

Effect of temperature on immatures of *Stiretrus decastigmus* (Hemiptera: Pentatomidae)

S. Poncio^{a,*}, *S. T. B. Dequech*^{a,b}, *A. Bolzan*^b, *C. Güths*^b, *M. P. Walker*^b,
V. S. Sturza^b and *D. E. Nava*^c

^aPrograma de Pós-graduação em Agrobiologia, Universidade Federal de Santa Maria – UFSM, Av. Roraima, 1000, Cidade Universitária, Camobi, CEP 97105-900, Santa Maria, RS, Brazil

^bDepartamento de Defesa Fitossanitária, Universidade Federal de Santa Maria – UFSM, Av. Roraima, 1000, Cidade Universitária, Camobi, CEP 97105-900, Santa Maria, RS, Brazil

^cLaboratório de Entomologia, Embrapa Clima Temperado, Rodovia BR 392, Km 78, CP 403, CEP 96010-971, Pelotas, RS, Brazil

*e-mail: soniaponcio@yahoo.com.br

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Abstract

Stiretrus decastigmus (Herrich-Schaeffer) (Hemiptera: Pentatomidae) is an important predator of the insect pest *Microtheca ochroloma* Stal (Coleoptera: Chrysomelidae). The present study investigated the pre-imaginal development of *S. decastigmus* at different temperatures. The temperatures were: 20, 25, and 30 °C, with a relative humidity of 70 ± 10% and a photofase of 12 h, and the nymphs were fed larvae of *M. ochroloma*. We evaluated the duration and viability of the egg and nymphal stages, the duration of each instar, and the predation potential. The incubation time decreased with increasing temperature, and the viability was highest at 25 °C. The duration of the nymphal stage was inversely proportional to the temperature, ranging from 18 days at 30 °C to 40.6 days at 20 °C. The highest *S. decastigmus* predation rates were found at 20 °C (90.4 larvae) and 30 °C (72.5 larvae). *S. decastigmus* showed the highest viability and lowest consumption of larvae of *M. ochroloma* at 25 °C.

Keywords: Asopinae, Chrysomelidae, biological control, predator.

Efeito da temperatura sobre imaturos de *Stiretrus decastigmus* (Hemiptera: Pentatomidae)

Resumo

Stiretrus decastigmus (Herrich-Schaeffer) (Hemiptera: Pentatomidae) é um importante predador do inseto-praga *Microtheca ochroloma* Stal (Coleoptera: Chrysomelidae). O objetivo deste trabalho foi estudar o desenvolvimento pré-imaginal de *S. decastigmus* em diferentes temperaturas. Foram utilizadas as temperaturas de 20, 25, e 30 °C, umidade relativa do ar de 70 ± 10% e fotofase de 12 h, sendo as ninfas alimentadas com larvas de *M. ochroloma*. Foram avaliados a duração e viabilidade dos estágios de ovo e ninfa, a duração de cada instar e o potencial de predação. O período de incubação diminuiu com o aumento da temperatura e apresentou maior viabilidade a 25 °C. A duração do estágio ninfal foi inversamente proporcional a temperatura com 18 dias a 30 °C e 40,6 dias a 20 °C. A maior taxa de predação de *S. decastigmus* foi encontrada a 20 °C (90,4 larvas) e 30 °C (72,5 larvas). *S. decastigmus* teve maior viabilidade e menor consumo de larvas de *M. ochroloma* a 25 °C.

Palavras-chave: Asopinae, Chrysomelidae, controle biológico, predador.

1. Introduction

Production of vegetables such as species of Brassicaceae is an excellent option for family farms, especially in organic systems (Pinto et al., 2001). However, the plants are attacked by the insect pest *Microtheca ochroloma* Stal (Coleoptera: Chrysomelidae), which defoliates the plants during both its larval and adult stages (Fasulo, 2005). The impacts of this pest on crops is well documented in South America, where it is indigenous, and in North America (Ameen

and Story, 1997). The extent of the damage may impel farmers, especially organic producers, to avoid cultivating Brassicaceae (Dequech et al., 2008).

As an alternative to chemical pesticides, biological control using natural enemies has been increasing in importance in integrated pest management programs (IPM), especially to promote sustainability of agriculture (Parra et al., 2002).

The natural enemy of *Microtheca* species, *Stiretrus decastigmus* (Herrich-Schaeffer) (Hemiptera: Pentatomidae), has been recorded in the state of Rio Grande do Sul, southern Brazil, preying on larvae and adults of *M. ochroloma* (Poncio et al., 2010) and *M. semilaevis* Stal (Sturza et al., 2012). However, little is known of the biology of this predator, a promising agent for biological control programs targeting this pest. In order to implement biological control, it is necessary to determine the effects of abiotic factors on the development of the predator. The present study examined the effect of different temperatures on the pre-imaginal development of *S. decastigmus*, with a view toward rearing the species in the laboratory for the purpose of biological control of *M. ochroloma*.

2. Material and Methods

2.1. Establishment and rearing of *Microtheca ochroloma*

Adults of *M. ochroloma* were collected from plots of Chinese cabbage (*Brassica chinensis* L.) (Brassicaceae) at the Universidade Federal de Santa Maria (UFSM) in the state of Rio Grande do Sul, municipality of Santa Maria, southern Brazil (29° 43' 28" S, 53° 43' 19" W, altitude: 95 m). The adults were kept in rearing cages (transparent plastic boxes measuring 30 × 30 × 20 cm), with mesh sides and cover) and fed with Chinese cabbage leaves pre-washed in running water. Water was provided to the insects by capillary action through cotton dental rolls that were placed in plastic containers containing 100 mL of water and changed every two days. The egg masses were removed from the leaves and placed in Petri dishes (diameter = 8.5 cm) with their bottoms covered with filter paper. The larvae from these egg masses were kept in cages similar to those used for adults, but smaller (17.7 × 26.2 × 14.7 cm), and were also fed with leaves of Chinese cabbage, which were replaced daily. After the adults emerged, they were transferred to the larger cages for mating. Part of the insects, in various stages of development, were used to maintain the rearing stock, and part were used for the experiments. The creations were maintained at 23 °C, with a relative humidity of 70 ± 10% and a photofase of 12 h.

2.2. Establishment and rearing of *Stiretrus decastigmus*

Nymphs and adults of *S. decastigmus* were collected from plots of Chinese cabbage (*Brassica chinensis* L.) (Brassicaceae) at the UFSM. The insects were taken to the laboratory and kept in cages (17.7 × 26.2 × 14.7 cm). Their eggs were removed daily and kept in Petri dishes (diameter = 8.5 cm) with the bottoms covered with filter paper moistened with distilled water, until they reached the second instar. The nymphs were then transferred to breeding cages (17.7 × 26.2 × 14.7 cm) because they begin to predate in this stage. Larvae and/or adults of *M. ochroloma* were used as prey for *S. decastigmus* nymphs and adults, in laboratory rearing. The creations

were maintained at 23 °C, with a relative humidity of 70 ± 10% and a photofase of 12 h.

2.3. Effect of temperature on the immatures of *Stiretrus decastigmus*

Biological parameters of *S. decastigmus* were evaluated at temperatures of 20, 25 and 30 °C. Forty eggs of this predator were used per temperature, in a Petri dish (9.0 cm diameter × 1.5 cm height) lined with filter paper moistened with distilled water to reduce egg dehydration. At each temperature, 20 second-instar nymphs were placed in individual cages prepared from plastic pots (350 mL). The experiments began with second-instar nymphs, because the first-instar nymphs do not feed (Zanuncio et al., 1991; Silva et al., 1996; Wanderley and Ramalho, 1999). The upper opening was closed with voile fabric to allow aeration. An acrylic container (2.5 cm diameter × 4.5 cm height) was placed inside the cage, containing moistened cotton dental rolls to supply water. The nymphs were fed with third- and fourth-instar *M. ochroloma* larvae, offered to the nymphs in similar proportions *ad libitum*, and the larvae were left inside the cage until the nymphs reached the final instar.

The duration and survival of the embryo and nymphal stages, the number and duration of instars, the total life-cycle duration, and the number of prey consumed were assessed. The duration and survival were determined daily, when nymph hatching was recorded. The number and durations of instars were determined based on the interval between ecdyses, determined by the presence of molts in the rearing container. The total duration of the nymphal stage was taken as the sum of the duration of each nymphal stage. The overall life cycle was the sum of the durations of the egg and nymphal stages.

2.4. Data analysis

The experiments used a fully randomized design with 20 replications. Data normality was checked with Shapiro-Wilk's test (k samples) before analysis of variance (ANOVA); means were compared using Tukey's test ($\alpha = 0.05$). The ASSISTAT 7.5 Beta (2008) software was used (Silva, 1996).

3. Results

The incubation period of *S. decastigmus* was inversely proportional to the temperature, i.e., 5.00 and 13.66 days at 30 and 20 °C, respectively (d.f. = 2; F = 127.5851; P < 0.001). Eggs kept at 25 °C had the highest survival, and more individuals reached the nymphal stage (95%). At this temperature, the egg incubation period of 8.57 days was intermediate between those at the other temperatures.

The temperature also affected the duration of all nymphal instars (Table 1). Nymphs kept at 20 °C had a longer instar duration than those at 25 and 30 °C. The duration of the final instar was longer at all temperatures. The duration of the nymphal stage varied from 18.00 days at 30 °C to 40.60 days at 20 °C (d.f. = 2; F = 174.4424; P < 0.001). Nymphal survival was highest (85.00%) at 25 °C, lasting

Table 1. Duration (days) of nymphal instars (mean \pm SE) and survival of *Stiretrus decastigmus* (Hemiptera: Pentatomidae) fed on *Microtheca ochroloma* (Coleoptera: Chrysomelidae) larvae at 20, 25, and 30 °C.

Temperature (°C)	Nymphal instar					Total
	1 st	2 nd	3 rd	4 th	5 th	
20	4.91 \pm 0.22aD	7.27 \pm 0.14aB	6.40 \pm 0.16aC	7.88 \pm 0.20aB	13.80 \pm 0.37aA	40.60 \pm 0.81a
25	2.55 \pm 0.12bC	4.00 \pm 0.14bB	3.11 \pm 0.08bC	4.70 \pm 0.25bB	8.35 \pm 0.33bA	22.70 \pm 0.47b
30	2.83 \pm 0.30bB	2.60 \pm 0.24cB	3.40 \pm 0.24bB	3.00 \pm 0.28cB	5.25 \pm 0.47cA	18.00 \pm 1.41c

Means followed by the same lowercase letter per column and uppercase letter per row did not differ by Tukey's test ($\alpha = 0.05$).

Table 2. Larval consumption (mean \pm SE) per instar and total consumption of *Microtheca ochroloma* (Coleoptera: Chrysomelidae) larvae by the *Stiretrus decastigmus* (Hemiptera: Pentatomidae) nymph stage fed at 20, 25, and 30 °C.

Temperature (°C)	Instar				Nymph consumption
	2 nd	3 rd	4 th	5 th	
20	4.63 \pm 0.45aC	8.80 \pm 0.71bC	18.00 \pm 1.39aB	58.20 \pm 4.11aA	90.40 \pm 4.23a
25	4.27 \pm 0.22aD	10.58 \pm 0.40bC	14.88 \pm 0.66aB	23.11 \pm 0.97cA	52.88 \pm 1.11c
30	4.00 \pm 0.89aC	14.40 \pm 2.14aBC	19.40 \pm 3.78aAB	33.25 \pm 6.71bA	72.50 \pm 9.46b

Means followed by the same lowercase letter per column and uppercase letter per row did not differ by Tukey's test ($\alpha = 0.05$).

22.70 days, whereas at 20 and 30 °C the duration was 18.0 and 40.6 days, with survival rates of 25 and 20% respectively. The total life cycle of *S. decastigmus* lasted 23.0, 32.2, and 54.0 days at 30, 25, and 20 °C, respectively (d.f. = 2; $F = 215.9122$; $P < 0.001$). The predation rate of *S. decastigmus* was highest at 20 °C, when each predator nymph preyed on a mean of 90.40 *M. ochroloma* larvae (d.f. = 3; $F = 38.0827$; $P < 0.001$) (Table 2). At all temperatures, consumption was proportional to the increase in size of the nymphs, i.e., the older nymphs consumed more prey (Table 2).

4. Discussion

The embryonic period of *S. decastigmus* was relatively long for rearing purposes; the duration of 8.57 days at 25 °C is longer than those reported for other members of Pentatomidae such as *Podisus nigrispinus* (Dallas) (4.00 days) (Vacari et al., 2007), *Podisus distinctus* (Dallas) (5.20 days) (Santos et al., 2004) and *Brontocoris tabidus* (Signoret) (3.50 days) (Fernandes et al., 1996) and 5.60 days (Jusselino-Filho et al., 2003). However, *S. decastigmus* showed higher survival for the embryonic period than these other predators. Probably longer periods of embryonic development is a characteristic of this species, because when the temperature was high, although this period decreased, it was still longer than those reported for other members of Pentatomidae at lower temperatures (Fernandes et al., 1996; Jusselino-Filho et al., 2003; Santos et al., 2004; Vacari et al., 2007).

As noted by Coursey and Esselbaugh (1962), pentatomids typically have five instars. The number of instars of *S. decastigmus* was not affected by the temperature, since the nymphs passed through five instars at all temperatures tested. However, the temperature was a determining factor in the duration of all nymphal instars. For nymphs exposed to temperatures of 20 °C the duration of each instar was

longer, and shorter durations predominated in nymphs exposed to 30 °C.

The survival of *S. decastigmus* nymphs at 25 °C was higher than that of *P. nigrispinus* reared on *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (68%) and *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (64%) (Oliveira et al., 2004), and of *P. distinctus* reared on *Musca domestica* (L.) (Diptera: Muscidae) larvae (60%) (Lacerda et al., 2004), and *T. molitor* (76%) (Santos et al., 2004). This difference may be related to food quality, since *S. decastigmus* was fed on prey that, despite being laboratory-reared, is natural; while the studies of these other predators used prey reared on artificial diets.

The nymphal period of 20 days at 25 °C found for *S. decastigmus* fed with *M. ochroloma* larvae indicates that this prey species may be appropriate for rearing this predator, because they provide suitable nutritional resources for its development, as reported for another pentatomid predator (Oliveira et al., 2004). Since at these temperatures the prey consumption was lowest, this translates to a reduction in production costs. The larger number of prey consumed at 20 °C is related to the longer developmental period at this temperature, since nymphs that take longer to develop require more food. However, this relationship did not obtain at the higher temperature (30 °C), where the development time was reduced; however, the number of prey consumed was higher than that consumed by nymphs kept at 25 °C. Possibly this is related to the accelerated metabolism at higher temperatures, causing the insects to require more energy to reach the next stage.

The total life-cycle duration of *S. decastigmus* is similar to that of *S. cincticeps* at 25 °C fed on *M. domestica* (30.2 days) and *T. molitor* (30.5 days) (Zanuncio et al., 1992).

The number of *M. ochroloma* larvae predated by *S. decastigmus* was higher than that of *P. nigrispinus* in the laboratory at 28 ± 1 °C, $53 \pm 5\%$ RH and a photophase of 14 h, which was 43.10 larvae of *Tuta absoluta* (Meyrick)

(Lepidoptera: Gelechiidae) (Vivan et al., 2002) and 15.2 larvae of *Plutella xylostella* (L.) (Lepidoptera: Pyralidae) at 25 ± 1 °C, 70 ± 10% RH, and a photophase of 12 h (Xavier et al., 2009). This larger amount of prey consumed is likely related to their size and to the amount of nutrients contained, since the larvae of *M. ochroloma* are smaller than the prey used by these investigators.

In summation, for the purpose of laboratory rearing with a view toward optimizing the production of predators, the temperature of 25 °C was optimum for nymphal development of *S. decastigmus*, as shown by their higher survival rate and lower consumption of *M. ochroloma* larvae. With respect to the use of *S. decastigmus* for the purpose of biological control under field conditions, lower rearing temperatures lead to a higher consumption of larvae and a longer nymphal developmental period.

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