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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.

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

* Corresponding author.
E-mail address: ander_bolzan@hotmail.com (A. Bolzan).

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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 ± 10% and a photophase of 12 h, until the emergence of adults.

The adults were kept in plastic cages (60 × 40 × 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 ± 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 × 40 × 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 × 10 × 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 ± 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 × 4.8 × 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