How does host quality affect oviposition rate and diapause in sexual and asexual *Trichogramma brassicae*?

Somayeh Rahimi-Kaldeh, Ahmad Ashouri* and Alireza Bandani

Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.

**Abstract:** The egg parasitoids of the genus *Trichogramma* have become one of the most important economic insects, for they are widely used in biological control of Lepidopteran key pests. For that reason, all aspects of their mass production including diapause have been considered over the last few years. In this study, the effect of host quality on the diapause induction of sexual and asexual *T. brassicae* Bezdenko (Hym: Trichogrammatidae) have been investigated at two temperatures. Maternal generation developed at two distinct temperatures (14 and 20 °C) and they oviposited on four different qualities of *Ephestia kuehniella* Zeller (Lep: Pyralidae) eggs including fresh eggs, frozen eggs, host eggs with developing embryo and low quality eggs. Data analysis revealed significant influence of temperature and host quality on diapause induction in both sexual and asexual strains. Maximum diapause happened in host eggs with developing embryo even though they were in fetal development. A higher level of diapause was observed in *T. brassicae* whose maternal generation had developed at 20 °C. The diapause induction in asexual *T. brassicae* was less than sexual wasps. The results imply that *Wolbachia* causes a disturbance in the diapause process. The outcomes of this study, which are new, indicate the complexity of diapause and the importance of factors such as host quality which have received less attention in diapause induction.

**Keywords:** Diapause, egg parasitoid, host quality, temperature, *Wolbachia*

**Introduction**

*Trichogramma* are minute polyphagous wasps which are the most widely used endoparasitoids of Lepidopteran pests (Andrade et al., 2011). These parasitoids often have two reproductive modes, arrhenotok (sexual) and thelytoky (asexual). Arrhenotoky is common in most parasitoid wasps where males develop from unfertilized eggs and females from fertilized eggs. Thelytoky is another reproductive mode where unfertilized eggs develop into females (Stouthamer and Werren, 1993). The main reason for thelytoky in the *Trichogramma* genus is the presence of an obligatory endosymbiotic bacterium called *Wolbachia* (Pinto and Stouthamer, 1994). In spite of all the benefits of being infected by *Wolbachia* such as a higher population growth rate, no energy waste for the production of males and no time wasted on searching for mates, it is estimated that few species of *Trichogramma* wasps are infected by the bacterium in nature (Pinto and Stouthamer, 1994). Kishani Farahani et al. (2015) demonstrated that lower ability for determination of host quality in asexual *T. brassicae* could be the reason for scarcity of *Wolbachia* infected *Trichogramma* species in nature.
As the host is the only available nutritional source for immature parasitoid stages, consequently sufficient resources and a high quality host guarantees the survival and reduces the mortality rate of parasitoids (Visser et al., 1992). Host quality has an important role in foraging behavior, remaining time in the patch and foraging success of parasitoids (Outreman et al., 2005). Several studies have shown that parasitoids are able to estimate the quality of their hosts (Schmidt, 1994; Louapre et al., 2011). Sexual *T. brassicae* oviposited in high quality host while asexual ones oviposited regardless of host quality in order to increase their population (Kishani Farahani, 2014). Therefore, we could expect that host quality affects other properties of parasitoids such as the diapause induction in *Trichogramma* offspring.

Diapause happens in the prepupal stage in *Trichogramma* wasps, though in all life stages (embryonic to adult) they are sensitive to environmental abiotic factors such as temperature and photoperiod (Boivin, 1994). In these parasitoid wasps, photoperiodic conditions of pupal and adult development have maternal effects on diapause of their progeny (Ivanov and Reznik, 2008) and temperature is the key factor inducing the diapause in *Trichogramma* during embryonic and larval development of the progeny generation (Reznik et al., 2008). Most studies, have concentrated on the roles of indirect photoperiod and direct temperature on diapause induction of *Trichogramma* wasps (Reznik, 2011) while other environmental factors, such as host quality have not received attention. The reason may be due to the fact that host quality has been often shown to be a major factor inducing diapause for some insects such as *Hippodamia convergens* (Stewart et al., 1967), *Semiadalia undecimnotata* (Rolley et al., 1974), *Chilo zonellus* and *C. argyrolepia* (Scheltes, 1976) that undergo aestival rather than hibernal diapause (Tauber et al., 1986).

This paper describes laboratory experiments designed to examine the maternal influence of host quality and maternal temperature on the facultative prepupal diapause of, on one side, the uninfected-sexual and, on the other, *Wolbachia*-infected asexual *T. brassicae* as the most abundant *Trichogramma* wasps in Iran (Poorjavad, 2011). The purpose of the present study was to assess the following predictions: (1) the proportion of prepupal stage of *T. brassicae* entering diapause varies among different host qualities, (2) the mechanism by which host quality influences diapause induction is by influencing maternal generation developmental conditions relative to changes in temperature and reproductive mode, (3) the asexual strain may produce offspring with lower diapause ability through oviposition in low quality hosts, which may decrease even more in low temperatures as we have previously shown that asexual *T. brassicae* has less ability to overwinter (Rahimi et al., 2017a).

**Materials and Methods**

**Insects**

In this study, sexual and asexual *T. brassicae* strains kept in “Ecology and Behavior” laboratory of University of Tehran were used. Both strains were reared under laboratory conditions, 20 ± 1 °C, 16L: 8D photoperiod and 70 ± 5% RH on the *Ephestia kuehniella* eggs. The offspring of one female was used in this study to generate the highest genetic similarity. The genetic background of the two strains was previously determined by Kishani Farahani et al. (2015) based on the nuclear ribosomal DNA (nrDNA) internal transcribed spacer 2 (ITS2) region. Their results showed a close similarity between them as well as with other *T. brassicae* strains from the northern part of Iran, south of the Caspian Sea (Mazandaran and Guilan province).

**Effect of host quality on the diapause percentage at two temperatures**

The maternal generation of sexual and asexual *T. brassicae* was reared under 10L: 14D photoperiod (Rahimi et al., 2017b), 70 ± 5% RH and at two different temperatures (14 and 20 °C). The maternal generation was reared at
20 °C because higher maternal temperatures would inhibit diapause in *Trichogramma* offspring (Dr. S. Ya. Reznik, personal communication, Zoological Institute RAS, St. Petersburg) and at 14 °C which makes the life cycle of maternal generation longer (about 45-50 days). Therefore, maternal females are more likely to produce offspring with more ability to withstand low temperatures. Maternal females were developed under a short photoperiod because of the maternal influence of photoperiod on the diapause induction in *Trichogramma* wasps (Reznik et al., 2011; Rahimi et al., 2017b). One day after mass emergence of maternal generation (about 17-18 days at 20 °C and 45-50 days at 14 °C), 160 cardboard paper strips were exposed for 4 h to parasitization by 50 asexual and 1000 mated-sexual *T. brassicae* (both 24 h old) in transparent, plastic cylinders (approximately 18 cm tall × 8 cm in diameter) with an opening on the side covered with a mesh in order to allow for ventilation (Rahimi et al., 2017b). The 20% honey water was sprayed on the walls of cylinders to feed *T. brassicae* adults. Then, 50 *E. kuehniella* eggs were glued by non-toxic and water-soluble glue (Canco) on each cardboard paper strip (5cm × 1cm). The ovipositing females were provided with four different quality eggs of *E. kuehniella*, simultaneously. Those included host eggs with high quality which were less than 24 h old (high quality-fresh eggs), *E. kuehniella* eggs which were stored at 20 °C for 24 h (frozen eggs, used usually as sterile eggs in the laboratories), *E. kuehniella* eggs which were stored at 4 °C for 40 days (low quality eggs, considered as low quality eggs by Kishani Farahani et al. (2015)) and *E. kuehniella* eggs which were stored at 14 °C for 10 days (eggs with developing embryo). The experiments were done with the aim to observe the effect of developing embryo of host eggs on the mean number of parasitized eggs/female and diapause induction in sexual and asexual *Trichogramma brassicae*. Eggs with different qualities were glued on separate cardboard paper strips (40 cardboard paper strips for each quality). Then the cards with parasitized host eggs were individually placed in glass tubes and incubated at 10 ± 1°C, 70 ± 5% RH and absolute darkness for two months (Rahimi et al., 2018). The parasitized host eggs were checked weekly.

At 10 °C, the darkening of parasitized eggs occurs after about one month but no emergence was observed at two months after parasitism whereas the darkening happens after about five days at 25 °C. We, therefore, considered these *T. brassicae* to be in diapause. All tubes were then transferred to 20 °C, L16: D8 and 70 ± 5% RH to break diapause and to facilitate the emergence of diapausing individuals. As *Trichogramma* females usually lay only one egg in each *E. kuehniella* egg (Garcia and Tavares, 2001), the number of diapausing individuals was estimated by counting the numbers of eggs with an emergence hole. The remaining eggs were then dissected to take into account the remaining living diapausing prepupae. The percentage of diapausing individuals was separately calculated for each card. We did not include cardboard paper strips with less than 10 parasitized *E. kuehniella* eggs in our experiments.

**Statistical analysis**

Chi-squared test was used to determine the influence of host quality on the oviposition rate (eggs/female) and the percentage of diapause induction in sexual and asexual *T. brassicae* at the two temperatures, separately with 40 replications. To determine which host quality differed from others in oviposition and diapause induction, we compared all pairwise combinations (six possible comparisons for four host qualities) using chi-squared test. The effect of Wolbachia infection (reproductive mode) and temperature on the oviposition rate and the diapause induction of *T. brassicae* were checked using *t*-test. All calculations were performed using SAS statistical software, version 9.2.

**Results and Discussion**

The results showed that differences in oviposition rate among host qualities were more obvious and statistically significant at 20 °C (χ² = 524.83, df =
3, \( P \leq 0.0001 \) than at 14 °C \( (\chi^2 = 65.89, \text{df} = 3, P \leq 0.0001) \) in asexual strain. Six pairwise comparisons among the four qualities resulted in three and five significant differences among qualities at 14 and 20 °C, respectively (Table 1), with lower levels of oviposition in frozen eggs than the other three qualities (Fig. 1A, B). Host quality influenced the oviposition rate of sexual strain more significantly at 14 °C \( (\chi^2 = 936.25, \text{df} = 3, P \leq 0.0001) \) than 20 °C \( (\chi^2 = 368.33, \text{df} = 3, P \leq 0.0001) \). Six and five of six pairwise comparisons among host qualities were significant after applying chi square test at 14 and 20 °C, respectively (Table 2). No significant difference was observed between fresh and eggs with developing embryo at 20 °C (Table 2). The lowest oviposition rate was shown on low host quality at both temperatures (Fig. 1A, B).

According to the results, sexual females oviposited more on host eggs with developing embryo and high quality eggs at both temperatures. The number of parasitized eggs/female by asexual females on the high and low quality eggs was similar at 14 °C (Fig. 1A) while it was completely different at 20 °C with maximum oviposition on eggs with developing embryo followed by high quality eggs (Fig. 1B). As a result, temperature could affect the oviposition of asexual females but not sexual ones which could be related to Wolbachia infection. As seen in Fig. 1B, the oviposition rate of asexual \textit{T. brassicae} on all the host qualities (except for frozen eggs) was greater than that of sexual wasps at 20 °C, while the oviposition rate of sexual strain in all the host qualities except low quality host was greater than that of asexual one at 14 °C (Fig. 1A). Our results confirm the importance of temperature in changing the oviposition behavior of \textit{Trichogramma} wasps which is controlled by \textit{Wolbachia} as the results showed that asexual wasps have lower oviposition rate in comparison to sexual ones at 14 °C while something completely different happened at 20 °C.

The results showed that differences in diapause percentage among host qualities were more obvious and statistically significant at 20 °C \( (\chi^2 = 289.75, \text{df} = 3, P \leq 0.0001) \) than 14 °C \( (\chi^2 = 42.69, \text{df} = 3, P \leq 0.0001) \) in asexual strain. Six pairwise comparisons among the four qualities resulted in five and six significant differences among qualities at 14 °C and 20 °C, respectively (Table 3), with lower levels of diapause induction in low host quality than the other three qualities. A significant difference was not observed between fresh and frozen eggs at 14 °C whereas it was observed at 20 °C in asexual strain (Table 3). Host quality influenced the percentage of prepupal diapause at both 14 °C \( (\chi^2 = 232.37, \text{df} = 3, P \leq 0.0001) \) and 20 °C \( (\chi^2 = 107.86, \text{df} = 3, P \leq 0.0001) \) in sexual strain.

Five and three of six pairwise comparisons among host qualities were significant after applying chi square test at 14 °C and 20 °C, respectively (Table 4). A significant difference was not observed between fresh eggs and eggs with developing embryo at both 14 °C and 20 °C whereas the significant difference was not observed either between fresh and frozen eggs and frozen and eggs with developing embryo at 20 °C in sexual strain (Table 4). The lowest percentage of diapause was shown on low host quality. These results suggest that nutritional quality significantly influences the induction of diapause in both sexual and asexual strains.

### Table 1

<table>
<thead>
<tr>
<th>Comparison</th>
<th>14 °C</th>
<th>20 °C</th>
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<tr>
<td></td>
<td>( \chi^2 )</td>
<td>( P )</td>
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<tr>
<td>Fresh vs. frozen eggs</td>
<td>59.57</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Fresh vs. low quality eggs</td>
<td>0.04</td>
<td>0.8379</td>
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<tr>
<td>Fresh vs. eggs with developing embryo</td>
<td>0.00</td>
<td>1.0000</td>
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<tr>
<td>Frozen vs. low quality eggs</td>
<td>56.89</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Frozen vs. eggs with developing embryo</td>
<td>59.57</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Low quality vs. eggs with developing embryo</td>
<td>0.04</td>
<td>0.8379</td>
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Each comparison represents a chi-square approximation with df = 1.
Figure 1 The mean number of parasitized eggs/female in sexual and asexual *Trichogramma brassicae* on four different host qualities, (HQE)-*Ephesia kuehniella* eggs which were 24 h old, (FE)-*E. kuehniella* eggs which were stored at 20 °C for 24 h, (LQE)-*E. kuehniella* eggs which were stored at 4 °C for 40 days, (DEE)-*E. kuehniella* eggs which were stored at 14 °C for 10 days. The maternal generation developed at (A) 14 °C and (B) 20 °C. Chi-square test was used to determine the influence of host quality on the mean number of parasitized eggs/female in sexual and asexual *T. brassicae*.

Maximum diapause happened on *E. kuehniella* eggs with developing embryo, which was followed by fresh, frozen and low quality eggs, respectively, in asexual strain whose maternal generation had developed at 14 and 20 °C, and in sexual strain whose maternal generation had developed at 14 °C. As expected, these results were in good agreement with the result of Hu *et al.* (1999). They stated that freezing may cause damage to the egg structure through a reduction in the host quality resulting from the denaturation of the large proteins naturally induced by freezing which directly impact the development of the parasitoid. Unlikely as it may be, the highest percentage of diapause was observed on frozen eggs in sexual strain whose maternal generation had developed at 20 °C. The lowest diapause percentage was observed in low quality eggs in all the treatments. Our results revealed that the mortality of individuals increased during the diapause process as a result of host quality reduction. Similarly, Ozder (2004) showed that the quality of *E. kuehniella* eggs kept at 0, 4 and 8 °C decreased as storage time increased.

Based on the results of this research, the question arises as to whether the concept of host quality for *Trichogramma* females is the same as our definition for host quality? We had expected higher diapause percentage in *E. kuehniella* eggs which were 24 h old (high quality-fresh eggs), but the results showed that *E. kuehniella* eggs which were stored at 14 °C for 10 days (eggs with developing embryo) had a higher diapause percentage. It is likely that host eggs with developing embryo attract more ovipositing females or they may have more nutrients in comparison with other host qualities. Similarly, Hunter and Mcneil (1997) have shown that the proportion of larvae entering diapause was two times higher on the low- than on the high-quality artificial diet under constant environmental conditions. Brodeur and Boivin (2004) revealed the fact that chemical components in insect eggs change from semi-liquid to solid in texture and this phenomenon can have negative effects on host acceptance and suitability, parasitism, sex ratio and mortality of parasitoid offspring. Exposure to 14 °C for 10 days will certainly cause fetal development of *E. kuehniella*. Jacob and Cox (1977) indicated that fetal development of *E. kuehniella* takes 14 days at 15 °C and 70% RH. Consequently, embryonic development was not completed after 10 days in our experiment. It seems that embryonic development of *E. kuehniella* eggs turns them to a better host for oviposition and diapause induction.
The oviposition rate of *T. brassicae* showed a statistically significant difference between two temperatures in both sexual ($t = -2.69, P = 0.009$) and asexual ($t = -7.09, P \leq 0.001$) strains. Similarly, the induction of diapause showed significant difference between the two temperatures in both sexual ($t = -2.39, P = 0.019$) and asexual ($t = -6.96, P \leq 0.001$) strains. The oviposition rate of both strains decreased at 14 °C compared to 20 °C, which was followed by reduction in diapause induction (Fig. 1, 2) while the pattern of diapause was the same at two temperatures (Fig. 2A,B). Similar result was obtained by Zaslavski and Umarova (1990), and they indicated that the induction of diapause increased by increasing maternal temperature from 15 to 25 °C.
Figure 2 The induction of diapause in sexual and asexual Trichogramma brassicae on four different host qualities, (HQE)- Ephestia kuehniella eggs which were 24 h old, (FE)- E. kuehniella eggs which were stored at 20 °C for 24 h, (LQE)- E. kuehniella eggs which were stored at 4 °C for 40 days, (DEE)- E. kuehniella eggs which were stored at 14 °C for 10 days. The maternal generation developed at (A) 14 °C and (B) 20 °C. Chi-square test was used to determine the influence of host quality on the induction of diapause in sexual and asexual T. brassicae.

The oviposition rate of T. brassicae showed a statistically significant difference between two strains at both 14 °C (\(t = -3.34, P \leq 0.001\)) and 20 °C (\(t = 3.29, P = 0.002\)). Similarly, the induction of diapause showed significant difference between two strains at both 14 °C (\(t = -5.13, P \leq 0.001\)) and 20 °C (\(t = -2.63, P = 0.01\)). Although both strains showed the same pattern of diapause in response to host quality yet the diapause percentage decreased by Wolbachia infection. Many studies have shown negative effects of Wolbachia infection on biological characteristics of parasitoids such as fecundity, survival, parasitism rate and body size (Grenier and De Clercq, 2003). For example, Wolbachia infection caused shorter longevity in Aedes aegypti (Moreira et al., 2009). Also, asexual T. kaykai showed a lower parasitism rate, emergence rate and longevity in comparison with sexual ones (Hohmann et al., 2001; Miura and Tagami, 2004). Moreover, asexual T. cacoeciae and T. evanescens indicated a higher mortality rate in the embryonic stage than sexual individuals (Tagami et al., 2001). Kishani Farahani et al. (2015) have also stated that asexual T. brassicae have a higher mortality rate in comparison with sexual individuals, which would reduce the fitness and number of asexual offspring. Our previous results also showed that Wolbachia infection has a negative effect on the overwintering of T. brassicae under outdoor conditions (Rahimi et al., 2017a) and long term storage of T. brassicae under laboratory conditions (Rahimi et al., 2017b) through disturbance of the clock gene expression (Rahimi et al., 2017c). As a result, it seems that even if some of the parasitoid characteristics were not affected by the Wolbachia infection; the fitness of parasitoids was reduced by it which could be the reason for scarcity of asexual strain in comparison with the sexual one in nature.

Kishani Farahani et al. (2015) showed that sexual T. brassicae which were reared on high quality host were larger than those reared on low quality host. Body size is directly related to the amount of lipid reserves, therefore larger insects will have more lipid reserves (Candy et al., 1997). It has been shown that larger individuals are generally more resistant to stresses such as cold temperatures in comparison with smaller ones (Rivero and West, 2002; Arnett and Gotelli, 2003). This could indicate greater ability of sexual wasps to survive at low temperatures.

Consequently, results of this study demonstrate that there is a direct effect of host quality on diapause induction of T. brassicae.
that is dependent on temperature and reproductive mode. It has also been shown that greater diapause induction occurred at higher temperature and sexual strain which indicates negative effects of *Wolbachia* infection on the diapause induction of *T. brassicae*. It is hoped that the results of the present study will encourage further work in this area.

**Acknowledgements**

The authors sincerely thank Dr. Nafiseh Poorjavad (Isfahan University of Technology, Isfahan, Iran) for the molecular and morphological identification of asexual and sexual *T. brassicae* and Petra Pribicevic (Faculty of Philology, University of Belgrade, Serbia) for her proofreading.

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چگونگی اثر کیفیت میزان بر میزان تخمگذاری و دیافروز زنبورهای دوجنسی و تکجنسی

Trichogramma brassicae

سمه رحمی کلده، احمد عاشوری و علیرضا بندانی

گروه گیاهپزشکی، بردسی کشاورزی و منابع طبیعی، دانشگاه تهران، کرج، ایران

پست الکترونیکی نویسندگان: مسندل مکاتیه: ashouri@ut.ac.ir

دریافت: 26 مهر 1396، پذیرش: 16 اردیبهشت 1397

چکیده: کاربرد گسترده زنبورهای پارازیتوید Trichogramma در کنترل پیلوژیک آفات مهم بال-پر پلاکدار، آنها را به یکی از مهم‌ترین حشرات اقتصادی تبدیل کرده است. بر این اساس، چنین‌های مختلف تولید آبیه این زنبورها در سراسر دنیا مورد توجه قرار گرفته است. مطالعه هم‌جنبه‌های وابسته این زنبورهای جنسی Trichogramma و تغییرات آنها در ایجاد و پژوهش اثر کیفیت میزان بر درصد دیافروز زنبورهای تکجنسی (نوعه به ولباکیا) و دوجنسی (غیرولباکی) با استفاده از Trichogramma brassicae Bezdenko در دو دمای 14 و 20 درجه سلسیوس مورد مطالعه قرار گرفت. نسل مادری در دو دمای فوق پرورش یافتند و روی جهان کیفیت متفاوت میزان شامل تخم‌های باکیفیت، تخم‌های مسئول حیات جنبیده جنبه‌های دیافروز در حاملی تخم‌های حاوی ژن‌های رشد و تخم‌های باکیفیت تعیین گردید. نتایج نشان داد که کیفیت میزان دارای اثر معنی‌داری بر درصد دیافروز هر دو دمای است. نسل‌های اول درصد دیافروز در حاملی تخم‌های حاوی ژن‌های رشد و تخم‌های باکیفیت مشاهده شد. همچنین نتایج نشان داد میزان دیافروز در نتایج که نسل مادری آنها در دمای 20 درجه سلسیوس پرورش یافتند بیش از نتایج بود که نسل مادری آنها در دمای 14 درجه سلسیوس پرورش یافتند. درصد دیافروز در زنبورهای تکجنسی کم‌تر از دوجنسی بود. این نتیجه با انگراییده باکتری Wolbachia ایجاد اختلال در روند دیافروز زنبور توسط باکتری Wolbachia ایجاد تأثیر قرار گرفت. نتایج این پژوهش، حاکی از پیچیدگی فرایند دیافروز و تحت تأثیر قرار گرفتن آن توسط عوامل (کیفیت میزان) است که تاکنون کمتر مورد توجه بوده‌اند.

واژگان کلیدی: دیافروز، پارازیتوید تخم، کیفیت میزان، دما

Wolbachia