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Irradiation of *Anastrepha fraterculus* (Diptera: Tephritidae) Eggs to Inhibit Fly Emergence in the Mass-Rearing of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae)

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Abstract

As the incidence of *Anastrepha fraterculus* (Wiedemann) has increased in Southern Brazil in the past 3 yr, an initiative to release sterile flies and parasitoids has started. In order to make feasible the mass-rearing of the parasitoid *Diachasmimorpha longicaudata* (Ashmed), this study investigated the suitability of *A. fraterculus* larvae derived from irradiated eggs as host for *D. longicaudata*. Two different ages of *A. fraterculus* eggs (24 and 48 h old) were analyzed for hatchability after the exposure to a range of radiation doses. The hatchability of 48-h-old eggs was not affected by radiation, and no fly emerged at doses higher than 27.5 Gy. The larvae derived from irradiated eggs proved to be suitable hosts for the parasitoid development, with observed parasitism rates higher than 70% and sex ratio values above 0.6. The parasitism capability and longevity of *D. longicaudata* reared on larvae derived from irradiated eggs were also assessed. During the 10 d of parasitism evaluated, *D. longicaudata* from the treatments were able to parasitize nonirradiated larvae similarly as the parasitoids from controls and the laboratory colony. The longevity of *D. longicaudata* from the treatments was not affected either, with survival rates higher than 80% after 20 d of evaluation. The age of 48 h and a dose of 30 Gy could be considered the best age and dose for *A. fraterculus* eggs to be used in the mass-rearing of *D. longicaudata*. The results of this study will decrease the costs of mass-rearing *D. longicaudata* on *A. fraterculus*.

Resumen

A medida que la incidencia de *Anastrepha fraterculus* (Wiedemann) se ha incrementado en el sur de Brasil en los últimos tres años, una iniciativa para liberar moscas estériles y parasitoides ha comenzado. Con el objetivo de hacer posible la cría masiva del parasitoide *Diachasmimorpha longicaudata* (Ashmed), fue estudiada la viabilidad del uso de larvas de *A. fraterculus* oriundas de huevos irradiados como hospedero para *D. longicaudata*. La viabilidad de huevos de 24 y 48 h de edad de *A. fraterculus* fue evaluada después de la exposición a un rango de dosis de radiación gama. La exposición a las diferentes dosis de radiación no afectó la eclosión de larvas oriundas de huevos irradiados con 48h de edad y la emergencia de moscas fue inhibida con dosis a partir de 27.5 Gy. Las larvas provenientes de huevecillos irradiados se mostraron hospederos adecuados para el desarrollo del parasitoide, con tasas de emergencia superiores a 70% y un cociente sexual mayor que 0,6. También se evaluó la capacidad de parasitismo y la longevidad de *D. longicaudata* criados sobre larvas derivadas de huevos irradiados. Adultos de *D. longicaudata* oriundos de los tratamientos evaluados parasitaron de forma eficiente larvas no irradiadas durante 10 consecutivos. La longevidad del parasitoide no fue afectada por la exposición de su hospedero al rango de dosis testadas, con tasas de sobrevivencia superiores a 80% después de 20 días de evaluación. Los resultados obtenidos sugieren que larvas de *A. fraterculus* oriundas de huevos de 48 h de edad expuestos a 30 Gy pueden ser usadas como hospedero en la cría masiva de *D. longicaudata*. Además, los resultados de este estudio permitirán reducir los costos del proceso de producción masiva de *D. longicaudata* a partir de *A. fraterculus*.

Key words: fruit fly, parasitoid, radiation

Many species of fruit flies occur in the agroecosystems of Southern Brazil. However, one species is considered pest of apple and other temperate fruits in the Sierra Gaucha (Kovaleski et al. 2000), the South American fruit fly, *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae). Management of this pest has relied heavily on the use of pesticides over the past four decades, especially organophosphates, without any cases of resistance reported (Kovaleski and Ribeiro 2003). As some of the active ingredients of this group of insecticides are being gradually banned and the maximum tolerable residue limits have been reduced (Scoz et al. 2004, Urbaneja et al. 2009), Brazilian fruit growers are urging the scientific community to find alternative methods to control fruit flies such as *A. fraterculus*, especially environmentally sound and sustainable tactics. This situation represents a significant opportunity to increase the use of augmentative biological control in Brazil through the massive release of natural enemies of fruit flies.

Biological control programs for *Anastrepha* species have been in operation for more than 20 yr in Central America, demonstrating the feasibility of integrating parasitoid and sterile insect releases in area wide (Montoya et al. 2007, Reyes et al. 2000, Rull et al. 1996). Research and field trials are underway for *A. fraterculus* in Southern Brazil. During the first phase of a pilot-program called MOSCASUL, sterile flies will be mass-reared at the Center for Nuclear Energy in Agriculture from the University of São Paulo (CENA/USP) at Piracicaba, São Paulo state (Mastrangelo et al. 2010, Walder et al. 2014) and shipped weekly to the Release Center from the *Estação Experimental de Fruticultura de Clima Temperado* of EMBRAPA at Vacaria, Rio Grande do Sul state. As the release of sterile flies together with parasitoids is proposed to have synergistic effects for population suppression when applied simultaneously (Knipling 1992, Wong et al. 1992), both growers and program managers are willing to start releasing fruit fly parasitoids as soon as possible. Among the fruit fly parasitoids available in Brazil for inundative releases, *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) would be a reliable choice.

D. longicaudata, a larval-pupal opiine braconid, was introduced to Brazil in 1994 (Walder et al. 1995). This Indo-Philippine wasp, originally a parasite of *Bactrocera* spp., has been used in innumerous inoculative releases in several regions of Brazil since 1995 to control a wide range of tephritid hosts (Alvarenga et al. 2005, Carvalho and Nacimento 2002, Matrangolo et al. 1998, Paranhos et al. 2007, Sugayama 2000, Walder 2002). In the beginning, *D. longicaudata* was reared on *Ceratitis capitata* (Wiedemann) third instar larvae at CENA/USP (Walder 2002, Walder et al. 1995), but since 2006, with the domestication of *A. fraterculus* (Walder et al. 2014), irradiated *A. fraterculus* larvae have been offered as host, allowing cohorts with bigger and more vigorous parasitoids.

Due to the absence of commercial irradiators and other radioactive sources close to the release sites from the Sierra Gaucha, the growers involved in the pilot-program intended initially to obtain not only sterile flies from the mass-rearing facility of CENA/USP but also *D. longicaudata*. However, some constraints were presented when considering the shipment of a few millions of pupae parasitized by *D. longicaudata*, like space limitations of CENA's facility to build up the rearing to generate extra larvae for the parasitoid colony and the high costs of shipping large volumes of parasitized pupae by plane.

An innovative way to apply radiation to solve these constraints and even to increase the efficiency of *D. longicaudata* rearing was explored by Cancino et al. (2009a). In many facilities where *D. longicaudata* is reared, third instar larvae are irradiated before exposure to the parasitoids, avoiding the emergence of unparasitized hosts (Cancino et al. 2002a, Sivinski and Smittle 1990). This use of radiation makes activities such as host exposure, packing and release easier to carry out, besides eliminating completely the risk of shipping fertile flies.

Cancino et al. (2009a) then evaluated the ability of *D. longicaudata* to develop in larvae derived from irradiated *Anastrepha ludens* (Loew) eggs. They verified that 72-h-old eggs irradiated at 25 Gy or more were found to be the best stage to be used in the mass-rearing of the parasitoids. Literature still provides very little guidance on irradiation of host eggs for parasitoids. So far, no study has investigated the effects that radiation might have on *A. fraterculus* eggs and its suitability for *D. longicaudata* mass-rearing. The aim of this study, therefore, was to investigate the possibility of rearing *D. longicaudata* on larvae derived from irradiated eggs of *A. fraterculus*, making feasible the weekly shipment of these eggs to a mass-rearing facility at Southern Brazil.

Materials 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* eggs used for the experiments were obtained from the colony maintained by the procedures described by Walder et al. (2014). 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. The adults were provided with water and a mixture of sugar and hydrolyzed yeast at 4:1 rate (Jaldo et al. 2001). The *D. longicaudata* colony was maintained using *A. fraterculus* third instar larvae as hosts, and the adults were provided with water and honey ad libitum. This *D. longicaudata* colony was maintained for 70 generations in the laboratory.

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°C. All irradiations were carried out in separate assays and performed under normal atmospheric conditions (free oxygen). The eggs of *A. fraterculus* were irradiated according to the methodology described by IAEA (1982) in a glass container with a lid (5 × 3 cm) with 1 ml of eggs suspended in 3 ml of water. For each lot of irradiated egg, dosimetry was performed with a Fricke Dosimeter (Fricke and Hart 1966).

Irradiation of *A. fraterculus* Eggs at Different Ages

The first experiment was conducted in order to evaluate the viability of *A. fraterculus* eggs at different ages after exposure to a range of gamma radiation doses. Eggs bubbled in water bath at 24°C for 24 and 48 h were irradiated at 0 (control), 5, 10, 15, 20, 25, 27.5, 30, 32.5, 35, 40, and 45 Gy. Hatching percentages were taken from samples of 200 eggs distributed on pieces of moistened black filter paper held in closed Petri dishes at 24°C. After 96 h, the number of eggs that did not hatch was recorded and used to calculate the percentage of eggs hatched for each age and dose of radiation. This test was replicated five times.

Exposure of Larvae Hatched From Irradiated Eggs to *D. longicaudata*

The second experiment aimed to analyze the effects of irradiation on the larvae derived from 48-h-old irradiated eggs and the quality of

these larvae for parasitism. Eggs of *A. fraterculus* were irradiated following the same procedures of the previous experiment. After irradiation, aliquots of 1 ml of eggs (approximately 11,700 eggs) were seeded in trays containing 1 liter of artificial diet (Walder et al. 2014), and larvae remained there for 12–14 d. After this period, the third instar larvae were separated from the diet medium by washing and then distributed in two lots: the first was exposed to *D. longicaudata* females, and the second was kept as a nonparasitized set of larvae. In the first set of larvae, parasitism units containing 200 larvae were placed on the top of the cages and exposed to 8-d-old parasitoid females (10 larvae per female rate) for 45 min. One treatment consisting of larvae that were irradiated at 40 Gy (routine procedure of the laboratory rearing) and parasitized (I × P treatment) and another where larvae were irradiated with the same dose but not parasitized (I × NP treatment) were also used as controls, but these larvae were derived from nonirradiated *A. fraterculus* eggs and the parasitoids used were obtained from the laboratory colony.

Both sets of larvae (parasitized and unparasitized) were then transferred to 500-ml plastic cups with moist vermiculite for pupation under laboratory conditions (24 ± 1°C, 65 ± 10% relative humidity). One day before emergence, samples of 100 pupae from the set of unparasitized larvae were weighed for each treatment and then returned to the cups for adult emergence.

After emergence, the number of adult flies and parasitoids was counted for each treatment to assess the percentages of emergence and sex ratios. Five replicates were performed for all treatments.

Parasitism and Longevity of Parasitoids Reared on Larvae Derived From Irradiated Eggs

In the third experiment, the quality of *D. longicaudata* parasitoids derived from *A. fraterculus* irradiated eggs was assessed. Four treatments, each of them replicated five times, were set consisting of 200 *A. fraterculus* larvae derived from 48-h-old eggs exposed to the following doses: 0 (control), 25, 27.5, and 30 Gy (as described previously). These larvae were placed in the parasitism units and exposed to 8-d-old parasitoids from the laboratory colony. After the parasitoid emergence, 20 couples of *D. longicaudata* recovered from each dose were separated for the assessment of their fecundity and longevity.

For evaluation of parasitism capability, approximately 200 nonirradiated *A. fraterculus* larvae (10 larvae per 1 parasitoid female rate) were offered for 45 min on the cages containing 20 parasitoid couples of each treatment when females were 5 d old. The larvae were exposed to the parasitoids during the following 10 d, with five replicates per treatment in each day. The parasitized larvae were kept in cups with fine vermiculite for 14–16 d, and the number of parasitoids that emerged each day was recorded in order to assess the parasitism rates (%) and sex ratios. One treatment consisting of larvae irradiated at 40 Gy (routine procedure of the laboratory rearing) exposed to parasitoids of the main colony, derived from nonirradiated eggs, was also used for the comparisons between treatments.

To evaluate longevity, 20 females and 20 males of parasitoids of each treatment were placed in 500-ml plastic cups with water and adult diet made of honey offered ad libitum. Mortality was recorded daily until the 20th day.

Statistical Analysis

For statistical analysis of mortality induced in *A. fraterculus* eggs by radiation doses in the first two experiments conducted, Probit regression following Sokal and Rohlf (1981) was performed and observed the adjustment of the mathematic model applied (R^2). For the data related to weight of pupae (mg), pupation (%), parasitoid emergence (%), and sex ratio (number of females/number of females + males), parasitism rates (number of parasitoids that emerged/ number of pupae) and longevity, the one-way analysis of variance *F*-test was calculated for the means at the 5% of significance, and when a significant difference was found, the Tukey's honestly significant difference test ($\alpha = 5\%$) was applied to compare averages. For the parasitism rates (number of parasitoids that emerged/number of pupae) and sex ratio (number of females/number of females + males) from the third experiment, a two-way analysis of variance *F*-test was applied at a 5% level of significance, considering the day and treatment (factorial scheme 10 by 5) as factors. The Bartlett (1937) and Shapiro and Wilk (1965) 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 (SAS Institute 2003).

Results

Effects of Radiation on *A. fraterculus* Eggs at Different Ages

The mean viability of the *A. fraterculus* eggs from the control groups at 24 and 48 h was 84.9 ± 0.6% and 91.9 ± 0.9%, respectively. The mortality of *A. fraterculus* eggs, analyzed by Probit regressions, was estimated from the number of eggs that did not hatch. The differences in radiosensitivity of the *A. fraterculus* eggs at two ages were very clear when increasing the radiation doses (Fig. 1). The 24-h-old eggs proved to be very sensitive to radiation, and egg hatch decreased significantly as irradiation doses were increased ($F = 240.9$; df = 1, 10; $P < 10^{-3}$). The dose required to induce 99% mortality (LD_{99}) in 24-h-old *A. fraterculus* eggs was estimated at 53.7 Gy, with upper and lower confidence limits (95%) estimated at 62.9 and 45.9 Gy, respectively.

For instance, the hatchability of the 48-h-old eggs was not adversely affected by the radiation doses ($F = 0.56^{ns}$; df = 1, 9; $P = 0.48$), and percent of egg hatch ranged between 85.7 and 92.5% among treatments.

Quality of Larvae Derived From Irradiated 48-h-Old Eggs for Parasitism by *D. longicaudata*

In the second experiment of this study, two sets of larvae derived from 48-h-old eggs irradiated at different doses were separated in order to assess the effects of radiation on the quality control parameters.

The emergence of *A. fraterculus* flies, sex ratio of the flies, and the pupal weight from the unparasitized set of larvae were significantly affected by the radiation doses (Table 1). Fly mortality, estimated from the data of fly emergence (%), increased significantly as the radiation doses increased ($F = 7.7$; df = 1, 5; $P = 0.05$). Flies did not emerge at 27.5 Gy or higher doses, even after the successful pupation of some larvae. The dose estimated with the obtained Probit regression (Table 1) to reduce fly emergence in 99% was 27.7 Gy (95% confidence interval = 12.9–59.2 Gy). Sex ratio of the emerged flies was also affected, with a few more females being detected at the group irradiated at 25 Gy, whereas no difference was found between means from the control group (0 Gy) to the dose of 20 Gy (means ranged from 0.51 to 0.61) (Table 1).

The weight of unparasitized pupae differed significantly among treatments (Table 1). The means between the control group (0 Gy) and the dose of 20 Gy ranged between 12.5 and 13.03 mg and did not differ significantly. However, pupal weight decreased significantly at doses higher than 27.5 Gy, reaching the minimum observed value of 5.35 ± 0.03 mg at 45 Gy.

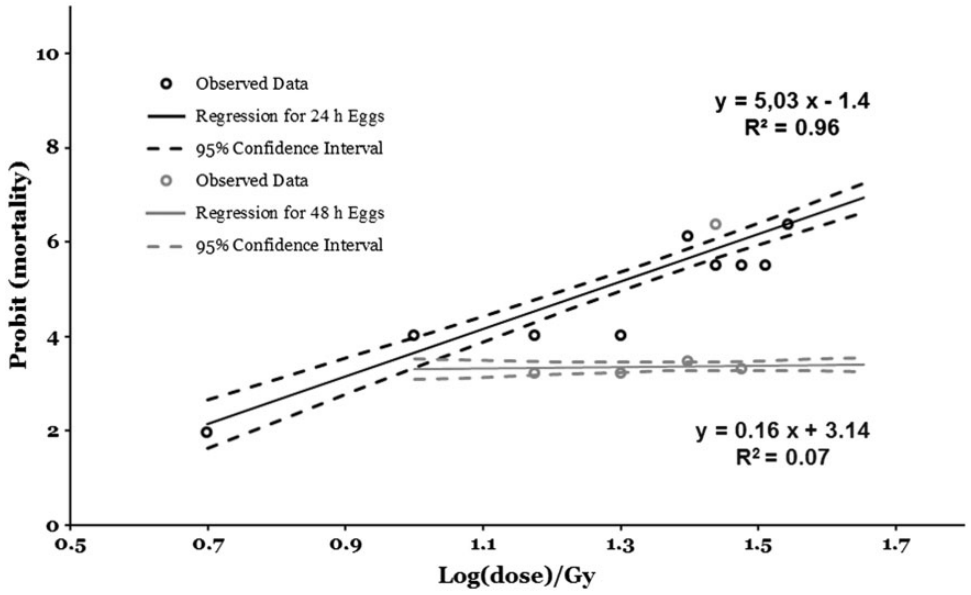

Fig. 1. Estimated Probit of mortality from 24- and 48-h *Anastrepha fraterculus* eggs irradiated at different doses.

Table 1. Means (±SE) of emergence of flies, sex ratio, and pupal weight from nonparasitized larvae obtained from 48-h *A. fraterculus* eggs irradiated at different doses

Doses (Gy)	Fly emergence (%)	Sex ratio (♀/♂+♀)	Weight of pupae (mg)
0	90.9 ± 0.9	0.56 ± 0.02 b†	13.03 ± 0.03 a
5	87.3 ± 1.8	0.51 ± 0.01 b	12.5 ± 0.03 a
10	92.8 ± 2.6	0.52 ± 0.01 b	12.9 ± 0.06 a
15	84.6 ± 1.9	0.54 ± 0.01 b	13.01 ± 0.05 a
20	50.3 ± 1.7	0.61 ± 0.01 b	12.6 ± 0.07 a
25	2.6 ± 0.58	0.77 ± 0.07 a	11.7 ± 0.04 b
27.5	0.0 ± 0.0	0.0 ± 0.0 c	9.54 ± 0.05 d
30	0.0 ± 0.0	0.0 ± 0.0 c	8.23 ± 0.35 e
32.5	0.0 ± 0.0	0.0 ± 0.0 c	6.59 ± 0.08 f
35	0.0 ± 0.0	0.0 ± 0.0 c	6.67 ± 0.07 f
40	0.0 ± 0.0	0.0 ± 0.0 c	5.63 ± 0.05 g
45	0.0 ± 0.0	0.0 ± 0.0 c	5.35 ± 0.03 g
I × NP treatment*	0.0 ± 0.0	0.0 ± 0.0 c	10.87±0.03 c
	Probit regression	Analyis of variance	
	$y = 6.88 \times -2.61$ $R^2 = 0.66$	$F_{12,64} = 225$; $P < 10^{-3}$; C.V.§ = 0.87%	$F_{12,64} = 775.6$; $P < 10^{-3}$; C.V. = 2.45%

*I × NP treatment = treatment that consisted of larvae, derived from nonirradiated *A. fraterculus* eggs, that were irradiated at 40 Gy (routine procedure of the laboratory rearing) and not parasitized.

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

§C.V. = Coefficient of variation.

All the quality control parameters (i.e., pupation, emergence of parasitoids and flies, sex ratios) from the set of parasitized larvae were significantly affected by the radiation doses (Table 2). Pupation rates of the parasitized larvae ranged from 87.7 ± 0.5 to 100%, but significant differences were detected. The mean pupation value of the control group of 0 Gy (100%) did not differ from the mean values from the treatments between 5 and 15 Gy and between 25 and 30 Gy, but the lowest pupation level was observed at 45 Gy.

In all treatments, the percentages of parasitoid emergence were all higher than 70%. The highest parasitism emergence was observed at 25 Gy (95.5 ± 1.2%), and this mean was not significantly different from the means observed at 5, 15, 20, 27.5, 30, and 35 Gy (Table 2). The parasitism from the control group (0 Gy) did not differ from most of the treatments either, and the means of all of them were higher than the mean from the treatment that consisted of larvae irradiated at 40 Gy, derived of nonirradiated eggs (I × P treatment). The sex ratio of the parasitoids that emerged ranged between 0.46 and 0.7, and no significant difference was found for the means of the treatments between 0 and 40 Gy (Table 2).

The emergence of *A. fraterculus* adults from the parasitized larvae was low (< 13%) for all treatments and no fly emerged at doses higher than 25 Gy. According to the Probit regression obtained ($F = 3.64$; df = 1, 4; $P = 0.15$) (Table 2), the LD_{99} was estimated at 23.9 Gy (95% confidence interval = 11.2–51.03 Gy). The sex ratios of the emerged flies were not significantly different between the control (0 Gy) and the treatments between 5 and 25 Gy. Pupal weight was not assessed for this set of larvae because the consumption of the pupal content by the parasitoid larvae alters the values of

Table 2. Means (±SE) of pupation, parasitoid and fly emergence, and sex ratios from parasitized larvae obtained from 48-h *A. fraterculus* eggs irradiated at different doses

Doses (Gy)	Pupation (%)	Parasitoid emergence (%)	Sex ratio (♀/♂+♀)	Fly emergence (%)	Sex ratio (♀/♂+♀)
0	100 ± 0.0 a*	84.8 ± 0.6 bcd	0.7 ± 0.0 a	12.1 ± 0.7	0.4 ± 0.01 a
5	99.0 ± 0.6 ab	86.8 ± 2.1 abc	0.68 ± 0.01 a	5.04 ± 1.04	0.5 ± 0.1 a
10	98.8 ± 0.1 ab	83.8 ± 0.8 cde	0.66 ± 0.02 ab	6.3 ± 1.2	0.51 ± 0.04 a
15	99.0 ± 0.2 ab	86.6 ± 2.8 abc	0.65 ± 0.02 ab	5.4 ± 1.2	0.32 ± 0.1 ab
20	97.1 ± 0.2 bc	86.8 ± 0.6 abc	0.64 ± 0.01 ab	3.8 ± 0.4	0.56 ± 0.1 a
25	99.2 ± 0.3 a	95.5 ± 1.2 a	0.69 ± 0.02 a	0.91 ± 0.3	0.37 ± 0.2 a
27.5	99.1 ± 0.2 ab	92.8 ± 0.9 ab	0.65 ± 0.01 ab	0.0 ± 0.0	0.0 ± 0.0 b
30	98.8 ± 0.2 ab	85.6 ± 0.7 abc	0.63 ± 0.01 ab	0.0 ± 0.0	0.0 ± 0.0 b
32.5	95.5 ± 0.3 cd	81.3 ± 2.4 cde	0.66 ± 0.02 ab	0.0 ± 0.0	0.0 ± 0.0 b
35	94.5 ± 0.4 d	86.5 ± 2.3 abc	0.69 ± 0.02 a	0.0 ± 0.0	0.0 ± 0.0 b
40	90.0 ± 1.0 f	80.6 ± 2.5 cde	0.64 ± 0.04 ab	0.0 ± 0.0	0.0 ± 0.0 b
45	87.7 ± 0.5 f	74.8 ± 4.2 de	0.62 ± 0.05 b	0.0 ± 0.0	0.0 ± 0.0 b
I × P treatment†	92.8 ± 0.9 e	73.7 ± 2.6 e	0.46 ± 0.02 c	0.0 ± 0.0	0.0 ± 0.0 b
	ANOVA			Probit regression	ANOVA
	$F_{12,64} = 131.4$; $P < 10^{-3}$; C.V.§ = 0.64%	$F_{12,64} = 8.2$; $P < 10^{-3}$; C.V. = 1.24%	$F_{12,64} = 11.3$; $P < 10^{-3}$; C.V. = 2.5%	y = 8.3 x – 4.12 $R^2 = 0.55$	$F_{12,64} = 12.4$; $P < 10^{-3}$; C.V. = 12.3%

ANOVA, analysis of variance.

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

†I × P treatment = treatment that consisted of larvae, derived from nonirradiated *A. fraterculus* eggs that were irradiated at 40 Gy (routine procedure of the laboratory rearing) and parasitized by *D. longicaudata* from the laboratory colony.

§C.V. = Coefficient of variation.

weight widely, not allowing accurate comparisons between the treatments.

Parasitism and Longevity of Parasitoids Developed From Larvae of Irradiated Eggs

As third instar nonirradiated larvae, not derived from irradiated eggs, were offered to the parasitoids in the third experiment, a few *A. fraterculus* flies were able to emerge. However, the percentages of emerged flies from unparasitized pupae were all lower than 21%, with the overall means of 11.6 ± 0.8% for the control group (0 Gy), 3.1 ± 0.4% for the treatment at 25 Gy, 2.1 ± 0.2% at 27.5 Gy, and 3.8 ± 2.4% at the 30 Gy treatment. In the I × P treatment, not a single fly emerged.

The significant differences observed in the parasitism rates ($F_{49,249} = 8.8$; $P < 10^{-3}$; coefficient of varation (C.V.) = 13.5%) and sex ratios ($F_{49,249} = 5.72$; $P < 10^{-3}$; C.V. = 0.06%) obtained in the third experiment are presented in Tables 3 and 4. The interaction (day × treatments) was significant for the parasitism of nonirradiated larvae by *D. longicaudata* couples derived from *A. fraterculus* larvae developed from irradiated eggs ($F = 4.2$; df = 36; $P < 10^{-3}$) with regard to day alone ($F = 21.5$; df = 9; $P < 10^{-3}$) and among treatments ($F = 21.2$; df = 4; $P < 10^{-3}$) (Table 3).

In Table 3, when parasitism was sorted by day, the *D. longicaudata* females derived from the treatments of 25, 27.5, and 30 Gy parasitized the nonirradiated larvae equally as the females from the control group (0 Gy) on 6 d (days 2, 5, 6, 8, 9, and 10), with parasitism rates ranging from 43 to 81%. The mean parasitism values demonstrated, therefore, that parasitism did not tend to decrease in parasitoids reared on larvae derived from irradiated eggs at high doses as 30 Gy.

Sorting parasitism by treatment, significant differences were observed in most of the days (Table 3). Only in the control (0 Gy) and the treatment of 30 Gy, parasitism seemed to be slightly reduced until the 10th day. Nevertheless, no significant differences between means were verified for 9, 6, and 8 d in the control group (0 Gy) and in the treatments at 25 and 27.5 Gy, respectively. No significant difference in parasitism was observed during the 10 consecutive days for the parasitoids reared on larvae derived from eggs irradiated at 30 Gy ($F_{9,49} = 2.7$; $P = 0.06$; C.V. = 16.2%). Along the 10 days of parasitism, the overall mean values ranged from 59.1 to 69% of parasitism, and the means from the I × P treatment (57 ± 2.4%) was slightly lower than the means from the other treatments.

Considering the sex ratios obtained, all mean values ranged between 0.49 and 0.76 (Table 4). Significant interaction (day × treatments) was also verified for the sex ratios of the parasitoid offspring obtained from the larvae offered to the *D. longicaudata* couples derived from the treatments of irradiated eggs ($F = 2.24$; df = 36; $P = 0.0002$), when sorting by day ($F = 20.4$; df = 9; $P < 10^{-3}$) or by treatment ($F = 3.99$; df = 4; $P = 0.004$). When the sex ratios were sorted by day, significant differences were observed only on the second and forth days of parasitism (Table 4), with a few more *D. longicaudata* females (sex ratio ≥ 0.69) being detected in the control group (0 Gy). When the sex ratio values were sorted by treatment, not significant differences were observed only for the treatment of 30 Gy ($P = 0.1$). The mean values of sex ratio from the 10th day did not differ significantly from other 9 d in the control group (0 Gy), at 25 Gy, and from seven others at 27.5 Gy and in the I × P treatment. No clear tendency in reduction or increase in sex ratios along the days was found in the third experiment of this study.

The longevity of the recovered parasitoids was not affected by radiation doses (Table 5). The percentages of live parasitoids at the 20th day were all high, ranging from 86 to 100%. Significant differences were not found in the longevity results ($P > 0.05$).

Discussion

In view of the impact that field augmentation trials and programs can have on fruit fly populations (Camacho 1993, Eitam et al. 2004, Messing et al. 1993, Montoya et al. 2007, Reyes et al. 2000, Rull

Table 3. Parasitism rates (%) over nonirradiated *A. fraterculus* larvae of *D. longicaudata* emerged from pupae derived from 48-h irradiated eggs at different doses

	Parasitism day											
Dose (Gy)	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	Mean ± SE	ANOVA
0	75.4 ± 3.3 Aab**	75.2 ± 3.4 Aab	70.3 ± 3.7 ABab	80.7 ± 1.6 Aa	72.5 ± 2.8 Aab	78.6 ± 1.9 Aab	71.8 ± 2.5 Aab	52.8 ± 2.1 ABc	66.8 ± 2.6 Ab	52.2 ± 2.9 Ac	69. ± 1.6	$F_{9,49} = 12.6$; $P < 10^{-3}$; C.V. = 8.9%
25	43.0 ± 1.8 Ce	69.6 ± 2.7 Aabc	80.0 ± 1.9 Aa	77.9 ± 1.3 Aab	79.7 ± 5.1 Aa	81.0 ± 3.0 Aa	75.6 ± 3.1 A abc	61,1 ± 4.4 Acd	63.7 ± 3.0 Abcd	51.5 ± 3.9 Ade	68.3 ± 2.0	$F_{9,49} = 16.5$; $P < 10^{-3}$; C.V. = 10.7%
27.5	53.9 ± 2.7 BCb	59.4 ± 4.2 ABab	60.2 ± 3.1 Bab	60.5 ± 2.2 Cab	64.0 ± 3.6 Aab	70.7 ± 2.5 Aa	56.6 ± 2.2 BCab	49.2 ± 2.8 ABb	55.9 ± 5.2 Aab	60.4 ± 2.7 Aab	59.1 ± 1.2	$F_{9,49} = 3.2$; $P = 0.01$; C.V. = 12.3%
30	66.2 ± 6.3 ABa	53,1 ± 7.3 ABa	52.2 ± 3.2 Ba	66.4 ± 3.5 BCa	71.9 ± 3.8 Aa	72.0 ± 3.3 Aa	68.4 ± 4.3 ABa	60.6 ± 3.5 Aa	58.0 ± 5.4 Aa	56.5 ± 1.8 Aa	62.5 ± 1.6	$F_{9,49} = 2.7$; $P = 0.06$; C.V. = 16.2%
I × P treatment*	49.4 ± 4.1 Cc	40.6 ± 7.4 Bc	58.6 ± 8.3 Babc	73.2 ± 1.6 ABab	74.6 ± 3.7 Aab	76.8 ± 2.8 Aa	46.9 ± 2.6 Cc	38.1 ± 4.5 Bc	59.0 ± 3.1 Aabc	52.7 ± 5.8 Abc	57 ± 2.4	$F_{9,49} = 8.4$; $P < 10^{-3}$; C.V. = 19.02%
ANOVA	$F_{4,24} = 11.01$; $P<10^{-3}$; C.V.§ = 15.3%	$F_{4,24} = 6.4$; $P = 0.002$; C.V. = 20.2%	$F_{4,24} = 5.6$; $P = 0.003$; C.V. = 16.1%	$F_{4,24} = 14.2$; $P<10^{-3}$; C.V. = 6.8%	$F_{4,24} = 2.1$; $P = 0.11$; C.V. = 11.9%	$F_{4,24} = 2.5$; $P = 0.08$; C.V. = 8.1%	$F_{4,24} = 15.1$; $P<10^{-3}$; C.V. = 10.7%	$F_{4,24} = 6.9$; $P = 0.01$; C.V. = 15.4%	$F_{4,24} = 1.2$; $P = 0.33$; C.V. = 14.9%	$F_{4,24} = 1.03$; $P = 0.4$; C.V. = 15.1%		

ANOVA, analysis of variance.

*I × P treatment = treatment that consisted of larvae, derived from nonirradiated *A. fraterculus* eggs, that were irradiated at 40 Gy (routine procedure of the laboratory rearing) and parasitized by *D. longicaudata* from the laboratory colony.

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

§C.V. = Coefficient of variation.

Table 4. Sex ratio of the parasitoids that emerged from nonirradiated third instar larvae parasitized by *D. longicaudata* derived from 48-h irradiated eggs at different doses

	Parasitism day											
Dose (Gy)	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	Mean ± SE	ANOVA
0	0.62 ± 0.03 Aab**	0.73 ± 0.02 Aa	0.58 ± 0.01 Ab	0.69 ± 0.02 Aa	0.7 ± 0.02 Aa	0.69 ± 0.01 Aa	0.72 ± 0.02 Aa	0.66 ± 0.03 Aab	0.68 ± 0.03 Aab	0.63 ± 0.02 Aab	0.67 ± 0.01	$F_{9,49} = 4.2$; $P < 10^{-3}$; C.V. = 0.05%
25	0.52 ± 0.02 Ab	0.63 ± 0.03 Bab	0.66 ± 0.02 Aa	0.65 ± 0.01 ABa	0.71 ± 0.02 Aa	0.7 ± 0.02 Aa	0.72 ± 0.02 Aa	0.66 ± 0.04 Aa	0.64 ± 0.03 Aa	0.6 ± 0.02 Aa	0.7 ± 0.02	$F_{9,49} = 5.6$; $P < 10^{-3}$; C.V. = 0.05%
27.5	0.49 ± 0.02 Ad	0.66 ± 0.01 ABabc	0.59 ± 0.01 Ac	0.67 ± 0.01 ABab	0.69 ± 0.03 Aab	0.71 ± 0.01 Aa	0.66 ± 0.02 Aabc	0.61 ± 0.02 Abc	0.67 ± 0.01 Aab	0.63 ± 0.02 Aabc	0.64 ± 0.02	$F_{9,49} = 13.4$; $P<10^{-3}$; C.V. = 0.04%
30	0.56 ± 0.05 Aa	0.59 ± 0.02 Ba	0.57 ± 0.02 Aa	0.6 ± 0.03 Ba	0.67 ± 0.04 Aa	0.6 ± 0.03 Aa	0.7 ± 0.03 Aa	0.61 ± 0.01 Aa	0.64 ± 0.02 Aa	0.68 ± 0.02 Aa	0.63 ± 0.01	$F_{9,49} = 2.9$; $P = 0.1$; C.V. = 0.06%
I × P treatment*	0.5 ± 0.03 Ad	0.57 ± 0.03 Bcd	0.6 ± 0.04 Abcd	0.71 ± 0.02 Aab	0.72 ± 0.02 Aab	0.76 ± 0.01 Aa	0.7 ± 0.04 Aabc	0.6 ± 0.03 Aabc	0.64 ± 0.02 Aabcd	0.72 ± 0.03 Aab	0.66 ± 0.01	$F_{9,49} = 7.3$; $P < 10^{-3}$; C.V. = 0.06%
ANOVA	$F_{4,24} = 2.8$; $P = 0.06$; C.V.§ = 0.08%	$F_{4,24} = 6.6$; $P = 0.001$; C.V. = 0.05%	$F_{4,24} = 2.7$; $P = 0.06$; C.V. = 0.05%	$F_{4,24} = 5.8$; $P = 0.003$; C.V. = 0.04%	$F_{4,24} = 0.5$; $P = 0.7$; C.V. = 0.06%	$F_{4,24} = 2.6$; $P = 0.07$; C.V. = 0.04%	$F_{4,24} = 1.3$; $P =0.3$; C.V. = 0.06%	$F_{4,24} = 1.04$; $P = 0.4$; C.V. = 0.06%	$F_{4,24} = 0.7$; $P = 0.6$; C.V. = 0.1%	$F_{4,24} = 2.4$; $P = 0.08$; C.V. = 0.06%		

ANOVA, analysis of variance.

*I × P treatment = treatment that consisted of larvae, derived from nonirradiated *A. fraterculus* eggs, that were irradiated at 40 Gy (routine procedure of the laboratory rearing) and parasitized by *D. longicaudata* from the laboratory colony.

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

§C.V. = Coefficient of variation.

Table 5. Longevity of *D. longicaudata* parasitoids emerged from 48-h-old eggs irradiated at different doses

Treatment	Live parasitoids at the 20th day (%)		Total
	♂	♀	
0 Gy	93 ± 2.6 a†	99 ± 1.0 a	96 ± 1.5 a
25 Gy	94 ± 2.9 a	100 ± 0.0 a	97 ± 1.5 a
27.5 Gy	94 ± 1.9 a	100 ± 0.0 a	97 ± 0.9 a
30 Gy	86 ± 5.6 a	98 ± 2.0 a	92 ± 2.6 a
I × P treatment*	96 ± 1.9 a	98 ± 1.2 a	97 ± 0.5 a
Analyis of variance	$F_{4,24} = 0.7$; $P = 0.63$; C.V. = 5.3%	$F_{4,24} = 1.5$; $P = 0.25$; C.V. = 1.6%	$F_{4,24} = 1.3$; $P = 0.3$; C.V. = 2.6%

*I × P treatment = treatment that consisted of larvae, derived from nonirradiated *A. fraterculus* eggs, that were irradiated at 40 Gy (routine procedure of the laboratory rearing) and parasitized by *D. longicaudata* from the laboratory colony.

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

et al. 1996, Sivinski et al. 1996, Yao 1989), *D. longicaudata* was chosen to be used against *A. fraterculus* populations that have attacked more frequently apple orchards of a pilot-project in Southern Brazil, called MOSCASUL. As the MOSCAFRUT facility from Chiapas sent 50 million parasitized pupae weekly via commercial flights to many different states of Mexico (Montoya et al. 2007), MOSCASUL managers thought initially that *D. longicaudata* from irradiated larvae could be supplied by the mass-rearing facility from CENA/USP at Piracicaba. However, due to space limitations of the facility and costs of shipment, it was necessary to find a cheaper alternative to make feasible the augmentative releases of *D. longicaudata* in the pilot-project.

According to Hendrichs et al. (2009), nuclear techniques can be applied in a way that the trade, safety, efficiency, and cost effectiveness of biological control programs can be improved. Larvae of several *Anastrepha* species have been successfully irradiated for the mass-rearing of fruit fly parasitoids because radiation make the host unable to develop or produce progeny (Sivinski and Smittle 1990). Larvae of *A. obliqua* (Macquart), *A. serpentina* (Wiedemann), and *A. ludens* (Loew) are routinely gamma rayed for the mass-rearing of tens of millions of parasitoids (Cancino et al. 2009b), ensuring shipments clean of adult flies. Viscarret et al. (2012) and Bachmann et al. (2015) showed that *C. capitata* and *A. fraterculus* larvae irradiated with X-rays are also suitable for the rearing of *D. longicaudata* in Argentina. The irradiation of host eggs instead of larvae is an important alternative in the mass-rearing of fruit fly parasitoids (Cancino et al. 2009a), but little work has been done with *Anastrepha* spp. hosts. This study investigated the possibility of using irradiated eggs of the South American fruit fly for the rearing of *D. longicaudata* in Brazil.

After exposing *A. fraterculus* eggs to different doses of gamma radiation, only the hatching of the 24 h-old-eggs was affected (Fig. 1), with a LD_{99} estimated at 53.7 Gy. Insect eggs are expected to be one of the most sensitive stages to irradiation, especially in the early stages (Bakri et al. 2005). *C. capitata* eggs irradiated at 4 h old are more radiosensitive than older eggs and, at this age, doses higher than 20 Gy are sufficient to prevent pupation and adult emergence (Rahman et al. 1990). MaCFarlane (1966) observed that 20 Gy was sufficient to prevent egg hatch of *Bactrocera tryoni* (Froggatt) 2–5-h-old eggs. Bughio et al. (1969), testing eggs of *Bactrocera zonata* (Saunders) at the same age, also found that 20 Gy could prevent eggs from hatching. For *A. ludens*, percent hatching of 24- and 48-h-old eggs irradiated at doses ≥ 12.5 Gy was lower than 40% (Cancino et al. 2009a).

The results obtained in this study suggests that certain systems of the embryo in development are extremely radiosensitive. Very few studies have been undertaken to correlate the stage of embryological development with radiosensitivity. Rahman et al. (1990) and Jessup et al. (1992) showed that the t ganglion (i.e., brain) of *C. capitata* and *Bactrocera* spp. was reduced significantly in size when eggs or young larvae (up to 96 h after eggs were laid) were irradiated with doses between 5 and 150 Gy. Nation et al. (1995) demonstrated that *A. suspensa* larvae irradiated at hatching with 50 Gy presented very small brains, small or undeveloped imaginal disks and an elongated sinuous nerve cord, what would explain the deaths and failure of adults to emerge from the larvae that survived through pupation. More recently, Nirmala et al. (2015) showed that when irradiated at 16 h after oviposition with 30 Gy, *A. suspensa* embryos exhibited significant levels of apoptosis, consistent with strong induction of transcript levels of two proapoptotic genes by gamma radiation in embryos with less than 24 h after oviposition. Selivon et al. (1997) observed four classes of embryos in *A. fraterculus*, but the genetic mechanisms involved during embryogenesis and how they are affected by radiation are yet to be elucidated.

Despite the alterations induced in irradiated eggs, the *A. fraterculus* larvae that hatched were suitable as hosts for *D. longicaudata* (Table 2). The parasitism rates were all higher than 70%, varying between 73 and 96%, and sex ratio values were higher than 0.6 (Table 2), demonstrating that the development of *D. longicaudata* was uninfluenced by the radiation dose received by its host. Similar values of parasitism by *D. longicaudata* have been found in other studies. Messing et al. (1993) verified a mean emergence rate of 68.6 ± 0.64% and sex ratio of 0.64 for *D. longicaudata* during the 1.5 yr that the USDA-ARS laboratory from Honolulu shipped this parasitoid to Kauai. The mean parasitism rate obtained in *D. longicaudata* reared on *C. capitata* larvae on the 1990s at CENA/USP was around 30% (Walder et al. 1995). At the parasitoid/host ratios of 1:4 and 1:8, Montoya et al. (2000) obtained emergence percentages of *D. longicaudata* of 67.3 ± 1.64% and 66.58 ± 3.01%, respectively. Gonzalez et al. (2007) observed a percentage of emergence and sex ratio of 83.1 ± 1.2% and 0.61, respectively, in *D. longicaudata* coming from pupae with 2–6 scars. Cancino et al. (2009a) reported parasitoid emergence and sex ratio values of *D. longicaudata* derived from 72-h-old irradiated eggs of *A. ludens* higher than 40% and ≥ 0.6, respectively.

The parasitism capability and longevity of *D. longicaudata* specimens reared on larvae derived from irradiated eggs were not negatively affected. During the 10 d of oviposition evaluated, *D. longicaudata* from the treatments were able to parasitize nonirradiated larvae similarly to the parasitoids from the control group (0 Gy) and the laboratory colony (I × P treatment) (Table 3). The parasitism rates varied between 43 and 81%, an interval that is overlapped by values found in literature (Cancino et al. 2009a, Gonzalez et al. 2007). Sex ratio of the parasitoids from the treatments were not negatively affected either (Table 5), maintaining mean values (0.63–0.7) similar to those reported by Gonzalez et al. (2007) and Cancino et al. (2009a). Longevity of the parasitoids emerged from larvae derived of 48-h-old eggs irradiated at different doses was not significantly affected, with survival rates higher than 80% after 20 days of evaluation (Table 5). Cancino et al. (2009a) found that the longevity and fecundity of *D. longicaudata* emerged from *A. ludens* irradiated eggs were not affected either by radiation doses.

As no significant reduction in hatchability was observed for the 48-h-old eggs at the dose range of 5–45 Gy, no adult fly emerged at doses higher than 27.5 Gy (Tables 1 and 2), and pupation was maintained greater than 90% up to 40 Gy, the age of 48 h and a dose of 30 Gy could be considered the best age and dose for *A. fraterculus* eggs to be used in the mass-rearing of *D. longicaudata*.

The irradiation of *A. fraterculus* eggs instead of larvae brings basically four major advantages for the rearing of *D. longicaudata*. The first one is related to the suppression of fly emergence from unparasitized pupae, allowing the shipment of clean lots of parasitoids to be released in the field. When *A. fraterculus* nonirradiated larvae are used as hosts, the emergence of *D. longicaudata* occurs at almost the same time as the adult flies, increasing the labor to separate the flies and contamination caused by starved and dead hosts. Collecting eggs from the colony cages is easier than removing larvae from diet, again decreasing the demands of labor, time and space. When larvae are irradiated, they are usually kept in small crowded containers and the increase of temperature can induce sometimes a mortality of hosts of 10% or greater (Cancino et al. 2002b), what is not verified for eggs diluted in water. The fourth advantage is related to the volume of eggs exposed to radiation. One milliliter of *A. fraterculus* eggs contains approximately 11,700 eggs, and as pupation is not affected by doses up to 30 Gy (Table 2), much lesser volumes of host material are required to be irradiated for the mass-rearing of the parasitoid. Small volumes containing thousands of irradiated eggs in water can be easily shipped in the same boxes containing sterile *A. fraterculus* pupae that will be sent weekly from CENA/USP to Southern Brazil, eliminating extra costs of transport with additional boxes.

With the reduction of residue limits and lack of new insecticides for the chemical control of *A. fraterculus* in temperate fruit orchards, time has come for the implementation of biological control through inundative release of fruit fly parasitoids in Southern Brazil. The apple growers, in particular, are eager to test this strategy because it has been considered a technique with great potential to achieve environmentally sound fruit fly control (Baranowski et al. 1993, Montoya et al. 2007, Suarez et al. 2014). Studies on shipment, handling and release techniques are underway in order to maintain the quality of the parasitoids released in the fields.

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