Article

Thermal Requirements of Two Egg Parasitoids of Brown Stinkbug *Euschistus heros* (Hem.: Pentatomidae)

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Simple Abstract: The brown stinkbug, *Euschistus heros*, is the most abundant species in soybean crops in Brazil, causing significant yield losses. The control of this stinkbug is done mostly with chemical insecticides; despite increasing use of biological control with the egg parasitoids *Ooencyrtus submetallicus* and *Telenomus podisi*. Our objective was to evaluate the development of *O. submetallicus* and *T. podisi* in *E. heros* eggs at different temperature regimes and to estimate the number of annual generations for seven representative soybean producing areas in Brazil. *Ooencyrtus submetallicus* produced more females and individuals per egg compared to *T. podisi*. Both parasitoids were reared under the temperatures of 16, 19, 22, 25, 28, 31, and 33 °C to assess their thermal requirements. The lower temperature thresholds (Tb) for *O. submetallicus* and *T. podisi* were 9.3° and 6.7 °C, while the thermal constants (K) were 336.9 and 272.7 degree days, respectively. The estimated mean number of generations per year of the parasitoids was greater than the pest *E. heros* for all regions. *O. submetallicus* and *T. podisi* have the same ability to reproduce using *E. heros* eggs. In addition, the thermal range for development was between 16° and 33°C, indicating that they can survive in this host in different soy-producing regions.

Abstract: Temperature is the abiotic factors that strongly influence the biology and behavior of insects. Thus, we assessed the development of the egg parasitoids, *Ooencyrtus submetallicus* and *Telenomus podisi*, parasitizing eggs of the brown stinkbug, *Euschistus heros*, at different temperatures, in addition to estimating the average number of generations using temperature records for seven representative soybean producing regions in Brazil. The comparative biology study conducted evidenced that *O. submetallicus* and *T. podisi* had similar percentages of parasitism and emergence, life cycle duration (egg-adult), and longevity. The sex ratio and the number of adult parasitoid emerged per parasitized egg was superior for *O. submetallicus*. The study of thermal requirements evaluated temperatures of 16, 19, 22, 25, 28, 31, and 33 °C. *Ooencyrtus submetallicus* and *T. podisi* developed at temperatures between 16 and 31°C. The lower temperature (Tb) threshold for *O. submetallicus* and *T. podisi* was 9.3° and 6.7°C, while the thermal constant (K) was 336.9 and 272.7 degree days, respectively. The estimated average number of generations per year for both parasitoids was greater than the pest *E. heros*. *Ooencyrtus submetallicus* and *T. podisi* exhibited the same biological capacity to parasitize and to develop parasitizing *E. heros* eggs under laboratory studied conditions. The temperature range between 16° and 33 °C was favourable for the development of both parasitoids parasitizing *E. heros* eggs.

Keywords: Biological control, egg parasitoid; biological characteristics; brown stink bug.

1. Introduction

The tropical climate in Brazil favors the cultivation of soybean, *Glycine max* (L.) Merrill, in different locations, likewise the incidence of herbivorous species reaching the status of pest [1]. The brown stink bug, *Euschistus heros* (Fabricius, 1794) (Hemiptera: Pentatomidae), stands out among the herbivores species. This species is the most abundant pentatomid (hereafter stinkbug) pest species in the soybean crop, with occurrence from northern to southern Brazil [2]. Nymphs and adults feed on grains in development causing a drastic reduction in grain quality, as well as a yield reduction. Furthermore, during stinkbugs' feeding, they deliver toxins into the plant, causing physiological disturbances, such as leaf retention, which hinder the harvesting process [3].

The control of stinkbugs is done almost exclusively with synthetic insecticides, both via seed treatment and spraying on plants, with implications for the environment and human health [4]. Therefore, more adequate methods based on the principles of integrated pest management (IPM) have been pursued. Biological control has become an important method to reduce the population of agricultural pests, for example, through the use of egg parasitoids [5]. The egg parasitoid *Ooencyrtus submetallicus* (Howard, 1897) (Hymenoptera: Encyrtidae) is reported in the literature as an egg parasitoid of stink bugs, such as *Nezara viridula* (Linnaeus, 1758) [6], *Edessa meditabunda* (Fabricius, 1794) [7] e *E. heros* [8]. Furthermore, *Telenomus podisi* (Ashmead, 1893) (Hymenoptera: Platygastridae) has been shown to be the most effective in controlling *E. heros* [9].

The efficiency, development, and reproduction of parasitoids are affected by abiotic factors including temperature [10, 11]. Insects exposed to very high temperatures increase body temperature to lethal levels, while lower temperatures can cause physical damage, negatively affecting insect development [12]. Therefore, knowing the ideal temperature regime is essential to develop mass rearing programs for parasitoids, and predict their establishment in the field [13].

The effects of temperature variation on natural enemies can be studied by determining the thermal requirements, which considers the needs of each parasitoid in their hosts, such as the lowest temperature threshold allowing development, thermal constant (degree-days), upper thermal threshold, and optimum temperature. After, based on the enviroment temperature, the number of generations that can be obtained under certain environmental conditions can be estimated [14, 15].

Several studies have been carried out with the parasitoid *T. podisi* to establish the ideal temperature for its rearing and release in the field, with an estimated number of generations of this species [16, 15, 17-19]. This parasitoid can be found in various crop ecosystems shwoing their adaptation to different climatic conditions and stink bug species as hosts [20]. On the other hand, the favourable temperatures for rearing and release of the parasitoid *O. submetallicus* have not been defined yet.

Therefore, the development of *O. submetallicus* and *T. podisi* parasitizing *E. heros* eggs in addition to determining the thermal requirements for these parasitoids were assessed. The outcome allowed estimation of the average number of generations per year for seven representative soy-producing locations in Central-Western and Southern Brazil.

2. Materials and Methods

The experiments were conducted in the "Laboratório de Controle Biológico de Insetos (LECOBIOL)" of the Faculdade de Ciências Biológicas e Ambientais (FCBA) da Universidade Federal da Grande Dourados (UFGD), in Dourados, Mato Grosso do Sul, Brazil.

2.1. Rearing and maintenance of insects used in the experiments

2.1.1. Ooencyrtus submetallicus

Ooencyrtus submetallicus was reared following the methodology described in Faca [21]. Briefly, the parasitoids were kept in glass tubes (2.0 ×15.0 cm in diam × Ht) closed with cotton, containing a droplet of pure bee honey as food. The colony of the parasitoid was maintained using *E. heros* eggs as host. Part of the colony was kept in a BOD-type air-conditioned chamber (model EL 222, ELETROLab, São Paulo, São Paulo, Brazil) and the other part in an air-conditioned room, both colonies maintained at a temperature of 25 ± 2 °C, 70 ± 10% relative humidity, and 14:10 h (L:D) photoperiod. Individuals were initially collected parasitizing eggs of *E. meditabunda* from tomato plants, *Solanum lycopersicum* (Linnaeus, 1753) (Solanales: Solanaceae) located in Dourados, MS (INSERE COORDENADAS). The collection was made by Antônio de Souza Silva, and specimens were identified by one of the authors (V.A.C.) according to Noyes (2010).

2.1.2. Telenomus podisi

Adult *T. podisi* were supplied by Embrapa Agropecuária Oeste, after from Koppert Biological Systems (Piracicaba, São Paulo, Brazil). The identity of the specimens were confirmed by one of the authors (V.A.C.) according to Johnson (1984). The parasitoids were produced following similar procedures used with *O. submetallicus.*

Voucher specimens of both parasitoid species were deposited in the "Coleção de Insetos Entomófagos Oscar Monte" (IBCBE, Instituto Biológico, Campinas, SP, curator Valmir A. Costa).

2.1.3. Euschistus heros

The brown stink bug was collected from the experimental farm of the UFGD using a sweeping net and manual collection. Nymphs and adults were reared in cages made with transparent plastic pots of 5 L-volume containing openings fixed with orgrandi fabric to ventilation. The diet for nymphs and adults consisted of fresh bean (*Phaseolus vulgaris* L.) pods, green privet (*Ligustrum* sp.) seeds, dry soybeans (*Glycine max* L.), and raw and shelled peanuts (*Arachis hypogaea* L.), with moisture source provide with cotton swab moistened with distilled water, placed in plastic Petri dishes perforated in the middle. A 30 × 30 cm diam filter paper folded in a fan shape was used as the oviposition substrate for the sink bugs [22]. Part of the eggs collected were separated daily to maintain the rearing colony of *E. heros*, the other part was destined to develop the parasitoids *O. submetallicus* and *T. podisi* and mount the experiment. Eggs separated to maintain the colony were placed in Petri dishes with moist cotton swab and bean pods until hatching and transference to rearing pots. The rearing was kept in an acclimatized room at 25 ± 2 °C, 70 ± 10% RH, and 14:10 (L:D) photoperiod. The species *E. heros* was identified by Dr. Jocélia Grazia, taxonomist of pentatomid stink bugs (Federal University of Rio Grande do Sul – UFRGS).

2.2. Experimental development and analysis

2.2.1. Comparative biology of O. submetallicus and T. podisi

Eggs 24-h old of *E. heros* were fixed on sky blue cardboard (0.50 × 1.00 cm Wd × L) with gum arabic diluted into water (20%) at the density of 10 eggs per card. Each card was inserted into glass tubes (1.0 × 9.50 cm in diam × Ht) containing a drop of pure bee honey on the inner wall of the tube, as food for the parasitoid. The parasitism was obtained releasing one female of *O. submetallicus* 120-h old [21]. The same procedure was performed for *T. podisi* females, however, with the female 24-h old [23]. Egg parasitism by females was allowed for 24 h. After this period, the females of both parasitoids were removed from the tubes and the cards containing eggs were transferred to the climate chamber set at 25 ± 2 °C, 70 ± 10% RH, and 14:10h (L:D) phosperiod until the possible emergence of the adult *O. submetallicus* and *T. podisi*, thus confirming the parasitism.

The characteristics evaluated during this study were developmental time (egg-adult), percentage of parasitism [(number of dark eggs/total number of eggs offered) × 100], emergence percentage [(number of eggs with emergence hole/number of dark eggs) × 100], number of individuals emerged per egg parasitized, sex ratio [(sr = number of females/total number of offspring)], and longevity of offspring with and without food (bee honey). The experimental design used was completely randomized (DIC), with two treatments (parasitoid species) and 20 replications (one female parasitizing 10 eggs each). Data were subjected to analysis of variance and Student t-test at 5% probability of error.

2.2.2. Development of O. submetallicus *and* T. podisi *as a function of the temperature regime.*

Eggs of *E. heros* less than 24-h old were individualized in glass tubes (1.0 × 9.50 cm in diam × Ht) containing a droplet of bee honey deposited on the inner wall of the tube, and closed with a cotton swab. Each tube contained a card with 10 eggs received two 120-h old female *O. submetallicus*. The same procedure was performed for *T. podisi*, but with 24-h old females. Parasitism was allowed for 5 h in an acclimatized room at 25 ± 2 °C and 70 ± 10% RH. At the end of this period, the females were discarded and the tubes holding the cards with eggs were transferred to climatized chambers regulated to 16, 19, 22, 25, 28, 31, or 33 °C, and 14:10h (L:D) photoperiod.

The experimental design was a completely randomized, with seven treatments (temperatures) with 20 replications each (10 eggs each). For each temperature, the following characteristics were determined: developmental time (egg-adult in days), performed through daily observations, always at the same time; percent of emergence [(nº. of eggs with emergence hole/nº. of dark eggs) × 100]; number of individuals emerged per egg parasitized; and sex ratio (sr= nº. of females/nº. total). The data obtained were subjected to analysis of variance, and when significant at 5% probability, regression analysis was performed. The equation that best fit the data was chosen based on the coefficient of determination (R^2) and the significance of the regression coefficients (βi).

2.2.3. Thermal requirements and estimate the number of generations of O. submetallicus *and* T. podisi.

The lowest temperature threshold (Tb) and the thermal constant (K) were estimated by the hyperbola method [24], based on the developmental time (egg-adult) of *O. submetallicus* and *T. podisi* at the evaluated temperatures com base em quem Campbell et al ???or ???. The number of annual generations of the two parasitoid species was estimated for the representative soybean producing regions in Central-Western and Southern Brazil, such as: Tupanciretã, Rio Grande do Sul, RS; Marshal Cândido Rondon, Paraná, PR; Maracaju, Mato Grosso do Sul, MS; Dourados, Mato Grosso do Sul, MS; Ponta Porã, Mato Grosso do Sul, MS; Sorriso, Mato Grosso, MT, and Rio Verde, Goiás, GO. The equation: NG= {T(Tm-Tb)/K} was employed where: K= thermal constant, Tm= the average temperature for each region, Tb = the lowest temperature threshold, and T = the time considered in days. Climatic data for the last ten years of the regions were provided by INMET (National Institute of Meteorology).

3. Results

3.1. Comparative biology of O. submetallicus *and* T. podisi

Both, *O. submetallicus* and *T. podisi*, exhibited similar percentages of parasitism and emergence rates, ranging from 59.0 to 62.5% and from 86.4 to 87.3, respectively. The developmental time (egg-adult) was also similar for both parasitoids (Table 1). The proportion of females in the offspring, however, was significantly different with 100% of females in the offspring production of *O. submetallicus* and 54% females of *T. podisi*. The number of parasitoids that emerged per parasitized egg was also superior for *O. submetallicus* (Table 1). The adult longevity was similar for the produced parasitoids either fed or unfed pure bee honey. On the other hand, adults of both species maintained on food lived, on average, 50% longer (Table 1).

Table 1. Biological characteristics (mean±SE) of *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae) and *Telenomus podisi* (Hymenoptera: Platygastridae) reared on eggs of *Euschistus heros* (Hemiptera: Pentatomidae), at 25 ± 2 °C, 70 ± 10% relative humidity, and 14:10h (L:D) photoperiod.

Characteristics	***O. submetallicus***	***T. podisi***	**CV (%)**
Cycle length (egg-adult) (days)	17.7 ± 0.10 a	17. 5 ± 0.11 a	2.72
Parasitism (%)	59.0 ± 1.43 a	62.5 ± 1.22 a	9.83
Emergence (%)	86.4 ± 2.26 a	87.3 ± 2.08 a	11.43
Sex ratio	1.0 ± 0.00 a	0.5 ± 0.02 b	6.76
Individuals emerged per egg (n)	1.9 ± 0.05 a	1.2± 0.04 b	12.11
Longevity without food (days)	9.6 ± 0.47 a	10.8 ± 0.51 a	21.57
Longevity with food (days)	18.1 ± 0.82 a	19.9 ± 0.83 a	19.60

Means followed by the same letter within row do not differ by the t-test at 5% probability.

3.2. Development of O. submetallicus *and* T. podisi *as a function of the temperature regime.*

The developmental time (egg-adult) of the parasitoids presented an inverse relationship with the temperature increase. For *O. submetallicus* the developmental time was 33.4 ± 0.25 days at 16 °C and 13.2 ± 0.09 days at 33 °C. The same pattern was observed for *T. podisi*, which was 35.4 ± 0.11 and 11.3 ± 0.11 days, when reared at 16 °C to 33 °C, respectively (Figure 1). The emergence of *O. submetallicus* was 74.2% and 88.8% at 28 °C and 33 °C, respectively; while *T. podisi* achieved 92.9% emergence at 25 °C and 72.5% at 31 °C (Figure 2).

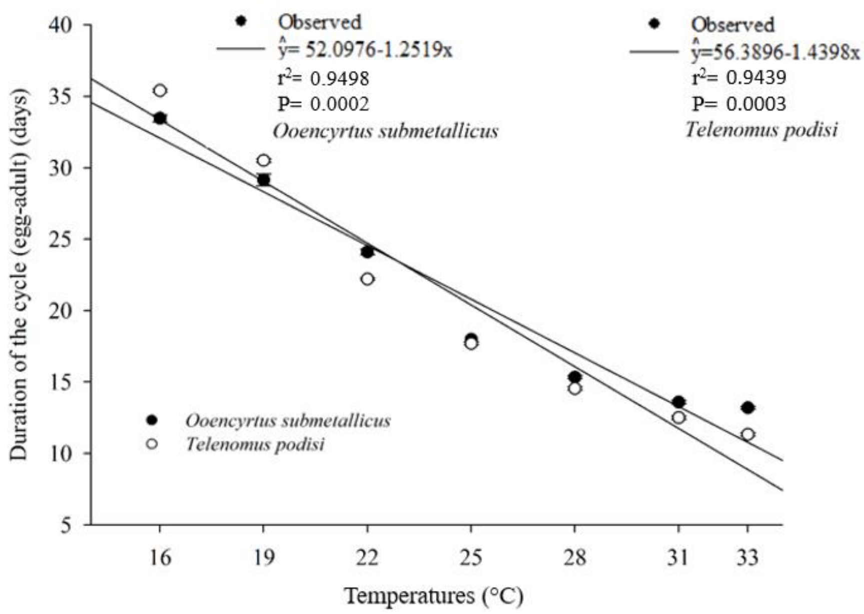

Figure 1. Egg-to-adult developmental time (days) of *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae) and *Telenomus podisi* (Hymenoptera: Platygastridae) parasitizing eggs of *Euschistus heros* (Hemiptera: Pentatomidae) at different temperatures.

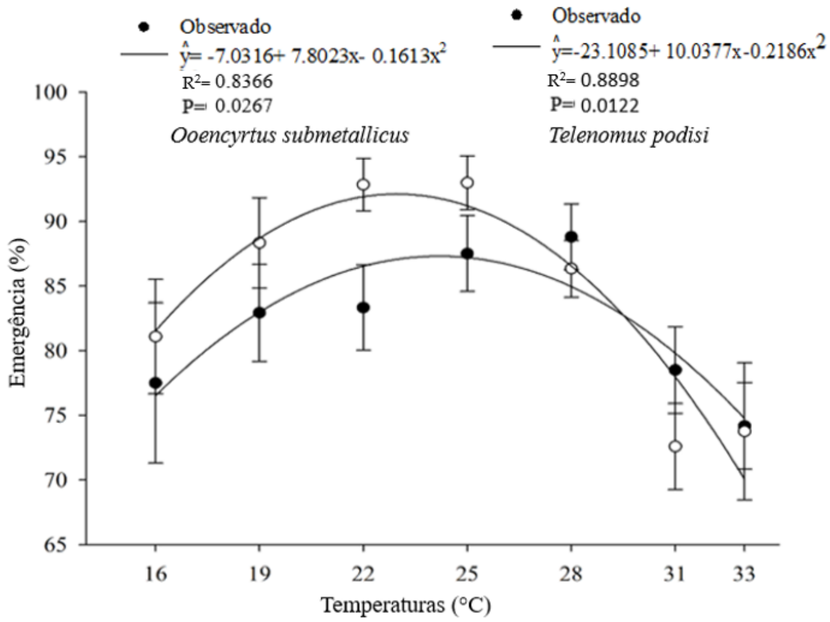

Figure 2. Emergence (%) (mean ± SE) of *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae) and *Telenomus podisi* (Hymenoptera: Platygastridae) parasitizing eggs of *Euschistus heros* (Hemiptera: Pentatomidae) at different temperatures.

The number of offspring produced per parasitized host egg by *O. submetallicus* was not influenced by the temperature regimes with an average of 1,31 parasitoids/egg. On the other hand, the temperature regime studied affected the number of individual *T. podisi* per host egg parasitized (Figure 3). At extreme temperatures was produced more offspring per egg parasitized (ca. 1.31 and X at 16 and 33°C, respectively). The proportion of females in the offspring of *O. submetallicus* was 100% for all temperatures studied, while for *T. podisi*, the proportion of females in the offspring, ranged from 54% to 67%, which were observed at temperatures 25° and 33 °C, respectively (Figure 4).

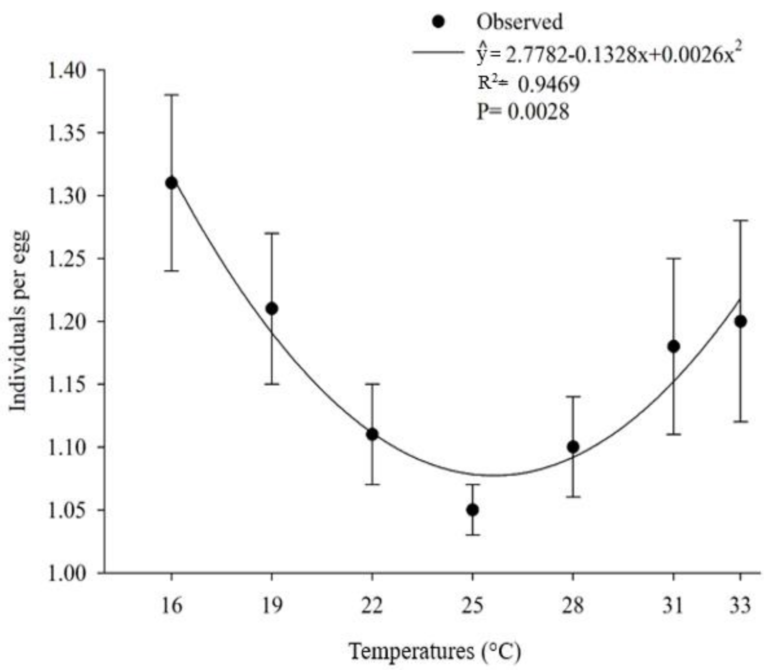

Figure 3. Number of parasitoids emerged per host egg parasitized (mean±SE) of *Telenomus podisi* (Hymenoptera: Platygastridae) in eggs of *Euschistus heros* (Hemiptera: Pentatomidae) at different temperatures. The results did not vary for *Ooencyrtus submetallicus*.

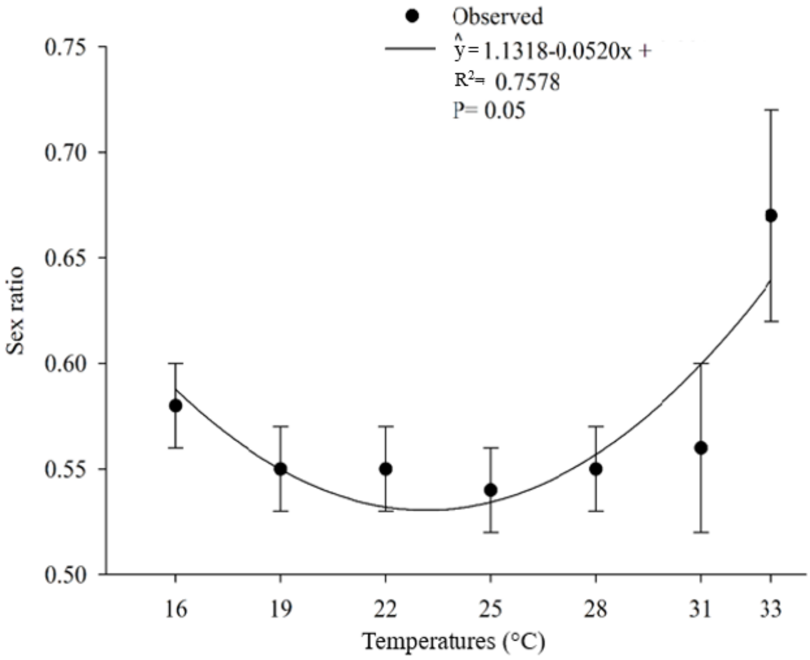

Figure 4. Sex ratio (%) (mean±SE) of *Telenomus podisi* (Hymenoptera: Platygastridae) in eggs of *Euschistus heros* (Hemiptera: Pentatomidae) at different temperatures. The offspring of *Ooencyrtus submetallicus* was 100% females.

3.3. Thermal requirements and estimate the number of generations of O. submetallicus *and* T. podisi.

The thermal requirements of *O. submetallicus* parasitizing *E. heros* eggs were based on the model y= (1/D) = -0.0020098 + 0.002968x (r^2 = 0.98), with values for the lowest temperature threshold (Tb) estimated as 6.77 °C, and thermal constant (K) of 336.97 degree-days (DD) (Figure 5). For *T. podisi*, y= (1/D) = - 0.034174 + 0.003670x (r^2 = 0.99), the lowest temperature threshold (Tb) was 9.31 °C, and thermal constant (K) 272.46 degree days (DD) (Figure 6).

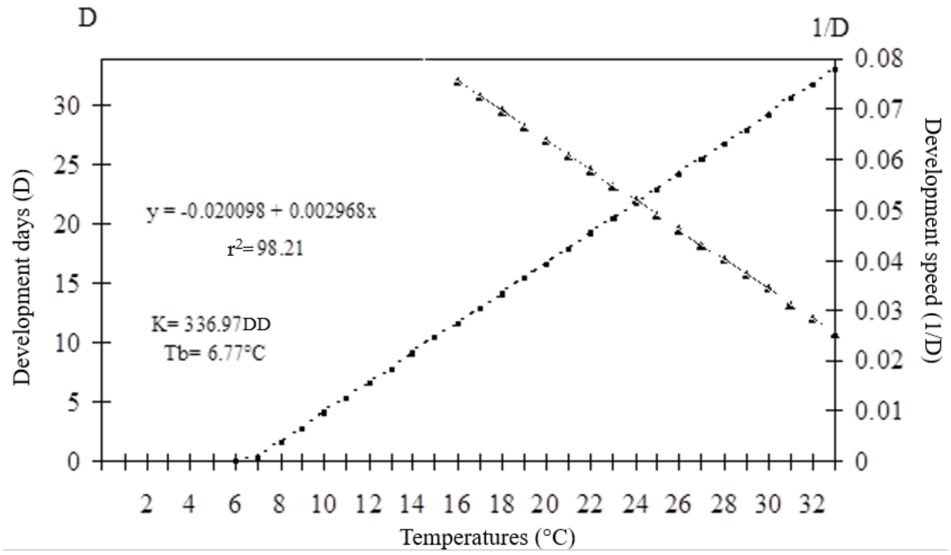

Figure 5. Duration (days) and developmental speed of *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae) parasitizing eggs of *Euschistus heros* (Hemiptera: Pentatomidae) at different temperatures.

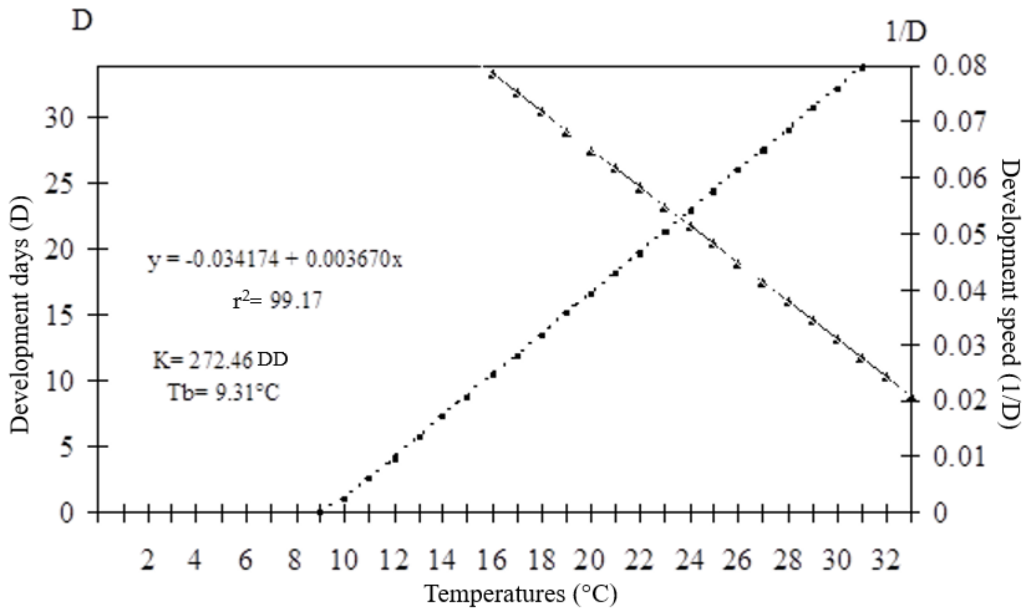

Figure 6. Duration (days) and developmental speed of *Telenomus podisi* (Hymenoptera: Platygastridae) parasitizing eggs of *Euschistus heros* (Hemiptera: Pentatomidae) at different temperatures.

The estimated annual generations of *O. submetallicus* for Dourados-MS, Ponta Porã-MS, Maracaju-MS, Sorriso-MT, Rio Verde-GO, Marechal Cândido Rondon-PR, and Tupanciretã-RS were: 16.9, 16.4, 15.3, 18.8, 14.9, 11.9, and 13.4 generations per year, while for *T. podisi* were: 18.3, 16.8, 15.5, 18.8, 15.0, 11.9, and 13.4 generations per year, respectively. For the host pest species, *E. heros*, using these same locations, the outcome were: 9.76, 8.57, 7.41, 11.03, 7.06, 4.05, and 5.46 generations per year, respectively (Table 2).

Table 2. Estimated number of generations per year of the host pest, *Euschistus heros* (Hemiptera: Pentatomidae), and its egg parasitoids, *Telenomus podisi* (Hymenoptera: Platigastridae), and *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae), considering climatic data for seven soybean producing areas of Brazil.

	Egg parasitoids		Host pest species
Localities	***E. heros***	***T. podisi***	***O. submetallicus***
Tupanciretã, Rio Grande do Sul	4.05	11.43	11.99
Mal. Cândido Rondon, Paraná	5.46	13.12	13.36
Maracaju, Mato Grosso do Sul	7.41	15.47	15.26
Dourados, Mato Grosso do Sul	9.76	18.29	18.33
Ponta Porã, Mato Grosso do Sul	8.57	15.29	16.39
Sorriso, Mato Grosso	11.03	18.82	18.78
Rio Verde, Goiás	7.06	15.05	14.92

4. Discussion

The results highlight the potential of the egg parasitoid *O. submetallicus* for use in the biological control of the brown stink bug, *E. heros*. The percentage of parasitism and other biological characteristics of *O. submetallicus* were similar, or superior (e.g., produced offspring and percentage of females in the offspring), compared to the commercial parasitoid, *T. podisi*, parasitizing *E. heros* eggs.

The emergence percentages observed for both species were considered adequate because above 72% is ideal for laboratory rearings [25]. A high emergence percentage of parasitized eggs indicates that the host was satisfactory for the parasitoids to complete their development becoming an important outcome for biological control programs [26]. Thus, *E. heros* eggs are suitable for rearing these parasitoids in the laboratory and future semi-field, and field studies are needed to confirm their ability to search for and parasitize the host.

The developmental time of the both parasitoid species was similar when reared in *E. heros* eggs (average of 17 days), requiring only 60% of the time to complete the development of their host, which corresponds to 28.4 days at 25 °C. This result is interesting feature for both parasitoids and a favourable factor in respect of field releases [27]. The genus *Ooencyrtus* is known to deposit more than one egg per host and is not selective about when it will parasitize or even superparasitizing its hosts. Furthermore, this parasitoid is smaller than other parasitoids including *T. podisi* [28].

The longevity of parasitoids consists of their survival period, from emergence to death. The knowledge of this characteristic, considering field stress conditions such as temperature and food scarcity, can provide information for the release interval aiming at the reduction of the pest population [29]. The parasitoids *O. submetallicus* and *T. podisi* survived longer when fed. Variations in the longevity of parasitoids may be related to several factors, ranging from the host's eggs used, environmental conditions to which they are exposed, energy expenditure during copulation, oviposition, and food deprivation [30].

The progeny of *O. submetallicus* was 100% female under the conditions of the study. For biological control purpose is an advantage for the population growth of the species regarding that females are directly responsible for the parasitism and, consequently, production of new offspring, and reduction of the pest population [12]. This pattern in the sex ratio of *O. submetallicus* can be explained by its type of reproduction, which occurs by thelytoky parthenogenesis, where the eggs develop only female offspring [6]. In insectareis, the predominance of females over male parasitoids is important for large-scale breeding, as they are mainly responsible for subsequent generations in the laboratory [31].

All temperatures evaluated influenced the development of both parasitoids. Therefore, this study determined that the ideal thermal range for the development of both parasitoids is between 25 and 28 °C. In other studies, similar results have been reported for *T. podisi* parasitizing eggs of *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae), in the thermal range between 20° and 28 °C, where it lasted from 21.8 to 11.6 days [20]. For *Telenomus pachycoris* (Costa Lima, 1928) (Hymenoptera: Platygastridae), the duration of the egg-adult period parasitizing eggs of *Pachicoris torridus* Scopoli, 1772 (Hemiptera: Scutelleridae) ranged from 24 to 10 days at temperatures from 20° to 28°C, respectively [15]. The inverse relationship between temperature and duration of the egg-adult period can be explained by the low metabolic activity of the parasitoids when maintained at low temperatures; therefore, they complete their development slowly. On the other hand, the increase in temperature increases metabolic activity and development is completed faster until a certain level, which can be an advantage for biological control as adults will appear earlier, but this can have negative effects on the parasitism of the natural enemies [18]. Fact that the information generated in this study are relevant for rearing and the use of the parasitoid in biological control programs. These type data, however, usually need complementary information about the influence of the host used and the adaptation of the parasitoid [11]. Again, in this study, the host used for yielding the data was the pest species target making the data valuable for future rearing and release of the parasitoids.

Both studied parasitoids exhibited high emergence rates at temperatures between 16 and 33 °C. Despite a rearing colony is not set for this large variation in temperature regimes, these information can be used for management of the colony when needing speed or slow the developmental time of the parasitoid produce to fit release demand, for example. Furthermore, the emergence of *O. submetallicus* and *T. podisi*, despite experiencing a slight reduction above 30 °C, remained greater than 70%. Some parasitoids, such as *Ooencyrtus mirus* (Triapitsyn & Power, 2020) (Hymenoptera: Encyrtidae) have been reported to exhibit 85% of emergence from their hosts at temperature ranges between 16 and 32 °C [32]. *Telenomus remus* (Nixon, 1937) (Hymenoptera: Platygastridae), parasitizing eggs of *Spodoptera* spp. (Lepidoptera: Noctuidae), also achieved a mean value of 80% emergence in the range of 19 to 28 °C [33]. The successful emergence of parasitoids can influence the number of generations and the ability of parasitoids to establish in the field [34]. The sex ratio of *T. podisi* had a small increase at 33 °C, indicating a higher proportion of females than males. The increase in sex ratio at more extreme temperatures may be related to the physiology of the parasitoid during the period of embryonic development [35]. Both parasitoids had higher proportions of females at all temperatures evaluated, which is important in biological control programs, since only females kill the target pest through parasitism [36].

Based on the developmental time (egg-adult) at the different temperatures, the thermal constant (K) and the lowest temperature threshold (Tb) were determined. For *O. submetallicus*, the thermal constant (K) was higher, indicating that it needs a greater thermal accumulation (degree-days) to complete its development, unlike *T. podisi*, which needs less heat accumulation. However, *O. submetallicus* has the potential for higher daily thermal accumulation, as *T. podisi* presented a higher base temperature (Tb). This means that there are no differences in the developmental time between the two species. In another study, the Tb and K of *T. podisi* were estimate as 11.1 °C and 205.3 DD when parasitizing eggs of *E. heros* [37]. The variation between the values found, both for Tb and K, indicates that both the host used and the adaptation of the parasitoid to it may influence the thermal requirements [31].

Ooencyrtus submetallicus and *T. podisi* developed faster than their host *E. heros* at the temperatures studied. The host pest, *E. heros*, exhibits higher thermal requirements than the parasitoids (Tb = 14.2 °C, and K = 327.8 DD) [38]. Therefore, taking in consideration the relationship of developmental duration and the influence of temperature, the number of generations of both parasitoids were also greater than those of the host, under the temperature conditions studied for selected soybean producing localities in Central-Western and Southern Brazil. Faster development and more generations of both parasitoids than their host pest species at the same temperature are important characteristics for biological control programs, as they can help in the stability and/or reduction of the pest population [31]. However, other factors can influence the number of generations, such as photoperiod, air humidity, and availability of the host, but when these conditions are favourable, temperature can become a limiting factor for insects [39, 32].

The results found for the biology and thermal requirements for *O. submetallicus* and compared to *T. podisi*, parasitizing *E. heros* eggs, are important for their rearing in the laboratory. *O. submetallicus* exhibited similar performance to *T. podisi* in the evaluated traits and may stand out for producing only female offspring at temperatures common for soybean crops and production areas in Brazil.

5. Conclusions

Ooencyrtus submetallicus and *T. podisi* have the same ability to parasitize and to develop parasitizing eggs of *E. heros* under the conditions studied.

The temperature regimes between 16 and 33 °C were favourable for the development of *O. submetallicus* and *T. podisi* parasitizing eggs of *E. heros*, indicating that both are able to survive in different soybean producing areas with this thermal range.

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References

1. WIEST, A.; BARRETO, M. R. Evolução dos Insetos-Praga na Cultura da Soja no Mato Grosso. *EntomoBrasilis.* **2012**, 5, 84-87. [CrossRef]
2. CORRÊA-FERREIRA B. S.; PANIZZI, A. R. Percevejos da soja e seu manejo. Londrina: EMBRAPA – CNPSo. (Circular Técnica, 24). **1999**, 45. [CrossRef]
3. CORRÊA-FERREIRA, B. S.; SOSA-GÓMEZ, D. R. Percevejos e o sistema de produção soja/milho. Londrina: Embrapa Soja. (Documentos, n. 397). **2017**, 98. [CrossRef]
4. CORRÊA-FERREIRA, B. S.; CASTRO L. C.; ROGGIA, S.; CESCONETTO, N. L.; DA COSTA J. M.; OLIVEIRA, M. C. N. MIP-Soja: resultados de uma tecnologia eficiente e sustentável no manejo de percevejos no atual sistema produtivo da soja. Londrina: Embrapa soja. (Documentos, n. 341). **2013**, 55. [CrossRef]
5. BUENO, A. F.; SOSA-GÓMEZ, D. R.; CORRÊA-FERREIRA, B. S.; MOSCARDI, F.; BUENO, R. C. O. F. Inimigos naturais das pragas da soja. In: HOFFMANN-CAMPO, C. B.; CORRÊA-FERREIRA, B. S.; MOSCARDI, F. (Ed.). Soja: manejo integrado de insetos e outros artrópodes-praga. Brasília, DF: Embrapa, **2012**, 493-629. [CrossRef]
6. WILSON, F.; WOOLCOCK, L. T.; Temperature determination of sex in a parthenogenetic parasite, *Ooencyrtus submetallicus* (Howard) (Hymenoptera: Encyrtidae). *Australian Journal of Zoology*. **1960**, 8, 153-169. [CrossRef]
7. GOLIN, V.; LOIÁCONO, M. S.; MARGARÍA, C. B.; AQUINO, D. A. Natural incidence of egg parasitoids of *Edessa meditabunda* (F.) (Hemiptera: Pentatomidae) on *Crotalaria spectabilis* in Campo Novo do Parecis, MT, Brazil. *Neotropical Entomology*. **2011**, 40, 617-618. [CrossRef]
8. SANOMIA, W. Y.; PEREIRA, F. F.; SILVA, I. F. Inseticide selectivity to *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae) under extended laboratory conditions. *Pesquisa Agropecuária Tropical*. **2020**, 50, 1-8. [CrossRef]
9. STECCA, C. S.; BUENO, A. F.; PASINI, A.; SILVA, D. M.; ANDRADE, K.; ZIRONDI FILHO, D. M. Impact of insecticides used in soybean crops to the egg parasitoid *Telenomus podisi* (Hymenoptera: Platygastroidae). *Neotropical Entomology*. **2018**, 47,281-291. [CrossRef]
10. PEREIRA, F. F.; ZANUNCIO, J. C.; OLIVEIRA, H. N.; GRANCE. E. L. V.; PASTORI, P. L.; GAVA-OLIVEIRA, M. D. Thermal requirements and estimate number of generations of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) in diferente *Eucalyptus* plantations regions**.** *Brazilian Journal Biological*. **2011**, 71, .431-436. [CrossRef]
11. SELVARAJ, S.; GANESHAMOORTHI, P.; PANDIARAJ, T. Potential impacts of recent climate change on biological control agents on agro-ecosystem: A review**.** *International Journal of Biodiversity and Conservation.* **2013**, 5, 845-852. [CrossRef]
12. BUENO, R. C. O. F.; CARNEIRO, T. R.; PRATISSOLI, D.; BUENO, A. F.; FERNANDES, O. A. Biology and thermal requirements of *Telenomus remus* reared on fall armyworm *Spodoptera frugiperda* eggs. *Ciência Rural*. **2008**, 38, 01–06. [CrossRef]

13. MAWELA, K. V.; KFIR, R.; KRÜGER, K. Effect of temperature and host species on parasitism, development time and sex ratio of the egg parasitoid *Trichogrammatoidea lutea* Girault (Hymenoptera: Trichogrammatidae). *Biological Control*. **2013,** 64, 211-216. [CrossRef]
14. PRATISSOLI, D., PEZZOPANE, J. E. M., ESPOSTI, M. D. D., BERTAZO, C. L., & FORNAZIE, J. M. Number of *Trichogramma pretiosum* Riley generations in *Tuta absoluta* (Meyrick), related to thermal requirements. *Anais da Sociedade Entomológica do Brasil*. **1998**, 27, 109-115. [CrossRef]
15. BORGES-FILHO. R. C.; NAVA, D. E.; POLANCZYK, R. A.; MARANGON, R. B.; LOIÁCONO, M. Biology of *Telenomus pachycoris* (Hymenoptera: Scelionidae), a parasitoid of eggs of *Pachycoris torridus* (Hemiptera: Scutelleridae): The effects of egg age, exposure time, and temperature. *Florida Entomologist*. **2017**, 100, 375-379. [CrossRef]
16. CIVIDANES, F. J.; FIGUEIREDO, J. G.; CARVALHO, D. R. Prediction of the emergence of *Trissolcus brochymenae* (Asgmead) and *Telenomus podisi* (Ashmead) (Hymenoptera: Scelionidae) in the field. *Scientia Agricola*. **1998**, 55, 1-5. [CrossRef]
17. SILVA, G, V.; BUENO, A. F.; NEVES, P. M. O. J.; FAVETTI, B. M. Biological characteristics and parasitism capacity of *Telenomus podisi* (Hymenoptera: Platygastridae) on eggs of *Euschistus heros* (Hemiptera: Pentatomidae). *Journal of Agricultural Science*. **2018,** 10, 210-220. [CrossRef]
18. TAGUTI, E. A.; GONÇALVES, J.; BUENO, A. F.; MARCHIORO, S. T. *Telenomus podisi* parasitism on *Dichelops melacanthus* and *Podisus nigrispinus* eggs at different temperatures. *Florida Entomologist*. **2019**, 102, 607-613. [CrossRef]
19. BRAZ, E. C.; BUENO, A.F.; COLOMBO, F. F.; QUEIROZ, A. P. Temperature impact on *Telenomus podisi* emergence in field releases of unprotected and encapsulated parasitoid pupae. *Neotropical Entomology*, **2021**, 50, 462-469. [CrossRef]
20. TORRES, J. B.; PRATISSOLI, D.; ZANUNCIO, J. C. Thermal requirements and development of the egg parasitoids *Telenomus podisi* Ashmead and *Trissolcus brochymenae* (Ashmead) on the predator stinkbug *Podisus nigrispinus* (Dallas). *Anais da Sociedade Entomológica do Brasil*. **1997**, 26, 445-453. [CrossRef]
21. FACA, E. C. Interações biológicas entre *Trissolcus urichi* (Hymenoptera:Scelionidae) e *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae) em ovos de *Nezara viridula* e *Chinavia pengue* (Hemiptera: Pentatomidae). Dissertação Mestrado, Universidade Federal da Grande Dourados, Dourados-MS. **2017**. [CrossRef]
22. PANIZZI, A. R.; MCPHERSON, J. E.; JAMES, D. G.; JAVAHERY, M.; MCPHERSON, R. M. Stink bugs (Pentatomidae). In: SCHAEFER, C.W.; PANIZZI, A.R. (Eds.). *Heteroptera of economic importance*. **2000**, 421-474. [CrossRef]
23. BUENO, A. F.; BRAZ, E. C.; FAVETTI, B. M.; FRANÇA-NETO, J. B.; SILVA, G. V. Release of the egg parasitoid *Telenomus podisi* to manage the Neotropical Stink Bug, *Euschistus heros*, in soybean production. *Crop Protection*. **2020,** 137, 1-7. [CrossRef]
24. HADDAD, M. L.; MORAES, R. C. B.; PARRA, J. R. P. MOBAE, Modelos bioestatísticos aplicados à entomologia. Manual. ESALQ/USP. **1995**, p.44.
25. OLIVEIRA, H. N.; PEREIRA, F. F.; PRATISSOLI, D. *Trichogramma* as an alternative control of *Heliothis virescens. Revista Brasileira de Agroecologia*. **2008**, 3, 191-194. Disponível em: [CrossRef]
26. BUENO, R. C. O. F.; PARRA, J. R. P.; BUENO, A. F.; HADDAD, M. Performance of trichogrammatids as biocontrol agents of *Pseudoplusia includens* Walker (Lepidoptera: Noctuidae). *Neotropical Entomology*. **2009**, 38, 389-394. [CrossRef]
27. PANIZZI, A. R. History and contemporary perspectives of the integrated pest management of soybean in Brazil. *Neotropical Entomology*. **2013**, 42, 119-127. [CrossRef]
28. TUNCA, H.; COLOMBEL, E.; VENARD, M.; TABONE, E. Incidence of superparasitism in the egg parasitoid, *Ooencyrtus kuvane* Howard (Hymenoptera: Encyrtidae). *Biocontrol Science and Technology*. **2017**, 27, 796-808. [CrossRef]
29. OLIVEIRA, F. A. L. D.; SILVA, R. O.; OLIVEIRA, N. R. X. D.; ANDRADE, G. S.; PEREIRA, F. F.; ZANUNCIO, J. C.; COUTINHO, C. R.; PASTORI, P. L. Reproduction of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) with Different Densities and Parasitism Periods in *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) Pupae. *Folia Biologica (Kraków)*. **2018**, 66, 103-110. [CrossRef]
30. PACHECO, D. J. P.; CORRÊA-FERREIRA, B. S. Reproductive potential and longevity of the parasitoid *Telenomus podisi* Ashmead in eggs of different stinkbug species. *Anais da Sociedade Entomológica do Brasil*. **1998**, 27, 585-591. [CrossRef]

31. FAVERO, K.; PEREIRA, F. F.; TORRES, J. B.; OLIVEIRA, H. N.; KASSAB, S. O.; ZANUNCIO, J. C. Reproduction of *Tetrastichus howardi* (Hymenoptera: Eulophidae) in *Diatraea saccharalis* (Lepidoptera: Crambidae) Pupae at Different Temperatures. *Florida Entomological Society*. **2015**, 98, 865- 869. [CrossRef]
32. POWER, N. A.; GANJISAFFAR, F.; PERRING, T. M. Effect of temperature on the survival and developmental rate of immature *Ooencyrtus mirus* (Hymenoptera: Encyrtidae). *Journal of Economic Entomology*. **2020**, 20, 1-10. [CrossRef]
33. POMARI, A. F.; BUENO, A. F.; BUENO, R. C. O. F.; JÚNIOR-MENEZES, A. O. Biological characteristics and thermal requirements of the biological control agente *Telenomus remus* (Hymenoptera: Platygastridae) reared on eggs of diferent species of the genus *Spodoptera* (Lepidoptera: Noctuidae), *Annals of the Entomological Society of America*. **2012**, 105, 73-81. [CrossRef]
34. PRATISSOLI, D.; OLIVEIRA, H. N.; POLANCZYC, R. A.; HOLTZ, R. A.; BUENO, R. C. O. F.; BUENO, A. F.; GONÇALVES, J. R. Adult feeding and matting effects on the biological potencial and parasitism of *Trichogramma pretiosum* and *T. accacioi* (Hym: Trichogrammatidae). *Brazilian Archives of Biology and Technology*. **2009**, 52, 1057-1052. [CrossRef]
35. HANCE, T.; VAN-BAAREN, J.; VERNON, P.; BOIVIN, G. Impact of extreme temperatures on parasitoids in a climate change perspective. *Annual Review of Entomology*. **2007,** 52, 107-126. [CrossRef]
36. ZANUNCIO, J. C.; SILVA, C. A. D.; LIMA, E. R.; PEREIRA, F. F.; RAMALHO, F. S.; SERRÃO, J. E. Predation rate of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae with and without defense by *Podissus nigrispinus* (Heteroptera: Pentatomidae). *Brazilian Archives of Biology and Technology*. **2008**, 51, 121-125. [CrossRef]
37. CHEVARRIA, V. V.; DEL PONTA, E. M.; JAHNKE, S. M. Number of generations of a stink bug and its parasitoid and severity of soybean rust, simulated under climate and crop management scenarios in northern RS State, Brazil. *Ciência Rural*. **2013**, 43, 571-578. [CrossRef]
38. CIVIDANES, F. J.; PARRA, J. R. P. Biology in different Temperatures and thermal requirements of stink bugs pests of soybean. *Pesquisa Agropecuária Brasileira*. **1994,** 29, 1841-1846. [CrossRef]
39. QUEIROZ, A. P.; FAVETTI, B. M.; HAYASHIDA, R.; GRANDE, M. L. M.; NEIVA, M. M.; PANIZZI, A. R.; BUENO, A. F. Effect of the ages parasitoid and host eggs on *Telenomus podisi* (Hymenoptera: Platygastridae) parasitism. *Neotropical Entomology***. 2019**, 48, 974-982. [CrossRef]
40. NOYES, J.S. Encyrtidae of Costa Rica (Hymenoptera: Chalcidoidea), 3. Subfamily Encyrtinae: Encyrtini, Echthroplexiellini, Discodini, Oobiini and Ixodiphagini, parasitoids associated with bugs (Hemiptera), insect eggs (Hemiptera, Lepidoptera, Coleoptera, Neuroptera) and ticks (Acari). Memoirs of the American Entomological Institute. **2010**, 84, 1-848. [CrossRef]
41. JOHNSON, N.F. Systematics of Nearctic *Telenomus*. Classification and revisions of the *podisi* and *phymatae* species groups (Hymenoptera: Scelionidae). *Bulletin of the Ohio Biological Survey*. **1984**, 6, 113. [CrossRef]