

Biological quality of *Tetrastichus howardi* (Hymenoptera: Eulophidae) reared with *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae after cold storage

Elison F. Tiago¹, Fabricio F. Pereira^{1,*}, Samir O. Kassab¹, Rogério H. Barbosa¹, Carlos Reinier G. Cardoso², Willian Y. Sanomia², Helter C. Pereira², Rosilda M. M. F. Silva¹, and José C. Zanuncio³

Abstract

The endoparasitoid *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae) can be reared with the alternative host *Tenebrio molitor* (Linnaeus) (Coleoptera: Tenebrionidae). Host storage at low temperatures can regulate parasitoid production and demand in biological control programs. The life cycle (egg to adult), parasitism and emergence percentage, number of parasitoids emerged per host pupae, sex ratio, and longevity of the *T. howardi* offspring per *T. molitor* pupa were evaluated after low temperature storage of this host for different periods, and its immature (pupae) in *T. molitor* pupae for 5 periods at 10.3 °C. *Tenebrio molitor* pupae stored at 0.5 ± 0.09 °C and 2.7 ± 0.11 °C for 10 and 20 d, respectively, were adequate to produce *T. howardi*. The biological characteristics of this parasitoid were better with *T. molitor* pupae stored at 0.5 ± 0.09 °C and 2.7 ± 0.11 °C for 10 and 20 d. *Tetrastichus howardi* immature (pupae) can be stored in *T. molitor* pupae for 10, 20, 30, 60, and 90 d at 10.3 °C, preferably in pupae of this host for 10 d to produce the adults of this parasitoid for biological control programs. These results contribute to overcoming 1 of the difficulties encountered in the mass production of parasitoids. Therefore, the conservation of *T. molitor* pupae in low temperatures can be used to increment of population of *T. howardi* in mass production for pest control programs.

Key Words: biological control; cold storage; parasitoids; progeny

Resumo

O endoparasitoide *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae) pode ser criado com o hospedeiro alternativo *Tenebrio molitor* (Linnaeus) (Coleoptera: Tenebrionidae). O armazenamento de hospedeiros em baixas temperaturas pode regular a produção e a oferta de parasitoides em programas de controle biológico. O ciclo de vida (ovo a adulto), parasitismo e porcentagem de emergência, número de parasitoides emergidos por pupa hospedeira, razão sexual e longevidade de *T. howardi* por pupa de *T. molitor* foram avaliados após o armazenamento em baixa temperatura deste hospedeiro por diferentes períodos e seu imaturo (pupa) em pupas de *T. molitor* por 5 períodos a 10,3 °C. Pupas de *Tenebrio molitor* armazenadas a 0,5 ± 0,09 °C e 2,7 ± 0,11 °C por 10 e 20 dias, respectivamente, foram adequadas para produção de *T. howardi*. As características biológicas deste parasitoide foram melhores com pupas de *T. molitor* armazenadas por 10 e 20 dias. Imaturos (pupas) de *T. howardi* podem ser armazenados em pupas de *T. molitor* por 10, 20, 30, 60 e 90 dias a 10,3 °C, preferencialmente em pupas deste hospedeiro por 10 dias para produzir adultos desse parasitoide para programas de controle biológico. Estes resultados contribuem para superar uma das dificuldades encontradas na produção em massa de parasitoides. Portanto, a conservação de pupas de *T. molitor* em baixas temperaturas pode ser utilizada para incremento da população de *T. howardi* na produção em massa para programas de controle de pragas.

Palavras Chave: controle biológico; armazenagem em frio; parasitoides; progênie

The use of parasitoids in most biological control programs requires that the parasitoids meet some requirements, such as high quality and low-cost techniques to rear these insects (Colinet & Boivin 2011; Vargas et al. 2013; Ferreira et al. 2016). Parasitoids, especially adult females, used in biological control programs, have a short life cycle (Barbosa et al. 2016) and, therefore, should be sent to release sites soon after being produced. The number of parasitoids produced depends on the demand for release and should

be controlled according to priority areas. The continuous parasitoid availability, for commercial experiments or release aiming to reduce insect pest populations, is 1 of the difficulties in biological control laboratories. Temperature affects insect life cycle (Pereira et al. 2009a), and its regulation can facilitate host and parasitoid storage over different periods (Pereira et al. 2013), adjusting production according to the demand for these agents in biological control programs (Pastori et al. 2013).

¹Departamento de Ciências Biológicas e Ambientais, Universidade Federal da Grande Dourados, Dourados, Mato Grosso do Sul, Brazil; E-mails: elisonfloriano@yahoo.com.br (E. F. T.), tetrastichus@gmail.com (F. F. P.), samirkassab@gmail.com (S. O. K.), hidalgo.rogerio@gmail.com (R. H. B.), maramussury@ufgd.edu.br (R. M. M. F. S.)

²Departamento de Ciências Agrárias, Universidade Federal da Grande Dourados, Dourados, Mato Grosso do Sul, Brazil; E-mail: cr.garcia.cardoso@gmail.com (C. R. G. C.), willian_sanomia@hotmail.com (W. Y. S.), heltercp@gmail.com (H. C. P.)

³Departamento de Entomologia/BIOAGRO, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil; E-mail: zanuncio@ufv.br (J. C. Z.)

*Corresponding author; E-mail: tetrastichus@gmail.com

Cold stress effects on parasitoids should be studied because storage at low temperatures can compromise the quality of these natural enemies (Lysyk 2004; Chen et al. 2008b). Biological characteristics such as parasitism, emergence, longevity, progeny, and sex ratio can be used to evaluate the performance of parasitoids after cold storage (Pereira et al. 2013). *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae) parasitize many lepidopteran pests (Prasad et al. 2007), including *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) caterpillar pupae and adults (Pereira et al. 2015). This natural host can damage sugar cane, corn, and sorghum crops (Cruz et al. 2011), and rice in Brazil (Lv et al. 2011). The *T. howardi* use in biological control programs depends on its low cost, large scale production and quality (Ferreira et al. 2016).

The objectives were to evaluate the life cycle (egg to adult), parasitism, and emergence percentages, number of parasitoids emerged per host pupae, sex ratio, and longevity of *T. howardi* offspring in *T. molitor* pupae after storage of this host at low temperatures.

Materials and Methods

The experiment was conducted at the Laboratory of Biological Control of Insects of the Faculty of Biological and Environmental Sciences of the Universidade Federal de Grande Dourados in Dourados, Mato Grosso do Sul, Brazil, with the following stages: *Tenebrio molitor* larvae were kept in plastic trays (29 × 23 × 11 cm), and kept air-conditioned at 25 ± 1 °C, 60 ± 10% RH, and a 14:10 h (L:D) photoperiod, with wheat bran (97%), beer yeast (3%), and chuchu slices (Zamperline & Zanuncio 1992) or cucumber to supplement the feeding of this insect (Oliveira 2013). Viable *D. saccharalis* eggs were placed in glass bottles (8.5 × 13 cm) with an artificial diet based on soybean meal, wheat germ, vitamins, minerals, and anti-pollutants to feed newly hatched caterpillars until the third instar. These caterpillars were transferred to Petri dishes (6.5 × 2.5 cm) with a 3 × 1.5 cm cube of artificial diet until the pupa stage. The pupae were collected, sexed, and 50 (20 males and 30 females) were placed per PVC cage in tubes (10 × 22 cm) internally coated with sulphite paper as an oviposition substrate, and sealed using voile type cloth and elastic to prevent escape and to allow air to circulate (Parra 2007).

REARING OF THE PARASITOID *TETRASTICHUS HOWARDI*

Fifty *D. saccharalis* pupae, 24 to 48 h old, were individually exposed for 24 h to parasitism by 5 *T. howardi* females, in glass tubes (2 × 15 cm), sealed with cotton and containing a honey droplet. Following this, they were individualized and maintained at 25 ± 2 °C, 70 ± 10% RH, and a photoperiod of 14:10 h (L:D) in a controlled climate chamber until the emergence of the adults (Pereira et al. 2009b, 2015).

TETRASTICHUS PARASITISM AND DEVELOPMENT ON *TENEBRIO MOLITOR* PUPAE STORED FOR DIFFERENT PERIODS AT LOW TEMPERATURES

Fifty 24-h-old *T. molitor* pupae weighing between 0.110 and 0.140 g (Oliveira 2013) were selected. Pupae of this weight range had better quality, and were stored at temperatures and relative air humidity of -28.2 °C and 53.8%; 0.5 °C and 55.6%; 2.7 °C and 52.0%; 10.5 °C and 56.7%; 14.8 °C and 62.5%, respectively, for 10, 20, 30, 60, and 90 d. After these periods, each pupa was parasitized for 72 h by seven 24-h-old *T. howardi* females in glass tubes (14 × 2.5 cm) sealed with cotton. At the end of the parasitism period, the *T. howardi* females were removed from the tubes, and the *T. molitor* females were kept at 25 ± 2 °C, 70 ± 10% RH, and a photoperiod of 14:10 h (L:D) until the emergence of

the parasitoid offspring. This procedure also was used in the control, where the pupae were cold stored. The pupae were submitted to parasitism at 25 ± 2 °C, 70 ± 10% RH, and a 14:10 h (L:D) photoperiod.

EXPERIMENT 1 – *TENEBRIO MOLITOR* PUPAE, PREVIOUSLY PARASITIZED BY *TETRASTICHUS HOWARDI* MAINTAINED AT 10.3 °C

Tenebrio molitor pupae 24-h-old, weighing 0.110 to 0.140 g, were individualized with seven 24-h-old *T. howardi* females (Oliveira 2013) in glass (14 × 2.5 cm) tubes sealed with cotton for 72 h at 25 °C, 70 ± 10% RH, and a 14:10 h (L:D) photoperiod.

The *T. howardi* females were removed at the end of the parasitism exposure period and *T. molitor* females were kept in the tubes at 25 ± 2 °C, 70 ± 10% RH, and a 14:10 h (L:D) photoperiod until the parasitoids reached the pupal stage. Ten pupae of this host were separated, opened with the help of a stylet, and the immature *T. howardi* counted to verify the pupal stage of the parasitoid. *Tetrastichus howardi* pupae were stored at 10.3 ± 0.48 °C in a Thermoelectric Wine Cellar Model No. 25123 (127)/25127 (220) (Eletrolab, São Paulo, São Paulo, Brazil) for 10, 20, 30, 60, and 90 d. This parasitism procedure also was performed in the control, but the parasitized pupae were maintained in the tubes at 25 ± 2 °C, 70 ± 10% RH, and a 14:10 h (L:D) photoperiod.

Assessment of biological characteristics. The parasitism percentage [(number of pupae without *T. molitor* adult emergence)/(number of pupae offered to parasitoid females)] × 100; emergence percentage [number of *T. molitor* pupae with emergence of parasitoid adults]/(number of pupae parasitized) × 100; life cycle duration (from pupa exposure to parasitism to adult emergence of parasitoid [not evaluated in experiment 2]); number of parasitoids emerged per pupa (progeny) (females + males); and female and male longevity (adult emergence period until death) were evaluated in experiments 1 and 2. *Tetrastichus howardi* males and females were separated according to the morphological characteristics of the antenna (females have antennae with dark brown flagellum, and males have pale flagellum, usually yellowish, with an extra segment of dark brown or black coloring) (La Salle & Polaszek 2007). The mortality of the host was observed with 20 *T. molitor* pupae individualized in glass tubes (1.5 × 10 cm) sealed with cotton without the parasitoid, and the number of mature *T. molitor* adults emerged. *Tetrastichus howardi* adult longevity was determined daily with 20 females and 20 males randomly chosen from the offspring of each treatment, individualized in glass tubes, and fed with droplets of pure honey dispersed inside the glass tube.

STATISTICAL METHODS OF EXPERIMENT 1

The design was completely randomized with 22 treatments and 10 replications, each one with 5 individualized *T. molitor* pupae, totaling 50 pupae per treatment. Data on parasitism, emergence, life cycle duration, number of parasitoids emerged per *T. molitor* (progeny) pupa, sex ratio, and longevity of *T. howardi* males and females were submitted to variance analysis and, when significant at 5% probability, to the Scott-Knott test. The lack of emergence of the parasitoid in some treatments prevented the performance of regression analysis. The data did not show trends; therefore, the means were submitted to the clustering test.

STATISTICAL METHODS OF EXPERIMENT 2

The experimental design was completely randomized with 5 treatments (storage and control periods), with 5 replications with *T. molitor* pupae per replication, totaling 50 pupae per treatment. The data for parasitism, emergence, life cycle duration, number of parasitoids

emerged per *T. molitor* (progeny) pupa, sex ratio, and longevity of *T. howardi* males and females were submitted to regression analysis.

The equation that best fit the data was chosen based on the determination coefficient (R^2), the significance of the regression coefficients (β_i) and the regression analysis by the F test (5% probability level).

Results

REARING OF *TENEBRIO MOLITOR* AND *DIATRAEA SACCHARALIS*

Tetrastichus howardi parasitized and developed on *T. molitor* pupae after storage at low temperatures (Fig. 1a). *Tetrastichus howardi* can be multiplied into pupae of the alternative *T. molitor* host after storage at 0.5 °C and 55.6% RH for 10 and 20 d. *Tenebrio molitor* pupae can be stored at 2.7 °C and 52.0% RH for *T. howardi* breeding in all periods except for 90 d (Table 1). Parasitism, with temperature and relative air humidity combinations of 10.5 °C and 56.7%, and 14.8 °C and 62.5% RH occurred only in pupae stored for 20, 30, and 10 d, respectively (Table 1). *Tetrastichus howardi* progeny in *T. molitor* pupae (Fig. 1b), stored at 14.8 °C and 62.5% RH, were similar to those of the control,

and more numerous than those of the other treatments (Table 1), an important fact to consider when managing this host to produce this parasitoid. The *T. howardi* sex ratio, with *T. molitor* pupae stored at low temperatures, was higher than 0.70 in all treatments with parasitoid emergence. The *T. howardi* life cycle (egg to adult) was similar between treatments with 19.75 ± 3.32 and 25.50 ± 1.85 d, with variation in the *T. howardi* male and female longevity, but always greater than 15 d.

REARING OF THE PARASITOID *TETRASTICHUS HOWARDI*

The percentage of *T. molitor* pupae, parasitized by *T. howardi* females after storage for different periods in the immature stage at 10.3 ± 0.48 °C, $54.20 \pm 2.52\%$, was lower than that of the control, and reached at the most 80% after 10 d of storage. The emergence and sex ratio of *T. howardi* were similar between treatments with $87.88 \pm 3.13\%$ and 0.94 ± 0.01 , respectively. *Tenebrio howardi* progeny was greater in the control, after 10 and 20 d of storage. The longevity of *T. howardi* females and males emerged from *T. molitor* pupae was inversely proportional to cold storage periods, which were higher at 0, 10 and 20 d (Fig. 2a, b).

Discussion

The parasitism percentage was lower than that of the control, but above 70% in the treatments and, therefore, adequate for the mass rearing of this parasitoid (Oliveira 2013). In addition, emergence was greater than 80% and similar to that of the control.

This may be due to the lower host nutritional quality, since refrigeration for long periods can cause biochemical, physical, and morpho-physiological modifications compromising the nutritional quality of the host pupae and, consequently, the parasitoid reproduction (Mainali & Lim 2013). *Tetrastichus howardi* did not parasitize *T. molitor* pupae stored at -28.2 °C and 53.8% RH due to their death, since the lethal minimum for insects is -15 to -25 °C (Fields 1992).

The *T. howardi* life cycle (egg to adult) was similar between treatments, but with variation in the *T. howardi* male and female longevity, but always greater than 15 d. Values for life cycle, over 10 d, are adequate as a period sufficient for parasitoids, released in the field, to find their hosts and reproduce (Pratissoli et al. 2005).

The multiplication of parasitism results by emergence, progeny and sex ratio shows 12.662 females per 7 *T. howardi* from 100 *T. molitor* pupae parasitized without storage (control). The total female progeny, closer to the control, was 5.704 at 0.5 °C and 55.6%, followed by the temperature and relative air humidity combination of 2.7 °C and 52.0%, 4.931 and 4.940 females with 1, 0, and 20 d storage, respectively. These combinations can be recommended for *T. molitor* pupae storage, for the *T. howardi* production, because one of the difficulties found in the mass parasitoid production is to obtain large numbers of suitable hosts when necessary. Thus, the possibility of conserving *T. molitor* pupae with viability for the multiplication of *T. howardi* will be very useful in biological control programs with this natural enemy.

The predominance of females over males is important for large-scale parasitoid rearing, because females are the main agent responsible for subsequent generations in the laboratory (Uçkan & Gülel 2002), and for preventing the continuity of the insect-pest cycle in the field (Fávero et al. 2014).

The lower percentage of *T. molitor* pupae, parasitized by *T. howardi* females after storage for different periods in the immature stage at 10.3 ± 0.48 °C and $54.20 \pm 2.52\%$ compared to the control, shows a reduction in the *T. howardi* reproduction as the exposure period to low temperatures increased, as observed for this parasitoid with *D. sac-*

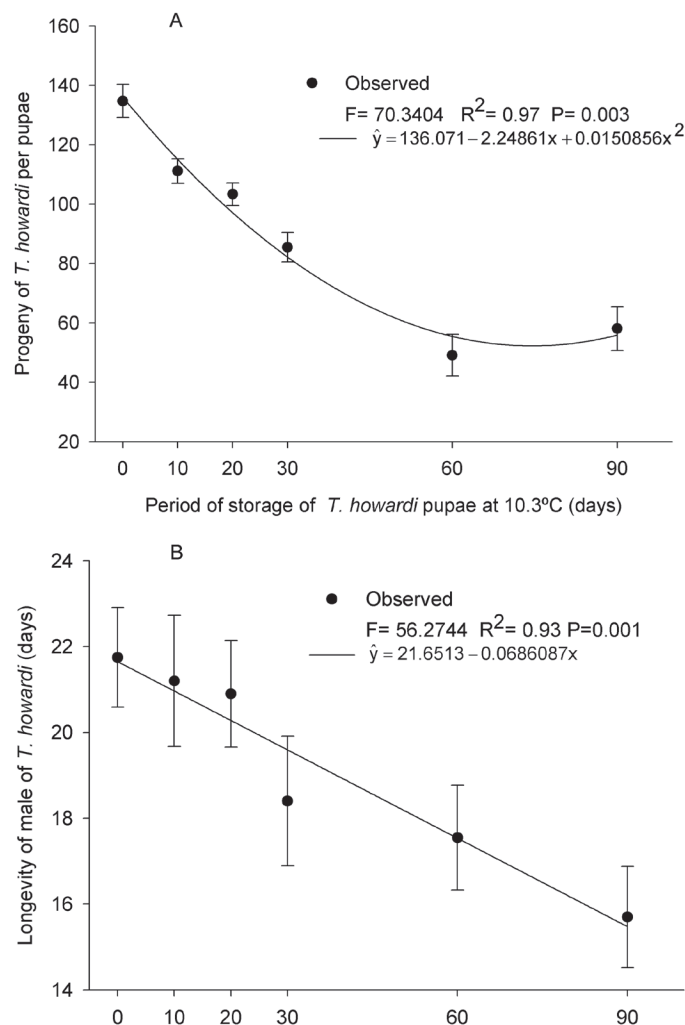


Fig. 1. Percentage of *Tenebrio molitor* pupae parasitized by female (A) and progeny (B) of *Tetrastichus howardi* after storage for different periods while immature at 10.3 ± 0.48 °C, $54.20 \pm 2.52\%$ RH, and photoperiod of 14:10 h (L:D).

Table 1. Period of storage (Ar), parasitism (Par), emergence (Emerg), progeny (Prog), sexual ratio (RS), egg to adult period (Per), and longevity (Long) of males and females (d) of *Tetrastichus howardi* (Hymenoptera: Eulophidae) grown with *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae stored for different periods at low temperatures and relative units (°C, RU).

| °C, RU | Ar | Par (%) | Emerg (%) | Prog (%) | RS (%) | Per (egg to adult) | Long ♂ | Long ♀ |
|-----------------------------|-----|---------------|-------------------------|-------------------------|------------------------|------------------------|-------------------------|-------------------------|
| -28.2 ± 0.67 / 53.8 ± 2.1 * | — | — | — | — | — | — | — | — |
| 0.5 ± 0.09/55.6 ± 1.8 | 10 | 72.0 ± 6.7 b | 92.5 ± 5.3 a | 68.6 ± 5.3 c | 0.8 ± 0.05 a | 22.5 ± 0.5 a | 22.8 ± 1.2 a | 16.2 ± 1.0 b |
| 0.5 ± 0.09/55.6 ± 1.8 | 20 | 82.0 ± 6.2 b | 80.6 ± 2.5 a | 96.9 ± 5.8 b | 0.8 ± 0.02 a | 21.8 ± 0.3 a | 15.8 ± 1.1 b | 19.2 ± 1.3 a |
| 0.5 ± 0.09/55.6 ± 1.8 | 30 | — | — | — | — | — | — | — |
| 0.5 ± 0.09/55.6 ± 1.8 | 60 | — | — | — | — | — | — | — |
| 0.5 ± 0.09/55.6 ± 1.8 | 90 | — | — | — | — | — | — | — |
| 2.7 ± 0.1/52.0 ± 1.7 | 10 | 68.0 ± 7.4 b | 83.3 ± 7.0 a | 95.6 ± 7.0 b | 0.9 ± 0.01 a | 20.2 ± 1.0 a | 15.0 ± 1.3 b | 15.0 ± 1.3 b |
| 2.7 ± 0.1/52.0 ± 1.7 | 20 | 68.0 ± 7.4 b | 97.5 ± 2.5 a | 80.1 ± 2.5 c | 0.9 ± 0.01 a | 23.6 ± 0.4 a | 15.8 ± 1.3 b | 15.8 ± 1.3 b |
| 2.7 ± 0.1/52.0 ± 1.7 | 30 | 38.0 ± 7.5 c | 80.0 ± 13.3 a | 56.6 ± 12.7 c | 0.7 ± 0.10 b | 19.7 ± 3.3 a | 17.6 ± 1.3 b | 17.6 ± 1.3 b |
| 2.7 ± 0.1/52.0 ± 1.7 | 60 | 40.0 ± 5.9 c | 54.3 ± 12.1 b | 64.7 ± 12.1 c | 0.8 ± 0.09 a | 19.9 ± 2.2 a | 17.8 ± 1.6 b | 17.8 ± 1.6 b |
| 2.7 ± 0.1/52.0 ± 1.7 | 90 | — | — | — | — | — | — | — |
| 10.5 ± 0.5/56.7 ± 2.6 | 10 | — | — | — | — | — | — | — |
| 10.5 ± 0.5/56.7 ± 2.6 | 20 | 54.0 ± 9.4 c | 79.9 ± 7.9 a | 60.1 ± 7.8 c | 0.7 ± 0.0 b | 25.5 ± 1.8 a | 19.5 ± 1.3 a | 21.4 ± 1.3 a |
| 10.5 ± 0.5/56.7 ± 2.6 | 30 | 66.0 ± 7.3 b | 79.9 ± 7.6 a | 47.5 ± 7.6 c | 0.7 ± 0.0 b | 23.4 ± 0.5 a | 19.9 ± 1.0 a | 19.7 ± 0.9 a |
| 10.5 ± 0.5/56.7 ± 2.6 | 60 | — | — | — | — | — | — | — |
| 10.5 ± 0.5/56.7 ± 2.6 | 90 | — | — | — | — | — | — | — |
| 14.8 ± 0.2/62.5 ± 3.2 | 10 | 58.0 ± 8.6 c | 83.3 ± 8.6 a | 120.6 ± 8.6 a | 0.9 ± 0.0 a | 22.7 ± 0.5 a | 18.6 ± 0.8 b | 20.2 ± 1.4 a |
| 14.8 ± 0.2/62.5 ± 3.2 | 20 | — | — | — | — | — | — | — |
| 14.8 ± 0.2/62.5 ± 3.2 | 30 | — | — | — | — | — | — | — |
| 14.8 ± 0.2/62.5 ± 3.2 | 60 | — | — | — | — | — | — | — |
| 14.8 ± 0.2/62.5 ± 3.2 | 90 | — | — | — | — | — | — | — |
| 25 | (0) | 100.0 ± 0.0 a | 100.0 ± 0.0 a | 134.7 ± 0.0 a | 0.9 ± 0.0 a | 21.5 ± 0.3 a | 21.7 ± 1.2 a | 20.4 ± 1.3 a |
| | | | F = 6.80 e P < 0.001 | F = 9.75 e P < 0.001 | F = 2.15 e P = 0.03 | F = 1.54 e P = 0.14 | F = 4.49 e P > 0.001 | F = 2.86 e P = 0.03P |

Averages per column followed by the same letter do not differ by a 5% probability by the Scott-Knott Test (ANOVA).

*On 10, 20, 30, 60, 90 d there was no emergence. There was no development of *T. howardi*.

EP = means ± standard error.

charalis pupae (Favero et al. 2015). Cold storage reduced reproduction of the parasitoids *Eretmocerus corni* Haldean and *Encarsia formosa* Gahan (both Hymenoptera: Aphelinidae) (López & Botto 2005) when stored as immature in their hosts (Chen et al. 2008a; Bourdais et al. 2012).

The similar emergence and sex ratio of *T. howardi* between treatments showed high values because stress can alter mating behavior and reproductive success (Shreve et al. 2004). Female *Anaglyphus ananatis* Gahan (Hymenoptera: Encyrtidae) exposed to low temperatures when immature produced only male offspring, with the effect of the cold making males incapable of copulating or producing viable spermatozooids (Pandey & Johnson 2005).

The greater *T. howardi* progeny in the control, after 10 and 20 d of storage, shows that it is possible to store this parasitoid in the cold, but the lower temperatures and the longer exposure period reduce the number and quality of the offspring. This also was confirmed with *Trissolcus basalis* Wollaston and *Telenomus podisi* Ashmead (both Hymenoptera: Scelionidae) pupae at 12 °C and 15 °C for 120 to 210 d, and with different parasitism periods at 18 °C without emergence of these parasitoids at 12 °C. The *T. basalis* and *T. podisi* emergence transferred at 15 °C at the beginning of the pupa stage was 1.5% and 26.3%, respectively, whereas those transferred 1 d before the expected adult emergence date at 18 °C presented indices of 86.4% in *T. basalis* and 59.9% in *T. podisi*. Similarly, the adult longevity of these parasitoids was lower at 15 °C at the beginning of the pupal stage (Foerster et al. 2004).

The inversely proportional *T. howardi* female and male longevity emerged from *T. molitor* pupae with cold storage periods confirms that exposing immatures to low temperatures may decrease adult parasitoid longevity, as reported for *Anaglyphus ananatis* Gahan (Hymenoptera: Encyrtidae) (Rundle et al. 2004; Pandey & Johnson 2005; Colinet &

Boivin 2011). *Trissolcus basalis* and *T. podisi* adults also were stored at 15 or 18 °C after their immature development had been completed at 18 or 25 °C. The longevity and fertility of these parasitoids, at different storage temperatures, were evaluated after returning them to 25 °C from different reproductive diapause periods. The temperature during immature development influenced female longevity being higher for those that reached adulthood at 25 °C and were then stored at 15 °C (13 mo for *T. basalis* and 10 mo for *T. podisi*).

The egg to adult period, parasitism and emergence percentages, number of parasitoids emerged per pupal host, sex ratio and longevity of adult *T. howardi* descendants in *T. molitor* pupae after storage of this host, and the descendants of the parasitoid as immature (pupae) were compromised. Exposure to low temperatures can affect physiological activity (Hance et al. 2007), reducing the body parasitoid mass and, consequently, their longevity as reported for *Trichogramma nerudai* Pintureau and Gerding (Hymenoptera: Trichogrammatidae) (Tezze & Botto 2004). This is due to the need for resources during long cold storage periods (Chen et al. 2013). In addition, absence of feeding and water loss during storage also may reduce parasitoid longevity (Chen et al. 2011).

Tetrastichus howardi biological characteristics were better with *T. molitor* pupae stored at 0.5 ± 0.09 °C and 2.7 ± 0.11 °C for 10 and 20 d. *Tetrastichus howardi* immatures (pupae) can be stored in *T. molitor* pupae for 10, 20, 30, 60, and 90 d at 10.3 °C and their emerged adults used in biological control programs, preferably pupae of this after 10 d of storage without deleterious effects for the population of this parasitoid.

These results contribute to overcoming one of the difficulties encountered in the mass production of parasitoids, which is to obtain large numbers of suitable hosts when they are needed. Therefore, the

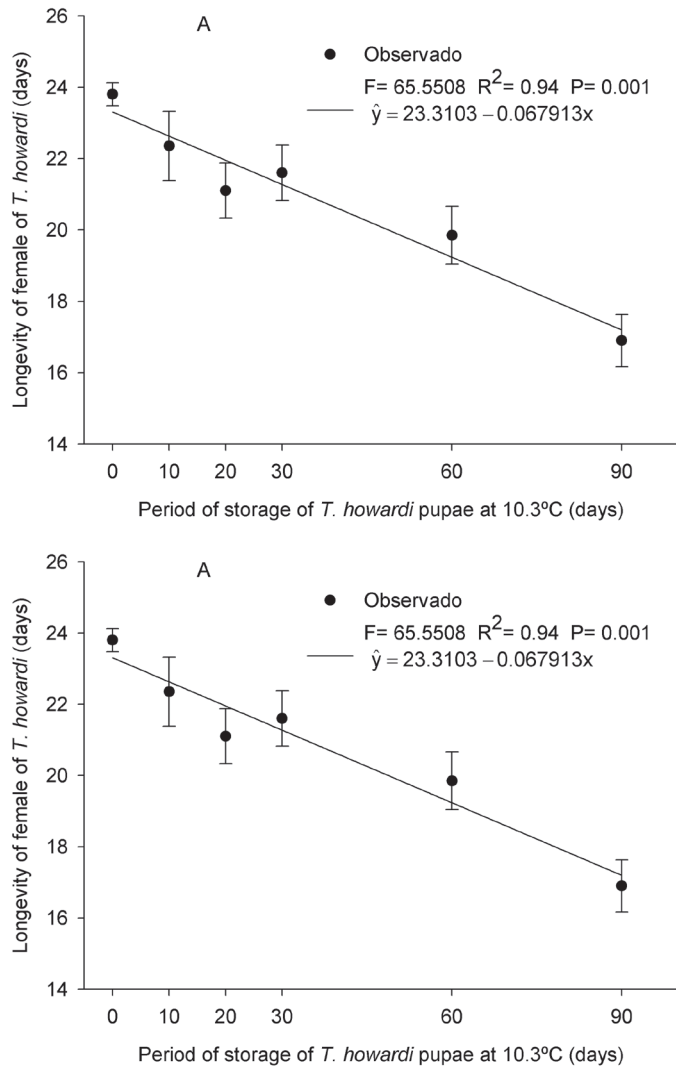


Fig. 2. Longevity of females (A) and males (B) of *Tetrastichus howardi* emerged from pupae of *Tenebrio molitor* after storage for different periods while immature at 10.3 ± 0.48 °C, $54.20 \pm 2.52\%$ RH, and photoperiod of 14:10 h (L:D).

possibility of conserving *T. molitor* pupae to rear *T. howardi* will be useful to use this natural enemy in biological pest control programs.

Acknowledgments

The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela concessão de bolsa de estudo e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT) e “Programa Cooperativo sobre Proteção Florestal/PROTEF do Instituto de Pesquisas e Estudos Florestais/IPEF” for financial support. Phillip John Villani (University of Melbourne, Australia) revised and corrected the English language used in this manuscript.

References Cited

Barbosa RH, Zanuncio JC, Pereira FF, Kassab SO, Rossoni C. 2016. Foraging activity of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) at various densities

- on pupae of the eucalyptus defoliator *Thyrintea arnobia* (Lepidoptera: Geometridae). *Florida Entomologist* 99: 121–127.
- Bourdais D, Vernon P, Krespi L, Baaren JV. 2012. Behavioural consequences of cold exposure on males and females of *Aphidius rhopalosiphii* De Stephani Perez (Hymenoptera: Braconidae). *Biological Control* 57: 349–360.
- Chen H, Opiit GP, Sheng P, Zhang H. 2011. Maternal and progeny quality of *Habrobracon hebetor* Say (Hymenoptera: Braconidae) after cold storage. *Biological Control* 58: 255–261.
- Chen H, Zhang H, Zhu KY, Throne J. 2013. Performance of diapausing parasitoid wasps *Habrobracon hebetor* after cold storage. *Biological Control* 64: 186–194.
- Chen W-L, Leopold RA, Boetel MA. 2008a. Cold storage of adult *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae) and effects on maternal and progeny fitness. *Journal of Economic Entomology* 101: 1760–1770.
- Chen W-L, Leopold R, Harris MO. 2008b. Cold storage effects on maternal and progeny quality of *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae). *Biological Control* 46: 122–132.
- Colinet H, Boivin G. 2011. Insect parasitoids cold storage: a comprehensive review of factors of variability and consequences. *Biological Control* 58: 83–95.
- Cruz I, Redoan AC, Silva RB, Figueiredo MLC, Pentead-Dias AM. 2011. New record of *Tetrastichus howardi* (Olliff) as a parasitoid of *Diatraea saccharalis* (Fabr.) on maize. *Scientia Agrícola* 68: 252–254.
- Favero K, Pereira FF, Kassab SO, Costa DP, Zanuncio JC. 2014. Life and fertility tables of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) with *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae. *Annals of the Entomological Society of America* 107: 621–626.
- Favero K, Pereira FF, Torres JB, Oliveira HN, Kassab SO, Zanuncio JC. 2015. Reproduction of *Tetrastichus howardi* (Hymenoptera: Eulophidae) in *Diatraea saccharalis* (Lepidoptera: Crambidae) pupae at different temperatures. *Florida Entomologist* 98: 865–869.
- Ferreira NGP, Pereira FF, Borges FLG, Rossoni C, Silva AS, Kassab SO. 2016. Multiplicar *Tetrastichus howardi* (Hymenoptera: Eulophidae) en la oruga de la seda afecta su biología? *Acta Biologica Colombiana* 21: 189–193.
- Fields PG. 1992. The control of stored product insects and mites with extreme temperatures. *Journal of Stored Products Research* 28: 89–118.
- Foerster LA, Doetzer AK, De Castro LCF. 2004. Emergence longevity and fecundity of *Trissolcus basalis* and *Telenomus podisi* after cold storage in the pupal stage. *Pesquisa Agropecuária Brasileira* 39: 841–845.
- Hance T, Van Baaren J, Vernon P, Boivin G. 2007. Impact of extreme temperatures on parasitoids in a climate change perspective. *Annual Review of Entomology* 52: 107–126.
- La Salle J, Polaszek A. 2007. Afrotropical species of the *Tetrastichus howardi* species group (Hymenoptera: Eulophidae). *African Entomology* 15: 45–56.
- López SN, Botto E. 2005. Effect of cold storage on some biological parameters of *Eretmocerus corni* and *Encarsia formosa* (Hymenoptera: Aphelinidae). *Biological Control* 33: 123–130.
- Lv J, Wilson LT, Beuzelin JM, White WH, Reagan TE, Way MO. 2011. Impact of *Cotesia flavipes* (Hymenoptera: Braconidae) as an augmentative biocontrol agent for the sugarcane borer (Lepidoptera: Crambidae) on rice. *Biological Control* 56: 159–169.
- Lysyk TJ. 2004. Effects of cold storage on development and survival of three species of parasitoids (Hymenoptera: Pteromalidae) of house fly, *Musca domestica* L. *Environmental Entomology* 33: 823–831.
- Mainali BP, Lim UT. 2013. Quality assessment of *Riptortus pedestris* (Hemiptera: Alydidae) eggs cold-stored at different temperature and RH regime. *Biological Control* 64: 132–137.
- Oliveira FG. 2013. Multiplicação de *Tetrastichus howardi* (Hymenoptera: Eulophidae) em pupas de *Tenebrio molitor* (Coleoptera: Tenebrionidae) e de *Diatraea saccharalis* (Lepidoptera: Crambidae). 63f. Dissertação (Mestrado em Entomologia e Conservação da Biodiversidade) – Universidade Federal da Grande Dourados, Dourados, Mato Grosso do Sul, Brazil.
- Pandey RR, Johnson MW. 2005. Effects of cool storage on *Anagyrus ananatis* Gahan (Hymenoptera: Encyrtidae). *Biological Control* 35: 9–16.
- Parra JRP. 2007. Técnicas de Criação de Insetos para Programa de Controle Biológico. 6ª ed. ESALQ/FEALQ. Piracicaba, São Paulo, Brazil.
- Pastori PL, Zanuncio JC, Pereira FF, Pratisoli D, Cecon PR, Serrão JE. 2013. Temperatura e tempo de refrigeração de pupas de *Anticarsia gemmatilis* (Lepidoptera: Noctuidae) afetam parâmetros biológicos de *Trichospilus diatraeae* (Hymenoptera: Eulophidae). *Semina: Ciências Agrárias* 34: 1493–1508.
- Pereira FF, Kassab SO, Vargas EL, Calado VRF, Oliveira HN, Zanuncio JC. 2015. Parasitism and emergence of *Tetrastichus howardi* (Hymenoptera: Eulophidae) on *Diatraea saccharalis* (Lepidoptera: Crambidae) larvae, pupae and adults. *Florida Entomologist* 98: 384–387.
- Pereira FF, Zanuncio JC, Kassab SO, Pastori PL, Barbosa RH, Rossoni C. 2013. Biological characteristics of *Palmistichus elaeisis* Delvare & LaSalle (Hymenoptera: Eulophidae) on refrigerated pupae of *Anticarsia gemmatilis* Hüb-

- ner (Lepidoptera: Noctuidae). Chilean Journal of Agricultural Research 73: 117–121.
- Pereira FF, Zanuncio JC, Serrão JE, Pastori PL, Ramalho FS. 2009b. Reproductive performance of *Palmistichus elaeisis* Delvare and LaSalle (Hymenoptera: Eulophidae) with previously refrigerated pupae of *Bombyx mori* L. (Lepidoptera: Bombycidae). Brazilian Journal of Biology 69: 865–869.
- Pereira FF, Zanuncio JC, Serrão JE, Oliveira HN, de Fávoro K, Grance ELV. 2009a. Progenie de *Palmistichus elaeisis* Delvare & LaSalle (Hymenoptera: Eulophidae) parasitando pupas de *Bombyx mori* L. (Lepidoptera: Bombycidae) de diferentes idades. Neotropical Entomology 38: 660–664.
- Prasad KS, Aruna AS, Kumar V, Kariappa BK. 2007. Feasibility of mass production of *Tetrastichus howardi* (Olliff) a parasitoid of leaf roller (*Diaphania pulverulentalis*) on *Musca domestica* (L.). Indian Journal of Sericulture 46: 89–91.
- Pratissoli D, Zanuncio JC, Vianna UR, Andrade JS, Pinon TBM, Andrade GS. 2005. Thermal requirements *Trichogramma pretiosum* and *T. acacioi* (Hym.: Trichogrammatidae), parasitoids of the avocado defoliator *Nipteria panacea* (Lep.: Geometridae), in eggs of two alternative hosts. Brazilian Archives of Biology and Technology 48: 523–529.
- Rundle BJ, Thomson LJ, Hoffmann AA. 2004. Effects of cold storage on field and laboratory performance of *Trichogramma carverae* (Hymenoptera: Trichogrammatidae) and the response of three *Trichogramma* spp. (*T. carverae*, *T. nr brassicae* and *T. funiculatum*) to cold. Journal of Economic Entomology 97: 213–221.
- Shreve SM, Kelty JD, Lee Jr RE. 2004. Preservation of reproductive behaviors during modest cooling: rapid cold hardening fine-tunes organismal response. Journal of Experimental Biology 207: 1797–1802.
- Tezze AA, Botto EN. 2004. Effect of cold storage on the quality of *Trichogramma nerudai* (Hymenoptera: Trichogrammatidae). Biological Control 30: 11–16.
- Uçkan F, Gulel A. 2002. Age-related fecundity and sex ratio variation in *Apanteles galleriae* (Braconidae) and host effect on fecundity and sex ratio of its hyperparasitoid *Dibrachys boarmiae* (Hym. Pteromalidae). Journal of Applied Entomology 126: 534–537.
- Vargas EL, Pereira FF, Glaeser DF, Calado VRF, Oliveira FG, Pastori PL. 2013. Searching and parasitism of *Diatraea saccharalis* (Lepidoptera: Crambidae) by *Trichospilus diatraeae* (Hymenoptera: Eulophidae). Acta Biológica Colombiana 18: 259–264.
- Zamperline B, Zanuncio JC. 1992. Influência da alimentação de *Tenebrio molitor* L. 1758 (Coleoptera: Tenebrionidae) no desenvolvimento ninfal de *Podisus connexivus* Bergroth. 1891 (Hemiptera: Pentatomidae). Revista Árvore 16: 224–230.