JAP&T TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2021, *27*(3), 284-290

*Journal of Agroalimentary Processes and Technologies*

# Factors Affecting Development and Reproduction of *Trichogramma cacoeciae* on *Ephestia kuehniella* Eggs

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***Abstract***

In this work, we examined the effects of temperature (15-35±1 ºC), host age, parasite age and food (honey) on reproduction and rate of development of the egg parasite, *Trichogramma cocaeciae*, reared on *Ephestia kuehniella* eggs. The results showed that temperature had a significant effect on the mean number of parasitized eggs/female, developmental time, survival to adults and female longevity. Survival of immatures to adults was best at 25±1 ºC, lowest at 15±1ºC and no survival was recorded at 35±1 ºC. Furthermore, parasitization was highest in the 1st day after emergence, continued (depending on the temperature) for up to 10 days with gradual decrease and peaked at 25±1 ºC. An inverse relationship was noted between temperature, mean female longevity and developmental time; longevity decreased and developmental rate increased (the time needed for development decreased) with increasing temperature. Food, in the form of honey, increased significantly female longevity and parasitization ability and young eggs (*E. kuehniella* eggs) were significantly more suitable for parasitism than older ones.

***Keywords***: *Trichogramma cocaeciae, Ephestia kuehniella*, Rearing, Egg parasitoids

## 1. Introduction

The codling moth, *Cydia pomonella* (L.), is a key pest of apple in Syria and a serious one on pears, walnuts and some other fruit trees [1]. Worldwide, chemical control of this pest caused a wide diversity of problems including developing resistance to most insecticides, reduction in populations of natural enemies and explosion in phytophagous mite populations [2, 3]. In addition, the intensive chemical spray program used to control this insect often leaves unacceptable levels of pesticide residues on fruits causing health problems and export difficulties [4, 5]. Concerns about these issues lead plant protection authorities to consider alternative tools to control this pest. The new policy aims at adapting the concept of an integrated approach for controlling this insect [5, 6, 7]. Consequently, the use of non-insecticidal methods such as insect growth regulators, agricultural practices, pheromones and natural enemies, particularly egg parasitoids, have been given serious consideration in controlling this pest.

The egg parasitoid, *Trichogramma cacoeciae* Marchal, was recovered from codling moth eggs collected from apple orchards in southern Syria [8].

The parasitoid was also found to be very common on codling moth eggs collected from different apple production areas in the country, particularly from neglected orchards [9]. Generally, this species attacks Tortricid eggs, more frequently, *C. pomonella* and *Lobesia botrana* (Denis & Schiffermmuller) [10, 11]. Augmentative releases of *T. cacoeciae* in apple orchards require mass rearing of this species. Because rearing on its natural host can be very costly, eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, are usually used as an alternative host to rear *Trichogramma* species.

Rearing *T. cacoeciae* on *E. kuehniella* eggs efficiently requires good knowledge of the factors affecting reproduction of this species under laboratory conditions. This study intended to examine the effects of certain environmental factors on development and reproduction of *T. cacoeciae* on *E. kuehniella* eggs. Specifically, it aimed to study the effects of temperature on the mean number of parasitized eggs/female, survival to the adult stage and rate (speed) of development. In addition, it studied the effects of food availability (honey) on female longevity and parasitization ability.

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Furthermore, it examined the effects of host age (age of *E. kuehniella* eggs) on its suitability for parasitzation by *T. cacoeciae* females and parasite age (age of *T. cacoeciae*) on the mean number of parasitized eggs/female/day.

## 2. Materials and Methods

### *2.1. Rearing E. kuehniella*

*E. kuehniella* colony was maintained at 25±1 ºC, 50 ±10 %RH, 16:8 L.D. cycle and the rearing procedure was done as reported by Mansour [7]. Collected eggs were incubated at the same temperature (25±1 ºC) for egg development and used, as needed, in the experiments and stock culture maintenance.

### *2.2. Rearing T. cacoeciae*

*T. cacoeciae* females used in this study came from a laboratory colony that originated from insects collected from apple orchards in southern Syria in the spring of 2005. The *T. cacoeciae* colony was reared, ever since, on *E. kuehniella* eggs at 25±1 ºC, 60-70 % RH and 16 h. photophase. *E. kuehniella* eggs (≤49 h-old) used in rearing *T. cacoeciae* were killed by storing them at 2±1 ºC for three weeks. The rearing procedure was carried out as reported by Mansour [12].

### *2.3. Preparing "egg cards"*

"Egg cards" were prepared from strips of cardboard paper (2 x 5 cm) brushed with a thin layer of Arabic gum and allowed to dry for few minutes. The "egg cards" were, then, sprinkled with *E. kuehniella* eggs at a density of about 20 eggs/cm$^2$ (about 200/card). These "egg cards" were used to study the effects of temperature, food, and host and parasite age on development and reproduction in *T. cacoeciae* females on *E. kuehniella* eggs.

### *2.4. Temperature effects on parasitization and survival to adults*

"Egg cards" carrying *E. kuehniella* eggs were prepared (as before), placed (each) in a clean culture tube (2.5 x 20 cm) and the inner surface of each tube was provided with a fine drop of honey to feed emerging wasps. One *T. cacoeciae* female was introduced into each tube and the tubes were plugged with cotton wads. Twenty four hours later, egg cards carrying exposed eggs were removed, incubated at 25 ±1ºC for *Trichogramma* development and new "egg cards" were introduced. This was repeated as long as the females lived. The eggs were evaluated for parasitism (parasitized eggs turn black) 4 days following incubation at 25 ºC. The number of parasitized eggs and emerging wasps was recorded and percentage adult emergence was calculated. This procedure was repeated at 15, 20, 30 and 35 ±1ºC and 25 replicates (25 culture tubes) were used for each temperature.

### *2.5. Effects of T. cacoeciae age on parasitism rate*

Parasitized eggs, in the previous experiment, were counted daily for every insect and their number was recorded. The mean number of parasitized eggs/female/day was calculated by dividing the number of parasitized eggs at a certain temperature on a particular day by the number of living females in that day.

### *2.6. Effects of temperature on developmental rate*

"Egg cards" carrying each about 200 *E. kuehniella* eggs (1-24 h. old), were placed in clean culture tubes (2.5 x 20 cm) each contains ten one-day-old *T. cacoeciae* females. The "egg cards" were withdrawn after 24 h. of exposure to the wasps, placed in new clean culture tubes and the tubes were plugged with cotton wads. The culture tubes were divided into 5 groups, 25 each and each group was incubated at one of 5 different temperatures (15, 20, 25, 30 and 35 ±1ºC). "Egg cards" were examined daily and the date and number of emerged wasps was noted.

### *2.7. Effects of age of E. kuehniella eggs on parasitism*

"Egg cards" (2 x 5 cm) carrying *E. kuehniella* eggs (about 200) were placed, each, in a clean culture tube (2.5 x 20 cm). The inner surface of each tub was provided with a fine drop of honey to feed emerged wasps. Three age groups of eggs (1-24, 25-48 and 49-72 h. old) were prepared and each group was replicated 25 times (25 culture tubes). One *T. cacoeciae* female was introduced into each culture tube and the tubes were plugged with cotton wads. Twenty four hours later, exposed eggs were removed, incubated at 25 ºC for *Trichogramma* development and the females were discarded. The "egg cards" were examined daily, the number of parasitized eggs was recorded and the mean number of parasitized eggs/female was figured.

### *2.8. Effects of food on T. cacoeciae longevity and parasitization ability*

Sixty clean culture tubes (2.5 x 20 cm) were prepared, one "egg card" carrying about 200 *E.*

*kuehniella* (1-24 h. old) was introduced into each tube and the tubes were divided into two groups, 30 each. The 1st group of tubes was provided with food (a fine drop of honey on the inner side of the tube) while the 2nd group was left as a control (without any food). One day old *T. cacoeciae* female was introduced into each tube and the tubes of both groups were incubated at 25±1 ºC, 60-70 % for 24 hours after which all cards were removed and new ones were introduced. This process was repeated as long as females lived. The "egg cards" were incubated at the same temperature (25±1 ºC), examined daily and the number of parasitized eggs/female/day was calculated. Dead females were removed daily, their number was noted and mean adult longevity was figured. This experiment was repeated at 15, 20, 30 and 35±1 ºC.

### *2.9. Data analysis*

The data of the various experiments were subjected to analysis of variance (ANOVA). The treatments were arranged in a completely randomized block design and Statview program (version 4.57) was used to analyze the data. Fisher's protected least significant difference test (PLSD) was used to separate means at the 5% level of probability.

## 3. Results and discussion

Egg parasitoids, particularly species belong to the family Trichogrammatidae, have long been considered as an important component of insect pest management programs, particularly against Lepidopterous pests [13, 14]. In addition to the fact that they are the only parasitoids that kill the pest before causing any damage (kill eggs before hatching), they are easy to rear at an acceptable cost [14]. Furthermore, species belong to this family are the only species that attack Tortricid eggs [10] and, in fact, the 1st experiments to use *Trichogramma* in insect pest control were done against *C. pomonella* [15].

Several species of *Trichogramma* have been investigated as potential candidates for codling moth control [16, 17, 18, 19, 20, 21]. Among them, *T. cacoeciae*, is cited as one of the most important egg parasitoids, particularly under warm climatic conditions [21, 22]. Consequently, this species has been suggested as a biological control agent for codling moth in Syria [9].

Rearing *Trichogramma* species for field releases is often done on *E. kuehniella* eggs. Efficient production of *T. cacoeciae* on *E. kuehniella* requires good knowledge of the factors affecting reproduction, particularly temperature, food availability and host and parasite age. This paper provides data on development and reproduction of *T. cacoeciae* on *E. kuehniella* eggs reared under different temperatures. In addition, it examines the effects of food (honey) and host (*E. kuehniella* eggs) and parasite (*T. cacoeciae* females) age on reproduction and longevity.

The results of examining temperature (15-35 ±1ºC) effects on parasitization rate (mean number of parasitized eggs/female) of *T. cacoeciae* females, survival of immatures to adults, time needed for completing development to the adult stage and female longevity (with and without food) are shown in Table 1. The results show that temperature had a significant ($P<0.0001$) effect on the rate of parasite attack; parasite attack was highest between 20 and 30º C with maximum effect at 25 ±1ºC. At 15 ±1ºC and 35 ±1ºC, however, parasitization rate was very low. This suggests that a temperature regimen around 25ºC may give the best results. Data on the effects of temperature (15-35 ±1ºC) on survival of *T. cacoeciae* immatures to the adulthood shows that percentage adult emergence from parasitized eggs at different temperatures was highest under the same temperature range suitable for parasitization (20-30 ±1ºC), low (about 25%) at 15 ±1ºC and no adult emergence was recorded at 35 ±1ºC. These results are consistent with those reported before on this species [12, 23] and other *Trichogramma* species [24, 25, 26, 27, 28].

Because *Trichogramma* species are usually released in the pupal stage, knowing the developmental time and adult longevity of the released parasite under certain environmental conditions is important in determining release frequencies in the field. The results of this study show that these two parameters (developmental time and adult longevity) are inversely related to temperature; developmental time and female longevity decreased with increasing temperature. The time needed to reach the adult stage decreased from about 23 days at 15 ±1ºC to about 8 days at 30 ±1ºC. At 35 ºC, however, all *T. cacoeciae* pupae died before adult emergence.

***Table 1.*** Effects of temperature on *Trichogramma cacoeciae* parasitization rate, longevity, development and survival to adults.

| Rearing temp. (°C) | Mean ± SD | | | | |
|---|---|---|---|---|---|
| | No. of parasitized eggs/female (lifetime fecundity) | Female longevity (day) | | Develop. time (day) | Surv. to adults % |
| | | With food | Without food | | |
| 15 ±1°C | 16.4 ± 8.2[a] | 21.5 ± 11.0[a] | 2.8 ± 0.9[a] | 23.3±3.1[a] | 39.6 ±17.5[a] |
| 20 ±1°C | 45.9± 13.6[b] | 19.4 ± 3.0[b] | 2.6 ± 1.0[b] | 17.2 ±1.1[b] | 91.1 ±3.6[b] |
| 25 ±1°C | 62.3 ± 21.4[c] | 13.7 ± 2.3[c] | 2.3 ± 1.0[c] | 12.0±1.0[c] | 97.6 ± 2.5[c] |
| 30 ±1°C | 58.3 ± 16.6[c] | 8.2 ± 2.1[d] | 1.9 ± 0.8[d] | 7.9±0.9[d] | 96.9 ± 4.0[c] |
| 35 ±1°C | 3.5 ± 2.2[d] | 3.0 ± 1.1[e] | 1.2 ± 0.4[e] | 0.0±0.0[e] | 0.0 ± 0.0[d] |

Means followed by the same letter within each column are not significantly different (p=0.05, Fisher's LSD test).

These results are in agreement with those reported before for *T. cacoeciae* on *C. pomonella* eggs [12] and some other *Trichogramma* species [14, 22, 29, 30].

The effects of *T. cacoeciae* female age on the mean number of parasitized eggs/female/day at different temperatures (15-35±1ºC) are presented in Fig. 1. The figure clearly shows that most eggs were laid during the first few days of life. The results also show that the rate of *T. cacoeciae* attack was at its maximum during the first day of life and decreased gradually afterwards. It also shows that parasitization continued (depending on the temperature) for 2-10 days. In addition, it shows that the mean number of parasitized eggs/female/day decreased continuously with increasing female's age.

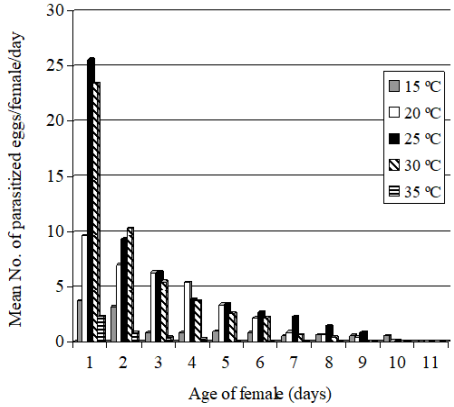


***Figure 1.*** Effects of age of *T. cacoeciae* females on the mean number of parasitized eggs/female/day at different temperatures

This suggests that, for laboratory rearing, it is probably not economical to rear *T. cacoeciae* for more than few days (depending on the rearing temperature).

Fig. 2 presents data on the effects of host age (age of *E. kuehniella* eggs) on parasitization rate (mean number of parasitized eggs/female). The figure shows that 1-24 h. old *E. kuehniella* eggs were the most suitable for parasitization by *T. cacoeciae* females. The acceptability of *E. kuehniella* eggs to *T. cacoeciae* females, however, decreased significantly (P>0.0001) with increasing their age and 49-72 h. old eggs (the oldest used eggs) were not much suitable for parasitization. In fact, it has been found before that host age is one of the most important factors in determining host acceptability by females in *Trichogramma* species [31]. Similar results were also reported by several other researchers before [12, 28, 32, 33, 34]. The decrease in acceptability of *E. kuehniella* eggs to parasitization with increasing age is probably due to the development of the embryo inside the egg or the hardening of the egg chorion [25, 31].

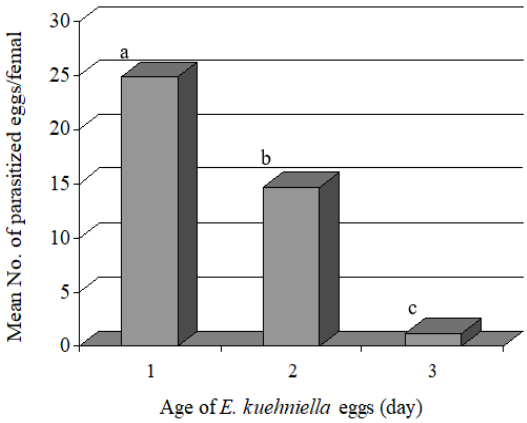


***Figure 2.*** Effects of host age (*E. kuehniella* eggs) on the mean number of parasitized eggs by *T. cacoeciae* females

Fig. 3 presents data on the effects of food (honey) on parasitization rate by *T. cacoeciae* females.

The results clearly show that food significantly increased female's parasitization rate (P>0.0001); the mean number of parasitzed eggs/female increased by about 53% when food was provided. In addition, food significantly increased (P>0.0001) female's longevity (Table 1). Female longevity, although decreased consistently with increasing temperature, was much higher when food was provided. Mean female longevity decreased from about 22 days at 15 ±1ºC to 3 days at 35 ±1ºC. In the case of not providing food (honey), longevity was much lower and ranged from about 3 days at 15 ±1ºC to about a day at 35 ±1ºC. These results are in agreement with data reported by other researchers [12, 35, 36, 37, 38].

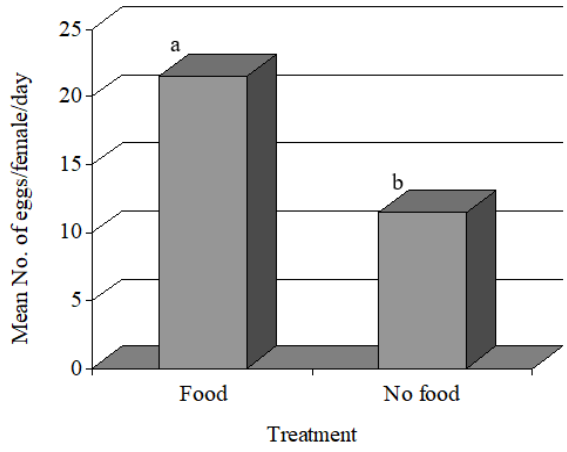


***Figure 3.*** Effects of food on the mean number of parasitized eggs by *T. cacoeciae* females

## 4. Conclusion

To summarize, this paper provides data on the development and reproduction of *T. cacoeciae* on *E. kuehniella* eggs under different laboratory conditions. The data clearly show that *E. kuehniella* eggs, particularly young ones, are good host for *T. cacoeciae* rearing and the best temperature for rearing is between 20 and 30±1°C with maximum results at 25±1°C. It also shows that food in the form of honey is important in increasing female longevity and egg production. Furthermore, the data shows that most *T. cacoeciae* eggs are laid in the 1st few days of life and, consequently, it may not be economical to maintain rearing for more than few days in mass rearing facilities.

**Compliance with Ethics Requirements.** Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

**Acknowledgements**: The author would like to thank Dr. I. Othman, Director General of the Syrian Atomic Energy Commission and Dr. F. Kurd-Ali, Head of the Department of Agriculture for supporting this project. This research was supported, in part, by the International Atomic Energy Agency, Vienna, Austria (Res. Contract No. SYR/15574).

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