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*Original article (Orijinal araştırma)*

# An evaluation on host discrimination and superparasitism in *Trissolcus semistriatus* (Nees, 1834) (Hymenoptera: Scelionidae), egg parasitoid of *Eurygaster integriceps* Put., 1881 (Hemiptera: Scutelleridae)[1]

## *Eurygaster integriceps* Put., 1881 (Hemiptera: Scutelleridae)'in yumurta parazitoiti *Trissolcus semistriatus* (Nees, 1834) (Hymenoptera: Scelionidae)'da konukçuyu ayırt etme ve süperparazitizm üzerine bir değerlendirme

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## Abstract

*Trissolcus semistriatus* (Nees, 1834) (Hymenoptera: Scelionidae) is the most common and important egg parasitoid of *Eurygaster integriceps* Put., 1881 (Hemiptera: Scutelleridae). This study was conducted to determine discrimination *E. integriceps* eggs parasitized by either self or a conspecific female of *T. semistriatus* in choice and no-choice tests, and to establish effect of adult parasitoid density and host density on parasitism in the laboratory in 2016. Female parasitoids did not superparasitize self-parasitized host eggs. However, superparasitism of 18% (significantly lower than the 81% parasitism rate) of the eggs parasitized by another female occurred within 1 h of first parasitism. Superparasitism was significantly lower at 1% in no-choice tests than the 23% recorded in choice tests within 24 h of first parasitism. Parasitism significantly decreased with increasing host egg number, but parasitism did not change with increasing parasitoid density. Therefore, it is concluded that *T. semistriatus* can discriminate between parasitized and unparasitized host eggs, with superparasitism infrequent when females encounter preparasitized host eggs within 48 h of first parasitism.

**Keywords:** *Eurygaster integriceps*, host discrimination, intraspecific competition, parasitism, *Trissolcus semistriatus*

## Öz

*Trissolcus semistriatus* (Nees, 1834) (Hymenoptera: Scelionidae) *Eurygaster integriceps* Put., 1881 (Hemiptera: Scutelleridae)'in en yaygın ve önemli yumurta parazitoitidir. Bu çalışma, 2016 yılında laboratuvarda tercihli ve tercihsiz denemelerle *T. semistriatus*'un kendisi ya da diğer bir dişisi tarafından parazitlenmiş *E. integriceps* yumurtasını ayırt etme durumunu tespit etmek ve ergin parazitoit ve konukçu yoğunluğunun parazitlenme üzerindeki etkisini saptamak için yürütülmüştür. Dişi parazitoit kendisi tarafından parazitlenmiş konukçu yumurtasını süperparazitlememiştir. Ancak ilk parazitlenmeden sonraki 1 saat içinde diğer bir dişi tarafından parazitlenmiş yumurtaların %18'inin (%81'lik parazitizm oranından önemli olarak daha düşük oranda) süperparazitlendiği görülmüştür. İlk parazitlenmeden 24 saat sonrasındaki tercihli denemelerde kaydedilen %23'lük orana göre tercihsiz denemelerde önemli olarak daha düşük %1'lik bir oranda süperparazitizm meydana gelmiştir. Parazitizm artan konukçu yumurtası sayısı ile önemli oranda düşmüş, ancak artan parazitoit yoğunluğu ile değişmemiştir. Bu nedenle, *T. semistriatus*'un parazitlenmiş konukçu yumurtasını ayırt edebildiği, dişilerin ilk parazitlenmeden 48 saat sonrasına kadar önceden parazitlenmiş konukçu yumurtalarıyla karşılaştığında nadiren bir süperparazitizm meydana geldiği kanısına varılmıştır.

**Anahtar sözcükler:** *Eurygaster integriceps*, konukçuyu ayırt etme, tür içi rekabet, parazitizm, *Trissolcus semistriatus*

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## Introduction

The Sunn pest, *Eurygaster integriceps* Put., 1881 (Hemiptera: Scutelleridae) has a wide distribution in the Palearctic Region, that is Eastern Europe, Near and Middle East, and Western Asia. It is the most important species in cereal fields and it causes severe qualitative and quantitative damage on wheat and barley. The scelionid egg parasitoids have demonstrated a great potential to suppress the Sunn pest below the economic threshold, but this can vary among regions and from year to year (Brown, 1962; Safavi, 1968; Lodos, 1982; Öncüer & Kıvan, 1995). *Trissolcus semistriatus* (Nees, 1834) (Hymenoptera: Scelionidae) is the most common egg parasitoid of the Sunn pest.

The scelionid egg parasitoids of certain species of Lepidoptera and Heteroptera have been used in both augmentative and classical biological control applications (Orr, 1988; Hoffmann et al., 1991; Corrêa-Ferreira & Moscardi, 1996; Weber et al., 1996; Ehler, 2002). They are solitary parasitoids and only one parasitoid can develop within one host egg (van Lenteren, 1981). When a female lays an egg in a host egg, she marks it with pheromone after oviposition so females can discriminate between parasitized and unparasitized eggs (Okuda & Yeargan, 1988; Roitberg & Mangel, 1988). These pheromone marks are generally species specific and reduce superparasitism, but sometimes they can affect related species (Agboka et al., 2002). However, it has been observed that parasitoid females frequently oviposited an egg into a host preparasitized by herself (self-superparasitism), by a conspecific female (superparasitism) or by another species (multiparasitism) (van Alphen & Visser, 1990; Visser et al., 1990, 1992; Godfray, 1994). Superparasitism is more likely to occur when the parasitoid to host ratio is high (van Alphen & Vet, 1986). When host eggs are fewer in number, more than one female *Trissolcus grandis* (Thomson, 1861) (Hymenoptera: Scelionidae) oviposits into the same host egg (Kozlov, 1968). In such cases, solitary parasitoids waste both time and eggs.

An earlier study of female behaviour in *Trissolcus viktorovi* Kozlov, 1968, *Trissolcus djadetshko* (Ryach., 1959) and *T. grandis* during attack on eggs of *Eurydema ornatum* (L., 1758) (Hemiptera: Pentatomidae) already parasitized by the same species showed that intraspecific reinfestation occurs only rarely, whereas interspecific multiple parasitism is frequent, but varies between the different species (Buleza, 1971a). Buleza (1971b) also researched the interspecific relationships between *T. viktorovi*, *T. djadetshko* and *T. grandis*. According to another study, *T. grandis, Trissolcus simoni* (Mayr, 1879) and *T. viktorovi* could distinguish between parasitized and non-parasitized eggs of *E. integriceps, Graphosoma lineatum* (L., 1758) and *Eurydema ventralis* (Kolenati, 1846) (Hemiptera: Pentatomidae); in the absence of non-parasitized eggs, once-parasitized eggs were preferred to twice-parasitized eggs (Kartsev, 1985). Todoroki & Numata (2017) recently studied about effects of mating experience on superparasitism by female *T. semistriatus*. It was observed that virgin females discriminated between parasitized *Eurydema rugosum* Motshulsky, 1861 (Hemiptera: Pentatomidae) eggs from unparasitized eggs and avoided complete superparasitism. In contrast, mated females superparasitized more parasitized eggs than virgin females did.

The primary aim of this study was to determine intraspecific host discrimination of *T. semistriatus* and if its females superparasitized host eggs. Similar studies (mentioned above) have been done on *T. grandis*. Recently, *T. semistriatus* was synonymized with *T. grandis* (Talamas et al., 2017). In this study, host discrimination of *T. semistriatus* will be detailed and compared with former findings from different *Trissolcus* spp. and different host species. The secondary aim was to determine the effect of parasitoid and host density on parasitism efficiency. Thus, intraspecific competition could be considered their adaptive value.

## Material and Methods

### Insect rearing

*Eurygaster integriceps* adults were collected from wheat fields in Tekirdağ at the end of April 2016 when they have migrated to fields after overwintering area. They were cultured on potted wheat in a growth room at 26±1ºC, 60±10% RH and 16:8 h L:D photoperiod (Kıvan & Kılıç, 2002). Eggs of *E. integriceps* were collected daily for use in the experiments.

Laboratory progeny of *T. semistriatus* were used in the experiments. They were reared on *E. integriceps* eggs in glass tubes in an incubator (26±1ºC, 60±10% RH and 16:8 h L:D photoperiod) and fed with a sugar solution (30%) absorbed into filter paper (1 x 5 cm) (Kıvan, 1998).

**In choice superparasitism experiments**

Egg masses of *E. integriceps* (1 d old) was placed individually in a Petri dishes (6 cm diameter), and a sketch of each egg mass was drawn under a stereomicroscope to record the number and location of its eggs (Mahmoud & Lim, 2008). For evaluation of self-discrimination, individual females (24 h old) previously mated and inexperienced were released onto an egg mass in a Petri dish, allowed to parasitize half of the eggs in the mass, and then removed. One h later, the same female was introduced again to the same preparasitized egg mass. For evaluation of conspecific discrimination, 1 h later another female of *T. semistriatus* was released onto the preparasitized egg mass. All observations were made under a stereomicroscope and ended when the female parasitoid had parasitized all the eggs in the mass or had stopped ovipositing for more than 30 min. The number of parasitized and self superparasitized eggs were counted then incubated at 26±1ºC for the development of parasitoids. The eggs marked after oviposition was considered to be parasitized (Rabb & Bradley, 1970; Weber et al., 1996; Mahmoud & Lim, 2008). The experiment was repeated 12 times.

**Effect of time after first oviposition on superparasitism in choice tests**

To examine the possible effects of the length of time after first oviposition, the female of *T. semistriatus* was allowed to parasitize half of the eggs in an egg mass. Another female of the same species was then introduced onto the same egg mass after 1, 24 and 48 h from first parasitism. Observations were under a stereomicroscope. The number of parasitized or conspecific superparasitized eggs were recorded, and eggs were incubated at 26±1ºC for the development of the host or the parasitoids. These procedures were repeated 10 times with different individual parasitoids.

**No-choice superparasitism experiments**

One female was allowed to parasitize all the eggs in an egg mass and, after 24 h from the first oviposition, another female of the same species was released on the same egg mass. Observations were under a stereomicroscope. The number of parasitized or conspecific superparasitized eggs were recorded, and eggs were incubated at 26±1ºC for the development of the host or the parasitoid. The experiment was repeated with 10 parasitoid females.

**Adult competition experiments**

Two treatments were used to examine adult competition and the effect of parasitoid and host density on parasitism by *T. semistriatus*. Firstly, the host density was kept constant and female parasitoid density was varied. Two, four and eight females were released on two egg masses. After 24 h of parasitism, the females were removed and parasitized eggs were kept for incubation. Secondly, female parasitoid density was kept constant and host density was varied. This time, two females were released on two, four and eight egg masses. Each treatment was replicated five times.

**Statistical analysis**

Parasitism rate was calculated by dividing the number of once-parasitized eggs by the total number of host eggs offered. Superparasitism rate was the proportion of the number of twice-parasitized eggs to total number of host eggs offered. Adult emergence rate was the proportion of the number of emerged adult parasitoids to number of parasitized eggs. Parasitism, superparasitism and adult emergence rate were analyzed as pooled data by the $X^2$ test of contingency table ($p < 0.05$), and to compare choice with no-choice test results, t-test was used ($p = 0.05$). To evaluate the effect of time for data collected after 1, 24 and 48 h from first parasitism and data on adult competition effects ANOVA was applied and Tukey's test for multiple comparing (SPSS, 2006).

## Results

### Intraspecific host discrimination and larval competition

There was no superparasitism when *T. semistriatus* female encountered host eggs preparasitized by herself after 1 h (Table 1). However, another female deposited a few eggs in the host eggs preparasitized by a conspecific female. The number of superparasitized eggs was significantly different from once-parasitized ($X^2$ = 66.5, p < 0.05). In choice tests, the superparasitism rate (18%) was significantly lower than the parasitism rate (81%) (Table 1). Although the rate of adult emergence from conspecific superparasitized eggs was seemed lower in compared to emergence from unparasitized eggs, it was not significant according to the t-test. When unparasitized and preparasitized host eggs in the same egg mass were provided to a female *T. semistriatus*, she did not usually oviposit eggs in parasitized host eggs but occasionally eggs were oviposited in host eggs previously parasitized by other females.

Table 1. Host discrimination of *Trissolcus semistriatus* on *Eurygaster integriceps* eggs previously parasitized by themselves or conspecific females (mean±SD)

| Host egg status | Number of eggs (masses) tested | Self-discrimination | | Conspecific discrimination | |
|---|---|---|---|---|---|
| | | Parasitism rate | Emergence rate | Parasitism rate | Emergence rate |
| Unparasitized | 167 (12) | 0.97±0.06 | 0.97±0.06 | 0.81±0.14 | 0.99±0.02 |
| Preparasitized | 137 (10) | 0 | - | 0.18±0.15 | 0.76±0.41 |

The number of superparasitized host eggs were significantly lower after 1 ($X^2$ = 66.5, p < 0.05), 24 ($X^2$ = 56.5, p < 0.05) and 48 h ($X^2$ = 65.5, p < 0.05) from first parasitism. The parasitism rates after 1, 24 and 48 h were similar at 8, 70% and 81%, respectively (Table 2). The parasitism and superparasitism rates, and also percentage adult emergence of once-parasitized eggs did not change with time elapsed after first parasitism. Although the emergence rate from superparasitized eggs (67%) decreased after 48 h from first oviposition, it was not significant. The survival of superparasitized eggs was relatively low.

Table 2. Effect of time after first oviposition on host discrimination of *Trissolcus semistriatus* on preparasitized *Eurygaster integriceps* eggs by conspecific females (mean±SD)

| Time (h) after pre-parasitization | Number of eggs (masses) tested | Parasitism rate | Superparasitism rate | Emergence rate | |
|---|---|---|---|---|---|
| | | | | Once parasitized | Superparasitized |
| 1 | 137 (10) | 0.81±0.14 | 0.18±0.15 | 0.99±0.02 | 0.76±0.41 ab |
| 24 | 123 (9) | 0.70±0.18 | 0.23±0.14 | 0.96±0.11 | 0.92±0.17 a |
| 48 | 137 (10) | 0.81±0.24 | 0.10±0.11 | 0.99±0.03 | 0.47±0.50 b |

*Same letters in the column indicate that means are not significantly different (p ≤ 0.05, Tukey's test).

In the no-choice test, the number of superparasitized host eggs was significantly low ($X^2$ = 65.3, p < 0.05) from first parasitism. The superparasitism rate in no-choice test was significantly low (0.01±0.04) than that of choice test (0.23±0.14) (*t* = 4.35, p = 0.05) (Figure 1). The females could discriminate between parasitized and unparasitized host eggs, and only one female deposited two eggs into two preparasitized eggs. As a result of hatching all two superparasitized eggs, the emergence rate was 1.0±0.00 and was not significantly different from that of choice test (0.92±0.17) (*t* = -0.232, p = 0.05).

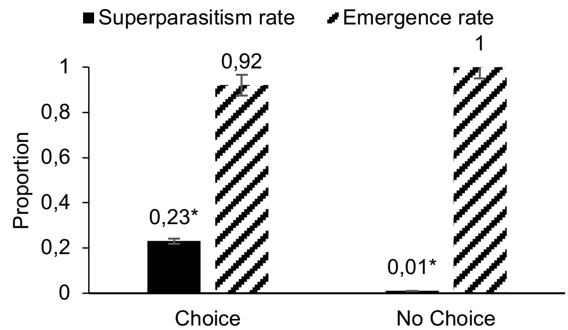


Figure 1. Comparison of the superparasitism and emergence rate of *Trissolcus semistriatus* in choice and no-choice tests. The asterisks indicate significant differences between proportions (p = 0.05, t-test).

### Adult competition

The parasitism and adult emergence significantly decreased increasing density when two females encountered with a high density of host eggs (Table 3).

Table 3. Influence of host density on parasitism and emergence rate of *Trissolcus semistriatus* (mean±SD)

| Number of host egg masses (Mean number of eggs tested) | Parasitism rate | Emergence rate |
|---|---|---|
| 2 (26.75) | 0.91±0.17 a | 0.97±0.05 a |
| 4 (53.00) | 0.51±0.28 b | 0.91±0.05 a |
| 8 (98.80) | 0.31±0.22 b | 0.66±0.25 b |

*Same letters in the same column indicate that means are not significantly different (p ≤ 0.5, Tukey's test).

When two egg masses were exposed to a different number of the female parasitoid, the parasitism rate was low (Table 4). Parasitism rate was 91% when two host egg masses were exposed to two females of *T. semistriatus* and 80% with eight females, but, this decrease was not significant. However, emergence rate was significantly affected at different parasitoid density (Table 4).

Table 4. Influence of parasitoid female density on host parasitism and emergence rate of *Trissolcus semistriatus* (mean±SD)

| Number of female parasitoids | Mean number of eggs tested | Parasitism rate | Emergence rate |
|---|---|---|---|
| 2 | 26.8 | 0.91±0.17 | 0.97±0.05 ab |
| 4 | 27.6 | 0.91±0.10 | 0.89±0.07 b |
| 8 | 25.3 | 0.80±0.21 | 1.00±0.00 a |

*Same letters in the same column indicate that means are not significantly different (p ≤ 0.5, Tukey's test).

## Discussion

*Trissolcus semistriatus* females could discriminate between host eggs that where unparasitized or preparasitized by self or conspecific parasitoids. No females deposited eggs in host eggs preparasitized by herself and self superparasitism was not observed. However, one female deposited a few eggs in host eggs preparasitized by conspecific another female. Thus, conspecific superparasitism was much lower in both choice and no-choice experiments. Intraspecific reinfestation of *E. ornatum* by *T. grandis* occurred very rarely (Buleza, 1971a). Mated females of *T. semistriatus* superparasitized more parasitized eggs of *E. rugosum* than virgin females (Todoroki & Numata, 2017). Agboka et al. (2002) also reported that self superparasitism was only 4% for *Telenomus busseolae* Gahan, 1922 and about 6% for *Telenomus isis* Polaszek, 1993 (Hymenoptera: Scelionidae). Self superparasitism was considerably lower than intraspecific superparasitism, suggesting that the females are able to discriminate between the marking of their own and conspecific females (Agboka et al., 2002). Mahmoud & Lim (2008) suggested that host discrimination has evolved to increase the fitness of female that marks the host eggs, and showed that *Trissolcus nigripedius* Nakagawa, 1900 and *Telenomus gifuensis* Ashmead, 1904 (Hymenoptera: Scelionidae) discriminated between host egg preparasitized by either self or conspecific. They reported that superparasitism rates were 19 and 31% for *T. nigripedius* and *T. gifuensis*, respectively. Female parasitoids avoid superparasitism and disperse to search unparasitized host eggs (Okuda & Yeargan, 1988).

Low emergence rates for conspecific superparasitized eggs was observed in this study, but it was not significant unlike some reports in the literature (Agboka et al., 2002, Mahmoud & Lim, 2008). The low rates for both *T. nigripedius* and *T. gifuensis* have been attributed to the intraspecific competition between similar aged larvae (Mahmoud & Lim, 2008).

*Trissolcus semistriatus* tended to avoid host eggs that had been parasitized by conspecifics 1, 24, and 48 h previously, so the superparasitism rate was low and did not change with increasing time after first oviposition. However, Agboka et al. (2002) determined that superparasitism was significantly higher when eggs were offered directly and after the 0 and 48 h than after 24 h for both *T. busseolae* and *T. isis*. They suggested that the parasitoids recognized eggs parasitized at least 24 h earlier via an internal marker; when they insert their ovipositor in parasitized eggs, they abruptly removed it as if startled.

Adult emergence from superparasitized eggs was not significantly different at 1, 24 or 48 h after first oviposition. The eggs of *Telenomus solitus*, Johnson, 1983 hatched less 20 h after deposition (Navasero & Oatman, 1989), for example *Trissolcus basalis* (Wollaston, 1858) (Hymenoptera: Scelionidae) requires, on average, 17 h for egg development (Corrêa-Ferreira, 1993). When a female deposits an egg in an already parasitized host, larval competition begins in the host (Bakker et al., 1985). The emergence was higher at 24 h after the first oviposition since the older larvae might outcompete the younger one (Okuda & Yeargan, 1988). However, Volkoff & Colazza (1992) reported that second instar platygastrid larvae, although larger, are less mobile and have no jaws, which makes them more susceptible to attack by first-instar larvae. So, second larvae have a better chance of surviving if the time between oviposition events is enough for the first larvae to molt to the second instar (Cingolani et al., 2013). The first instar of the larvae has well-developed mandibles and is very mobile, so it is possible for older instars to be killed by younger, first instar larvae. It was not possible to determine whether first larvae or second larvae were dominant in the study because they are from the same species. So adult emergence from host eggs superparasitized at the different interval time was a similar and high ratio.

Superparasitism occurred at low percentage when female exposed to parasitized eggs in no-choice test, it was even lower than that observed in the choice test. A single female superparasitized just two eggs in a whole parasitized egg mass in no-choice test and the emergence was 100% in these eggs. If females had no chance to choose unparasitized host egg, they did not prefer to oviposit in the host. Female showed different behavior in choice and no-choice tests when a female encountered preparasitized egg masses. She spent a shorter time on the mass in no-choice tests, although she stayed for a longer time on the eggs and searched for suitable host eggs in the choice tests. Adult emergence rates have been reported to be 93 and 75% for *T. nigripedius* and *T. gifuensis*, respectively, in no-choice tests (Mahmoud & Lim, 2008), which is similar to the current study.

In adult competition tests, parasitism rate decreased with increasing host egg numbers because females encountered many host eggs which were preparasitized by herself. It is known that a female can parasitize 12 to 24 host eggs per day (Kıvan & Kılıç, 2006a, b). Emergence rate also decreased with increasing host egg numbers. It is suggested that host eggs may be multiparasitized two, three or more times when the number of females is increased, however, emergence rate can decrease. However, parasitism rate was not changed when parasitoid density was increased. This may be due to the female *T. semistriatus* discriminating between host eggs parasitized by other conspecific females and avoids parasitizing these eggs resulting in higher adult emergence from parasitized eggs. In this experiment, all emergence rates were close to one however there was some significant difference. It appears that a smaller number of host eggs parasitized by eight females but all of these parasitized eggs hatched while similar rate of emergence.

In summary, the present study indicates that *T. semistriatus* had intraspecific host discrimination abilities, so superparasitism was very low. Future studies should aim to determine host discrimination and interspecific competition of different *Trissolcus* species under both laboratory and field conditions.

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