



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

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

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**The effects of host cold storage on *O. pityocampae* and *O. kuvanae***

**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.

Key words: Cold storage, host, egg parasitoid, *Ooencyrtus pityocampae*, *Ooencyrtus kuvanae*, *Philosamia ricini*

## 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*

46 *halys* (Stål) (Hemiptera: Pentatomidae) and *Eurygaster maura* (Linnaeus) (Hemiptera:  
 47 Scutelleridae) (Tiberi et al., 1991; Tiberi et al., 1993; Federico et al., 2016).  
 48 *Ooencyrtus kuvanae* (Howard) (Hymenoptera: Encyrtidae) is a primary egg parasitoid of  
 49 *Lymantria dispar* (L.) (Lepidoptera, Lymantriidae) (Howard, 1910; Tadic and Bincev, 1959;  
 50 Brown, 1984; Hofstetter and Raffa, 1998; Wang et al., 2013) but it can also parasitize other hosts,  
 51 such as *Dendrolimus spectabilis* Butler (Lepidoptera: Lasiocampidae), *Malacosoma americana*  
 52 (Fabricius), *M. neustria tartacea* (Motschulsky), *Euproctis chrysorrhoea* (Linnaeus),  
 53 *Hemerocampa leucostigma* (Abbot & Smith), *Hemerocampa definata* (Packard), *Lymantria*  
 54 *fumida* (Butler), *Nygmia phaeorrhoea* (Donovan), *Stilpnotia salicis* (Linnaeus) *Eriogyna*  
 55 *pyreterom* (Westwood) (Lepidoptera: Saturniidae), *Lycorma delicatula* (White) (Hemiptera:  
 56 Fulgoridae) and *H. halys* (Stål) (Hemiptera: Pentatomidae) (Huang and Noyes, 1994; Hofstetter  
 57 and Raffa, 1998; Liu, 2019; Tunca et al., 2019).  
 58 To reduce the costs associated with biological control programmes and to ensure the supply of  
 59 high-quality natural enemies at times of high demand, it is important to improve parasitoid mass-  
 60 rearing techniques (Spínola-Filho, 2014). The encyrtid egg parasitoids *O. pityocampae* and *O.*  
 61 *kuvanae* can be successfully raised on the host *Philosamia ricini* (Donovan) (Lepidoptera:  
 62 Saturnidae) (Tunca et al., 2016, 2017). *Philosamia ricini* can be reared on *Ligustrum vulgare* (L.)  
 63 (Amaranthaceae) and *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae) (Venard, 2016), and  
 64 rearing is straightforward and inexpensive. In addition, *P. ricini* oviposits more often, and its  
 65 eggs are larger than those produced using two other known laboratory hosts, *N. viridula* and *H.*  
 66 *halys*. Although methods for breeding this host are well known and have been optimized, they  
 67 can be affected by external problems, such as the natural contamination of host plants by  
 68 bacterial species. Consequently, there are occasional problems related to the production of *P.*  
 69 *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 Jr, 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, b; Bayram et al., 2005; Tunca et al., 2014; Liu 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 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, 2010; de Carvalho Spínola-Filho et al., 2014; Singhamuni et al., 2015; Wong et al., 2020).

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  $25 \pm 1^\circ\text{C}$  and  $60 \pm 5\%$  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)  $\times$  10 (1, 5, 10, 15, 30, 45, 60, 75, and 90 days cold storage and control)  $\times$  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  $3^\circ\text{C}$  in a refrigerator during the cold storage period and 40 host eggs were placed in a test tube (1 x 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  $25 \pm 1^\circ\text{C}$ , with a RH of  $65\% \pm 5\%$  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 x 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 analysed 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, SAS Institute, 2003).

## Results

The cold storage period  $\times$  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. pityocampae* and *O. kuvanae* were 1.37 days and 1.28 days, respectively. At the same time, both have a post-oviposition periods. The post-oviposition times of *O. pityocampae* and *O. kuvanae* were 20.25 days and 14.71 days, respectively.

## Discussion

It is important to rear egg parasitoid species on suitable hosts to ensure the success of biocontrol programmes (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., 2020). 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., 2020).

Lethal effects lead to decreased parasitoid progeny emergence (Wong et al., 2020; Mahmoud and Lim, 2007; Mainali and Lim, 2013; McIntosh et al., 2019). 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. 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., 2020). 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) (de Carvalho 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 in host eggs, and the other is the level of tolerance to changes in physical characteristics, such as the colour, 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, Karaborklu 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. (2020) 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. 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 (Ozder and Saglam 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 unfavourable 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., 2019; Tunca et al., 2017). Similar results were obtained for *T. cacoeciae*, *T. brassicae*, *T. evanescens* (Ozder and Saglam, 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.

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