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ŞENER TARLA

SERPİL KORNOŞOR

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Reproduction and survival of overwintered and F₁ generation of two egg parasitoids of sunn pest, *Eurygaster integriceps* Put. (Heteroptera: Scutelleridae)

Şener TARLA^{1,*}, Serpil KORNOŞOR²

¹Uşak University, Vocational High School, Department of Organic Agriculture Program, 64800 Sivasslı, Uşak - TURKEY

²Department of Plant Protection, Faculty of Agricultural, Çukurova University, 01330 Adana - TURKEY

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Abstract: The differences in the reproductive biology of overwintered (OW) and F₁ generation of *Trissolcus semistriatus* Nees and *T. festivae* Victorov (Hymenoptera: Scelionidae) were investigated under laboratory conditions. The experiments were conducted to evaluate oviposition period, lifetime fecundity, female progeny, sex ratio, and longevity of OW and F₁ generation with the two species as factors. The results of this study showed that the oviposition period of F₁ generation was longer than that of OW females for both of the two parasitoid species. With the exception of F₁ generation of *T. festivae*, the highest daily average progeny production of females was recorded on the first day. The mean lifetime fecundity of ovipositing females were calculated as 88.8 ± 6.13 and 111.9 ± 6.66 for OW and F₁ generation of *T. semistriatus*, 85.6 ± 6.83; 104.7 ± 4.66 for OW and F₁ generation of *T. festivae*, respectively. The sex ratios of progeny for both of the species were distinctly female-biased. The effects of species, factors (OW and F₁ generation), and their interactions were significant on the sex ratio of progeny. The average longevity of OW females was 16.2 ± 1.76 days for *T. semistriatus* and 16.9 ± 1.21 for *T. festivae*. The average longevity of F₁ generation females was 17.5 ± 1.46 days for *T. semistriatus* and 28.5 ± 1.94 for *T. festivae*. Thus, some several biological characteristics of both species were compared and discussed with regard to the use of OW populations for the successful implantation of biological control and mass production.

Key words: Egg parasitoid, overwintering, progeny, sunn pest, *Trissolcus*

Süne, *Eurygaster integriceps* Put. (Heteroptera: Scutelleridae)'in iki yumurta parazitoitinin kışlamış ve F₁ neslinin yaşam süreleri ve üremeleri

Özet: Kışlamış (OW) ve F₁ nesline ait *Trissolcus semistriatus* Nees ve *T. festivae* Victorov (Hymenoptera: Scelionidae)'nın üreme biyolojisi laboratuvar koşullarında incelenmiştir. Faktör olarak iki türün, OW ve F₁ neslinin ovipozisyon süresi, yaşam süresince verdiği birey sayısı, dişi sayısı, cinsiyet oranı ve yaşam sürelerini belirlemek için denemeler kurulmuştur. Bu çalışmanın sonuçları her iki parazitoit türü için F₁ neslinde ovipozisyon süresinin kışlamış olan dişilerinkinden daha uzun olduğunu göstermektedir. Günlük olarak en yüksek ortalama verdikleri birey sayısı, *T. festivae*'in F₁ nesli hariç, dişilerin günlük olarak ortalama verdikleri birey sayısı en yüksek birinci günde kaydedilmiştir. Dişi bireylerin yaşamları

* E-mail: sener.tarla@usak.edu.tr

süresince verdikleri ortalama birey sayıları sırayla *T. semistriatus*'un kışlamış ve F₁ nesli için 88.8 ± 6.13 ve 111.9 ± 6.66 ve *T. festivaе*'nin kışlamış ve F₁ nesli için 85.6 ± 6.83 ve 104.7 ± 4.66 birey olarak hesaplanmıştır. Cinsiyet oranı her iki tür için de belirgin olarak dişi ağırlıklı olmuştur. Türlerin, faktörler (OW ve F₁ nesli) ve bunların interaksiyonlarının cinsiyet oranı üzerine önemli bir etkisi olmamıştır. Kışlamış dişilerin ortalama yaşam süreleri *T. semistriatus* için 16.2 ± 1.76 ve *T. festivaе* için 16.9 ± 1.21 ve ayrıca F₁ nesline ait ortalama yaşam süresi *T. semistriatus* dişileri için 17.5 ± 1.46 ve *T. festivaе* için 28.5 ± 1.94 gün olduğu belirlenmiştir. Böylece, kitle üretimi ve biyolojik mücadele uygulamalarının başarısı için her iki türün bazı biyolojik özellikleri kışlamış popülasyonların kullanılması bakımından karşılaştırılmış ve tartışılmıştır.

Anahtar sözcükler: Yumurta parazitoiti, kışlama, nesil, süne, *Trissolcus*

Introduction

The sunn pest, *Eurygaster integriceps* Puton (Heteroptera: Scutelleridae), is one of the most important pests of wheat in Western and Central Asia. Both nymphs and adults of this species have been linked to plant damage, where they feed on leaves, stems, and grains of cereals (Critchely, 1998). The economic loss can reach up to 100% if control measures are not implemented (Kıvan and Kılıç, 2006b).

Among natural enemies, egg parasitoids belonging to the Scelionidae family are the most important biological control agents that suppress sunn pest density. By using these parasitoids, the sunn pest population could be reduced without resorting to chemical insecticides (Rosca et al., 1996). Egg parasitoids haven been used against sunn pest in Iran, Morocco and the former USSR (Voegelé, 1961; Safavi, 1968; Lazarov et al., 1969; Laraichi and Voegele' 1975). However, these early attempts were never successful given the difficulties of rearing them continuously under controlled conditions and the problems in establishing them in the field due to the widespread aerial spraying of broad spectrum insecticides (Critchley, 1998). Recently, where *T. semistriatus* Nees was released under natural conditions in the wheat fields, the parasitization of sunn pest eggs increased by 8-16% in the first generation as compared with control plots where parasitoids were not released (Tarla and Kornoşor, 2003). Parasitism is the main mortality factor acting on the eggs of sunn pest. *T. semistriatus* and *T. festivaе* Victorov are responsible for about 87% of the natural parasitism in Southern Turkey (Tarla and Kornoşor, 2003). Since 2004, both species have been used in the National Sunn Pest Project to control sunn pest in Turkey.

There is some literature on the reproductive biology of *T. semistriatus* (Alexandrov, 1948; Safavi, 1968; Tarla, 2002; Kıvan and Kılıç, 2006b; Tarla and Kornoşor, 2007). However, the biology of *T. festivaе* has not been fully studied in detail. Furthermore, to the best of our knowledge, there are no studies concerning the reproductive biology of overwintered (OW) Scelionidae species, but many laboratory studies at low temperatures on cold stored conditions have been reported (Bayram et al., 2005; Foerster and Doetzer, 2006; Foerster et al., 2004).

The sunn pest is not a suitable host for year-round production of egg parasitoids, because it belongs to the univoltine species. Many pentatomid species, such as *Dolycoris baccarum* (L.), *Eurydema ornatum* (L.), *Graphosoma lineatum* L. and *Holcostethus vernalis* Wolf. (Heteroptera: Pentatomidae), exist in alternative hosts for egg parasitoids, but until now, it has been impossible to produce sufficient host eggs for mass culture of these parasitoids. The release of parasitoids in the field should be managed at the initial onset of sunn pest oviposition.

Working continuously on parasitoid cultures throughout the year in the laboratory is very difficult and time consuming. For instance, under crowded laboratory conditions, a significant increase in the percentage of male progeny has been observed in other scelionids (Waage, 1982). The performance of the parasitoids can change over time for many generations as a result of changes in laboratory conditions or as a consequence of genetic changes in the colony (Mackauer, 1972; Hopper et al., 1993). This shows that inadvertent selection under artificial conditions, or random genetic drift, may lead to changes in sex ratio, fecundity and lifespan (Mangan, 1992). Quality deterioration during laboratory rearing of insects is of general concern. Hopper et al. (1993)

has recommended that the culture time should not exceed five generations, if possible, prior to field release of entomophages. For reasons mentioned above, OW female wasps collected from fields have been used in our laboratory to mass-cultivate the egg parasitoids for augmentative biological control of sunn pest for many years.

The aim of this study is to compare several reproduction characteristics and the longevity of OW and F₁ generation in order to facilitate cultural practices and improve the efficacy of these wasps in biological control programs.

Materials and methods

Host culture

The eggs of *E. integriceps* were used as host. To obtain the eggs, OW adults of sunn pest, which were collected from wheat fields, were fed wheat plants and the eggs were obtained according to the method described by Tarla and Kornoşor (2007). Overwintered adults of sunn pest were collected by sweep nets and by hand from wheat fields. They were released on wheat plants mown close to the roots into 8-liter plastic rearing cups covered by organdy cloth tops. To encourage breeding and obtain enough eggs from adult sunn pest, the plastic rearing cups were placed in a chamber maintained at 29 ± 2 °C, $65 \pm 10\%$ relative humidity (RH) and under a light: dark (L:D) cycle of 16:8 hours (hrs). It was observed that sunn pest preferred to lay its eggs on paper strips than on the leaf of wheat plants in the laboratory. Paper strips were removed from the plastic rearing cups on the wheat plants in order to collect egg masses easily. Freshly laid egg masses (one egg mass = 14 eggs), collected daily, were used in the experiments. Every two days, plants were removed from the plastic rearing cups and new wheat plants mown close to the roots were placed.

Obtaining overwintered female parasitoids

On March 7, 2005, before the start of lifespan of the sunn pest and alternative host pentatomid species oviposition under natural conditions, the overwintered females of *T. semistriatus* and *T. festiva* were obtained by sweeping nets in wheat fields located in Adana (37° 02' N 35° 21' E). The overwintered females were separated for the species in the laboratory. They were then individually transferred into cotton-plugged glass

tubes (1.6 cm diameter, 10 cm long) and streaked inside with a diluted honey and water solution to provide a food source. The glass tubes were kept at 20 ± 0.5 °C, $65 \pm 10\%$ RH, L16 : D8 hrs photoperiod for 17 days; tests were started because there were no host eggs during this period.

F₁ generation culture

The F₁ cultures of the parasitoid in the laboratory were obtained from parasitoids that emerged from sunn pest egg masses collected in wheat fields during late March in 2005. Virgin wasps of the F₁ generation were used and cultured at 26 ± 0.5 °C, $65 \pm 10\%$ RH and: L16 : D8 hrs photoperiod. Prior to the experiment, pairs of females and males of the F₁ generation were placed together in glass tubes for one day to breed. Voucher specimens of both species were deposited in the ICMKU collection, Insect Museum of Plant Protection Department, Agriculture Faculty, Mustafa Kemal University, Hatay, Turkey.

Experimental procedures

The experiments were carried out under controlled environmental conditions (26 ± 0.5 °C, $65 \pm 10\%$ RH and L16: D8 hrs photoperiod) with the OW and F₁ generation of parasitoids. To determine reproduction characteristics and longevity of OW and F₁ generations of the two parasitoid species, twenty females of each species were tested. Given that the species laid more eggs at the beginning of the oviposition period, in the first two days, four, and the remaining days two egg masses (< one day old) of *E. integriceps*, glued to a labeled index card strip (1 x 3 cm), were exposed to one female per treatment until the parasitoid females died. Egg masses were provided every 24 hrs and then the old egg masses were transferred to new tubes under the same conditions until their fate could be determined. When nymphs of *E. integriceps* hatched from unparasitized eggs, they were removed from the glass tubes. The mortality of female parasitoids was recorded twice daily. The sex of the progeny that emerged from the egg masses was determined and recorded. Males and females are distinguishable by the shape of their antennae. Thus, reproduction characteristics and longevity of both species were recorded for OW and F₁ generations as factors. The values for longevity of OW females were calculated after providing host eggs to parasitoids for oviposition, because they were post-overwintered.

Statistical analysis

Differences in reproduction and longevity of *T. semistriatus* and *T. festiva* were compared by two-way ANOVA with OW and F₁ generation as factors. To reduce variance differences, the transformation radical, $\sqrt{(x + 0.5)}$, for the data concerning the lifetime fecundity and daughter progeny and arcsin $\sqrt{(x)}$ for sex ratio were calculated before the statistical analysis (Zar, 1984). The factors (OW and F₁) was tested by the F statistic and was considered to be significant when $P < 0.05$. The reported values are untransformed means and standard errors. We used the statistical software SPSS 11.0 for Windows (SPSS 11.0, SPSS Inc., Chicago, IL) for statistical analysis.

Results

Oviposition period and lifetime fecundity

The data for the oviposition period, lifetime fecundity, female progeny, sex ratio and longevity of OW and F₁ generation of *T. semistriatus* and *T. festiva* are shown in Table 1.

The females of OW and F₁ generation began ovipositing when host eggs were provided. Statistical analyses indicated that there was not any significant difference between species' oviposition periods ($F_{1,76} = 1.703$, $P = 0.196$). The oviposition period of F₁ generation was longer than OW females ($F_{1,76} = 12.948$, $P = 0.01$). No significant differences on the oviposition periods for the interactions between the species and factors were observed ($F_{1,76} = 1.287$, $P = 0.260$).

Except for F₁ generation of *T. festiva*, age-specific fecundity per female peaked on the first day of oviposition for the OW and F₁ generation of both species and it declined as female age progressed ($F_{1,76} = 4.658$, $P = 0.034$) (Figures 1a, b and 2a, b). However, the mean fecundity of OW females of both species was greater than fecundity of F₁ generation in the first day of oviposition, whereas in the following day of oviposition the mean fecundity decreased ($F_{1,76} = 12.147$, $P = 0.001$). On average, lifetime fecundity of OW and F₁ generation females was approximately 80% and 54% for *T. semistriatus*, 69% and 33% for *T. festiva* during the first four days of oviposition, respectively.

Table 1. Descriptive statistics (two-way ANOVA) results for mean oviposition period, lifetime fecundity, female progeny, sex ratio and longevity of OW and F₁ generation of *T. semistriatus* and *T. festiva*.

Species	Factors	Oviposition period (day) ^a (means ± SE)	Fecundity ^{a,b} (means ± SE)	Female progeny ^{a,b} (means ± SE)	Sex ratio ^{a,c} (means ± SE)	Longevity (day) ^{a,d} (means ± SE)
<i>T. semistriatus</i> (n = 20)	OW	10.5 ± 1.15	88.8 ± 6.13	62.5 ± 6.00	71.4 ± 5.16	16.2 ± 1.76
	F ₁	13.0 ± 0.95	111.9 ± 6.66	84.5 ± 5.57	75.4 ± 2.26	17.5 ± 1.46
<i>T. festiva</i> (n = 20)	OW	10.7 ± 1.07	85.6 ± 6.83	67.1 ± 6.89	78.8 ± 5.77	16.9 ± 1.21
	F ₁	15.5 ± 0.91	104.7 ± 4.66	80.7 ± 4.97	78.9 ± 4.17	28.5 ± 1.94
Statistical tests for species		$F_{1,76} = 1.703$ $P = 0.196$	$F_{1,76} = 0.525$ $P = 0.471$	$F_{1,76} = 0.000$ $P = 0.991$	$F_{1,76} = 1.440$ $P = 0.234$	$F_{1,76} = 13.295$ $P = 0.01$
Statistical tests for factors		$F_{1,76} = 12.948$ $P = 0.01$	$F_{1,76} = 11.808$ $P = 0.01$	$F_{1,76} = 7.398$ $P = 0.008$	$F_{1,76} = 0.107$ $P = 0.744$	$F_{1,76} = 16.028$ $P = 0.00$

^a The differences of mean oviposition period, lifetime fecundity, female progeny, sex ratio and longevity were tested by two-way ANOVA for statistical significance ($P \leq 0.05$)

^b The lifetime fecundity and female progeny data were transformed by $\sqrt{(x + 0.5)}$ before statistical analysis .

^c Data transformed using arcsin $\sqrt{(x)}$ before statistical analysis.

^d Values for longevities of OW females calculated after host eggs providing because they are post-overwintered individuals.

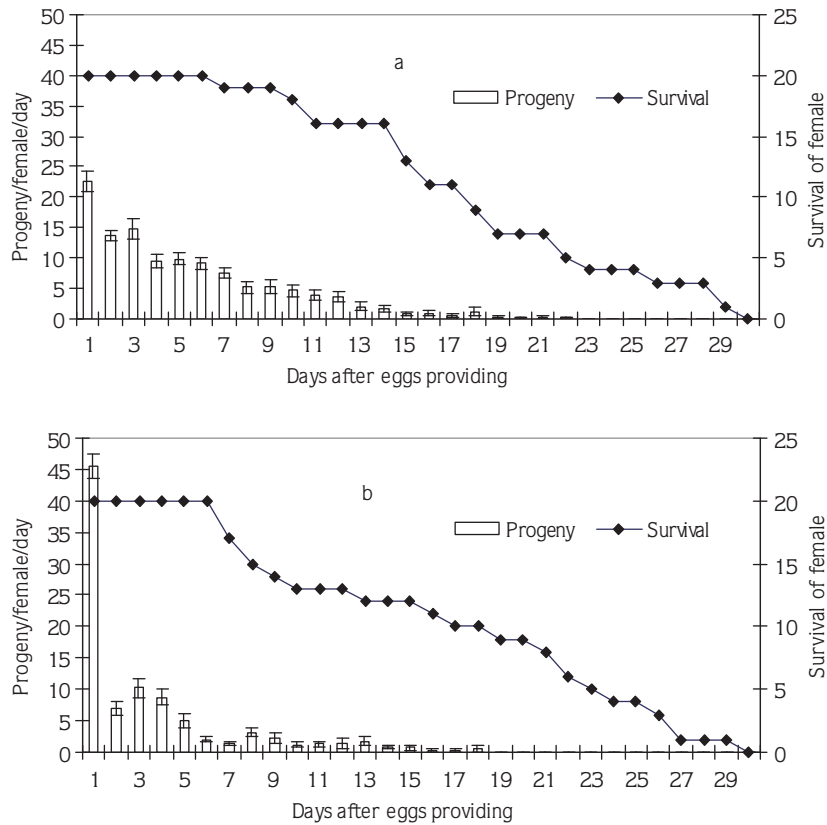


Figure 1. Age-specific fecundity (mean \pm SE) and survival females of OW (a) and F1 generation (b) of *T. semistriatus*.

There was no significant difference between species ($F_{1,76} = 0.525$, $P = 0.471$) for the lifetime fecundity, which was considerably different for OW and F₁ generation ($F_{1,76} = 11.808$, $P = 0.01$). No significant differences in lifetime fecundity for the interactions between the species and factors were observed ($F_{1,76} = 0.014$, $P = 0.907$; Table 1), as well.

Number of female progeny and sex ratio

Between the species, there was no significant difference on the number of females progeny ($F_{1,76} = 0.00$, $P = 0.991$), but the female progeny of OW and F₁ generation was significantly different ($F_{1,76} = 7.398$, $P = 0.008$). Furthermore, no significant differences were found for the interaction of species and factors on the female progeny of lifetime ($F_{1,76} = 0.326$, $P = 0.570$; Table 1).

The sex ratio of progeny was not significant between species and factors ($F_{1,76} = 1.440$, $P = 0.234$;

$F_{1,76} = 0.107$, $P = 0.744$). The interaction between species and factors on the sex ratio of progeny of lifetime ($F_{1,76} = 0.231$, $P = 0.632$; Table 1) was also not significant.

Longevity after providing host eggs

When the longevity of females of the species were compared, it was found to be significant ($F_{1,76} = 13.295$, $P = 0.01$). The effect of factors on the longevity of females were also significant ($F_{1,76} = 16.028$, $P = 0.00$). Finally, significant differences were found in the interaction of species and factors on the longevity of females ($F_{1,76} = 10.041$, $P = 0.02$; Table 1). The F₁ generation of *T. semistriatus* lived longer than the others, but OW wasps that were collected after emergence from winter refuges in the field are post-overwintered. The survival of females was high during oviposition period for the species, after which, it declined gradually as female age increased (Figures 1a, b and 2a, b).

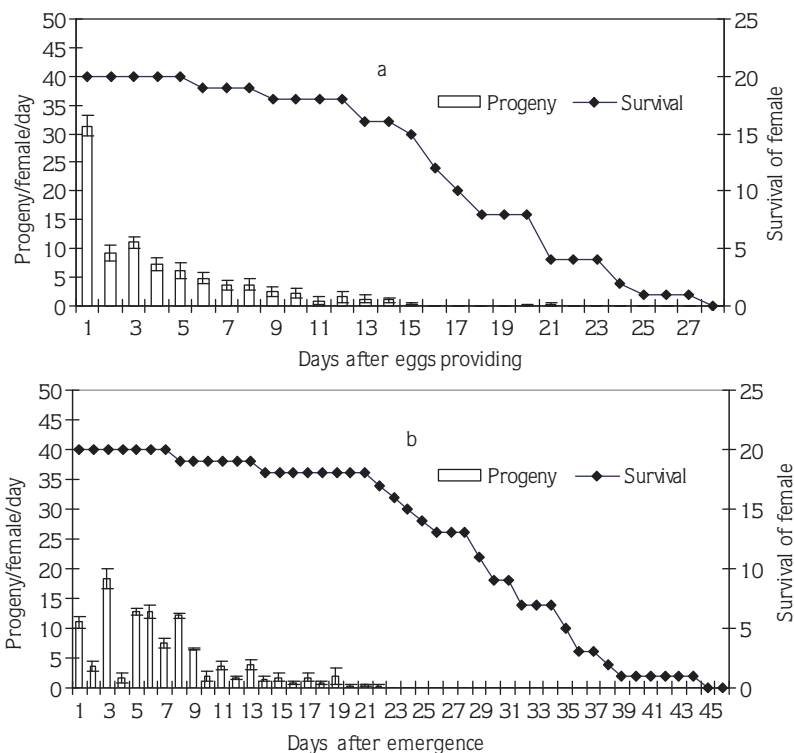


Figure 2. Age-specific fecundity (mean ± SE) and survival females of OW (a) and F₁ generation (b) of *T. festivaе*.

A linear relationship was measured between lifetime fecundity and female longevity and this was a significantly positive relation only for OW *T. semistriatus* ($y = 2.7622x + 44.003$, $R^2 = 0.63$; $F_{1,18} = 30.652$, $P = 0.000$; Figure 3).

Discussion

Life history traits in relation to reproductive biology and longevity of parasitoids are important as efficiency parameters in mass culture for biological control programs based on augmentative or inoculative releases. The reproduction characteristics and longevity of OW wasps that were collected in the field were compared to F₁ generation of laboratory-reared parasitoids on the sunn pest eggs.

The females of both species produced a greater number of progeny during the first days of oviposition. Like adults of these species, other Scelionidae females usually produce the majority of offspring in the first few days after emergence (Powell

and Shepard, 1982; Yergan, 1982; Vogt and Nechols, 1993; Saber et al., 2005; Canto-Silva et al., 2006). Relatively a few detailed studies of the reproductive biology of *T. semistriatus* are available in the literature. The fecundity per female was determined to be 84-122 offspring by Alexandrov (1948) and ca. 100 by Safavi (1968). Other studies have shown that the mean number of progeny was 117.5 ± 3.99 at 26 °C (Tarla and Kornoşor, 2007) and 88.15 ± 4.80 at 26 °C (Kivan and Kılıç, 2006b). Kivan and Kılıç (2006b) have documented similar results regarding the fecundity of various scelionid egg parasitoids. However, to our knowledge, no previous report has been presented on the reproductive biology of scelionid species overwintering populations under natural conditions in the field.

Unmated *T. semistriatus* females produced only male progeny (Tarla, 2002). In the current experiment, one female of the F₁ generation of *T. semistriatus*, produced only male progeny. This result showed that this parasitoid has arrhenotokous parthenogenetic

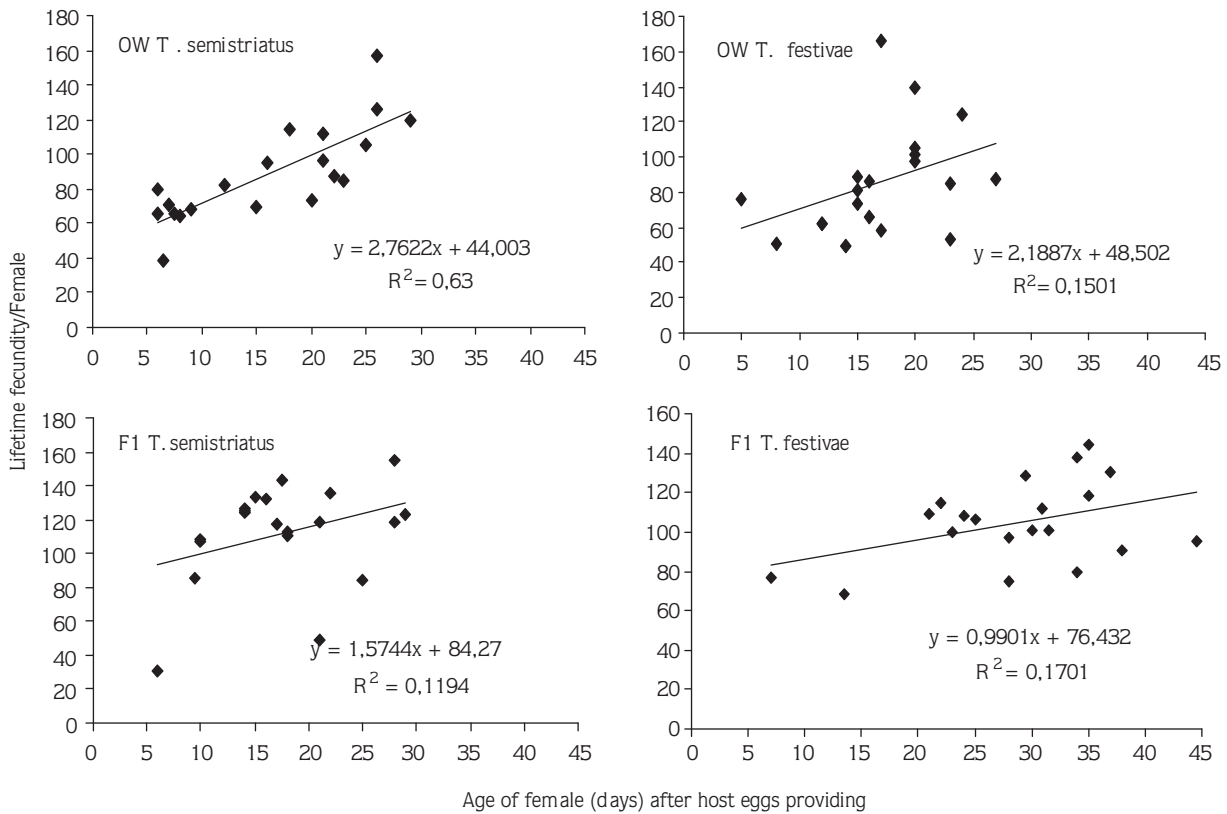


Figure 3. Relationship between the lifetime fecundity and longevity of OW and F1 generation of *T. semistriatus* and *T. festivae*.

reproduction. Since the females of both species produced a greater number of females during their lifetime, the sex ratio was distinctly female-biased. Given that sex is determined by haplodiploidy in parasitoid wasps, adult females are impregnated before overwintering, and the sperm stored in the spermatheca is used for fertilization in the spring. Only females of scelionid egg parasitoids of the sunn pest overwinter under the bark of various trees and migrate to wheat fields earlier than the pest from overwintering sites (Lodos, 1961). Voronin (1981) has reported that adults of *Trissolcus grandis* Thomson, *T. simoni* Mayr, *T. basalis* Wollaston, *T. histani* Voegelé and *Telenomus chloropus* Nixon (Hymenoptera: Scelionidae) become inactive at 9.0 °C, increasingly active from 10.0 to 11.0 °C, and start to parasitize their host eggs at 13.0 °C. Similarly, *Telenomus remus* Nixon become inactive at 10 °C (Schwartz and Gerling, 1974). The theoretical lower developmental threshold and thermal requirements were calculated as 11.8 °C, 138.8 degree-days (DD) for males and 11.6 °C, 161.3 DD for females

(Tarla, 2002) and 13.1 °C, 142.9 DD for males and 11.8 °C, 166.7 DD for females of *T. semistriatus*, respectively (Kıvan and Kılıç, 2006b). Kıvan and Kılıç (2006a; 2006b) and Tarla and Kornoşor (2007) have detailed in many studies theoretical lower developmental threshold and thermal requirements for various scelionid egg parasitoids.

The main effect of species, factors and interaction of species on the sex ratio of progeny was not significant. On average, sex ratio produced by *Trissolcus basalis* (Wollaston) (0.86) was significantly higher than that for *Telenomus podisi* Ashmead (0.78) and progeny sex ratio of the parasitoids was not affected by both storage temperature and period (Foerster and Doetzer, 2006).

In conclusion, at latitudes specific to Turkey, parasitoids and sunn pest migration times to wheat fields are different from one region to another. The migration to wheat fields first occurs in the Mediterranean areas, including Adana in Southern

Turkey, because of the climate of this region. Due to the migration of OW parasitoids and sunn pest adults to young cereal fields, this area is very important for mass-culture of egg parasitoids. Continuing parasitoid cultures throughout the year in the laboratory is very difficult. For this reason, they can be easily collected from the fields for mass-culture of parasitoids that can be sent to higher latitude areas at the onset of sunn pest oviposition. The present results show that F₁ generation females have a greater reproductive potential than OW females. However, OW female parasitoids can be used to initiate mass-culture for use in biological control against sunn pest without the need of continuing parasitoid culturing in the laboratory throughout the year. Thus, release of egg

parasitoids can be managed at the beginning of sunn pest oviposition, which is a very critical period. Otherwise, from the time when the host eggs are in progressed embryonic stages, the results will be unsatisfying. Synchronization of life cycles of natural enemies' populations with that of their hosts can be very important in determining the enemies' effectiveness (Coppel and Mertins, 1977). The natural overwintering wasps of both species have been successfully used in our laboratory for several years and have been suggested for biological control of sunn pests. The only potential constraint to the use of OW wasps to increase parasitoid numbers might be simply having inadequate parasitoids at times when they are needed to be distributed.

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