic cylinder (8.0 cm x 15.0 cm). After 24 hours, wasps were removed and shoots bearing aphids were placed in small vials of fertilizer solution in a growth chamber at 21.0 ºC. The level of parasitism obtained in each replicate was tallied as the number of mummies formed.

Statistical analysis

Data from the first experiment were analyzed for each storage temperature using a factorial 2-way ANOVA with life stage (larva / pupa) and storage period (control, 1, 2, or 3 weeks) as independent fixed factors. Means were separated using the Bonferroni test (α

= 0.05) when three or more treatments were compared. Data for emergence rate and sex ratio in the cold storage experiment were analyzed using generalized linear models. Data for number of mummies formed, emergence rate, and sex ratio were also analyzed using generalized linear models. For these ratio-scaled data, a binomial error distribution with log link function was used (SPSS, 1988; Briffa and Hardy, 2013).

Results

Survival and sex ratio at 6.0 ⁰C

The logistic regression was significant overall for percent emergence ($G = 24.99$, $P = 0.001$), but not for sex ratio ($G = 10.82$, $P = 0.147$). The main effect of life stage was not significant, but that of storage period was, although the interaction term was not (Table 1). Survival of wasps did not vary between larvae and pupae, but larval survival was reduced after three weeks of storage, and that of pupae after one week (Table 2). Sex ratios of wasps were similar to controls across storage periods, whether stored as larvae or pupae, but pupal storage yielded a higher sex ratio than larval storage after three weeks.

Survival and sex ratio at 8.0 ⁰C

The logistic regression was significant overall for percent emergence ($G = 332.80$, $P < 0.001$), and sex ratio ($G = 23.73$, $P = 0.001$). The main effect of life stage and storage period, as well as the interaction between them, were all significant (Table 1). Survival of larvae was significantly reduced after two weeks of storage, whereas that of pupae was reduced after one week (Table 2). Larval survival was better than pupal survival after both one and three weeks of storage.

Sex ratios of wasps stored as larvae were lower than those stored as pupae after two weeks storage. Whereas there was no significant variation in sex ratio after pupal storage for different periods, sex ratios were more variable over storage periods for wasps stored as larvae, being highest after one week and lowest after two.

Post-storage development and body size at at 6.0 ºC

The 2-way ANOVA was significant overall for post-storage developmental time ($F_{7,423}$ = 128.50, $P < 0.001$), hind tibia length ($F_{7,250}$ = 38.44, $P < 0.001$), and antennal length ($F_{7,249}$ = 41.87, $P < 0.001$). The main effects of life stage, storage period, and the interaction term, were all significant (Table 3). Post-storage development was slower after larval storage than after pupal storage for all storage periods (Fig. 1). Although one week of larval storage increased developmental time relative to controls, longer periods decreased it. Pupal storage decreased developmental time relative to controls after all storage periods, and more so than larval storage for each storage period.

Cold storage of wasps as larvae resulted in smaller wasps relative to controls over all storage periods, but the size of wasps stored as pupae was not affected (Figs. 2 & 3). Wasps stored as larvae were smaller than those stored as pupae over all storage periods.

Post-storage development and body size at at 8.0 ºC

The 2-way ANOVA was significant overall for post-storage developmental time ($F_{7,431}$ = 92.62; $P < 0.001$), hind tibia length ($F_{7,273}$ = 61.48, $P < 0.001$), and antennal length ($F_{7,255}$ = 59.51, $P < 0.001$). The main effect of life stage, storage period, and the interaction between them were all significant (Table 3). Once again, larval storage for one week produced a longer developmental time than controls, whereas two weeks storage yielded values similar to controls, and three weeks, values significantly shorter (Fig. 1). Pupal storage resulted in faster development than controls only after three weeks. Post-storage development was slower for larval storage than for pupal storage over all storage periods.

Cold storage of wasps for all storage periods resulted in smaller females relative to controls regardless of life stage stored (Figs. 2 & 3). Two weeks of pupal storage produced wasps with shorter hind antennae than those stored as larvae, and three weeks produced wasps with shorter hind tibia.

Egg load and egg size at 6.0 ºC

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The 2-way ANOVA was significant overall for both egg load ($F_{7,248} = 61.54$, $P < 0.001$), and egg size ($F_{7,228} = 32.38$, $P < 0.001$). The main effects of life stage, storage period, and the interaction term between them were all significant (Table 4). Egg load was progressively diminished as a function of storage period, regardless of the life stage stored (Fig. 4). With the except of one week of storage, egg loads of wasps stored as larvae was reduced more than that of wasps stored as pupae.

For wasps stored as larvae, egg size was diminished relative to controls after one week of storage, but did not decrease further after longer periods of storage (Fig. 5). Egg size decreased relative to controls only after three weeks of pupal storage. Larval storage decreased egg size more than pupal storage after one and two weeks of storage, but the situation was reversed after three weeks.

Egg load and egg size at 8.0 ºC

The 2-way ANOVA was significant overall for both egg load ($F_{7,264} = 138.04$, $P < 0.001$) and egg size ($F_{7,225} = 29.56$, $P < 0.001$). The main effect of life stage, storage period, and the interaction term between them were all significant (Table 4). Once again, egg load was progessively diminished as a function of storage period regardless of the life stage stored, and always more so for larval storage than for pupal storage (Fig. 4).

For wasps stored as larvae, egg size was diminished relative to control, although there was no significant difference between storage treatments (Fig. 5). For wasps stored as pupae, egg size was progessively diminished as a function of storage period. Egg size of wasps stored as pupae, compared with larval storage, was significantly higher for all storage period (Fig. 5).

Parasitoid performance following cold storage

Wasps stored as pupae for two weeks at 6.0 °C successfully mummified an average of 23.1 ± 1.9 aphids per replicate in 24 h, not significantly different from the 24.1 ± 2.0 aphids mummified by controls ($G = 1.30$, $P = 0.254$). However, a lower percentage of mummies yielded adult wasps from the stored treatment compared to controls (79.8 ± 3.2 vs. 82.9 ± 6.4, respectively, $G = 13.39$, $P < 0.001$). The percentage female was not sig-

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nificantly different between treatments (63.7 ± 5.1 vs. 67.0 ± 5.2, respectively, $G = 0.97$, $P = 0.325$).

Discussion

Wasp mortality tended to increase with storage period for both life stages at both storage temperatures, and more so for pupal storage than for larval storage. This result is consistent with the analogy of storage period as 'cold dosage' and observations on many other species (Bale, 1996; Tezze & Botto, 2004; Kostal et al., 2006). However, in these experiments we employed a fluctuating temperature regime which has been used successfully to reduce cold storage mortality for other Aphidiine species including *Aphidius colemani* Viereck, *Aphidius matricariae* (Haliday), *Ephedrus cerasicola* Stary, *Proan volucre* Haliday, and *Aphidus ervi* (Haliday) (Colinet & Hance, 2010; Ismail et al., 2013) and other sexual populations of *L. fabarum* (Jarry & Tremblay, 1989).

There was a clear trend for post-storage developmental time to decrease with storage period at both storage temperatures, the exception being larvae stored for only one week. This reflects some progession of developmental under all the cold storage conditions employed, likely facilitated by the fluctuating temperature regimes.

Wasp sex ratios were not significantly reduced by cold storage relative to controls, except for larval storage at 8.0 °C, and they did not vary in any consistent manner across storage periods. Overall, mean differences in sex ratio were relatively small in magnitude and a female bias was retained in all cases (Table 2), indicating little or no concern for biocontrol applications. These results are consistent with the findings of Ismail et al. (2013) for *A. ervi* and Jarry and Tremblay (1989) for *L. fabarum*. A sex ratio shift toward males has been reported after cold storage in other studies, suggesting differential mortality of females (Ballal et al., 1989; Okine et al., 1996; Amice et al., 2008; Chen et al., 2008). However, other studies have reported that males were more susceptible than females (Jayanth & Nagarkatti, 1985; Jackson, 1986; Uckan & Gulel, 2001) so, the relative cold tolerance of each sex would seem to be a species-specific trait.

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Cold storage treatments tended to result in smaller wasps, the reductions increasing with storage period, as typically observed for Aphidiines (e.g., Ismail et al., 2013). Body mass often correlates with many other fitness components in insects (Honek, 1993) including parasitoid wasps (Cloutier et al., 1981; Roitberg et al., 2001; Ameri et al., 2013a). In general, tibia length appeared similar to antennal length in sensitivity to treatment at both storage temperatures. Ismail et al. (2013) reported that size of the male and female emerged from mummies under constant temperature 0 °C were significantly lower than control and fluctuating temperature regime. However, cold storage did not affect antennal asymmetry, as was observed for *Aphidius picipes* Nees (Amice et al., 2008) and *A. rhopalosiphi* (Bourdais et al., 2006).

Egg load declined progressively with increasing storage period for both larvae and pupae stored at both temperatures. Interestingly, egg load reductions over time occurred more rapidly in larvae than in pupae when stored at 6.0 °C, but more rapidly in pupae than in larvae when stored at 8.0 °C. Whereas egg size was reduced by larval storage at 6.0 °C for all storage periods, it was only diminished after three weeks of pupal storage. At 8.0 °C, a gradual reduction in egg size with storage period occurred for both life stages, the reductions being consistently greater for larvae than for pupae.

In parasitoids as in other insects, trade-offs exist between survival and reproduction (Jervis et al., 2003; Colinet et al., 2007) and fat reserves are the currency of stored resources (Ellers & van Alphen, 1997), especially in parasitoids that are unable to synthesize lipids (Visser & Ellers, 2008). The diversion of lipid reserves to survival processes may thus account for the progressive reduction of egg load with increasing storage period (Colinet et al., 2006). Egg size can be a proxy for offspring fitness (Smith & Fretwell, 1974; Bernardo, 1996; Giron & Casas, 2003), but is rarely measured in cold storage studies. The impacts of cold storage on egg loads and egg size observed in this study suggest that body size alone may not be a sufficient measure of storage impact on wasp fitness.

The results of the post-storage performance test with wasps that had been stored as pupae at 6.0 °C for two weeks revealed no significant difference in the numbers of aphids parasitized per female in 24 h, nor in the sex ratio of adult progeny. However,

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there was a slight reduction in the emergence rate of mummies that may reflect some transgenerational impact of cold storage on offspring viability. Cold storage can also impair male function, although thermal fluctuations may alleviate the impact (e.g., in *A. colemani*, Colinet & Hance, 2009), but the lack of any sex ratio departure suggests that no such impairment occurred in this treatment. Therefore, this particular regime is a highly suitable means of preserving developing wasps for a two week period. However, wasps emerged in less than 24 h following this treatment, so storage of either larvae or pupae for one week under a constant regime may be preferable if a longer post-storage developmental period is required, e.g., if material cannot be distributed to release sites under refrigeration. .

In summary, the storage of *L. fabarum* pupae at 6 ^{0}C under a fluctuating TR for a two week period reduced survival by less than 20%, had no measurable impact on body size, egg size or sex ratio, and only moderate impact on egg number. Furthermore, females emerging from this treatment mumified similar numbers of aphids as control wasps in 24 h and their progeny did not differ significantly in sex ratio, although mummy emergence was slightly reduced. These results can be used to calibrate cold storage conditions for *L. fabarum* that are mass-reared for use in augmentation biological control programs.

Acknowledgements

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Tables

Table 1. Logistic regression of effects of life stage (late instar larva / pupa) and storage period (0, 1, 2, or 3 weeks) on emergence rate and sex ratio of *Lysiphlebus fabarum* reared on *Aphis fabae* at 21 ºC and 16:8 L:D photoperiod. Photoperiod during storage was 10:14 L:D.

Source of variation	df	Emergence (%)		Sex ratio (% female)	
		G	P	G	P
		6.0 °C			
Life stage (LS)	1	0.65	0.420	0.82	0.366
Storage period (SP)	3	17.15	0.001	2.30	0.513
LS*SP	3	7.53	0.057	7.38	0.061
		8.0 °C			
Life stage (LS)	1	46.33	< 0.001	1.67	0.196
Storage period (SP)	3	232.03	< 0.001	16.47	0.001
LS*SP	3	78.32	< 0.001	6.63	0.085

Table 2. Mean (± SEM) emergence rate and sex ratio of *Lysiphlebus fabarum* reared in *Aphis fabae* at 21.0 ℃, L:D 16:8 photoperiod and then stored as either late instar larvae or pupae at either 6.0 or 8.0 ℃ for various periods. Values bearing the same upper case letters were not significantly different between life stages within a storage treatment (TEST ; α = 0.05); values bearing the same lower case letters were not significantly different between storage treatments within a life stage (TEST ; α = 0.05).

Dependent variable	Storage time (weeks)	6.0 °C		8.0 °C	
		Larvae	Pupae	Larvae	Pupae
Emergence (%)	0 (control	0.92 ± 0.02 Aa	0.92 ± 0.02 Aa	0.92 ± 0.02 Aa	0.92 ± 0.02 Aa
	1	0.86 ± 0.02 Aa	0.80 ± 0.02 Ab	0.89 ± 0.02 Aa	0.73 ± 0.02 Bb
	2	0.86 ± 0.02 Aa	0.79 ± 0.02 Ab	0.77 ± 0.02 Ab	0.82 ± 0.02 Ab
	3	0.81 ± 0.02 Ab	0.89 ± 0.02 Aab	0.80 ± 0.02 Ab	0.49 ± 0.02 Bc
Sex ratio (% female)	0 (control	70.1 ± 4.9 Aa	72.0 ± 5.6 Aa	70.1 ± 4.9 Ab	72.0 ± 5.6 Aa
	1	65.4 ± 4.3 Aa	62.0 ± 2.7 Aa	77.7 ± 4.1 Aa	76.2 ± 2.1 Aa
	2	68.7 ± 5.3 Aa	62.7 ± 3.3 Aa	62.2 ± 1.7 Bc	73.3 ± 1.7 Aa
	3	59.5 ± 3.4 Ba	75.6 ± 1.4 Aa	75.9 ± 1.9 Aab	77.1 ± 2.6 Aa

Table 3. Two-way ANOVA of effects of life stage (late instar larva / pupa) and storage period (0, 1, 2, or 3 weeks) on post-storage development and morphometric measurements of *Lysiphlebus fabarum* reared on *Aphis fabae* at 21 ºC and 16:8 L:D photoperiod. Photoperiod during storage was 10:14 L:D.

Source of variation	df	Post-storage development time (days)		Hind tibia length (mm)		Antennal length (mm)	
		F	P	F	P	F	P
				6.0 °C			
Life stage (LS)	1	191.37	< 0.001	120.24	< 0.001	118.12	< 0.001
Storage period (SP)	3	188.05	< 0.001	32.53	< 0.001	37.39	< 0.001
LS*SP		25.45	< 0.001	11.50	< 0.001	14.33	0.573
Df (error)	3	432		250		249	
				8.0 °C			
Life stage (LS)	1	91.45	< 0.001	10.41	0.001	33.87	< 0.001
Storage period (SP)	3	157.55	< 0.001	136.67	< 0.001	118.80	< 0.001
LS*SP		15.71	< 0.001	5.29	< 0.001	10.83	< 0.001
Df (error)	3	431		265		255	

Table 4. Two-way ANOVA of effects of life stage (late instar larva / pupa) and storage period (0, 1, 2, or 3 weeks) on egg load and egg size (2D image area) of *Lysiphlebus fabarum* females reared on *Aphis fabae* at 21 °C and 16:8 L:D photoperiod.

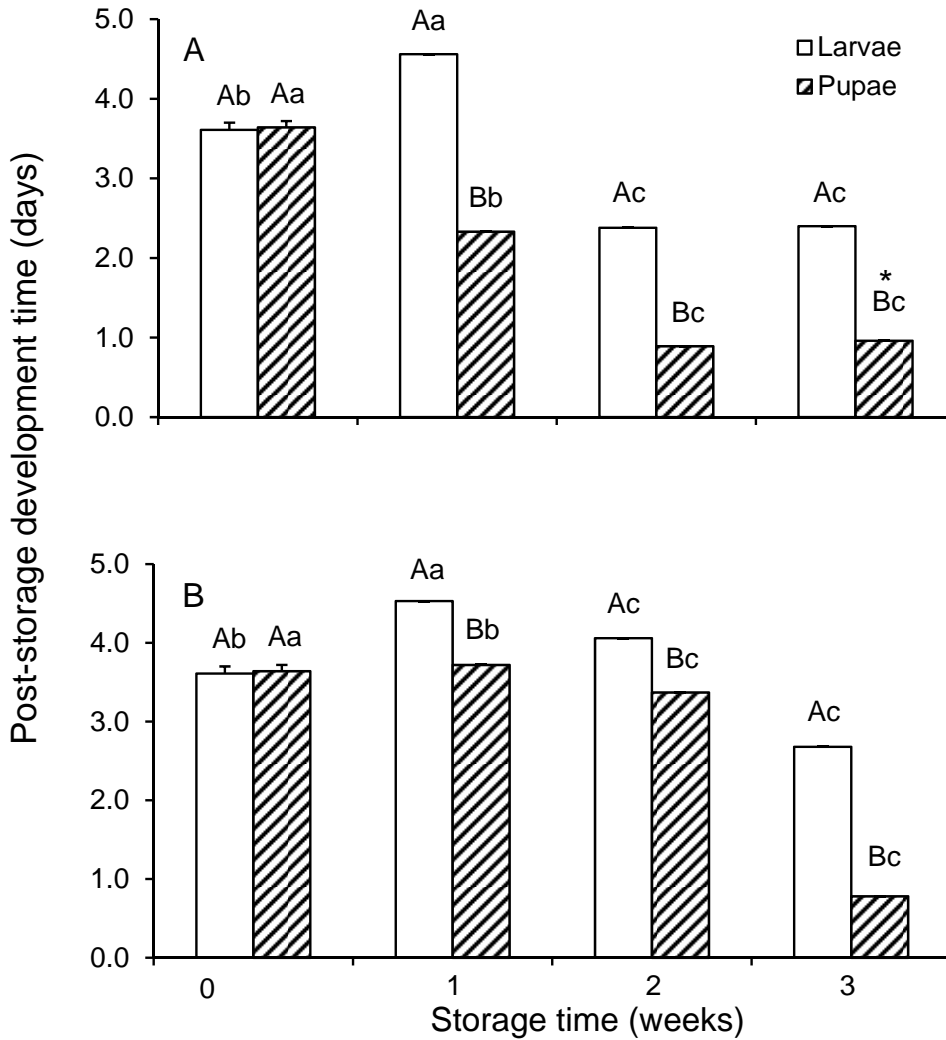| Source of variation	df	Egg load (no. mature eggs)		Egg area (μm^2)	
		F	P	F	P
		6.0 °C			
Life stage (LS)	1	30.22	< 0.001	0.68	0.410
Storage period (SP)	3	125.43	< 0.001	55.73	< 0.001
LS*SP	3	4.15	< 0.001	21.45	< 0.001
Df (error)		248		228	
		8.0 °C			
Life stage (LS)	1	70.88	< 0.001	43.95	< 0.001
Storage period (SP)	3	286.74	< 0.001	46.24	< 0.001
LS*SP	3	13.68	< 0.001	5.66	< 0.001
Df (error)		264		225	

Figures

Fig. 1. Mean (± SE) post-storage developmental time (days) of *Lysiphlebus fabarum* reared on *Aphis fabae* at 21.0 °C, 16:8 L:D photoperiod, and then stored as either late instar larvae (open columns) or pupae (hatched columns) for various periods at either 6.0 °C (A) or 8.0 °C (B). Values bearing the same upper case letters were not significantly different between life stages within a storage period (ANOVA, $\alpha = 0.05$); values bearing the same lower case letters were not significantly different among storage periods within a life stage (Bonferroni, $\alpha = 0.05$). *Some adult emergence occurred during the storage period.
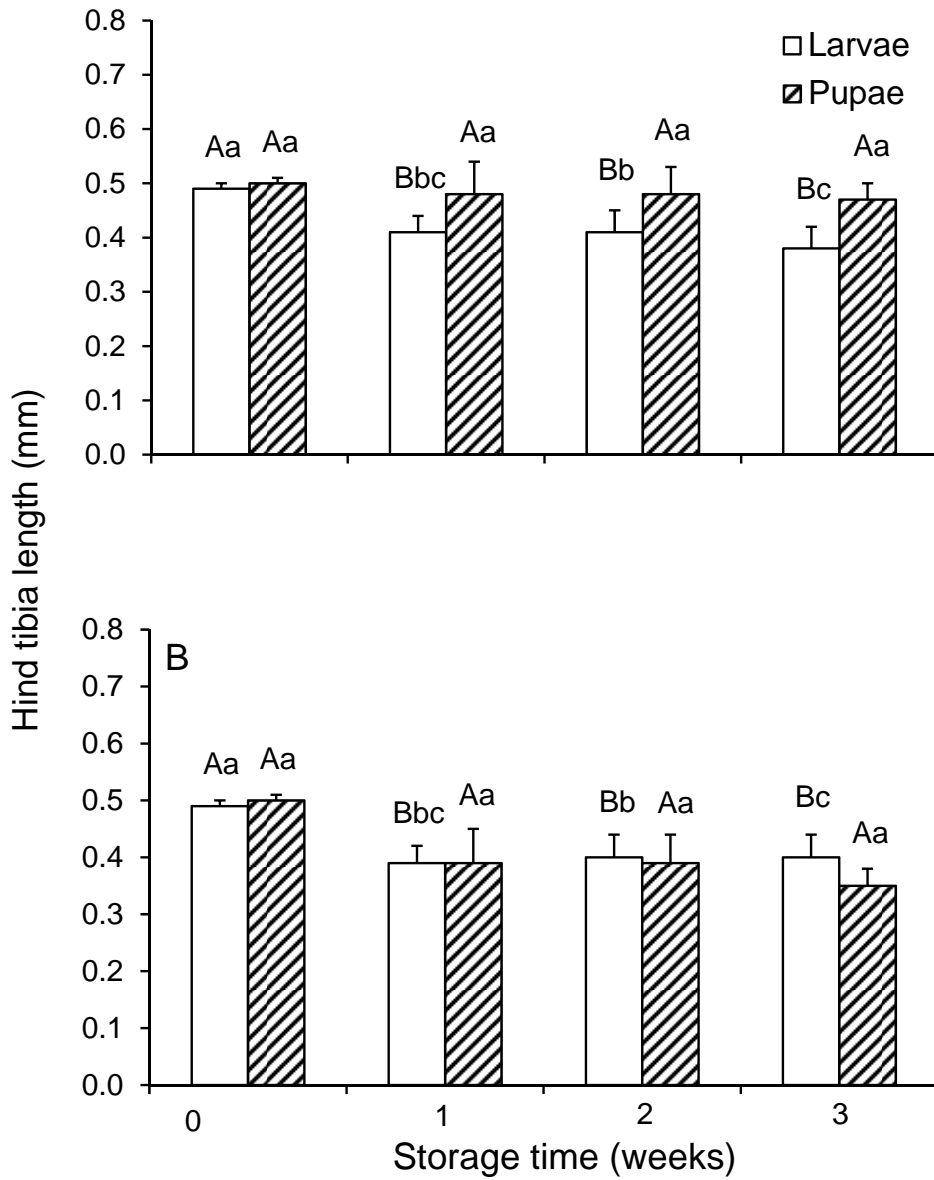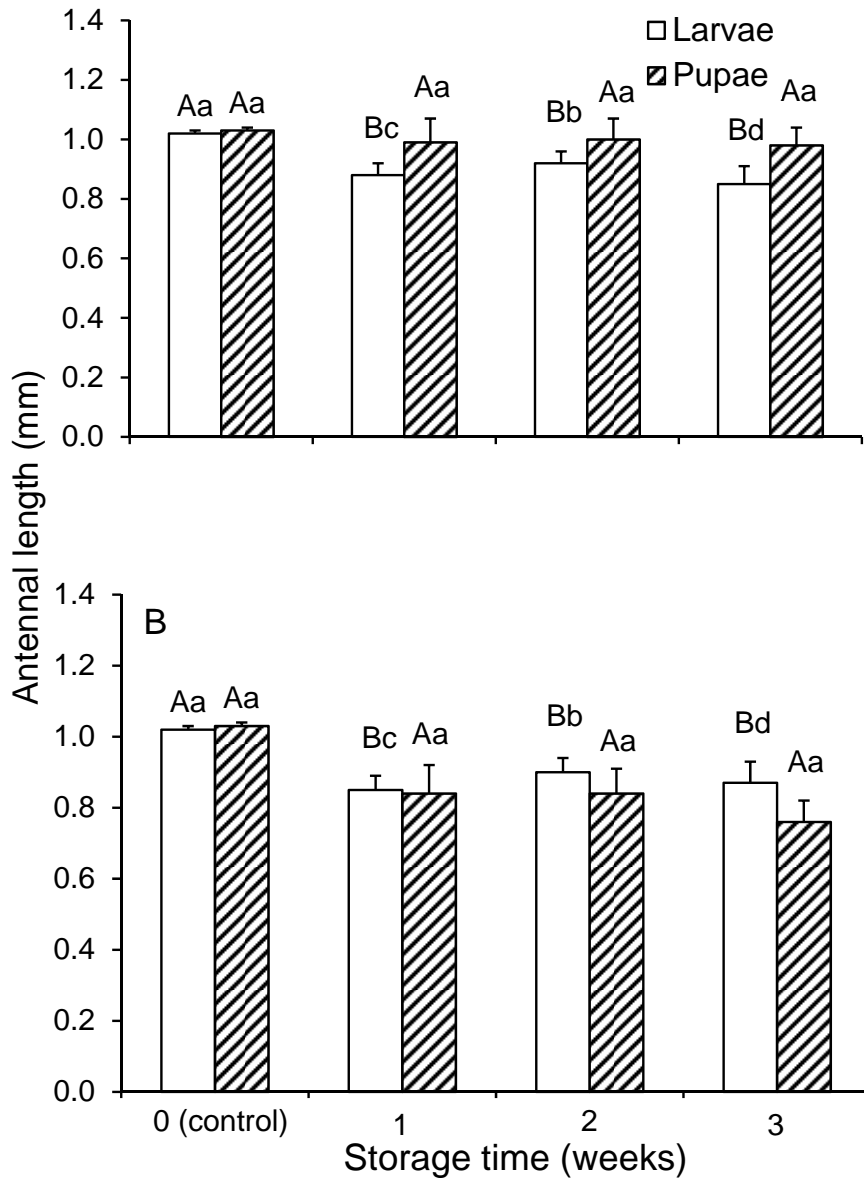
Fig. 2. Mean (± SE) hind tibia lengths (mm) of *Lysiphlebus fabarum* reared on *Aphis fabae* at 21.0 ℃, 16:8 L:D photoperiod and then stored as either late instar larvae (open columns) or pupae (hatched columns) for various periods at either 6.0 ℃ (A) or 8.0 ℃ (B). Values bearing the same upper case letters were not significantly different between life stages, within a storage period (ANOVA, α = 0.05); values bearing the same lower case letters were not significantly different among storage periods within a life stage (Bonferroni, α = 0.05).

Fig. 3. Mean (± SE) antennal lengths (mm) of *Lysiphlebus fabarum* reared on *Aphis fabae* at 21.0 ℃, 16:8 L:D photoperiod and then stored as either late instar larvae (open columns) or pupae (hatched columns) for various periods at either 6.0 ℃ (A) or 8.0 ℃ (B). Values bearing the same upper case letters were not significantly different between life stages within a storage period (ANOVA, α = 0.05); values bearing the same lower case letters were not significantly different among storage periods within a life stage (Bonferroni, α = 0.05).
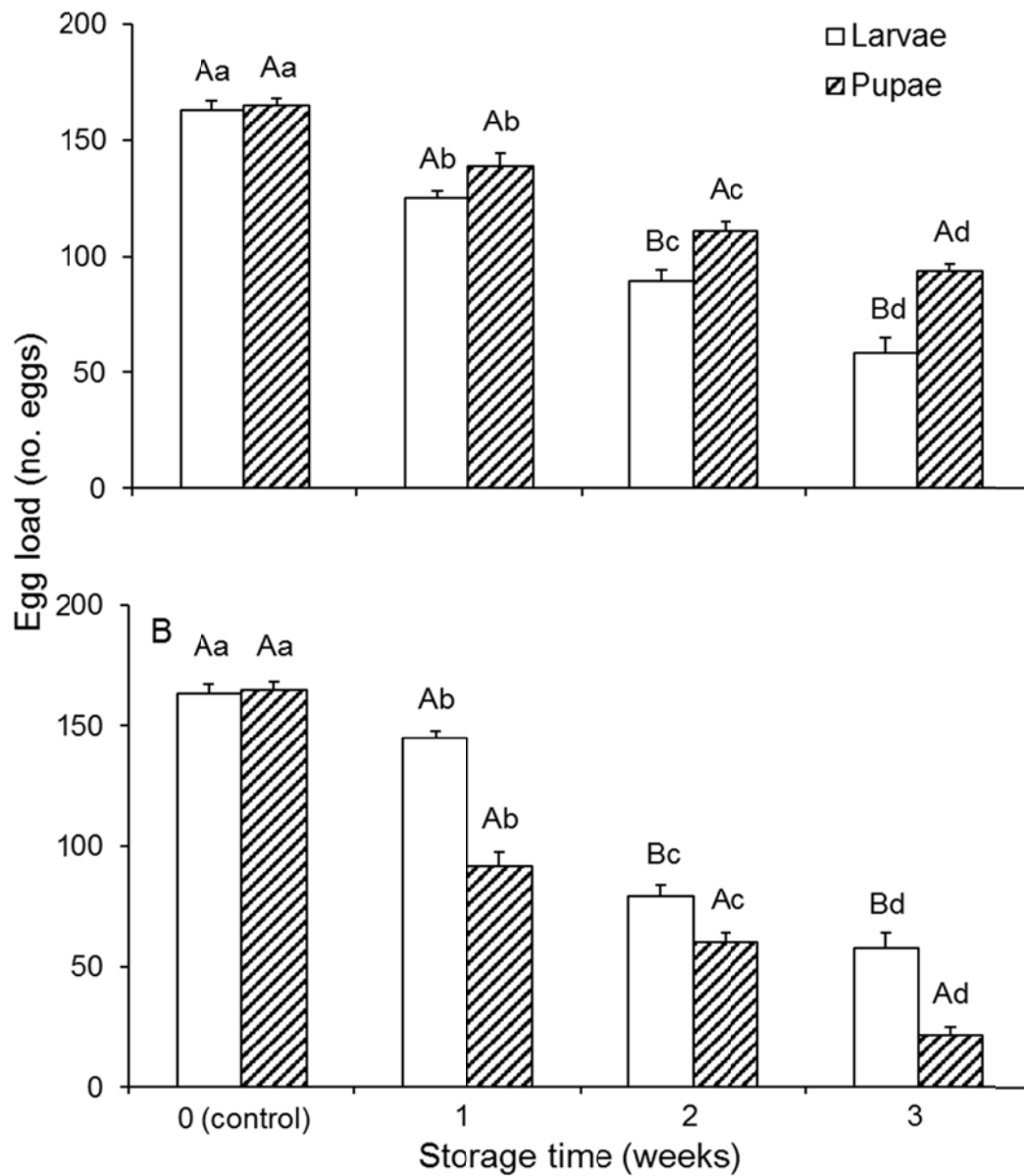
25

Fig. 4. Mean (± SE) egg load (no. mature eggs) in overies of *Lysiphlebus fabarum* females reared on *Aphis fabae* at 21.0 °C, 16:8 L:D photoperiod and then stored as either late instar larvae (open columns) or pupae (hatched columns) for various periods at either 6.0 °C (A) or 8.0 °C (B). Values bearing the same upper case letters were not significantly different between life stages within a storage period (ANOVA, α = 0.05). Values bearing the same lower case letters were not significantly different among storage periods within a life stage (Bonferroni, α = 0.05).
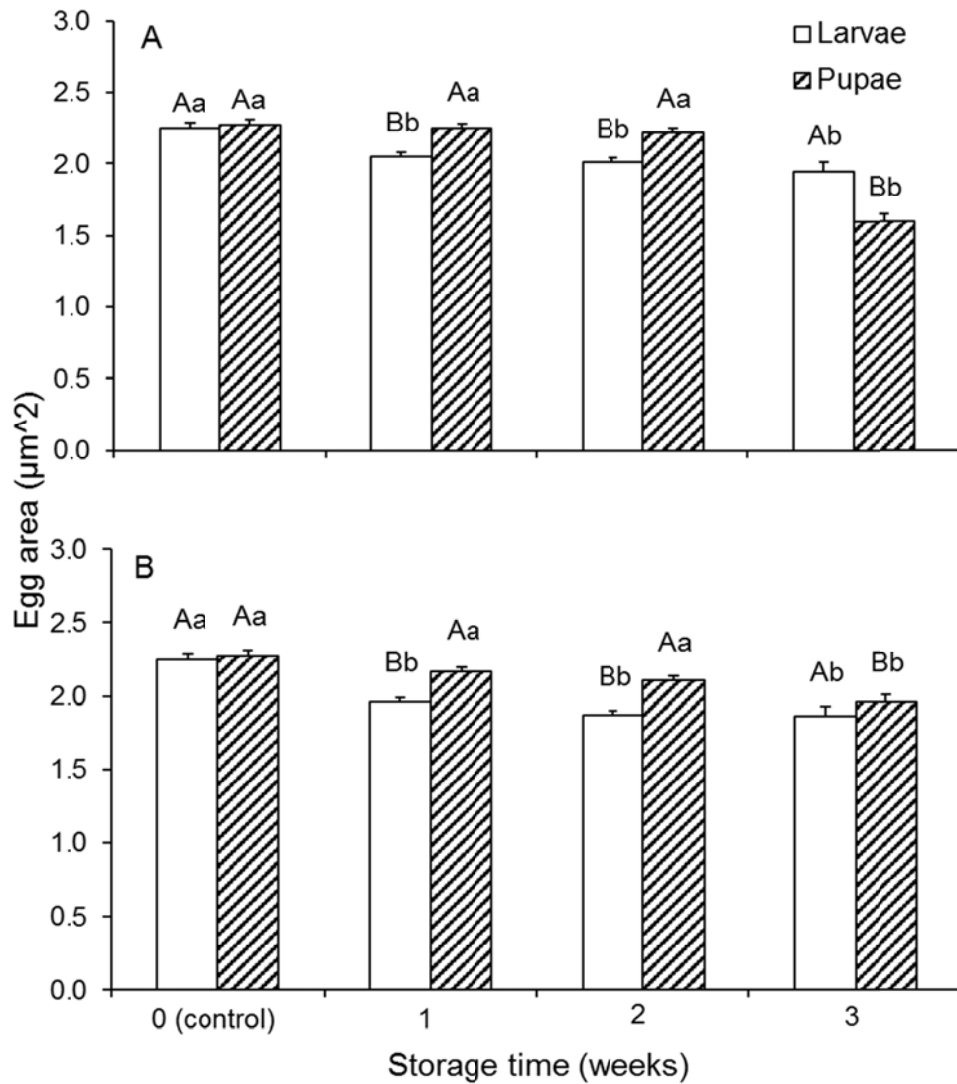
Fig. 5. Mean (± SE) egg size (2D area in µm^2) of *Lysiphlebus fabarum* females reared on *Aphis fabae* at 21.0 ℃, 16:8 L:D photoperiod and then stored as either late instar larvae (open columns) or pupae (hatched colums) for various periods at either 6.0 ℃ (A) or 8.0 ℃ (B). Values bearing the same upper case letters were not significantly different between life stages within a storage period (ANOVA, $\alpha = 0.05$); values bearing the same lower case letters were not significantly different among storage periods within a life stage (Bonferroni, $\alpha = 0.05$).