

Biology and fertility life table of the South American fruit fly *Anastrepha fraterculus* on grape

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Abstract

The South American fruit fly *Anastrepha fraterculus* (Wiedemann) (Diptera Tephritidae) was grown in the laboratory on two different table grape cultivars, 'Italia' (*Vitis vinifera* L.) and 'Niagara Rosada' (*Vitis labrusca* L.). In 'Niagara Rosada', oviposition occurred but larvae did not develop. In 'Italia', the duration in days (mean \pm SE) and viability (%), given in parentheses) of the egg, larval and pupal stages were 3.01 ± 0.04 (61.75%), 21.95 ± 0.33 (8.25%) and 14.60 ± 0.09 (65.10%), respectively. The duration of the egg-to-adult period was 39.10 ± 0.45 days and the viability was 3.28%. The pupal weight was 15.10 ± 0.31 mg with a sex ratio (male:female) of 0.64. The pre-oviposition period was 11.77 ± 0.58 days with an average of 129.47 ± 16.38 eggs per female. Oviposition puncture resulted in two injuries to the skin of the 'Italia' grapes. Through the fertility life table, the net reproductive rate (R₀) and finite rate of increase (λ) were 1.71 and 1.01, respectively. The grape cultivar 'Italia' allowed *A. fraterculus* to complete its life cycle and is considered a host for this pest.

Key words: Vitaceae, *Vitis vinifera*, *Vitis labrusca*, vineyard, damage, fruit fly, *Anastrepha fraterculus*.

Introduction

Fruit flies (Diptera Tephritidae) are economically important fruit pests worldwide and are responsible for direct and indirect damage (Follett and Neven, 2006). In Brazil, two genera of fruit flies are considered pests in commercial orchards: *Anastrepha* Schiner, a native genus from America that includes 99 identified species in Brazil (Zucchi, 2007), and *Ceratitis* Macleay, which is represented by only one species, *Ceratitis capitata* (Wiedemann), that originates on the continent of Africa and is also found in South America (Zucchi, 2000).

The South American fruit fly, *Anastrepha fraterculus* (Wiedemann), is considered the main fruit pest in the southeastern and southern regions of Brazil and is distributed from the southern USA to northern Argentina (Salles, 1995; Aluja, 1999). This species occurs on fruit trees in the Southern Cone countries (Argentina, Brazil, Paraguay, and Uruguay), and it is most damaging to peaches (*Prunus persica* L.), plums (*Prunus domestica* L.), apples (*Malus domestica* Borkh.) and citrus fruits (*Citrus* spp.) (Salles, 1995; Segura *et al.*, 2006). *A. fraterculus* has been implicated in damage to white skin grapes (*Vitis vinifera* L.) due to the visibility of the galleries caused by larvae when feeding on the pulp of the grape berry (Soria, 1985; Botton *et al.*, 2003).

The principal species of commercial grapes cultivated in Brazil are *Vitis labrusca* L., or American grapes ('Isabel', 'Niagara', 'Bordo'), and *V. vinifera*, or European grapes ('Italia', 'Cabernet', 'Merlot'). In both cases, the product may be destined for processing (juice, wine, etc.) or direct consumption (Mello, 2007). Little information is available on the biology of fruit flies associated with grapevines (Chu and Tung, 1996; Barnes, 2006). Furthermore, no data are available on the development of *A. fraterculus* in association with grapevines or the current state of colonization of vineyards by this pest.

Our limited knowledge of the incidence of *A. fraterculus* on grapes is mainly due to a lack of study of the damage that this species causes to grape cultivars that are either destined for processing or are to be consumed as fine table grapes in Brazil (*V. vinifera*). Fine table grapes are recognizably more susceptible to puncture by *A. fraterculus* (Soria, 1985; Botton *et al.*, 2003); however, they are concentrated in the San Francisco River valley region (states of Bahia and Pernambuco, Northeast of Brazil) where damage caused by *C. capitata* is prevalent (Habibi *et al.*, 2006). On the other hand, the recent expansion of the region in which fine table grapes are cultivated (Protas *et al.*, 2002) to include areas where *A. fraterculus* is present reinforces the need to investigate aspects of the biology of this species in culture. The objective of this work was to study the biology of the South American fruit fly in the laboratory and to develop a fertility life table of this species when grown on grape berries of 'Niagara Rosada' (*V. labrusca*) and 'Italia' (*V. vinifera*), which are the main table grapes cultivars in Brazil.

Materials and methods

This work was conducted from January to August of 2007 at the Laboratory of Entomology of Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) located in Bento Gonçalves, Rio Grande do Sul State, Brazil (29°09'S, 51°31'W and an altitude of 640 m). The original source of the insect colony used in the study was from peaches collected from an orchard located in Bento Gonçalves in 2001. Genetic diversity of the culture was maintained by the annual addition of field-collected insects from strawberry guava fruits (*Psidium cattleianum* Sabine). The culture was maintained in a plastic cage (30 x 30 x 30 cm) in laboratory

(temperature 23 ± 2 °C, $70 \pm 10\%$ UR and 14 h of photo phase). The rearing facility used papaya (*Carica papaya* L.) as the substrate for oviposition and subsequent larval development. Adults were fed with a solid diet (soy extract, wheat germ, and brown sugar, at a ratio of 3:1:1) and water, in accordance with Taufer *et al.* (2000).

Infestation and individualization of berries

Mature grape berries from 'Italia' and 'Niagara Rosada' cultivars were cut with the aid of scissors, leaving the pedicel to avoid rupturing the skin. The grape berries were placed inside plastic rearing cages containing approximately 100 flies 15 to 25 days after emergence (reproductive age). In each cage, 40 grape berries were offered for oviposition using a photoperiod of 14L:10D. After 6 hours of oviposition, grape berries were removed and isolated in plastic containers (100 ml) with lids and then placed on a layer of sterilized vermiculite (0.5 cm). An opening (1 cm²) was made in the lid, which was then closed with filter paper and adhesive tape, allowing air and humidity to exchange with the environment.

Rating egg stage

The oviposition site of the fruit fly was identified by the presence of a peculiar puncture on the grape berry (figure 1). Thirty grape berries (fifteen for each cultivar) with 25-35 punctures each were used to evaluate egg development. The infestation of the berries in each cultivar was conducted in separate containers in order to eliminate the possibility of free choice of berries at the moment of oviposition. The site of oviposition on each grape berry was marked with a pen (Pilot®) by drawing a circle around the puncture. The grape berries were evaluated for larval eclosion every 12 hours after being withdrawn from the cages. Eclosion was characterized by the formation of a gallery that could be visualized by the darkening of the fruit pulp below the skin of the grape. In the puncture, where the gallery was observed, another mark was made with a different colour pen to avoid duplication of the eclosion record in the same grape



Figure 1. Injuries caused by *A. fraterculus* by perforation of aculeus (a) and the pressure of the sheath of the ovipositor (b) in the berry of the grape cultivar 'Italia'.

berry. At the end of the fifth day of evaluation (120 h), all grape berries were opened to confirm larval eclosion and to determine the number of eggs using a stereomicroscope (10 X). Because fruit flies may create punctures without laying eggs, it was necessary to remove the skin of the berries to confirm the lay (puncture test). With this procedure, it was possible to calculate the duration and viability of the egg stage.

Rating larval stage

The larval stage was evaluated in two distinct experiments. In order to evaluate the larval stage, 800 grape berries were utilized (400 for each cultivar). These berries were infested in adult cages and maintained individually in the same plastic containers. The viability of the larval period was evaluated using 150 grape berries that had been infested for only two hours. The shorter period of infestation reduced the number of punctures per grape berry, facilitating puncture counting. Five days after withdrawing the grape berries, the number of galleries was counted per grape berry. The viability was calculated according to the number of larvae that pupated in the vermiculite relative to the number of initial galleries.

Rating pupal stage

During larval development, the time at which the larvae left the grape berries was recorded (pre-pupae). Then, the larvae were collected and separated in flat-bottomed test tubes (h 8.5 cm x diameter 1.5 cm) with sterilized vermiculite and wet filter paper. These tubes were closed with plastic film paper (Magipac®) to allow observation of the pupal stage. The pupal mass was evaluated by weighing the pupae after 24 hours. Water was added to the tubes containing pupae every three days to maintain humidity.

Rating adult stage

The adults that emerged were separated by gender and flies with malformations were discarded. The adults were placed in cages constructed from transparent plastic cups (h 11 cm x larger diameter 7 cm x diameter smaller 5 cm), one couple per cage. A total of 30 pairs (repetitions) were observed and the dates of their emergence and death were recorded. The cups were open on the side with the smallest diameter and were fitted with a wad of cotton for air circulation. They were placed under sulphite paper with the larger opening facing down. Every three days throughout each adult's life, food and water were added through the downward facing opening. The diet offered was the same as that which was used during laboratory rearing. The grape berries provided for oviposition were exchanged daily throughout each female's life. After they were removed, the grape berries were numbered according to date and fruit fly pair, and they were then held in plastic containers (h 5 cm x larger diameter 6.5 cm x diameter smaller 4.5 cm). After five days, the number of punctures and the larval galleries were evaluated and the grape berries were dissected for egg counting. We also used a binocular magnifier to observe the percentage of hatched larvae by counting the number of whole eggs (non-hatched) and those with a ruptured chorion (hatched).

Rating the life table

Using biological data, we determined the fertility life table according to Silveira Neto *et al.*, (1976) based on the net reproduction rate (Ro), mean generation time (T), intrinsic rate of increase (rm) and finite rate of increase (λ).

Results and discussions

The total number of eggs oviposited by *A. fraterculus* on the berries of 'Niagara Rosada' (*V. labrusca*) cultivar (55 eggs) was less than in the 'Italia' (*V. vinifera*) cultivar (217 eggs). However, the duration (3 days) and viability (61.82%) observed in 'Niagara Rosada' was similar to that observed in 'Italia' (table 1).

In the 'Niagara Rosada' cultivar, the oviposition and larval eclosion were difficult to observe; however, no flies developed up to the pupal stage, indicating the inability of this cultivar as a host for *A. fraterculus*. For this reason, the evaluation was not carried out for the larval, pupal or adult stages of the pest in the 'Niagara Rosada' cultivar. Baker (1945) suggested that the grapevine is a good host for obtaining eggs of *A. fraterculus* in the laboratory. However, he recorded no larval development and did not mention the species or the vine cultivar he evaluated. In the 'Italia' cultivar, development of post-embryonic stages through adult emergence was observed (table 1). The existence of different populations of *A. fraterculus* (Alberti *et al.*, 1999) may justify the suitability of the grape as a host for *A. fraterculus* under Brazilian conditions. This may result in greater adaptation among introduced fruit trees when compared with those grown in the presence of Mexican fruit fly populations. Similar observations have been recorded for citrus, where the Mexican population does not present sufficient adaptation to colonize citrus fruits (Aluja *et al.*, 2003) when compared with the Brazilian population that can (Raga *et al.*, 2004).

The duration of the egg stage in the 'Italia' cultivar was 3.01 ± 0.13 days, a value close to what has been found for this species when feeding on other substrates (Baker, 1945; Gonzalez *et al.*, 1971; Martins, 1986; Machado *et al.*, 1995; Jaldo, 2001). Based on this result, we show that *A. fraterculus* does not alter its egg incubation time when grown on the 'Italia' cultivar. The viability rate found (61.75%) in this study was also close to rates that have been reported in studies of *A. fraterculus* grown on plums (57.7%) (Baker, 1945), guavas

(*Psidium guajava* L.) (67.7%) and apples (cv. 'Fuji', 70.4%) (Sugayama *et al.*, 1998). In China, Chu and Tung (1996) observed egg viability rates close to 20% for *Bactrocera dorsalis* (Hendel) in grape berries at 60 to 80 days after blooming.

In the present study, the larval stage lasted 21.95 ± 0.33 days on average with a viability rate of 8.25%. These values are close to those observed by Sugayama *et al.* (1998) in a 'Fuji' apple cultivar (25.5 days and 11.6%). Sugayama *et al.* (1998) observed a higher larval survival in guava cultivars when compared with apple cultivars. The characteristic "meaty" pulp of the 'Italia' strain may be responsible for the adaptation of *A. fraterculus* in this cultivar. This was not observed in 'Niagara Rosada', which has a "juicy" pulp. Grape berries from American cultivars (*V. labrusca*) present pellicles with thicker skin and "juicy" pulp, resulting in a lower susceptibility of these cultivars to fruit fly attack (Botton *et al.*, 2003). Field observations in the southern region of Brazil are consistent with this finding, considering that other *V. vinifera* cultivars ('Semillon', 'Muscat Alexandria') and hybrids of the species ('Moscato Embrapa', 'Seyve Vilard') with white skin have consistently shown evidence of damage caused by the development of larvae in grape berries (Soria, 1985). Chu and Tung (1996) observed that larval viability of *B. dorsalis* in grapes was dependent on the maturation of the grape. They reported egg-to-pupa survival rates of 6, 15 and 25% for infestation periods of 60, 70 and 80 days after blooming, respectively. In addition, Barnes (2006) observed that the South African species *Ceratitidis rosa* Karsch and *C. capitata* completed their life cycles during the "harvest-ripe berries" stage in *V. vinifera* cultivars used for wine processing.

We found that pre-pupae periods lasted less than 24 hours on average (0.85 ± 0.03 days), with a maximum of two days. The viability rate was 98.96%, with a pre-pupal mortality rate of 1.04% during the stage where larval characteristics were present. Observations of this period have not been reported in other studies of this species (Baker, 1945; Gonzalez *et al.*, 1971; Martins, 1986). This may be because in previous studies, flies were provided with artificial diets making it difficult to determine the moment when the larvae stop feeding and begin pupating. The short duration of this period most likely increases the chance of survival of fruit fly larvae in the environment, mainly because the insect may find non-favorable conditions with respect to predators (Galli and Rampazzo, 1996) and soil conditions (Jackson *et al.*, 1998).

Table 1. Mean (\pm SE) and range of variation in days of immature stages of *A. fraterculus* in berries of the 'Italia' grape cultivar (*V. vinifera*) and their viability (%) in the laboratory (temperature 23 ± 2 °C, $70 \pm 10\%$ RH and 14 h of photophase). Bento Gonçalves, RS, Brazil, 2007.

Stage	N	Mean \pm SE	Range of variation	Viability (%)
Egg ¹	217	3.01 ± 0.13	(2 - 4)	61.75
Larva	192	21.95 ± 0.33	(14 - 32)	8.25 ²
Pupa	125	14.60 ± 0.09	(13 - 17)	65.10
Egg-Adult	125	39.10 ± 0.45	(31 - 50)	3.28

¹Observation of 15 separate berries.

²Viability of 412 larvae developed measured at 150 berries.

Table 2. Biological parameters evaluated in adults (30 male-female pairs) of *A. fraterculus* in the laboratory (temperature 23 ± 2 °C, $70 \pm 10\%$ RH, and 14 h photophase). Bento Gonçalves, RS, Brazil, 2007.

Biological parameters	(Mean \pm SE)	Range of variation
Pre-oviposition (days)	11.77 \pm 0.58	(7 - 20)
Oviposition (days)	20.70 \pm 2.76	(1 - 61)
Longevity total (days)	43.13 \pm 2.95	(12 - 106)
Longevity male (days)	52.00 \pm 4.71	(12 - 106)
Longevity female (days)	34.27 \pm 2.79	(16 - 77)
Eggs/female/day	6.25 \pm 0.85	(0 - 38)
Eggs/female/total	129.47 \pm 16.38	(5 - 323)
Punctures/female/day	21.24 \pm 16.72	(0 - 215)
Punctures/female/total	510.40 \pm 56.27	(13 - 1109)

In 2.4% of the population observed, fly emergence did not occur due to the adult being trapped in the pupal case. The mean duration of the pupal period was 14.6 ± 0.09 days and the viability rate was 65.10%. Sugayama *et al.* (1998) observed durations of 15.5 ± 0.7 and 14.5 ± 0.7 days in apple ('Gala') and guava with viability rates of 26.5% and 53.4%, respectively.

The mean weight of the pupae was 15.10 ± 0.31 mg, with a maximum and minimum of 21 and 9.1 mg, respectively. In native hosts (Myrtaceae), Salles and Leonel (1996) observed similar values for the weight of *Anastrepha* sp. pupae with heavier pupae obtained from fruits with smaller numbers of pupae per fruit. In the case of the 'Italia' cultivar, a ratio of 0.97 pupae per fruit was observed with a variation ranging from zero to three.

The mean longevity observed for adults was 43.13 \pm 2.95 days (table 2), a value less than that observed by Gonzalez *et al.* (1971) in Peru, who reported a mean duration rate of 67.3 days for adults fed on a 4:1 mixture of sugar and soy protein. Sugayama *et al.* (1998) observed a survival rate of 50% for males and females of *A. fraterculus* after 31 and 35 days in apple ('Gala') and 45 and 60 days in guava, respectively. Joachim-Bravo *et al.* (2003) observed a lifespan for *A. fraterculus* of up to 190 days in guava fruits with 50% population survival recorded within 115 days. In 90% of male-female pairs, the date of the first puncture did not coincide with the first oviposition, and it generally occurred within a period of 8.77 ± 0.35 days. The period of pre-oviposition was 11.77 days, which is similar to that observed by Gonzalez *et al.* (1971) who recorded 9 days on average. Salles (1995) observed that temperature may cause variation in the survival and the period of pre-oviposition in *A. fraterculus*, which would prolong the time of ovarian maturation in the females kept at low temperatures (Taufe *et al.*, 2000).

The mean number of punctures per grape berry was 21.24 ± 16.72 , with 74.6% of punctures lacking eggs. The occurrence of punctures made by *A. fraterculus* without the presence of eggs has also been described by Sugayama *et al.* (1997) in apple trees. However, in a situation where the fly can choose the host (quantity and quality), the number of punctures in each fruit tends to decrease (Sugayama *et al.*, 1997; Aluja *et al.*, 2003), which may explain the high rate of punctures without egg deposits in grapes in closed conditions. The maxi-

imum number of punctures per grape berry was observed on the 15th day (36.73 ± 1.11 punctures), and the largest number of eggs per female was also recorded at this time (9.97 ± 0.58 eggs) (figure 2). In total, an average of 129.47 eggs were recorded per female (table 2). Sugayama *et al.* (1998) observed that the *A. fraterculus* that had developed in guava showed a better reproductive performance than those that developed in a 'Gala' apple cultivar. We also observed that *A. fraterculus* caused two punctures in the berry skin: one was characterized by the entry of the ovipositor, and the other was characterized by the pressure of the sheathing of the ovipositor at the moment of introduction into the grape berry (figure 1). These punctures may facilitate infection by pathogenic microorganisms in fruits (Engelbrecht *et al.*, 2004). This possibility still needs to be studied for *A. fraterculus* in different grape cultivars.

The calculated net reproduction rate (Ro) was 1.71. This value, compared with those reported by Martins (1986) (16.6 on an artificial diet), reflects the low suitability of the 'Italia' grape as a host for *A. fraterculus*. The finite reason of increase (λ) was 1.01, which is less than the values observed by Sugayama *et al.* (1998) who reported $\lambda = 1.058$ and $\lambda = 1.031$ for *A. fraterculus* in guavas and apples ('Gala'), respectively. The net growth rate was 0.01 and the mean time of generation (T) was 72.1 days.

Due to the host succession necessary for *A. fraterculus* to reproduce in nature, the adaptation of the insect to different hosts has been an important aspect for the survival of the species in different Brazilian regions. In southern Brazil, different botanical species are found in the same environment. These species include stone fruits (peaches, plums, nectarines), apples, citrus and native fruits (Myrtaceae) in domestic orchards and native forests. In vineyards, the crop is characterized by grape ripening at the end of December with late cultivars being harvested in the beginning of April. At the end of the harvest of other fruits, the presence of hosts with a greater tolerance for the breeding of *A. fraterculus* in the region decreases, increasing the chance infestation in the vine. Additionally, table grape (*V. vinifera*) cultivation in southern Brazil is expanding (Protas *et al.*, 2002) and creating a susceptible host. The incidence of *A. fraterculus* on grapes may select for adapted individuals that also cause damage to processing wines. However, the difference in *A. fraterculus* survival with

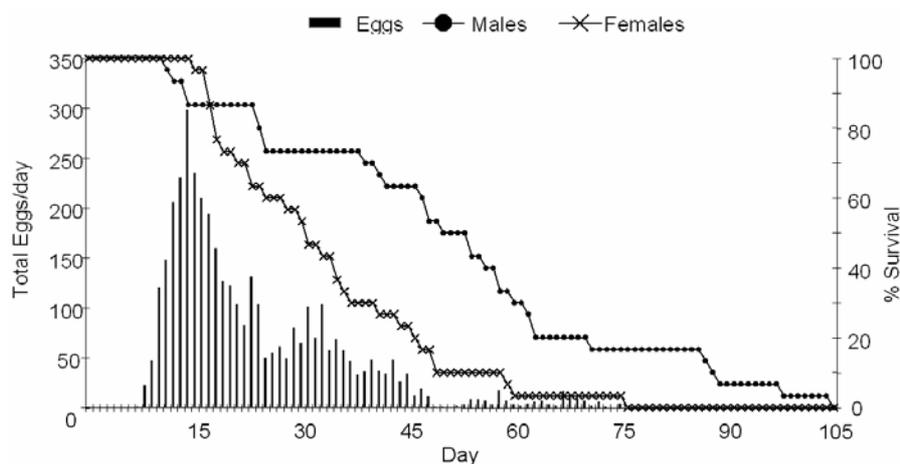


Figure 2. Number of eggs per day deposited in berries of the 'Italia' grape cultivar by 30 females and survival curve of males and females (n = 30) fed with artificial diets in the laboratory (temperature 23 ± 2 °C, $70 \pm 10\%$ RH and 14 h of photophase). Bento Gonçalves, RS, Brazil, 2007.

respect to the species of vine demonstrates a necessity for studies of the physical/chemical conditions of *V. labrusca* and *V. vinifera* grape berries. Such studies would make it possible to use more resistant cultivars in areas with high prevalence of *A. fraterculus*.

Conclusions

South American fruit flies, *A. fraterculus*, may complete the biological cycle in the 'Italia' cultivar (*V. vinifera*) but not in 'Niagara Rosada' cultivar (*V. labrusca*).

The 'Italia' grape cultivar is a potential multiplier host of *A. fraterculus*.

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