

## ORIGINAL CONTRIBUTION

**Biology of *Doryctobracon brasiliensis* at different temperatures: development of life table and determining thermal requirements**S. Poncio<sup>1</sup>, A. Medeiros Nunes<sup>2</sup>, R. Silva Gonçalves<sup>1</sup>, H. Lisboa<sup>1</sup>, R. Manica-Berto<sup>3</sup>, M. Silveira Garcia<sup>1</sup> & D. E. Nava<sup>3</sup><sup>1</sup> Programa de Pós-Graduação em Fitossanidade, Universidade Federal de Pelotas, Pelotas, RS, Brazil<sup>2</sup> Programa de Pós-Graduação em Entomologia, Universidade Federal de Pelotas, Pelotas, RS, Brazil<sup>3</sup> Laboratório de Entomologia, Embrapa Clima Temperado, Pelotas, RS, Brazil**Keywords***Anastrepha fraterculus*, biological control, braconidae, fruit production, native parasitoids**Correspondence**

Dori Edson Nava (corresponding author), Laboratório de Entomologia, Embrapa Clima Temperado, CEP 96010-971, Pelotas, RS, Brazil.

E-mail: dori.edson-nava@embrapa.br

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**Abstract**

*Doryctobracon brasiliensis* (Szépligeti) is a parasitoid larval–pupal of fruit flies and has great potential to be used in biological control programmes as it feeds on other *Anastrepha* species in addition to *Anastrepha fraterculus* (Wiedemann). This study investigated the biology of *D. brasiliensis* at different temperatures to design a life fertility table and determine thermal requirements. The parasitoids were multiplied in larvae of *A. fraterculus* in air-conditioned chambers at 15, 18, 20, 22, 25, 28 and 30°C, 70 ± 20% RH and photophase of 12 h. We determined the number of offspring, sex ratio, longevity of males and females and duration of egg–adult period. The temperature range 18–22°C ensures higher fecundity and at 20°C, and the average number of offspring per female was 152.77 parasitoids. The sex ratio of offspring produced was reduced with increasing temperatures. Longevity of males and females of *D. brasiliensis* was reduced by increasing temperatures. At 15, 28 and 30°C, there was no development of immature stages. For the temperature range 18–25°C, the duration of egg–adult period of *D. brasiliensis* was inversely proportional to temperature. At 20 and 22°C, we observed the highest values of net reproduction rate ( $R_0$ ) and finite reason of increase ( $\lambda$ ), meaning that at the estimated optimum temperature (21°C), the population of *D. brasiliensis* increased 47 times each generation. The lower temperature threshold for development was 10.01°C and the thermal constant (K) 303.21 degree/days. This information confirms that *D. brasiliensis* is better suited to temperate environments, which implies a significant potential for the use of *D. brasiliensis* in the control of *A. fraterculus*, because most areas occupied by this pest are in temperate regions. In addition, *D. brasiliensis* is useful in mass rearing systems in laboratory.

**Introduction**

*Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) is a native species of fruit flies of great economic importance in South America with distribution between two latitudinal extremes occurring in quite distinct environments (Malavasi et al. 2000). In Brazil, *A. fraterculus* is considered the main pest of tem-

perate fruit trees and accounts for almost all of species in orchards in southern Brazil (Salles 1995; Kovaleski 1997; Nunes et al. 2011).

Currently, control measures use organophosphate and pyrethroid insecticides applied on cover (total area) or as toxic baits (Harter et al. 2010; Nava and Botton 2010). However, insecticides kill predators, parasitoids and pollinators and contaminate the soil

and groundwater, besides posing a risk to health of agricultural workers and food quality (Nava and Botton 2010). Therefore, the biological control of fruit flies is a viable management and promising alternative, especially with the use of native parasitoids of families Braconidae and Figitidae through inoculation releases. In Brazil, there are few publications on biological parameters of native parasitoids of fruit flies reared in the laboratory, which makes it difficult to determine the best technique (Garcia and Ricalde 2013).

Among these parasitoids, *Doryctobracon brasiliensis* (Szépligeti) (Hymenoptera: Braconidae) is reported in six Brazilian states (Garcia and Ricalde 2013), in the south-east (Leonel et al. 1995; Aguiar-Menezes and Menezes 1997; Raga et al. 2004) and in the south (Leonel et al. 1995; Salles 1996; Garcia and Corseuil 2004). *D. brasiliensis* occurs in northern Argentina (Ovruski et al. 2000; Ovruski and Schliserman 2003) and parasitism occurs in larvae of different species of fruit flies; however, mostly associated with *A. fraterculus* in different fruit species (Salles 1996; Marinho et al. 2009; Machado et al. 2012).

Temperature is one of the main factors for insect survival and reproduction (Hallman and Denlinger 1998); therefore, determining its effect on the development rate of insects is an important ecological tool to better understand population dynamics of insects (Parra 1997). The knowledge of such relationships is important to determine the occurrence period of insects in nature, serving as a strategy for integrated pest management (IPM) allowing to know thermal requirements and helping to establish models to predict occurrence of insect pests and predators and parasitoids in biofactory (Haddad et al. 1999; Cividanis 2000). This information is important for the establishment of mass rearing of *D. brasiliensis* for biological control programmes applied to *A. fraterculus*. In this sense, this study investigated the biology of *D. brasiliensis* at different temperatures to design a fertility life table and establish thermal requirements.

## Material and Methods

### Insect rearing

The species *A. fraterculus* and *D. brasiliensis* were reared in the Laboratory of Entomology of Embrapa Clima Temperado, in air-conditioned rooms with temperature  $25 \pm 1^\circ\text{C}$ ,  $70 \pm 20\%$  RH and photophase of 12 h. The methodology of

Nunes et al. (2013) was used for the maintenance rearing of *A. fraterculus*. To establish the maintenance rearing of *D. brasiliensis*, fruit of cerejeira-domato (*Eugenia involucrata* D.C – Myrtaceae) was collected in the municipality of Chiapeta, Rio Grande do Sul (RS) State ( $27^\circ55'S$ ,  $53^\circ56'W$ ), and peach fruits (*Prunus persica* L. – Rosaceae) in the municipalities of Rodeio Bonito, RS ( $27^\circ28'S$ ,  $53^\circ10'W$ ), and Pelotas, RS ( $31^\circ37'S$ ,  $53^\circ31'W$ ). In the laboratory, the fruits were packed in plastic trays ( $11 \times 12 \times 19$  cm) containing extra-fine vermiculite to absorb excess moisture and provide environment for pupation. The vermiculite was sifted weekly, and puparia were separated from the substrate and packed in a Gerbox<sup>®</sup> container ( $11 \times 11 \times 3.5$  cm) with extra-fine vermiculite (JProLab, São José dos Pinhais, Paraná, Brasil). After emergence, some adults were used for the maintenance rearing and others were stored in plastic containers (5 ml) containing 70% alcohol. The identification was performed by Dr Valmir Antônio Costa from the Biological Institute of the Agência Paulista de Tecnologia dos Agronegócios in Campinas, São Paulo, Brazil.

The parasitoids used for the laboratory rearing were kept in plastic cages ( $30 \times 50 \times 30$  cm) lined with nylon woven ( $0.5 \times 0.5$  mm). The adult insects were fed with a honey solution 30% in glass containers (5 ml) that was offered to parasitoids through a dental roller with capillarity solution. Three guava fruits (*Psidium guajava* L., Myrtaceae) were cut at the top to remove part of the pulp, and about 200 larvae of *A. fraterculus* of third instar reared on artificial diet according to Nunes et al. (2013) were placed inside the fruits.

The guava fruits containing larvae were arranged at the bottom of the rearing cages for adult females of *D. brasiliensis* to feed on larvae inside the fruit. The fruits remained inside the cage for 24 h. Afterwards, the larvae were removed from the fruit and returned to the artificial diet, where they remained until they reached the larval development. The pupae were transferred to a Gerbox<sup>®</sup> container ( $11 \times 11 \times 3.5$  cm) on a vermiculite layer, where they remained until emergence of parasitoids that formed the next generations and the insects were used in the experiments.

### Biology of adults of *D. brasiliensis*

Newly emerged couples of *D. brasiliensis* were individualized in plastic cages of acrylic cups (500 ml) covered with nylon fabric ( $0.5 \times 0.5$  mm) to allow aeration and parasitism of the larvae of *A. fraterculus*

that were housed in parasitism units inside the cages. Inside the cages, two vials were arranged, one containing 30% of honey solution and another containing distilled water. The insects were kept in air-conditioned chambers at 15, 18, 20, 22, 25, 28 and  $30 \pm 1^\circ\text{C}$ ,  $70 \pm 20\%$  RH and photophase of 12 h.

After forming the couples, 30 larvae of *A. fraterculus* in the third instar were offered every day (2 days before pupation) in parasitism units until the death of females. The parasitism units were composed of larvae placed in one of the parts of an acrylic plate (1.7 cm in diameter  $\times$  0.5 cm height) wrapped in *voile* fabric containing guava pulp. After exposure for 24 h, the larvae were kept in acrylic bottles (5 cm diameter  $\times$  6 cm high) containing extra-fine moistened vermiculite. About 20 days after parasitism, the number of flies and parasitoid emerged was measured. The puparia that remained intact were opened to check for the presence of flies or parasitoids to determine the actual parasitism rate.

We evaluated the number of offspring (ND), daily rate and cumulative rate of parasitism, emergence percentage (*E*), sex ratio (sr) and longevity of males and females. To calculate the ND, we considered the number of emerged and non-emerged parasitoids. To determine the daily parasitism rate, we considered only females that generated offspring at each temperature.

To determine emergence percentage, we used the following equation:  $E = (\text{number of parasitoids emerged} \times 100) / \text{number total of parasitoids}$ . The sex ratio was determined using the equation:  $sr = (\text{number of females}) / (\text{number of females} + \text{number of males})$ .

The experiment was conducted in a completely randomized design in unifactorial scheme. The treatment factor was the temperature with seven levels (15, 18, 20, 22, 25, 28 and  $30^\circ\text{C}$ ) with 15 repetitions and each repetition consisted of a couple of parasitoids.

#### Biology of immature of *D. brasiliensis*

Larvae of *A. fraterculus* of third instar (2 days before pupation) were exposed to parasitism for 24 h. Then, they were transferred to plastic bottles (5 cm diameter  $\times$  6 cm high) containing extra-fine moistened vermiculite for pupation. The bottles were kept in air-conditioned chambers at 15, 18, 20, 22, 25, 28 and  $30 \pm 1^\circ\text{C}$ ,  $70 \pm 20\%$  RH and photophase of 12 h. Observations were carried out daily to determine the emergence date of parasitoids (males and females) and record the egg–adult period.

The experiment was conducted in a completely randomized design in a unifactorial scheme. The treatment factor was the temperature with seven levels, and six repetitions were used with each repetition consisting of 20 larvae of *A. fraterculus*.

#### Fertility life table and thermal requirements

The fertility life table of *D. brasiliensis* was calculated using data from the egg–adult period, fertility, sex ratio, viability (parasitism rate) and longevity. We estimated the gap between generations (T) that represents the average time between oviposition of one generation and oviposition of the next generation, net reproductive rate ( $R_0$ ), which is the estimated average number of females from females generated over the oviposition period that will reach the next generation, intrinsic rate of increase (MRI) that is related to the growth speed of the population and the finite growth rate ( $\lambda$ ) that is the multiplication factor of daily growth of the population. The basis temperature ( $T_b$ ), or lower thermal threshold development, and thermal constant (K) were determined by the hyperbole method from the average duration of egg–adult periods of *D. brasiliensis* at different temperatures.

#### Statistical analyses

Parameters of the fertility life table were estimated through the technique of ‘jackknife’ (Meyer et al. 1986) using the programming ‘Lifetable.sas’ (Maia et al. 2013) in the SAS System. These data, along with the number of offspring and sex ratio, were analysed by parametric methods, once the data corresponded to the normality likelihood in the Shapiro–Wilk test. Homoscedasticity was analysed by the Hartley test (table 1) and residue independence in the graphical analysis. Afterwards, the data were subjected to analysis of variance using the *F* test ( $P \leq 0.05$ ). If statistical significance occurred, temperature effects were evaluated by regression models ( $P \leq 0.05$ ) represented by equations:  $y = y_0 + ax$  or  $y = y_0 + ax + bx^2$ , where  $y$  = variable response;  $y_0$  = response variable corresponding to the minimum point of the curve;  $a$  = maximum estimated value for the response variable;  $b$  = curve slope; and  $x$  = temperature (SAS Institute, 2002). Model selection was based on low residuals, a low P-value, and high  $R^2$  and  $R^2$  adj. To evaluate the variable longevity, survival curves were constructed through the Kaplan–Meier estimator and after that these curves were

**Table 1** Values of tests Shapiro–Wilk (*W*) and Hartley (*H*) for the likelihood of normality of homoscedasticity, respectively, of biological parameters where parametric methods were used

Parametric methods	Normality <sup>1</sup>		Homoscedasticity <sup>2</sup>	
	<i>W</i>	<i>P</i>	<i>F</i> <sub>max</sub>	Tabled value
Net reproduction rate ( <i>R</i> <sub>0</sub> )	0.94	0.3238	4.00	4.7
Intrinsic growth rate ( <i>R</i> <sub>m</sub> )	0.91	0.1035	4.00	
Average interval between generations (IMG)	0.94	0.3111	4.00	
Doubling time (TD)	0.95	0.3974	2.40	
Finite growth ratio ( $\lambda$ )	0.97	0.7654	1.20	
Sex ratio	0.93	0.1324	1.10	
Number of offspring	0.95	0.0700	1.06	

<sup>1</sup>Use of the Shapiro–Wilk (*W*) test.<sup>2</sup>Use of the Hartley (*H*) test.

compared using the log-rank test (Francis et al. 1993).

The duration of the egg–adult period was analysed by the analysis of variance (ANOVA) and averages compared by *t*-test at 5% of significance using the JMP statistical program (version 5.0.1; SAS, Cary, NC,

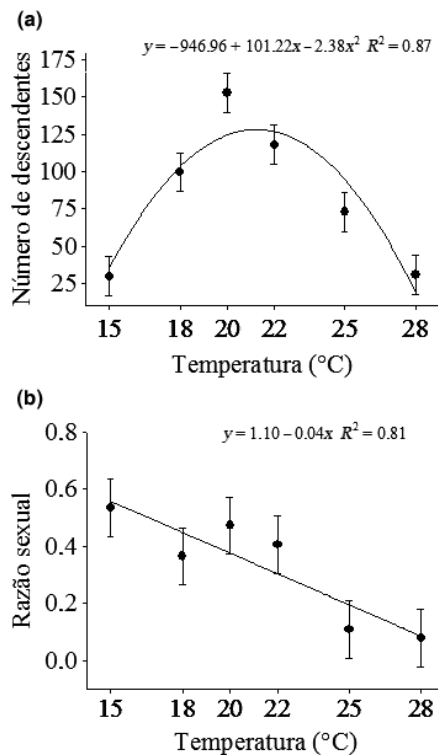
USA). Thermal requirements were determined using the MOBAE software (FEALQ - Fundação de Estudos Agrários Luiz de Queiroz, Piracicaba, São Paulo, Brasil) (Bioestatistic Models Applied to Entomology) (Haddad et al. 1999).

## Results

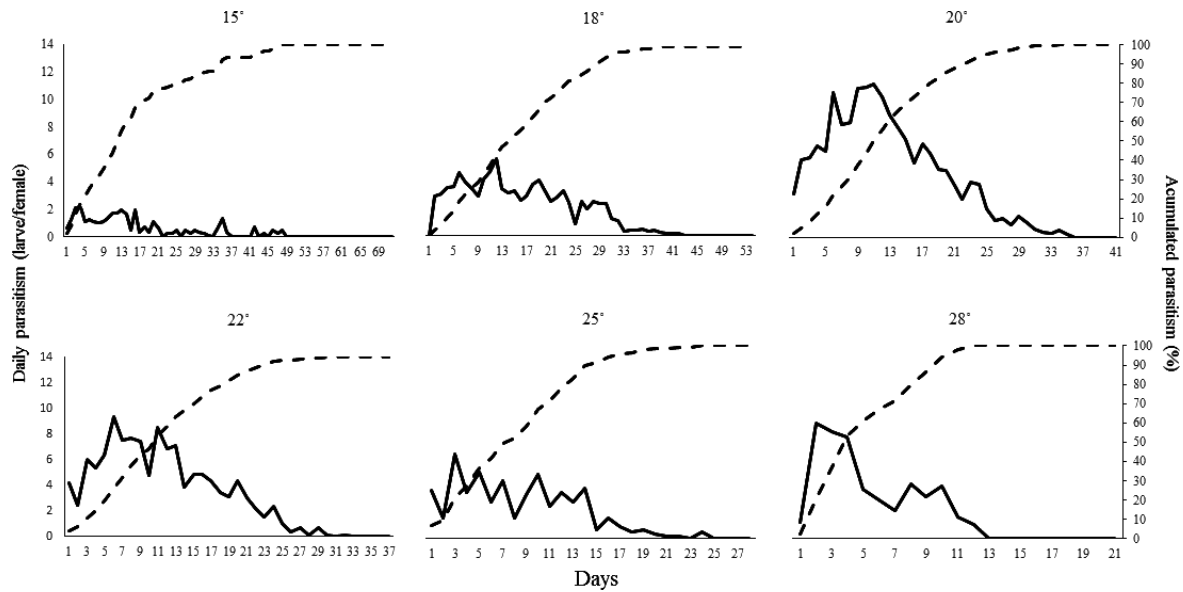
### Biology of adults of *D. brasiliensis*

The data on the total number of offspring produced by females at the studied temperatures adjusted to the quadratic polynomial regression equation ( $F = 10.3010$ ;  $gl = 5$ ;  $P = 0.0453$ ) obtained a determination coefficient ( $R^2$ ) of 0.87. Females kept at 20°C generated the largest number of offspring with an increase of 250% of individuals compared to 15°C (fig. 1a). Between the 6th and 12th day after emergence, the largest number of offspring was generated at 20°C (fig. 2). The maximum number of offspring per female was estimated by the model to 129.25 at 21.26°C (fig. 1a).

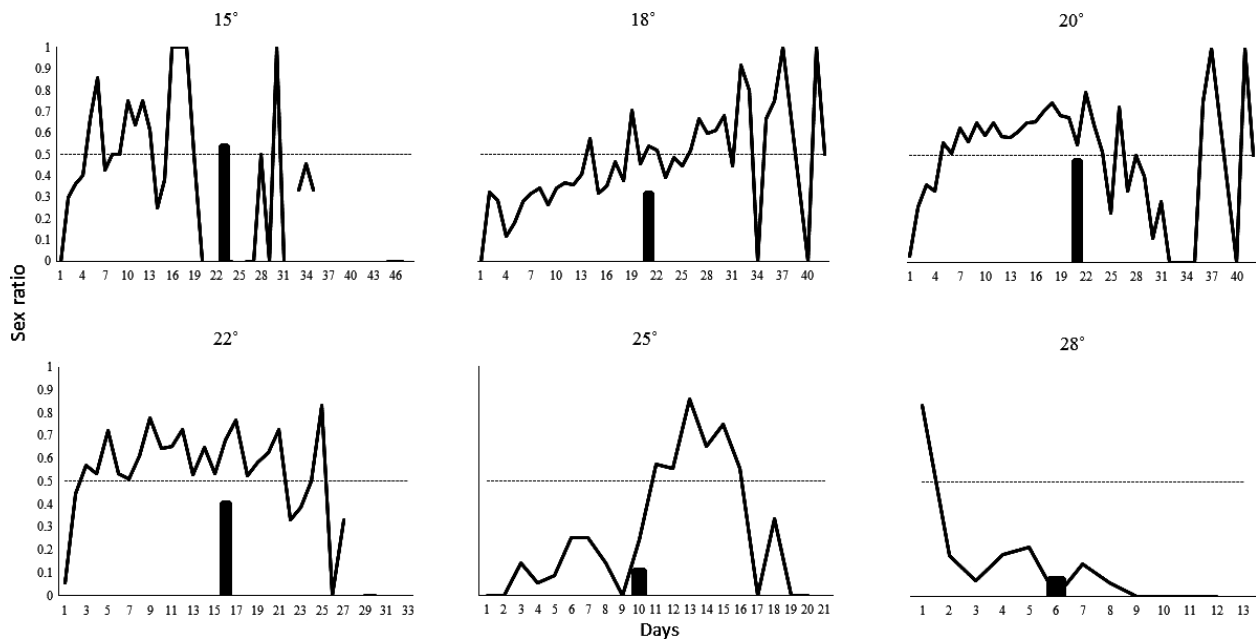
As temperature increased, sex ratio decreased and within the temperature range studied (15–28°C), this response was linear ( $F = 16.9122$ ;  $gl = 5$ ;  $P = 0.0147$ ) with  $R^2$  0.81, showing appropriate adjustment of data to the model (fig. 1b). Females kept at 15, 20 and 22°C had the largest number of female offspring. The greatest sex ratio was recorded at 15°C, and when it was compared at 20 and 22°C, there was an estimated decrease of 40 and 56%, respectively (fig. 1b). However, when we examined the sex ratio over the oviposition period, we observed that in the period between the 4th and 22nd day at 20 and 22°C, the sex ratio was equal to or greater than 0.5, indicating that within this temperature range, considered optimal for



**Fig. 1** Average number of offspring per female (a) and (b) sex ratio of *Doryctobracon brasiliensis* in larvae of *Anastrepha fraterculus* at six different temperatures. RH 70 ± 20% and photophase of 12 h (vertical bars represent the confidence intervals at 95%).



**Fig. 2** Number of offspring generated daily (—) and accumulated (...) per female of *Doryctobracon brasiliensis* in larvae of *Anastrepha fraterculus* at six different temperatures. RH 70 ± 20% and photophase of 12 h.



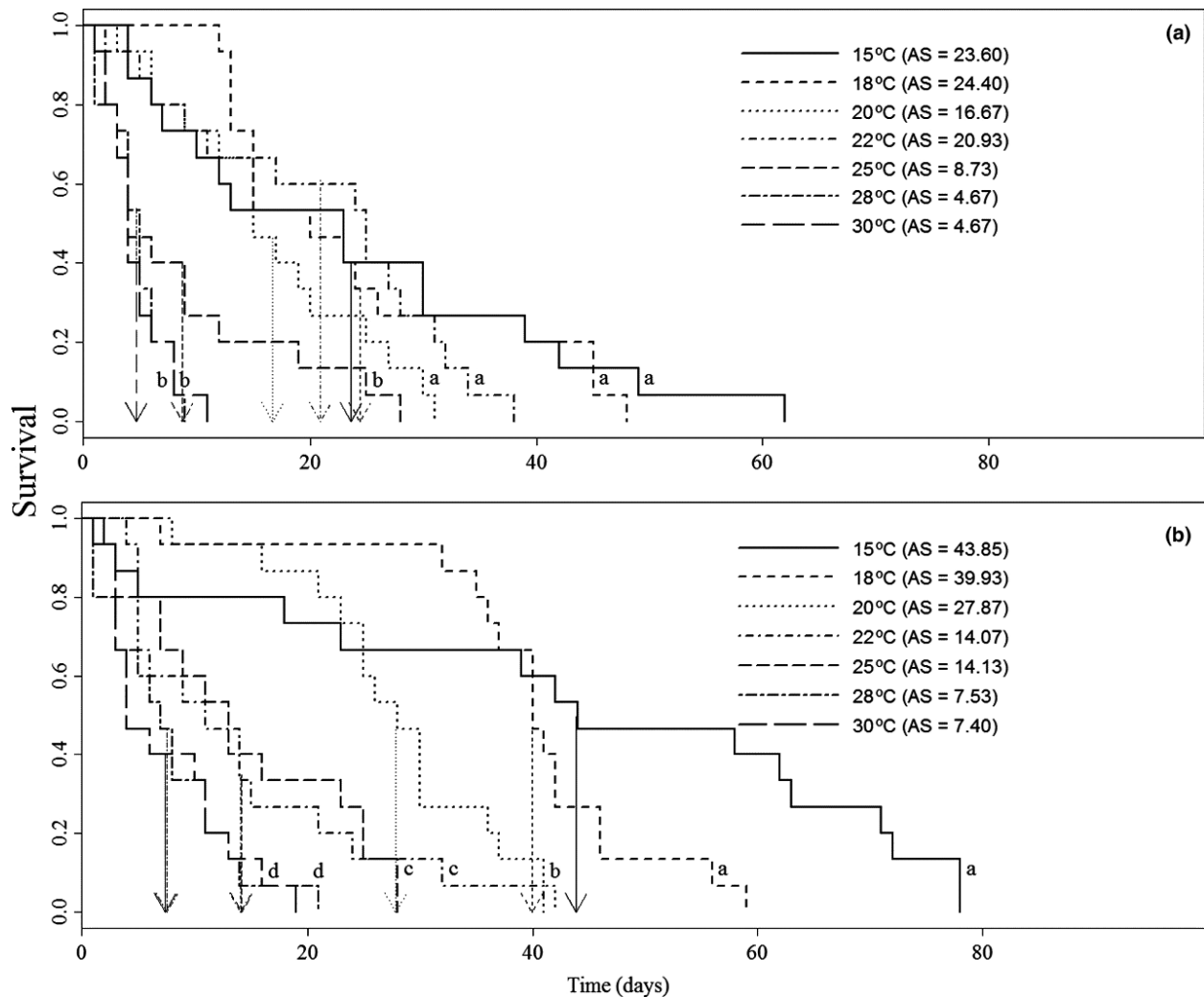
**Fig. 3** Average sex ratio (■) and daily (—) of *Doryctobracon brasiliensis* in larvae of *Anastrepha fraterculus* at six different temperatures. RH 70 ± 20% and photophase of 12 h.

*D. brasiliensis*, the greatest production of females occurred (fig. 3).

Longevity of males ( $\chi^2 = 56.76$ ; gl = 6;  $P < 0.0001$ ) and females ( $\chi^2 = 93.55$ ; gl = 6;  $P < 0.0001$ ) was also significantly affected by temperature (fig. 4). Males had longer longevity within the range 15–20°C, while females showed the highest longevity at the temperature range 15–18°C (fig. 4).

#### Biology of immature of *D. brasiliensis*

The duration of the egg–adult period of *D. brasiliensis* was inversely proportional to temperature, ranging from 20.91 to 41.17 d at temperatures ranging from 25 to 18°C, respectively ( $F = 768.33$ ; gl = 3;  $P < 0.001$ ) (fig. 5). At 15, 28 and 30°C, there was no development of immature.



**Fig. 4** Survival curves of males (a) and females (b) of *Doryctobracon brasiliensis* in larvae of *Anastrepha fraterculus* kept at seven different temperatures. AS = average survival. RH 70 ± 20% and photophase of 12 h. Curves marked with the same letters do not differ significantly from each other.

#### Fertility life table and temperature requirements

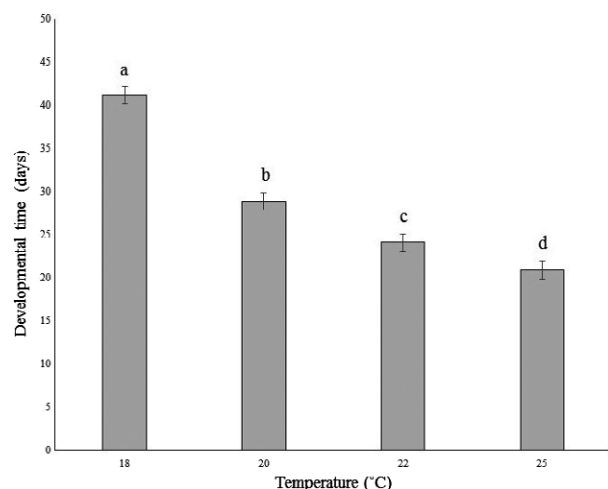
Data on net reproduction rate ( $R_0$ ) of *D. brasiliensis* were adjusted to the quadratic polynomial regression model ( $F = 574.7266$ ;  $gl = 3$ ;  $P = 0.0295$ ;  $R^2 = 0.99$ ) (fig. 6a). The highest reproduction rate occurred at 20 and 22°C, and the greatest  $R_0$  estimated (47.68) at 21°C. This same behaviour also occurred for the intrinsic growth rate ( $r_m$ ). The data were adjusted to the quadratic polynomial regression model ( $F = 1109.1046$ ;  $gl = 3$ ;  $P = 0.0212$ ;  $R^2 = 0.99$ ) (fig. 6b).

Data on average interval between generations (IMG) of *D. brasiliensis* adjusted to the quadratic polynomial model ( $F = 1731.6664$ ;  $gl = 3$ ;  $P = 0.0170$ ;  $R^2 = 0.99$ ) (fig. 6c). Comparison of temperatures showed that females exposed to 20 and 22°C had

lower IMG, 25.33 and 36.2%, respectively, compared to temperature at 18°C. Based on equation, the smallest gap between generations occurred at 22.5°C.

The doubling time between generations (TD) of *D. brasiliensis* presented quadratic behaviour with data adjustment to the model ( $F = 485.4176$ ;  $gl = 3$ ;  $P < 0.0001$ ;  $R^2 = 0.95$ ) (fig. 6d). Females exposed to 18, 20 and 22°C doubled the generation more quickly compared to those kept at 25°C. Comparison of temperatures showed that females exposed to 18, 20 and 22°C decreased TD, compared to those kept at 25°C. Based on equation, the minimum TD was obtained at 19.9°C.

Similar to the previous parameters, data on finite growth ratio ( $\lambda$ ) of *D. brasiliensis* were also adjusted to the quadratic polynomial model ( $F = 253.0979$ ;



**Fig. 5** Duration of egg–adult development of *Doryctobracon brasiliensis* in larvae of *Anastrepha fraterculus* kept at four different temperatures. RH 70 ± 20% and photophase of 12 h. Columns followed by different letters differ in the *t*-test ( $P \leq 0.05$ ).

gl = 3;  $P = 0.0444$ ;  $R^2 = 0.99$ ) (fig. 6e). The highest values occurred at 20 and 22°C, while at 18 and 25°C, the values were reduced. The comparison of temperatures showed that females exposed to 20 and 22°C increased the finite growth ratio ( $\lambda$ ) of 19.8 and 25.6%, respectively, compared to females kept at 18°C. The maximum finite growth ratio ( $\lambda$ ) was obtained at 21.8°C.

Based on the average duration of egg–adult period of *D. brasiliensis* at 18, 20, 22 and 25°C, the lowest temperature limit ( $T_b$ ) was determined at 10.0°C and temperature constant ( $K$ ) at 303.2° per day (fig. 7).

## Discussion

The information obtained in this study shows that temperature influences the biological parameters of *D. brasiliensis* both in pre-imaginal period and in adult life, affecting its development. In general, temperatures 20 and 22°C allowed better parasitoid development and reproduction.

Greater reproductive capacity of *D. brasiliensis* occurred at the beginning of the adult stage of females at all temperatures, except for 30°C, where reproduction did not occur. Temperatures 15, 28 and 30°C were lethal to the young stages of *D. brasiliensis*. The temperature 20°C generated the highest number of offspring, combined with adequate sex ratio and high survival rate. According to Hance et al. (2007), extreme temperatures can decrease longevity, survival and fecundity of parasitoids. The results

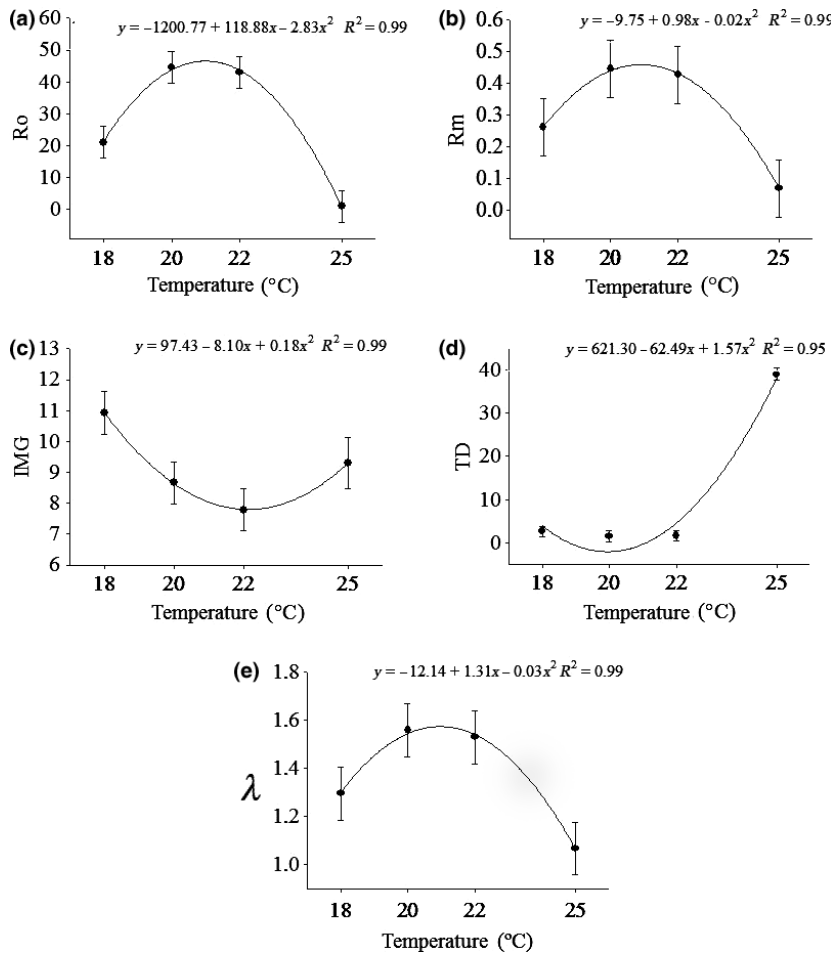
obtained in this study are similar to those reported for other parasitoids of fruit flies, such as *Psytalia cosyrae* (Wilkinson) (Hymenoptera: Braconidae) reared in *Ceratitis cosyra* (Walker) (Diptera: Tephritidae) (Mohamed et al. 2006) *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae) and *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) reared in *Bactrocera invadens* Drew (Diptera: Tephritidae) (Appiah et al. 2013).

The most suitable temperature range to obtain the highest number of offspring of *D. brasiliensis* was 18–22°C. This result is similar to that obtained by Gonçalves et al. (2014), who reported the largest number of offspring for *Aganaspis pelleranoi* (Brèthes) (Hymenoptera: Figitidae) on larvae of *A. fraterculus* within this same temperature range. On the other hand, studies on parasitoids of fruit flies show that temperature range of 20–25°C is best suited for parasitism (Hurtrel et al. 2001; Appiah et al. 2013). *D. longicaudata* reared in larvae of *B. invadens* at 25°C generates the largest number of offspring (21.8) and *F. arisanus* reared on eggs of this host also features as many offspring (97.5) at 25°C (Appiah et al. 2013).

The daily parasitism rate showed oscillations at all temperatures with visible reduction throughout the life of females. However, at 20°C, the highest number of offspring was generated from the 5th to the 12th day. Cancino et al. (2002) observed that oviposition of *D. longicaudata* intensifies from the 5th day onward and extends for a period of 10–15 days. This observation allowed optimizing the mass rearing of *D. longicaudata* on Moscafrut also offering larvae in the period of greater parasitism (Cancino et al. 2006). After the oviposition peak, which occurs in the first days, oviposition capacity of females is reduced dramatically (Cancino et al. 2002), as observed for *D. brasiliensis*.

The accumulated parasitism rate of *D. brasiliensis* at 20°C was 80% at the 18th day. This result is higher than that described for parasitoid *D. longicaudata* that showed 70% accumulated parasitism at 26°C at the 18th day (López et al. 2009). Possibly, *D. brasiliensis* shows the highest parasitism rate in the field of subtropical and temperate climate regions in southern Brazil, where *D. longicaudata* displayed adaptations problems (Sugayama 2000).

The temperature greatly influenced the offspring sex ratio, because females kept at 20°C generated offspring with high sex ratio (>0.5) and stability for longer time (5th–23rd day). According to Cancino et al. (2002), the sex ratio of fruit fly parasitoids can be affected by factors such as host quality, temperature and photoperiod. The sex ratio of parasitoids is a

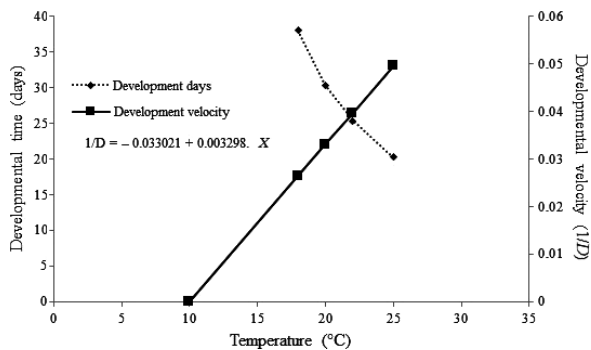


**Fig. 6** Parameters of fertility life table of *Doryctobracon brasiliensis* in larvae of *Anastrepha fraterculus*. (a) Net reproduction rate ( $R_0$ ), (b) intrinsic growth rate ( $R_m$ ), (c) average interval between generations (IMG), (d) doubling time (TD) and (e) finite growth ratio ( $\lambda$ ) at different temperatures. Vertical bars represent confidence intervals at 95%.

limiting factor in mass rearing of parasitoids in biological control programmes, which should be female-biased to ensure higher rate of population growth and because males do not contribute to pest mortality (Heimpel and Lundgren 2000). In studies carried out

on *D. areolatus* at 25°C, the sex ratio was 0.62 (Nunes et al. 2011), showing that, probably, factors related to the method used for *D. brasiliensis* may influence this biological parameter.

Longevity of *D. brasiliensis* was influenced by temperature, and in general, it was lower for parasitoids kept at higher temperatures. The negative effect of high temperature on longevity of braconid parasitoids is widely studied. For example, Mohamed et al. (2006) reported that longevity of *P. cosyrae* was higher at 25°C than at 27 and 30°C for both sexes. Males and females of *F. arisanus* and *D. longicaudata* also had longer longevity at 15°C than at 25, 30 and 35°C (Appiah et al. 2013). For Figitidae *A. pelleranoi*, Gonçalves et al. (2014) observed the same effect where males and females had longer longevity at 18°C than at 25, 28 and 30°C. This possibly occurs because insects kept at high temperature constantly suffer from water loss (Denlinger and Yocum 1998). However, under field conditions, the insect is likely to survive these temperatures because extreme tempera-



**Fig. 7** Speed curve of egg–adult development period of *Doryctobracon brasiliensis* in larvae of *Anastrepha fraterculus* kept at four different temperatures. RH 70 ± 20% and photophase of 12 h.



tures for long periods are rare and temperature variations generally occur during the day. Although insects survived longer at 15 and 18°C, the greater number of offspring was obtained from females kept at 20 and 22°C.

The egg–adult development time of *D. brasiliensis* was inversely proportional within the temperature range 18–25°C. Constant extreme temperatures were harmful to this parasitoid, mostly temperatures of 15, 28 and 30°C. This was possibly attributed to the fact that *D. brasiliensis* was not adapted to these conditions, because this is a species from temperate regions. Similar behaviour was observed for *Diachasmimorpha tryoni* (Cameron) (Hymenoptera: Braconidae), where pre-imaginal development does not occur at 29 and 30°C (Hurtrel et al. 2001). Comparison to other species of *Doryctobracon* shows that the duration of egg–adult period of *D. brasiliensis* was similar to that observed for *Doryctobracon areolatus* (Szépligeti) (Hymenoptera: Braconidae) (25 days at 25°C) reared in larvae of *A. fraterculus* (Nunes et al. 2011). Parasitoids from tropical regions such as *F. arisanus* and *D. longicaudata* resist longer to higher temperatures and complete their development at 30°C (Appiah et al. 2013). However, as the pre-imaginal development of parasitoids of fruit flies occurs inside the fruits, which are protected from excessive temperatures (Fletcher 1987), possibly *D. brasiliensis* may be used in tropical climate regions.

Based on net reproduction rate, the more favourable temperatures for *D. brasiliensis* reproduction were 20 and 22°C. At these temperatures, population increased every generation by 102% compared to 18°C and the finite growth ratio was higher at 20 and 22°C. These temperatures represented the shortest time for the population doubling. Thus, temperatures 20 and 22°C promote greater population growth of the parasitoid as it depends on the number of surviving females and individual production at each time interval. However, the results of this work show that *D. brasiliensis* can expand its population within the temperature range 18–25°C. Thus, this parasitoid seems to be better adapted to temperate and subtropical climate. The results indicate that *D. brasiliensis* shows potential to control *A. fraterculus*, mainly in regions with mild temperatures where this pest is responsible for the major losses of the fruit production. However, further studies should be carried out to discriminate its potential with other parasitoid species.

Although there are no data for temperature requirements of *D. brasiliensis*, we observe that the estimated value is higher than that reported for other parasitoid species of fruit flies. For example,

Hurtrel et al. (2001) studied the development of *D. tryoni* at different temperatures and determined values of Tb at 9.19°C and K at 322.6 degrees/days. For *F. arisanus*, Tb values were 10.1°C and K 359.59° per day, and for *D. longicaudata*, Tb and K values were 10.4°C and 281.6° per day, respectively (Appiah et al. 2013).

The base temperature 10.01°C and temperature constant 303.21° per day for *D. brasiliensis* were slightly lower than the values found by Salles (2000) for *A. fraterculus* (10.7°C and 430.6° per day, respectively), considered the main host of this parasitoids. Possibly, *D. brasiliensis* is able to influence pest population, because this parasitoid displays its optimum temperature for development within the estimated range for *A. fraterculus*. The development speed showed a positive linear relationship with temperature, corroborating Bursell (1974) who stated that low temperatures affect the speed of biological development of insects, extending life cycle.

A precondition for biological control of an insect pest population is related to co-occurrence (density-dependent) of biological control agents with the pests (Zhou et al. 2010). Normally, insect pests occur in the field sooner than their natural enemies do. Thus, for a biological control to effectively prevent economic losses caused by pests, one alternative is to use biological control through mass rearing and augmentative release of the parasitoid (Pu 1978). The results obtained in this study show that the biological control of *A. fraterculus* with the parasitoid *D. brasiliensis* could be viable and that the best temperature conditions for its rearing in the laboratory are near 21°C.

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