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Sterilization doses under hypoxia for strains of *Anastrepha fraterculus* (Diptera: Tephritidae)

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1. Introduction

Of the 250 species of the genus *Anastrepha* Schiner present in the Neotropics, the South American fruit fly *Anastrepha fraterculus* (Wiedemann) is one of the most economically important tephritids [1]. The availability of modern environment-friendly control methods for this pest, such as the Sterile Insect Technique (SIT), is very important for fruit growers. A common strategy used to maintain sterile fly quality in mass-rearing facilities without sacrificing sterility is to irradiate the insects under an oxygen-reduced atmosphere. So far, sterilizing doses for *A. fraterculus* have only been determined under normoxia [2, 3, 4]. Therefore, our study reported the dose-sterility response under hypoxia for two different *A. fraterculus* strains. Recently, a genetic sexing strain (GSS) of *A. fraterculus* (named GSS-89) derived from a Brazilian-1 morphotype population that was based on a pupa color dimorphism (i.e., males emerge from brown pupae while the females emerge from black pupae) was developed through a partnership between the Center for Nuclear Energy in Agriculture (CENA/USP) and the Insect Pest Control Laboratory (IPCL) of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Seibersdorf [5]. The irradiated pupae were derived from a bisexual strain (a Brazilian-1 population) and the GSS-89 [5, 6].

2. Methodology

The *A. fraterculus* pupae used for the sterilization tests under hypoxia were derived from colonies of a bisexual strain and the GSS-89 maintained at the IPCL [7]. All pupae were irradiated 24 h or less before adult emergence in a ^{60}Co irradiator (GammaCell-220, Nordion, Canada) that had an activity of 203.8 TBq and a dose rate of 80 Gy/min. Two hours prior to the irradiation, pupae were transferred to sealed glass bottles (25 mL) and the O_2 and CO_2 levels were monitored at 22 ºC using a gas-sensor device (Dansensor® CheckMate3, Ringsted, Denmark). When the oxygen concentration was lower than 3% inside the vials, they were taken to the irradiator. To provide dose uniformity, the sealed vials were placed at the center of the radiation chamber. For each exposure, dosimetry was performed using the Gafchromic® dosimetry system [8]. Pupae were treated with eight doses (30, 40, 50, 60, 70, 80, 90, and 100 Gy) in addition to the untreated control. After irradiation, the vials were opened and treated pupae were placed in cages until adult emergence.

Emerged flies of the GSS-89 and the bisexual strain, henceforth named GSS and VAC, respectively, were manually sexed and the sterility was assessed for each radiation dose on 4 types of crosses: (1) 50 irradiated GSS males x 50 non-irradiated VAC females; (2) 50 irradiated VAC males x 50 non-irradiated VAC females; (3) 50 non-irradiated VAC males x 50 irradiated GSS females; and (4) 50 non-irradiated VAC males x 50 irradiated VAC females. Two cages that contained 50 pairs of non-irradiated flies from both strains were kept separately as untreated controls. Females oviposited their eggs through the netting on the top of the cage into a water-filled Petri dish with a black silicon panel. The oviposition device was deployed every morning, and after 3 h, the eggs were collected and the oviposition devices cleaned. Each cross was replicated 3 times and eggs were collected 5 times from each cage.

For data modeling, the relationships between the proportion of egg hatch, egg-to-pupa recovery and recovery of adults for males were analyzed with functions *drm()* and *mselect()* of the 'DRC' package in R (Dose-Response Curves, Version 3.0-1) [9] in order to find the best fitted models by comparing the log-likelihood values, Akaike's Information Criteria (AIC), lack of fit and residual variance of all models. The datasets were fitted to the non-linear 4-parameter Weibull model (W1.4), $y = c + (d - c)\exp\left(-\exp\left(b(\log(x) - \log(e))\right)\right)$, with binomial distribution for errors and including as fixed effects the radiation dose and fly strain in the linear predictor. Doses providing sterility levels of 50, 90 and 99% were determined with their respective 95% confidence intervals and then the estimated doses from both strains were compared using Student's t-test ($P < 0.05$). All graphs were made in the statistical environment R [10].

3. Results and Discussion

The relationship between irradiation dose and the proportion of egg hatch, egg-to-pupa recovery and recovery of adults were determined (Table 1 and Figure 1). The mean egg hatch and pupal and adult recovery decreased significantly with increasing radiation doses. No egg hatch was observed with doses of 90 and 100 Gy (Table 1). The four-parameter Weibull model was the best fitting model for describing the dose response curves for egg hatch, egg-to-pupa recovery and adult recovery (Figure 1). The lower fertility of untreated GSS males (i.e., egg hatch around 50% and recovery of pupae and adults be-low 40%) and a possible combination between this natural sterility and irradiation effects resulted in a distance between the curves of the two strains when the males were treated with doses lower than 40 Gy. However, the trend between the two strains was similar and the curves tended to overlap at higher doses (Figure 1).

Table I: Biological parameters (mean ± SE) from crosses between fertile females and irradiated *Anastrepha fraterculus* males from a bisexual strain (VAC) and a genetic sexing strain (GSS) under hypoxia.

Dose (Gy)	**Biological Parameters**					
	Egg hatch (%)		**Egg-to-pupa Recovery (%)**		**Recovery of Adults (%)**	
	VAC	**GSS**	**VAC**	**GSS**	**VAC**	**GSS**
0	80.3±2.8	51.5±1.2	76.6±4.1	35.5±1.9	73.5±3.8	32.1±1.5
30	18.03±2.1	13.9±1.6	12.1±1.5	9.7±1.8	10.9±1.3	9.2±1.7
40	9.3±0.8	7.9±1.2	5.2±0.9	4.4±0.8	4.8±0.8	4.0±0.7
50	5.2±0.7	5.2±0.7	2.6±0.5	2.4±0.5	2.3±0.4	2.0±0.4
60	3.9±0.7	3.9±0.6	2.03±0.5	2.4±0.7	1.7±0.6	1.9±0.6
70	1.7±0.4	1.1±0.3	0.73±0.3	0.4±0.2	0.6±0.2	0.2±0.15
80	0.13±0.1	0.17±0.06	0.07±0.05	0	0.03±0.03	0
90	0	0	0	0	0	0
100	0	0	0	0	0	0
Weibull model	y= (0.78)exp(exp(0.92(log(x)-log(20.6))))	y= (0.55)exp(exp(0.96(log(x)- log(23.1))))	y= (0.77)exp(exp(0.77(log(x)- log(13.9))))	y= (0.35)exp(exp(0.82(log(x)- log(19.1))))	y= (0.74)exp(exp(1.18(log(x)- log(17.1))))	y= (0.32) exp(exp (1.26 (log(x)- log(24.7))))

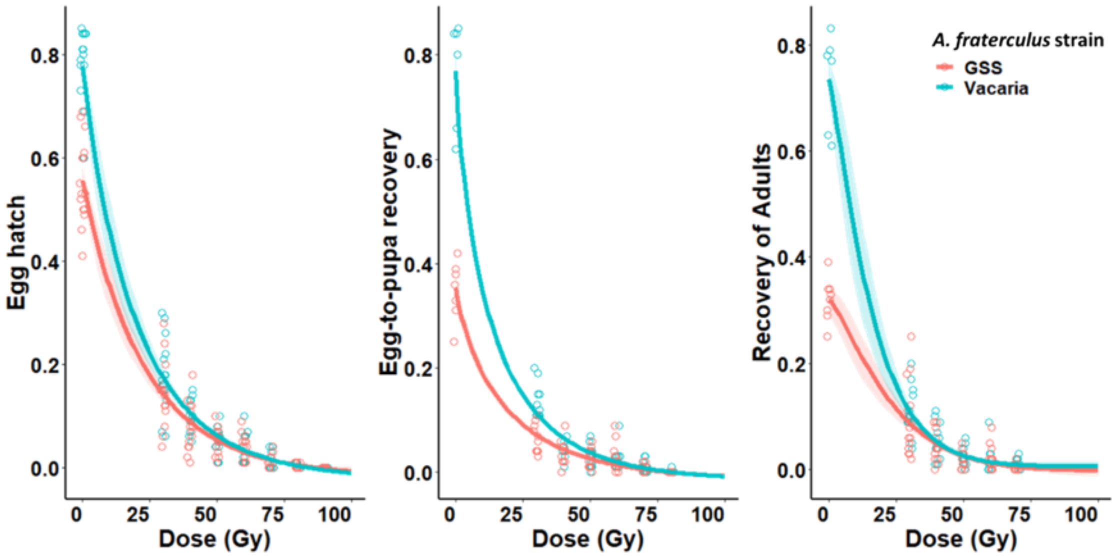

Figure 1: Weibull dose-response curves with 95% confidence intervals for three biological parameters resulting from crosses between fertile female *Anastrepha fraterculus* and males from a genetic sexing strain (GSS) and a bisexual strain (Vacaria) irradiated under hypoxia.

Females from both strains irradiated with 50 Gy were not able to lay eggs, and females treated with a dose of 40 Gy showed a mean egg-to-pupa recovery and recovery of adults of 0.1-0.2%. GSS females from the control group presented a fertility similar to VAC females (~74-77%) when crossed with fertile VAC males.

4. Conclusions

The dose-sterility response under hypoxia for a new black pupae GSS and a bisexual strain of *A. fraterculus* was assessed. The dose of 74 Gy applied under hypoxic conditions can induce 99% sterility in males from both strains, while complete sterility can be achieved with 80-90 Gy.

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