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Research Article

Effects of egg age in *Sesamia cretica* (Lepidoptera: Noctuidae) on parasitism, development and reproduction of *Telenomus busseolae* (Hymenoptera: Platygastridae)

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Abstract The sugarcane stem borer, *Sesamia cretica* Lederer, is one of the most economically important pests of sugarcane in Khuzestan province, southwest Iran. The egg parasitoid *Telenomus busseolae* Gahan significantly affect *S. cretica* population in the field. Host age is an important factor of host acceptance and suitability for egg parasitoids. We examined the ability of *T. busseolae* to parasitize and develop in *S. cretica* eggs of different ages. In a no choice laboratory experiment, we measured the effect of host age (12, 36, or 60 h old) on parasitism rate and offspring fitness characteristics such as survival, development time, sex ratio, size, and progeny longevity and fecundity. Results revealed that the suitability of host eggs decreased as host age increased. Exposure of parasitoids to 12h old eggs resulted in higher percentage parasitism, higher acceptance, higher survival and larger body size of parasitoid progeny. Progeny that emerged from 12h old eggs had also higher longevity and fecundity than those emerged from other host ages tested.

Keywords: Sugarcane pink borer, Egg parasitoid, Egg age

Introduction

Sugarcane is one of the most important cash crops in Iran. The crop occupies nearly 120,000 ha in Khuzestan province, southwestern Iran. Sugarcane is planted between September and October as "plant cane", and this is harvested after one year with the root system still left in the soil. The crop grows again for another year as "first ratoon". Growers may continue to ratoon their crops for three or four years after which the crop is ploughed-out, and new canes replanted. According to the Iranian sugarcane industry report total growing surface in 2015-2016 was 115000 ha and the annual sugar production from cane was 7.5 million tons (ISFS, 2017). These data place the sugarcane crop in the third rank among economically important crops in Iran.

The most damaging pest of sugarcane in Khuzestan province is the sugarcane pink borer, *Sesamia cretica* Lederer (Lepidoptera: Noctuidae). The larvae tunnel vertically within stalks producing a hollow cavity that may be invaded by different fungi, weakening the plant and causing death (Daniali, 1976, 1985). It has been found that sugarcane borer causes severe damage to the crop (Mathes *et al*., 1969; Askarianzadeh *et al*., 2008).

Telenomus busseolae Gahan (Hymenoptera: Platygastridae) is an arrhenotokous, proovigenic parasitoid wasp and one of the most important biological control agents of

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sugarcane pink borers, including *S. cretica* and *S. nonagrioides* (Lefebvre) in Iran (Baniabbasi, 1981). *T. busseolae* is able to parasitize 90% of *Sesamia* spp. eggs in sugarcane (Jamshidnia, 2010) and corn fields (Abbasipour, 2004; Alexandri and Tsitsipis, 1990). *T. busseolae* is widely distributed in southern Africa, southern Europe and Middle East (Polaszek *et al*. 1993).

Although the life history traits and life table parameters of *T. busseolae* parasitizing *S. cretica* eggs have already been studied (Jamshidnia and Sadeghi, 2014), the effect of egg age on preference and life history of *T. busseolae* is unknown. Thus, we studied parasitism rate, development time, sex ratio, longevity and fecundity of *T. busseolae* parasitizing eggs of different ages of *S. cretica.* Understanding the effect of host age on preference and life history of *T. busseolae* will assist in determining the most suitable egg age for its mass production and augmentation for biological control programs.

Materials and Methods

Insect colonies

Larvae of *S. cretica* were collected from sugarcane crops in Amirkabir Agro-industry located in Khuzestan province, southwestern Iran (48° 10' E, 30° 4' N), and reared on sugarcane stalks in plastic rearing cages (16 cm diameter × 25 cm height) at 28 ± 1 °C, 50 ± 5% relative humidity and 16:8 (L: D) photoperiod until pupation. Pupae were collected from rearing cages and placed in plastic containers (10 cm diameters × 5 cm height) until adult emergence. Four pairs of *S. cretica* were confined in plastic oviposition cages (16 cm diameter × 25 cm height) together with four young sugarcane shoots. The moths laid their eggs under the leaf sheath. Shoots were checked daily and egg masses used for experiments.

Colony of *T. busseolae* was established using individuals collected from egg batches of *S. cretica* in the same sugarcane field where *S. cretica* larvae were obtained. Parasitoids were reared on *S. cretica* eggs for several generations in a growth chamber at 28 ± 1 °C, 50 ± 5% relative humidity and 16:8 (L: D) photoperiod. The adult parasitoids were maintained in a glass tube (3cm diameter × 17 cm length) and fed honey-water solution (20%) for mating, before use in experiments.

Experimental procedure

Three ages of eggs were used in the experiment: 12, 36 and 60h. At 28 °C, *S. cretica* eggs hatch almost four days after oviposition (Cheraghi, unpublished data). Thus, we restricted our experiments to hosts that were less than four days old. Eggs of the appropriate age were obtained by collecting eggs laid under leaf sheath by moths during a 12h period and maintaining them at 28 ± 1 °C until they were the suitable age. Eggs were carefully removed from leaf sheath using a fine blade. The midpoint of the 12 hours period was used as the starting point for ascertaining egg age.

One hundred eggs of each age were attached with water-sugar solution (10%) to a rectangular radiological used film (15 cm long × 2 cm wide) each film was placed in a glass tube described above. A single, less than 24 hours old mated, naïve *T. busseolae* female was introduced into each glass tube containing egg batches of 12, 36 and 60h old eggs of *S. cretica*. The glass tubes were plugged with cotton wool. All tubes were kept in a growth chamber at 28 ± 1 °C, 50 ± 5% R.H. and a photoperiod of 16: 8 (L: D). After 24 hours, the optimal exposure time determined in earlier experiments, the *T. busseolae* female were removed, and the glass tubes kept in the growth chamber until adult emergence. Larvae that hatched from unparasitized eggs were removed and counted twice a day. The parasitoid development time, survival, sex ratio (number of female/total number of individuals) and size (right hind tibiae) were assessed. The rate of parasitism was evaluated as the number of emerged parasitoids plus obviously parasitized but unhatched eggs over total number of eggs exposed. Rate of emergence was determined by dividing the number of emerged adults by the number of clearly parasitized eggs. Each

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treatment (host age) was replicated 15 times in a completely randomized design.

The effect of different host age on longevity and fecundity of progenies was studied by placing one adult female and male (less than 24h old) obtained from previous experiment in a glass tube containing batches of 100 1-day-old *S. cretica* eggs and honey. New eggs were provided every 24h, and the number of *T. busseolae* females still alive was recorded daily until death. A completely randomized design with 15 replications for each treatment (host age) was used. This experiment was also conducted in a growth chamber (28 ± 1 °C, 50 ± 5% R. H. and 16: 8 L: D). Exposed egg batches were maintained in the growth chamber until parasitoid emergence. Longevity and fecundity of adult parasitoids and sex ratio of offspring of *T. busseolae* were calculated.

Statistical analysis

Percentage of parasitism, emergence, survival, sex ratio, also developmental time of premature stage, length of hind tibia and longevity and fecundity of progenies were analyzed using one-way analysis of variance (ANOVA). When significant differences among treatments were detected by ANOVA, comparisons were done by Least Significant Difference (LSD) at $P \leq 0.05$ level. Percentages were transformed to GLM method.

The life history data from each host age were analyzed based on the age-stage, two sex life table (Chi and Liu, 1985; Chi, 1988; Yu *et al*, 2013) using TWOSEX MSchart computer program (Chi, 2016). For each host age, we determined the age-stage specific survival rate (*lx*), the age-stage specific fecundity (m_x) and other stage and age specific parameters as described in Chi and Liu (1985) and Chi (1988). These data were used to determine the intrinsic rate of increase (r_m), finite rate of increase (λ), net reproductive rate (R_0), mean generation time (T) and the gross reproductive rate (GRR). The intrinsic rate of increase was determined by iteratively solving the Euler-Lotka equation with age indexed from 0 (Goodman, 1982):

$$\sum_{\chi=0}^{\infty} e^{-r(x+1)} l_x m_{x=1}$$

The finite rate of increase (λ) and R_0 were calculated as follows:

$$\lambda = e^{\gamma}$$

The mean generation time (T) is then calculated using the following equation:

$$T = \frac{1n(R)}{\gamma}$$

The bootstrap technique was used to estimate the means, variances, and standard errors of the population parameters (Efron and Tibshirani, 1993). As bootsraping uses random resampling, a small number of replications will generate variable means and standard errors. To generate less variable results, 100,000 replications were used in this study (Huang and Chi, 2013; Chi, 2016).

Results

Mean percentage parasitism was significantly affected by host age ($F = 22.36$; $df = 2, 44$; $P < 0.0001$). The highest rate of parasitism was observed on 12h old eggs (Table 1).

Table 1 Mean parasitism, survival, sex ratio, development time and tibia size (± SE) of *Telenomus busseolae* parasitizing *Sesamia cretica* eggs of different ages.

Host ages (hours)	Parasitism %	Development time (days)	Sex ratio (%female)	Survival rate (%)	hind tibia length in female (mm)	hind tibia length in male (mm)
12	$59.98^a \pm 2.46^a$	13.09 ± 0.41^a	0.58 ± 0.012^a	99.48 ± 3.24^a	0.1393 ± 0.00009^a	0.1327 ± 0.00010^a
36	$53.23^b \pm 2.90^b$	13.17 ± 0.51^a	0.57 ± 0.007^a	99.55 ± 4.12^a	0.1294 ± 0.00016^b	0.1216 ± 0.00017^b
60	$31.44^c \pm 2.92^c$	13.97 ± 0.83^b	0.53 ± 0.006^b	99.06 ± 4.35^a	0.1254 ± 0.00024^c	0.1152 ± 0.00034^c

Means in a column followed by the same letter are not significantly different at $p \leq 0.05$ (LSD test).

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The egg-to-adult developmental times for *T. busseolae* varied significantly with host age (F = 12.46; df = 2, 44; P = 0.005). Developmental time was shortest on 12 h old host eggs. There were no significant differences in developmental times between 36 and 60h old host eggs (Table 1).

The percentage of females was significantly affected by host age (F = 6.51; df = 2, 44; P = 0.006). A female-biased sex ratio was observed on all host ages tested (Table 1).

T. busseolae was able to complete its development on all tested egg ages. Host age had no significant effect on egg-to-adult survivorship (F = 43.54; df = 2, 44; P = 0.083) (Table 1).

Tibial length of emerging parasitoids was significantly affected by host age for females (F = 2.53; df = 2, 44; P < 0.0001) and males (F = 3.82; df = 2, 44; P < 0.0001) (Table 1).

Host age had significant effect on longevities of males (F = 5.57; df = 2, 44; P = 0.007) and females (F = 21.94; df = 2, 44; P < 0.0001). Longest lived parasitoid emerged from 12h old eggs (Table 2).

The mean daily and total number of eggs laid by *T. busseolae* females emerged from different host ages varied significantly (daily: F = 21.62; df = 2, 44; P = 0.006; total: F = 121.07; df = 2, 44; P < 0.0001). The highest life time fecundity (eggs/female) was recorded for females emerged from 12 h old eggs (Table 2). Secondary sex ratio (female %) was significantly affected by host age (F = 8.93; df = 2, 44; P = 0.006) (Table 2).

The highest r_m value of *T. busseolae* was found on 12 h old eggs (0.26 female/female/day), followed by 36h (0.24 female/female/day) and 60 h (0.19 female/female/day) (Table 3). However, there were no significant differences in the r_m values of *T. busseolae* reared on 12 and 36h old host eggs. Because of rapid developmental time and high fecundity, *T. busseolae* reared on 12h old eggs had a significantly greater net reproductive rate (R_0) and finite rate of increase (λ) than those reared on other host ages (Table 3). The mean generation time and population doubling time were also significantly shorter when 12h old eggs were used compared with other host ages tested.

Table 2 Mean longevity, daily fecundity, total fecundity and secondary sex ratio ((± SE) of *Telenomus busseolae* offspring emerging from *Sesamia cretica* eggs of different ages.

Host ages (hours)	Female longevity (days)	Male longevity (days)	Daily fecundity	Total fecundity	Secondary sex ratio (%female)
12	5.80 ± 0.17^{a}	4.73 ± 0.18^{a}	17.32 ± 0.56^{a}	98.6 ± 1.03^{a}	0.63 ± 0.017^{a}
36	4.80 ± 0.20^{b}	4.53 ± 0.17^{ab}	17.03 ± 0.62^{a}	80.3 ± 1.30^{b}	0.56 ± 0.014^{b}
60	4.07 ± 0.18^{c}	3.93 ± 0.18^{b}	11.24 ± 0.68^{b}	44.8 ± 0.8^{7}c	0.50 ± 0.016^{c}

Means in a column followed by the same letter are not significantly different at $p \leq 0.05$ (LSD test).

Table 3 Life table parameters of *Telenomus busseolae* offspring emerging from *Sesamia cretica* eggs of different ages.

Host ages (hours)	r_m (day^{-1})	λ (day^{-1})	*R* offspring/individual	*T* (days)
12	0.26^{a}	1.30^{a}	49.30^{a}	14.81^{b}
36	0.24^{a}	1.27^{a}	40.16^{a}	15.38^{ab}
60	0.19^{b}	1.22^{b}	22.40^{b}	15.59^{a}

Means in a column followed by the same letter are not significantly different.

Discussion

As we expected and similar to the pattern commonly reported for egg parasitoids, most fitness characteristics of *T. busseolae* were negatively affected by increasing host age. Our study shows that *T. busseolae* can successfully parasitize and develop in all stages of embryonic development of *S. cretica*; however, their developmental time increased and their fecundity decreased in older host eggs.

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The proportion of *S. cretica* eggs parasitized by *T. busseolae* varied with the host age. Our results are similar to those of most studies of scelionids, where parasitism level decreases as host age increases (Olaye *et al*., 1997; Sousa and Spence, 2001; Hirose *et al*., 2003; Legault *et al*., 2013). These authors suggested that parasitism level were lower in old host eggs either because female failed to oviposit owing to change in the hardening of chorion as egg aged or their offspring failed to successfully exploit the nutritional resources in older host eggs.

T. busseolae could complete its development in all age groups of host eggs tested, but took longer to develop in older host eggs. Similar results have been reported for *T. busseolae* parasitizing eggs of *Sesamia calamistis* Hampton (Okoth *et al*., 2006) and other scelionids (Hirose *et al*., 2003; Kivan and Kilic, 2004; Zhou *et al*., 2014). Strand (1986) stated that the longer development time in older host eggs could be due to existence of a greater amount of embryonic substances which probably needs more time to metabolize than yolk. This phenomenon was more noticeable for males than females, which suggested sex-related physiological differences between immature parasitoids that in turn could affect the ability of processing embryonic tissues. Increasing the development time not only extends the time interval of vulnerability to predation or hyperparasitism to developing *T. busseolae* offspring, but also the sex-dependent increase in development time with advancing host egg age could decline the level of protandry (the appearance of the males earlier in the season than the females), and cause a large number of females leaving the patch with no chance of mating.

This study showed that sex ratio of *T. busseolae* was always female biased and significantly influenced by host age. In contrast to our findings, it was reported that sex ratio of *T. busseolae* was not significantly affected by egg age of *Sesamia calamistis* (Hampton) (Olaye *et al*., 1997) and *Busseola fusca* Fuller (Okoth *et al*., 2006). Our study indicated that maximum percentage female offspring (58%) was produced from eggs 12 h of age. We ponder that *T. busseolae* sex ratio was more profoundly female skewed when females were presented eggs 12 h of age because these eggs were most suitable and acceptable for oviposition, giving rise to higher parasitism and longer oviposition courses, which in turn increased proportion of fertilized eggs laid into hosts (Waage, 1982).

Similar to the current results female biased sex ratio have been reported for *T. busseolae* parasitizing eggs of *S. calamistis* (Olaye *et al*., 1997) and *B. fusca* (Okoth *et al*., 2006) and other scelionid egg parasitoids such as *Typhodytes gerriphagus* Marchal (Sousa and Spence, 2001), *Gryon gallardoi* (Berthes) (Da Rocha *et al*., 2006), *Telenomus isis* Polaszek (Bruce *et al*., 2009), *Telenomus remus* Nixon (Penaflor *et al*., 2012) and *Telenomus podisi* Ashmead (Zhou *et al*., 2014) across the host egg ages tested.

Consistent inverse relationship between host age and tibial length of *T. busseolae* (in both male and female) across all three ages showed that suitability declines steadily as the host ages. These results are in agreement with studies of other species of Scelionidae, *Gryon philippinease* (Ashmead) the egg parasitoid of *Acanthocoris sordidus* Thunberg (Dasilao and Arakawa, 2005), a member of Eulophidae, *Edovum puttleri* Grissell with eggs of *Leptinotarsa decemlineata* (Say) (Ruberson *et al*., 1987) and a species of Trichogrammatidae, *Trichogramma pretiosum* Riley with eggs of *Helicoverpa zea* (Boddie) (Ruberson and Kring, 1993). Size of egg parasitoids has profound effect on fecundity and or longevity (Waage and Ng, 1984; Ruberson *et al*., 1988; van den Assem *et al*., 1989; Godfray, 1994); therefore the age of the eggs in which *T. busseolae* develop may have long-term effect on female fitness.

The r_m values of *T. busseolae* are reported to be 0.298 on *S. cretica* eggs at 30 °C (Jamshidnia, 2010), 0.303 on *S. calamistis* eggs at 27 °C (Olaye *et al*., 1997), and 0.239 on *Busseolae fusca* Fuller eggs at 27 °C (Okoth *et al*., 2006); which are in the same

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range as our estimate on 12h old eggs (0.260). It should be reminded that all these authors used 24h old eggs of *Sesamia* spp. in their experiments.

Results from this study showed that the growth, development, and survival of a solitary egg parasitoid, *T. busseolae* varied with the host egg age. In current study, *T. busseolae* females oviposited on all host ages that were presented and they reproduced to some degree on all host ages. Generally, host age suitability for *T. busseolae* is identified by response data such as parasitoid survival to adulthood, development time, mean daily fecundity and longevity of offsprings. Also, this study showed that 12 h old eggs were most suitable host age followed by 36h old eggs, which were marginally suitable age, and 60h old eggs, which were the least suitable egg age of those tested. Reproductive fitness of *T. busseolae* is obviously maximized through employment of 12-36h old host eggs which permit the highest level of reproduction and parasitoid progeny survival.

Conclusion

Host-age effects have several implications for augmentative biological control using egg parasitoids. Ruberson *et al*. (1987) stated that the mass production phase of such a project should use younger hosts in order to increase the fitness of the progeny that are to be released in the field. In the case of *T. busseolae*, the current findings suggest that 12-36h old egg masses would be suitable for mass rearing.

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اثر سن تخم *Sesamia cretica* (Lepidoptera: Noctuidae) روی پارازیتیسم، نمو و تولیدمثل زنبور *Telenomus busseolae* (Hymenoptera: Platygastridae)

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چکیده: ساقه‌خوار نیشکر *Sesamia cretica* Lederer یکی از مهم‌ترین آفات اقتصادی مزارع نیشکر استان خوزستان در جنوب غربی ایران می‌باشد. پارازیتویید تخم *Telenomus busseolae* Gahan به‌طور عمده‌ای روی جمعیت *S. cretica* در مزرعه تأثیر می‌گذارد. سن میزبان فاکتور مهم پذیرش آن و عامل پایداری پارازیتوییدهای تخم است. در این مطالعه ما توانایی *T. busseolae* را برای پارازیته کردن و نمو در تخم‌های سنین مختلف *S. cretica* مورد آزمایش قرار دادیم. در آزمایش غیرانتخابی، اثر سن میزبان (۱۲، ۳۶ یا ۶۰ ساعته) در میزان پارازیتیسم و خصوصیات سازگاری نتاج مانند بقاء، زمان نمو، نسبت جنسی، اندازه، طول عمر و باروری نتاج را اندازه‌گیری کردیم. نتایج نشان می‌دهد که با افزایش سن تخم میزبان، مطلوبیت آن کاهش می‌یابد. قرار دادن تخم‌های ۱۲ ساعته میزبان در اختیار زنبور پارازیتویید منجر به درصد بالاتر پارازیتیسم، پذیرش و بقاء بیش‌تر و اندازه بزرگ‌تر نتاج پارازیتویید گردید. هم‌چنین نتاج خارج شده از تخم‌های ۱۲ ساعته میزبان بیش‌ترین طول عمر و باروری را نسبت به دیگر سنین تخم میزبان نشان دادند.

واژگان کلیدی: ساقه‌خوار نیشکر، پارازیتویید تخم، سن تخم

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