Bulletin of Entomological Research

cambridge.org/ber

Effects of different *Philosamia ricini* (Lepidoptera: Saturniidae) cold storage periods on *Ooencyrtus pityocampae* and *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae) rearing

Hilal Tunca[1], Benjamin Cosic[2], Marine Venard[2], Mathilde Capelli[2], Etty-Ambre Colombel[2] and Elisabeth Tabone[2]

[1]Department of Plant Protection, Faculty of Agriculture, Ankara University, 06110, Ankara Dıskapı, Turkey and [2]Laboratoire BioContrôle, INRAE, UEFM site Villa Thuret, 90 Chemin Raymond, 06160, Antibes, France

Research Paper

Cite this article: Tunca H, Cosic B, Venard M, Capelli M, Colombel E-A, Tabone E (2022). Effects of different *Philosamia ricini* (Lepidoptera: Saturniidae) cold storage periods on *Ooencyrtus pityocampae* and *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae) rearing. *Bulletin of Entomological Research* **112**, 667–673. https://doi.org/10.1017/S0007485322000074

Received: 3 February 2020
Revised: 2 February 2022
Accepted: 2 March 2022
First published online: 8 April 2022

Keywords:
Cold storage; egg parasitoid; host; *Ooencyrtus kuvanae*; *Ooencyrtus pityocampae*; *Philosamia ricini*

Author for correspondence:
Hilal Tunca, Email: htunca@ankara.edu.tr

Abstract

Ooencyrtus pityocampae and *Ooencyrtus kuvanae* are egg parasitoids that are considered potential candidates for the control of different pest species through inundative release. The aim of this study was to assess the effects of different cold-storage periods of *Philosamia ricini* eggs (host) on the rearing parameters of *O. pityocampae* and *O. kuvanae*. Host eggs were stored at 3 °C, and a factorial experiment involving two parasitoid species, nine host storage periods (1, 5, 10, 15, 30, 45, 60, 75 and 90 days) and a control, and two host ages (1 and 2 days) was conducted, with 10 replications including 40-*P. ricini* eggs each. Adult emergence, development time, longevity, and fecundity were investigated. The parasitoid adult emergence percentage significantly varied with storage duration. These values were lower in *O. kuvanae* than in *O. pityocampae*. The development time of *O. kuvanae* progeny increased in both host age groups except in the 1-day storage period subgroup. However, the development times of the progeny of *O. pityocampae* reared on one-day-old eggs stored for 5, 10, 60, and 75 days were increased, and the development times of the progeny of *O. pityocampae* reared on 2-day-old eggs stored for 45 and 90 days were increased. The longevity of the F1 progeny of *O. kuvanae* was negatively affected by storage time. There was no difference in the longevity of the F1 progeny of *O. pityocampae* compared to that of the control. Additionally, the fecundities of the F1 progeny of *O. pityocampae* and *O. kuvanae* were 54.7 and 47.0 offspring/female, respectively. These results provide useful information for guiding the development of mass rearing methodologies for both parasitoid species.

Introduction

Ooencyrtus pityocampae (Mercet) (Hymenoptera: Encyrtidae) is an efficient biological control agent of *Thaumetopoea pityocampa* (Den. & Schiff.) and *T. wilkinsoni* (Tams) (Lepidoptera: Notodontidae), which are among the most important defoliators of pine forests throughout the Mediterranean Basin (Buxton, 1983; Battisti *et al.*, 1990; Masutti *et al.*, 1993; Tiberi *et al.*, 1994; Hódar and Zamora, 2004; Zhang *et al.*, 2005; Binazzi *et al.*, 2013; Samra *et al.*, 2015). Other known hosts of *O. pityocampae* include *Nezara viridula* (Linnaeus) *Aelia rostrata* (Boh), *Carpocoris* sp., *Dolycris baccarum* (Linnaeus), *Rhaphigaster nebulosa* (Poda), *Eurydema ventrale* (Kolenati), *E. oleracea* (Linnaeus), *Graphosoma lineatum* (Linnaeus) *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) and *Eurygaster maura* (Linnaeus) (Hemiptera: Scutelleridae) (Tiberi *et al.*, 1991, 1993; Federico *et al.*, 2016).

Ooencyrtus kuvanae (Howard) (Hymenoptera: Encyrtidae) is a primary egg parasitoid of *Lymantria dispar* (L.) (Lepidoptera, Lymantriidae) (Howard, 1910; Tadic and Bincev, 1959; Brown, 1984; Hofstetter and Raffa, 1998; Wang *et al.*, 2013) but it can also parasitize other hosts, such as *Dendrolimus spectabilis* Butler (Lepidoptera: Lasiocampidae), *Malacosoma americana* (Fabricius), *M. neustria tartacea* (Motschulsky), *Euproctis chrysorrhoea* (Linnaeus), *Hemerocampa leucostigma* (Abbot & Smith), *Hemerocampa definata* (Packard), *Lymantria fumida* (Butler), *Nygmia phaeorrhoea* (Donovan), *Stilpnotia salicis* (Linnaeus) *Eriogyna pyreterom* (Westwood) (Lepidoptera: Saturniidae), *Lycorma delicatula* (White) (Hemiptera: Fulgoridae) and *H. halys* (Stål) (Hemiptera: Pentatomidae) (Huang and Noyes, 1994; Hofstetter and Raffa, 1998; Liu, 2019; Tunca *et al.*, 2020).

To reduce the costs associated with biological control programs and to ensure the supply of high-quality natural enemies at times of high demand, it is important to improve parasitoid mass-rearing techniques (Spínola-Filho *et al.*, 2014). The encyrtid egg parasitoids *O. pityocampae* and *O. kuvanae* can be successfully raised on the host *Philosamia ricini* (Donovan) (Lepidoptera: Saturnidae) (Tunca *et al.*, 2016, 2017). *Philosamia ricini* can be

reared on *Ligustrum vulgare* (L.) (Amaranthaceae) and *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae) (Venard *et al*., 2016), and rearing is straightforward and inexpensive. In addition, *P. ricini* oviposits more often, and its eggs are larger than those produced using two other known laboratory hosts, *N. viridula* and *H. halys*. Although methods for breeding this host are well known and have been optimized, they can be affected by external problems, such as the natural contamination of host plants by bacterial species. Consequently, there are occasional problems related to the production of *P. ricini* eggs due to high larval mortality, and the rearing of the two parasitoids can be affected.

When inundative release is used as a biological pest control strategy, large numbers of parasitoids are required to rapidly reduce the damaging pest population. However, the major challenge associated with this biological control strategy is the production of a large number of parasitoids of adequate quality (Orr, 1988). Several techniques have been used to optimize the large-scale mass production of parasitoids in laboratories. Some of these approaches include, the in vitro development of parasitoids (Strand *et al*., 1988; Nettles, 1990; Consoli and Vinson, 2004; Shirazi, 2006; Paladino *et al*., 2010; Kim *et al*., 2018), cold storage of parasitized hosts (Noble, 1937; Dass and Ram, 1983; Gautam, 1986; Ganteaume *et al*., 1995*a*, 1995*b*; Bayram *et al*., 2005; Liu *et al*., 2014; Tunca *et al*., 2014; Kidane *et al*., 2015), cold storage of parasitoids as pupae or adults (Gautam, 1986; Foerster *et al*., 2004; Foerster and Doetzer, 2006; Yılmaz *et al*., 2007; Mousapour *et al*., 2014; Afshari and Nazari Fandokht, 2019; Cira *et al*., 2021) and cold storage of unparasitized hosts (Corrêa-Ferreira and Moscardi, 1993; Kıvan and Kılıç, 2005; Mahmoud and Lim, 2007; Alim and Lim, 2011; Spínola-Filho *et al*., 2014; Singhamuni *et al*., 2015; Wong *et al*., 2021).

Storing a host at a low temperature can arrest its development at the desired stage and contribute to the rearing of parasitoids. The cold storage technique allows the synchronization of parasitoid release with outbreaks of insect pests (Leopold, 1998; Pitcher *et al*., 2002; Colinet and Boivin, 2011). Cold storage of *P. ricini* eggs is important, as it allows for a sufficient number of egg hosts for the rearing of *O. pityocampae* and *O. kuvanae*. The objective of this study was to investigate the optimum cold storage conditions of host eggs and to assess the performance of *O. pityocampae* and *O. kuvanae* reared on stored *P. ricini* eggs.

Materials and methods

This study was conducted at the INRAE-PACA Mediterranean Forest and Entomology Unit, Laboratory of Biological Control, Antibes, France. The experimental trials were conducted under laboratory conditions at 25 ± 1 °C and 60 ± 5% relative humidity (RH), with a photoperiod of 18:6 h L:D. *P. ricini* and *O. pityocampae* were reared according to Tunca *et al*. (2016). *O. kuvanae* was reared according to Tunca *et al*. (2017).

Experimental setup

To determine the effect of cold storage of unparasitized *P. ricini* eggs on *O. pityocampae* and *O. kuvanae* rearing parameters, an experiment was carried out in a completely randomized 2 (1- and 2- day old hosts) × 10 (1, 5, 10, 15, 30, 45, 60, 75, and 90 days cold storage and control) × 2 (2 parasitoids, *O. pityocampae* and *O. kuvanae*) factorial design, with 10 replicates of each treatment combination. One- and two-day-old eggs were kept at 3°C in a refrigerator during the cold storage period and 40 host eggs were placed in a test tube (1 × 7 cm) with a single mated *O. pityocampae* or *O. kuvanae* female for 24 h for oviposition. Five-day-old mated females of *O. pityocampae* and *O. kuvanae* were fed honey and used for the oviposition experiments. After oviposition, *O. pityocampae* and *O. kuvanae* females were removed from the test tubes. The tubes were incubated at 25 ± 1 °C, with a RH of 65 ± 5% and a 16:8 h L:D photoperiod until parasitoid offspring emerged. Host larvae that hatched from unparasitized eggs were removed, and parasitized eggs were left in the tube.

Exposed eggs were monitored on a daily basis, and the number of emerged adults was recorded. Similarly, the time that elapsed from the exposure until adult emergence of the parasitoids was recorded to account for the developmental time. The emergence rate was calculated as the proportion of parasitized eggs in the tube and expressed as a percentage. To determine adult longevity, the parasitoids were placed in a test tube (1 × 7 cm) with a drop of bio-honey. Longevity was recorded daily until all of the parasitoids died. The fecundities of *O. pityocampae* and *O. kuvanae* were determined using one-day-old *P. ricini* eggs. For this analysis, *O. pityocampae* and *O. kuvanae* females (8 and 7 females, respectively) that emerged from stored eggs were chosen randomly. Thirty eggs were supplied on a daily basis to each newly emerged female until the females died, and the parasitoids that emerged from the parasitized eggs were counted every day.

The emergence rate, the development time and adult longevity were analyzed using a general linear model (GLM). Percentage data were normalized using an arcsine transformation (Zar, 1999). The means were compared with Duncan's test at a significance level of $\alpha = 0.05$ (McKenzie and Goldman, 2005; Minitab Release 14).

Results

The cold storage period × parasitoid species interaction showed a significant ($P < 0.001$) effect on the emergence rates of *O. pityocampae* and *O. kuvanae* (Table 1). Increasing cold storage periods significantly reduced the emergence rate in both parasitoid species ($F = 8.76$, df = 9, $P < 0.001$). The emergence rates of *O. pityocampae* were higher than those of *O. kuvanae* at 10, 15, 30, 45, 60, 75 and 90 days of storage (Table 2). The development times of the parasitoids were significantly affected by the interaction of three factors: cold storage period, host age, and parasitoid species ($F = 3.58$, df = 9, $P < 0.001$) (Table 3). The development times of both parasitoid species increased compared to those of the corresponding controls for some storage periods (Table 4). The longevity of the parasitoids was affected by the interaction of the F1 condition and parasitoid species ($F = 13.68$, df = 1, $P < 0.001$) (Table 5). The longevity of *O. pityocampae* (43.6 days) was significantly longer than that of *O. kuvanae* (36.6 days) when reared on stored eggs. Compared with the life span of *O. kuvanae* reared on unstored eggs (49.5 days), of *O. kuvanae* reared on stored eggs was shorter (36.6 days) (Table 6). The fecundities of O. *pityocampae* and *O. kuvanae* were 54.7 (progeny/per female) and 47.0 (progeny/per female), respectively. The pre-oviposition times of *O. pityocapae* and *O. kuvanae* were 1.37 days and 1.28 days, respectively. At the same time, both have post-oviposition periods. The post-oviposition times of *O. pityocampae* and *O. kuvanae* were 20.25 days and 14.71 days, respectively.

https://doi.org/10.1017/S0007485322000074 Published online by Cambridge University Press

Table 1. Results of the GLM analysis of the emergence rate of *Ooencyrtus pityocampae* and *Ooencyrtus kuvanae*.

Source of variation	DF	SS	*F*	*P*-value
Cold storage period	9	19.76	194.53	$P < 0.001$
Host age	1	0.04	4.25	0.040
Parasitoid species	1	2.59	229.57	$P < 0.001$
Cold storage period × host age	9	0.18	1.86	0.057
Cold storage period × parasitoid species	9	0.88	8.76	$P < 0.001$
Host age × parasitoid species	1	0.00	0.01	0.934
Cold storage period × host age × parasitoid species	9	0.17	1.67	0.094
Error	339	3.82		

Table 2. Emergence rate (%) of *Ooencyrtus pityocampae* and *Ooencyrtus kuvanae* (Cold storage period × parasitoid species).

	Parasitoid species	
Cold storage period	*Ooencyrtus pityocampae*	*Ooencyrtus kuvanae*
1	77.59Aa	71.95Aa
5	68.86Ba	63.10Ba
10	55.19Ca	42.82Cb
15	52.94Ca	28.06Db
30	43.94Da	10.20Eb
45	34.00Ea	12.31Eb
60	37.39Ea	18.29Eb
75	38.20Ea	16.46Eb
90	31.32Ea	7.29Fb
Control	82.74Aa	78.48Aa

Means in each row followed by same lowercase letter do not differ statistically.
Means in each column followed by same capital letter do not differ statistically.

Table 3. Results of the GLM analysis of the development time of *Ooencyrtus pityocampae* and *Ooencyrtus kuvanae*.

Source of variation	DF	SS	*F*	*P*-value
Cold storage period	9	831.089	43.48	$P < 0.001$
Host age	1	28.089	13.23	$P < 0.001$
Parasitoid species	1	455.758	214.62	$P < 0.001$
Cold storage period × host age	9	83.403	4.36	$P < 0.001$
Cold storage period × parasitoid species	9	258.906	13.55	$P < 0.001$
Host age × parasitoid species	1	0.534	0.25	0.616
Cold storage period × host age × parasitoid species	9	68.334	3.58	$P < 0.001$
Error	1617	3287.110		

Discussion

It is important to rear egg parasitoid species on suitable hosts to ensure the success of biocontrol programs (Consoli *et al.*, 2010; Masry and El-Wakeil, 2020). Additionally, the ability to mass produce a large number of parasitoids is required storing host eggs for different periods could contribute positively to the mass rearing of parasitoids (Bigler, 1986; Vieira and Tavares, 1995; Lalitha *et al.*, 2010; Masry and El-Wakeil, 2020).

However, longer storage times may result in a decrease in the nutritional quality of host eggs; therefore the performance of parasitoids reared on refrigerated eggs may be reduced (Flanders, 1938; Kostal *et al.*, 2004, 2006). For this reason, it is important to take into consideration the host storage period during the rearing of parasitoids (Wong *et al.*, 2021). Our results showed that the storage of *P. ricini* eggs caused adverse effects on in the biological parameters of the F1 generation of *O. pityocampae* and *O. kuvanae* adults, represented by the rate of adult emergence, development time, longevity and fecundity. These results can be explained as follows: first, lethal effects occur during parasitoid development in low-quality stored eggs, and second parasitoids fail to accept stored eggs as hosts (Wong *et al.*, 2021).

Lethal effects lead to decreased parasitoid progeny emergence (Mainali and Lim, 2013; Mahmoud and Lim, 2007; McIntosh *et al.*, 2019; Wong *et al.*, 2021). In this study, exposure of *P. ricini* eggs at different ages to low temperatures led to a reduction in the emergence rates of the both parasitoids. Siam *et al.* (2019) reported that low-temperature storage of host eggs for different periods had an effect on the efficiency of *Trichogramma* parasitoids. After 10, 15, 20 and 30 days of storage at 5 °C *T. evanescens* emergence percentages decreased by 84.91, 80.48, 61.17, and 50.73%, respectively. Rundle Bradely *et al.* (2004) and Ozder (2004) noted that the prolongation of cold storage led to a reduction in the efficiency of F1 female parasitoids. In our study, all the cold storage periods except for 1 day of cold storage (5, 10, 15, 30, 45, 60, 75 and 90 days) affected adult emergence in both parasitoids. In another study, when refrigerated *H. halys* eggs were stored at 8 °C for up to two months, the emergence rate of *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) decreased significantly (Wong *et al.*, 2021). Similar results have been reported in other studies for *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae) (Chen and Leopold, 2007), *Trichogramma acacioi* (Brun), *T. atopovirilia* (Oatman & Platner), *T. benneti* (Nagaraja & Nagarkatti), *T. brasiliensis* (Ashmead), *T. bruni* (Nagaraja), *T. demoraesi* (Nagaraja), *T. galloi* (Zucchi), *T. pretiosum* (Riley), *T. soaresi* (Nagaraja) (Hymenoptera: Trichogrammatidae) (Spínola-Filho *et al.*, 2014), *T. chilonis* and *T. achaeae* (Singhamuni *et al.*, 2015). Tunca *et al.* (2014) noted that *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae) could not develop on *Ephestia kuehniella* (Zeller) or *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) stored at 5 °C for 5, 7 and 15 days. Additionally, they did not develop on *P. interpunctella* larvae stored at 10 °C for 15 days. The low rate of emergence can additionally be explained by the low rate of parasitism by *O. pityocampae* and *O. kuvanae*. Neither species may not accept stored *P. ricini* eggs for parasitization. There were significant reductions in the emergence rates of *O. pityocampae* and *O. kuvanae* after 5-days of host storage.

There are two important factors that influence the parasitism of stored host eggs: one is the ability to recognize chemical signals

https://doi.org/10.1017/S0007485322000074 Published online by Cambridge University Press

Table 4. Development time (days) of *Ooencyrtus pityocampae* and *Ooencyrtus kuvanae*.

Parasitoid species	Cold storage period	Host age																				
		One day										Two days										
	1	5	10	15	30	45	60	75	90	control	1	5	10	15	30	45	60	75	90	control
Ooencyrtus pityocampae	19.6 ± 0.2bA *n* = 44	20.6 ± 0.1aA *n* = 50	21.2 ± 0.1aA *n* = 31	20.1 ± 0.2bA *n* = 55	20.0 ± 0.1bA *n* = 49	19.5 ± 0.1bA *n* = 55	20.6 ± 0.1aA *n* = 66	21.0 ± 0.1aA *n* = 43	19.7 ± 0.2bA *n* = 56	19.6 ± 0.1bA *n* = 80	19.7 ± 0.2bA *n* = 40	20.5 ± 0.2bA *n* = 61	20.7 ± 0.1bA *n* = 38	20.2 ± 0.2bA *n* = 56	20.0 ± 0.2bA *n* = 47	21.1 ± 0.2aA *n* = 61	20.3 ± 0.1bA *n* = 52	20.4 ± 0.1bA *n* = 62	21.5 ± 0.1aA *n* = 59	19.9 ± 0.1bA *n* = 74
Ooencyrtus kuvanae	17.2 ± 0.1cB *n* = 46	19.3 ± 0.2bB *n* = 40	19.7 ± 0.1aB *n* = 20	19.3 ± 0.2bA *n* = 35	19.4 ± 0.3bA *n* = 17	20.0 ± 0.3aA *n* = 20	19.2 ± 0.2bB *n* = 34	20.1 ± 0.1aB *n* = 65	18.8 ± 0.1bB *n* = 26	16.9 ± 0.1cB *n* = 64	17.5 ± 0.2cB *n* = 36	19.4 ± 0.2bB *n* = 37	19.2 ± 0.3bB *n* = 14	19.9 ± 0.2aA *n* = 16	20.5 ± 0.3aA *n* = 23	19.2 ± 0.4bB *n* = 25	20.0 ± 0.2aA *n* = 38	19.9 ± 0.1aA *n* = 33	20.3 ± 0.4aB *n* = 9	17.2 ± 0.1cB *n* = 50

Means in each row followed by same lowercase letter do not differ statistically.
Means in each column followed by same capital letter do not differ statistically.

Table 5. Results of the GLM analysis of the longevity of *Ooencyrtus pityocampae* and *Ooencyrtus kuvanae*.

Source of variation	DF	SS	*F*	*P*-value
Condition F1 (Stored eggs and unstored eggs)	1	128.49	3.63	0.060
Parasitoid species	1	1556.42	44.02	$P < 0.001$
Condition F1 × parasitoid species	1	483.62	13.68	$P < 0.001$
Error	87	3040.99		

Table 6. Longevity (days) of *Ooencyrtus pityocampae* and *Ooencyrtus kuvanae*.

	Parasitoid species	
Condition F1	*Ooencyrtus pityocampae*	*Ooencyrtus kuvanae*
Stored eggs	43.64 ± 1.06Aa *n* = 22	36.60 ± 0.60Bb *n* = 23
Unstored eggs (Control)	47.32 ± 1.83Aa *n* = 22	49.57 ± 1.25Aa *n* = 23

Means in each row followed by same lowercase letter do not differ statistically.
Means in each column followed by same capital letter do not differ statistically.

in host eggs, and the other is the level of tolerance to changes in physical characteristics, such as the color, size and shape of the eggs (Stoepler *et al.*, 2011). Female parasitoids may refuse cold-stored eggs with modified chemical and physical characteristics (Soares *et al.*, 2009; Goubault *et al.*, 2011; Penaflor *et al.*, 2011; Stoepler *et al.*, 2011) Relatedly, Conti *et al.* (1996) reported that low-temperature storage modified egg shape and affected host recognition by parasitoids. The parasitism rates of *T. semistriatus* were decreased when exposed to *Dolycoris baccarum* (L.), *Graphosoma lineatum* (L.) and *Eurydema ornatum* (L.) (Heteroptera: Pentatomidae) eggs stored for three months at 6 °C (Kıvan and Kılıç, 2005). Chen and Leopold (2007) reported that parasitism by *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae) on eggs of *Coagulata homalodisca* (Say) (Hemiptera: Cicadellidae) decreased with an increasing cold storage period. Similarly, Karabörklü and Ayvaz (2007) noted that the emergence rate of and parasitism by *T. evanescens* adults that emerged from stored host eggs decreased depending on the storage period at 4 °C. A similar result was also obtained for *Trichogramma olea* reared on *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs stored at 4 °C (Gharbi, 2014).

Compared with those reared on unstored eggs, *O. pityocampae* and *O. kuvanae* reared on eggs subjected to cold storage for different periods had longer development times. Chen and Leopold (2007) reported that after 70 days of *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae) egg storage, the developmental time of the parasitoid *G. ashmeadi* was delayed. Similarly, the development time of *V. canescens* was negatively affected by low temperature and storage time (Tunca *et al.*, 2014). Wong *et al.* (2021) noted that the development time of *T. japonicus* increased when eggs were refrigerated for long periods. Relatedly, the development time of parasitoids obviously decreased when reared on eggs of *H. halys* refrigerated for short periods.

The longevity of adult *O. pityocampae* that emerged from cold-stored eggs did not differ from that of the adult control group.

https://doi.org/10.1017/S0007485322000074 Published online by Cambridge University Press

However, the longevity of *O. kuvanae* was significantly reduced. Kidane *et al*. (2015) reported that the longevity of *Encarsia sophia* (Hymenoptera: Aphelinidae) that emerged from host pupae stored for one week at 12 and 8 °C was not affected, although longevity decreased to 66–72% with increasing storage period. The longevity of adult *T. evanescens* decreased significantly with increased host storage time (Özder and Sağlam, 2004). Similarly, Gharbi (2014) reported that the longevity of *T. oleae* adults that emerged from stored pupae decreased significantly with increasing cold storage duration. Siam *et al*. (2019), showed that the longevity of female *T. evanescens* decreased with prolonged *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) cold storage periods.

The cold storage of *P. ricini* eggs creates unfavorable conditions for the development of both *O. pityocampae* and *O. kuvanae*. *Ooencytus pityocampae* and *O. kuvanae* showed decreases in fecundity of 18.1 and 31.7%, respectively, when reared on cold-stored *P. ricini* eggs compared to those reared on fresh eggs (Tunca *et al*., 2017, 2019). Similar results were obtained for *T. cacoeciae*, *T. brassicae*, *T. evanescens* (Özder and Sağlam, 2004), *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae) (Chen and Leopold, 2007) and *T. evanescens* (Siam *et al*., 2019).

The healthy storage of host insects is extremely important for mass production. However, storing *P. ricini* eggs did not lead to the successful rearing of parasitoids. *Ooencyrtus kuvanae* was more sensitive than *O. pityocampae* in terms of development on stored eggs. However, the results of this study showed that one- and two- day-old *P. ricini* eggs could be stored for up to 30 days for the rearing of *O. pityocampae* and that those stored for up to 10 days at 3 °C could be used for rearing *O. kuvanae* for the sustainable production of these parasitoids**.** These results should be considered in the mass production of these two parasitoid species during autumn and winter and for their release in the field during the critical periods of natural pest hosts outbreaks.

Acknowledgements. Great appreciation is extended to Jean Claude Martin (INRAE-Unité Expérimentale Forestière Méditerranéenne) for his critical review of the manuscript.

References

Afshari A and Nazari Fandokht E (2019) Effect of sugar concentration and feeding duration on the cold tolerance of *Bracon hebetor* Say adults. *Plant Pest Research* **8**, 55–69.

Alim MA and Lim UT (2011) Refrigerated eggs of *Riptortus pedestris* (Hemiptera: Alydidae) added to aggregation pheromone traps increase field parasitism in soybean. *Journal of Economic Entomology* **104**, 1833–1839.

Battisti A, Ianne P, Milani N and Zanata M (1990) Preliminary accounts on the rearing of *Ooencyrtus pityocampae* (Mercet) (Hym., Encyrtidae). *Journal of Applied Entomology* **110**, 121–127.

Bayram A, Ozcan H and 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). *Biological Control* **35**, 68–77.

Bigler F (1986) Mass production of *Trichogramma maidis* Pint et Voeg. and its field application against *Ostrinia nubilalis* Hbn. in Switzerland. *Journal of Applied Entomology* **101**, 23–29.

Binazzi F, Benassai D, Peverieri SG and Roversi PF (2013) Effects of *Leptoglossus occidentalis* Heidemann (Heteroptera: Coreidae) egg age on the indigenous parasitoid *Ooencyrtus pityocampae* Mercet (Hymenoptera Encyrtidae). *Redia* **96**, 79–84.

Brown MW (1984) Literature review of *Ooencyrtus kuvanae* (Hym.: Encyrtidae), an egg parasite of *Lymantria dispar* (Lep.: Lymantriidae). *Entomophaga* **29**, 249–265.

Buxton RD (1983) Forest management and the pine processionary moth. *Outlook Agric***12**, 34–39.

Chen WL and Leopold RA (2007) Progeny quality of *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae) reared on stored eggs of *Homalodisca coagulate* (Hemiptera: Cicadellidae). *Journal of Economic Entomology* **100**, 685–694.

Cira T, Santacruz EN and Koch RL (2021) Optimization of *Trissolcus japonicus* cold storage methods for biological control of *Halyomorpha halys*. *Biological Control* **156**, 104534.

Colinet H and Boivin G (2011) Insect parasitoids cold storage: a comprehensive review of factors of variability and consequences. *Biological Control* **58**, 83–95.

Consoli FL and Vinson SB (2004) Host regulation and the embryonic development of the endoparasitoid *Toxoneuron nigriceps* (Hymenoptera: Braconidae). *Comparative Biochemistry and Physiology B Biochemistry and Molecular Biology* **137**, 463–473.

Consoli FL, Parra JRP and Zucchi RA (2010) *Egg Parasitoids in Agroecosystems with Emphasis on Trichogramma*. Heidelberg, London, New York: Springer, 479 pp.

Conti E, Jones WA, Bin F and Vinson SB (1996) Physical and chemical factors involved in host recognition behavior of *Anophes iole* Girault (Hymenoptera: Mymaridae), an egg parasitoid of *Lygus hesperus* Knight (Heteroptera: Miridae). *Biological Control* **7**, 10–16.

Corrêa-Ferreira BS and Moscardi F (1993) Storage techniques of stink bug eggs for laboratory production of the parasitoid. *Trissolcus basalis (Wollaston) Pesquisa Agropecuária Brasileira* **28**, 1247–1253.

Dass R and Ram A (1983) Effect of frozen eggs of *Corcyra cepahlonica* Staiton (Pyralidae: Lepidoptera) on parasitism on *Trichogramma exiguum* (Pinto and Platner) (Trichogrammatidae: Hymenoptera). *Indian Journal of Entomology* **45**, 345–347.

Federico RP, Francesco B, Leonardo M, Elena C, Lara M and Sabbatini Peverieri G (2016) Searching for native egg-parasitoids of the invasive alien species *Halyomorpha halys* Stål (Heteroptera Pentatomidae) in Southern Europe. *Journal of Zoology* **XCIX**, 1–8.

Flanders SE (1938) The effect of cold storage on the reproduction of parasitic Hymenoptera. *Journal Economic Entomology* **31**, 633–634.

Foerster LA and Doetzer AK (2006) Cold storage of the egg parasitoids *Trissolcus basalis* (Wollaston) and *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae). *Biological Control* **36**, 232–237.

Foerster LA, Doetzer AK and Castro LCF (2004) Emergence, longevity and fecundity of *Trissolcus basalis* and *Telenomus podisi* after cold storage in the pupal stage. *Pesquisa Agropecuaria Brasileira* **39**, 841–845.

Ganteaume A, Tabone E and Poinsot-Balaguer N (1995*a*) Variation du potentiel biotique de l'auxiliaire parasite *Encarsia formosa* G. (Hym., Aphelinidae) en fonction du stockage au froid 2. Du point de vue de la fecondité journalière et de la longevité. *Journal of Applied Entomology* **119**, 547–551.

Ganteaume A, Tabone E and Poinsot-Balaguer N (1995*b*) Variation du potentiel biotique de l'auxiliaire parasite *Encarsia formosa* G. (Hym., Aphelinidae) en fonction du stockage au froid: I. Du point de vue du taux d'emergence. *Journal of Applied Entomology* **119**, 419–422.

Gautam RD (1986) Effect of cold storage on the adult parasitoid *Telenomus remus* Nixon Scelionidae: Hymenoptera and the parasitised eggs of *Spodoptera litura* Fabr. Noctuidae Lepidoptera. *Journal of Entomological Research* **10**, 125–131.

Gharbi N (2014) Influences of cold storage period and rearing temperature on the biological traits of *Trichogramma oleae*. *Tunisian Journal of Plant Protection* **9**, 143–153.

Goubault M, Cortesero AM, Paty C, Fourrier J, Dourlot S and Le Ralec A (2011) Abdominal sensory equipment involved in external host discrimination in a solitary parasitoid wasp. *Microscopy Research and Technique* **74**, 1145–1153.

Hódar JA and Zamora R (2004) Herbivory and climatic warming: a Mediterranean outbreaking caterpillar attacks a relict, boreal pine species. *Biodiversity and Conservation* **13**, 493–500.

Hofstetter RW and Raffa KF (1998) Endogenous and exogenous factors affecting the orientation and development of the gypsy moth egg parasite, *Ooencyrtus kuvanae*. *Entomologia Experimentalis et Applicata* **88**, 123–135.

Howard LO (1910) Technical results from the gypsy moth parasite laboratory. I. The parasites reared or supposed to have been reared from

https://doi.org/10.1017/S0007485322000074 Published online by Cambridge University Press

the eggs of gypsy moth. *U.S. Department Agriculture Technical Service Bulletin* **19**, 1–12.

Huang DW and Noyes JS (1994) A revision of the Indo-Pacific species of Ooencyrtus (Hymenoptera: Encyrtidae), parasitoids of the immature stages of economically important insect species (mainly Hemiptera and Lepidoptera). *Bulletin Natural History Museum London* **63**, 1–136.

Karabörklü S and Ayvaz A (2007) Soğukta depolamanın farklı konukçularda yetişen *Trichogramma evanescens* Westwood (Hym: Trichogrammatidae)'in farklı evreleri üzerine etkileri. *Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi* **23**, 30–36.

Kidane D, Yang N-W and Wan F-H (2015) Effect of cold storage on the biological fitness of *Encarsia sophia* (Hymenoptera: Aphelinidae), a parasitoid of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *European Journal Entomology* **112**, 460–469.

Kim K, Lee K, Lim UT and Hong K-J (2018) In vitro rearing of *Ooencyrtus nezarae* (Hymenoptera: Encyrtidae), an egg parasitoid of *Riptortus pedestris* (Hemiptera: Alydidae). *Biocontrol Science and Technology* **28**, 837–847.

Kıvan M and Kılıç N (2005) Effects of storage at low-temperature of various heteropteran host eggs on the egg parasitoid, *Trissolcus semistriatus*. *Biocontrol* **50**, 589–600.

Kostal V, Vambera J and Bastl J (2004) On the nature of pre-freeze mortality in insects: water balance, ion homeostasis and energy charge in the adults of *Pyrrhocoris apterus*. *Journal of Experimental Biology* **207**, 1509–1521.

Kostal V, Yanagimoto M and Bastl J (2006) Chilling-injury and disturbance of ion homeostasis in the coxal muscle of the tropical cockroach (*Nauphoeta cinerea*). *Comparative Biochemistry and Physiology A: Molecular & Integrative Physiology* **143**, 171–179.

Lalitha Y, Jalali SK, Venkatesan T and Sriram S (2010) Production attributes of Trichogramma reared on Eri silkworm eggs vis-à-vis Corcyra eggs and economics of rearing system. In *Blueprint for the Future of Arthropod Rearing and Quality Assurance–Abstracts of 12th Workshop of the IOBC Global Working Group on Arthropod Mass Rearing and Quality Control (IOBC–AMRQC) Held at IAEA, Vienna, 18–22 Oct.* IOBC-AMQRC, p. 47.

Leopold RA (1998) Cold storage of insects for integrated pest management. In Hallman GJ and Denlinger DL (eds), *Temperature Sensitivity in Insects and Application in Integrated Pest Management*. Boulder: Westview Press, pp. 235–267.

Liu H (2019) Occurrence, seasonal abundance, and superparasitism of *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae) as an egg parasitoid of the spotted lanternfly (*Lycorma delicatula*) in North America. *Forests* **10**, 1–17.

Liu K, Fu B, Lin J, Fu Y, Peng Z and Jin Q (2014) Effect of temperatures and cold storage on performance of *Tetrastichus brontispae* (Hymenoptera: Eulophidae), a parasitoid of *Brontispa longissimi* (Coleptera: Chrysomelidae). *Journal of Insect Science* **14**, 1–6.

Mahmoud AMA and Lim UT (2007) Evaluation of cold-stored eggs of *Dolycoris baccarum* (Hemiptera: Pentatomidae) for parasitization by *Trissolcus nigripedius* (Hymenoptera: Scelionidae). *Biological Control* **43**, 287–293.

Mainali BP and Lim UT (2013) Quality assessment of *Riptortus pedestris* (Hemiptera: Alydidae) eggs cold-stored at different temperature and relative humidity regime. *Biological Control* **64**, 132–137.

Masry SHD and El-Wakeil N (2020) Egg parasitoid production and their role in controlling insect pests. In El-Wakeil N, Saleh M and Abu-Hashim M (eds), *Cottage Industry of Biocontrol Agents and Their Applications*. Cham: Springer, pp. 3–47.

Masutti L, Battisti A, Milani N, Zanata M and Zanazzo G (1993) In vitro rearing of *Ooencyrtus pityocampae* (Hym., Encyrtidae), an egg parasitoid of *Thaumetopoea pityocampa* (Lep., Thaumetopoeidae]. *Entomophaga* **38**, 327–333.

McIntosh H, Lowenstein DM, Wiman NG, Wong JS and Lee JC (2019) Parasitism of frozen *Halyomorpha halys* eggs by *Trissolcus japonicus*: applications for rearing and experimentation. *Biocontrol Science Technology* **29**, 478–493.

Mckenzie JD and Goldman R (2005) *The Student Guide to Minitab Release 14 Manual*. Boston, MA: Pearson Education.

Mousapour Z, Askarianzadeh A and Abbasipour H (2014) Effect of cold storage of pupae parasitoid wasp, *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae), on its efficiency. *Archives of Phytopathology and Plant Protection* **47**, 966–972.

Nettles Jr. WC (1990) In vitro rearing of parasitoids: role of host factors in nutrition. *Archives of Insect Biochemistry and Physiology* **13**, 167–175.

Noble NS (1937) An Egg parasite of the green vegetable Bug. *Agricultural Gazette New South Wales* **48**, 337–334.

Orr DB (1988) Scelionid wasps as biological control agents: a review. *The Florida Entomologist* **71**, 506–528.

Ozder N (2004) Effect of different cold storage periods on parasitization performance of *Trichogramma cacoeciae* (Hymenoptera, Trichogrammatidae) on eggs of *Ephestia kuehniella* (Lepidoptera: Pyralidae). *Biocontrol Science and Technology* **14**, 441–447.

Özder N and Sağlam Ö (2004) Effect of short term storage on the quality of *Trichogramma brassicae*, T. cacoeciae and *T. evanescens* (Hmymenoptera: Trichogrammatidae). *The Great Lakes Entomologist* **37**, 183–187.

Paladino LZC, Papeschi AG and Cladera JL (2010) Immature stages of development in the parasitoid wasp, *Diachasmimorpha longicaudata*. *Journal of Insect Science* **10**, 56.

Penaflor MFGV, Erb M, Miranda LA, Werneburg AG and Bento JMS (2011) Herbivore induced plant volatiles can serve as host location cues for a generalist and a specialist egg parasitoid. *Journal of Chemical Ecology* **37**, 1304–1313.

Pitcher SA, Hoffmann MP, Gardner J, Wright MG and Kuhar TP (2002) Cold storage of *Trichogramma ostriniae* reared on *Sitotroga cerealella* eggs. *BioControl* **47**, 525–535.

Rundle Bradley J, Thomson Linda J and Hoffmann Ary A (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. *Journal of Economic Entomology* **97**, 213–221.

Samra S, Ghanim M, Protasov A, Branco M and Mendel Z (2015) Genetic diversity and host alternation of the egg parasitoid *Ooencyrtus pityocampae* between the pine processionary moth and the caper bug. *PLoS One* **10**, 1–21.

Shirazi J (2006) Investigation on the in vitro rearing of *Trissolcus grandis* an egg parasitoid of *Eurygaster integriceps* by use of artificial diet. *Pakistan Journal of Biological Sciences* · **9**, 2040–2047.

Siam A, Zohdy NZM, Abd ELHafez AM, Eid Moursy L and Sherif HAEL (2019) Effect of different cold storage periods of rearing host eggs on the performance of the parasitoid *Trichogramma evanescens* (Westwood) (Hymenoptera: Trichogrammatidae). *Egyptian Journal of Biological Pest Control* **29**, 1–4.

Singhamuni SAA, Hemachandra KS and Sirisena UGAI (2015) Potential for mass rearing of the egg parasitoids, *Trichogramma chilonis* and *Tricogramma achaeae* (Hymenoptera: Trichogrammatidae) on *Corcyra cephalonica* eggs. *Tropical Agricultural Research* **27**, 1–12.

Soares MA, Torres-Gutierrez C, Zanuncio JC, Pedrosa ARP and Lorenzon AS (2009) Superparasitismo de *Palmistichus elaeisis* (Hymenoptera: Eulophidae) y comportamiento de defensa de dos hospederos. *Reviewe Colombiana Entomology* **35**, 62–65.

Spínola-Filho PR, Leite GLD, Soares MA, Alvarenga AC, de Paulo PD, Tuffi-Santos LD and Zanuncio JC (2014) Effects of duration of cold storage of the eggs on percent parasitism and adult emergence of each of ten Trichogrammatidae (Hymenoptera) species. *Florida Entomologist* **97**, 14–21.

Stoepler TM, Lill JT and Murphy SM (2011) Cascading effects of host size and host plant species on parasitoid resource allocation. *Ecological Entomology* **36**, 742–735.

Strand MR, Vinson SB, Nettles WC Jr. and Xie ZN (1988) In vitro culture of the egg parasitoid *Telenomus heliothidis*: the role of teratocytes and medium consumption in development. *Entomologia Experimentalis et Applicata* **46**, 71–78.

Tadic MD and Bincev B (1959) *Ooencyrtus kuvanae* How in Yugoslavia. *Zaštita Bilja* **10**, 51–59.

Tiberi R, Niccoli A, Roversi PF and Saccetti P (1991) Laboratory rearing of *Ooencyrtus pityocampae* (Mercet) on eggs of *Nezara viridula* (L.) and other pentamonid eggs. In *Insect Parasitoids, 4th Eur Workshop-Perugia, 3–5 April*. Redia, vol. 74, pp. 467–469.

Tiberi R, Niccoli A and Sacchetti P (1993) Allevamento di *Ooencyrtus pityocampae* (Mercet) su uova di rincori pentatomidi. *MA.F. Convegno 'Piante*

https://doi.org/10.1017/S0007485322000074 Published online by Cambridge University Press

forestali', Frienze, 5 marzo 1992 (Coord. M. Covassi). Roma: Ist Sper Pat Veg, pp. 78–84.

Tiberi R, Niccoli A and Sacchetti P (1994) Parassitizzazione delle Uova di *Thaumetopoea pityocampa*: Modificazioni Conseguenti Al Potenziamento Artificiale Di *Ooencyrtus pityocampae*. *Atti XVII Congressonazionale Italiano di Entomologia Udine 13–18 giugno 1994*, pp. 763–766.

Tunca H, Yeşil AN and Çalişkan TF (2014) Cold storage possibilities of a larval parasitoid, *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae). *Turkiye Entomoloji Dergisi* **38**, 19–29.

Tunca H, Colombel E-A, Ben Soussan T, Buradino M, Galio F and Tabone E (2016) Optimal biological parameters for rearing *Ooencyrtus pityocampae* on the new laboratory host *Philosamia ricini*. *Journal of Applied Entomology* **140**, 527–535.

Tunca H, Venard M, Colombel E-A and Tabone E (2017) A new substitute host and its effects on some biological properties of *Ooencyrtus kuvanae*. *Bulletin of Entomological Research* **107**, 742–748.

Tunca H, Venard M, Colombel E-A and Tabone É (2019) Étude de la performance d'un parasitoïde oophage Ooencyrtus pityocampae (Mercet) (Hymenoptera : Encyrtidae) pour lutter contre des ravageurs d'un écosystème méditerranéen. *BASE* **23**, 153–159.

Tunca H, Cosic B, Colombel EA, Venard M, Capelli M and Tabone E (2020) Performance of *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae) on two host species, *Halyomorpha halys* and *Philosamia ricini*. *Journal of Applied Entomology* **144**, 961–967.

Venard M, Tunca H, Colombel E, Martin JC and Tabone E (2016) Implementation of a new rearing technique of *Ooencyrtus pityocampae* and *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae) to develop the biocontrol of lepidopterous pests in forest. *Plant Biology Europe EPSO/FESPB 2016 Congress, Jun 2016, Prague, Czech Republic*.

Vieira V and Tavares J (1995) Rearing of *Trichogramma cordubensis* Vargas & Cabello (Hymenoptera: Trichogrammatidae) on Mediterranean flour moth cold-stored eggs. *Les Colloques de l'INRA* **73**, 177–180.

Wang JJ, Liu XB, Zhang YA, Wen C and Wei JR (2013) The reproductive capability of *Ooencyrtus kuvanae* reared on eggs of the factitious host *Antheraea pernyi*. *Journal of Applied Entomology* **138**, 267–272.

Wong WHL, Walz MA, Oscienny AB, Sherwood JL and Abram PK (2021) An effective cold storage method for stockpiling *Halyomorpha halys* eggs for field surveys and laboratory rearing of *Trissolcus japonicas*. *Journal of Economic Entomology* **114**, 571–581.

Yılmaz S, Karabörklü S and Ayvaz A (2007) Effect of cold temperature durations on the performance of the adult *Trichogramma evanescens* (Westwood, 1833) (Hymenoptera: Trichogrammatidae). *Türkiye Entomologi. Dergisi* **31**, 269–278.

Zar JH (1999) *Biostatistical Analysis*, 4th Edn. Upper Saddle River, NJ: Prentice-Hall.

Zhang YZ, Li W and Huang DW (2005) A taxonomic study of Chinese species of Ooencyrtus (Insecta: Hymenoptera: Encyrtidae). *Zoological Studies* **44**, 347–360.

https://doi.org/10.1017/S0007485322000074 Published online by Cambridge University Press