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Suitability of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) Eggs for Parasitisation by *Trichogramma evanescens* Westw and *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae)

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Abstract: Suitability of *Ephestia kuehniella* (Zeller) eggs at various ages (2, 4, 8, 12, 24, 48 or 72-h-old eggs), as a substitute host for rearing *Trichogramma evanescens* Westw. and *Trichogramma cacoeciae* Marchal, were tested. Also, the effect of chilling on hatching and parasitism of *E. kuehniella* eggs (>24-h-old) at 2, 4, 8, 12, 24, 48 and 72 chilling hours by the two *Trichogramma* species was studied. Eggs, at 28°C and 50-60% R.H., only 2 hold were not very attractive to the two parasitoid species. In general, the number of host eggs attacked, decreased with increasing age. The highest rate of parasitism occurred at 4 h and the lowest at 72 h. However, eggs of all ages proved suitable for the development of *T. evanescens* and *T. cacoeciae*, with no significant differences in the emergence rate from *E. kuehniella* eggs. Moreover, *T. evanescens* was more suitable for biological studies on of *E. kuehniella* eggs than *T. cacoeciae*. When *E. kuehniella* chilled eggs (>24-h-old) for 2, 4, 8, 12, 24, 48 or 72 h, at -1°C, were exposed to the female of the two-trichogrammatid species, the rate of parasitism was diminished as the period of chilling increased. Prolonged exposure of eggs to cold resulted to the death of embryos and reduced hatching.

Key words: Ages, chilling, parasitism, hatching, *Ephestia kuehniella*, *Trichogramma evanescens*, *Trichogramma cacoeciae*

Introduction

The egg parasitoids, *Trichogramma* spp. have gained in biological control, widespread interest in many countries, partly because they are easy to mass rear and attack eggs of many economic crop insect pests (Li-Ying, 1994). Despite the fact that intensive studies have been conducted on *Trichogramma*, the identification and selection of suitable time for controlling lepidopterous pests are still one of the important issues.

Host age is a limited factor for selecting the suitable laboratory host for different species of *Trichogramma*. It has a great influence upon biological activity and fertility of the adult emergence (Schmidt, 1970). For example, *Trichogramma australicum* Girault preferred eggs of *Corcyra cephalonica* Stainton among 24 and 48 hours of age. But the percentage of eggs of *Trichoplusia neither* Hübner parasitized by *Trichogramma minutum* Riley was similar in all the ages tested, while the young one (1-11 h.) guaranteed the best development for the parasitoid (Marston and Ertle, 1969).

Cold injury of host's eggs has been used to improve parasitoid rearing programs for egg parasitoids, where the increase demand for the supply of *Trichogramma* parasitoids, prolonged storage of eggs under chilled conditions would be necessary to have continuous supply of host eggs (i.e. *E. kuehniella* and *C. cephalonica* eggs). The host eggs exposed to temperatures (i.e., super-cooling points) distinctly below 0°C supported parasitoid survival at later stages of egg development than fresh eggs (Drooze and Weems, 1982). Many authors (Hu and Xu, 1988; Hugar *et al*., 1990; Burks *et al*., 1997) studied the effect of chilling host eggs on parasitism of *Trichogramma* spp.

The objective of this investigation is to test the eggs of *Ephestia kuehniella* (Zeller) at different ages for their suitability to rear the two parasitoid species *Trichogramma evanescens* Westw. and *Trichogramma cacoeciae* Marchal through the estimation of their percentage parasitization and percentage emergence. Also, to study the effect of chilling period on *E. kuehniella* egg hatching, rate of parasitism and parasitoid emergence.

Materials and Methods

Rearing of host and parasitoid species: The Mediterranean flour moth, *E. kuehniella* was reared in glass jars (volume = 1 Kg) containing sterilized soft wheat flour as food for larvae under the laboratory conditions of 28°C and 50-60% R.H.

The egg parasitoids, *T. evanescens* and *T. cacoeciae* were obtained from the Department of Biological Control, A.R.C., reared in glass cylinders (25 cm long and 10 cm

diameter) and kept under the controlled conditions of 28°C and 50-60% R.H. To ensure uniformity of *Trichogramma* age for the experiments, *E. kuehniella* egg stripes were taken out after 24 hours and exposed to the females of each parasitoid species. Parasitized eggs were then left in separate containers, under the same laboratory conditions, to allow development of parasitoids. Days before emergence, the eggs containing parasitoid pupae were kept in emergence tubes for the experiments.

Tests

Parasitoid tests

For host ages: Ten replicates of eighty of 2, 4, 8, 12, 24, 48 and 72-h-old *E. kuehniella* eggs were distributed on the sticky strips. Each of these strips was exposed to 20 females from each of *T. evanescens* or *T. cacoeciae* to allow parasitism for a period of 12 hours. Parasitized eggs were stored in glass tubes (10 cm length x 4 cm diameter) covered with muslin. The rate of parasitism (number of blackened host eggs /total eggs on the strip) and percentage emergence for each treatment were estimated.

For host chilling: Ten replicates of eighty chilled *E. kuehniella* eggs (>24-h-old) for 2, 4, 8, 12, 24, 48 or 72 h, at -1°C were exposed to 20 females of the two parasitoid species to allow parasitism for a period of 12 hours. The parasitized eggs were kept in the glass tubes to calculate the rate of parasitism and percentage emergence in each treatment.

Host tests: Three replicates of *E. kuehniella* (>24-h-old) egg strips (100 eggs / strip) were chilled at -1°C for 2, 4, 8, 12, 24, 48 or 72 h as compared to control (strip of eggs kept at rearing temperature). The strips were left individually in glass tubes until hatching.

- All tests were kept under the controlled laboratory conditions of 28°C and 50-60% R.H.

Statistical analysis: Factorial analyses of variances (ANOVA) with Duncan's multiple range tests were used (Snedecor and Cochran, 1980).

Results and Discussion

Effect of *E. kuehniella* eggs at various ages on parasitism: Percentage of parasitism by *T. evanescens* and *T. cacoeciae* reached (92.00 and 94.88%) and (83.25 and 88.25%) for 2 and 4 h eggs' age, respectively. While for 8 and 12 h eggs the parasitism by *T. cacoeciae* attained 86.63 and 84.38%, respectively, (Table 1). Correspondent parasitism rates by *T. evanescens* for the same ages were 93.75 and 92.50%, respectively. The rate of parasitism decreased in the host eggs from 24 to 48 h old (89.00 and 85.88% & 79.48 and 74.25%) and from eggs of 72 h old (76.13 and 65.25%) for *T. evanescens* and *T. cacoeciae*, respectively. The percent of *E. kuehniella* eggs parasitized by *T. evanescens* and *T. cacoeciae* were significantly affected at the three last ages tested (24, 48 and 72 h old).

The results indicated that the 2 h old *E. kuehniella* eggs were not very attractive to the two parasitoid females and it was observed a diminish parasitism towards the eggs from 48 to 72 hours, next to the moment of the eclosion. Our results agree with those obtained by Benoit and Voegele (1979) who decided that the parasitism of *E. kuehniella* eggs by *T. evanescens* was smaller at the beginning and to the end of the higher and embryonic development. Also, Sivapragasam and Ahmad (1986) who indicated that *C. cephalonica* eggs attractiveness at various ages as host of *T. australicum*, decreased with increasing age. Remund and Bigler (1986) found that the eggs of *Eupoecilia ambiguella*, less than 4 days old (with the head capsule not visible) were equally preferred to the species *T. dendrolimi*; while the percentage parasitism decreased sharply almost to zero when the head capsule had developed.

In general, there was none differentiates among the adults emergency percentages of *T. cacoeciae* and *T. evanescens*, among the eggs of different ages. These results are similar to those reported by Parker and Pinnel (1974) in case of the eggs of *Pieris rapae* at different ages where they did not observe differentiates significant as for percentages of emergency of *T. evanescens*.

Data shows that *T. evanescens* was more suitable for biological studies on *E. kuehniella* eggs than *T. cacoeciae* as the foundation of Schöller (1999).

Effect of chilled *E. kuehniella* eggs on parasitism: Treatments of chilled *E. kuehniella* eggs (>24-h-old) for 2, 4, 8, 12, 24, 48 or 72 h before parasitization by *T. evanescens* or *T. cacoeciae* are given in (Table 2). Maximum percentages of parasitism were 90.38 or 81.98%, for eggs chilled for a period of 2 h, for *T. evanescens* or *T. cacoeciae*, respectively. Whereas the minimum of 59.13% or 50.00 of eggs were parasitized by the two *Trichogramma* species, when the host eggs chilled for a period of 72 h, respectively. The results showed significant differentiates for percentages of parasitism of *T. cacoeciae* and *T. evanescens* among eggs chilled for different periods; except those chilled for a period of 8 and 12 h appeared none differences. Concerning, the percent of parasitism between *T. cacoeciae* and *T. evanescens* significant differences were observed. On

Table 1: Rate of parasitization by *T. evanescens* and *T. cacoeciae* and percent emergence of the parasitoid adults, at various ages of *E. kuehniella* eggs

E. kuehniella ages age (ages/ h)	% of parasitization		% of emerged parasitoid adults	
	T. evanescens	*T. cacoeciae*	*T. evanescens*	*T. cacoeciae*
2 h	92.00±3.13^{b*} a**	83.25±5.82^{a*} b**	96.49±1.37	96.19 ±1.80
4 h	94.88±2.73^{b} a	88.25±3.75^{a} a	95.76±2.26	95.35 ±3.29
8 h	93.75±2.70^{b} a	86.63±3.27^{a} ab	95.30±2.05	95.11 ±2.45
12 h	92.50±3.23^{b} a	84.38±3.10^{a} b	94.99±2.73	94.86 ±2.30
24 h	89.00±3.37^{b} b	79.48±4.38^{a} c	94.43±2.41	94.51 ±2.02
48 h	85.88±4.15^{b} c	74.25±3.73^{a} d	93.85±2.85	93.29±3.24
72 h	76.13±2.91^{b} d	65.25±3.32^{a} e	93.01±2.94	92.15±2.29
L.S.D.	2.9295	3.6594	-----	-----

Table 2: Rate of parasitization by *T. evanescens* and *T. cacoeciae* and percent emergence of the parasitoid adults, at chilling *E. kuehniella* eggs.

Chilled period of *E. kuehniella* >24 h-old eggs (hours)	% of parasitization±S.D.		% of emerged parasitoid adults	
	T. evanescens	*T. cacoeciae*	*T. evanescens*	*T. cacoeciae*
2 h	90.38±4.18^{b*} a**	81.98±4.10^{a*} a**	95.46± 1.87	95.31±2. 80
4 h	84.00±4.63^{b} b	73.38±3.81^{a} b	94.82±3.67	94.53±2.11
8 h	75.05±4.82^{b} c	66.00±4.28^{a} c	94.38±2.02	94.17± 2.94
12 h	72.18±4.08^{b} c	62.63±3.64^{a} c	93.86±2.87	93.63± 2.99
24 h	68.00±3.95^{b} d	58.5 0± 3.85^{a} d	93.19±2.63	93.18± 4.2 7
48 h	63.50±2.85^{b} e	54.00±4.59^{a} e	92.65±5.36	92.21± 3.15
72 h	59.13±4.02^{b} f	50.00±5.21^{a} f	92.55±2.60	91.57±2.77
L.S.D.	3.7622	3.8736	-----	-----

Equal letters in column do not present differentiates significant (P> 0.05). Test of Duncan.
* In each row indicates differentiates significant among species.
**Indicates differentiates significant differences among different treatments for the same species.

Table 3: Effect of chilling on the hatchability of *E. kuehniella* eggs, at their various ages.

E. kuehniella eggs at chilled period (hours)	% of hatching after chilling eggs Mean±S.D.	Range
Control	92.60±3.05 a	89.00-97.00
2 h	82.80±2.28 b	80.00-85.00
4 h	75.28±2.78 c	70.00-7 6 .00
8 h	61.40±2.07 d	5 9.00- 64 .00
12 h	37.40±3.21 e	33.00-40.00
24 h	21.80±1.79 f	20.00-24.00
48 h	11.00±2.00 g	9.00-14.00
72 h	3.60±2.41 h	1.00-7.00
L.S.D.	3.2222	-.------

Percentage which are not significantly different are followed by the same number (Significance = 0.05)

the other hand, adult emergence percentage of both parasitoid species, among the eggs chilled for different periods did not present significant differentiates. In conclusion, the eggs of *E. kuehniella* when chilled for limited time were found to be preferred by the two trichogrammatid parasitoid species, where the rate of parasitism was diminished as the period of chilling increase. These results are similar to that of Torre *et al.* (1972) who found that the percentage of *C. cephalonica* parasitism reduced drastically as the chilling period advanced. Dass and Ram (1985) described that the storage of eggs of *C. cephalonica* at -6 °C for longer than 8 days adversely affected percentage parasitism by *T. exiguum*, while no effects were observed on percentage emergence. Also, Hugar *et al.* (1990) observed that when the chilling period of *C. cephalonica* eggs increased the parasitism by *T. chilonis* decreased.

Chilling effect on the hatchability of *E. kuehniella* eggs: Among the results of chilling injury treatments (at -1 °C) on *E. kuehniella* eggs for a period of 2, 4, 8, 12, 24, 48 or 72 h, the maximum hatchability of the eggs was observed at eggs chilled for 2 h (82.8%) comparing to that of the optimum temperature (92.6%). While the percent of hatching reached to 11.00 and 3.60%, when eggs were chilled for 48 and 72 h, respectively, (Table 3). These results indicated significant differences for the percentage of hatchability at different periods of chilling.

It was noticed that by increasing the period of chilling, the mortality rate of embryos increased and the hatching rate of eggs decreased. Our results are in agreement with Singh (1969) who observed that the advancement of chilling period of *Corcyra* eggs at -4°C for 16 to 76 h caused a gradual decrease in hatching which may be due to adverse effect of cold on developing embryos.

Finally, we concluded that the age of the host egg and the chilling period are of the important factors affecting parasitism. The chilled for limited times were found to be preferred by the parasitoids, but host eggs chilling supported parasitoid survival at later stages of egg development than fresh eggs.

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