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Reproduction of *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae) and *Trissolcus* sp. aff. *urichi* (Hymenoptera: Scelionidae) in Eggs of *Nezara viridula* (Hemiptera: Pentatomidae) of Different Ages

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Abstract

The study of the interaction between parasitoid and host, especially the age of these organisms, is an important step towards the implementation of biological control programs. Therefore, we investigated the performance of *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae) and *Trissolcus* sp. aff. *urichi* (Hymenoptera: Scelionidae) parasitizing eggs of *Nezara viridula* (Hemiptera: Pentatomidae), considering different ages of the parasitoids and the host. We performed four laboratory bioassays: two using females of *O. submetallicus* and *Trissolcus* sp. aff. *urichi* at 24, 48, 72, 96, 120, or 144 hours of age exposed to parasitism in *N. viridula* eggs (24 h) and two trials with *N. viridula* eggs at 24, 48, 72, 96, 120, or 144 hours exposed to the parasitism of *O. submetallicus* and *Trissolcus* sp. aff. *urichi* (24 h). We evaluated the percentage of parasitism and emergence, life cycle length, progeny, sex ratio, and the longevity of the parasitoids. The parasitism of *O. submetallicus* in *N. viridula* eggs was influenced by the age of the parasitoid, 120 hours being the minimum to obtain better parasitism. From this age on, there is interference in the longevity of the progeny. *Trisolcus* sp. aff. *urichi*, at all ages, parasitized *N. viridula* eggs relatively well, but with almost no emergence of the parasitized eggs. Females of *O. submetallicus* parasitized and developed in eggs of *N. viridula* of all ages. Females of *Trissolcus* sp. aff. *urichi* parasitized their host, but there was barely any emergence. These pieces of information regarding the breeding methodology contribute to the implementation of new protocols for the multiplication of these parasitoids in the laboratory, and later, their release in the field.

Keywords: biological control, host-parasitoid interaction, breeding methodology, egg parasitoid, green stink bug

1. Introduction

Biological control is an environmentally friendly and affordable management alternative, adopted to reduce insect populations considered pests for their natural enemies (Parra et al., 2002). In addition, this type of control is used to minimize excessive applications of synthetic insecticides, being a viable tool when associated with other strategies adopted by Integrated Pest Management (IPM) (Parra, 2014; Van Lenteren et al., 2018).

For the success of a biological control program, studies involving the interaction between the natural enemy and its host are needed, in order to understand the biology of these organisms and then adopt them as biological control agents (Parra & Zucchi, 2004; Siqueira et al., 2012). Several egg parasitoids have been reported, studied, and used as biological control agents (Crouzel & Saini, 1981; Corrêa-Ferreira & Moscardi, 1996; Queiroz et al., 2018; Martel et al., 2019; Stahl et al., 2019; Zerbino & Panizzi, 2019; Scaccini et al., 2020).

Among these, the parasitoids *Ooenycyrtus submetallicus* (Howard) (Hymenoptera: Encyrtidae) and *Trissolcus urichi* (Crawford) (Hymenoptera: Scelinidae) parasitize eggs of several species of stink bugs (Zanuncio et al., 2000; Maciel et al., 2007; Laumann et al., 2010; Riffel et al., 2010; Golin et al., 2011; Sousa et al., 2019). Like those of *Nezara viridula* (L.) (Hemiptera: Pentatomidae), known as the green bug (Lee, 1979; Sujii et al., 2002; Paz-Neto et al., 2015; Zerbino & Panizzi, 2019).

The green stink bug and other species of the complex group of phytophagous of the Pentatomidae family cause irreversible damage to the soybean crops from the beginning of pod formation to the final grain filling stage, affecting both yield and final quality (Panizzi & Slansky Jr, 1985; Soares et al., 2018). Commonly, species of the Pentatomidae complex are controlled with synthetic insecticides, both in seed treatment and in aerial application at the initial stages of crop development (Chiesa et al., 2016). However, this management method has been associated with the emergence of insect populations resistant to the main groups of insecticides adopted, the reduction of natural control agents, as well as other implications for the agroecosystem (Sosa-Gómez et al., 2001; Sosa-Gómez et al., 2010, Turchen et al., 2016).

Therefore, having knowledge and development of research involving the interaction between the parasitoid and the host is necessary to increase the efficiency and success of biological control programs (Parra & Zucchi, 2004; Parra et al., 2019). Among these interactions, studying the age of both parasitoid and host females is not only important for analyzing the performance of biological control agents but also for predicting their establishment in the field (Cingolani et al., 2014; Queiroz et al., 2019; Hill et al., 2019; Queiroz et al., 2020a). Considering this, we determined the age of the parasitoids *O. submetallicus* and *Trissolcus* sp. aff. *urichi* that are considered optimal for parasitism in eggs of *N. viridula*. Furthermore, we evaluated the influence of the host egg age on the multiplication of both parasitoids and the influence of these factors on their biological characteristics.

2. Method

2.1 Laboratory Breeding of O. submetallicus, Trissolcus sp. aff. urichi and N. viridula

The experiments were set up based on stock cultures of the parasitoids *O. submetallicus* and *Trissolcus* sp. aff. *urichi* and host *N. viridula*, kept in an acclimatized room with temperature of 25±2 ºC, relative humidity of 70±10% RH, and a 12-hour photophase. The parasitoids *O. submetallicus* and *Trissolcus* sp. aff. *urichi* were identified according to Noyes (2010) and Johnson (1987), respectively. Voucher specimens were deposited at Coleção de Insetos Entomófagos "Oscar Monte" (Instituto Biológico, Campinas, SP, Brazil; curator Valmir A. Costa).

Nymphs and adults of *N. viridula* were placed in a 5 L transparent plastic containers and fed with fresh pods of *Phaseolus vulgaris* (Fabaceae), seeds of *Ligustrum* sp. (Oleaceae), raw grains of *Arachis hypogaea* L. (Fabaceae), and dry grains of *Glycine max* (Fabaceae) (Silva et al., 2008). In addition to portions of cotton moistened in distilled water to supply the insect's water needs. Filter paper (30 cm × 30 cm) folded in a fan shape and voile fabric (30 cm × 30 cm) were placed as a substrate for the stink bugs' oviposition. Adults of *O. submetallicus* and *Trissolcus* sp. aff. *urichi* were kept in glass tubes (15 cm high × 2 cm in diameter) closed with plastic film, fed with a droplet of honey (100%), and then multiplied into egg masses of *N. viridula* and *Chinavia pengue* (Rolston) (Hemiptera: Pentatomidae), respectively. The identification of the species *N. viridula* and *C. pengue* was carried out by Dr. Jocélia Grazia, (Universidade Federal do Rio Grande do Sul) Porto Alegre, Rio Grande do Sul.

2.2 Experimental Development

2.2.1 Age of *O. submetallicus* and *Trissolcus* sp. aff. *urichi* Reared in Eggs of *N. viridula*

Five eggs of the host *N. viridula* (yellow and not deformed, 24 hours of age) were offered to each female of *O. submetallicus* at 24, 48, 72, 96, 120, or 144 hours of age, as well as to each female of *O. submetallicus* at 24, 48, 72, 96, 120, or 144 hours of age. *Trisolcus* sp. aff. *urichi*, and they were kept separately. These eggs were placed on cardboard cartons (1 × 5 cm), fixed with 20% gum arabic, and inserted into glass tubes (2 cm in diameter × 15 cm in height) (Figure 1A). After 24 hours of parasitism, the females of the parasitoids were removed from the tubes and the hosts were transferred to the climatized chamber (BOD) at 25±2 ºC, 70±10% relative humidity, and 12-hour photophase until the emergence of the parasitoid adults.

2.2.2 Age of *N. viridula* Eggs Offered to the Parasitoids *O. submetallicus* and *Trissolcus* sp. aff. *urichi*

Five eggs of the host *N. viridula* (yellow and not deformed) at 24, 48, 72, 96, 120, or 144 hours of age were placed on cardboard sheets (1 × 5 cm), fixed with 20% gum arabic, and inserted in glass tubes (2 cm in diameter × 15 cm in height). Later, they were offered to females of *O. submetallicus* and *Trissolcus* sp. aff. *urichi*, both at 24 hours of age, in separate tubes (Figure 1B). After 24 hours of parasitism, the females of each species were removed from the tubes and the cardboards containing the host's eggs were transferred to a climatized chamber (BOD) at 25±2 ºC, 70±10% relative humidity, and a 12-hour photophase until possible emergence of adults of the parasitoids.

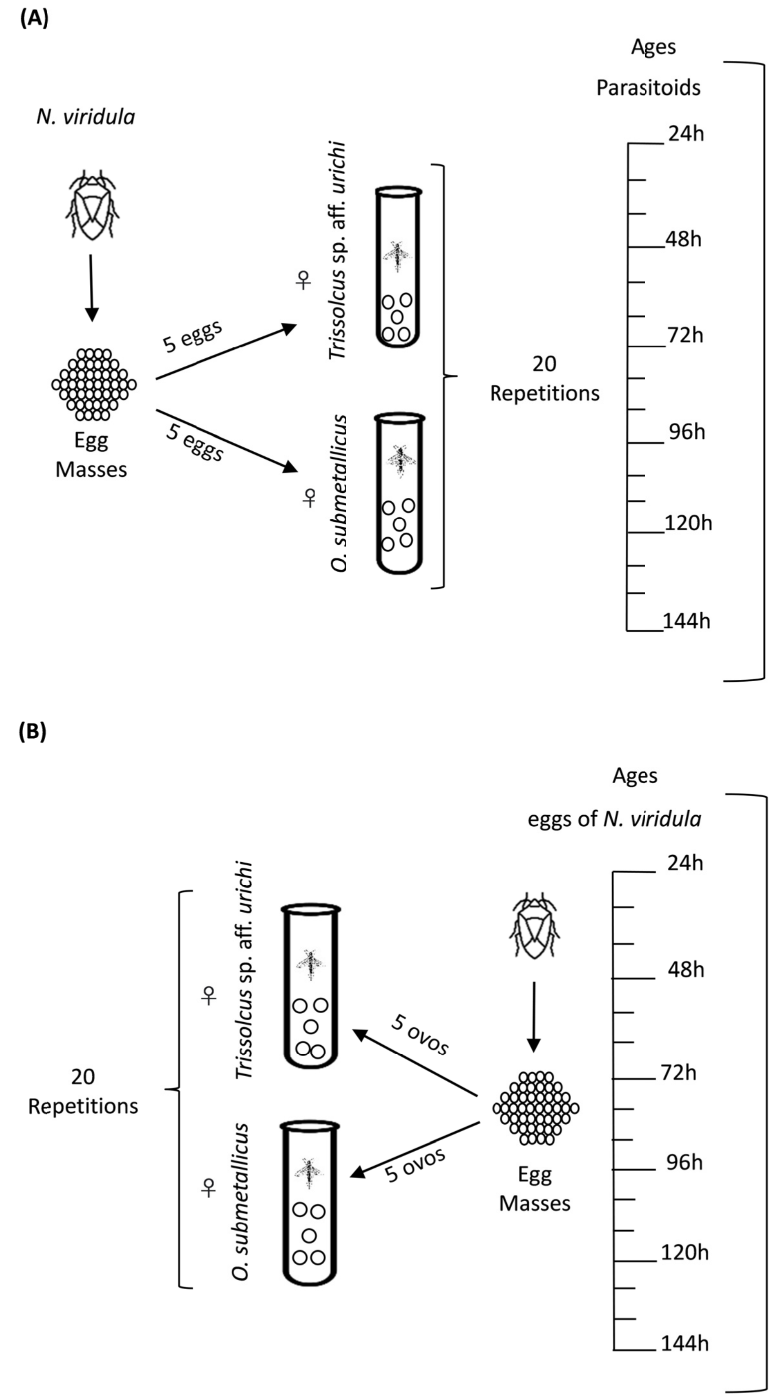

Figure 1. Scheme of experimental development. (A) ages of females of the parasitoids *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae) and *Trissolcus* sp. aff. *urichi* (Hymenoptera: Scelionidae) at 24, 48, 72, 96, 120, or 144 hours exposed to parasitism in eggs of the host *Nezara viridula* (Hemiptera: Pentatomidae) (n = 5). (B) ages of *N. viridula* host eggs (n = 5) at 24, 48, 72, 96, 120, or 144 hours exposed to parasitism of females of *O. submetallicus* and *Trissolcus* sp. aff. *urichi*. Kept at 25±2 °C, 70±10% relative humidity, and 12-hour photophase. Dourados, Mato Grosso do Sul, Brazil

2.3 Experimental Evaluation

The biological characteristics evaluated were: percentage of parasitism and parasitoid emergence; life cycle length (egg-adult); progeny (number of individuals per egg); adult longevity (with food) and sex ratio (number of females/total number of individuals). The experimental design used was completely randomized, with 20 repetitions per treatment (age of parasitoids and age of host eggs). Data on life cycle length, percentage of parasitism and parasitoid emergence, number of individuals per egg, sex ratio, and longevity were submitted to analysis of variance, and, when significant, of regression with 5% probability (Software Sigmaplot 12.0).

The choice of the best suitable equation was based on the Coefficient of Determination (R^2), the significance of the Regression Coefficients ($^{\beta}$i), and the Regression by Test F (at 5% probability).

3. Results

3.1 Age of O. submetallicus and Trissolcus sp. aff. urichi in eggs of N. virirula

Parasitism progressively increased with the age of females of *O. submetallicus* in *N. viridula* eggs, going from 40.00±20.00% at 24 hours to 97.33±1.82% at 144 hours (F = 41.2610, p ≤ 0.0001 and Rtrat = 0.83) (Figure 2). The emergence percentage of *O. submetallicus* in *N. viridula* eggs was not a significant factor when related to the ages of this parasitoid, although it remained high during the six days (144 h), presenting an overall average of 94.52±1.61 % (p > 0.05) (Table 1).

The length of the life cycle (egg-adult), progeny, and sex ratio of *O. submetallicus* was also not influenced by the different ages of the parasitoid (p > 0.05), presenting overall average values of 17.85±0.07 days; 1.39±0.07 individuals per egg and 1.00±0.00, respectively (Table 1).

Similar to parasitism, the progeny longevity was influenced by the different ages of *O. submetallicus* females in *N. viridula eggs* (F = 10.4012, p ≤ 0.0001 and Rtrat = 0.82) (Figure 3). Longevity decreased as parasitoid age increased, from 18.13±1.72 days (with females aged 24 hours) to 8.50±0.44 days (with females aged 144 hours) (Table 1).

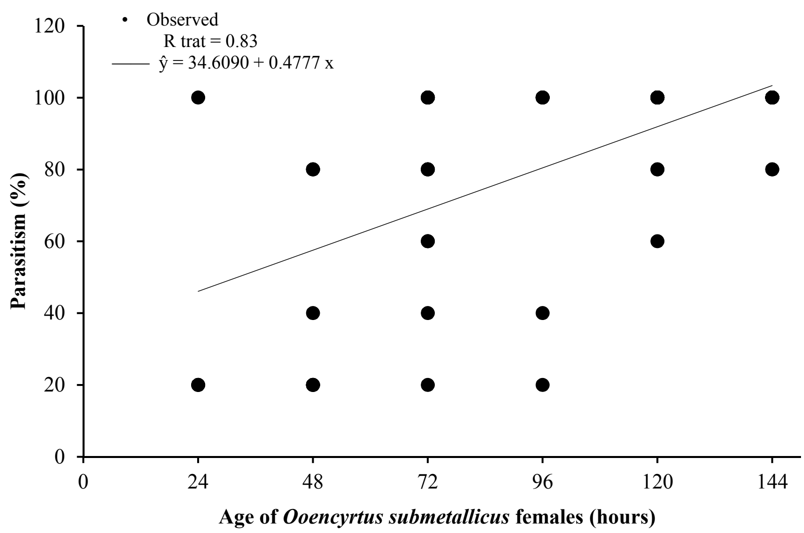

Figure 2. Parasitism (%) of females of *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae) at 24, 48, 72, 96, 120, and 144 hours in eggs of *Nezara viridula* (Hemiptera: Pentatomidae) at 25±2 °C, 70±10 % relative humidity and 12-hour photophase (F = 41.2610 and p ≤ 0.0001). Dourados, Mato Grosso do Sul, Brazil

Table 1. Biological characteristics (mean±SE) of *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae) at 24, 48, 72, 96, 120, and 144 hours of age, emerged from eggs of *Nezara viridula* (Hemiptera: Pentatomidae), under laboratory conditions at 25±2 °C, 70±10% (RH) and 12-hour photophase. Dourados, Mato Grosso do Sul, Brazil

Biological characteristics	Age of the parasitoid *Ooencyrtus submetallicus* (hours)							Overall average
	24 h	48 h	72 h	96 h	120 h	144 h		
Emergency (%)	100.00±1.00	89.29±4.40	94.00±3.63	91.00±6.06	96.84±3.16	96.00±2.14	n.s.	95.00±1.61
Life cycle[1] (days)	18.00±0.00	17.69±0.11	17.84±0.10	17.59±0.19	18.04±0.17	17.93±0.15	n.s.	17.85±0.07
Progeny[2]	1.60±0.00	1.39±0.17	1.22±0.08	1.22±0.10	1.59±0.15	1.34±0.11	n.s.	1.39±0.07
Sex ratio[3]	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	n.s.	1.00±0.00

Note. n.s. = not significant (p > 0.05); [1] total egg duration-adult; [2] number of individuals per egg; [3] n° of females/(n° of males + n° of females).

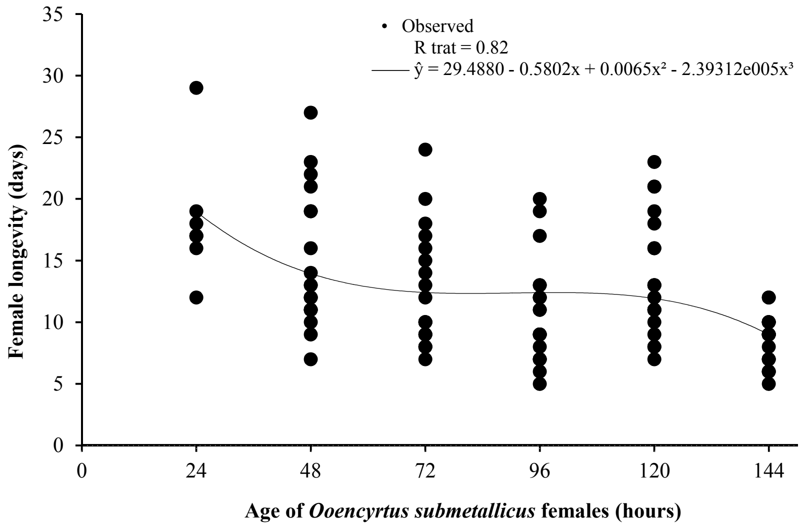

Figure 3. Longevity (days) of *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae) aged 24, 48, 72, 96, 120, and 144 hours emerged from eggs of *Nezara viridula* (Hemiptera: Pentatomidae) at 25±2 °C, 70±10% relative humidity and 12-hour photophase ($F = 10.4012$ and $p \leq 0.0001$). Dourados, Mato Grosso do Sul, Brazil

The different ages of *Trissolcus* sp. aff. *urichi* in *N. viridula* eggs did not influence the biological characteristics of this parasitoid ($p > 0.05$). Nevertheless, a parasitism percentage higher than 75% was observed at all ages of females of *Trissolcus* sp. aff. *urichi* (24 to 144 h). However, the percentage of emergence was absolutely low, presenting an overall average of 3.32±0.71%, as only three individuals emerged in these treatments (24 to 144 h) (Table 2). The average life cycle length (24 to 144 h) (egg to adult) of *Trissolcus* sp. aff. *urichi* was 15.00±1.53 days with 1.00±0.00 progeny emerged per egg of *N. viridula* and the sex ratio was 1.00±0.00. Although this parasitoid presents sexual reproduction and generate both males and females, only female individuals emerged from *N. viridula* eggs in our results, with overall average longevity of 2.33±1.33 days (Table 2).

Table 2. Biological characteristics (mean±SE) of *Trissolcus* sp. aff. *urichi* (Hymenoptera: Scelionidae) at 24, 48, 72, 96, 120, and 144 hours of age, emerged from eggs of Nezara viridula (Hemiptera: Pentatomidae), at 25±2 °C, 70±10% of (RH) and photophase of 12 hours. Dourados, Mato Grosso do Sul, Brazil

Biological characteristics	Age of the parasitoid *Trissolcus* sp. aff. *urichi* (hours)							Overall average
	24 h	48 h	72 h	96 h	120 h	144 h		
Parasitism (%)	75.00±12.25	98.33±1.67	90.91±5.63	93.75±5.07	88.33±6.72	97.78±2.22	n.s.	90.68±3.51
Emergency (%)	3.33±3.33	-	4.55±4.55	-	2.08±2.08	-	n.s.	3.32±0.71
Life cycle¹ (days)	14.00±0.00	-	13.00±0.00	-	18.00±0.00	-	n.s.	15.00±1.53
Progeny²	1.00±0.00	-	1.00±0.00	-	1.00±0.00	-	n.s.	1.00±0.00
sex ratio³	1.00±0.00	-	1.00±0.00	-	1.00±0.00	-	n.s.	1.00±0.00
Longevity ♀ (days)	5.00±0.00	-	1.00±0.00	-	1.00±0.00	-	n.s.	2.33±1.33

Note. n.s. = not significant ($p > 0.05$); ¹ total egg duration-adult; ² number of individuals per egg; ³ n° of females/(n° of males + n° of females).

The parasitism and the emergence of *O. submetallicus* did not vary with the age of the eggs of the host *N. viridula* (24, 48, 72, 96, 120, or 144 h), with an overall average of 63.26±7.92% and 9.91±1.69%, respectively ($p > 0.05$). The average length of the life cycle of *O. submetallicus* was also not influenced by the ages of the host, with an overall average of 18.81±0.25 days and with a number of individuals per egg of 1.79±0.12 ($p > 0.05$). The sex ratio of *O. submetallicus* was 1.00±0.00, not being a factor influenced by the different ages evaluated, as well as the longevity of adult females, which had an overall average of 13.43±0.79 days ($p > 0.05$) (Table 3).

Table 3. Biological characteristics (mean±SE) of *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae) emerged from eggs of *Nezara viridula* (Hemiptera: Pentatomidae) at 24, 48, 72, 96, 120, and 144 hours of age, at 25±2 °C, 70±10% (RH) and 12-hour photophase. Dourados, Mato Grosso do Sul, Brazil

Biological characteristics	Age of host *Nezara viridula* (hours)							Overall average
	24 h	48 h	72 h	96 h	120 h	144 h		
Parasitism (%)	37.78±6.19	73.33±8.43	67.69±10.15	60.00±6.29	48.00±12.00	92.73±4.88	n.s.	63.26±7.92
Emergency (%)	100.00±0.00	91.67±8.33	98.18±1.82	91.58±6.03	100.00±0.00	100.00±0.00	n.s.	96.91±1.69
Life cycle[1] (days)	18.82±0.12	18.87±0.11	18.12±0.21	18.62±0.12	19.95±0.45	18.50±0.23	n.s.	18.81±0.25
Progeny[2]	2.32±0.31	1.43±0.17	1.75±0.17	1.75±0.15	1.81±0.42	1.65±0.11	n.s.	1.79±0.12
sex ratio[3]	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	n.s.	1.00±0.00
Longevity ♀ (days)	13.05±1.34	11.15±1.83	16.90±1.55	12.60±1.74	14.05±2.58	12.80±1.46	n.s.	13.43±0.79

Note. n.s. = not significant ($p > 0.05$); [1] egg duration-adult; [2] number of individuals per egg; [3] n° of females/(n° of males + n° of females).

Similar to *O. submetallicus*, the evaluated ages of *N. viridula* eggs did not significantly affect the biological characteristics of *Trissolcus* sp. aff. *urichi* ($p > 0.05$). The parasitism of *Trissolcus* sp. aff. *urichi* in eggs of *N. viridula* at up to six days old (144 h) was 87.57±1.93% and only seven individuals emerged in the entire experiment (24 to 144 h) (Table 4).

The average life cycle length (egg-adult) of *Trissolcus* sp. aff. *urichi* was 14.50±0.29 days, with a number of 1.00±0.00 individuals emerged per *N. viridula* egg. The overall average for sex ratio was 0.54±0.21, because female and male parasitoids emerged, with average longevities of 15.83±6.09 and 18.33±5.55 days, respectively (Table 4).

Table 4. Biological characteristics (mean±SE) of *Trissolcus* sp. aff. *urichi* (Hymenoptera: Scelionidae) emerged from eggs of *Nezara viridula* (Hemiptera: Pentatomidae) at 24, 48, 72, 96, 120, or 144 hours of age, at 25±2 °C, 70±10% of (RH) and 12-hour photophase. Dourados, Mato Grosso do Sul, Brazil

Biological characteristics	Age of host *Nezara viridula* (hours)							Overall average
	24 h	48 h	72 h	96 h	120 h	144 h		
Parasitism (%)	85.00±9.82	80.00±9.61	89.23±6.25	86.67±8.82	92.86±3.39	91.67±5.20	n.s.	87.57±1.93
Emergency (%)	-	40.00±0.00	20.00±0.00	-	20.00±0.00	35.00±15.00	n.s.	28.75±5.15
Life cycle[1] (days)	-	15.00±0.00	15.00±0.00	-	14.00±0.00	14.00±1.00	n.s.	14.50±0.29
Progeny[2]	-	1.00±0.00	1.00±0.00	-	1.00±0.00	1.00±0.00	n.s.	1.00±0.00
sex ratio[3]	-	1.00±0.00	0.00±0.00	-	0.67±0.33	0.50±0.50	n.s.	0.54±0.21
Longevity ♀	-	9.50±1.50	-	-	28.00±0.00	10.00±0.00	n.s.	15.83±6.09
Longevity ♂	-	-	20.00±0.00	-	8.00±0.00	27.00±0.00	n.s.	18.33±5.55

Note. n.s. = not significant ($p > 0.05$); [1] total egg duration-adult; [2] number of individuals per egg; [3] n° of females/(n° of males + n° of females).

4. Discussion

In general, it was observed that the percentage of *O. submetallicus* parasitism increased according to the age of the females. This fact is easily explained because the representatives of the Encytidae family present synovigenic maturation, that is, the eggs are produced and, consequently, matured throughout their adult life and in general the females need a few days to start oviposition (Flanders, 1950; Papaj, 2000; Kapranas & Tena, 2015). Similar to our study, Aung et al. (2012) and Tunca (2016) verified an increase in the reproductive efficiency of Encyrtidae parasitoids from their fifth day of life.

Unlike *O. submetallicus*, the female ages of the parasitoid *Trissolcus* sp. aff. *urichi* did not influence the percentage of parasitism, remaining constant during the six days, possibly due to the viability of spermatozoa, guaranteed by the female's spermatheca during her reproductive phase (Damiens et al., 2003; Pascini & Martins, 2017). Regarding the age of the eggs of the host *N. viridula*, this factor was not significant for the percentage of parasitism, once females of *O. submetallicus* parasitized eggs up to 144 hours of age, as verified by Binazzi et al. (2013), where the parasitoid *Ooencyrtus pityocampae* (Mercet) (Hymenoptera Encyrtidae) parasitized eggs at up to seven days old (168 h), with no interference in their reproductive capacity. The host age did not generate any significant response in the parasitism of *Trissolcus* sp. aff. *urichi*, remaining above 80% at all ages of *N. viridula* eggs, however, some Scelionidae parasitoids prefer younger eggs for reproduction, because as the eggs age their embryonic content may change, compromising the quality of their nutritional status (Peñaflor et al., 2012; Da Wedge et al., 2017).

The percentage of emergence was not influenced by the ages of *O. submetallicus*, presenting a rate above 85%, as observed by Lee (1979) in his study on *O. submetallicus* and *N. viridula*. Similar to the age of the parasitoid, the various ages of *N. viridula* eggs did not cause variations in emergence since *O. submetallicus* emerged in more than 90% of the eggs at all ages of the host, similarly to what was described by Catalán and Verdú (2005), through the emergence of the progeny of the parasitoid Encyrtidae in more than 80% of the parasitized eggs of *N. viridula*.

Despite the multiple ages of *Trissolcus* sp. aff. *urichi* and *N. viridula* eggs not interfering in the percentage of emergence, few adults emerged in these bioassays. Possibly, the host does not have characteristics considered nutritionally suitable for the perfect development of the progenies, or even, due to non-reproductive effects such as the abortion of the parasitoid's eggs performed by the host's own immune system (Strand & Pech, 1995; Roversi, 2017; Abram et al., 2019). Botch and Delfosse (2018) also verified low percentage of the emergence of the parasitoid *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) in eggs of non-target Pentatomidae hosts, and the authors associated this factor to the absence of interaction and/or non-suitability of the parasitoid to the host.

Both the age of *O. submetallicus* females and the age of *N. viridula* eggs did not interfere in the life cycle length of this parasitoid or in the number of individuals per egg, discriminating that the parasitoid can develop normally regardless of the age of the progenitor or host, which is similar to what was observed by Power et al. (2020) who, when studying alternative hosts for the reproduction of *Ooencyrtus mirus* (Triapitsyn and Power) (Hymenoptera: Encyrtidae), noted that the development time of this parasitoid in *N. viridula* eggs was approximately 15 days, being a suitable host for the reproduction of this parasitoid Encyrtidae. In our study, there was the emergence of more than one individual per egg, possibly due to the low density of eggs provided by females of *O. submetallicus*, with more than one adult emerging from a single host (Böckmann et al., 2012; Tunca et al., 2017). However, superparasitism tends to to lead to intraspecific competition within the host, which limits the nutrition of the progeny and can compromise the development of these parasitoids (Tunca et al., 2017).

The ages of *Trissolcus* sp. aff. *urichi* and *N. viridula* eggs did not influence the life cycle, the number of individuals per egg, and the longevity of males and females of this parasitoid, however, these characteristics were compromised by the non-target host, possibly due to its limited nutritional resources, inadequate egg size or even by the barriers of its immune system, as already mentioned (Vinson, 1976; Strand & Vinson, 1983; Abram et al., 2019). Other authors also report that *T. urichi* does not always develop in *N. viridula* eggs (Sujii et al., 2002), although its biological characteristics may undergo variations when subjected to other hosts of the Pentatomidae family (Laumann et al., 2008, 2010; Barakat et al., 2020).

The longevity of *O. submetallicus* progeny decreased as the age of the female increased, because as the parasitoid females age, there is a limitation of their nutritional resources that would be destined to the process of ovogenesis, resulting in the deficiency of proteins, carbohydrates, and lipids in their eggs (Giron & Casas, 2003; Muller et al., 2017). Therefore, the reduction in the number of eggs resulting from the increase in the age of the female progenitor may affect the process of nutrition of the offspring, thereby decreasing their aptitude and longevity (Lansing, 1947; Kindsvater & Otto, 2014; Plaistow et al., 2015; Muller et al., 2017). Unlike this, the age of the eggs of *N. viridula* did not have any effect on the longevity of *O. submetallicus* and it survived more than ten days, however, when compared to other host species and submitted to different diets, the longevity of the parasitoid may suffer several variations (Roversi et al., 2018).

Regardless of the age of *O. submetallicus* or *N. viridula* eggs, the sex ratio was characterized by the absolute presence of female individuals. The parasitoid *O. submetallicus* reproduces by thelytokous parthenogenesis, generating only female parasitoids at temperatures below 29.44 °C, however, males can also be produced at particular temperature variations (Wilson & Wooloo, 1952). This characteristic was also not influenced by the ages of *Trissolcus* sp. aff. *urichi* and neither by the different ages of the eggs of *N. viridula*, being characterized by the presence of male and female individuals, which is similar to what was observed by Queiroz et al. (2020b) through the emergence of individuals of both sexes of *T. urichi* in eggs of Pentatomidae hosts. From the results obtained and the discussion raised, this work provides grounds and opens the possibility for future studies about other stink bugs of the Pentatomidae complex, with the possibility of implementing new protocols for multiplication and for subsequent release of these species of parasitoids in a synchronized manner with the occurrence of stink bug populations in the field.

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