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The suitability of *Anastrepha* spp. and *Ceratitis capitata* larvae as hosts of *Diachasmimorpha longicaudata* and *Diachasmimorpha tryoni*: Effects of host age and radiation dose and implications for quality control in mass rearing

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The emergence of parasitoids from irradiated tephritid host larvae of different species and ages was evaluated. Parasitoid and fly longevity and fecundity resulting from each treatment were also assessed. Doses of 5, 10, 20, 30, 40, 50, 80, 100 and 150 Gy were applied to samples (100 larvae) of 6-, 7-, 8- and 9-day-old *Anastrepha* spp. larvae (*A. ludens* (Loew), *A. obliqua* (Mcquart) and *A. serpentina* (Wiedemann)) and 6- and 7-day-old *Ceratitis capitata* (Wiedemann) larvae. *Anastrepha* larvae were exposed to *Diachasmimorpha longicaudata* (Ashmead), and *C. capitata* larvae to *D. tryoni* (Cameron). Following larval exposures of 20 Gy, fly emergence was totally suppressed in all larval ages of *A. ludens* and *A. serpentina*, while in *A. obliqua* and *C. capitata*, total suppression was achieved at 30 Gy. In all species, fly emergence decreased with increasing radiation dosages from 5 to 20 Gy. Emerged fly fertility and longevity also decreased as the radiation increased. On the other hand, parasitoids did not suffer decreases in longevity or fecundity as host radiation dose increased. Larval age at the time of irradiation did not influence emergence, longevity and fecundity of either flies or parasitoids. When the irradiated cohort size was raised to one liter of larvae (about 32,000 *Anastrepha* or 50,000 *C. capitata* larvae) a dose of 40 Gy in *A. ludens*, *A. serpentina* and *A. obliqua* totally suppressed fly emergence but permitted *D. longicaudata* emergence, while for *C. capitata* larvae, it was necessary to increase the dose to 60 Gy. Quality control tests under mass rearing conditions were applied to *D. longicaudata* reared using irradiated *A. ludens* larvae. There was no statistical difference between parasitoids derived from irradiated or non-irradiated host for most parameters. Only percent pupation after 72 h differed, along with the consequent differences between the percent emergence and pupal weight. The conclusions drawn from this study lead to a greater flexibility in the use of irradiated hosts in the mass rearing of the fruit fly parasitoids *D. longicaudata* and *D. tryoni*.

Keywords: *Diachasmimorpha longicaudata*; *Diachasmimorpha tryoni*; irradiated host; fruit fly parasitoids; mass-rearing parasitoids; quality control; *Anastrepha*; *Ceratitis*

Introduction

Radiation is an important and novel technique in the mass rearing of natural enemies. Radiation has led to advances such as host storage, emergence suppression,

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and ease of host manipulation (Morgan, Smittle, and Patterson 1986; Sivinski and Smittle 1990; Roth, Fincher, and Summerlin 1991). The mass rearing of fruit fly parasitoids has taken an important step forward with the use of radiation. Host irradiation has permitted the suppression of emergence from unparasitized hosts without affecting the fecundity or longevity of the adult parasitoids that emerge (Sivinski and Smittle 1990; Cancino, Ruíz, Gómez, and Toledo 2002). The earliest experiments applied radiation to *Bactrocera dorsalis* (Hendel) pupae that had been previously parasitized by *Diachasmimorpha longicaudata* (Ashmead) in order to obtain parasitoids and sterile flies at the same time. Unfortunately, sterility was found in both species (Ramadan and Wong 1989). Sivinski and Smittle (1990) reported the emergence of *D. longicaudata* parasitoids and the suppression of *Anastrepha suspensa* (Loew) emergence when host larvae were irradiated before exposure to parasitoids. As a result, irradiation was incorporated into mass-rearing procedures for various species of fruit fly parasitoids. Some adjustments and applications were proposed by Cancino et al. (2002) using *Anastrepha ludens* (Loew) as host to *D. longicaudata* that further simplified the management of large quantities of parasitoids. Host irradiation in the mass rearing of fruit fly parasitoids is currently found useful in many laboratories (Brazil, Florida, Guatemala, Mexico and Peru) (Sivinski et al. 1996; Baeza, Sivinski, Holler, and Aluja 2002; Cancino et al. 2002).

However, some effects of radiation on different species remain to be analyzed. There is little known about the effects of the radiation on larval hosts of different ages. This information is vital because the age of a host larva is a determinant of host size and weight, percent parasitism, and emergence of adult parasitoids (Wong and Ramadan 1992; Wong 1993). Other aspects, such as the optimal dose for mass-rearing in the different host species and the effects of dose on parasitoid quality control parameters must also be assessed.

In this study, larvae of three species of the genus *Anastrepha*: *A. ludens*, (Loew) *A. obliqua* (Mcquart) and *A. serpentina* (Wiedemann) were evaluated at different ages as hosts to *D. longicaudata*. In the same fashion, different larval ages of *Ceratitidis capitata* (Wiedemann) were compared as hosts for *Diachasmimorpha tryoni* (Cameron). Various radiation doses were then evaluated in each host species with their respective parasitoids. The effects of host irradiation on parameters of quality control in *D. longicaudata* were also determined. All these appraisals will ultimately improve the use of radiation in the mass rearing of larval fruit fly parasitoids.

Materials and methods

Parasitoids and flies evaluated were taken from their respective colonies maintained in Moscamed-Moscafrut Program ubicated in Metapa de Domínguez, Chiapas, Mexico. Adult parasitoids of *D. longicaudata* and *D. tryoni* were obtained from the Moscafrut Plant and the Biological Control Department. These strains have been maintained under laboratory conditions for over 300 generations. Larvae of *A. ludens*, *A. obliqua* and *A. serpentina* were obtained from the laboratory colonies of the Rearing and Colonization Department. *Ceratitidis capitata* larvae were taken from the strain kept under mass rearing conditions.

The irradiation of larvae was performed in a Gammacell 220 with a Co 60 source of γ radiation. The doses were applied with a range of 2.5–3.0 Gy/min in free oxygen. Exposure times were determined with Fricke dosimetry (IAEA 2001).

Irradiating host *Anastrepha* and *C. capitata* larvae at different ages and radiation effects on emergence of parasitoids

Anastrepha ludens, *A. obliqua* and *A. serpentina* larvae aged 6, 7, 8 and 9 days old and *C. capitata* larvae aged 6 and 7 days old were subjected to irradiation doses of: 5, 10, 20, 30, 40, 50, 80, 100 and 150 Gy. The irradiated larvae of *Anastrepha* were then exposed to *D. longicaudata* and *C. capitata* larvae to *D. tryoni* as described below. Non-irradiated larvae were used as the control.

A sample of 100 larvae per species, age and dose were exposed to 30♀:15♂ parasitoids for 2 h. Five- to 10-day-old parasitoids were put into a 'Hawaii-type' screen cage (27 × 27 × 27 cm) (Wong and Ramadan 1992). The larval exposure unit consisted of a Petri dish cover containing larvae and diet, and then covered with a piece of organza cloth held in place with a circular plastic top. Following exposure a cylindrical plastic container (8 × 4 cm) with vermiculite was placed into each treatment and held at 26°C and 60–80% R.H. The data taken consisted of the number of emerged adults (parasitoids and flies), larval, and pupae weight.

Fecundity and longevity in adults derived from irradiated hosts

Samples of 10♀:5♂ adults from each treatment were evaluated for longevity and fecundity. For each *D. longicaudata* treatment, 50 *A. ludens* larvae were exposed to parasitoids that had emerged from *A. ludens*, *A. obliqua* and *A. serpentina* larvae. Fifty *C. capitata* larvae were exposed to *D. tryoni* parasitoids emerged from *C. capitata* larvae. The larval exposure and holding were as described above. Following adult emergence, daily fecundity was calculated as the number of offspring eclosed daily per female (offspring/female/day). Fecundity of females from age 5 to 15 days was evaluated. Dead parasitoids found in each cage were removed and counted daily for 15 days.

The fertility of the emerged flies was also evaluated with samples of 10♀:10♂. When females reached 12 days of age, an oviposition substrate was provided per cage for 2 h daily. The oviposition unit was a green agar ball (2 cm diameter) covered with parafilm paper (Boller 1968). The eggs oviposited in the unit were collected with a scalpel. A sample of 100 of the collected eggs was then immediately incubated inside a Petri dish with a piece of filter paper saturated with water. After 5 days, the eggs were observed with a stereoscope to count the number hatched. Fertility was calculated as the number of hatched eggs/female/day.

Longevity, fecundity and fertility of adults emerged from irradiated larvae were compared with the control treatments of parasitoids and flies emerged from non-irradiated larvae.

Radiation dose and host emergence under mass rearing conditions

In these experiments, the radiation doses that suppress emergence of unparasitized flies were analyzed. Again, larvae of *A. ludens*, *A. obliqua* and *A. serpentina* (8 days old) were exposed to *D. longicaudata* and *C. capitata* larvae (7 days old) were exposed to *D. tryoni*. For *A. ludens* and *A. serpentina* larvae, doses of 20, 30, 40 and 50 Gy were used. In *A. obliqua* and *C. capitata* larvae, 30, 40, 50 and 60 Gy were

applied. In each treatment, 1 L of larvae (approximately 32,000 *Anastrepha* spp. larvae and 50,000 *C. capitata* larvae) were irradiated. A sample of 200 larvae from each liter was then exposed to parasitism. Exposure periods lasted 2 h and the subsequent procedures were similar to those used in mass rearing (Cancino 2000). After exposure, larvae were removed from their diet and placed in trays (8 × 4 cm) with vermiculite. Before adult emergence, 14 days following pupation, three samples of 100 pupae per treatment were taken and put into a cylindrical plastic container (8 × 4 cm). The number of emerged parasitoids and flies was counted.

Quality parameters in the mass rearing of D. longicaudata with irradiated

A. ludens larvae

Ten lots of mass produced *D. longicaudata* from irradiated and non-irradiated larvae were evaluated by applying various quality control tests. The parameters were:

Percent mortality and pupation of the host at 72 h. Three samples of 100 larvae per lot were taken 72 h after exposure to parasitoids. In each sample, the numbers of pupae, live and dead larvae were counted and the data were used to calculate the percent pupation and mortality.

Emergence and sex-ratio. Three samples of 100 pupae were taken per lot and put into cylindrical plastic containers (8 × 4 cm). Following emergence, the numbers of parasitoids and flies and their sex-ratio were obtained.

Percentage of adults capable of flight. Three samples of 100 pupae per lot were individually introduced into black PVC tubes (10 × 10 cm). The inside walls were covered with talcum powder. Each tube was put into a cage (0.5 × 1 × 1 m) fitted with a light source. Following emergence, the parasitoids walking inside the tube were considered 'non-fliers' and those outside the tube 'fliers'.

Longevity with and without food. 30♀:15♂ newly emerged adult parasitoids per lot were held in screen cage and were either provided with honey and water or deprived of both. Daily mortality inside the cages was registered from day one. The test was carried out until the 15th day when honey and water were present and only until the 7th day when no food or water were provided.

Fecundity. One sample per lot of 30♀:15♂ was placed into a screen cage. When the females were 5 days old and continuing until they were 15 days old, daily host exposures were carried out with a Petri dish-type oviposition unit containing 50 *A. ludens* larvae. Following exposure, larvae were maintained in a container with vermiculite for 15 days at 26°C and 60–80% R.H. The number of offspring per day was related to the number of live females.

Olfatometric measurements. Thirty groups of three parasitoid females per lot were introduced into a 'Y' glass tube olfactometer with arms 21 cm long and 3 cm in diameter, and separated by an angle of 85°. Air flow (400 ml/min) was provided by a pressurized tank. The air flow in each arm bore volatiles from one of two sources: mango fruit infested with *A. ludens* larvae and uninfested mango. A positive response was defined as a walk of at least 10 cm into an arm within 5 min.

Onset of oviposition activity. Samples of 10 female (5 days old) parasitoids per lot were introduced into a screen cage with an oviposition unit containing 50 *A. ludens*

larvae. Over three consecutive days, for a period of 4 h, the number of females posing and ovipositing on the artificial unit was observed and recorded hourly.

Longevity and fecundity under field conditions. A sample of 100♀:50♂ parasitoids per lot were placed into a cage (1×1×0.5 m). Over 15 days (from the first to the 15th day of age), the daily mortality of both females and males was recorded, and for a period of 10 days (from the 5th to the 15th day) a fresh oviposition unit with 50 *A. ludens* larvae was placed inside daily. A leafy branch was added to the cage to simulate environmental conditions. This test was carried out at 25–30°C and 70–90% H.R. The fecundity data were calculated from offspring emergence per day and its relationship to the number of live females. Longevity data were obtained from the proportion of live parasitoids per day.

Data analysis

Twenty replicates were performed for the evaluations of the effects of radiation on different aged larvae and their subsequent suitability as hosts. The data were obtained by applying a bifactorial design (factors: age of larvae and doses) and analyzed by two-way ANOVA. Data with zero value were compared using Bonferroni's test. Fecundity of the progeny was also analyzed with two-way ANOVA and Tukey's multiple range test was used to distinguish means. In the comparison of various radiation doses, 10 replicates were carried out and the results were analyzed using ANOVA and Tukey's multiple range test. The means for the quality control parameters were obtained from 10 lots of *D. longicaudata*, and a Student's *t*-test was applied to these parameters for statistical analysis. Prior to statistical analysis, data were checked for ANOVA assumptions and transformed, if needed, by $\log(x+1)$, arcsine and Box-cox transformation.

Results

Irradiating host Anastrepha and C. capitata larvae at different ages and radiation effects on the emergence of parasitoids

The percentage emergence and sex-ratio of adult *D. longicaudata* and *D. tryoni* parasitoids emerged respectively from *Anastrepha* and *C. capitata* larvae are shown in Table 1. The parasitoid emergence in *A. ludens* and *C. capitata* decreased with the age of larvae (two-way ANOVA *A. ludens*: $df=3$, $F=26.98$, $P<0.000$; *C. capitata*: $df=1$, $F=49.42$, $P<0.000$). This inverse relationship was not observed in the other species. However, there were significant differences between particular ages in *A. obliqua* and *A. serpentina* (two-way ANOVA *A. obliqua*: $df=3$, $F=4.56$, $P=0.004$; *A. serpentina*: $df=3$, $F=2.70$, $P=0.045$). Parasitoid sex ratios were not different statistically in *A. obliqua* and *C. capitata* (two-way ANOVA *A. obliqua*: $df=3$, $F=0.40$, $P=0.755$; *C. capitata*: $df=1$, $F=3.30$, $P=0.070$). In *A. ludens* and *A. serpentina*, there were significant differences (two-way ANOVA *A. ludens*: $df=3$, $F=6.22$, $P=0.000$; *A. serpentina*: $df=3$, $F=13.09$, $P<0.000$), but these had no clear relationship with larval age. There were no interactions between irradiation doses and larval age in any host species.

Table 1. Mean (\pm S.E.) of emergence and sex-ratio of parasitoids reared on *Anastrepha* spp. and *C. capitata* irradiated larvae at different ages.

Age of larva (days)	n	Parasitoid emergence (%)	Sex-ratio (female/males)
<i>A. ludens</i>			
6	158	67.51 \pm 1.10 a	2.17 \pm 0.12 ab
7	168	60.90 \pm 1.07 b	2.44 \pm 0.11 a
8	158	57.39 \pm 1.11 bc	1.76 \pm 0.11 b
9	175	57.09 \pm 1.05 c	2.03 \pm 0.11 ab
<i>A. obliqua</i>			
6	93	38.33 \pm 1.06 a	1.51 \pm 0.07 a
7	100	36.28 \pm 1.01 ab	1.42 \pm 0.07 a
8	99	32.72 \pm 1.02 b	1.48 \pm 0.07 a
9	96	36.91 \pm 1.04 a	1.41 \pm 0.06 a
<i>A. serpentina</i>			
6	102	50.10 \pm 1.29 ab	5.12 \pm 0.07 c
7	118	48.92 \pm 1.20 ab	6.07 \pm 0.64 bc
8	111	48.44 \pm 1.25 b	7.97 \pm 0.70 a
9	88	52.66 \pm 1.41 a	7.27 \pm 0.76 ab
<i>C. capitata</i>			
6	206	36.39 \pm 0.79 a	7.09 \pm 0.41 a
7	183	28.33 \pm 0.84 b	5.97 \pm 0.42 a

Means followed by different letters into the same column indicate a significant difference. Data was analyzed through two-way ANOVA followed by Tuckey Multiple Range test ($\alpha = 0.05$)

Irradiating host Anastrepha and C. capitata larvae at different ages and radiation's effects on the emergence of flies

Only in *C. capitata* was the fly emergence between ages statistically different. Averages of 13.17 \pm 0.99 and 8.68 \pm 1.05 flies emerged from 6- and 7-day-old larvae, respectively ($df = 1$, $F = 6.31$, $P = 0.013$). The fly emergence from irradiated larvae of *Anastrepha* spp. were not different between ages (*A. ludens*: $df = 3$, $F = 1.69$, $P = 0.169$; *A. serpentina*: $df = 3$, $F = 0.29$, $P = 0.833$; *A. obliqua*: $df = 3$, $F = 2.07$, $P = 0.107$). In

Table 2. Mean (\pm S.E.) of fly emergence from unparasitized hosts of different species of *Anastrepha* and *C. capitata*, exposed to various radiation doses.

Irradiation	Fly emergence			
	<i>A. ludens</i>	<i>A. obliqua</i>	<i>A. serpentina</i>	<i>C. capitata</i>
Dose (Gy)				
0	6.88 \pm 0.72 a	25.45 \pm 2.04 a	3.21 \pm 1.03 a	16.24 \pm 1.55 a
5	7.5 \pm 0.72 a	25.69 \pm 2.07 a	4.61 \pm 1.17 a	21.25 \pm 1.55 a
10	1.23 \pm 0.72 b	8.4 \pm 2.04 b	0.14 \pm 1.19 b	18.32 \pm 1.61 a
20	0 c	0.53 \pm 2.04 c	0 c	0.12 \pm 1.50 b
30	0 c	0 d	0 c	0.10 \pm 1.54 b
40 to 150	0 c	0 d	0 c	0 c

Means followed by different letters into the same column indicate a significant difference. Data was analyzed through a two-way ANOVA followed by Tuckey Multiple Range test ($\alpha = 0.05$).

general, *Anastrepha* fly emergence began to be reduced at 10 Gy. In all species, emergence at 10 Gy was significantly less than at 5 Gy. Fewer flies emerged at each progressively higher dose (*A. ludens*: $df=2$, $F=57.67$, $P<0.000$; *A. obliqua*: $df=3$, $F=80.93$, $P<0.000$; *A. serpentina*: $df=2$, $F=18.04$, $P<0.000$). At 30 Gy, fly emergence was totally suppressed in the three species of *Anastrepha*. This complete suppression was obtained above 40 Gy in *C. capitata*. There were statistical differences between doses in *C. capitata* ($df=4$, $F=220.39$, $P>0.000$) (Table 2).

Longevity, fecundity and fertility in adults derived from irradiated hosts

Neither the longevity (Table 3) nor fecundity of parasitoids (Figure 1 and 2) were affected consistently by host irradiation dose, although there were some significant differences among host ages. Fertility in flies emerged from unparasitized irradiated larvae was very sensitive to dosage (Table 4). In *Anastrepha*, fertility decreased significantly at 5 Gy (*A. ludens*: $df=7$, $F=5.95$, $P<0.0001$; *A. serpentina*: $df=7$, $F=4.37$, $P=0.0004$; *A. obliqua*: $df=7$, $F=22.56$; $P<0.0001$). A few flies emerged at 10 Gy but they showed abnormalities in their body (wings, legs, antennae, etc.) and it was not possible to evaluate their fertility. A clear reduction of fertility was obtained in *A. ludens* and *A. obliqua*; this was variable with *A. serpentina* because the oviposition unit (green ball of agar) was not an efficient substrate for this species. The larvae of *C. capitata* were more resistant to radiation at 10 Gy than were those of *Anastrepha* spp. But beyond this level, fertility was notably reduced (*C. capitata*: $df=5$, $F=13.68$, $P<0.0001$). For practical reasons, the number of eggs oviposited by the offspring was

Table 3. Results of statistical analysis of longevity of *D. longicaudata* and *D. tryoni* adults emerged from different aged larvae of *Anastrepha* spp. and *C. capitata* exposed to different irradiation doses.

Age of larva (days)	d.f.	χ^2	P
<i>A. ludens</i>			
6	9	4.513	0.875
7	9	23.33	0.005
8	9	8.52	0.482
9	9	19.97	0.018
<i>A. obliqua</i>			
6	9	13.74	0.132
7	9	10.04	0.348
8	9	29.82	0.000
9	9	49.37	<0.0001
<i>A. serpentina</i>			
6	9	23.78	0.005
7	9	25.53	0.002
8	9	35.79	<0.000
9	9	33.25	0.000
<i>C. capitata</i>			
6	9	19.58	0.021
7	9	16.49	0.057

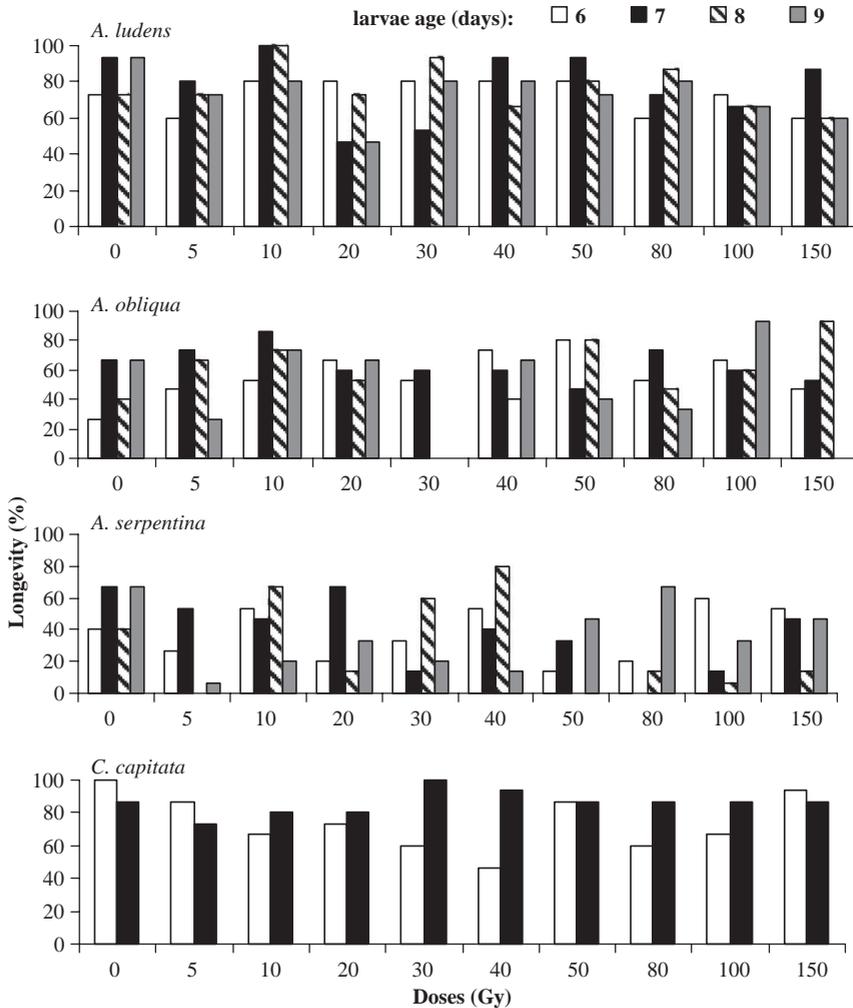


Figure 1. Percent adults surviving to the 15th day of *Diachasmimorpha longicaudata* and *D. tryoni* emerged from different aged larvae of *Anastrepha* spp. and *Ceratitidis capitata* exposed to different irradiation doses.

not recorded. Nonetheless, we observed that fewer fly eggs were oviposited by adults which had been subjected to higher doses of radiation.

Adjusting dose to avoid host emergence under mass rearing conditions

The emergence of adult flies from 1 L of *Anastrepha* spp. and *C. capitata* larvae was totally suppressed at higher doses (Table 5) (*A. ludens*: $df = 2,95$, $F = 529.22$, $P < 0.0001$; *A. obliqua*: $df = 1, 23$, $F = 359.47$, $P < 0.0001$; *A. serpentina*: $df = 1,21$, $F = 452.55$, $P < 0.0001$; *C. capitata*: $df = 2,79$, $F = 219.53$, $P < 0.0001$). In *Anastrepha* larvae, doses above 30 Gy were necessary to suppress fly emergence and complete suppression of *C. capitata* was obtained at 50 Gy. Again, there were no negative side-effects of higher doses on parasitoid emergence or sex ratio.

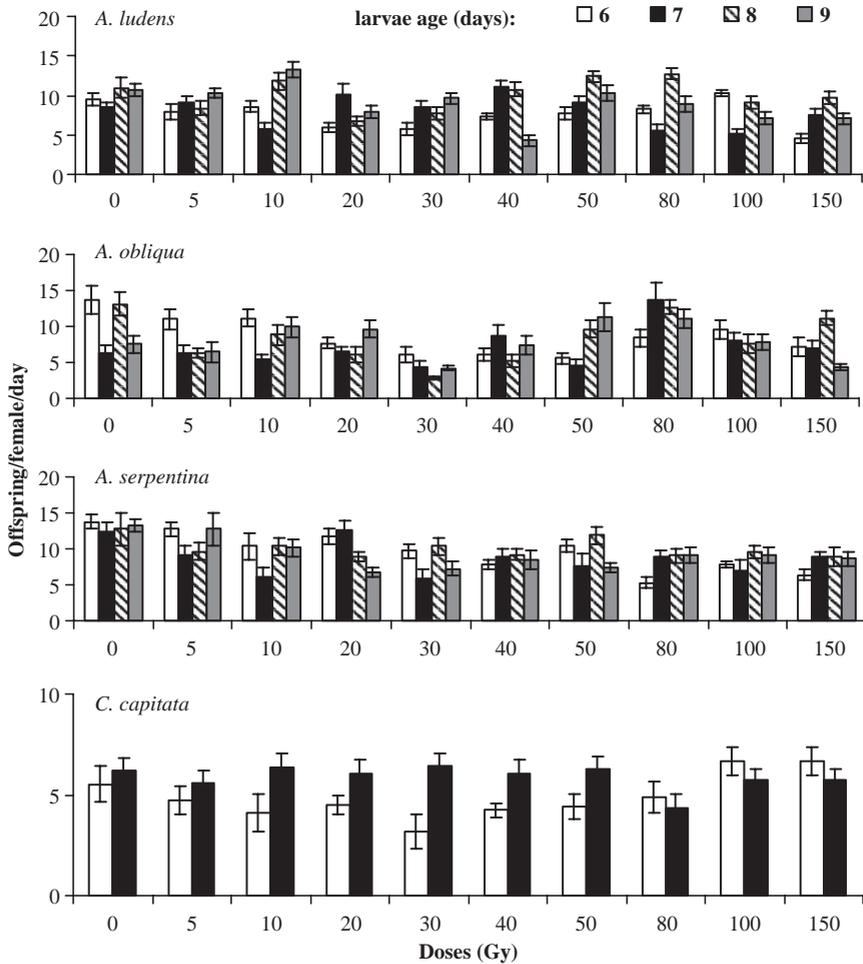


Figure 2. Means (\pm SE) of fecundity (offspring/female/day) of *Diachasmimorpha longicaudata* and *D. tryoni* emerged from different aged larvae of *Anastrepha* spp. and *Ceratitidis capitata* exposed to different irradiation doses.

Quality parameters in the mass rearing of *D. longicaudata* parasitoids with irradiated hosts

Only the percentage of host pupation at 72 h, pupal weight, and the percentage of *D. longicaudata* parasitoid emergence were statistically different between the parasitoids that developed in 45 Gy irradiated versus non-irradiated *A. ludens* larvae (percentage of host pupation at 72 h: $t = 2.35$, $P = 0.03$; pupae weight: $t = 2.72$, $P = 0.008$, and the percentage of parasitoid emergence: $t = 2.48$, $P = 0.015$). No significant difference was found for the other parameters evaluated (Table 6).

Discussion

These results indicated broad tolerances in the use of irradiation for the rearing of fruit fly parasitoids. There were no effects of radiation dosage on host-suitability nor

Table 4. Means (\pm S.E.) of fertility (percent egg hatch) of flies that emerged from irradiated larvae.

Dose (Gy)	Host age (days)	Percent egg hatch			
		<i>A. ludens</i>	<i>A. obliqua</i>	<i>A. serpentina</i>	<i>C. capitata</i>
0	6	85.27 \pm 3.07 ab	73.31 \pm 5.97 ab	5.58 \pm 3.63 b	73.04 \pm 9.71 a
	7	89.27 \pm 2.66 a	78.75 \pm 2.24 ab	34.86 \pm 6.46 a	90.43 \pm 1.52 a
	8	83.27 \pm 3.31 ab	85.57 \pm 2.51 a	9.14 \pm 6.05 b	
5	9	80.6 \pm 2.92 ab	92.62 \pm 1.20 a	7.15 \pm 3.05 ab	
	6	58.8 \pm 5.71 c	42.83 \pm 10.26 c	0 \pm 0 b	74.76 \pm 9.26 a
	7	75.46 \pm 3.71 abc	12.63 \pm 5.86 d	11.63 \pm 4 ab	82.82 \pm 5.72 a
	8	77.6 \pm 3.75 abc	51.75 \pm 6.80 bc	0 \pm 0 b	
10	9	68 \pm 5.95 bc	28.94 \pm 7.27 cd	7.10 \pm 3. ab	
	6				11.85 \pm 4.55 b
	7				28.61 \pm 12.21b

Means followed by different letters within each column are significantly different. Data was analyzed through a ANOVA followed by Tuckey Multiple Range test ($\alpha=0.05$).

did the age of the various larvae substantially interact with dosage. The major difference was found between *Anastrepha* species and *C. capitata*, i.e. a higher dose was required to suppress adult-host emergence in the latter, and this may be related with the larval size. During these evaluations, the mean larval weights for all ages of *A. ludens* and *A. serpentina* were above 21 mg. In *A. obliqua*, the minimum weight was 17.4 mg, while in *C. capitata*, weight never exceeded 13 mg. The effects of radiation appear related to the size of the receiving body (Balock, Burditt, and Christenson 1963; Bustos, Enkerlin, Toledo, Reyes, and Casimiro 1992). Fertility of emerged flies was affected at a lower dose, 5 Gy, in the larger *Anastrepha* spp. Fertility was maintained in the smaller *C. capitata* up to 10 Gy. Similar results have been published in diverse evaluations of fruit fly larvae irradiated during post-harvest treatments (Arthur and Wiendl 1996; Hallman and Worley 1999; Toledo, Bustos, and Liedo 2001). The studies performed by Bustos et al. (1992) provided important support for the irradiation of larval hosts prior to exposition to *D. longicaudata* parasitoids.

Parasitoids which emerged from irradiated host larvae demonstrated adequate levels of longevity and fecundity. The results obtained in these evaluations demonstrate that the use of an irradiated host does not have any negative repercussions on parasitoid development. The irradiation affects fly pupal development and it is independent of parasitoid physiology (Nation, Smittle, Milne, and Dykistra 1995). The availability of irradiated larvae in *Anastrepha* spp. and *C. capitata* could be extended to other fruit fly larval parasitoids particularly larval-prepupal parasitoids of the braconid subfamily Opinae (Cancino, Ruíz, Sivinski, Gálvez, and Aluja, 2009).

The radiation doses that were effective for suppression of fly emergence in small lots were not effective, however, when large lots of mass reared larvae were exposed. The radiation doses used for 1 L of larvae (about 32,000 larvae in *Anastrepha* spp. and 50,000 in *C. capitata*) had to be raised to 40 Gy for *A. serpentina*, *A. ludens* and *A. obliqua*, and 60 Gy were necessary for *C. capitata*. In addition to species/size

Table 5. Means (\pm S.E.) of fly and parasitoid emergence and sex-ratio of *D. longicaudata* and *D. tryoni* reared on *Anastrepha* spp. and *C. capitata* from 1 L of larvae irradiated at different doses.

Irradiation Dose (Gy)	Fly emergence (%)	Parasitoid emergence (%)	Sex-ratio (female/males)
<i>A. ludens</i>			
0	31.13 \pm 1.37 a	65.47 \pm 1.39 a	1.13 \pm 0.03 a
20	5.39 \pm 0.46 b	67.64 \pm 1.33 a	1.16 \pm 0.02 a
30	0.66 \pm 0.21 c	67.82 \pm 1.32 a	1.20 \pm 0.01 a
40	0 d	68.36 \pm 1.51 a	1.16 \pm 0.01 a
50	0 d	68.59 \pm 1.18 a	1.17 \pm 0.02 a
<i>A. obliqua</i>			
0	30.93 \pm 3.71 a	20.93 \pm 1.77 a	1.12 \pm 0.12 a
30	0.36 \pm 0.28 b	20.18 \pm 2.23 a	1.55 \pm 0.31 a
40	0 b	22.33 \pm 2.70 a	1.03 \pm 0.31 a
50	0 b	20.20 \pm 1.67 a	1.81 \pm 0.35 a
60	0 b	21.62 \pm 3.03 a	2.18 \pm 0.61 a
<i>A. serpentina</i>			
0	19.08 \pm 4.15 a	34.50 \pm 3.66 a	1.94 \pm 0.29 a
20	0.08 \pm 0.08 b	41.58 \pm 3.68 a	1.68 \pm 0.26 a
30	0 b	41.75 \pm 3.57 a	1.47 \pm 0.09 a
40	0 b	40.00 \pm 3.91 a	1.40 \pm 0.12 a
50	0 b	43.17 \pm 3.88 a	1.61 \pm 0.11 a
<i>C. capitata</i>			
0	23.15 \pm 1.62 a	27.52 \pm 1.61 a	1.66 \pm 0.20 a
30	5.23 \pm 1.21 b	25.87 \pm 1.73 a	1.96 \pm 0.27 a
40	1.60 \pm 0.30 c	25.43 \pm 1.69 a	2.21 \pm 0.33 a
50	0 d	26.93 \pm 2.07 a	2.40 \pm 0.38 a
60	0 d	24.31 \pm 1.93 a	2.58 \pm 0.36 a

Means followed by different letters in the same column indicate statistical difference. Data was analyzed through a ANOVA followed by Tuckey Multiple Range test ($\alpha = 0.05$).

affects, other factors, such as the physical condition of the larvae after they have been taken off their diet, the number and volume of larvae, and the status of the irradiator, influence the required radiation dose. Prior evaluations to determine the optimal doses for large quantities of larvae have been carried out. For example, in Mexico, in the mass rearing of *D. longicaudata* at the Moscafrut Plant in Metapa de Domínguez, Chiapas, 45 Gy are routinely applied to 10 million *A. ludens* larvae daily with the objective of suppressing adult fly emergence (Cancino et al. 2002). In the mass production of *D. tryoni* using *C. capitata* larvae as hosts, 70 Gy were necessary to avoid the adult emergence of the non-parasitized flies.

Several mass release field studies in Mexico have reported a high efficiency of parasitoids mass reared in irradiated hosts (Montoya et al. 2000). The longevity and fecundity data obtained from parasitoids emerged from irradiated larvae in this study confirm that these attributes do not suffer any reduction. Moreover, the analysis of quality control parameters of parasitoids reared with irradiated and non-irradiated larvae did not show any significant difference.

Table 6. Means (\pm S.E.) of quality control parameters of *D. longicaudata* reared on irradiated to 45 Gy and non-irradiated larvae of *A. ludens*.

Parameter	Irradiated host	Non irradiated host
QUALITY OF THE PROCESS		
Host mortality at 72 h (%)	1.17 \pm 0.20 a	1.25 \pm 0.22 a
Host pupation at 72 h (%)	97.03 \pm 0.28 b	98.31 \pm 0.24 a
Pupae weight (mg)	11.61 \pm 0.12 b	12.06 \pm 0.12 a
QUALITY OF THE PRODUCT		
Emergence of flies from non-exposed larvae (%)	0 b	90.16 \pm 1.15 a
Emergence of flies from exposed larvae (%)	0 b	4.44 \pm 0.60 a
Parasitoid emergence (%)	65.94 \pm 1.63 a	59.69 \pm 1.91 b
Sex-ratio of parasitoids (female/male)	3.95 \pm 0.30 a	3.86 \pm 0.25 a
Parasitoid fliers (%)	88.32 \pm 1.18 a	88.79 \pm 0.90 a
Longevity and fecundity with food at 20 th day (percent of alive adults)		
Females	69.70 \pm 5.97 a	66.67 \pm 5.02 a
Males	66.67 \pm 3.68 a	60.00 \pm 4.87 a
Offspring/female/day	4.26 \pm 0.13 a	4.31 \pm 0.12 a
Longevity without food at 7 th day (percent of alive adults)		
Female	21.52 \pm 8.18 a	36.37 \pm 9.65 a
Male	15.04 \pm 5.98 a	21.62 \pm 7.07 a
Behavior test		
Female with positive response to infested mango	61.11 \pm 7.35 a	51.39 \pm 6.24 a
Search and oviposition activity of female		
Female posing	40.00 \pm 2.67 a	39.33 \pm 2.91 a
Female ovipositing	20.00 \pm 2.30 a	20.67 \pm 2.75 a
Evaluation under field conditions		
Longevity at female at 20 th day (percent of alive females)	53.67 \pm 7.82 a	50 \pm 6.18 a
Longevity of male at 20 th day (percent of alive male)	45 \pm 6.13 a	37 \pm 7.35 a
Offspring/female/day	4.61 \pm 0.38 a	4.44 \pm 0.25 a

Means followed by different letters by row implicate significant differences. Data was analyzed through a ANOVA followed by Tuckey Multiple Range test ($\alpha=0.05$).

Only the percentage of pupation at 72 h was higher in the non-irradiated larval groups. This suggests that irradiated larvae were slower in their transformation to pupae, perhaps due to damage of some component(s), structures, glands, hormones, etc., that are crucial to the pupation process. This effect was mentioned by Zderek (1985), who included irradiation as an important abiotic factor in affecting the pupation time.

Fortunately, this delay in the pupation of irradiated larvae is not a problem in the mass rearing process of these parasitoids. The proper temperature and the artificial vermiculite medium promote a high percentage of pupation 72 h after the exposure. In general, the use of irradiation in larval hosts of fruit fly parasitoids does not cause negative effects to the mass rearing process. The use of irradiation in the mass rearing process of fruit fly parasitoids is clearly positive and even indispensable to the application of augmentative parasitoid releases as part of a fruit fly integrated pest management program.

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