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Effects of Gamma Radiation on the Ovarian Development of the South American Fruit Fly, *Anastrepha fraterculus* (Wiedemann, 1830)

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ABSTRACT

The South American fruit fly, *Anastrepha fraterculus* (Wied.) represents 98.5% of the flies captured in commercial apple orchards from Brazil. Looking for environmentally friendly alternatives to suppress this fruit fly pest, researchers from USP and EMBRAPA, along with the Brazilian Association of Apple Producers, created the MOSCASUL *Biological Control Center*, which intends to apply an Area-Wide Integrated Pest Management Program that integrates the Sterile Insect Technique (SIT). The assurance of sterility of the released flies is a critical assumption of an SIT program, since the unintentional release of thousands of fertile females could cause severe damages to the orchards nearby. Females of *A. fraterculus* are more radiosensitive than males, but the morphology of the ovaries of the sterile females was not investigated so far. The objective of this study, therefore, was to characterize the morphology of the ovaries of *A. fraterculus* treated with different doses of gamma radiation. Pupae with 48 h before adult emergence were irradiated with 0 (control), 15, 25, 35, and 45 Gy at the *Gammabeam*-650® irradiator from CENA/USP. Twelve hours after initial emergence, adults were sorted by sex, and for each radiation dose, 1 day old nonirradiated males were placed with irradiated females of the same age in cages. Adult females were then dissected in a saline solution at 1, 7, and 15 days of age, and the ovaries were examined under stereomicroscope. The length, width and area of each ovary were measured from the images made. On the 1st day after the emergence of the adults, no morphological differences were observed among irradiated and nonirradiated ovaries. On the 7th day, however, the nonirradiated ovaries showed to be larger than the irradiated ones. Doses of 15 Gy or higher induced complete atrophy of the ovaries and, on the 15th day, no signs of regeneration of the ovaries were observed. On the last day of observation, the irradiated ovaries presented mean area values 9-10 fold smaller than the values of the nonirradiated ovaries. This study proved that the dose of 40 Gy would guarantee the release of fully sterilized females in the field, and that for adults more than 7 days old, the ovarian dissection technique could be used in laboratory as a quick confirmation of sterility of trapped females that were not adequately marked with fluorescent dyes.

1. INTRODUCTION

The South region of Brazil concentrates 99% of the apple growing areas, with a harvest of more than 1.1 million tons of fruits and a supply chain moving up to USD 3 billion annually [1]. Despite the occurrence of several species of fruit flies in the region, the South American fruit fly, *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae) represents 98.5% of the flies captured in commercial apple orchards [2]. For example, the gross value of yield losses associated with the chemical control of this pest were estimated at more than USD 25 million per year [3]. Looking for environmentally friendly alternatives to suppress this fruit fly pest, Brazilian researchers from USP and EMBRAPA, along with the Brazilian Association of Apple Producers, created the MOSCASUL *Biological Control Center* located at the *Estação Experimental* de *Vacaria* of Embrapa Uva & Vinho, Rio Grande do Sul state [4]. This center received an investment of US$ 600,000 from the Ministry of Agriculture, Livestock and Food Supply (MAPA) at the end of 2014 to build a mass-rearing facility to produce millions of sterile insects and parasitoids for the biological control of *A. fraterculus* in Southern Brazil.

An Area-Wide Integrated Pest Management (AW-IPM) Program that integrates the Sterile Insect Technique (SIT) as a control tactic depends fundamentally on good quality mass-reared insects to succeed [5]. Mass-reared sterile flies and parasitoids will be released in forests and areas adjacent to commercial groves to keep fruit fly populations from expanding into the commercial apple orchards.

The assurance of sterility of the released flies is a critical assumption of an SIT program, since the unintentional release of thousands of fertile females could cause severe damages to the orchards nearby. Most fruit fly species are often irradiated at the late pupal stage [6], which must receive an appropriate dose for complete female sterilization. A dose of only 40Gy applied on *A. fraterculus* pupae at one or two days prior to adult emergence can result in flies 99% sterile with minimal effects on quality or sexual competitiveness [7]. Females of *A. fraterculus* are more radiosensitive [Mastrangelo, unpublished data], but the morphology of the ovaries of sterile females has not been investigated so far.

Furthermore, sterile-released flies are usually marked with fluorescent dyes before release to distinguish them from the wild flies caught in surveillance traps [8]. However, a small number of flies (less than 1%) can escape from being marked [5]. It is very important to be able to distinguish these sterile unmarked flies from wild fertile flies if they are captured in commercial apple orchards, since false detections could trigger unnecessary and costly control actions over the surveyed area.

The objective of this study, therefore, was to characterize the morphology of the ovaries of the South American Fruit Fly treated with different doses of gamma radiation, also aiming to develop an additional tool that could serve to determine the sterility of trapped unmarked females.

2. MATERIAL AND METHODS

The study was conducted at the Laboratório de Irradiação de Alimentos e Radioentomologia (LIARE) of CENA/USP at Piracicaba, Brazil. The *A. fraterculus* adults used for the experiments were obtained from the colony maintained by the procedures described by Walder et al. [9]. The *A. fraterculus* larvae of this colony were reared using an artificial diet based on wheat germ, yeast, sugar, hydrochloric acid, sodium benzoate, nipagin, agar and water [9]. Larvae collected on the 8th day, after they began to crawl from the diet, were allowed to pupate on moistened vermiculite and kept in controlled environment room (25 ºC, 65% RH, and a photoperiod of 4:20 [L:D] h).

The batches of pupae with 48 h before adult emergence destined for the tests were irradiated with 0 (control), 15, 25, 35, and 45 Gy. The source of radiation was a *Gammabeam*-650® irradiator (MDS Nordion International Inc., Canada) with an activity of 6.5 TBq (177.5 Ci) and dose rate of 4 Gy/min. (or 0.24 kGy/h) at 25 ºC (Fig. 1). All irradiations were performed under normal atmospheric conditions (free oxygen). For each irradiation, dosimetry was performed with a Fricke Dosimeter [10].

Figure 1. *Gammabeam*-650® irradiator from CENA/USP used to sterilize the flies as pupae 48 h before the emergence of the adults.

Twelve hours after initial emergence, adults were sorted by sex. For each radiation dose, one day old nonirradiated males were placed with irradiated females of the same age in cages made of a 2-liter cylinder flask (*Plaszom* model PRT2000-TL150). Control cages with

nonirradiated flies were also set up. In each cage, the adults were fed *ad libitum* with water and a mixture of sugar and hydrolyzed yeast at 4:1 rate [11]. These cages were distributed in a randomized design with 4 replicates for each treatment, with 25 couples per cage.

Adult females were dissected in 0.85% sodium chloride (NaCl) solution [12] at 1, 7, and 15 days of age. After removal of the reproductive system, the ovaries were transferred to a clean microscope slide and examined as whole mounts under a Leica MDG41® stereomicroscope (Leica Microsystems, Heerbrugg, Switzerland). Images of the freshly dissected organs were made with a Leica Digital DFC450® camera on the stereomicroscope for further characterization (Fig. 2).

Figure 2. Characterization of the ovaries from irradiated and nonirradiated females of *Anastrepha fraterculus* using a Leica MDG41® stereomicroscope.

The length (mm), width (mm) and area (mm^2) of each ovary on the three different days were measured from the images. For these measurements, the one-way analysis of variance *F*-test was calculated for the means at the 5% of significance (ANOVA), and, when a significant difference was found, the Tukey's honestly significant difference (HSD) test ($\alpha = 5\%$) was applied to compare the means. The Bartlett and Shapiro-Wilk tests were performed to verify the homocedasticity assumptions and the normality of the errors, respectively. The analyses were performed by the statistical program SAS 9.1 [13].

3. RESULTS

The effects of gamma radiation on the ovarian development of *A. fraterculus* (Fig. 3) are presented in Table 1. As the flies were irradiated during the mature pupae stage, the reproductive system of the females was in the initial stages of development, which is characterized by an intensive proliferation of germ cells, when cell differentiation and cell migration are extremely radiosensitive [6].

On the 1st day after the emergence of the adults, no morphological differences were observed among irradiated and nonirradiated ovaries (Fig. 3). The overall mean values for the length, width and area of the ovaries from the treatments were 0.4±0.03 mm ($F_{3, 20}$ = 0.3; C.V. = 2.8%; P = 0.9), 0.32±0.02 ($F_{3, 19}$ = 2.5; C.V. = 3.5%; P = 0.1) and 0.1±0.007 mm^2 ($F_{3, 20}$ = 3.02; C.V. = 17.1%; P = 0.06).

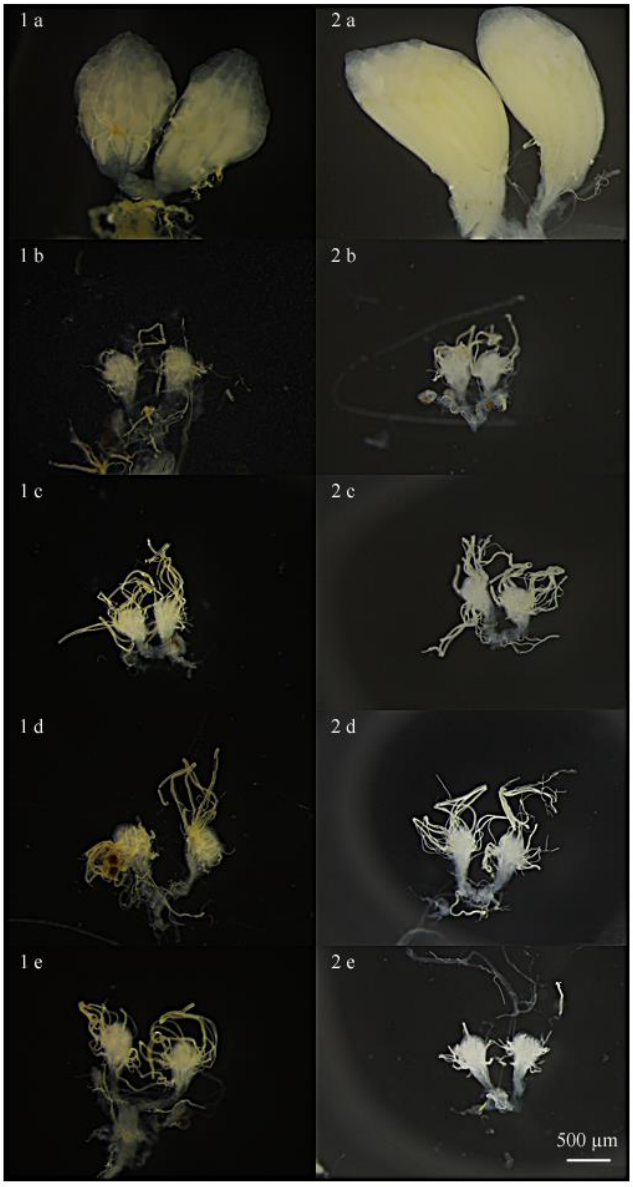

Figure 3. Ovarian development of *A. fraterculus* irradiated as pupae with different doses of gamma radiation (a-Control; b-15 Gy; c-25 Gy; d-35 Gy; e-45 Gy) and observed at different times (1 - 7 day old flies; 2 - 15 day old flies).

On the 7th day, however, the nonirradiated (control) ovaries showed to be larger than the irradiated ones, and abnormally developed ovaries with few ovarioles could already be observed in females exposed to 15 Gy of gamma radiation. The doses of 15 Gy and higher caused damage to the ovaries, making them smaller as demonstrated by the variables evaluated. The length, width and area of the ovaries from the flies irradiated with 15 Gy or

higher did not differ significantly among them, but they did differ significantly from the means of the control group (Table 1). On the 7th day (Fig. 3), the irradiated ovaries presented mean area values 9-10 fold smaller than the values of the nonirradiated ovaries.

Table 1. Measurements (means ± standard errors) of the ovaries from fertile and irradiated females of *Anastrepha fraterculus* at two different times (7 and 15 days after the emergence of the adults).

Measurement	Age of the Fly (days old)	Treatment					ANOVA
		Control	15 Gy	25 Gy	35 Gy	45 Gy	
Length (mm)	7	1.49 ± 0.08 a	0.47 ±0.01 b	0.45 ± 0.02 b	0.44 ± 0.02 b	0.44 ± 0.01 b	$F_{4,24}$ = 166.8; C.V.= 4.8%; $P<10^{-3}$
	15	2.64 ± 0.13 a	0.5 ± 0.02 b	0.51 ± 0.01 b	0.41 ± 0.03 b	0.48 ± 0.01 b	$F_{4,27}$ = 247.5; C.V.= 7.7%; $P<10^{-3}$
Width (mm)	7	0.71 ± 0.1 a	0.27 ±0.01 b	0.28 ± 0.01 b	0.32 ± 0.01 b	0.29 ± 0.01 b	$F_{4,24}$ = 15.9; C.V.= 7.6%; $P<10^{-3}$
	15	0.99 ± 0.06 a	0.39 ±0.06 b	0.28 ±0.01 b	0.25 ± 0.01 b	0.29 ± 0.02 b	$F_{4,27}$ = 63.1; C.V.= 6.5%; $P<10^{-3}$
Area (mm^2)	7	0.92 ± 0.05 a	0.09 ± 0.002 b	0.093 ± 0.005 b	0.106 ± 0.005 b	0.097 ± 0.01 b	$F_{4,24}$ = 221.9; C.V.= 5.4%; $P<10^{-3}$
	15	1.99 ± 0.09 a	0.15 ±0.02 b	0.11±0.004 b	0.10 ± 0.01 b	0.12 ± 0.002 b	$F_{4,27}$ = 321.4; C.V.= 7.5%; $P<10^{-3}$

* Means (± SE) followed by the same letters in the lines do not differ significantly by the Tukey's test ($P > 0.05$).

Doses of 15 Gy or higher induced complete atrophy of the germinal cell structures and, on the 15th day of observation, no female showed ovaries with signs of regeneration (Fig. 3). This was corroborated by a sterilization experiment where no eggs were laid by females treated with doses higher than 20 Gy [Mastrangelo, unpublished data].

The CENA/USP has established a sterilizing radiation dose applied to mature South American Fruit Fly pupae at 40 Gy [Mastrangelo, personal communication] for use in field releases in South Brazil. As demonstrated by this study, radiation doses higher than 15 Gy cause complete and irreversible ovary atrophy.

4. CONCLUSION

As the atrophied ovaries are easily distinguished from nonirradiated ovaries after 7 days of adult life, the ovarian dissection technique can be used in laboratory as a quick confirmation of sterility of trapped females that are not adequately marked with fluorescent dyes. However, flies younger than 7 days cannot be distinguished on the basis of ovary development, regardless of the radiation treatment.

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