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BIOLOGICAL CONTROL

Biology and Thermal Requirements of *Fopius arisanus* (Sonan, 1932) (Hymenoptera: Braconidae) Reared on *Ceratitis capitata* Eggs (Wiedemann) (Diptera: Tephritidae)

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Abstract

Fopius arisanus (Sonan) is a solitary parasitoid of eggs and the first instar larvae of Tephritidae. Due to the occurrence of *Ceratitis capitata* (Wiedemann) in various regions and under several climatic conditions, this study aimed to evaluate the effect of different temperatures on the embryonic development (egg–adult) and determine thermal requirements and the number of annual generations *F. arisanus* on eggs of *C. capitata*. In the laboratory, eggs of *C. capitata* (24 h) were submitted to parasitism of *F. arisanus* during 6 h. Later, the eggs were placed in plastic containers (50 mL) (50 eggs/container) on a layer of artificial diet and packed in chambers at temperatures 15, 18, 20, 22, 25, 28, 30, and 32 ± 1°C, RH 70 ± 10%, and a photophase of 12 h. The largest number of offspring, emergence rate, and weight of adults of *F. arisanus* were observed at 25°C. The highest sex ratios (sr > 0.75) were recorded at 15 and 18°C, being statistically higher than the temperatures 20°C (0.65), 22°C (0.64), 25°C (0.65), 28°C (0.49), and 30°C (0.47). At 32°C, there was no embryonic development of *F. arisanus*. The egg–adult period was inversely proportional to temperature. Based on the development of the biological cycle (egg–adult), the temperature threshold (T_t) was 10.3°C and thermal constant (K) of 488.34 degree-days, being the number of generations/year directly proportional to the temperature increase. The data show the ability of *F. arisanus* to adapt to different thermal conditions, which is important for biological control programs of *C. capitata*.

Introduction

Fopius arisanus (Sonan, 1932) (Hymenoptera: Braconidae) is originally from Indo–Australian regions and is considered the main egg parasitoid of fruit flies species *Bactrocera dorsalis* (Hendel) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Vargas *et al* 2012) with the ability to parasitize on the first instar larvae of *C. capitata* (Manoukis *et al* 2011, Murillo *et al* 2015).

Historically, in 1940, *F. arisanus* was introduced in Hawaii (USA) for control of *B. dorsalis* and *C. capitata* (Clausen *et al* 1965). Later, it was introduced in various regions of Australia, Central America, and the islands of the Indian and Pacific Oceans for control and elimination of fruit flies species of the Tephritidae family (Harris *et al* 2007, Vargas *et al* 2007, Manoukis *et al* 2011). In Brazil, *F. arisanus* was introduced from insects obtained originally from Hawaii in 2012 by Embrapa (Brazilian Agricultural Research Corporation) in the municipality in Jaguariúna, São Paulo State, and by the Ministry of Agriculture, Livestock and Food Supply (MAPA) for biological control of carambola fly *Bactrocera carambolae* (Drew & Hancock, 1994) (Diptera: Tephritidae) in the State of Amapá (Groth *et al* 2016).

Due to its capacity of parasitism and development in eggs of *C. capitata* (Harris *et al* 2007, Vargas *et al* 2007, Manoukis *et al* 2011, Vargas *et al* 2012, Groth *et al* 2016), *F. arisanus* can

be an alternative for the management of *C. capitata*, which is considered one of the main fruit fly species in Brazil (Malavasi *et al* 2000, Nava and Botton 2010, Zucchi 2015), which control is carried out with chemical insecticides (e.g., phosphorous, pyrethroids, and spinosyns) (Nava and Botton 2010). Due to the factors related to its biotic potential, polyphagy, and ability to infest a variety of native and exotic hosts (Aluja and Mangan 2008), the Mediterranean fruit fly has its occurrence reported in almost all Brazilian regions throughout the year (Malavasi *et al* 2000, Zucchi 2015).

The high adaptability of the pest to different environmental conditions can provide to *F. arisanus* unfavorable thermal conditions for distribution, adaptation, survival, and reproduction (Damos and Savopoulou-Soultani 2012, Khaliq *et al* 2014). Therefore, the understanding of the relative importance of temperature and development of *F. arisanus* will help to determine models the occurrence and survival of the natural enemy in the environment (Haddad *et al* 1999). As well as, the knowledge of the thermal requirements of *F. arisanus* will provide information about its ability to develop on the *C. capitata* under different thermal conditions and help to create strategies for mass production programs aimed at biological control. In this sense, the objective of this work was to study the parasitoid potential to develop under different temperatures, determine thermal requirements, and estimate the number of offspring of *F. arisanus* reared on eggs of *C. capitata*.

Material and Methods

Rearing of *C. capitata* and *F. arisanus*

The species of *C. capitata* and *F. arisanus* were obtained from colonies rearing at the Entomology Laboratory of Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil, in controlled room conditions of 25 ± 2°C, 70 ± 10% RH, and a photophase of 12 h.

For rearing and maintenance of *C. capitata*, adults were kept in plastic cages (48 cm long by 30 cm wide and 30 cm tall) and fed with refined sugar, wheat germ, and yeast at ratio 3:1:1, respectively, provided in a plastic Gerbox™ (11.5 cm long by 11.5 cm wide by 3.5 cm tall) (J. Prolab Ltda., São José dos Pinhais, Paraná, Brazil) and distilled water in plastic containers (250 mL) provided via capillarity with a sponge vegetable cloth (Spontex™, Ilheús, Bahia, Brazil) (Nunes *et al* 2013). The eggs were obtained and the aeration and inoculum processes in artificial diet were carried out according to the methodologies proposed by Kamiya (2010) and Gonçalves *et al* (2013). The larval development of *C. capitata* was carried out in artificial diet proposed by Salles (1992) and modified by Nunes *et al* (2013).

After the emergence of *F. arisanus* adults, approximately 400 females and 100 males from the sixth generation in *C. capitata* eggs reared at the laboratory were placed in plastic cages (23 cm long by 27 cm wide and 40 cm tall) according to Gonçalves *et al* (2013). Adults were fed with a honey-based paste (30%) and distilled water supplied via capillarity through a sponge vegetable cloth (Spontex™), which was replaced every 72 h. Four days after parasitoid emergence, eggs of *C. capitata* (apx. 2500 eggs) obtained from the lab colony were deposited on a piece of filter paper (4 cm diameter) with the aid of a LabMate™ micropipette on top of a piece of sponge cloth (Spontex™) and placed in an acrylic plate (4 cm diameter by 0.2 cm tall), being offered for 6 h to females of *F. arisanus* (Groth *et al* 2016). Later, the eggs were removed and placed on a layer of artificial diet for larval development. After 9 days, the larvae were removed from the artificial diet with a sieve (0.22 mm) and running water and placed in plastic containers (200 mL) on a layer of extra-fine vermiculite (3 cm) where pupation occurred. They remained there until the parasitoids emerged to form a new generation (Groth *et al* 2016).

Effect of temperature on the development of immature stages of F. arisanus *and determining thermal requirements*

Approximately 7500 eggs of *C. capitata* (24 h old) obtained from the lab colony were deposited on a piece of filter paper (4 cm wide) on a sponge cloth (Spontex™) (4 cm diameter) and placed in acrylic plates (4 cm wide by 0.2 cm tall) as described previously. Posteriorly, these plates were placed inside cages (1 plate/cage 23 cm × 27 cm × 40 cm) containing adults of *F. arisanus* (300 females and 100 males) in a proportion of approximately 25–30 eggs of *C. capitata* per female (13 days old) according to peak *F. arisanus* oviposition rate (Groth *et al* 2016). After 6 h of exposure, the plates were removed and, with the aid of a fine brush, 50 eggs were randomly removed and inoculated in artificial diet (25 mL) in plastic pots (50 mL) for larval development, according to the diet of Salles (1992), modified by Nunes *et al* (2013). Subsequently, the pots were placed in a climatized room at 15, 18, 20, 22, 25, 28, 30, and 32°C, RH 70 ± 10%, and a photophase of 12 h. After emerging, the first pupae of each treatment were removed from the artificial diet with a sieve (0.2 mm mesh) and running water and transferred to acrylic containers (2.5 cm wide by 4.5 cm tall) containing a layer of extra-fine vermiculite (1 cm) to allow pupation. The pupae were checked daily for the emergence of adults to determine the egg–adult period (days). The intact puparia in which emergence of insects (*C. capitata* or *F. arisanus*) was not observed were dissected with the aid of tweezers to check the actual parasitism rate. The biological parameters

evaluated for each temperature (treatment) were (i) egg–adult period (days); (ii) number of offspring (NO) obtained by the equation: NO = number of parasitoids emerged + number of parasitoids that did not emerge; (iii) emergence rate (E%) determined by the equation: E (%) = (number of parasitoids emerged)/(number of offspring) × 100; (iv) weight of adults at 24 h old, and (v) sex ratio (sr) by the equation: sr = (number of females)/(number of females + number of males).

Statistical analysis

The experiment was conducted in a completely randomized design with eight temperatures (treatments) with 20 replicates per temperature, being each replicate composed of 50 eggs. The data for the egg–adult period (days), NO, and E (%) were submitted to the analysis of variance by the GLM procedure (PROC GLM, SAS® 9.1) (SAS Institute 2002) and the means were compared by the Tukey test at 5% significance (SAS Institute 2002). The values for sex ratio were analyzed by the chi-square test (χ^2) ($P < 0.05$) (SAS Institute 2002). For number of offspring, if statistical significance occurred, temperature effects were evaluated by regression models ($P < 0.05$) represented by equations: $y = bx^2 + ax + y_0$, where y = variable response; y_0 = response variable corresponding to the minimum point of the curve; a = maximum estimated value for the variable response; b = curve slope; and x = temperature (SAS Institute 2002). The thermal requirements of the immature stages of *F. arisanus* were estimated by the hyperbola method (Haddad *et al* 1999), calculating the lower limit for temperature or the temperature threshold (T_t) and the thermal constant (K) (SAS Institute 2002). The number of *F. arisanus* generations in eggs of *C. capitata* per year in laboratory conditions was estimated using the equation NG = {T(Tm – Tb)/K} where: K = thermal constant; Tm = average temperature; Tb = inferior thermal threshold, and T = time in days.

Results

There was no development of *F. arisanus* at constant temperature of 32°C, indicating that this should be the maximum tolerable limit for parasitoid development. The emergence rate of *F. arisanus* was greater at temperatures 22°C (83%) and 25°C (83%), differing significantly ($F_{6, 136} = 88.34$; $P < 0.0007$) at the other temperatures (Table 1). The data on the total number of offspring produced by females at the studied temperatures adjusted to the quadratic polynomial regression equation ($F_{6, 136} = 88.34$; $P < 0.0007$) obtained a determination coefficient ($R^2 = 0.88$). The maximum number of offspring per female estimated by the model was 22.5 at 25°C (Fig 1). However, the number of females of *F. arisanus* in relation to the number of males was statistically higher ($\chi^2 = 263.40$, g. $l = 6$, $P < 0.0001$) at lower temperatures (15 and 18°C) (Table 1). Adult body mass of males ($F_{6, 154} = 54.31$, $P < 0.0001$) and females ($F_{6, 136} = 53.71$, $P < 0.0001$) was higher in intermediate temperatures (20, 22, and 25°C) (Table 1).

Average developmental time (egg–adult) of *F. arisanus* was shortened by temperature increase ($F_{6, 136} = 316.28$, $P < 0.0001$) (Fig 2), showing that 99% of the reduction of the development time was explained by the temperature increase ($R^2 = 0.99$) (Fig 3). According to the average duration of the egg–adult period (days) of *F. arisanus*, the lower thermal limit or temperature threshold was 10.3°C, corresponding to a thermal constant (K) of 488.34 degree-days when reared in *C. capitata* eggs (Fig 3). Based on these values, the number of annual generations of *F. arisanus* was estimated to range from 2.69 to 6.43 within the thermal range 15 to 30°C (Table 2).

Discussion

An important aspect to determine population dynamics of insects is based on the estimation of the optimal

Table 1 Means (± SE) of emergence (%), sex ratio, and weight (mg) of *Fopius arisanus* reared in *Ceratitis capitata* eggs at different temperatures.

Temperature (°C)	Emergence (%)[a]	Sex ratio[b]	Weight (mg)[a]	
			Male	Female
15	36 ± 5.87d	0.78a	1.43 ± 0.09c	1.56 ± 0.62c
18	53 ± 4.09c	0.73a	2.16 ± 0.14b	2.25 ± 0.17b
20	70 ± 3.12b	0.65b	2.66 ± 0.69a	2.70 ± 0.69a
22	85 ± 5.10a	0.64b	2.67 ± 0.63a	2.80 ± 0.35a
25	83 ± 3.12a	0.63b	2.78 ± 0.02a	2.86 ± 0.03a
28	57 ± 5.98c	0.49c	1.98 ± 0.04b	2.05 ± 0.01b
30	21 ± 2.18e	0.47c	1.44 ± 0.04c	1.48 ± 0.04c

[a] Columns followed by different letters differ by the Tukey test ($P \leq 0.05$).

[b] Significant difference by the chi-square test (a female-biased sex ratio).

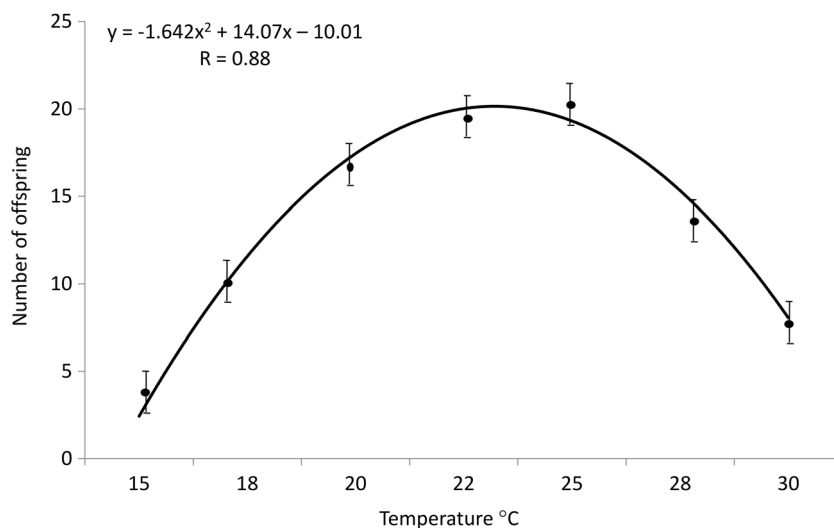$$y = -1.642x^2 + 14.07x - 10.01$$
$$R = 0.88$$

Fig 1 Average number of offspring per female of *Fopius arisanus* reared in eggs of *Ceratitis capitata* at different temperatures. (*Vertical bars* represent the confidence intervals at 95%).

temperature for the development of the host and the natural enemy (Khaliq *et al* 2014). In this study, the biological characteristics of egg–adult development of *F. arisanus* on eggs of *C. capitata* were significantly influenced by temperature. Favorable thermal conditions to obtain the highest number of offspring and emergence of parasitoids were obtained at temperature 25°C. Similar values were observed with the same species in eggs of *Bactrocera invadens* (Drew, Tsuruta & White) (Diptera: Tephritidae) (Appiah *et al* 2013) and *C. capitata* (Ramadan 2004, Zenil *et al* 2004, Kroder and Messing 2010), as well as for parasitoids *Aganaspis pelleranoi* (Brèthes, 1924) (Hymenoptera: Figitidae) (Gonçalves *et al*

2014), *Diachasmimorpha longicaudata* (Ashmead, 1905) (Hymenoptera: Braconidae) (Meirelles *et al* 2013), and *Doryctobracon brasiliensis* (Szépligeti) (Hymenoptera: Braconidae) (Poncio *et al* 2016) in larvae of *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae).

One of the factors that contribute to the increase of parasitism and success of biological control is the presence of a larger number of females in the insect population. In this work, the largest number of females was obtained at temperatures 15 and 18°C. In contrast, there was a reduction (sr < 0.50) at temperatures above 28°C, showing that temperature variation affects the ratio between the sexes
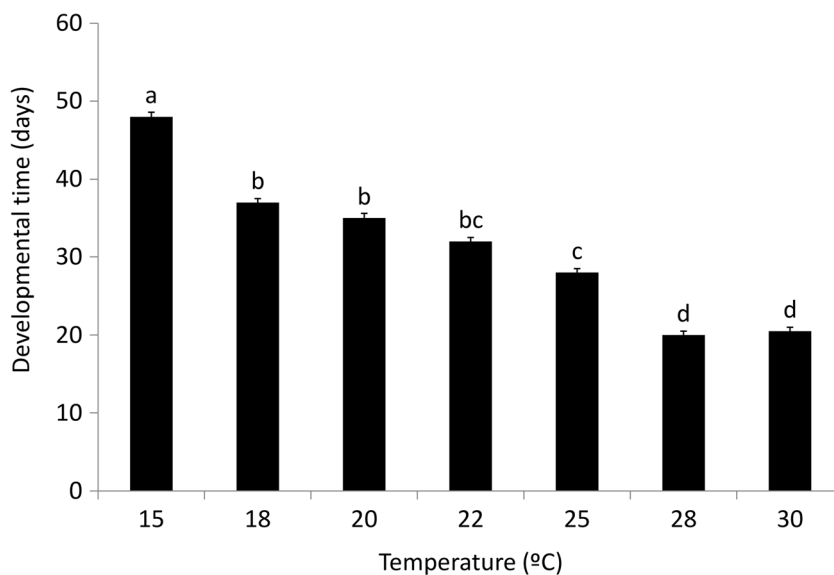

Fig 2 Duration of egg–adult development of *Fopius arisanus* in eggs of *Ceratitis capitata* at different temperatures. Columns followed by *different letters* differ by the Tukey test ($P \leq 0.05$).

$$y = 0.0033x - 0.0348$$
$$R^2 = 0.9999$$
$$T_b = 10.58$$

■ Developmental speed (1/D)
● Developmental time (days)

Fig 3 Curve of development rate of the egg–adult period of *Fopius arisanus* reared in eggs of *Ceratitis capitata* at different temperatures.
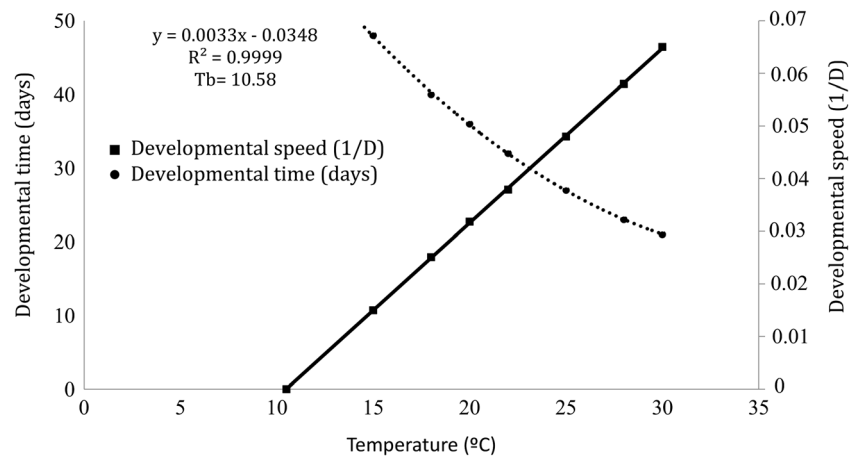
(Damos and Savopoulou-Soultani 2012). According to these authors, the sex ratio does not change in isolation only by the temperature variation, but also by the host quality, since under the same thermal conditions, a larger number of females of *Doryctobracon areolatus* (Szépligeti) (Hymenoptera: Braconidae), *A. pelleranoi*, and *D. brasiliensis* are observed at temperature 25°C in larvae of *A. fraterculus* (Nunes *et al* 2011, Gonçalves *et al* 2014, Poncio *et al* 2016). The sex ratio is a limiting factor in mass rearing of parasitoids and the success in biological control programs. Therefore, the presence of a larger number of females could increase the population growth rate in field conditions, given that males do not contribute to host mortality (Cancino *et al* 2002, Rousse *et al* 2005). However, a large number of females in the population can also provide superparasitism, providing early death of the host and cause negative effect on the population density of the natural enemy (Cancino *et al* 2002).

Table 2 Estimated number of generations of *Fopius arisanus* based on the thermal threshold (Tb) and thermal constant (*K*) reared in eggs of *Ceratitis capitata*.

Temperature (°C)	Number of generations
15	2.69
18	3.83
20	4.43
22	4.95
25	5.60
28	6.13
30	6.43

The adequate temperature range (22–25°C) to the embryonic development of *F. arisanus* observed in this study was equivalent to the optimum temperature for development and survival of *C. capitata* (Ricalde *et al* 2011). Similar developmental times between *F. arisanus* and *C. capitata* under the same thermal conditions (temperatures 22 and 25°C) can provide positive effects on the increase population dynamics of parasitoids in field conditions (Rwomushana *et al* 2008). The co-occurrence between parasitoids and hosts, associated with preference for parasitism of egg and the first larvae of *C. capitata* offers *F. arisanus* greater host foraging resources (Vargas *et al* 2012, Montoya *et al* 2009) offers *F. arisanus* greater foraging in the search for the host, increasing the chances of parasitism and suppression of the pest (Rousse *et al* 2009).

The egg–adult development time of *F. arisanus* was inversely proportional within the temperature range of 18–30°C. Constant extreme temperatures (15–32°C) were harmful to this parasitoid. This was possible due to the fact that *F. arisanus* was not adapted to these conditions. In general, extreme constant temperatures (15 and 32°C) were detrimental to the embryonic development of *F. arisanus*, as observed for *Diachasmimorpha tryoni* (Cameron) (Hymenoptera: Braconidae) (Hurtrel *et al* 2001), *D. longicaudata* (Meirelles *et al* 2013), *A. pelleranoi* (Gonçalves *et al* 2014), and *D. brasiliensis* (Poncio *et al* 2016). However, in the field, the insect can survive under these thermal conditions, as extreme temperatures for extended periods are rare and usually occur during the day (Haddad *et al* 1999). This factor, along with the pre-imaginal development of the parasitoid inside the fruit,

provides insect protection from extreme temperatures and ideal conditions for survival, as observed in hot regions of Hawaii (USA) in *B. dorsalis* and *C. capitata* (Wong *et al* 1992, Bokonon-Ganta *et al* 2007, Appiah *et al* 2013).

Although the number of generations per year of *F. arisanus* was calculated based on thermal requirements (T_t and K) observed in the laboratory. This information allows us to make inferences for different locations and climatic conditions, given that *C. capitata* has its occurrence reported in practically all Brazilian regions (Malavasi *et al* 2000, Zucchi 2015). However, it is possible that in some regions of southern Brazil, *F. arisanus* presents difficulties surviving the winter (June to August) due to the low temperatures and mainly because of the reduction of *C. capitata* in the field during this time of the year (Kovaleski *et al* 2000). The occurrence with the South American fruit fly *A. fraterculus* in southern Brazil (Malavasi *et al* 2000, Nava and Botton 2010, Zucchi 2015), along with the parasitism capacity of *F. arisanus* in species of the genus *Anastrepha* (Bautista *et al* 2004, Zenil *et al* 2004, Montoya *et al* 2009, Groth *et al* 2016), in this host helps meet the needs in the absence of preferred host and facilitates its stay in the field (Groth *et al* 2016). Although more studies are needed to verify the adaptability of the parasitoid to field conditions, the observations in this study provide important information to design strategies for future releases and implementations of biological control programs in *C. capitata* at regional scale.

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