

## ORIGINAL CONTRIBUTION

# Influence of larval rearing temperature on the quality of cold-stored *Oomyzus sokolowskii* Kurdjumov (Hymenoptera: Eulophidae)

Y.-X. Sun, C. Tong &amp; T.-X. Liu

State Key Laboratory of Crop Stress Biology for Arid Areas, Key Laboratory of Northwest Loess Plateau Crop Pest Management of Ministry of Agriculture, Northwest A&amp;F University, Yangling, Shaanxi, China

**Keywords**

biological control, cold storage, development temperature, mass rearing, parasitoid

**Correspondence**

Tong-Xian Liu (corresponding author), State Key Laboratory of Crop Stress Biology for the Arid Areas, and Key Laboratory of Northwest Loess Plateau Crop Pest Management of Ministry of Agriculture of China, Northwest A&amp;F University, Yangling 712100, Shaanxi, China. E-mail: txliu@nwsuaf.edu.cn

Received: July 17, 2015; accepted: March 19, 2016.

doi: 10.1111/jen.12326

**Abstract**

*Oomyzus sokolowskii*, an important parasitoid of *Plutella xylostella*, has great potential for use in biological control. Storage at suboptimal temperature is valuable for increasing the shelf-life of insect parasitoids. In this study, *O. sokolowskii* larvae were reared at 30/25, 25/25 and 25/20°C light/dark (65 ± 5% RH, 16 : 8 h L : D) until pupation. The pupae were then cold-stored at 4 ± 1°C (60 ± 5% RH, full darkness). The pupae were removed out from the storage at 10, 20, 30 and 40 days after storage (DAS) and maintained at 25 ± 2°C until adults emerged or pupae died. Quality of the emerging adults and their F<sub>1</sub> offspring were assessed. Incidence of parasitism by *O. sokolowskii* was higher at 30/25°C than at 25/20°C. Cold storage of *O. sokolowskii* pupae greatly affected the fitness of the parasitoid: adult emergence rates were lower in the 40 DAS treatment than in other treatments; when *O. sokolowskii* larvae developed at 25/25°C, female proportions of the emerged adults were lower in the 40 DAS treatment than in the 0 and 10 DAS treatments. Larval rearing temperature mildly affected the adult emergence rate, post-storage developmental time and female proportion with a few exceptions. Number of parasitoids emerged per host pupa, and incidence of parasitism by the females were neither affected by larval rearing temperature nor cold storage duration. Trans-generational effects on F<sub>1</sub> offspring were evident in adult emergence rate, egg-adult developmental time and female proportion which were negatively affected by long duration of storage (40 days), but not by larval rearing temperature with a few exceptions. In conclusion, *O. sokolowskii* pupae could be stored at 4°C for up to 30 days without significant fitness loss.

**Introduction**

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is known throughout the world as a destructive pest of cruciferous crops (Grzywacz et al. 2010). At present, synthetic insecticides are frequently used to control it. This approach cannot only eliminate natural enemies, but can also result in insecticide resistance and management failure (Sarraz et al. 2006). Increasing efforts have thus been

made to develop ecologically based integrated pest management (IPM) programmes, including manipulation, conservation and augmentation of natural enemies.

More than 130 species of hymenopteran parasitoids have been known to attack *P. xylostella* (Sarraz et al. 2005). One of the most abundant species in many parts of the world is *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) (Waterhouse and Norris 1987; Liu et al. 2000; Mahmood et al. 2004). *Oomyzus*

*sokolowskii* is a gregarious endoparasitoid of *P. xylostella*, feeding on larvae and pupae, and has been introduced as a biological control agent in several countries (Yaseen 1978; Lima and Harten 1985; Ooi 1988; Talekar and Hu 1996).

The mass production of natural enemies is necessary for many biological control programmes (van Lenteren and Tommasini 2003). Rearing and releasing natural enemies in the field is undertaken for the biological control of pests, in helping to increase local populations of natural enemies or to establish a new natural enemy population before a key pest occurs (van Lenteren 2000). However, it is not easy to make commercial production cost-effective and, meanwhile, maintain a high quality of organisms (Greenberg et al. 1996; Hance et al. 2007). Long-term storage of biological control products may help to reduce costs by allowing customized production schedules to synchronize a desired life stage of the natural enemy with the occurrence of the target pest (Greenberg et al. 1996; Hance et al. 2007).

Storage of natural enemies, particularly parasitoids, at suboptimal temperatures can be valuable for increasing their shelf-life, but entails risks of compromised performance of both the stored parental generation and their offspring (Okine et al. 1996; Häckermann et al. 2008; Colinet and Boivin 2011). For example, storage of the pupae of *Telenomus busseola* (Gahan) (Hymenoptera: Scelionidae) in the eggs of *Sesamia nonagrioides* (Lefebvre) (Lepidoptera: Noctuidae) at 4°C (60 ± 5% RH) for 1 week had a significant adverse effect on adult emergence, and for 2 weeks, percentage parasitism of host eggs by emerged females was significantly reduced (Bayram et al. 2005). Meanwhile, the female proportion of F<sub>1</sub> progeny was significantly reduced after more than 1 week of storage (Bayram et al. 2005); while the emergence of *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae), a parasitoid of *P. xylostella*, declined steadily with increased duration of storage at 4°C (70 ± 10% RH, 24 : 0 h L : D), and no adults emerged at 49 days after storage (DAS) (Okine et al. 1996). Improving the storage techniques to minimize quality loss is therefore important in the mass production and commercialization of parasitoids and other beneficial insects.

Cold tolerance of parasitoids is influenced by a wide range of endogenous (biotic) and exogenous (abiotic) factors. The exogenous factors include rearing environmental conditions and the duration of cold storage (Hance et al. 2007; Colinet and Boivin 2011). Rearing temperature has a great impact on *O. sokolowskii* development (Talekar and Hu 1996; Wang et al.

1999; Ferreira et al. 2003); the duration of development was shortened as temperature increased from 18 to 28°C, with the optimum temperature range for development, survival and reproduction was 20–30°C (Ferreira et al. 2003). Parasitism rate was positively correlated with temperature from 10 to 35°C. *Oomyzus sokolowskii* thus demonstrated a great potential for population growth at relatively high temperatures (Talekar and Hu 1996; Wang et al. 1999).

In previous studies, *O. sokolowskii* development was measured at constant temperatures, but in practice, temperature during daytime is generally higher than that at night. Therefore, our present objectives were to determine the influence of larval rearing temperature on the quality of cold-stored *O. sokolowskii*. The parasitoid pupae were stored at 4 ± 1°C (60 ± 5% RH, full darkness) for 10, 20, 30 and 40 days, following larval rearing under three temperature regimes: 30/25°C, 25/25°C, and 25/20°C (light/dark) and a photoperiod of 16 : 8 h L : D until they pupated. Specifically, we assessed (i) incidence of parasitism by *O. sokolowskii* at the three temperatures; (ii) post-storage fitness of the parasitoids; and (iii) trans-generational effects of cold storage on F<sub>1</sub> offspring.

## Materials and Methods

### Host and parasitoid cultures

*Plutella xylostella* larvae and pupae parasitized by *O. sokolowskii* were collected from a canola field in Yangling, China (34°17'00"N, 108°03'42"E). The insects were reared on cabbage (var. 'Qingan 70') for about 20 generations in an insectary at the Key Laboratory of Applied Entomology, Northwest A&F University. The environmental conditions were set at 25 ± 2°C, 65 ± 5% RH and a photoperiod of 16 : 8 h L : D.

### Rearing temperature and parasitism

*Oomyzus sokolowskii* adults emerged within a 24-h period were collected from the laboratory colony and allowed to mate for 48 h at 25 ± 2°C, 65 ± 5% RH and a photoperiod of 16 : 8 h L : D. Forty females and 10 males (all 2–3 days old) were released into each screened container containing approximately 200 fourth-instar *P. xylostella* larvae. Six fresh cabbage leaves (approximately 150 cm<sup>2</sup>) were placed in plastic containers (9 cm in diameter × 12 cm in height, with a 100 mesh plastic screen lid) as food for the larvae. A cotton ball saturated with a 10% honey solution was placed in each cage as food for the parasitoids. The parasitoids were allowed to parasitize larvae for 48 h in

bioclimatic chambers set to 30/25, 25/25 and 25/20°C (light/dark), 65 ± 5% RH and a photoperiod of 16 : 8 h L : D, respectively. The *P. xylostella* larvae were supplied daily with fresh cabbage leaves and monitored until parasitoids pupated. Most non-parasitized *P. xylostella* emerged as adults by the time when the parasitoids were at pupal stage. The incidence of parasitism (number of host parasitized/number of test host) was assessed (parasitized host pupae could be easily distinguished from unparasitized pupae). Five replicates were conducted for each temperature regime.

### Cold storage of parasitoid pupae

Parasitoid pupae obtained from the above experiment were carefully collected with forceps and placed in 5-ml centrifuge tubes with small holes for ventilation ( $n = 20$  per tube). The tubes were marked and stored in the upper section of a refrigerator in complete darkness at 4 ± 1°C and 60 ± 5% RH for 10, 20, 30 or 40 days. In the untreated control, the pupae were directly held at 25 ± 2°C, 65 ± 5% RH and 16 : 8 h L : D. There were five replicates in each treatment.

Every 10 DAS, samples of parasitoid pupae were transferred from the refrigerator to a climatic chamber set to 25 ± 2°C, 65 ± 5% RH and 16 : 8 h L : D. The stored pupae in 5-ml centrifuge tubes were individually placed in 1.5-ml centrifuge tube to monitor adult emergence. Parasitoid emergence was checked daily until all had either emerged or died. The following parameters were measured: adult emergence rate (number of parasitized host pupae with wasp emergence/total number of parasitized host pupae × 100%), developmental time (number of days from the date of maintenance at 25 ± 2°C until adult emergence), number of parasitoids emerged per host pupa and female proportion (number of female wasps/total number of adults emerged per host pupa).

### Cold storage and F<sub>1</sub> offspring performance

To evaluate trans-generational effects of cold storage on F<sub>1</sub> progeny, newly emerged parasitoid adults from the 1.5-ml centrifuge tubes with same parental regime were collected in a small plastic container (3.0 cm in diameter × 6.0 cm in height) with a 100-mesh plastic screen lid, and allowed to mate for 48 h, a cotton ball saturated with a 10% honey solution was supplied as food for the parasitoids. Five fourth-instar *P. xylostella* larvae were placed in each of a series of Petri dishes (10.0 cm in diameter × 1.5 cm in height). A hole (5 cm in diameter) was cut in the middle of the lid and covered with plastic screen (100 mesh) for

ventilation. One mated female was randomly selected from each container and introduced into the Petri dish to oviposit for 48 h. A fresh cabbage leaf (approximately 25 cm<sup>2</sup>) and a cotton ball saturated with a 10% honey solution were supplied. The cabbage leaf was changed daily until all *P. xylostella* larvae had pupated, and parasitized larvae were counted to assess incidence of parasitism (number of *P. xylostella* larvae parasitized by each female in 48 h). Parasitized host pupae were then isolated in 1.5-ml centrifuge tubes, as described above, and monitored daily until adult emergence. The experiment was held in a climatic chamber set to 25 ± 2°C, 65 ± 5% RH and a photoperiod of 16 : 8 h L : D. A total of 15–18 females were tested in each treatment, and the same parameters were measured as for the parental generation.

### Data analysis

Incidence of parasitism by *O. sokolowskii* under three larval rearing temperature regimes was analysed by one-way analysis of variance (ANOVA). After storage, all biological parameters of *O. sokolowskii* and their F<sub>1</sub> offspring were compared among treatments by two-way analysis of variance (ANOVA) (larval rearing temperature and cold storage duration). As the data of adults emerged from each host pupa and developmental time of the F<sub>1</sub> offspring did not meet the assumptions of normality and homoscedasticity, these variables were analysed by a Kruskal–Wallis test using the combined levels of the two factors. Means were separated with Bonferroni test ( $\alpha < 0.05$ ). All data were analysed using the SAS program (SAS Institute 2010).

## Results

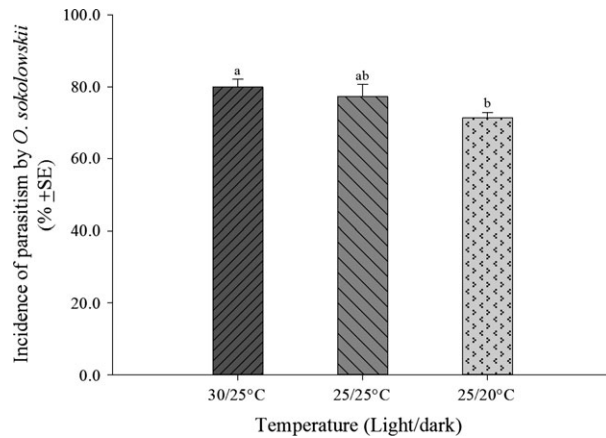
### Rearing temperature and parasitism

Incidence of parasitism by *O. sokolowskii* differed significantly among the three rearing temperatures ( $F_{2,14} = 21.39$ ,  $P = 0.0019$ ). The percentage of *P. xylostella* parasitized was lower at 25/20°C compared to 30/25°C, but was intermediate and not significantly differed from either when parasitism occurred at the 25/25°C regime (fig. 1).

### Effect of cold storage on the quality of *Oomyzus sokolowskii*

#### Adult emergence rate

*Oomyzus sokolowskii* adult emergence rate varied greatly among the treatments (larval rearing temperature:

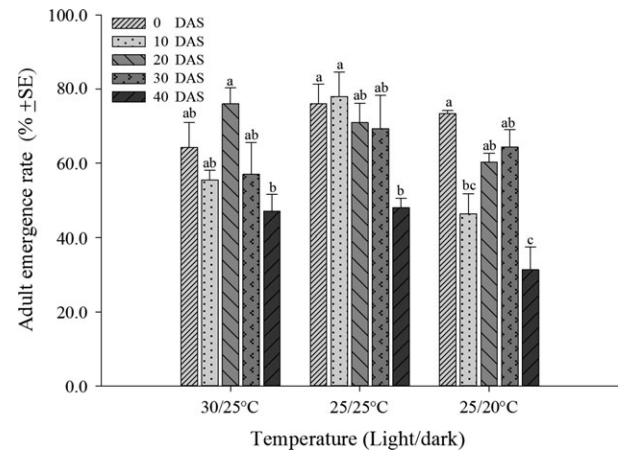


**Fig. 1** Parasitism incidence of *Plutella xylostella* by *Oomyzus sokolowskii* under 30/25, 25/25 and 25/20°C.

$F_{2,74} = 8.04$ ,  $P = 0.0014$ ; cold storage duration:  $F_{4,74} = 13.04$ ,  $P < 0.0001$ ; larval rearing temperature-by-cold storage interaction:  $F_{8,74} = 1.98$ ,  $P = 0.0798$ . When *O. sokolowskii* larvae developed at 30/25°C, adult emergence of *O. sokolowskii* differed among the cold storage treatments ( $F_{4,24} = 3.37$ ,  $P = 0.0456$ ), and was significantly greater in the 20 DAS treatment than that in the 40 DAS treatment, whereas no significant differences were detected in 0, 10 and 30 DAS treatments. When *O. sokolowskii* larvae developed at 25/25°C, survival of *O. sokolowskii* gradually decreased with the extension of storage duration ( $F_{4,24} = 3.82$ ,  $P = 0.0348$ ), and adult emergence rates were higher in the 0 and 10 DAS treatments than that in the 40 DAS treatment, whereas those in other treatments were not significantly different. When *O. sokolowskii* larvae developed at 25/20°C, the adult emergence rates significantly lower in the 40 DAS treatment than those in 0, 20 and 30 DAS treatments ( $F_{4,24} = 14.17$ ,  $P = 0.0004$ ). The larval rearing temperatures (30/25, 25/25 and 25/20°C) did not affect the adult emergence of *O. sokolowskii* ( $P > 0.05$ ) except for the 10 DAS treatment, and in which, higher adult emergence rate was obtained at 25/25°C compared to 30/25 and 25/20°C ( $F_{2,14} = 11.02$ ,  $P = 0.0069$ ) (fig. 2).

#### Developmental time

The post-storage developmental time of *O. sokolowskii* was significantly affected by the larval rearing temperature ( $F_{2,313} = 19.45$ ,  $P < 0.0001$ ), but not by cold storage duration ( $F_{4,313} = 0.51$ ,  $P = 0.7310$ ). There was a significant larval rearing temperature-by-cold storage interaction ( $F_{8,313} = 2.07$ ,  $P = 0.0393$ ). After storage, when *O. sokolowskii* larvae developed at 30/25 and 25/25°C, the developmental time until adult

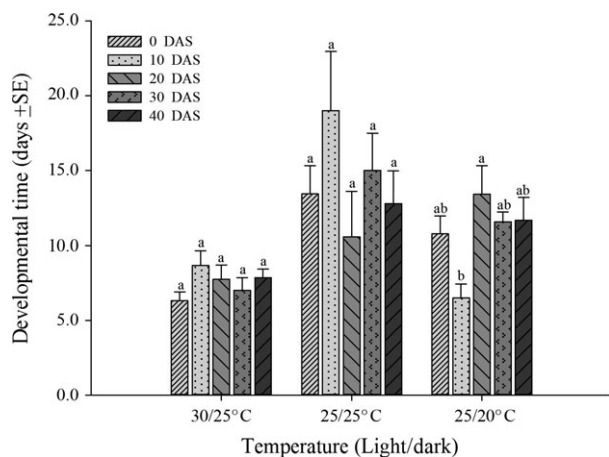


**Fig. 2** Adult emergence rate of *Oomyzus sokolowskii* when the pupae were stored at 4°C for different periods following larval rearing at 30/25, 25/25 and 25/20°C, respectively. Letters indicate differences within the bars of each temperature regime ( $P < 0.05$ , Bonferroni test).

emergence was not significantly different among the cold storage treatments, whereas for the 25/20°C regime, less time was found at 10 DAS than at 20 DAS ( $F_{4,99} = 3.66$ ,  $P = 0.0097$ ). Larval rearing temperature greatly affected the post-storage developmental time of *O. sokolowskii*. Generally, less time was needed to complete the development when *O. sokolowskii* larvae developed at 30/25°C than at other temperature treatments (0 DAS:  $F_{2,61} = 9.17$ ,  $P = 0.0004$ ; 10 DAS:  $F_{2,61} = 7.45$ ,  $P = 0.0013$ ; 30 DAS:  $F_{2,63} = 5.42$ ,  $P = 0.0077$ ) except for those at 20 DAS ( $F_{2,63} = 2.81$ ,  $P = 0.0711$ ) and 40 DAS ( $F_{2,61} = 2.85$ ,  $P = 0.0701$ ) (fig. 3).

#### Adults emerged per host pupa and female proportion

Overall numbers of parasitoids emerged from each host pupa were not differed among the treatments (Kruskal–Wallis statistic = 18.95,  $P = 0.1852$ ). An average of 7.9–11.1 adults emerged from each host pupa (table 1). However, female proportions of the emerged adults were greatly affected by the cold storage duration ( $F_{4,313} = 4.91$ ,  $P = 0.0008$ ) and larval rearing temperature ( $F_{2,313} = 6.73$ ,  $P = 0.0014$ ). A significant larval rearing temperature-by-cold storage interaction was also detected ( $F_{8,313} = 2.71$ ,  $P = 0.0072$ ). Among the storage duration treatments, significant differences only existed when *O. sokolowskii* larvae developed at 25/25°C, in which the female proportions were lower in 40 DAS treatment than in 0 and 10 DAS treatments ( $F_{4,105} = 5.82$ ,  $P = 0.0004$ ). Among the temperature treatments, at 20 or 40 DAS, the female proportions were significantly greater when *O. sokolowskii* larvae developed at



**Fig. 3** Post-storage developmental time of *Oomyzus sokolowskii* when the pupae were stored at 4°C for different periods following larval rearing at 30/25, 25/25 and 25/20°C, respectively. Letters indicate differences within the bars of each temperature regime ( $P < 0.05$ , Bonferroni test).

30/25°C than at 25/25°C (20 DAS:  $F_{2,63} = 5.64$ ,  $P = 0.0065$ ; 40 DAS:  $F_{2,59} = 5.23$ ,  $P = 0.0099$ ) (table 1).

#### Parasitism incidence

Number of *P. xylostella* larvae parasitized by the post-storage emerging *O. sokolowskii* females was influenced neither by cold storage duration ( $F_{4,241} = 1.59$ ,  $P = 0.1778$ ) nor by larval rearing temperature ( $F_{2,241} = 0.93$ ,  $P = 0.3951$ ). There was also no significant interaction among larval rearing temperature and cold storage duration ( $F_{8,241} = 0.73$ ,  $P = 0.6690$ ). Almost 1.1–2.3 of the *P. xylostella* larvae (total number of 5) were parasitized by each *O. sokolowskii* female in 48 h (table 1).

#### Effect of cold storage on progeny ( $F_1$ ) performance

##### Adult emergence rate

In the  $F_1$  generation, adult emergence rate varied significantly among the treatments of their parents (larval rearing temperature:  $F_{2,74} = 14.74$ ,  $P < 0.0001$ ; cold storage duration:  $F_{4,74} = 25.18$ ,  $P < 0.0001$ ). No larval rearing temperature-by-cold storage interaction was detected ( $F_{8,74} = 1.30$ ,  $P = 0.2578$ ). When *O. sokolowskii* larvae developed at 30/25 and 25/25°C, adult emergence rates of the  $F_1$  offspring were lower in the 40 DAS treatment than those in 0, 20 and 30 DAS treatments. Unexpectedly, at 10 DAS, adult emergence rates of the  $F_1$  offspring were even lower than those with longer storage duration (i.e. 0 and 20 DAS treatments for 30/25°C; 20 and 30 DAS treatments for 25/25°C) (30/25°C:  $F_{4,24} = 17.14$ ,  $P < 0.0001$ ; 25/25°C:  $F_{4,24} = 10.80$ ,  $P < 0.0001$ ). Larval rearing temperature also affected the adult emergence rate in the  $F_1$  generation. When *O. sokolowskii* larvae developed at 25/20°C, adult emergence rates of the  $F_1$  offspring were lower than those at 30/25°C (0, 10 and 20 DAS treatments) or 25/25°C (10, 20 and 40 DAS treatments) ( $F_{2,14} = 4.18$ – $6.24$ ,  $P = 0.0107$ – $0.0335$ ) (fig. 4).

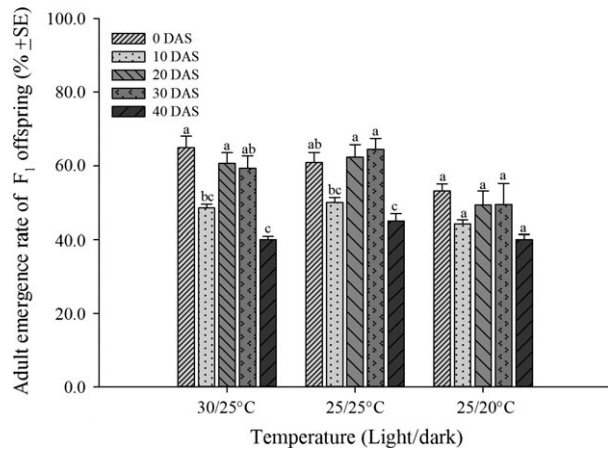
##### Developmental time

Developmental time from egg to adult of  $F_1$  offspring varied significantly according to their parental treatments (Kruskal–Wallis statistic = 43.95,  $P < 0.0001$ ). The developmental time of the  $F_1$  offspring was not affected by cold storage when *O. sokolowskii* larvae developed either at 30/25°C ( $F_{4,77} = 2.29$ ,  $P = 0.0678$ ) or 25/25°C ( $F_{4,58} = 0.76$ ,  $P = 0.5575$ ), but was different among the cold storage durations at 25/20°C ( $F_{4,58} = 2.69$ ,  $P = 0.0437$ ), in which the developmental time of  $F_1$  offspring was shorter in the

**Table 1** Mean ( $\pm$ SE) number of adults emerging from each *Plutella xylostella* pupa, female proportion and incidence of parasitism by the emerged female when *Oomyzus sokolowskii* pupae were stored at 4°C for different periods following larval rearing at 30/25, 25/25 and 25/20°C, respectively

	Larval rearing temperature (Light/Dark)	Cold storage duration (days)				
		0	10	20	30	40
Number of parasitoids emerged per host pupa	30/25°C	8.4 $\pm$ 0.6 a	8.6 $\pm$ 0.7 a	8.5 $\pm$ 0.6 a	7.9 $\pm$ 0.7 a	9.9 $\pm$ 0.8 a
	25/25°C	10.3 $\pm$ 0.9 a	9.6 $\pm$ 0.8 a	11.1 $\pm$ 1.5 a	8.6 $\pm$ 0.9 a	9.9 $\pm$ 0.8 a
	25/20°C	10.6 $\pm$ 0.8 a	11.0 $\pm$ 0.9 a	9.2 $\pm$ 0.9 a	10.1 $\pm$ 1.4 a	8.8 $\pm$ 1.3 a
Female proportion	30/25°C	0.81 $\pm$ 0.01 a	0.81 $\pm$ 0.02 a	0.85 $\pm$ 0.02 a	0.81 $\pm$ 0.02 a	0.80 $\pm$ 0.03 a
	25/25°C	0.82 $\pm$ 0.02 a	0.82 $\pm$ 0.02 a	0.74 $\pm$ 0.02 ab	0.74 $\pm$ 0.04 ab	0.59 $\pm$ 0.07 b
	25/20°C	0.83 $\pm$ 0.02 a	0.78 $\pm$ 0.03 a	0.78 $\pm$ 0.03 a	0.71 $\pm$ 0.06 a	0.81 $\pm$ 0.04 a
Incidence of parasitism	30/25°C	2.0 $\pm$ 0.2 a	1.6 $\pm$ 0.4 a	1.5 $\pm$ 0.2 a	1.7 $\pm$ 0.2 a	1.2 $\pm$ 0.3 a
	25/25°C	2.3 $\pm$ 0.4 a	2.0 $\pm$ 0.3 a	1.2 $\pm$ 0.4 a	2.0 $\pm$ 0.4 a	1.6 $\pm$ 0.2 a
	25/20°C	2.0 $\pm$ 0.4 a	1.5 $\pm$ 0.3 a	1.7 $\pm$ 0.3 a	1.1 $\pm$ 0.3 a	1.6 $\pm$ 0.3 a

For each evaluated parameter, means within rows followed by different letters are significantly different ( $P < 0.05$ , Bonferroni test).



**Fig. 4** Adult emergence rate of F<sub>1</sub> offspring of *Oomyzus sokolowskii* when the pupae of their parents have experienced different periods of cold storage at 4°C following larval rearing at 30/25, 25/25 and 25/20°C, respectively. Letters indicate differences within the bars of each temperature regime ( $P < 0.05$ , Bonferroni test).

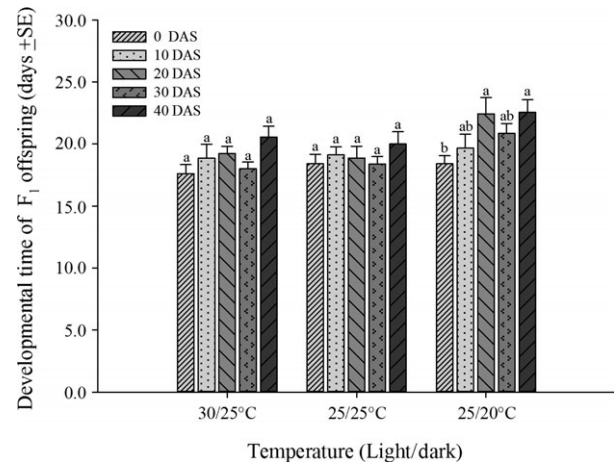
0 DAS treatment (18.4 days) than those in the 20 and 40 DAS treatments (22.4 and 22.5 days, respectively). In the 20 DAS treatment, larval rearing temperature greatly affected the growth rate of the F<sub>1</sub> offspring ( $F_{2,39} = 3.88$ ,  $P = 0.0299$ ). Compared to 30/25 and 25/25°C, when *O. sokolowskii* larvae developed at 25/20°C, more time was needed for the F<sub>1</sub> offspring to complete their development from egg to adult (fig. 5).

#### Adults emerged per host pupa and female proportion

Number of parasitoids emerged per host pupa was not affected by the parental experiences (larval rearing temperature:  $F_{2,195} = 1.02$ ,  $P = 0.3630$ ; cold storage duration:  $F_{4,195} = 0.67$ ,  $P = 0.6149$ ). There was no significant interaction between larval rearing temperature and cold storage ( $F_{8,195} = 1.28$ ,  $P = 0.2581$ ). An average of 8.2–11.8 of adults emerged from each host pupa (table 2). Female proportion of the F<sub>1</sub> offspring was not affected by their parents' larval rearing temperature ( $F_{2,195} = 0.15$ ,  $P = 0.8615$ ) and cold storage duration ( $F_{4,195} = 1.27$ ,  $P = 0.2823$ ). However, there was a significant interaction between larval rearing temperature and cold storage duration ( $F_{8,195} = 2.58$ ,  $P = 0.0109$ ). When *O. sokolowskii* larvae developed at 25/25°C, female proportion of the F<sub>1</sub> offspring was significantly lower in the 40 DAS treatment than those in 20 and 30 DAS treatments ( $F_{4,58} = 3.62$ ,  $P = 0.0109$ ) (table 2).

## Discussion

The favourable development temperatures for rearing *O. sokolowskii* range from 20 to 30°C (Talekar and Hu



**Fig. 5** Development time from egg to adult of F<sub>1</sub> offspring of *Oomyzus sokolowskii* when the pupae of their parents have experienced different periods of cold storage at 4°C following larval rearing at 30/25, 25/25 and 25/20°C, respectively. Letters indicate differences within the bars of each temperature regime ( $P < 0.05$ , Bonferroni test).

1996; Wang et al. 1999; Liu et al. 2000; Li et al. 2014). We found that of the three temperature regimes (30/25, 25/25 and 25/20°C), higher parasitism rates (77–80%) were obtained at 30/25°C, which were similar to that as previously reported (80.7% at 26°C) (Silva-Torres et al. 2010). Our results also showed that larval development temperature affected the post-storage fitness of *O. sokolowskii*, although these effects were not always significant. These findings indicate that cold tolerance of *O. sokolowskii* might be enhanced by regulating the development temperature before storage.

It has been reported that cold exposure greatly affect the survival of parasitoids, and the detrimental effects increase with prolonged low-temperature exposure (Rundle et al. 2004; Chen et al. 2008; Ghazy et al. 2012). In our study, the adult emergence rate of *O. sokolowskii* was notably decreased when the pupae were cold-stored for 40 days. Actually, cold tolerance varied among different species, even between closely related sibling species (Colinet and Boivin 2011). Under similar cold storage conditions ( $4 \pm 1^\circ\text{C}$  and  $75 \pm 5\%$  RH, and in full darkness) to ours, the emergence rate of another synovigenic species *Trichogramma nerudai* (Pintureau & Gerding) (Hymenoptera: Trichogrammatidae) was not greatly reduced in storage for almost 50 days (Botto et al. 2004; Tezze and Botto 2004).

The effects of cold storage to parasitoids could be caused by indirect injuries and exhaustion of energy reserves (Colinet et al. 2006, 2007). During larval development, energy is reserved in the parasitoid

**Table 2** Mean ( $\pm$ SE) number of *Oomyzus sokolowskii* adults emerging from each *Plutella xylostella* pupa and female proportion in the F<sub>1</sub> generation when the pupae of their parents have experienced different periods of cold storage at 4°C following larval rearing at 30/25, 25/25 and 25/20°C, respectively

	Larval rearing temperature (Light/Dark)	Cold storage duration (days)				
		0	10	20	30	40
Number of parasitoids emerged per host pupa	30/25°C	10.5 $\pm$ 0.7 a	11.4 $\pm$ 0.7 a	11.1 $\pm$ 0.7 a	10.2 $\pm$ 0.6 a	8.2 $\pm$ 0.7 a
	25/25°C	10.0 $\pm$ 1.1 a	11.8 $\pm$ 0.6 a	9.8 $\pm$ 1.2 a	11.1 $\pm$ 0.8 a	11.8 $\pm$ 1.2 a
	25/20°C	10.7 $\pm$ 1.0 a	10.2 $\pm$ 0.6 a	11.0 $\pm$ 0.9 a	8.9 $\pm$ 1.5 a	9.5 $\pm$ 0.8 a
Female proportion	30/25°C	0.83 $\pm$ 0.02 a	0.80 $\pm$ 0.04 a	0.82 $\pm$ 0.02 a	0.84 $\pm$ 0.02 a	0.77 $\pm$ 0.05 a
	25/25°C	0.80 $\pm$ 0.03 ab	0.85 $\pm$ 0.02 ab	0.87 $\pm$ 0.03 a	0.86 $\pm$ 0.02 a	0.76 $\pm$ 0.03 b
	25/20°C	0.80 $\pm$ 0.03 a	0.87 $\pm$ 0.02 a	0.85 $\pm$ 0.03 a	0.70 $\pm$ 0.10 a	0.84 $\pm$ 0.02 a

For each evaluated parameter, means within rows followed by different letters are significantly different ( $P < 0.05$ , Bonferroni test).

body and partly determined by the development temperature (Lessard and Boivin 2013). When *O. sokolowskii* larvae developed at higher temperature regimes (30/25 and 25/25°C), after cold storage, adult emergence rate was relatively higher compared to those at 25/20°C. Meanwhile, after storage, the time cost for developing to adult was generally shorter when *O. sokolowskii* larvae developed at 30/25°C than at 25/25 and 25/20°C. These findings indicate that higher rearing temperature regimes might be better for energy reserves of *O. sokolowskii*. But on the contrary, more mummies of *Aphidius colemani* (Viereck) (Hymenoptera: Aphidiidae) could survive after storage at 4°C when they were rearing at 15°C than at 25°C (Colinet et al. 2007). In addition, after being transferred from ambient environmental condition to low temperature, the normal metabolism of parasitoids could be changed, and some toxic wastes rapidly accumulated (Renault et al. 2002). Practically, before cold storage, many studies conducted some acclimation process by pre-exposure the parasitoids to sublethal low temperature (Levie et al. 2005; Pandey and Johnson 2005; Marwan and Tawfiq 2006; Luczynski et al. 2007). Thus, future works should pay more attention to similar approaches that enhance the cold tolerance of *O. sokolowskii*.

Chilling temperature also results in muscular dysfunction of the insect pupae that causes the difficulties in post-storage eclosion. When the flesh fly pupae, *Sarcophaga crassipalpis* (Macquart) (Diptera: Sarcophagidae), were exposed to low temperatures, the strength of muscle contraction was greatly reduced and the adults were unable to escape from the puparium (Žd'arek et al. 1986; Žd'arek and Denlinger 1992). In our study, when we dissected the dead parasitized host pupae after cold storage (data not shown), we found that many parasitoids were dead in the pharate stage. Mortality during pharate phase

caused by muscular perturbation has been also found in other parasitoid species (Okine et al. 1996; Colinet et al. 2006; Luczynski et al. 2007).

Apart from the detrimental effects caused by low temperature, survival of *O. sokolowskii* pre-storage phase might also influence the post-storage adult emergence rate. Actually, previous study has shown that survival of *O. sokolowskii* immature phase (egg–larvae–pupae) was influenced by development temperature, being higher (77.2%) at 28°C and no adults emerged at 15 and 33°C (Ferreira et al. 2003). When *O. sokolowskii* larvae developed at 25/25°C, the female proportion was greatly decreased at 40 DAS, but not for those at 30/25 and 25/20°C. This further highlights the potential influences of development temperature on parasitoids post-storage quality.

Exposure to low temperature would modify other life history traits of parasitoids, including decreasing longevity and fecundity and reducing female proportion (Hance et al. 2007). However, adults emerged per host pupa and incidence of parasitism by each *O. sokolowskii* female was not influenced by the cold storage. The progeny number is even greater than those at 20–30°C (8.5–9.4 adults per host) as reported (Wang et al. 1999; Uematsu and Yamashita 2000).

For many parasitoids, there is a trans-generational effect caused by temperature (Boivin 2010). Heat stress treatment could negatively affect adults of *Aphidius ervi* (Haliday) (Hymenoptera: Aphidiinae) with a negative effect on some quality traits of their F<sub>1</sub> progeny (Ismael et al. 2013). In our study, the larval rearing temperature of *O. sokolowskii* and the cold storage duration had little or no significant effect on the quality of the F<sub>1</sub> offspring, including incidence of parasitism and number of parasitoids emerged per host pupa. These results were similar to the results reported in the literature. For instance, for *Gonatocerus ashmeadi* (Girault) (Hymenoptera: Mymaridae), cold

storage duration did not have significant effect on their  $F_1$  offspring (Chen et al. 2008). However, the development time in our study varied from 17.6 days at 30/25°C in the 0 DAS treatment to 22.5 days at 25/20°C in the 40 DAS, which were slower than the results (17 days at 25.5°C) reported by Silva-Torres et al. (2009) and Li et al. (2014). The adult emergence rate was relatively higher when their parents were reared under higher temperatures (30/25 and 25/25°C) and shorter cold storage duration (<40 days).

In conclusion, *O. sokolowskii* pupae could be stored for up to 30 days at 4°C without significant quality loss and negative effect on the parasitoids and the  $F_1$  offspring. Thus, the results from this study are useful for mass rearing of *O. sokolowskii* for biological control of *P. xylostella*.

### Acknowledgements

This work was partially supported by the National Basic Research Program of Ministry of Science and Technology, China (973 Programs, 2013CB127604 and 2012CB114105), Special Fund for Agro-scientific Research in the Public Interest (201103022) and China Agriculture Research System (CARS-25-B-06). We greatly appreciate Prof. D. Gerling and N. Paz (Tel Aviv University, Israel) for linguistic editing and comments. We are grateful for the assistance of all staff and students in the Key Laboratory of Applied Entomology, Northwest A&F University at Yangling, Shaanxi, China.

### References

- Bayram A, Ozcan H, Kornosor S, 2005. Effect of cold storage on the performance of *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae), an egg parasitoid of *Sesamia nonagrioides* (Lefebvre) (Lepidoptera: Noctuidae). *Biol Control*, 35, 68–77.
- Boivin G, 2010. Phenotypic plasticity and fitness in egg parasitoids. *Neotrop Entomol*, 39, 457–463.
- Botto EN, Horny C, Klasmer P, Gerding M, 2004. Biological studies on two neotropical egg parasitoid species: *Trichogramma nerudai* and *Trichogramma* sp. (Hymenoptera: Trichogrammatidae). *Biocontrol Sci Technol*, 14, 449–457.
- Chen WL, Leopold RA, Harris MO, 2008. Cold storage effects on maternal and progeny quality of *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae). *Biol Control*, 46, 122–132.
- Colinet H, Boivin G, 2011. Insect parasitoids cold storage: a comprehensive review of factors of variability and consequences. *Biol Control*, 58, 83–95.
- Colinet H, Hance T, Vernon P, 2006. Water relations, fat reserves, survival, and longevity of a cold-exposed parasitic adult *Aphidius colemani* (Hymenoptera: Aphidiinae). *Environ Entomol*, 35, 228–236.
- Colinet H, Vernon P, Hance T, 2007. Does thermal-related plasticity in size and fat reserves influence supercooling abilities and cold-tolerance in *Aphidius colemani* (Hymenoptera: Aphidiinae) mummies? *J Therm Biol*, 32, 374–382.
- Ferreira SW, Barros R, Torres JB, 2003. Thermal requirements of *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) and estimation of the number of generations in crucifers in Pernambuco State, Brazil. *Neotrop Entomol*, 32, 407–411.
- Ghazy N, Suzuki T, Shah M, Amano H, Ohyama K, 2012. Effect of long-term cold storage of the predatory mite *Neoseiulus californicus* at high relative humidity on post-storage biological traits. *Biocontrol*, 57, 635–641.
- Greenberg SM, Nordlund D, King E, 1996. Mass production of *Trichogramma* spp.: experiences in the former Soviet Union, China, the United States and western Europe. *Biocontrol News Inform*, 17, 51N–60N.
- Grzywacz D, Rossbach A, Rauf A, Russell DA, Srinivasan R, Shelton AM, 2010. Current control methods for diamondback moth and other *Brassica* insect pests and the prospects for improved management with lepidopteran-resistant Bt vegetable *Brassicaceae* in Asia and Africa. *Crop Prot*, 29, 68–79.
- Häckermann J, Rott A, Tschudi-Rein K, Dorn S, 2008. Cold stored ectoparasitoid of *Cydia* fruit moths released under different temperature regimes. *Biocontrol*, 53, 857–867.
- Hance T, Van Baaren J, Vernon P, Boivin G, 2007. Impact of extreme temperatures on parasitoids in a climate change perspective. *Annu Rev Entomol*, 52, 107–126.
- Ismaeil I, Doury G, Desouhant E, Dubois F, Prevost G, Couty A, 2013. Trans-generational effects of mild heat stress on the life history traits of an aphid parasitoid. *PLoS One*, 8, e54306.
- van Lenteren JC, 2000. Success in biological control of arthropods by augmentation of natural enemies. In: *Biological control: measures of success*. Ed. by Gurr G, Wratten S, Springer, Netherlands, 77–103.
- van Lenteren JC, Tommasini M, 2003. Mass production, storage, shipment and release of natural enemies. In: *Quality control and production of biological control agents: theory and testing procedures*. Ed. by van Lenteren JC, CABI Publishing, Oxon, UK, 181–190.
- Lessard E, Boivin G, 2013. Effect of low temperature on emergence, fecundity, longevity and host-feeding by *Trichogramma brassicae*. *Biocontrol*, 58, 319–329.
- Levie A, Vernon P, Hance T, 2005. Consequences of acclimation on survival and reproductive capacities of cold-stored mummies of *Aphidius rhopalosiphii* (Hymenoptera: Aphidiinae). *J Econ Entomol*, 98, 704–708.



- Li XW, Niu YQ, Liu TX, 2014. The performance of *Oomyzus sokolowskii* (Hymenoptera: Eulophidae) parasitizing *Plutella xylostella* (Lepidoptera: Plutellidae) on different host plants. *Appl Entomol Zool*, 49, 67–75.
- Lima M, Harten AV, 1985. Biological control of crop pests in Cape Verde. Current situation and future programmes. *Revista Investigacao Agraria, Centro de Estudos Agrarios, A*, 1, 3–12.
- Liu SS, Wang XG, Guo SJ, He JH, Shi ZH, 2000. Seasonal abundance of the parasitoid complex associated with the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) in Hangzhou, China. *Bull Entomol Res*, 90, 221–231.
- Luczynski A, Nyrop JP, Shi A, 2007. Influence of cold storage on pupal development and mortality during storage and on post-storage performance of *Encarsia formosa* and *Eretmocerus eremicus* (Hymenoptera: Aphelinidae). *Biol Control*, 40, 107–117.
- Mahmood AR, Liu SS, Shi ZH, Song XH, Zalucki MP, 2004. Lack of intraspecific biological variation between two geographical populations of *Oomyzus sokolowskii* (Hymenoptera: Eulophidae), a gregarious larval-pupal parasitoid of *Plutella xylostella* (Lepidoptera: Plutellidae). *Bull Entomol Res*, 94, 169–177.
- Marwan IA, Tawfiq MM, 2006. Response of *Aphidius matriariae* Haliday (Hymenoptera: Aphidiidae) from mummified *Myzus persicae* Sulzer (Homoptera: Aphididae) to short term cold storage. *Int J Pest Control*, 48, 262–265.
- Okine J, Mitchell E, Hu G, 1996. Low temperature effect on viability of *Diadegma insulare* (Hymenoptera: Ichneumonidae) pupae and effect of this parasitoid on feeding rate of diamondback moth larvae (Lepidoptera: Plutellidae). *Fla Entomol*, 79, 503–509.
- Ooi PA, 1988. Laboratory studies of *Tetrastichus sokolowskii*. *Entomophaga*, 33, 145–152.
- Pandey RR, Johnson MW, 2005. Effects of cool storage on *Anagyrus ananatis* Gahan (Hymenoptera: Encyrtidae). *Biol Control*, 35, 9–16.
- Renault D, Salin C, Vannier G, Vernon P, 2002. Survival at low temperatures in insects: what is the ecological significance of the supercooling point? *Cryo Letters*, 23, 217–228.
- Rundle BJ, Thomson LJ, Hoffmann AA, 2004. Effects of cold storage on field and laboratory performance of *Trichogramma carverae* (Hymenoptera: Trichogrammatidae) and the response of three *Trichogramma* spp. (*T. carverae*, *T. nr. brassicae*, and *T. funiculatum*) to cold. *J Econ Entomol*, 97, 213–221.
- Sarfraz M, Keddie AB, Dossall LM, 2005. Biological control of the diamondback moth, *Plutella xylostella*: a review. *Biocontrol Sci Technol*, 15, 763–789.
- Sarfraz M, Dossall LM, Keddie AB, 2006. Diamondback moth-host plant interactions: implications for pest management. *Crop Prot*, 25, 625–639.
- SAS Institute, 2010. The SAS system for windows, release 10.1. SAS Institute, Cary, NC.
- Silva-Torres CSA, Ramos Filho IT, Torres JB, Barros R, 2009. Superparasitism and host size effects in *Oomyzus sokolowskii*, a parasitoid of diamondback moth. *Entomol Exp Appl*, 133, 65–73.
- Silva-Torres CSA, Torres JB, Barros R, Pallini A, 2010. Parasitism of diamondback moth by *Oomyzus sokolowskii*. *Pesqui Agropecu Bras*, 45, 638–645.
- Talekar N, Hu W, 1996. Characteristics of parasitism of *Plutella xylostella* (Lep., Plutellidae) by *Oomyzus sokolowskii* (Hym., Eulophidae). *Biocontrol*, 41, 45–52.
- Tezze AA, Botto EN, 2004. Effect of cold storage on the quality of *Trichogramma nerudai* (Hymenoptera: Trichogrammatidae). *Biol Control*, 30, 11–16.
- Uematsu H, Yamashita T, 2000. Number and sex ratio of adult wasps, *Oomyzus sokolowskii* (Hymenoptera: Eulophidae), emerging from diamondback moth pupae collected in cabbage fields. *Appl Entomol Zool*, 44, 197–200.
- Wang XG, Liu SS, Guo S, Lin W, 1999. Effects of host stages and temperature on population parameters of *Oomyzus sokolowskii*, a larval-pupal parasitoid of *Plutella xylostella*. *Biocontrol*, 44, 391–403.
- Waterhouse D, Norris K, 1987. Biological control-pacific prospects. Inkata Press, Melbourne.
- Yaseen M, 1978. The establishment of two parasites of the diamondback moth *Plutella xylostella* (Lep.: Plutellidae) in Trinidad, WI. *Entomophaga*, 23, 111–114.
- Žd'arek J, Denlinger DL, 1992. Eclosion behavior in tsetse (Diptera: Glossinidae): extrication from the puparium and expansion of the adult. *J Insect Behav*, 5, 657–668.
- Žd'arek J, Reid S, Fraenkel G, 1986. How does an eclosing fly deal with obstacles? *Physiol Entomol*, 11, 107–114.