JOURNAL OF APPLIED ENTOMOLOGY

J. Appl. Entomol.

ORIGINAL CONTRIBUTION

# Conservation of *Ephestia kuehniella* eggs as hosts for *Trichogramma ostriniae*

M. St-Onge[1], D. Cormier[2], S. Todorova[3] & É. Lucas[1]

1 Département des Sciences Biologiques, Université du Québec à Montréal, Montréal, QC, Canada
2 Institut de recherche et de développement en agroenvironnement, Saint-Bruno-de-Montarville, QC, Canada
3 Anatis Bioprotection Inc., Saint-Jacques-le-Mineur, QC, Canada

**Keywords**
freezing, liquid nitrogen, sterilization, UV, vacuum packing

**Correspondence**
Mylène St-Onge (corresponding author), Département des Sciences Biologiques Université du Québec à Montréal, Montréal, QC H3C 3P8, Canada.
E-mail: st-onge.mylene@courrier.uqam.ca

Received: December 17, 2014; accepted: March 24, 2015.

doi: 10.1111/jen.12227

## Abstract

Mass rearing of numerous biological control agents depends on large amounts of factitious hosts like *Ephestia kuehniella* eggs. Moreover, production of some parasitoids, such as *Trichogramma,* requires hosts of high quality. The objective of this study was to determine the optimal conservation method that allows *E. kuehniella* eggs to remain suitable for parasitism and development of *Trichogramma ostriniae* for the longest period of time. Fifteen sterilization–conservation treatments were compared: nine consisting of submersion of the eggs in liquid nitrogen as a conservation mode, two consisting of sterilization of the eggs with UV and four consisting of sterilization with freezing at −15°C. Liquid nitrogen submersion of *E. kuehniella* eggs did not allow the production of *T. ostriniae*. The sterilization by exposition to UV light followed by conservation using vacuum packing and refrigeration at 4°C provided the longest conservation of *E. kuehniella* eggs for *T. ostriniae* rearing. It was the only treatment (75.4 ± 4.62%) for which the parasitism rate remained over 70% after 2 weeks.

## Introduction

The mass rearing of biological control agents depends on large amount of factitious hosts. Parasitoids like *Trichogramma* can evaluate the quality of host eggs based on their size, age, nutritional suitability and previous parasitism (Schmidt 1994). For this reason, mass rearing of *Trichogramma* requires large amounts of high-quality host eggs. Those eggs must be available at the precise time when growers need *Trichogramma*. In order to respond to grower demand, optimal storage conditions need to be developed.

Different methods have been evaluated for the conservation of host eggs. Liquid nitrogen offers various levels of success depending on the host and the technique. Parasitism of *Nezara viridula* Linnaeus (Hemiptera: Pentatomidae) eggs stored during 12 months in liquid nitrogen was not significantly different than parasitism of fresh eggs by the parasitoid *Trissolcus basalis* Wollaston (Hymenoptera: Scelionidae) (Corrêa-Ferreira and de Oliveira 1998). Similarly, Ma (1988) observed no differences in the parasitism and emergence rates of *Trichogramma dendrolimi* Matsumara and *Trichogramma confusum* Viggiani (Hymenoptera: Trichogrammatidae) when these exploited tussah moth, *Antheraea pernyi* Guerin-Meneville (Lepidoptera: Saturniidae) eggs, whether fresh or stored in liquid nitrogen followed by thawing in water at 30°C. Greco and Stilinovic (1998) observed a significant decrease in the parasitism by *Trichogramma pretiosum* Riley of *Sitotroga cerealella* Oliver (Lepidoptera: Gelechiidae) eggs stored in liquid nitrogen when compared to fresh eggs. However, the parasitism rate was significantly higher for eggs stored in liquid nitrogen and thawed in a water bath at 50°C compared to eggs thawed at room temperature. The main advantage of liquid nitrogen conservation is that it can extend over several months.

Freezing at −15°C can be a suitable sterilization method of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs for *Trichogramma* parasitism (St-Onge et al. 2014), but long-term conservation of host eggs at freezing temperatures has not been found to be suitable for parasitoid rearing. Hu et al. (1999)

found a significant difference in the parasitism and emergence rates of *Edovum puttleri* Grissell (Hymenoptera: Eulophidae) on Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) eggs stored at −20°C for more than 5 min and fresh eggs. They were however optimistic that freezing could be, with improvement, used for long-term conservation of host eggs because the parasitism rate was over 40% after host eggs were stored for 15 days at −20°C. Freezing as a conservation method should be examined thoroughly because it does not require specialized equipment and time-consuming manipulations.

Cold storage at temperatures ranging from −1 to 8°C has been proven to be an effective conservation method for host eggs in parasitoid rearing. Nevertheless, Özder (2004) found that parasitism by *Trichogramma cacoeciae* Marchal of *E. kuehniella* eggs kept at 0, 4 and 8°C decreased as storage time increased. Voegelé et al. (1974) have successfully kept *E. kuehniella* eggs for 60 days between −1 and 4°C for *Trichogramma* multiplication.

Shelf life of host eggs is not only influenced by the temperature of conservation but also by the packaging. Vacuum packing, for example, has led to longer shelf life of host eggs for *Trichogramma* rearing. *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) eggs that were vacuum packed stayed suitable for *Trichogramma chilonis* Westwood parasitism for a period three to four times longer than eggs that were not (Jalali et al. 2007). Ramos and Jiménez (1993) also found that vacuum packing was beneficial for the conservation of *S. cerealella* eggs. They obtained 2400 parasitized eggs by *Trichogramma* per square inch for 35 days versus 25 days for non-vacuum packing eggs (minimal parasitism rate required by the Instituto Colombiano Agropecurio).

All these conservation methods were tested individually, but they have not been compared. The aim of this study was to compare 15 different sterilization–conservation methods of *E. kuehniella* eggs to obtain the longest conservation period while remaining suitable for *Trichogramma ostriniae* Pang and Chen parasitism and therefore for mass rearing of that biological control agent.

## Material and Methods

### Insect material

*Ephestia kuehniella* eggs were obtained from Anatis Bioprotection Inc. (St-Jacques le Mineur, QC, Canada) rearing. *Trichogramma ostriniae* were obtained from IPM Laboratory Inc. (Locke, NY). The species was imported by the USDA APHIS Mission Biological Control Center, Mission, Texas from Jilin Province in Northern China (Wang et al. 1997).

### *Ephestia kuehniella* eggs treatments

*Ephestia kuehniella* eggs less than 24 h old were used for the experiment. Fifteen treatments of *E. kuehniella* egg conservation were carried out. Nine treatments involved liquid nitrogen conservation: (i) eggs alone; (ii) eggs with water; (iii) eggs in a 5% glycerol solution; (iv) eggs in a 10% glycerol solution; (v) eggs in a 20% glycerol solution; (vi) eggs in a 30% glycerol solution; (vii) eggs in a 5% dimethyl sulfoxide (DMSO) solution; (viii) eggs in a 10% DMSO solution; and (ix) eggs in a 20% DMSO solution. For each treatment, one gram of *E. kuehniella* eggs in a 1.5-ml tube filled with the solutions was kept at 4°C for 30 min, then transferred at −20°C for 2 h and finally immerged in liquid nitrogen at −196°C for 1–4 weeks. Then, eggs were taken out of the liquid nitrogen, thawed in a 40°C water bath, rinsed 3 times with water and dried for 4 h at 30°C before they were exposed to *Trichogramma* parasitism.

Two treatments involved UV egg sterilization and storage at 4°C: (x) eggs UV sterilized and vacuum packed (UV vacuum 4°C) and (xi) eggs UV sterilized and non-vacuum packed (UV non-vacuum 4°C). For UV sterilization treatments, the eggs were put in an UV Germicidal Sterilizer Mini 209 (YCC Products Inc., Placentia, CA, USA) at 10 cm from an 8 W lamp producing light at a 254-nm wavelength for 15 min (St-Onge et al. 2014). For the vacuum packing, 5 g of eggs was placed in a 200-ml jar and vacuum was done at a 500 mm/Hg pressure with a V2240 pump (Jarden Corporation, Boca Raton, FL, USA).

For four treatments, eggs were sterilized by freezing at −15°C: (xii) eggs frozen, vacuum packed and then stored at 4°C (Frozen vacuum 4°C), (xiii) eggs frozen, non-vacuum packed and stored at 4°C (Frozen non-vacuum 4°C), (xiv) eggs frozen, vacuum packed and stored at −15°C (Frozen vacuum −15°C) and (xv) eggs frozen, non-vacuum packed and stored at −15°C (Frozen non-vacuum −15°C). For freezing treatments, eggs were put in a freezer at −15°C for 4 h (St-Onge et al. 2014). Eggs were vacuum packed as described above. Eggs were treated differently; eggs sterilized at −15°C and by UV irradiation were vacuumized before and after they were sterilized, respectively.

For each of the 15 treatments, five replicates were performed for the four durations (1–4 weeks) of

 

conservation (n = 15 × 5 × 4 = 300). Each week, 75 samples were removed from their conservation conditions for evaluation.

### Treatment evaluation for parasitism by *T. ostriniae*

The evaluation of the treatments was done under laboratory controlled conditions (24 ± 1°C, 60 ± 5% R.H., 16L:8D photoperiod) after 1–4 weeks of conservation. First, the visual aspect of the eggs was noted after they were removed from their conservation condition and it was noted again 7 days later. The colour and the shape of the eggs were observed under magnification each week for each treatment. Secondly, for each treatment, 0.2 g (~7400 eggs) was glued on cardboard with non-toxic mucilage glue by Elmer's Products Inc. (Columbus, OH, USA). Treated eggs were placed in a glass jar with 0.002 g of parasitized *T. ostriniae* eggs about to emerge. Emerging parasitoids, that is ~250 females, were allowed to parasitize treated eggs for 24 h, and then, the eggs were removed and placed in a new jar. The parasitism rate (i.e. the number of black eggs over the total number of eggs) was calculated 7 days after the contact of the females with the treated eggs. Eggs sterilized by UV and by freezing at −15°C were also submitted to the parasitism of *T. ostriniae* without conservation as a control. Data were analysed using a two-way ANOVA for the duration of conservation and the treatment, followed by a HSD Tukey's test ($\alpha = 0.05$). All statistical analyses were carried out using JMP 11 (SAS Institute Inc., Cary, NC, USA).

## Results

### Visual aspect of *E. kuehniella* eggs

Immediately after the eggs were removed from liquid nitrogen, they had the same colour and shape as fresh eggs whether after one or 4 weeks of conservation. Over time, eggs removed from liquid nitrogen did not change colour but collapsed less than 48 h later. For the other treatments, the egg aspect changed according to the conservation time. *Ephestia kuehniella* eggs sterilized by UV irradiation or by freezing and kept at 4°C or at −15°C became darker over the weeks. After the first week of conservation, the eggs had the same colour as that of fresh eggs except for those kept at −15°C, which had already become darker. After 2 weeks of conservation, only UV sterilized eggs that were vacuum packed and kept at 4°C, had the same colour as that of fresh eggs. Moreover, eggs sterilized by UV irradiation or by freezing and kept at 4°C or −15°C continued to become darker and slowly began to dry after they were removed from their conservation conditions.

### Conservation of *E. kuehniella* eggs in liquid nitrogen

None of the nine treatments involving the conservation of *E. kuehniella* eggs in liquid nitrogen resulted in any *T. ostriniae* production. No *E. kuehniella* eggs turned black after they were exposed to *T. ostriniae* parasitism.

### Conservation of *E. kuehniella* eggs sterilized by UV irradiation and freezing

The parasitism rates of *E. kuehniella* eggs sterilized by UV and by freezing at −15°C without conservation (control) by *T. ostriniae* were 81.2 ± 3.77% and 82.7 ± 4.35%, respectively.

The parasitism rate of *E. kuehniella* eggs by *T. ostriniae* was significantly different in relation to the number of weeks of conservation and the treatment the eggs had undergone ($F_{15,\ 96} = 90.18$; $P < 0.0001$) (fig. 1). The *T. ostriniae* parasitism rate decreased with increasing duration of the *E. kuehniella* eggs conservation for all treatments. After 1 week of conservation, three treatments had significantly higher parasitism rates: UV vacuum 4°C (83.0 ± 2.92%), UV non-vacuum 4°C (77.4 ± 3.36%) and frozen vacuum 4°C (83.2 ± 3.7%) (fig. 1). After 2 weeks of conversation, one treatment had a significantly higher parasitism rate: UV vacuum 4°C (75.4 ± 4.62%). This treatment was the only one that was not significantly lower after 2 weeks of conservation compared to 1 week. After

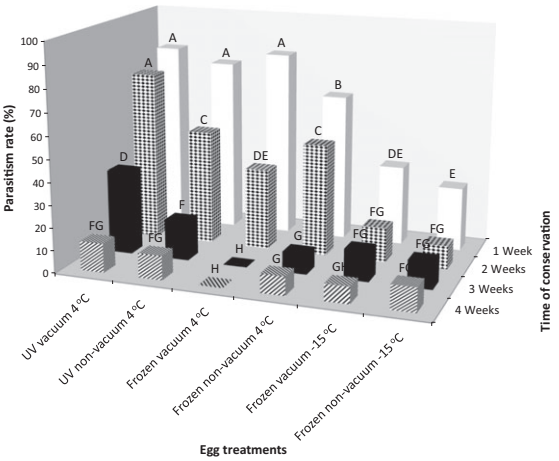


**Fig. 1** Parasitism rate by *Trichogramma ostriniae* of *Ephestia kuehniella* eggs sterilized and conserved under six different treatments during 4 weeks. Bars sharing the same letter are not significantly different (Tukey, $\alpha = 0.05$).

 

3 weeks of conservation, the parasitism rates of all the treatments were under 50% but again the parasitism rate of the UV vacuum 4°C treatment was significantly higher (37.2 ± 5.81%) than in the other treatments. After 4 weeks of conservation, the parasitism rates of all treatments were under 20%. Three treatments had significantly higher parasitism rates: UV vacuum 4°C (13.0 ± 1.87%), UV non-vacuum 4°C (10.8 ± 0.84%) and frozen non-vacuum −15°C (10.8 ± 1.92%) (fig. 1).

## Discussion

Although a quick immersion of *E. kuehniella* eggs in liquid nitrogen allowed 33.65% parasitism by *T. ostriniae* (St-Onge et al. 2014), the long-term conservation of *E. kuehniella* eggs in liquid nitrogen was not possible. After their removal from the liquid nitrogen, eggs had the appearance of fresh eggs but collapsed, that is the rounded eggs became flat within 48 h. It is possible that the thin chorion of *E. kuehniella* eggs (Schmidt 1994) is responsible for their collapse. Liquid nitrogen conservation has been the most successful for eggs with a very thick chorion such as *T. basalis* eggs. For eggs with a thin chorion like *E. kuehniella* eggs, we should rely on other techniques like vitrification (the transformation of a material into an amorphous solid that is free of any crystalline structure), which has been proven as effective as or even better than traditional slow-rate freezing (Kuwayama 2007). When the cooling occurs very rapidly at extremely low temperatures without freezing, this is known as vitrification. In vitrification, smaller samples are beneficial by increasing both cooling and warming rates, decreasing the chance of ice crystal nucleation/formation (Kuwayama 2007). Because vitrification is more effective with small samples, this limits its potential as a conservation method for the large quantity of host eggs needed in mass rearing.

Despite the fact that some parasitism occurs with eggs stored at −15°C, freezing is not a suitable conservation method of *E. kuehniella* eggs for *T. ostriniae* parasitism. Freezing may cause damage to the egg structure (Hu et al. 1999). The development of the parasitoid can also be compromised by the reduced nutrient quality resulting from the denaturation of the large proteins typically induced by freezing (Hu et al. 1999). Although freezing is not suitable for *Trichogramma* host egg storage, warmer temperatures seem to allow higher parasitism rates. Özder (2002) found that the parasitism rate of *E. kuehniella* eggs by *T. cacoeciae*, *Trichogramma brassicae* and *Trichogramma evanescens* decreased significantly when they were treated for 1 h at −20°C. St-Onge et al. (2014) found that the parasitism rate of *E. kuehniella* eggs by *T. ostriniae* was not significantly different after 4 h at −15°C compared to that of fresh eggs. A higher freezing temperature seemed less damaging to *E. kuehniella* eggs. Although parasitism was significantly reduced, after 4 weeks of storage at −15°C, *T. ostriniae* still parasitized 7.6% and 10.8% of the eggs (fig 1).

UV irradiation of eggs followed by vacuum packing and storage at 4°C was the most suitable treatment to conserve *E. kuehniella* eggs for *T. ostriniae* parasitism for 1–3 weeks. A percentage of parasitism over 70% (75.4%) was still observed for these eggs after 2 weeks of conservation. Two weeks is not a long shelf life compared to what was reported by Jalali et al. (2007). They observed that the *T. chilonis* parasitism rate was over 70% for *C. cephalonica* eggs conserved in vacuum packing at 8 ± 1°C for 42 days. Jalali et al. (2007) suggested that the vacuum packing may have induced the diapause of this host which, in turn, preserved their suitability for parasitism. In the present study, diapause of the host eggs could not occur because the eggs were sterilized. Voegelé et al. (1974) for their part worked with *E. kuehniella* eggs sterilized by UV irradiation and stored between −1 and 4°C. They found that the eggs remain suitable for *Trichogramma* multiplication for 60 days. In their study, they used *Trichogramma* species other than ours: *T. evanescens* and *T. brasilienses*. This may explain the difference between the shelf life of the same host eggs species. The acceptation of a host egg varies with the species of *Trichogramma* (Leopold 1998; de Carvalho Spínola-Filho et al. 2014). *Trichogramma ostriniae* may be a more selective species when it comes to the quality of its host eggs.

In conclusion, liquid nitrogen, although not suitable for conservation of *E. kuehniella* eggs, should be considered for other host eggs as with this method conservation may last for months. Freezing at −15°C was the easiest method, requiring very little manipulation and no specific equipment. However, higher freezing temperatures should be tested as they seem to improve the suitability of *E. kuehniella* eggs to *Trichogramma* parasitism. We think that the most suitable method for the short-term conservation of *E. kuehniella* eggs for *T. ostriniae* rearing would be sterilization by UV irradiation followed by vacuum packing and storage at 4°C.

## Acknowledgements

We thank Catherine Jumarie for her help and for providing the equipment for the liquid nitrogen

 

conservation treatments. We also thank Jill Vandermeerschen for the revision of the statistical analysis. This research was supported by the Fonds de recherche du Québec – Nature et technologie FQRNT, Natural Sciences and Engineering Research Council of Canada NSERC and Anatis Bioprotection Inc.

## References

de Carvalho Spínola-Filho PR, Demolin Leite GL, Alvarenga Soares M, Costa Alvarenga A, de Paulo PD, Tuffi-Santos LD, Cola Zanuncio J, 2014. Effects of duration of cold storage of host eggs on percent parasitism and adult emergence of each of ten Trichogrammatidae (Hymenoptera) species. Fla Entomol, 97, 14–21.

Corrêa-Ferreira BS, de Oliveira MCN, 1998. Viability of *Nezara viridula* (L.) eggs for parasitism by *Trissolcus basalis* (Woll.), under different storage techniques in liquid nitrogen. An Soc Entomol Brasil, 27, 101–107.

Greco CF, Stilinovic D, 1998. Parasitization performance of *Trichogramma* spp. (Hym., Trichogrammatidae) reared on eggs of *Sititroga cerealella* Oliver (Lep., Gelechiidae), stored at freezing and subfreezing conditions. J Appl Entomol, 122, 311–314.

Hu JS, Gelman DB, Bell RA, 1999. Effects of selected physical and chemical treatments of Colorado potato beetle eggs on host acceptance and development of the parasitic wasp, *Edovum puttleri*. Entomol Exp Appl, 90, 237–245.

Jalali SK, Venkatesan T, Srinivasa Murthy K, Rabindra RJ, Lalitha Y, 2007. Vacuum packaging of *Corcyra cephalonica* (Stainton) eggs to enhance shelf life for parasitization by the egg parasitoid *Trichogramma chilonis*. Biol Control, 41, 64–67.

Kuwayama M, 2007. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. Theriogenology, 67, 73–80.

Leopold RA, 1998. Cold storage of insects for integrated pest management. In: Temperature sensitivity in insects and application in integrated pest management. Ed. by Hallman GJ, Denlinger DL, Westview Press, Boulder, CO, 235–267.

Ma HY, 1988. Studies on long-term storage of hosts for propagating *Trichogramma*. In: *Trichogramma* and other egg parasites, 2nd International Symposium Guangzhou, Les Colloques de L'INRA, China, Paris, 43, 369–371.

Özder N, 2002. Parasitation performance of *Trichogramma cacoeciae*, *T. evanescens* and *T. brassicae* (Hym. Trichogrammatidae) reared on the embryos of *Ephestia kuehniella* Zell. (Lep. Pyralidae) killed by freezing. Great Lakes Entomol, 35, 107–111.

Özder N, 2004. Effect of different cold storage periods on parasitization performance of *Trichogramma cacoeciae* (Hymenoptera, Trichogrammatidae) on eggs of *Ephestia kuehniella* (Lepidoptera, Pyralidae). Biocontrol Sci Techn, 14, 441–447.

Ramos JP, Jiménez JV, 1993. Conservacion en frio de huevos de Sitotroga cerealella (Olivier) en refrigeracion y empacados al vacio a diferentes presiones. Rev Colomb Entomol, 19, 64–71.

Schmidt JM, 1994. Host recognition and acceptance by *Trichogramma*. In: Biological control with egg parasitoids. Ed. by Wajnberg E, Hassan SA, CAB International, Oxon, 165–200.

St-Onge M, Cormier D, Todorova S, Lucas É, 2014. Comparison of *Ephestia kuehniella* eggs sterilization methods for *Trichogramma* rearing. Biol Control, 70, 73–77.

Voegelé J, Daumal J, Brun P, Onillon J, 1974. Action du Traitement au Froid et aux Ultraviolets de l'œuf d'*Ephestia kuehniella* (Pyralidae) sur le Taux de Multiplecation de *Trichogramma evanescens* et *T. brasiliensis* (Hymenoptera: Trichogrammatidae). Entomophaga, 19, 341–348.

Wang B, Ferro DF, Hosmer DW, 1997. Importance of plant size, distribution of egg masses, and weather conditions on egg parasitism of the European corn borer, *Ostrinia nubilalis* by *Trichogramma ostriniae* in sweet corn. Entomol Exp Appl, 83, 337–345.

 