Ephestia kuehniella eggs sterilization for *Trichogramma ostriniae* Pang et Chen (Hymenoptera: Trichogrammatidae) mass production

M. St-Onge¹, D. Cormier², S. Todorova³, É. Lucas¹

¹ Département des sciences biologiques, Université du Québec à Montréal, ² Institut de recherche et de développement en agroenvironnement (IRDA), ³ Anatis Bioprotection

Introduction

Trichogramma ostriniae, a parasitoid of *Ostrinia furnacalis*, was introduced into the United States in 1990 for evaluation of its potential to parasitize eggs of the European corn borer *Ostrinia nubilalis* (Wang *et al.*, 1997). *Trichogramma ostriniae* appeared to be one of the most effective species against this pest. The rearing host *Ephestia kuehniella* eggs need to be sterilized because the voracious larvae of this species can affect the quantity of produced trichogramma by consuming parasitized eggs. Sterilization can be achieved by several ways: cold, heat, ultraviolet radiation, gamma rays and chemical (Vogelé et al., 1974). This study evaluates three different modes of sterilization: UV irradiation which is generally used, freezing at -15° C which has not been well studied, and liquid nitrogen (-196° C) where, if suitable, we can store the eggs for a long period. For each sterilization mode, we determined the minimal exposure time that prevented the *E. kuehniella* larvae emergence and the suitability of these eggs to be used in trichogramma production. Our hypothesis was that sterilization by liquid nitrogen would provide the best parasitism rate and trichogramma quality by *T. ostriniae* since vitrification generated by this temperature causes less damage than freezing process at higher temperature and UV irradiation.

Methods

Minimal exposure time: UV Irradiation (253,7nm) - 200 eggs each 5 minutes for 60 minutes Freezing (-15° C) - 200 eggs each hour for 8 hours

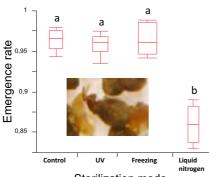
Freezing (-15° C) - 200 eggs each hour for 8 hours Liquid nitrogen (-196° C) - 200 eggs each 15 seconds for one minute then thawed in 40° C water for 5 minutes

 200 eggs each 15 seconds for one minute then thawed in 40° C water for 5 minutes Variable: larvae emergence rate Not sterilized eggs control; 5 replicates

Results

Table 1: Percentage of *E. kuehniella* larvae emergence after different time of sterilization by UV irradiation, Freezing at -15° C and Liquid nitrogen (Mean ± SE)

	UV	- 15º C	Liquid nitrogen
0	98,4 ± 1,3	98,4 ± 1,3	98,4 ± 1,3
5 min., 1 hour, 15 sec.	63,2 ± 2,2	74,6 ± 4,5	32,6 ± 1,6
10 min., 2 hours, 30 sec.	0,8 ± 0,1	57,9 ± 3,5	0
15 min., 3 hours	0	25,8 ± 2,8	
4 hours		0	



Sterilization mode

Fig. 2: Emergence rate of *T. ostriniae* as a function of sterilization mode. Boxes sharing the same letter are not significantly different (P > 0,05; Tukey test).

Sterilization mode comparison: 24° C; 16L: 8D; 60 % RH 10 000 eggs of each sterilization mo



24° C; 16L: 8D; 60 % RH 10 000 eggs of each sterilization mode for 500 females *T. ostriniae* (1/20) for 24 hours Not sterilized eggs control; 5 replicates

Variables: parasitism rate and emergence rate (500 eggs/replicate), % of female and % of deformed adults (100 individuals/replicate), female fecundity for 24 hours and walking speed (30 females/replicate)

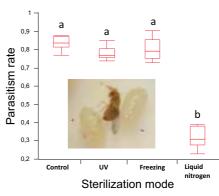


Fig. 1: Parasitism rate of *E. kuehniella* eggs by *T. ostriniae* as a function of sterilization mode. Boxes sharing the same letter are not significantly different (P > 0,05; Tukey test).

Table 2: Quality control variables for each sterilization mode (Mean ± SE)

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	% Female	% Deformed adults	Female fecundity (24 h)	Walking speed (mm/s)
Control	63,8 ± 9,3	4,8 ± 1,3	46,8 ± 5,5	4,8 ± 1,1
UV	62,4 ± 8,3	5,2 ± 1,3	34,3 ± 11,8	5,2 ± 0,9
Freezing	62,4 ± 9,2	5,2 ± 1,3	41,8 ± 8,4	4,9 ± 1,0
Liquid nitrogen	60,7 ± 9,5	6,6 ± 1,1	35,4 ± 7,3	4,6 ± 1,1
	ns	ns	ns	ns

Conclusions

- > Regardless of the sterilization mode, the quality of trichogramma produced was similar.
- Although sterilization with liquid nitrogen was the fastest method to prevent larvae emergence, it is unsuitable for E. kuehniella eggs used to trichogramma production. The vitrification didn't preserve the quality of the eggs.
- Freezing at -15° C requires more time and, although not significantly different than UV irradiation, is the sterilization method to favour because, in mass production, a difference of 2 % represent million of trichogramma.







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