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Full Length Article

Effect of emergence time on the reproductive parameters of mass-reared *Anastrepha ludens* (Diptera: Tephritidae) flies

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ABSTRACT

In the mass rearing of fruit flies, the adult emergence time is gradual, due to factors such as variation in food consumption in the larval phase, the female oviposition behavior and environmental conditions, among others. Here, we investigate the effect of emergence time on sex ratio, fecundity, fertility, body size and mating performance in mass-reared *Anastrepha ludens* (Diptera: Tephritidae) flies. The results of the study indicated that the emergence of the flies is gradual and lasts approximately 96 h, but the highest proportion of emerged flies was observed at 48 h. The flies emerging at 24 and 48 h showed higher fecundity than those emerging at 72 and 96 h, but there were no significant differences in the sex ratio, fertility and male mating performance. Body size was significant, the flies that emerged at 24, 48 and 72 h were bigger than those emerged at 96 h. The discussion focuses on the effect of emergence time on the rearing processes and release of the Mexican fruit fly.

Introduction

The mass rearing of insects to apply the sterile insect technique (SIT) represents a great advance in pest management (Dyck et al., 2005). In addition to being environmentally friendly, the technique is a fundamental method for suppressing infestations of fruit flies that attack a great variety of economically important fruits (Hendrichs et al., 2002). The SIT consists of the rearing, sterilization and release of a large number of insects into the target pest population (Knipling, 1955, 1979), and it requires both the ability to produce large quantities of insects at a reasonable cost as well as the preservation of a behavioral repertoire in reared insects that allows them to successfully compete with wild males and transfer their sperm to wild females, thus inducing sterility in the target population (Rull and Barreda-Landa, 2007). This implies the maintenance of high quality standards for the rearing plants that generate competitive insects in the field. Quality control in rearing processes has generally been limited to the evaluation of insect attributes such as larval weight, pupal weight, pupation percentage, flight ability, propensity at mating and adult longevity (FAO/IAEA/USDA, 2014), all of which provide a general overview of the quality of the insects. However, there are other important parameters that should also be considered, including the physiological development time of insects (Miyatake, 1995; Meza et al., 2005; Telles-Romero et al., 2011).

In *Anastrepha ludens* (Loew), the Mexican fruit fly, pupation time has been documented as having a significant effect on pupal weight and the sexual performance of adults (Meza et al., 2005). Male *Anastrepha obliqua* (Macquart) with a longer pupal development time exhibit greater sexual competitiveness with wild flies (Telles-Romero et al., 2011). In *Bactrocera cucurbitae* (Coquillett), an association was also documented between a short larval development time and circadian rhythm and age at sexual maturity (Miyatake, 1997) as well as with body size and survival rate (Miyatake, 1995). These studies support the idea that physiological development time has a significant effect on insect behavior and may be related to changes at the genetic level (Shimizu et al., 1997).

The Mexican fruit fly is an economically damaging pest of citrus and mango crops in Mexico and Central America, where seasonal infestations disrupt production, resulting in high economic losses each year (Aluja, 1994). This species is widely distributed in the tropical and subtropical regions of Mexico and Central America (Hernández-Ortiz, 1993), and the SIT is used to control this pest in Mexico. The Moscafrut facility (Planta Moscafrut) in Metapa de Dominguez, Chiapas produces hundreds of millions of flies that have been artificially bred, sterilized and sent for weekly release since 1992 (Orozco-Dávila et al., 2017).

The objective of this study was to evaluate the effect of emergence time on the parameters of emergence, sex ratio, fecundity, fertility and mating performance in *A. ludens* rearing flies through laboratory and field experiments. The discussion focuses on the effect of emergence

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time on the mass-rearing and release of the Mexican fruit fly.

Materials and methods

Study site

Experiments were conducted under field and laboratory conditions. The laboratory tests were carried out in the Moscafrut facility in Metapa de Dominguez, Chiapas, Mexico under the following environmental conditions: temperature of 25 ± 1°C, relative humidity of 65 ± 5%, light intensity of 300 ± 50 lx with a 12 L: 12 D photoperiod. Field trials were conducted in field cages in an Ataulfo variety mango orchard (14°49′47.2"N 92°11′42.8"W and an altitude of 100 masl) under variable temperature conditions from 24 to 29 °C, relative humidity from 68 to 85% and light intensity from 1283 to 0 lx. The field cages (3 m in diameter by 2 m in height) were supported by a metal structure (Calkins and Webb, 1983; Chambers et al., 1983) with a bitter orange tree (*Citrus x aurantium* L.) approximately 1.8 m high.

Insects

The fertile, mass-reared flies were obtained as pupae from the Moscafrut Plant. The wild flies were collected as fruit larvae infesting bitter orange from the Soconusco Region of Chiapas. The larvae and pupae were managed as described in Orozco et al. (2013).

The pupae were placed in 30x30x30-cm wooden cages covered with Tull mesh (2 mm) until emergence. The emerged flies were separated by sex and given water and a dry mixture of sugar (standard brown) and enzymatically hydrolyzed yeast (MP Biomedicals, LLC) *ad libitum* in a 3:1 ratio as a food until they reached sexual maturity (10–12 d for reared flies and 18–20 d for wild flies).

Emergence and sex ratio

The flies used for this test were reared as described in Orozco-Dávila et al. (2017). Eggs were collected of the egging cages within a period of 10 h (7:00 to 17:00 h). Each cage produces an average of 3.031 million eggs per day (140,000 flies per cage). The eggs produced were mixed and sent to the incubation room where they stay for *ca.* 3–4 days. The new-born larvae were placed on an artificial diet formulated according to the Moscafrut facility. After nine days of larval development were placed in trays with wet vermiculite to promote pupation, the which lasts 13 days at a temperature of 25 °C (Orozco-Dávila et al., 2017). Two days before adult emergence, a total of 1000 fertile pupae were randomly selected from a production batch and placed in 30 × 30 × 30-cm wooden cages covered on each side by a 2-mm Tull mesh. At the beginning of emergence, the adults were separated every 24 h and placed in the wooden cages as described above. For each emergence time, the number of flies that emerged were counted, and the sex ratio was determined according to the FAO/IAEA/USDA (2014). Each batch of 1000 pupae was considered one experimental unit, and there were 20 replicates of ten different batches of flies.

Fecundity and fertility

To determine the degree of fertility and fecundity of the flies at each emergence time, ten pairs of flies were placed in a wooden 30x30x30-cm cage with water and optimal food as described above.

Egg collection was carried out by means of an "oviposition device" (a cylindrical vessel 5 cm high and 6.5 cm in diameter covered at the base with tergal cloth and coated with silicone on the inside) placed at the top of the cage and moistened with water. The eggs were collected and quantified daily for 12 d and then placed in humid chambers (plastic Petri dishes with water-moistened sponges) and incubated at 25 °C until the larvae hatched. The number of oviposited eggs/female/d was a measure of fecundity (fecundity = number of oviposited eggs/number of females), and the hatching percentage was used as a measure of fertility (% fertility = number of hatched eggs/oviposited eggsx100). The estimated age of the flies at the time of evaluation was 9 to 22 d. The experimental unit consisted of a cage with ten pairs of flies, and ten replicates consisting of ten different batches of insects were performed at each emergence time.

Adults size

Body size was measured at each emergence time according to the methodology porposed by Rodriguero et al. (2002). Two morphometric characters highly related to the body size were measured: Thorax length (TL) and wing length (WL). Each of the structures was separated of the individuals with entomological tweezers and were placed in a cavity slide (26 × 76 mm) with a drop of saline water measurements were performed by means of photographs taken on a stereoscope (SMZ745T NIKON, Japan) with a 3 megapixel digital camera (ProgRes CT3, JENOPTIK Optical Systems, Jena, Germany). The measurements were made using ImagenJ software 1.37 (US National Institute of Health, Bethesda, MD, USA). A total of 45 individual were dissected from each emergence time. Each individual was considered as an experimental unit. Three different production batches were evaluated, with fifteen replicates for each batch.

Mating performance

Tests were carried out between mass-reared flies at each emergence time and wild flies, all of which were sexually mature (flies with capability to reproduce). To individually identify the flies in the field cages to which they were introduced, the flies were marked with a small paper label (2 mm in diameter) with a printed number (Arial type size 3) glued on the thorax 48 h before the test (Meza et al., 2005). A total of 10 males from each emergence time (24, 48, 72 and 96 h), 10 wild males and 25 wild virgin females were released into a field cage; a sex ratio of 2:1 was always maintained.

The number of mating was recorded from 4:00 pm to 7:00 pm, a range that covered the period of peak sexual activity for this species (Aluja et al., 2000). Each cage was considered an experimental unit, and 15 cages were evaluated (replicas) using three different batches of flies.

Data analysis

A completely randomized experimental design was applied in all experiments. The emergence and sex ratio, prior to analysis were normalized using the Box-Cox transformation. A linear model was adjusted through generalized least squares with serial autocorrelation of order 1.The fertility data that were anticipated to be analyzed were normalized by arcsine transformation. The normality of the residuals and the homogeneity of the variance were evaluated with a Shapiro-Wilk test and a Bartlett's test, respectively. The fecundity, fertility, body size and sexual performance data were analyzed using an analysis of variance (ANOVA) followed by a means comparison using Tukey's test ($P < 0.05$). A linear Pearson (r) correlation was calculated to determine the correlation between body size and emergence time. All analyses were performed using the statistical software Minitab 18 (2017).

Results

Emergence and sex ratio

The results of the study indicated that the emergence of the reared flies lasts 96 h. The highest percentage of emerged flies was observed at 48 h (59%) ($X^2 = 0.1600$; df = 1,79; $P = 0.689$). The 24-h flies (20%) were not significantly different from the 72-h flies (19%), and the