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Development of *Anastrepha grandis* (Diptera: Tephritidae) under constant temperatures and field validation of a laboratory model for temperature requirements

Anderson Bolzan [a,*], Dori E. Nava [b], Giovani Smaniotto [a], Ricardo A. Valgas [b], Flávio R.M. Garcia [a]

[a] *Departamento de Ecologia, Zoologia e Genética, Instituto de Biologia, Universidade Federal de Pelotas, Avenida Eliseu Maciel s/n, 96010-900, Capão do Leão, Rio Grande do Sul, Brazil*
[b] *Laboratório de Entomologia, Embrapa Clima Temperado, Rodovia Br 392 km 78, 96010-970, Pelotas, Rio Grande do Sul, Brazil*

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ABSTRACT

Anastrepha grandis (Macquart) is one of the main pests of cucurbits in the countries of Central and South America. Besides direct damage caused to fruits, *A. grandis* occurrence in producing regions can lead to export embargos. Despite its economic importance, little is known of the effects of temperature on its biology. This study investigated the development of *A. grandis* under different temperatures to estimate thermal requirements and then validated the model developed in the field. Development time was inversely proportional to temperature and greater fecundity and fertility were observed at 25 °C. Greater egg and pupa viabilities as well as a greater number of insects per fruit were also observed at 25 °C. The thermal threshold and the thermal constant for egg and pupal stages were 8.3 °C for both stages and 132.3 degree-days (DD) for the egg stage and 347.0 DD for the pupal stage. For the egg-to-adult period the values were 5.2 °C and 858.7 DD. Data collected in the field showed DD (937.9) and duration (79.7 d) values of the egg-to-adult period similar to those estimated in the laboratory. This information could support management of *A. grandis*, since the model for temperature requirements can be used to predict pest occurrence in crops and estimate the number of generations per year.

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1. Introduction

The South American cucurbit fruit fly, *Anastrepha grandis* (Macquart) (Diptera: Tephritidae) is one of the main pests in plantations of native and exotic cucurbits in the countries of South and Central America (Norrbom, 2000). In Brazil, *A. grandis* occurs primarily in the South, Southeast and Midwest (Zucchi, 2000a).

The main hosts of *A. grandis* are melon (*Cucumis melo* L.), zucchini (*Cucurbita pepo* L.), squash (*Cucurbita moschata* Duchesne), pumpkins (*Cucurbita maxima* Duchesne), watermelon (*Citrullus* spp.), cucumber (*Cucumisa sativus* L.) and chayote (*Sechium edule* (Jacq.) Swartz) (Costa Lima, 1926; Silva et al., 1968; Malavasi et al., 1980; Silva and Malavasi, 1993). However, among the hosts, the genus *Cucurbita* allows greater viability of *A. grandis* and shorter duration of immature stages, consequently, generating a greater

number of insects than hosts from other genera (Bolzan et al., 2015).

Anastrepha grandis can cause damage to fruit at different stages of development. After oviposition, when up to 30 eggs are laid per puncture, the larvae hatch and feed on the fruit pulp, building galleries. In addition, the puncture for oviposition allows microorganisms to enter the fruit, leading to fruit rot. The damage makes the fruit unfit for consumption, marketing and industrialization (Malavasi and Barros, 1988).

In Brazil, *A. grandis* is one of seven species of genus *Anastrepha* of economic importance (Zucchi, 2000b). In addition to direct damage caused to fruits, *A. grandis* occurrence is directly linked to quarantine restrictions imposed by various importing countries (Paranhos, 2008; NAPPO, 2009). Because of the embargo on exportations of Brazilian cucurbits, the Ministry of Agriculture, Livestock and Supply (Ministério da Agricultura, Pecuária e Abastecimento (MAPA) in Brazil) along with Secretariats of Agriculture established Pest-Free Areas (PFA) and Risk Mitigation Systems (RMS) in different regions in Brazil undertook to monitor the areas

designated for exports of cucurbits (MAPA, 2006; Paranhos, 2008; Bolzan et al., 2014, 2016).

Development, reproduction and behavior of insects are directly related to abiotic factors, such as temperature (Nava and Parra, 2003). Knowing temperature effects on the insect biological cycle requires determination of the base temperature and temperature requirements for development. Correlating this data according to local temperature of a given location allows the forecasting of the developmental stage of the insect, prediction of the occurrence of population spikes and estimation of the number of generations that may occur in a given time period, facilitating pest management (Rabb et al., 1984). However, temperature experiments carried out in the laboratory may sometimes show different results in the field, mainly due to the decrease in genetic variability of insects reared under controlled conditions and the action of abiotic factors (Parra, 2002). Thus, validation of the model in the field for temperature requirements obtained in the laboratory is necessary for an effective use of the model in systems to forecast pest occurrence.

Despite the economic importance of A. grandis in cucurbit crops, little is known about the effects of temperature variation on the development of this insect. This information can provide support to management procedures of this pest in different regions and ecosystems. Furthermore, it can help explain the presence or absence of this insect in certain regions. Thus, this study investigated A. grandis development at different temperatures to estimate temperature requirements and validate the model obtained in the field.

2. Materials and methods

2.1. Maintenance rearing

To initiate the rearing of A. grandis, infested fruits were collected in the municipalities of Aratiba (27° 26′ S, 52° 19′ W) and Flores da Cunha (29° 2′ S, 51° 13′ W), located in the state of Rio Grande do Sul, Brazil. The geographic coordinates were taken using a navigation GPS (Garmin International Inc. model Montana 650, Olathe, KS). In the Entomology Laboratory of Embrapa Temperate Agriculture, Pelotas, Rio Grande do Sul (RS), Brazil, the infested fruits were kept in room with controlled temperature at 25 ± 2 °C, RH $70 \pm 10\%$ and a photophase of 12 h, until the emergence of adults.

The adults were kept in plastic cages ($60 \times 40 \times 40$ cm), and were fed an artificial diet (Bionis YE MF and NS) based on yeast, wheat germ, and powdered sugar at the ratio of 1:1:3, respectively. The water was offered by capillarity, as described by Nunes et al. (2013) for the rearing of Anastrepha fraterculus (Wiedemann) (Diptera: Tephritidae).

As described by Bolzan et al. (2015), fruits of squash (Cucurbita pepo L.) were offered to females for oviposition. Every 48 h, the squash was replaced and placed in a pot containing vermiculite for moisture absorption and substrate for pupation. Later, the pupae were removed and placed in Petri dishes (10 cm in diameter and 1 cm in height) until the emergence of adults. All rearing and maintenance of insects was made in a room with temperature controlled at 25 ± 2 °C, RH $70 \pm 10\%$ and a photophase of 12 h.

2.2. Development of A. grandis under different temperature conditions

In this experiment, we used five plastic cages ($60 \times 40 \times 40$ cm) containing 25 couples at 25 d of age. In each cage, six squash fruits were exposed for oviposition. The cages with the adults were kept in a temperature-controlled room, under the conditions described for the maintenance rearing. After 24 h of exposure to females, the fruits were removed from cages and individualized in plastic

containers ($15 \times 10 \times 10$ cm), containing a vermiculite layer at the bottom. Randomly, the fruits were separated into five groups containing six fruits, each group being kept at a different temperature. The temperatures used (treatments) were 15, 20, 25, 30 and 35 ± 1 °C, RH $70 \pm 10\%$ and a photophase of 12 h. After the 10th day, the fruits were checked daily to remove and count the puparia, which were weighed 24 h after collection. Up to 30 pupae per fruit were individualized in acrylic tubes ($2.5 \times 4.8 \times 2.5$ cm) containing moist vermiculite. The pupae were kept at the same temperatures described for larval development until emergence of adults. The periods of the egg-to-pupa (from the egg laying to pupation) and of the pupal stage, viability and weight of pupae were estimated, sex ratio (rs) was determined by the formula rs = female/(female + male), proposed by Silveira Neto et al. (1976).

After emergence, 25 couples per treatment were formed and each couple was kept in a cage made of a 500 mL transparent plastic cup, with a 1-cm^2 hole on top, covered with nylon screen of 1 mm in diameter for air circulation. The couples were kept at constant temperatures used for the immature stages and were fed with the same diet described for maintenance and rearing. The number of eggs and mortality were registered daily to determine the periods of pre-oviposition, oviposition and post-oviposition, fecundity, fertility and longevity of females.

Fecundity of couples was determined using epicarp (skin) circles of squash (40 mm in diameter and approximately 3.2 mm thick) on the bottom of a Petri dish (36.4 mm × 8.0 mm diameter). A moistened vegetable sponge cloth was placed inside the Petri dish, which filled the entire bottom of the dish. The epicarp circles of squash were replaced every 48 h to prevent contamination due to decomposition. Eggs in the 2nd or 3rd oviposition of each female, obtained in artificial substrates, were used to evaluate fertility. Eggs retained in the lower part of the fruit skin or on moistened sponge were carefully removed with a brush, counted and placed on filter paper previously prepared on a moistened vegetable sponge cloth inside Petri dishes (36.4 mm × 8.0 mm diameter). The plates were kept in a temperature-controlled chamber (25 ± 1 °C). The hatched larvae were counted and removed from the plates daily. Then, the number of viable and unviable eggs were counted and the percentage of fertile eggs per couple was determined.

To evaluate the effect of constant temperatures (15, 20, 25, 30 and 35 ± 1 °C) on the percentage egg hatch (viability) and incubation period, 180 eggs per temperature (6 replicates with 30 eggs) were used. The eggs were placed on a filter paper previously prepared on a moistened vegetable sponge cloth, inside Petri dishes and kept at their respective temperatures, until the larvae hatched. The larvae were counted and removed daily to determine duration and viability. To standardize the origin, the eggs were obtained from couples that were kept at 25 ± 1 °C, due to the higher female fertility at this temperature.

Data on the duration of periods of egg-to-pupa, pre-oviposition, oviposition and post-oviposition, pupal stage and longevity of females were analyzed using the survival analysis techniques (R Development Core Team, 2013). For each period, survival curves of each treatment were determined considering the Kaplan-Meier estimator and compared by the Log-Rank test. Data on pupa viability, sex ratio and fertility were compared by a Tukey test ($P < 0.05$), based on the binomial distribution, according to the methodology described by Pimentel-Gomes (2009). For the egg-to-adult period (from the egg laying to adult emergence) and pupal weight, data were subjected to the analysis of variance (ANOVA) and means were compared by a Tukey test ($P < 0.05$). Data concerning the number of pupae per fruit and fecundity were submitted to the analysis of generalized linear models through the SAS GENMOD procedure (SAS Institute, 2002), as the data showed a Poisson distribution and the likelihood ratio (95% confidence) was

used to compare the means.

2.3. Determining thermal requirements

Data from different developmental stages of *A. grandis* at different temperatures were used to determine thermal requirements by the coefficient of variation method (Haddad and Parra, 1984). To determine the lower threshold temperatures (LTT) by the coefficient of variation method, the thermal constant (K) values were estimated using the following equation, $K = D^*(T-LTT)$, where D is the developmental period in days and T is the temperature at which the insect develops (Haddad and Parra, 1984). To find the K values, LTT was stablished with temperatures varying from −5 to +20 °C, considering intervals of 0.1 (−5, 4.9, 4.8, …, 19.8, 19.9, 20). The LTT established for each developmental stage presented lower CV (%) between the K values (K_{15}, K_{20}, K_{25}, K_{30}) at the temperatures evaluated in laboratory.

2.4. Field validation of the model for temperature requirements obtained in laboratory

Six squash fruits (*C. pepo*) were exposed to oviposition during a period of 24 h in plastic cages (60 × 40 × 40 cm) containing 25 couples of *A. grandis* of approximately 25 d of age. Afterward, the fruits were placed on a tray containing a vermiculite layer of approximately 5 cm. Next, the fruits were placed inside a screen cage with 1 mm diameter holes on the side for air circulation and installed in a cucurbit crop (31°40′47″ S; 52°26″21″ W) between April 2 and June 23, 2014.

A data logger device (Campbell Scientific, Inc. model CR 100, Lohan, UT) was installed inside each cage and was programmed to record the temperature every 10 min. Data on relative air humidity and precipitation were obtained from the meteorological station of Embrapa Clima Temperado, Pelotas, Rio Grande do Sul State, Brazil, installed 200 m from the experiment site. Daily assessments were made and when no more larvae were observed in any of the fruits, the vermiculite was sifted to check the number of puparia. After, the puparia were again placed in vermiculite where they remained until emergence of adults.

Data on daily temperature obtained in the field, subtracted from the estimated LTT in the laboratory, allowed estimation of the degree-days required for the insect to complete its biological cycle (egg-to-adult) under field conditions and to compare the estimated mean number of degree-days in laboratory with those observed in the field using a *t*-test (P < 0.05). Data on temperature and egg-to-adult period registered in the laboratory allowed estimation of the egg-to-adult period for average temperatures recorded in the field, through the second order polynomial regression ($y = 0.404x^2 − 21.7x + 327.2$; $r^2 = 0.995$). Thus, the estimated period in the laboratory was compared to the period observed in the field using t tests (P < 0.05).

3. Results and discussion

3.1. Development of A. grandis under different temperature conditions

The durations of the egg and pupa stages, egg-to-pupa and egg-to-adult periods was inversely proportional to temperature within the temperature range from 15 to 30 °C, with the exception of the larval stage in which duration had a linear behavior within the temperature range 15–25 °C. At 35 °C, there was no development of *A. grandis* (Table 1).

Duration of the egg stage ranged from 21 to 6.9 d within the temperature range 15 to 30 °C and the smallest durations recorded

at 25 and 30 °C did not differ significantly. The greatest eggs viability was observed at 25 °C (91.7%) within the temperature range studied, differing significantly at the other temperatures (P < 0.05) (Table 1). Within the temperature limits assessed, low viability (12.2%) was recorded at 15 °C, while at 35 °C, there was no embryonic development of *A. grandis*. Silva and Malavasi (1996) recorded egg viability of 16.6% for *A. grandis* at 25 °C, however, the authors assessed this parameter after the larvae had hatched in the oviposition puncture, which may not have been the best methodology, because larvae are typically difficult to be visualized in the substrate. Thus, 25 °C is the most suitable temperature for embryonic development.

Survival curves for the egg-to-pupa period differed significantly. The shortest duration was found at 25 °C (19.0 d), meaning that this temperature is more suitable for larval and pupal development, since at 30 °C, there was an increase in duration (22.6 d) ($\chi^2 = 3939$; df = 3; P < 0.0001). This may show that a temperature of 30 °C can affect larval development of *A. grandis* since this behavior did not occur at the embryonic and pupal stages (Table 1). For *A. grandis* in pumpkin (*Cucurbita* sp.), Silva and Malavasi (1996) recorded average times of larval and pupal development of 17.7 and 19.7 d, respectively, at 25 °C, totaling 37.4 d. This period is about 5 d longer than that recorded in this work. This difference is possibly related to the methodology used to assess the duration of embryonic period by Silva and Malavasi (1996) and because the host was different.

The duration of the pupal stage ranged from 52.3 to 16.5 d, when pupae were exposed to constant temperatures of 15 and 30 °C, respectively, differing significantly ($\chi^2 = 1460$; df = 3; P < 0.0001). At 25 °C, the average duration of the pupal stage was 20.3 d, close to the value found by Silva and Malavasi (1996), 19.7 d at 25 °C. The greater pupae viability (96.1%) occurred at 25 ± 1 °C, similar to values observed at 15 and 20 ± 1 °C (P < 0.05). However, at 30 ± 1 °C, there was a reduction in viability (43.0%), which means that more than half of the pupae were not viable at this temperature (Table 1).

The duration of the biological cycle (egg-to-adult) ranged from 93.3 to 39.1 d within the temperature range between 15 and 30 ± 1 °C, respectively. The duration values differed significantly at distinct temperatures, with the exception of values recorded at 25 and 30 °C (F = 9.696; df = 3; P = 0.0005). This value is about 2 d lower than that registered by Silva and Malavasi (1996) (41.3 d at 25 °C) and approximately 5.2 d lower than that observed by Bolzan et al. (2015) (44.3 d at 23.7 °C).

With respect to infestation of *A. grandis* per fruit, we observed a higher number of pupae at 25 °C (227.8), differing from the other treatments ($\chi^2 = 2495.79$; df = 4; P < 0.0001). At 15 and 20 °C, the number of pupae was similar (±144 pupae), while at 30 °C, a smaller number of pupae (38.8 pupae) was obtained, compared to the other treatments (Table 1), indicating that at this temperature development is compromised.

Sex ratio did not differ significantly between treatments and was around 0.5 (P < 0.05). For other fruit fly species of the genus *Anastrepha*, the sex ratio is also close to 0.5 (Garcia and Corseuil, 1998; Silva et al., 2007; Nunes et al., 2013), showing that the sex ratio of this group of insects is one female for one male, which is not affected by temperature.

The puparia weight within the temperature range evaluated showed significant differences (F = 18.73; df = 3; P < 0.0001) (Table 1). The highest values were recorded at 20 and 15 °C, although at 15 °C, the value did not differ from that at 25 °C. The lowest puparia weight was observed at 30 °C. Evaluating the results for viability, weight and number of pupae, we observed that the temperature that provided the best development of *A. grandis* was 25 °C, and temperatures between 15 and 25 °C were more favorable

Table 1
Biological parameters of immature stages of *Anastrepha grandis* reared in *Cucurbita pepo*. Mean values (±standard error), relative air humidity 70± 10% and photophase of 12 h.

Biological parameters	Temperatures (°C)				
	15	20	25	30	35
	[403 g]	[393 g]	[440 g]	[386 g]	[450 g]
Duration of egg stage (days)[1]	21.0 ± 0.45 a (18−22)	10.4 ± 0.09 b (9−13)	7.3 ± 0.05 c (6−9)	6.9 ± 0.08 c (5−9)	−
Eggs viability (%)[2]	12.2 ± 6.00 c	53.9 ± 5.67 b	91.7 ± 3.73 a	56.1 ± 14.23 b	0.00 ± 0.00 d
Duration of egg-to-pupa period (days)[1,4]	41.0 ± 0.11 a (33−48)	23.6 ± 0.06 b (20−32)	19.0 ± 0.06 d (15−26)	22.6 ± 0.24 c (19−28)	−
Duration of pupa stage (days)[1,4]	52.3 ± 0.26 a (46−61)	29.1 ± 0.13 b (24−33)	20.3 ± 0.06 c (17−23)	16.5 ± 0.2 d (14−26)	−
Pupa viability (%)[2]	89.4 ± 1.59 a	95.6 ± 1.11 a	96.1 ± 1.59 a	43.0 ± 3.97 b	0.00 ± 0.00 c
Duration of egg-to-adult period (days)[2]	93.3 ± 1.98 a (79−109)	52.7 ± 1.04 b (44−65)	39.3 ± 0.86 c (32−49)	39.1 ± 1.11 c (33−54)	−
Sex ratio[2]	0.45 b	0.51 a	0.53 a	0.54 a	−
Puparia weight (mg)[2]	22.5 ± 0.70 ab	22.9 ± 0.90 a	20.2 ± 0.80 b	13.6 ± 0.90 c	−
Average number of pupae per fruit[3]	144.7 ± 21.74 b	144.8 ± 28.51 b	227.8 ± 51.79 a	30.8 ± 19.56 c	0 ± 0.00 d

[1] Values represent the survival curves that do not differ by Log Rank test when followed by the same letter in the row.
[2] Values followed by the same letter in the row do not differ by the Tukey test (P < 0.05).
[3] Values followed by the same letter in the row also test differ by the Likelihood ratio (95% confidence).
[4] The duration of egg-to-pupa period covers the egg and larval stages. The duration of egg-to-adult period represents the egg laying to adult emergence.
Values in parentheses represent the minimum and maximum duration of stages and periods in each treatment.
Values in brackets represent the weight average (g) of the hosts tested.

than 30 °C.

In terms of fecundity, at 25 °C, females laid a greater number of eggs (232.2), differing significantly from the other temperatures tested (χ^2 = 1630.43; df = 3; P < 0.0001). Using the same host and the same temperature, Bolzan et al. (2015) observed fecundity almost twice as large, although there is a wide variation in fecundity of *A. grandis* in different hosts (106−538 eggs per female). At 15 and 20 °C, fecundity was similar, but it reduced at 30 °C (Table 2). These values show that as the temperature distances from 25 °C, fertility tends to decrease. The same behavior occurred for egg viability, where the highest percentage of viable eggs was registered at 25 °C (77.2%), differing significantly from the other temperatures tested (P < 0.05) (Table 2).

For the periods of pre-oviposition, oviposition and post-oviposition, we observed that as temperature increased within the range studied, the period duration decreases (Table 2). For the pre-oviposition period, the duration ranged from 75.6 to 27.3 d at 15 and 30 °C, respectively, with significant difference between survival curves (χ^2 = 75.5; df = 3; P = 0.0001). This shows that temperature influences development not only in the pre-imaginal period, but also in ovarian maturation. Similar behavior was observed for *A. fraterculus*, when it was determined that ovarian maturation is significantly dependent on temperature, meaning that the higher the temperature, the greater the number of mature

oocytes (Taufer et al., 2000). The same authors reported that, at 25 °C, 80% of females presented mature ovaries at 30 d of age and at 20 °C, to achieve frequency similar to 80%, the period was twice as long, 60 d.

The duration of the oviposition period ranged from 22.3 to 60.2 d at 30 and 15 °C, respectively, with significant difference between survival curves between 15 and 25 °C and those at 30 °C, during which, duration did not differ from that at 25 °C (χ^2 = 11.6; df = 3; P = 0.0087).

Female longevity was inversely proportional to temperature, ranging from 9.1 to 119.9 d at 35 and 15 °C, respectively. The longevity period differed significantly between temperatures, with exception of values registered for 20 and 25 °C (χ^2 = 144; df = 4; P < 0.0001) (Fig. 1). Average longevity of females found by Silva and Malavasi (1996) was 52.2 d exposed to constant temperature of 25 °C, similar to the longevity found at 30 °C in our study (Fig. 1).

3.2. Thermal requirements of A. grandis and model validation in the field

Obtaining the duration values of developmental stages allowed estimating a lower threshold temperature of 8.3 °C and thermal constant of 132.3 and 347.0 DD, respectively, for egg and pupa stages (Table 3). For the egg-to-adult period, the threshold

Table 2
Reproductive parameters of *Anastrepha grandis* reared in *Cucurbita pepo* at different temperatures. Mean values (±standard error), relative air humidity 70± 10% and photophase of 12 h.

Biological parameters	Temperatures (°C)				
	15 [17]	20 [18]	25 [20]	30 [20]	35 [0]
Fecundity (eggs)[1]	135.9 ± 21.20 c	147.3 ± 27.63 b	232.2 ± 51.61 a	78.2 ± 10.44 d	−
Fertility (%)[2]	57.0 ± 12.51 b	54.5 ± 20.70 b	77.2 ± 5.47 a	58.4 ± 7.16 b	−
Period of pre-oviposition (days)[3]	75.6 ± 3.67 a (37−101)	50.1 ± 3.90 b (16−79)	29.6 ± 1.61 c (18−45)	27.3 ± 1.87 c (17−54)	−
Period of oviposition (days)[3]	60.2 ± 13.51 a (1−156)	47.5 ± 8.81 a (1−120)	40.4 ± 9.05 ab (1−134)	22.3 ± 4.01 b (1−59)	−
Period of post-oviposition (days)[3]	20.4 ± 3.75 a (3−58)	13.3 ± 2.26 ab (0−33)	7.9 ± 1.37 b (0−22)	11.1 ± 1.67 b (1−31)	−

Values followed by the same letter in the row do not differ by the Likelihood ratio test (95% confidence)[1] and the Tukey test (P < 0.05)[2].
Values represent the survival curves that do not differ by Log Rank[3] test when followed by the same letter in the row.
Values in parentheses represent the minimum and maximum duration periods in each treatment.
Values in brackets represent the number of females that oviposited.
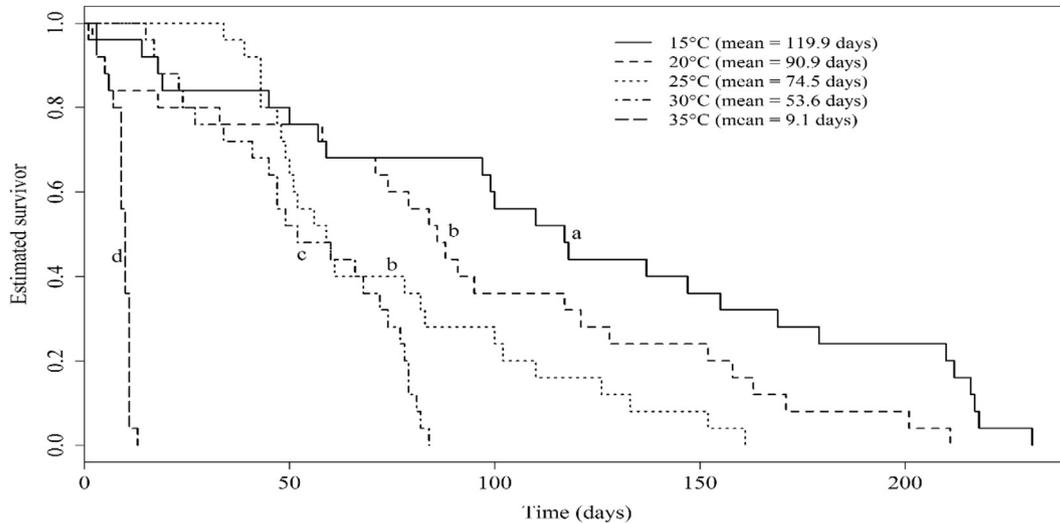
Fig. 1. Survival curve of *Anastrepha grandis* reared in *Cucurbita pepo* at different temperatures. Relative air humidity 70± 10% and photophase of 12 h. Survival curves followed by the same letter do not differ by the Log Rank test.

Table 3
Lower temperature threshold (LTT) and thermal constant (K) of *Anastrepha grandis* estimated in the laboratory.

Thermal requirements	Stages			Period
	Egg	Larva	Pupa	Egg-to-adult period
Lower temperature threshold (°C)[a]	8.3	–	8.3	5.2
Thermal constant (DD)[a]	132.3	–	347.0	858.7

[a] Values obtained from the method of Coefficient of Variation.

temperature was 5.2 °C and the thermal constant 858.7 DD (Table 3). For *A. fraterculus*, Machado et al. (1995) determined the threshold temperature and thermal constant for egg, larva and pupa stages and were 9.2 °C and 52.2 DD; 10.3 °C and 161.45 DD; 10.8 °C and 227.8 DD, respectively. This indicates that *A. grandis* is able to develop at lower temperatures and requires greater heat accumulation to complete its cycle; therefore, a longer developmental period was obtained compared to *A. fraterculus*.

When squash infested with eggs of *A. grandis* was exposed to varying temperature conditions, we obtained pupae viability of 54.8%. The lower pupae viability in field conditions, compared to

viability found in laboratory at constant temperatures, may be related to high relative humidity recorded during the developmental period of the insect, remaining above 80% virtually throughout the period. Data on relative humidity, average daily temperature and rainfall are shown in Fig. 2.

We recorded for the developmental period of *A. grandis* reared in squash under field conditions, in the period between April 2 and June 26, 2014, daily average temperature of 17 °C, ranging from 24.3 °C at the beginning of the experiment to 15.5 °C at the end, once temperature decreased in the study period (Fig. 2). Thus, the average duration of the egg-to-adult period for *A. grandis* kept
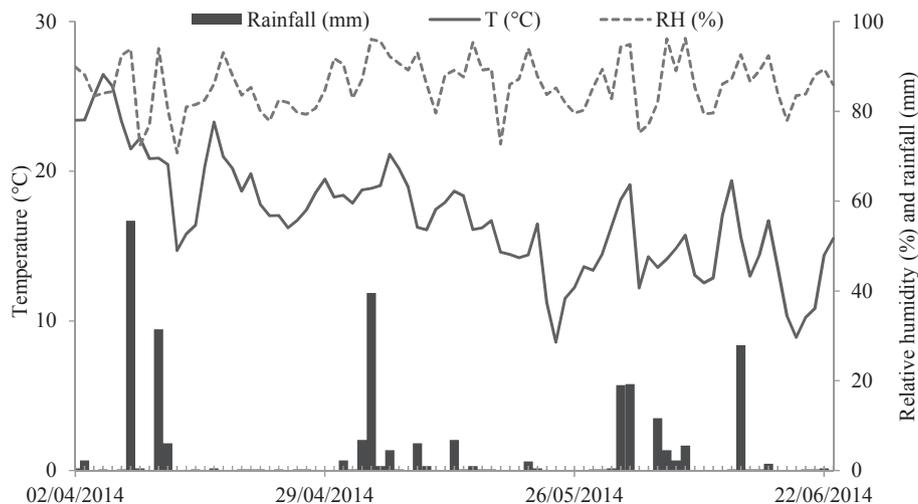
Fig. 2. Daily records of rainfall and mean air temperature (°C) and humidity (%) for the period when the development of *Anastrepha grandis* was observed under field conditions.

Table 4
Comparison of degree-days accumulation and egg-to-adult period observed (field) and estimated (laboratory). Accumulated degrees-days and average duration of egg-to-adult period (±standard error) of *Anastrepha grandis* based on the average daily temperature obtained in the field (17 °C) compared to respective values estimated in laboratory.

Degrees-day (DD)				Egg-to-adult period (days)			
Observed (field)	Estimated (laboratory)	Difference		Observed (field)	Estimated (laboratory)	Difference	
		DD	%			days	%
937.9 ± 8.16 a	858.7 ± 49.43 a	79.2	9.2	79.7 ± 1.15 a	74.8 ± 1.28 b	4.8	6.4

Means followed by the same letter does not differ significantly from each other by the *t*-test (P < 0.05).

under field conditions was 79.7 d.

Data estimated in the laboratory with constant temperature allowed comparison, through the *t*-test (P < 0.05) of the thermal constant 927 DD observed in field and the constant estimated in laboratory of 858.7 DD. The data also allowed comparison of the egg-to-adult period observed in the field (79.7 d) with that estimated in laboratory (74.8 d) (Table 4). The number of degree-days necessary for *A. grandis* to complete its biological cycle (egg-to-adult) did not differ significantly between the observed and estimated values; however, the duration of the egg-to-adult period showed significant difference from the estimated period in laboratory (P < 0.05). The difference percentage between degree-days and the egg-to-adult period was below 15%. For Higley et al. (1986) degree-days models with up to 15% of difference between estimated and observed values could be used in strategies for pest control.

Determining the thermal requirements plays an important role in pest management, such as the forecast of population peaks and sampling times. The degree-days methods allow predicting occurrence of insect pests, therefore, determining with greater precision the adoption of a control method. The results obtained in this study could be used in the field to assist in the management of *A. grandis*, important pest of cucurbits in South America.

The results show that temperature significantly affects the development and reproduction of *A. grandis*, thus, greater viability of immature stages was obtained at 25 °C, providing greater fecundity and fertility, in addition to a larger number of insects per fruit. Temperatures at 15 and 20 °C provide better reproductive conditions, compared to 30 °C. These data could be linked to low threshold temperatures found in the studied stages, showing that this insect has ability to develop satisfactorily in tropical and temperate regions. The study also showed that *A. grandis* has lower reproductive capacity and low viability of immature when exposed to higher constant temperatures. Therefore, the data presented in this study provide subsidies for the management of *A. grandis* in cucurbit crops in different production areas, allowing to predict the time needed to for the insect to complete its biological cycle, the occurrence of population peaks throughout the year and the number of generations in a given time period.

Acknowledgments

References

Bolzan, A., Nava, D.E., Garcia, F.R.M., Valgas, R.A., Smaniotto, G., 2015. Biology of *Anastrepha grandis* (Diptera: Tephritidae) in different cucurbits. J. Econ. Entomol. 108, 1034—1039. http://dx.doi.org/10.1093/jee/tov056.

Bolzan, A., Diez-Rodríguez, G.I., Garcia, F.R.M., Nava, D.E., 2016. *Anastrepha grandis*: Bioecologia e Manejo. Embrapa Clima Temperado (Documentos/Embrapa Clima Temperado, 404).

Bolzan, A., Smaniotto, G., Garcia, F.R.M., Nava, D.E., 2014. Risco mitigado. Cultiv. Hortaliças Frutas 12, 17—19.

Costa Lima, A., 1926. Sobre as moscas das frutas que vivem no Brasil. Chácaras Quintais 34, 20—24.

Garcia, F.R.M., Corseuil, E., 1998. Análise faunística de moscas-das-frutas (Diptera: Tephritidae) em pomares de pessegueiro em Porto Alegre. Rio Gd. do sul. Rev. Bras. Zool. 15, 1111—1117. http://scielo.br/pdf/rbzool/v15n4/v15n4a28.pdf.

Haddad, M.L., Parra, J.R.P., 1984. Métodos para estimar as exigências térmicas e os limites de desenvolvimento dos insetos. Fealq, Piracicaba, SP, Brazil, p. 45.

Higley, L.G., Pedigo, L.P., Ostlie, K.R., 1986. Degday: a program for calculating degree-days, and assumptions behind the degree-day approach. Environ. Entomol. 15, 999—1016. http://dx.doi.org/10.1093/ee/15.5.999.

Machado, A.E., Salles, L.A.B., Loeck, A., 1995. Exigências térmicas de *Anastrepha fraterculus* (Wied.) e estimativa do número de gerações anuais em Pelotas, RS. An. Soc. Entomol. Bras. 24, 573—578.

Malavasi, A., Barros, M.D., 1988. Comportamento sexual e de oviposição em moscas-das-frutas (Tephritidae). In: Souza, H.M.L. (Ed.), Moscas-das-frutas no Brasil. Fundação Cargill, Campinas, SP, Brazil, pp. 25—53.

Malavasi, A., Morgante, J.S., Zucchi, R.A., 1980. Biologia de "moscas-das-frutas" (Diptera: Tephritidae): lista de hospedeiros e ocorrência. Rev. Bras. Biol. 40, 9—16.

(MAPA) Ministério da Agricultura, Pecuária e Abastecimento Secretaria de Defesa Agropecuária, 2006. Áreas Livres de Pragas: Áreas livres de *Anastrepha grandis*. Brasilia, DF, Brazil. http://www.agricultura.gov.br/arq_editor/file/vegetal/ Importacao/Requisitos%20Sanit%C3%A1rios/Rela%C3%A7%C3%A3o%20de% 20Pragas/pragasvegetaisv1.pdf.

(NAPPO) North American Plant Protection Organization, 2009. Phytosanitary Alert System. Outbreak of *Anastrepha Grandis* (South American Cucurbit Fruit Fly) in Panama. http://www.pestalert.org/viewNewsAlert.cfm?naid=76.

Nava, D.E., Parra, J.R.P., 2003. Biology of *Cerotoma arcuatus* (Coleoptera: chrysomelidae) and field validation of a laboratory model for temperature requirements. J. Econ. Entomol. 96, 609—614. http://dx.doi.org/10.1603/0022-0493-96.3.609.

Norrbom, A.L., 2000. Host Plant Database for Anastrepha and Toxotrypana (Diptera: Tephritidae: Toxotrypani), Diptera Data Dissemination Disk 2. USDA-APHIS, Washington, DC, USA.

Nunes, A.M., Costa, K.Z., Faggioni, K.M., Costa, M.L.Z., Gonçalves, R.S., Walder, J.M.M., Garcia, M.S., Nava, D.E., 2013. Dietas artificiais para a criação de larvas e adultos da mosca-das-frutas sul-americana. Pesq. Agropec. Bras. 48, 1—6. http://dx.doi.org/10.1590/S0100-204X2013001000001.

Paranhos, B.A.J., 2008. Moscas-das-frutas que oferecem riscos à fruticultura brasileira. In: Simpósio Internacional de Vitivinicultura, Anais do Simpósio Internacional de Vitivinicultura, Embrapa Semi-Árido, Petrolina, PE, Brazil.

Parra, J.P.R., 2002. Criação massal de inimigos naturais. In: Parra, J.P.R., Botelho, P.S.M., Botelho, B.S., Bento, J.M.S. (Eds.), Controle Biológico No Brasil. Monole, São Paulo, SP, Brazil, pp. 143—163.

Pimentel-Gomes, F., 2009. Estatística Experimental, fifteenth ed. Fealq, Piracicaba, SP, Brazil, p. 451.

R Development Core Team, 2013. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rabb, R.L., Defoliari, G.R., Kennedy, G.G., 1984. An ecological approach to managing insect populations. In: Huffaker, C.B., Rabb, R.L. (Eds.), Ecological Entomology. John Wiley, New York, NY, USA, pp. 697—728.

SAS Institute, 2002. SAS System — SAS/STAT. Computer Program, Version 9.2. By SAS Institute, Cary, NC, USA.

Silva, A.G.D.'.A., Gonçalves, C.R., Galvão, D.M., Gonçalves, A.J.L., Gomes, J., Silva, N.M., Simoni, L., 1968. Quarto catálogo dos insetos que vivem nas plantas do Brasil, seus parasitos e predadores. Tomo 1. Parte 2. Ministério da Agricultura, Rio de Janeiro, RJ, Brazil.

Silva, J.G., Malavasi, A., 1993. The status of honeydew melon as a host of *Anastrepha grandis* (Diptera: Tephritidae). Fla. Entomol. 76, 516—519. http://dx.doi.org/10.2307/3495652.

Silva, J.G., Malavasi, A., 1996. Life cycle of *Anastrepha grandis*. In: McPheron, B.A., Steck, G.J. (Eds.), Fruit Fly Pests: a World Assessment of Their Biology and Management. St. Lucie Press, Delray Beach, FL, USA, pp. 347—351.

Silva, R.A. da, Nascimento, D.B. do, Deus, E. da G. de, Souza, G.D. de, Oliveira, L.P.S. de, 2007. Hospedeiros e parasitóides de Anastrepha spp. (Diptera: Tephritidae) em Itaubal do Piririm, Estado do Amapá, Brasil. Cienc. Rural. 37, 557—560.

http://dx.doi.org/10.1590/S0103-84782007000200041.

Silveira Neto, S., Nakano, O., Barbin, D., Villa-Nova, N.A., 1976. Manual de ecologia dos insetos. Agronômica Ceres, Piracicaba, SP, Brazil, p. 419.

Taufer, M., Nascimento, J.C. do, Cruz, I.B.M. da, Oliveira, A.K. de, 2000. Efeito da temperatura na maturação ovariana e longevidade de *Anastrepha fraterculus* (Wied.) (Diptera: Tephritidae). An. Soc. Entomol. Bras. 29, 639–648. http://dx.doi.org/10.1590/S0301-80592000000400003.

Zucchi, R.A., 2000a. Espécies de Anastrepha, sinonímias e plantas hospedeiras e parasitoides. In: Malavasi, A., Zucchi, R.A. (Eds.), Moscas-das-frutas de importância econômica no Brasil. Conhecimento básico e aplicado. Holos, Ribeirão Preto, SP, Brazil, pp. 41–48.

Zucchi, R.A., 2000b. Taxonomia. In: Malavasi, A., Zucchi, R.A. (Eds.), Moscas-das-frutas de importância econômica no Brasil. Conhecimento básico e aplicado. Holos, Ribeirão Preto, SP, Brazil, pp. 13–24.