Biology of Telenomus pachycoris (Hymenoptera: Scelionidae), a Parasitoid of Eggs of Pachycoris torridus (Hemiptera: Scutelleridae): The Effects of Egg Age, Exposure Time, and Temperature

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Biology of *Telenomus pachycoris* (Hymenoptera: Scelionidae), a parasitoid of eggs of *Pachycoris torridus* (Hemiptera: Scutelleridae): the effects of egg age, exposure time, and temperature

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Abstract

*Telenomus pachycoris* (Johnson) (Hymenoptera: Scelionidae) is a parasitoid of eggs of *Pachycoris torridus* (Scopoli) (Hemiptera: Scutelleridae), a main pest of physic nut (*Jatropha curcas* L.; Euphorbiaceae). The objective of this work was to know the biology of *T. pachycoris* in *P. torridus* eggs under various conditions in order to develop a rearing technique for this parasitoid in the laboratory. We offered eggs of *P. torridus* to *T. pachycoris* during 4 exposition periods (6, 12, 18, and 24 h), as well as eggs of different ages (1 to 11 d), to evaluate, in both experiments, the number of parasitized eggs, duration of the egg-to-adult period, percentage of emergence, and sex ratio. We also evaluated the effect of constant temperatures (18, 20, 22, 25, 28, and 30 °C) and determined the duration of the egg-to-adult period, percentage of emergence, and sex ratio and estimated the thermal requirements and the number of generations per yr of *T. pachycoris* at each temperature. Parasitism of eggs was the highest at 12 h of exposure. Eggs up to 3 d old were the most parasitized, and the parasitism was zero on day 11. The duration of the egg-to-adult period was inversely proportional to temperature, ranging from 33.6 d at 18 °C to 9.8 d at 30 °C. The threshold temperature estimated for *T. pachycoris* was 12.9 °C, and the estimated thermal constant was 163.9 degree-days. The number of generations of *T. pachycoris* ranged from 11.3 to 38.1 per yr at 18 and 30 °C, respectively. The results may contribute to developing techniques for rearing *T. pachycoris* in the laboratory.

Key Words: biological control; parasitism; physic nut; rearing technique; temperature requirement

Resumo

*Telenomus pachycoris* (Johnson) (Hymenoptera: Scelionidae) é um parasitoide de ovos de *Pachycoris torridus* (Scopoli) (Hemiptera: Scutelleridae), principal praga do pinhão-manso (*Jatropha curcas* L.; Euphorbiaceae). O objetivo do trabalho foi conhecer a biologia de *T. pachycoris* em ovos de *P. torridus* sob diferentes condições, visando subsidiar uma técnica de criação em laboratório. Foram oferecidos ovos de *P. torridus* a *T. pachycoris* durante quatro períodos de exposição (6, 12, 18 e 24 h), bem como de diferentes idades (1 a 11 dias), avaliando em ambos os experimentos o número de ovos parasitados, duração do período ovo-adulto, porcentagem de emergência e razão sexual. Também foi avaliado o efeito das temperaturas constantes (18, 20, 22, 25, 28 e 30 °C) e determinados a duração do período ovo-adulto, porcentagem de emergência, razão sexual e estimado as exigências térmicas e o número de gerações por ano de *T. pachycoris* em cada temperatura. O parasitismo de ovos foi superior em 12 h de exposição. Ovos de 3 dias foram mais parasitados e o parasitismo foi nulo no 11º dia. A duração do período ovo-adulto foi inversamente proporcional à temperatura, variando de 33,6 d em 18 °C a 9,8 d em 30 °C. A temperatura base estimada de *T. pachycoris* foi 12,9 °C e a constante térmica foi 163,9 graus-dia. O número de gerações variou de 11,3 a 38,1 por ano a 18 e 30 °C, respectivamente. Os resultados podem contribuir para o desenvolvimento de técnicas de criação de *T. pachycoris* em laboratório.

Palavras Chave: controle biológico; parasitismo; pinhão-manso; técnicas de criação; exigências térmicas

*Pachycoris torridus* (Scopoli) (Hemiptera: Scutelleridae), known in Brazil as “percevejo-do-pinhão-manso” (stink bug of jatropha) (Silva et al. 1968), is one of the main pests of physic nut (*Jatropha curcas* L.; Euphorbiaceae). This insect has received different names due to its diverse color polymorphism (Souza et al. 2012). The nymphs and adults suck the sap of all the soft tissues of the aerial part but mainly feed on fruits, which become seared and will have unviable seeds, resulting in damage to the culture (Carvalho et al. 2009). During feeding, *P. torridus* injects toxins and causes minor injuries to the plant, which becomes vulnerable to phytopathogen entry. These damages hinder plant growth and affect the production of fruit and oil (Borges Filho et al. 2013).
The control of *P. torridus* is hampered by the lack of management strategies for this pest. Currently, there are no pesticides registered for its control in Brazil. In addition, planted areas with physic nut have been increasing in recent years with incentives of the Brazilian government for bioenergy production (AGROFIT 2014). Thus, natural biological control and applied biological control have become alternative procedures for the management of *P. torridus*. Natural biological control is the most viable option because of the large number of natural enemies of eggs, nymphs, and adults of *P. torridus* (Costa Lima 1940; Gabriel et al. 1988), including micro-hymenopterans such as *Hexacladia smithii* Ashmead (Hymenoptera: Encyrtidae), *Trichopodopsis pennipes* (F.) (Diptera: Gymnosomatidae), *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae), and *Telenomus pachycoris* (Johnson) (syn.: *Pseudotelenomus pachycoris* [Costa Lima]) (Hymenoptera: Scelionidae), which are the main parasitoids of *P. torridus* (Costa Lima 1930, 1940). *Telenomus pachycoris* has a wide distribution, occurring in all regions of Brazil (Costa Lima 1940; Gabriel et al. 1988; Oliveira & Silva 2011).

The females of *P. torridus* cover their eggs with their own body to protect them from attack by natural enemies. Thus, the efficiency of *T. pachycoris* in the field is close to 30% because only the eggs laid in the outer part of the egg mass can be parasitized (Gabriel et al. 1988). However, the preservation of these parasitoids in crops becomes important because in places of occurrence of *T. pachycoris* virtually all egg masses suffer parasitism (Costa Lima 1928), contributing to reduction of the pest population. In addition, the multiplication of *T. pachycoris* in the laboratory and in sites without or with low presence of parasitoids may also be a strategy to assist in the management of *P. torridus*.

The study of the biology of control agents and pests is one of the first steps to establish a biological control program (Parra 2000). Much information about the biology of *P. torridus* is available (Borges Filho et al. 2013); however, little is known about the biology of *T. pachycoris*. Thus, this study assessed the biology of *T. pachycoris* in eggs of *P. torridus* at different egg ages and rearing temperatures to define exposure time to the parasitoids and to know the effect of temperature on the development during the pre-imaginal period of *T. pachycoris*, in order to establish a technique of rearing in the laboratory.

## Materials and Methods

### SAMPLE COLLECTION AND REARING OF *P. TORRIDUS* AND *T. PACHYCORIS*

*Pachycoris torridus* eggs, nymphs, and adults were collected during periodic visits to experimental physic nut plantations at the Agricultural Research Center of Temperate Climate (CPACT), Embrapa Temperate Climate, Rio Grande do Sul, Brazil (31.6811'S, 52.4406'W; 58 m asl), and stored in glass tubes (2.5 × 8.5 cm). The insects were transferred to plastic boxes (10 × 15 × 10 cm) and transported to the Laboratory of Entomology at the same institution.

In the laboratory under controlled conditions of temperature (25 ± 2 °C), relative humidity (RH) (70 ± 10%), and photoperiod (12:12 h L:D), the *P. torridus* eggs, nymphs, and adults were placed in cages with wooden frames (27 × 27 × 35 cm) and sides lined with nylon mesh. For rearing, the insects were separated into cages according to immature and adult stages. Physic nut and Brazilian peppertree (*Schinus terbinthifolius* Raddi; Anacardiaceae) branches, leaves, and fruits were placed inside the cages to feed the insects. The branches and leaves were placed in plastic pots (250 mL) containing water to keep them moistened, and the fruits were available in Petri dishes (8.5 × 1.5 cm). Both foods were replaced every 4 d. A Petri dish (8.5 × 1.5 cm) with hydrophilic cotton soaked with water was also provided. In cages with adults, branches and leaves also served as substrate for oviposition, and the eggs were collected daily.

After hatching, *P. torridus* nymphs were kept in separate cages exclusive for insects obtained from laboratory rearing. The *P. torridus* eggs collected in the field were put in Gerbox’ cages and placed in a temperature-controlled chamber to obtain *P. torridus* nymphs or parasitoids. After hatching, the *P. torridus* nymphs were placed in cages for nymphal development as described above, and, after emergence, *T. pachycoris* adults were placed in Petri dishes (8.5 × 1.5 cm) in chambers with the temperature adjusted to 25 ± 1 °C at 70 ± 10% RH and a photoperiod of 12:12 h L:D.

The parasitoids were fed with small honey drops offered on a plate lid. For reproduction, 30 *P. torridus* eggs up to 24 h old were offered for every 5 *T. pachycoris* females based on previous observations. After 24 h, the *P. torridus* eggs were removed and transferred to air-conditioned chambers to obtain the adults. Parasitoids that emerged from the eggs collected in the field were preserved in 70% alcohol and identified by Dr. Marta Loiácono from the Divisão Entomologia, Museo De La Plata, Argentina. Voucher specimens were deposited in the entomological collection of Bertels of Embrapa Temperate Climate, Pelotas, Rio Grande do Sul, Brazil.

### EXPOSURE TIME OF *P. TORRIDUS* EGGS TO *T. PACHYCORIS*

*Pachycoris torridus* eggs were fixed on blue cardboard strips (2.2 × 4.0 cm) with gum arabic diluted in water to 20% and placed in glass tubes (8.5 × 2.4 cm). Twenty-five eggs up to 24 h old were offered to *T. pachycoris* females up to 48 h old for different periods of exposure (6, 12, 18, and 24 h). The glass tubes were sealed with plastic film to prevent the parasitoids from escaping, and after 24 h, the females were removed from the tubes, and the tubes were kept at a temperature of 25 ± 1 °C, 70 ± 10% RH, and a photoperiod of 12:12 h L:D.

Daily observations were carried out to determine the number of parasitized eggs, duration of the egg-to-adult period of *T. pachycoris*, emergence rate, and sex ratio (SR) determined by the formula: SR = number of females/(number of females + number of males). The experimental design was completely randomized with 4 treatments (different exposure times) and 6 replicates (25 eggs each) in order to investigate the influence of exposure time on the parasitism capacity.

### INFLUENCE OF HOST AGE ON PARASITISM BY *T. PACHYCORIS*

*Telenomus pachycoris* females up to 48 h old were placed individually in glass tubes (8.5 × 2.4 cm) with 25 *P. torridus* eggs at ages 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 d of embryonic development. The holding of eggs and parasitoids in glass tubes for parasitoid oviposition was as described for the exposure time experiment. Eggs were exposed to the parasitoids for 24 h, and then the parasitoids were removed. The experiment was carried out under controlled conditions of temperature (25 ± 1 °C), RH (70 ± 10%), and photoperiod (12:12 h L:D).

Daily observations were carried out to record the number of parasitized eggs, duration of the egg-to-adult period, emergence rate, and sex ratio. The experimental design was completely randomized with 11 treatments (age of the eggs) and 6 replicates (approximately 25 eggs per replicate) to investigate the influence of the host embryonic development on parasitism capacity.

### DEVELOPMENT OF *T. PACHYCORIS* AT VARIOUS TEMPERATURES AND DETERMINATION OF TEMPERATURE REQUIREMENTS

Twenty-five *P. torridus* eggs, fixed and sealed in glass tubes as in the previous assay, were offered to *T. pachycoris* females up to 48 h
old. The tubes with the eggs were kept under controlled conditions of temperature \(25 \pm 1^\circ C\), RH \(70 \pm 10\%\), and photoperiod (12:12 h L:D). After 24 h of exposure, the females were removed with a brush, and the eggs were placed in air-conditioned chambers with temperatures adjusted to 18, 20, 22, 25, 28, 30 \pm 1^\circ C at 70 \pm 10\% RH and a photoperiod of 12:12 h L:D.

Daily observations were conducted to assess the duration of the egg-to-adult period of males and females and emergence rate. Based on the data of the egg-to-adult period at different temperatures, temperature requirements were determined for females, males, and both with the hyperbolic method (Haddad et al. 1999). From the threshold temperature and the degree-days required for the egg-to-adult period, we estimated the number of generations of \(T. pachycoris\) for each temperature studied. The experimental design was completely randomized with 6 treatments (temperatures) and 6 replicates (25 eggs per replicate).

**STATISTICAL ANALYSES**

The data from the experiments were subjected to analysis of variance, and the means were compared within the Student \(t\)-test, protected by the significance of the \(F\)-test at 5% probability, using SAS® software version 9.2 (SAS Institute 2002–2008).

**Results**

**EXPOSURE TIME OF \(P. torridus\) TO \(T. pachycoris\)**

The number of \(P. torridus\) eggs parasitized by \(T. pachycoris\) differed significantly between treatments \(F = 5.93, df = 3, P = 0.0079\), ranging from 5.5 to 11.3 when parasitoid females remained in contact with the eggs for 6 and 24 h, respectively. No significant differences were observed for the other biological parameters evaluated: emergence rate \(F = 0.56, df = 3, P = 0.6478\), duration of egg-to-adult period \(F = 0.28, df = 3, P = 0.8368\), and sex ratio \(F = 0.09, df = 3, P = 0.9660\). The emergence rate was greater than 80%, except for the 6 h period, in which the emergence rate was close to 70%. The duration of the egg-to-adult period was close to 15 d, and the sex ratio was greater than 0.8 (Table 1).

**INFLUENCE OF AGE OF \(P. torridus\) EGGS ON PARASITISM BY \(T. pachycoris\)**

The embryonic development period of \(P. torridus\) affected the parasitism capacity of \(T. pachycoris\). Significant differences were observed for the number of eggs parasitized \(F = 14.38, df = 10, P = 0.0001\). Eggs exposed at 1, 2, and 3 d of age were more parasitized than older eggs. Parasitism of 1- to 2-d-old eggs did not differ significantly from that of 4-d-old eggs. The number of eggs parasitized was significantly smaller in 5- to 10-d-old eggs, and no development of \(T. pachycoris\) occurred in eggs exposed at 11 d of age. The emergence rate of \(T. pachycoris\) did not differ significantly in relation to the egg age and was greater than 66\% \(F = 1.04, df = 9, P \geq 0.6270\). The duration of the egg-to-adult period of \(T. pachycoris\) differed significantly between host egg ages, ranging from 13.24 to 14.83 d \(F = 3.37, df = 9, P = 0.044\). The sex ratio was not affected by the egg age and was greater than 0.69 \(F = 0.20, df = 9, P = 0.9920\) (Table 2).

**DEVELOPMENT OF \(T. pachycoris\) AT VARIOUS TEMPERATURES AND DETERMINATION OF TEMPERATURE REQUIREMENTS**

Temperature influenced the duration of the egg-to-adult period of \(T. pachycoris\), but no significant differences were observed for the other variables analyzed. The duration of the egg-to-adult period of \(T. pachycoris\) differed significantly among temperatures for females \(F = 1,005.66, df = 3, P = 0.0001\), males \(F = 715.67, df = 3, P = 0.0001\), and for both sexes together \(F = 925.63, df = 3, P = 0.0001\). Within the temperature range from 18 to 30 °C, the duration was inversely proportional to temperature, ranging from 34.6 to 10.1 d for females, from 32.3 to 9.4 d for males, and from 33.6 to 9.8 d for both. The emergence rate of \(T. pachycoris\) did not differ significantly among the temperatures studied and was greater than 50\% \(F = 1.61, df = 5, P = 0.1864\) (Table 3). The sex ratio of \(T. pachycoris\) did not show significant differences at the different temperatures studied and was greater than 0.6 \(F = 0.24, df = 5, P = 0.9426\).

The threshold temperature and the thermal constant were 12.9 °C and 166.7 degree-days for females, 12.9 °C and 158.7 degree-days for males, and 12.9 °C and 163.9 degree-days for both sexes together (Table 4). The coefficients of determination were greater than 0.98 indicating a correlation between temperature and \(T. pachycoris\) development. The results show that the temperature requirements of males and females are similar; however, the females need to accumulate slightly more degree-days to complete the development from egg to adult.

Based on the temperature requirements of \(T. pachycoris\) and the constant temperatures studied, this parasitoid can complete between 11.4 to 38.1 generations per year within the range from 18 to 30 °C (Fig. 1).

**Discussion**

A period of 12 h was enough for \(T. pachycoris\) females to parasitize average 8.2 eggs, but when the parasitoids remained in contact with the eggs for 24 h, they parasitized on average 11.3 eggs. Although there are no previous studies that determined the parasitism capacity of \(T. pachycoris\) by different exposure periods to eggs of \(P. torridus\), Pacheco & Corrêa-Ferreira (1998) reported that 24-h-old \(T. podisi\) females parasitized about 14 eggs of Euschiistus heros (F.) (Hemiptera: Pentatomidae) and 8 eggs of Piezodorus guildinii (Westwood) (Hemiptera: Pentatomidae) in 1 d. This indicates that the parasitism capacity of \(T. pachycoris\) is similar to that of \(T. podisi\). In the present work, the influence of host egg age on the development of \(T. pachycoris\) was assessed, and we observed that for \(P. torridus\) eggs of 1 to 3 d of age, parasitism ranged from 8.8 to 11 eggs per female. Based on our results, 6 h of exposure time is enough to obtain good parasitism (on average 5.5
parasitized eggs per female) with an emergence rate greater than 70%, a sex ratio above 0.8, and a pre-imaginal period of approximately 15 d.

*Telenomus pachycoris* parasitism of *P. torridus* eggs decreased with host embryonic development after day 4 until day 11, when no successful parasitism occurred. This reduction in the number of parasitized eggs with increasing host age also occurs with other parasitoids, for example, those in the genus *Trichogramma* (Navarajan 1979; Mellini 1986; Pratissoli & Oliveira 1999; Soares et al. 2012). The parasitism by *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) decreased with increasing egg age of *Grapholitha molesta* (Busck) (Lepidoptera: Tortricidae) (Rodrigues et al. 2011). Food availability (carbohydrates) could affect the parasitism (Soares et al. 2012). Probably, the change of the embryonic content of nutrients in the host influenced the pre-imaginal development of *T. pachycoris*. For *P. torridus*, the hatching of nymphs at 25 °C occurs, on average, between 10 and 11 d (Borges Filho et al. 2013); therefore, 11-d-old eggs were not parasitized. The *T. pachycoris* females may have oviposited in the egg, but there was lack of time for parasitoid development; alternatively, they did not find the eggs viable for parasitism. This condition was also observed for *Trichogramma maxacalii* Vogege & Pointel (Hymenoptera: Trichogrammatidae) parasitizing eggs of *Oxydia vesuilia* (Cramer) (Lepidoptera: Geometridae): no parasitism occurred in older eggs or when host larvae began to hatch (Oliveira et al. 2003).

The age of the host eggs also influenced the duration of the egg-to-adult period of *T. pachycoris*, ranging from 14.8 d in 24-h-old eggs to 13.2 d in 6-d-old eggs. Although statistically significant, these differences are biologically not important, because no extension of the egg-to-adult period was observed with increasing egg age. Similar durations of the egg-to-adult period at 25 °C were observed for *T. pachycoris* (Oliveira & Silva 2011), *T. podisi*, *Trissolcus brochymene* Ashmead (Hymenoptera: Scelionidae) (Torres et al. 1996/1997). Probably, in the case of *T. pachycoris*, changes in the physical characteristics of the host egg with increasing age, such as the thickness and texture of the chorion, may also limit parasitism. In a study by Vinson (1997), the host egg quality decreased with embryonic development affecting the sex ratio in the emerging parasitoid generation. However, an effect of host age on the sex ratio was not observed in our study, and the sex ratio remained above 0.75.

The duration of the pre-imaginal period of *T. pachycoris* at different temperatures was previously not studied. Oliveira & Silva (2011) reported durations between 13 and 14 d at 25 °C, similar to the period observed in this study (14.1, 12.9, and 13.7 d for females, males, and both sexes together, respectively) at the same temperature. Similar values were also reported for other species of Scelionidae, such as *T. podisi*, parasitoid of *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae), with durations from 21.8 to 11.6 d at 20 to 28 °C (Torres et al. 1997). For *Telenomus cyamophylax* Polaszek (Hymenoptera: Scelionidae), a parasitoid of *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), the duration of the egg-to-adult period ranged from 25.4 to 12.6 d at 20 to 30 °C, respectively (Foerster & Butnariu 2004). For *Telenomus remus* Nixon (Hymenoptera: Scelionidae), the duration of the egg-to-adult period in eggs of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) at temperatures from 20 to 31 °C ranged from 24.2 to 8.3 d for females and from 23.8 to 8.1 d for males (Bueno et al. 2008), similar to the periods we observed for *T. pachycoris*.

The results of the temperature requirements of males and females were similar. However, females needed to accumulate more degree-days than males to complete the egg-to-adult period. The threshold temperatures estimated in the present study are similar to those determined for *T. remus* females and males in *S. frugiperda* eggs, where the sex ratio remained above 0.75.

### Table 2. Influence of the temperature on the duration of the egg-to-adult period (d) and emergence rate of *Telenomus pachycoris* reared in *Pachycoris torridus* eggs at RH of 70 ± 10% and a photoperiod of 12:12 h L:D.

| Temp. (°C) | Duration of egg-to-adult period (d) | Emergence (%) |
|-----------|-------------------------------------|----------------|
|           | Female | Male | Average |                       |
| 18        | 34.6 ± 0.4 a | 32.3 ± 0.2 a | 33.6 ± 0.3 a | 71.3 ± 6.0 a |
| 20        | 24.4 ± 0.4 b | 22.4 ± 0.6 b | 23.6 ± 0.4 b | 70.9 ± 3.0 a |
| 22        | 17.4 ± 0.2 c | 16.2 ± 0.3 c | 17.0 ± 0.2 c | 70.7 ± 7.3 a |
| 25        | 14.1 ± 0.3 d | 12.9 ± 0.5 d | 13.7 ± 0.4 d | 66.4 ± 7.9 a |
| 28        | 10.7 ± 0.2 e | 10.3 ± 0.3 e | 10.5 ± 0.3 e | 64.0 ± 12.4 a |
| 30        | 10.1 ± 0.4 e | 9.4 ± 0.4 e | 9.8 ± 0.4 e | 58.2 ± 12.4 a |

Means ± SE followed by the same letter per column did not differ by the Student *t*-test with significance of the *F*-test at 5% probability.
the threshold temperature was 12.5 °C and 12.6 °C, and the thermal constant was 158.9 and 154.1 degree-days, respectively (Bueno et al. 2008). However, for T. podisi in P. nigrispinus eggs, temperature requirements were greater and differed between the sexes (Torres et al. 1997).

These results are the basis for the development of a biological control program. The data obtained will help in establishing a rearing technique of T. pachycoris in the laboratory.

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