Biology of *Fopius arisanus* (Hymenoptera: Braconidae) in Two Species of Fruit Flies

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Abstract

*Fopius arisanus* (Sonan, 1932) (Hymenoptera: Braconidae) is an egg–larval parasitoid used in control programs of *Bactrocera dorsalis* (Hendel) and *Ceratitis capitata* (Wiedemann). In Brazil, *C. capitata* and *Anastrepha fraterculus* (Wiedemann) are considered the main tephritid pests of exotic and indigenous fruits. The objective of this study was to study the biology of *F. arisanus* in *C. capitata* and *A. fraterculus*. Eggs of the two fruit fly species were used to determine the parasitism rate, number of offspring, emergence rate, sex ratio, adult weight and longevity of male and female *F. arisanus*. These biological parameters were used to develop a fertility life table. We observed higher parasitism and emergence rates of adults, a shorter duration of the egg–adult period and a sex ratio biased to females when *F. arisanus* was reared in eggs of *C. capitata* than in those of *A. fraterculus*. However, adults of *F. arisanus* from eggs of *A. fraterculus* were heavier and had greater longevity than those obtained from *C. capitata* eggs. The fertility life table showed better biological and reproductive performance for *F. arisanus* reared in eggs of *C. capitata*, although eggs of *A. fraterculus* also provided positive values for population increase.

Key words: biological control, fruit fly, parasitoid, parasitism

Fruit flies (Diptera: Tephritidae) are a serious problem in fruit production worldwide because not only do they cause damage to fruit, but they also lead to the establishment of quarantine barriers (Aluja et al. 2008). In Brazil, *Anastrepha fraterculus* (Wiedemann), *A. obliqua* (Macquart), *A. grandis* (Macquart), *Ceratitis capitata* (Wiedemann), and *Bactrocera carambolae* (Drew and Hancock) are all listed as being of quarantine importance by several fruit-importing countries (Malavasi and Nascimento 2003, Uchoa 2012). Among these species of fruit fly, *B. carambolae* is also classified as a quarantine pest in Brazil. Although its occurrence is restricted to states in northern Brazil, it is feared that this pest will migrate to fruit-producing regions, such as Vale do São Francisco, the main production center of fruits for export. Thus, in an aim to establish a set of measures to prevent the spread of *B. carambolae*, in 2012, the parasitoid *Fopius arisanus* (Sonan 1932) (Hymenoptera: Braconidae) was imported from Hawaii (USA) for release in the areas of occurrence of this pest (Paranhos et al. 2013).

*F. arisanus* is a parasitoid that oviposits preferentially in the eggs of many tephritid fly pests (Carmichael et al. 2005), however, first-instar larvae can also be used (Manoukis et al. 2011). Indigenous to the Asian continent, *F. arisanus* is considered one of the main parasitoids of *C. capitata* fruit flies and of the *Bactrocera* fruit fly genus (Vargas et al. 2001). It was introduced to Hawaii (USA) in 1949 for the control of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), where it also showed good development in *C. capitata* and was thus able to be used in the control of this species (Vargas et al. 2001, Wang and Messing 2003, Yokoyama et al. 2006, Pokonon-Ganta et al. 2007, Manoukis et al. 2011). After its success in Hawaii, *F. arisanus* was introduced for the control and suppression of tephritids in Australia, Central America and various Pacific and Indian Ocean islands, and in the Mediterranean basin (Rousse et al. 2005, Vargas et al. 2001). However, several studies demonstrate that the development and success of parasitism depends on the development of the host (Bautista et al. 2004, Harris et al. 2007, Montoya et al. 2009).

Harris et al. (2007) studied the reproduction of *F. arisanus* in papaya fruits [*Carica papaya* (L.)] (Caricaceae) infested by *C. capitata* and *B. dorsalis* and observed that the parasitoids reared in *B. dorsalis* produced 28% more offspring than in *C. capitata*, even though *C. capitata* is considered a good host for *F. arisanus*. In species of the *Anastrepha* genus, the development of *F. arisanus* is not well-characterized. However, studies based on its development show that *Anastrepha* larvae were not suitable hosts for *F. arisanus* compared with *C. capitata* (Zenil et al. 2004). In species of the *Anastrepha* genus, the development of *F. arisanus* is not well characterized; however, studies show some development in larvae of *Anastrepha suspensa* (Loew, 1862), *Anastrepha ludens* (Loew,
1873), Anastrepha serpentina (Wiedemann, 1830), and Anastrepha striata (Schiner) (Diptera: Tephritidae) (Zenil et al. 2004, Montoya et al. 2009).

In Brazil, to date, it has not been observed egg parasitoids for the complex fruit fly species, but recently it has been found that the species Doryctobracon areolatus (Szépligeti) (Hymenoptera: Braconidae) showed the capacity to parasitize eggs and larvae of A. obliqua in regions of Mexico (Murillo et al. 2015). Therefore, it is feared that the possible release of F. arisanus for the control of B. carambolae in the Brazilian states of Pará, Amapá, and Roraima (Mapa 2015) could affect the populations of native larval parasitoids of A. fraterculus and other species of this genus, there by harming natural biological control. This study investigated the biology of F. arisanus in C. capitata and A. fraterculus to obtain information to support the biological control programs of fruit flies in Brazil.

Materials and Methods
Establishing Maintenance Rearing
The maintenance rearing of C. capitata, A. fraterculus and the parasitoid F. arisanus, as well as the bioassays to evaluate the biological parameters of the parasitoid on the two hosts, were performed at the Entomology Laboratory of Embrapa Clima Temperado in rooms with a temperature of 25 ± 2 °C, RH of 70 ± 10%, and a photoperiod of 12h.

Adults of A. fraterculus were kept in 57 by 39 by 37 cm (length × width × height) plastic cages. Water and food composed of refined sugar, wheat germ, and yeast at a ratio of 3:1:1 were offered (Nunes et al. 2013). The eggs were collected from oviposition screens placed on the cage sides and were transferred to Erlenmeyer-type glass containers (500 ml), where they remained for a 24h aeration process. Afterwards, with the aid of a 30 μl micropipette, the eggs were deposited onto a 0.8 by 8 cm (width × length) strip of filter paper that was placed in a 24 by 15 by 6 cm (length × width × height) plastic container over 300 ml of artificial diet, where larval development occurred. The ingredients used and the preparation procedures for the artificial diet followed the methodologies described by Salles (1992) and Nunes et al. (2013). For every 300 ml of the artificial diet, 0.8 ml of the water + eggs solution, totaling 9,200 eggs per container, were inoculated. The processes to collect the larvae and to condition the prepupae and pupae in vermiculite were the same as those proposed by Salles (1992) and Nunes et al. (2013).

For the rearing of C. capitata, adults were kept in 48 by 30 by 30 cm (length × width × height) plastic cages containing water and food as described for the rearing of A. fraterculus. The methodologies used for collecting eggs and for the aeration and inoculation processes were the same as those described by Gonçalves et al. (2013) and Kamiya (2010). Approximately 2,500 eggs per container (0.5 ml of solution) were inoculated into 300 ml of artificial diet. The diet used for larval development as well as the methodology used in rearing the insects in the prepupal and pupal stages were the same as those proposed by Salles (1992) and Nunes et al. (2013).

The rearing of F. arisanus from C. capitata eggs began with parasitoids imported on Hawaii (USA) by the Quarantine Laboratory of Costa Lima at the Embrapa Meio Ambiente in the municipality of Jaguariúna, São Paulo State, Brazil. In the Entomology Laboratory of Embrapa Clima Temperado, the rearing of F. arisanus was established in eggs of C. capitata (two generations) before the establishment of the bioassay. Approximately 2,500 C. capitata eggs (0.2 ml) of 24h of age (Paranhos 2015, personal communication) were placed on a piece of filter paper (4 cm) and over a piece of sponge cloth (Spontex, Ilhéus, Bahia, Brazil) inside an acrylic plate (4 cm in diameter and 0.2 cm in height) using a LabMate micropipettor (mono-channel variable volume of 50–250 μl). Next, the eggs were exposed to parasitism by F. arisanus inside a 23 by 27 by 24 cm (length × width × height) rearing cage containing ~400 females and 100 males. After 6 h of parasitism, the eggs of C. capitata were removed from the cages and were placed on the artificial diet (300 ml) in a 1.2-liter plastic container. After 9 d, the larvae were removed from the diet, washed under running water through a sieve (0.22 mm mesh), and then packed in 17 by 27.6 by 7 cm (length × width × height) plastic jars over an extra-fine 3-cm vermiculite layer for a period of 9 d. At pupation, the vermiculite was sifted through a galvanized sieve (0.29 mm mesh), and the insects were stored in 13.5 by 12.5 by 6.5 cm (length × width × depth) plastic jars until the emergence of parasitoids occurred. As the first adults emerged, the jar lids were replaced by a screen with 0.25 mm openings to allow only the parasitoids to pass. The parasitoids were packed in rearing cages as described above, containing distilled water supplied via capillarity through a strip of vegetal sponge cloth that was replaced every 72 h, following the methodology proposed by Gonçalves et al. (2013). The food offered was a paste based on honey and shredded toilet paper, placed inside a Petri dish (2 cm in diameter).

For the rearing of F. arisanus in A. fraterculus, the parasitoids were produced over two generations in A. fraterculus before conducting the bioassays. The same parasitism procedure described for C. capitata was used; however, the larvae obtained from the eggs subjected to parasitism were removed from the artificial diet at 12 d of age and the pupae were removed from the vermiculite at 11 d.

Biological of F. arisanus
Twenty pairs of F. arisanus that were up to 24h in age and were reared in eggs of C. capitata and A. fraterculus were separated into cages made of plastic cups (300 ml) closed at the top with voile fabric to prevent the escape of the parasitoids and to allow aeration to be maintained in a climatized room (temperature 25 ± 2 °C, 70 ± 10% RH, and a photoperiod of 12 h). The parasitoids were fed a honey drop on a piece of Parafilm (Bemis Company, Inc., USA), and distilled water was offered via capillarity by cotton wool; both were replaced every 7 d. Thirty eggs of each host obtained from maintenance rearing were placed on a strip of filter paper with a 4 cm in length, which was placed on a sponge cloth moistened with distilled water. The paper filter and the sponge cloth were arranged on an acrylic plate (4 cm in diameter × 0.2 cm depth). After 24 h of exposure to the parasitoids, the eggs were removed and were placed onto the surface of an artificial diet (50 ml), prepared according to Salles (1992), in plastic containers of 100 ml. After 9 d of larval development for C. capitata and 12 d for A. fraterculus, the larvae were separated from the diet under running water through a sieve (0.22 mm mesh) and were packaged in acrylic bottles (50 ml) containing extra-fine vermiculite, where they pupated and remained until emergence. After obtaining the first adult insects (fruit fly or parasitoid), the puparia were assessed daily. At the end of the experiment, the puparia that remained intact were dissected to check for the presence of flies or parasitoids that did not emerge to determine the true parasitism rate. We evaluated the duration of the egg-to-adult period (days), the parasitism rate (P), the number of offspring (ND), the parasitoid emergence rate (E), the sex ratio (rs), adult fresh weight (24h after emergence) (g), and the longevity of males and females (days). The number of offspring was calculated.
Table 1. Biological parameters of *F. arisanus* reared in eggs of *C. capitata* and *A. fraterculus*

<table>
<thead>
<tr>
<th></th>
<th><em>C. capitata</em></th>
<th><em>A. fraterculus</em></th>
</tr>
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<tbody>
<tr>
<td>Parasitism (%)</td>
<td>42.1 ± 3.16 a</td>
<td>16.0 ± 2.12 b</td>
</tr>
<tr>
<td>Number of offspring</td>
<td>123.4 ± 1.16 a</td>
<td>53.2 ± 1.98 b</td>
</tr>
<tr>
<td>Emergence (%)</td>
<td>83.3 ± 0.76 a</td>
<td>62.8 ± 1.65 b</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>0.63</td>
<td>0.49</td>
</tr>
<tr>
<td>Adult weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males (g)</td>
<td>0.00197 b</td>
<td>0.00278 a</td>
</tr>
<tr>
<td>Females (g)</td>
<td>0.00243 b</td>
<td>0.00301 a</td>
</tr>
</tbody>
</table>

Temp., 25 ± 2°C; RH: 70 ± 10%; photoperiod of 12 h.

*Means (±SE) followed by the same letter in the same row are not significantly different (LSMEANS with Tukey adjustment; *P* < 0.05).*

*Significant for the Chi-square test (a female-biased sex ratio).*

by the equation \( ND = \text{number of emerged parasitoids} + \text{number of parasitoids that did not emerge (inside the container)} \), the parasitism rate was calculated using the equation \( P(\%) = \left(\text{number of offspring} / \text{total number of pupae obtained}\right) \times 100 \), and the emergence rate was calculated using the equation \( E(\%) = \left(\text{number of emerged parasitoids} / \text{number of offspring}\right) \times 100 \). The sex ratio was determined using the equation \( r = \left(\text{number of females} / \text{number of males}\right) \).

Statistical Analysis

The experiment was conducted using a completely randomized design with two treatments (hosts *C. capitata* and *A. fraterculus*) and 20 replicates (pair of parasitoids/treatment). The data on parasitism and emergence rates, the number of offspring, adult weight and egg-to-adult period (days) were evaluated for normality using the Shapiro-Wilk test and for homoscedasticity using the Hartley test (*Chi-square* test). The averages were compared using the *t*-tests at 5% significance. The results for sex ratio were analyzed by the Chi-square test (*\( \chi^2 \)*; *P* < 0.05) (PROC FREQ, SAS Institute 2000). The longevity of adults of *F. arisanus* was analyzed using survival curves through the Kaplan-Meier estimator and were then compared using the log-rank test with the aid of the R program (R Development Core Team 2011). The parameters in the fertility life table were estimated by the jackknife method using lifetable programming in SAS (Maia et al. 2000) and the averages were compared by the *t*-bilateral test (*P* < 0.05) in the SAS software (SAS Institute 2000).

Results

The highest parasitism rate was observed in eggs of *C. capitata* (42%), which significantly differed (*F* = 240.12; df = 1, 36; *P* < 0.0001) from the parasitism rate observed in *A. fraterculus* eggs (16.3%) (Table 1). The peak of parasitism occurred at day 13 for *C. capitata* eggs and at day 19 in eggs of *A. fraterculus* (Fig. 1). The higher parasitism rate in the eggs of *C. capitata* produced a greater number of offspring of *F. arisanus* (∼123 offspring during its lifetime), differing significantly (*F* = 210.09; df = 1, 36; *P* = 0.0001) from the number of offspring obtained from eggs of *A. fraterculus* (∼53 offspring) (Table 1), with emergence rates of 83.3 and 62.8%, respectively (Table 1). Regarding the sex ratio, adults from the eggs of *C. capitata* presented a sex ratio (0.63), which was statistically higher (*F* = 53.69; df = 1, 36; *P* = 0.0001) that that observed in adults from eggs of *A. fraterculus* (0.49) (Table 1). However, adult males emerging from eggs of *A. fraterculus* showed a greater weight (0.00278 g) compared with males from eggs of *C. capitata* (0.00197 g) (*F* = 11.33; df = 1, 36; *P* = 0.0001; Table 1). The same pattern was also seen in female adults emerging from *A. fraterculus* (0.00301 g) compared with those emerging from *C. capitata* (0.00243 g) (*F* = 15.36; df = 1, 36; *P* < 0.0001) than males obtained from eggs of *C. capitata* (50.7 d) (Fig. 2).

The higher parasitism rate, number of offspring and sex ratio of *F. arisanus* reared in eggs of *C. capitata* had positive effects on the fertility life table in all biological parameters evaluated. The *T* values (egg-to-adult period) (∼3 d) were significantly lower (*P* < 0.05) for *F. arisanus* reared in eggs of *C. capitata* than in eggs of *A. fraterculus* (Table 2). Based on these results, after ∼37 d (T) for *F. arisanus* from eggs of *C. capitata* and 40 d (T) for *F. arisanus* from eggs of *A. fraterculus*, 65.2 and 19.46 females were expected to be produced by each female in the breeding phase (Table 2). In relation to the net reproductive rate (*Rₙ*), eggs of *A. fraterculus* negatively affected (*P* < 0.05) population growth in every generation, indicating a reduction of ∼62% in the capacity of females to generate female offspring compared with *F. arisanus* reared in eggs of *C. capitata* (Table 2). Despite significant differences in the life table parameters that are related to fertility, regardless of the host in which the parasitoid developed, the values of the intrinsic growth rate (*rₘ*) and the finite rate of population increase (*λ*) were positive, indicating population growth of *F. arisanus* occurs in both hosts (Table 2).

Discussion

The success of biological control programs involving the use of parasitoids to suppress fruit fly populations depends, among other things, on the knowledge of the biology of the parasitoid and its relationships with possible hosts (Carmichael et al. 2003, Rousse et al. 2006, Pérez et al. 2013). In this study, better biological performance was observed for *F. arisanus* when developing in eggs of *C. capitata* compared with eggs of *A. fraterculus*, showing a better adaptation of the parasitoid to the host. This better performance of *F. arisanus* in eggs of *C. capitata* compared with *A. fraterculus* was mainly due to a higher parasitism rate, corroborating results observed for *F. arisanus* that were reared in eggs of *C. capitata* compared with *A. serpentina* and other various species of *Anastrepha* (Zenil et al. 2004, Montoya et al. 2009). One reason for the low rates of parasitism in *Anastrepha* species may be associated with a high parasitoid encapsulation rate (Zenil et al. 2004), as reported in *Bactrocera cucurbitae* (Coquillet) (Diptera: Tephritidae) (Rousse et al. 2006) and *A. suspensa*. However, in this study, the encapsulation rate was low (<3%) when evaluated at the pupal stage for *C. capitata* and *A. fraterculus*.

The better biological conditions found in the eggs of *C. capitata* led to a greater number of female offspring over time. This indicates that the host provides the biological and physiological conditions...
necessary to provide all the nutritional requirements for the insects to develop offspring and, thus, generate a greater number of females (Quimio and Walter 2001; Ramadan et al. 2004; Zenil et al. 2004, Johnson et al. 2006, Montoya et al. 2009).

However, in the case of adults, both males and females of *F. arisanus* from the eggs of *A. fraterculus* were heavier than the parasitoids from the eggs of *C. capitata*. The lower longevity of females of *F. arisanus* that were exposed to eggs of *C. capitata* can be attributed to increased biological efforts of the parasitoids in searching for hosts to parasitize and may be linked to a higher fertility rate in the preferred host (Zenil et al. 2004, Bokonon-Ganta et al. 2007, Meirelles et al. 2013, Fletcher et al. 2015).

Although most biological parameters evaluated for *F. arisanus* that developed in *A. fraterculus* are lower than for those that developed in *C. capitata*, these values should be considered for studies aimed at the biological control of fruit flies as pointed out by Montoya et al. (2009). In Brazil, the parasitoid used so far in biological control programs for fruit flies of the genera *Anastrepha* and *Ceratitis* is the exotic *Diachasmimorpha longicaudata* (Ashmead, 1905) (Hymenoptera: Braconidae), which was introduced to the

Fig. 1. Relationship between age-specific fecundity (mx) and age-specific survival (lx) of *F. arisanus* reared in eggs of *C. capitata* (A) and *A. fraterculus* (B). Temp., 25 ± 2°C; RH: 70 ± 10%; photoperiod of 12 h.
country in the 1990s (Carvalho and Nascimento 2002). However, with the introduction of *F. arisanus* for the biological control of *B. carambolae* in northern Brazil in the year 2012, one of the main concerns is related to the possibility of intra- or interspecific competition among these species (Paranhos et al. 2013) and native species of braconids and figitids (Wang and Messing 2003, Wang et al. 2008). Although this possibility can occur, it is minimized by the fact that *A. fraterculus* does not offer the best growth conditions as a host, which was also observed by Zenil et al. (2004) and Montoya et al. (2009) in the *Anastrepha* species. However, further studies parasitism preferences of *F. arisanus* in fruits infested with eggs of *A. fraterculus* should be carried out, also taking into account possible interspecific competition among larval parasitoids because *F. arisanus* has the ability to detect pheromones released during oviposition by females of tephritids (Rousse et al. 2007, Pérez et al. 2013).

The fertility life table allowed the parameters of population growth of *F. arisanus* in *C. capitata* and *A. fraterculus* to be obtained. In both hosts assessed, the average interval between generations (T), that is, the average length of the period between generations, was significantly lower (3 d) when the parasitoids were obtained from eggs of *C. capitata* compared with from *A. fraterculus*. Similarly, the net reproductive rate ($R_o$) was 2.7 times higher in parasitoids from eggs of *C. capitata* than in eggs of *A. fraterculus*. Parasitoids have a lower capacity to generate offspring throughout their life, similar to what is observed for the population growth rate ($r_m$) and the finite rate of population increase ($\lambda$). According to Pedigo and Zeiss (1996), the higher the $r_m$ value, the

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**Fig. 2.** Survival curves of female (A) and male (B) adults of *F. arisanus* reared in eggs of *C. capitata* and *A. fraterculus*. Curves identified with the same letters do not differ significantly ($P < 0.05$). The arrows indicate mean survival time. Temp., 25 ± 2°C; RH: 70 ± 10%; photoperiod of 12 h.

**Table 2.** Parameters for fertility life table of *F. arisanus* reared in eggs of *C. capitata* and *A. fraterculus*

<table>
<thead>
<tr>
<th>Host</th>
<th>T (days)</th>
<th>Td (days)</th>
<th>$R_o(\frac{\alpha}{\beta})$</th>
<th>$r_m(\frac{\alpha}{\beta} + \text{day})$</th>
<th>$\lambda$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. capitata</em></td>
<td>37.47 ± 0.38 a</td>
<td>7.93 ± 0.35 a</td>
<td>43.79 ± 2.18 a</td>
<td>0.39 ± 0.295 a</td>
<td>1.490 ± 0.017 a</td>
</tr>
<tr>
<td><em>A. fraterculus</em></td>
<td>40.48 ± 0.37 b</td>
<td>27.45 ± 6.17 b</td>
<td>16.05 ± 3.13 b</td>
<td>0.19 ± 0.115 b</td>
<td>1.213 ± 0.153 b</td>
</tr>
</tbody>
</table>

T, mean length of a generation (eggs–adults); Td, doubling time; $R_o =$ net reproductive rate; $r_m =$ intrinsic rate of population increase; $\lambda =$ finite rate of population increase.

Means within a column followed by the same letter are not significantly different (t-tests for pairwise group comparisons, $P < 0.05$).
better adapted the species is to a particular environment or host. In this study, \textit{F. arisanus} that were exposed to eggs of \textit{C. capitata} presented an \( r_m \) that was approximately two times higher than that observed for eggs of \textit{A. fraterculus}. This indicates that in eggs of \textit{C. capitata}, \textit{F. arisanus} experiences greater population growth and a greater number of females in each generation, which is beneficial for mass rearing in the laboratory aimed at biological control programs. However, positive values were also observed in the population growth of \textit{F. arisanus} in eggs of \textit{A. fraterculus}, showing an apparent adaptation of this parasitoid species to this host.

The data presented and discussed in this article are preliminary studies that show better development of \textit{F. arisanus} in eggs of \textit{C. capitata} compared with in eggs of \textit{A. fraterculus}, indicating that the \textit{C. capitata} is the host most suitable for mass rearing of the parasitoid in the laboratory and that this host should be used for the augmentation of biological programs to control fruit flies.

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