

## Effect of gamma radiation on the male sterility and other quality parameters of peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae)

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### Abstract

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*Bactrocera zonata*, a serious pest of fruits in many parts of the world, has recently been recorded in Northern Africa. Even though it has not been introduced to the European continent yet, a strong emphasis is being placed on developing effective measures to suppress this pest and to prevent it from establishing in neighbouring European countries. The sterile insect technique is widely used in integrated programmes against tephritid fruit flies and, in this paper, quality parameters of irradiated *B. zonata* were evaluated for possible use of sterile insect technique within the management of this pest. Pupae were irradiated (<sup>60</sup>Co) 48 h before adult emergence (in an air atmosphere) with doses of 10, 30, 50, 70 or 90 Gy. While adult emergence and egg hatch decreased with increasing dose, no significant differences in female fecundity were found among doses. Exposure of pupae to 90 Gy resulted in a total sterility of eggs laid by non-treated females crossed with treated males. Only insignificant difference in the radiation effect on female fecundity was found. Moderate effects on sex ratio and size were recorded, as they decreased gradually by increasing doses. No considerable effect on flying capability was observed, but generally, the percentage of fliers decreased with increasing radiation doses. Fried's competitiveness values of treated males (30 and 70 Gy) suggest that irradiated males compete successfully with non-irradiated ones.

**Keywords:** gamma radiation; tephritid fruit flies; sterile insect technique; sterility; mating competitiveness

The peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), is a serious polyphagous pest that attacks over 50 cultivated and wild plants in many parts of the world (e.g. DREW 1989; WHITE, ELSON-HARRIS 1992; PEÑA et al. 1998). This fruit fly is native to Asia and mainly occurs in Southeast Asia, India, Pakistan, Mauritius, the Maluku Islands, Réunion, Sri Lanka, and Thailand. At present, it is a significant horticultural pest in India and Pakistan (QURESHI et al. 1991) and it primarily attacks peach, mango, guava, apricot, fig, and citrus (DREW 1989; WHITE, ELSON-HARRIS 1992).

*B. zonata* was recorded for the first time in Egypt in 1998. Today, it is well established in most Egyptian provinces and it causes severe damage to a wide range of fruits such as guava, peach, mango, and apricot (EL-MINSHAWY et al. 1999; KHAN et al. 2005). Development of the pest's population in Egypt proves its potential to establish populations in a relative short time and to compete successfully in new environments, particularly in the Mediterranean climate (IWAHASHI, ROUTHIER 2001; DUYCK et al. 2004). Annual losses due to the peach fruit fly are estimated at 190 million € in Egypt

(EPPO 2005). Although the species has not been introduced to the European continent yet, it has been included in the A1 list of pests, which the European and Mediterranean Plant Protection Organization (EPPO) recommends to be regulated as quarantine pests (EPPO 2005). Therefore, efficient methods need to be developed for suppressing the peach fruit fly populations in the affected areas and preventing the pest from expanding to other regions.

Traditional control measures using chemical insecticides experience disadvantages such as residual problems and inability of insecticides to penetrate infested fruits to kill larvae. Moreover, the public demand for insecticide-free fresh fruit is encouraging the use of environment-friendly methods of pest control. Sterile insect technique (SIT) is a promising environment-friendly method for control or eradication of a number of insect pests. It is rapidly becoming a major component of integrated pest management for fruit fly control (DYCK et al. 2005). The aim of SIT is to reduce the growth rate of target population by saturating wild females with released mass-reared sterilised males (KNIPLING 1955). Gamma irradiation is currently the most common method used to sterilize mass reared males for SIT (BAKRI et al. 2005) and effectiveness of SIT depends greatly on the production of good quality sterile males that are released into target wild populations. To ensure that released males are effective at inducing reproductive failure in their mates, it is important that irradiation procedures achieve an adequate level of sterility. For instance, 99.5% sterility from crosses between sterile males and fertile females is usually required in *Ceratitis capitata* (Wiedemann) (FAO/IAEA/USDA 2003). Quality of sterile males is assured through a system of bioassays of quality parameters that primarily reflect the male's ability to survive, interact with its environment, locate, mate, and fertilize females of target wild populations (COLLINS et al. 2008, 2009).

Recently, the effect of gamma radiation on development, morphology and anatomy of the peach fruit fly gonads was studied (SHEHATA et al. 2006; YOUNES et al. 2007, 2009); in addition, the influence of radiation dose on selected biological aspects of the peach fruit fly was evaluated (DRAZ et al. 2008). In the present study, we analyzed the effects of gamma radiation on selected quality control parameters of *B. zonata*, including adult emergence, female fecundity, pupal size, flight ability, male sterility, and sexual competitiveness.

## MATERIAL AND METHODS

### Insects

The laboratory culture of *B. zonata* was developed from pupae collected from infested mango fruits in Ismailia Governorate (Egypt). These collected pupae were placed in a cage (wire frame of 75 × 28 × 28 cm) coated with muslin fabric. A plastic dish (40 cm in a diameter) filled with tap water (1 l) was positioned under the rearing cage. Emerged adults were provided with a diet consisted of protein hydrolysate and sugar (1:3, w/w), a water soaked cotton clump in a small cup served as a water source. Females laid their eggs through muslin fabric and the eggs fell down to tap water in the plastic dish. The deposited eggs were collected daily and placed into plastic trays (15 × 5 × 3 cm) half-filled with an artificial diet for hatched larvae. The larval diet consisted of wheat bran (100 g), brewer's yeast (17 g), granulated sugar (33 g), agar (3.5 g), nipagin (0.5 g), hydrochloric acid (20 ml), and water (400 ml) (QURESHI et al. 1974). During the first three days of larval development, the plastic trays were covered with lids to provide sufficient humidity and then the lids were replaced by muslin fabric. On the 9<sup>th</sup> day of larval development, muslin fabric was removed to allow full-developed maggots to jump out of the trays and pupate in a layer of fine sand spread out around the trays. After the pupation terminated, the sand was sieved and pupae collected. These pupae were transferred into the rearing cages to start a new generation. Rearing of *B. zonata* and all experiments were conducted in a climatic chamber at 25 ± 2°C, 65–75% relative humidity and a photoperiod of 12:12 h (L:D).

### Treatment by gamma radiation

Samples of 7-day-old pupae (48 h before adult eclosion) obtained from the laboratory culture were irradiated using a <sup>60</sup>Co source (Gammacell irradiator, model 4000A, Bhabha Atomic Research Centre, Trombay Maharashtra, India) located at the Atomic Energy Authority, Gamma Irradiation Unit, Naser City, Cairo, Egypt. The gamma irradiation was carried out at a dose rate of 66 Gy/min and 5 different doses (10, 30, 50, 70, and 90 Gy) were used for the treatment of pupae in air atmosphere. Five hundred pupae in five batches (5 × 100 pupae), 1,600 pupae in four batches (4 × 400 pupae) and 2,400 pupae in four batches

(4 × 600 pupae) per each dose were treated for emergence evaluation, male sexual competitiveness test, and adult flight ability determination, respectively. Single batch of pupae corresponded to a repetition and each repetition was treated separately. During the irradiation process, the individual batches were held in ventilated plastic containers and an alanine reference dosimeter was attached to the irradiation chamber to measure the total delivered dose to the pupae. The treated batches of pupae were confined in separate rearing cages and used in following experiments.

### Emergence of adults and sex ratio determination

Emergence of adults was determined by placing the irradiated pupae in Petri dish bottoms inside the rearing cages. Altogether 500 pupae in five replications (5 × 100 pupae) per each dose and control variant were evaluated for emergence. In control variant the pupae were maintained in the same manner but no radiation was applied. Each batch of pupae (a replication) was placed in a separate rearing cage, emergence of adults was checked daily for five consecutive days and the number of emerged flies was recorded. The percentage of adult emergence, based on the number of adults emerging from pupal samples, was calculated.

Fruit flies from the emergence experiment were used for a sex ratio determination (males/total). Emerged adults from all the irradiation treatments and the control variant were collected using an aspirator, temporarily paralysed by chilling, and sexed. Sexes of the adults were recorded and a sex ratio was calculated.

### Female fecundity, egg hatchability, and male sexual competitiveness

Four mating trials were designed to evaluate female fecundity, egg hatchability, and sexual competitiveness of males:

- (i) 10 treated males/0 non-treated males/10 non-treated females,
- (ii) 0 treated males/10 non-treated males/10 non-treated females,
- (iii) 50 treated males/10 non-treated males/10 non-treated females,
- (iv) 100 treated males/10 non-treated males/10 non-treated females.

For the mating trials 10 non-treated virgin females and 0–10 non-treated males with 0–100 treated males were released into a rearing cage according to the specific trials. The non-treated females and males were obtained directly from the laboratory culture and the treated males were obtained from the cohorts of 400 pupae irradiated with different doses (10, 30, 50, 70, and 90 Gy for (i) trial and 30 and 70 Gy for (iii) and (iv) trial). Each mating trial was replicated 4 times per each dose and flies in the trials were allowed to mate and feed *ad libitum*.

Female fecundity and egg hatchability were estimated from mating trials (i) and (ii). In the trial (i) males from pupae treated with five different doses of gamma-radiation (10, 30, 50, 70, and 90 Gy) were used and in the trial (ii) (control variant) non-treated males were only used. Eggs laid by females were counted daily till their death in both trials and a mean fecundity was estimated as a number of eggs laid per single female in each trial. For evaluation of egg hatchability a sample of eggs laid by females from the mating trials were incubated in Petri dishes on fine black cloth placed on a moist cotton pad. On the third day of incubation the evaluation of egg hatching started and the eggs were observed daily for five days. Egg hatchability was determined using a light-stereo microscope and altogether 4 × 250 eggs ( $n = 1,000$ ) were evaluated per each dose and mating trial.

Competitiveness of treated males, a competition experiment between treated and non-treated males for mating with non-treated females, was studied in the above mentioned four mating trials. In the competitiveness evaluation only two doses were used, 30 and 70 Gy. We did not use a dose of 90 Gy because this dose of gamma radiation significantly affected quality of insects during preliminary bioassay. In the mating trials percent egg hatch (observed egg hatch –  $EH_o$ ) was evaluated as above and for the trials (iii) and (iv) expected egg hatch ( $EH_e$ ) was calculated using an equation by FRIED (1971):

$$EH_e = \frac{N(H_{nn}) + S(H_{sn})}{N + S}$$

where:

$N$  – number of non-treated males

$S$  – number of treated males

$H_{nn}$  – %  $EH_o$  for the mating trial (iv)

$H_{sn}$  – %  $EH_o$  for the mating trial (i)

The total competitiveness value (CV) for treated males was calculated according to Fried's equation (FRIED 1971):

$$CV = \frac{(H_a - H_b)}{(H_b - H_c)} \times \frac{N}{S}$$

where:

$H_a$  – rate of egg hatch in mating of non-treated females and non-treated males

$H_b$  – rate of egg hatch at a given ratio of treated vs. non-treated males in the presence of non-treated females

$H_c$  – rate of egg hatch in mating of non-treated females and treated males

$N$  – number of non-treated males

$S$  – number of treated males

### Pupal size

Seven-day-old pupae obtained from the mating trials (i) and (ii) were used to determine pupal size. To obtain a mean weight of pupa, altogether  $4 \times 25$  pupae ( $n = 100$ ) were weighed individually on a microbalance (Mettler Toledo, Mumbai, India,  $d = 0.1$  mg) for each mating trial and each dose (10, 30, 50, 70, and 90 Gy) in case of treated males. In addition, the total length and diameter of individual pupae were measured (FAO/IAEA/USDA 2003).

### Flight ability test

Flight ability test was performed according to the standard procedures developed for product quality control of sterile tephritid fruit flies (FAO/IAEA/USDA 2003). Flight ability of adults emerged from pupae exposed to different doses of gamma radiation (10, 30, 50, 70, and 90 Gy) was evaluated using a black plastic tubes (9 cm in a diameter) of different heights placed individually on Petri dish bottoms (10 cm in a diameter) inside the rearing cage. Altogether, six different heights of tubes were used in this test (5, 10, 15, 20, 25, and 30 cm). One hundred pupae were centred in the bottom of the Petri dish 24 h after their irradiation and the black tube was put up on the Petri dish bottom, thus the pupae were confined in the tube. Each black tube was lightly coated with unscented talcum powder, except for lower 10 mm, to prevent the flies from walking out. All flies that emerged and flew from the tube were counted and removed from the cage daily to minimise fly-back (or fall-back) into the tubes. The removed adults were separated by sex. The test terminated after 72 h and remaining contents of the

plastic tubes (dead flies and non-emerged pupae) were evaluated. The flight ability was based on a number of flies escaping from the black tube. The experiment was conducted four times per each tube height and each dose with 100 pupae in each replication. In a control variant non-irradiated pupae ( $4 \times 100$ ) were used for each tube height.

### Data analysis

Data obtained in all experiments were subjected to Pearson's correlation or analysis of variance (ANOVA) with the honestly significant difference value calculated as Tukey's statistic at  $\alpha = 0.05$  (SAS Institute 2002). Before correlation analysis, the data for adult emergence and egg hatching were corrected for control flight ability and hatching, respectively. The chi-square test was used to test the null hypothesis (1 *df*,  $P = 0.05$ ) that there is no significant difference between the expected ( $EH_e$ ) and observed ( $EH_o$ ) egg hatch in the competitiveness test. If the hypothesis could not be rejected, the observed, and expected values were significantly different.

## RESULTS AND DISCUSSION

Effects of gamma radiation on adult's emergence, sex ratio of emerged adults, female fecundity, egg hatching, and pupal size of *B. zonata* are shown in Table 1. The results indicated that adult's emergence decreased significantly with increasing radiation dose at constant dose rate (Pearson's  $r = -0.80$ ,  $P < 0.01$ ). The highest adult's emergence (82.2%) was recorded for the lowest dose tested (10 Gy). ANOVA revealed significant differences in emergence of adults developed from pupae treated with different doses of gamma radiation ( $F_{3,89} = 9.42$ ,  $P < 0.01$ ). The adult's emergence in the control (0 Gy) reached 88.7% what was significantly greater ( $P < 0.05$ ) than the percent emergence at doses of 50, 70, and 90 Gy. Although our results show lower emergence rate by about 10% for each dose that those presented recently by DRAZ et al. (2008) for *B. zonata*, we can conclude that even high doses do not have strong deleterious effects on the pupal viability. At the dose of 70 Gy nearly 3/4 of exposed pupae maintained their viability and produced adults.

The sex ratio (males/total) was also affected with gamma radiation; however, the effect was not prominent. At the lowest dose (10 Gy), the sex ratio was

Table 1. Effects (means  $\pm$  SE) of different doses of gamma radiation on adult emergence, sex ratio, egg hatching, female fecundity, and pupal size of peach fruit fly

Radiation dose (Gy)		0 (control)	10	30	50	70	90
Adult emergence (%) ( $n = 500$ )		88.7 $\pm$ 2.12 <sup>a1</sup>	82.2 $\pm$ 5.94 <sup>ab</sup>	80.0 $\pm$ 4.66 <sup>ab</sup>	74.7 $\pm$ 6.12 <sup>bc</sup>	74.5 $\pm$ 5.36 <sup>bc</sup>	68.0 $\pm$ 4.74 <sup>c</sup>
Sex ratio (males/total) <sup>2</sup>		0.50 $\pm$ 0.01 <sup>a</sup>	0.50 $\pm$ 0.01 <sup>a</sup>	0.45 $\pm$ 0.01 <sup>b</sup>	0.46 $\pm$ 0.01 <sup>b</sup>	0.46 $\pm$ 0.03 <sup>b</sup>	0.44 $\pm$ 0.02 <sup>b</sup>
Total number of eggs/female ( $n = 40$ )		413 $\pm$ 14.4 <sup>a</sup>	455 $\pm$ 43.8 <sup>a</sup>	414 $\pm$ 39.02 <sup>a</sup>	407 $\pm$ 29.9 <sup>a</sup>	401 $\pm$ 30.8 <sup>a</sup>	398 $\pm$ 29.50 <sup>a</sup>
Observed egg hatching (%) ( $n = 1000$ )		93.2 $\pm$ 6.02 <sup>a</sup>	52.9 $\pm$ 8.67 <sup>b</sup>	39.1 $\pm$ 8.76 <sup>c</sup>	20.0 $\pm$ 9.72	5.5 $\pm$ 6.97	0.0 <sup>f</sup>
Pupal size	weight (mg) ( $n = 100$ )	10.3 $\pm$ 0.6 <sup>a</sup>	10.1 $\pm$ 0.6 <sup>ab</sup>	9.5 $\pm$ 0.77 <sup>b</sup>	8.6 $\pm$ 0.33 <sup>c</sup>	8.7 $\pm$ 0.45 <sup>c</sup>	–
	length (mm) ( $n = 100$ )	4.8 $\pm$ 0.18 <sup>a</sup>	4.7 $\pm$ 0.14 <sup>a</sup>	4.4 $\pm$ 0.18 <sup>bc</sup>	4.2 $\pm$ 0.22 <sup>c</sup>	4.1 $\pm$ 0.22 <sup>c</sup>	–
	diameter (mm) ( $n = 100$ )	2.0 $\pm$ 0.08 <sup>a</sup>	2.0 $\pm$ 0.14 <sup>ab</sup>	1.9 $\pm$ 0.08 <sup>abc</sup>	1.8 $\pm$ 0.09 <sup>bc</sup>	1.8 $\pm$ 0.04 <sup>bc</sup>	–

<sup>1</sup>Values followed by the same letter within a row are not significantly different at the 5% level (Tukey's HSD test); <sup>2</sup>Number of evaluated adults for a particular dose was:  $n = 500 \times$  adult emergence/100

unaffected and at higher doses ( $\geq 30$  Gy) the proportion of emerged males significantly decreased ( $P < 0.05$ ) when compared with the control and the 10 Gy dose. The lowest ratio (0.44) was observed at 90 Gy. The effect of gamma radiation on sex ratio was also observed in other studies. Greater variability in sex ratios for *B. zonata* was observed in the study by DRAZ et al. (2008). They recorded the highest sex ratio at a dose of 30 Gy (0.60), followed by 0.56 and 0.47 for flies irradiated with 50 and 10 Gy, respectively. Percentage of males in rearing colonies of tephritid species should generally be within the range of 45–55% for bisexual strains (FAO/IAEA/USDA 2003). Significant deviation from a rearing colony's natural sex ratio gives an indication of rearing or genetic problems. Although our results showed significant differences in sex ratio among the control and higher doses, we can conclude that the deviation was not considerable.

Male fertility of *B. zonata*, evaluated after treatment with different doses of gamma radiation at constant dose rate, is determined here by a percent egg hatch. Our results showed that mating non-irradiated females with treated males (males developed from irradiated pupae) did not affect the production of eggs ( $P > 0.05$ ), but it seriously reduced their hatchability (Table 1). Treatment of pupae with 10 Gy reduced egg hatch nearly to a half (52.9%) and the percent egg hatch continued to decline with increasing dose. The exposure of pupae to 90 Gy even produced total sterility of eggs. According to the Tukey's HSD test, the differences in percent egg hatch among the treatments were highly significant ( $F_{4,25} = 558.63$ ,  $P < 0.01$ ). There was a strong negative relationship (Pearson's  $r = -0.94$ ,

$P < 0.01$ ) between dose and egg hatchability. It can be concluded from the results that doses  $\leq 70$  Gy did not prevent egg hatch, however, when the dose increased to 90 Gy, the egg hatch was completely suppressed. These results are in accordance with SHEHATA et al. (2006), whose anatomical and biometrical study determined a dose of 60–90 Gy having the most deleterious effect on male gonads of *B. zonata*. Results comparable to ours were presented for *Bactrocera philippinensis* Drew and Hancock with the most effective irradiation range 67–74 Gy (RESILVA et al. 2007), *Anastrepha fraterculus* (Wiedemann) (90–100 Gy) (ALLINGHI et al. 2007), or *Bactrocera cucurbitae* (Coquillett), and *B. zonata* (70–90 Gy) (HUQUE, AHMAD 1966). In other studies, however, a dose to induce total sterility of fruit fly males was a little lower ranging from 40–60 Gy, for example 40 Gy was recorded for *B. cucurbitae* (NAHAR et al. 2006), 50 Gy for *Anastrepha suspense* Loew (WALDER, CALKINS 1993) and *B. zonata* (DRAZ et al. 2008), or 60 Gy for *Anastrepha obliqua* (Macquart) (TOLEDO 1993). In general, the sterility dose of males seems to differ from laboratory to laboratory. These differences may be due to a type of irradiator cells, methodology of assay, genus of flies, age of irradiated pupae, as well as fitness of laboratory strains tested. Mean sterility doses also differ among families of Diptera ranging from 20–160 Gy (BAKRI et al. 2005), but tephritids have relatively homogeneous sensitivity to gamma irradiation, with most major pest species requiring  $< 100$  Gy to achieve suitably high levels of sterility (BAKRI et al. 2005). As mentioned above for *B. zonata*, based on anatomical observations of radiation effects on male gonads, a dose range between

Table 2. Egg hatchability and sexual competitiveness of peach fruit fly males emerged from pupae treated with gamma radiation (30 or 70 Gy) at different ratios of treated/non-treated males in cage trials

Radiation dose (Gy)	Egg hatch and competitiveness parameters	Sex ratio (S♂: N♂: N♀) <sup>1</sup>			
		0:1:1	1:0:1	5:1:1	10:1:1
30	$EH_o$ (%) <sup>2</sup>	93.2 <sup>a6</sup>	39.1 <sup>d</sup>	65.7 <sup>b</sup>	50.5 <sup>c</sup>
	$EH_e$ (%) <sup>3</sup>	–	–	48.1	44.0
	$\chi^2$ (1 <i>df</i> ; <i>P</i> = 0.05) <sup>4</sup>	–	–	6.20*	1.71
	CV <sup>5</sup>	–	–	0.21	0.37
70	$EH_o$ (%)	93.2 <sup>a</sup>	5.5 <sup>d</sup>	25.1 <sup>b</sup>	16.8 <sup>c</sup>
	$EH_e$ (%)	–	–	20.1	13.5
	$\chi^2$ (1 <i>df</i> ; <i>P</i> = 0.05)	–	–	0.78	0.93
	CV	–	–	0.69	0.68

<sup>1</sup>S♂ – males emerged from irradiated pupae, N♂ and N♀ – males and females emerged from non-irradiated pupae; <sup>2</sup>% egg hatch observed in the experiments; <sup>3</sup>% egg hatch calculated according to FRIED (1971); <sup>4</sup> $\chi^2$  values – asterisk (\*) indicate significant (*P* < 0.05) difference between observed and expected egg hatch; <sup>5</sup>CV – total competitiveness value (FRIED 1971); <sup>6</sup>Values followed by different letter within a row are significantly different at the 5% level (Tukey's HSD test)

60 and 90 Gy is considered sub-sterilizing and sterilizing (SHEHATA et al. 2006). Taken as a whole the results obtained in the present work support the use of a dose equal to or above 70 Gy, applied to pupae 48 h before adults' emergence, effective to induce sub-sterilization of *B. zonata* males.

Pupal size parameters (weight, length, and diameter) of filial generation, produced by a cross of non-treated females and treated males, gradually decreased with increasing radiation dose and differences in a pupal size were statistically significant among doses ( $F_{4.89} = 8.00$ , *P* < 0.01 for weight;  $F_{4.89} = 11.52$ , *P* < 0.01 for length;  $F_{3.06} = 4.40$ , *P* < 0.05 for diameter) (Table 1). Lower doses (10 Gy for pupal weight and length; 10 and 30 Gy for pupal diameter) did not affect significantly the measured pupal parameters (*P* > 0.05) when compared to control individuals. Pupal size is usually measured by diameter and weight (CALKINS, PARKER 2005) and it is a valuable indicator of overall viability of pupae and correlates positively with a size of resulting adult flies. In general, larger male tephritids are stronger fliers, live longer, have higher mating propensity and produce longer refractory periods in female flies than smaller males (BURK, WEBB 1983; CHURCHILL-STANLAND et al. 1986). In SIT programmes, sub-sterilizing doses are preferred for irradiation of insects resulting in a certain low frequency of successful fertilization of females with treated males in field populations. Therefore, it is important to know what effect irradiation has on pupal parameters of a filial generation, which, in

turn, may imply fitness of resulting adults. Our results show a significant effect of irradiation on pupal weight and dimensions. Pupae were significantly smaller at doses equal to and above 30 Gy when compared with control individuals (0 Gy). Other studies also demonstrated detrimental effect of gamma radiation on *B. zonata* pupae. DRAZ et al. (2008) studied effect of gamma rays on deformation of *B. zonata* pupae and irradiation had a significant effect on the occurrence of deformed pupae in populations.

The observed and expected percent egg hatch for different mating trials and the estimated total competitiveness values of treated males for particular sex ratio are presented in Table 2. Observed egg hatch varied significantly depending on a proportion of treated males in mating trials. The results indicate that percent egg hatch of non-irradiated females declined with increasing ratio of treated males in mating trials. However, the decline was not as prominent as that for a trial with absence of non-irradiated males (1:0:1 sex ratio), when the lowest percent egg hatch was observed. ANOVA revealed significant differences in percent egg hatch among trials for both doses ( $F_{5.95} = 68.58$ , *P* < 0.01 for 30 Gy and  $F_{5.95} = 568.26$ , *P* < 0.01 for 70 Gy). At a constant number of non-treated males in mating trials, egg hatch decreased significantly when a proportion of treated males increased. The observed values of egg hatch were greater than those of expected ones in all variants, however, significant difference (1 *df*; *P* < 0.05) was only observed for the 5:1:1 sex ratio

Table 3. Flight ability (%) (means  $\pm$  SE,  $n = 400$ ) of peach fruit flies emerged from pupae irradiated with different doses of gamma radiation

Radiation dose (Gy)	Tube height (cm)					
	5	10	15	20	25	30
0 (control)	80.2 $\pm$ 2.5 <sup>a1</sup>	68.7 $\pm$ 5.77 <sup>a</sup>	58.0 $\pm$ 1.30 <sup>a</sup>	38.0 $\pm$ 2.26 <sup>a</sup>	19.5 $\pm$ 1.19 <sup>a</sup>	7.5 $\pm$ 0.53 <sup>a</sup>
10	73.7 $\pm$ 6.90 <sup>a</sup>	67.0 $\pm$ 6.27 <sup>a</sup>	56.7 $\pm$ 2.43 <sup>a</sup>	37.2 $\pm$ 2.43 <sup>ab</sup>	19.5 $\pm$ 1.92 <sup>a</sup>	6.2 $\pm$ 1.38 <sup>a</sup>
30	71.0 $\pm$ 5.90 <sup>a</sup>	67.5 $\pm$ 5.15 <sup>a</sup>	53.7 $\pm$ 5.25 <sup>ab</sup>	36.2 $\pm$ 2.05 <sup>ab</sup>	16.5 $\pm$ 2.20 <sup>a</sup>	5.5 $\pm$ 1.19 <sup>ab</sup>
50	72.2 $\pm$ 8.53 <sup>a</sup>	63.0 $\pm$ 3.92 <sup>a</sup>	52.0 $\pm$ 6.27 <sup>abc</sup>	32.7 $\pm$ 3.88 <sup>bc</sup>	16.5 $\pm$ 2.44 <sup>a</sup>	5.5 $\pm$ 1.60 <sup>ab</sup>
70	66.2 $\pm$ 4.43 <sup>a</sup>	62.7 $\pm$ 6.06 <sup>a</sup>	49.2 $\pm$ 4.92 <sup>bc</sup>	28.0 $\pm$ 1.69 <sup>c</sup>	12.7 $\pm$ 1.16 <sup>b</sup>	4.0 $\pm$ 0.75 <sup>bc</sup>
90	68.0 $\pm$ 4.07 <sup>a</sup>	59.2 $\pm$ 3.24 <sup>a</sup>	45.0 $\pm$ 2.61 <sup>c</sup>	27.7 $\pm$ 4.55 <sup>c</sup>	10.2 $\pm$ 1.75 <sup>b</sup>	2.7 $\pm$ 2.05 <sup>c</sup>

<sup>1</sup>Values followed by the same letter within a column are not significantly different at the 5% level (Tukey' HSD test)

at 30 Gy. Values for the Fried's CV ranged from 0.21–0.69. While the values were nearly equivalent for both sex ratios at 70 Gy, the CV at 30 Gy was 1.85 times greater in the mating trial with a higher portion of sterile males. Normally, values for the Fried's CV range from 1–0. Values of 1 indicate an equivalent level of competitiveness between irradiated and non-irradiated males, while values close to zero indicate superior competitiveness of the non-irradiated males (FRIED 1971). Values between 0.2 and 0.4 are normal for sterile laboratory males and values less than 0.2 are a reason for a concern about the male competitiveness (FAO/IAEA/USDA 2003). The total competitiveness values obtained in our experiments suggest that irradiated males successfully competed with non-irradiated males and they correspond with the results of other authors (e.g. CALCAGNO 2001; LUX et al. 2002). In the competitiveness test, we did not use a dose of 90 Gy, because at this dose total sterility was observed and SIT depends on releases of sub-sterilized, not completely sterile males.

Results on flight ability test of fruit flies are shown in Table 3. Flight ability is expressed as a percentage of individuals that could fly based on the number of pupae put into a flight tube. The results demonstrate no considerable effect of different gamma radiation doses (10, 30, 50, 70 or 90 Gy) on flying capabilities of adults, particularly at lower heights of tubes ( $F_{2,77} = 2.25$ ,  $P > 0.05$  for a 5 cm tube height, and  $F_{2,77} = 1.67$ ,  $P > 0.05$  for 10 cm). However, there was significant heterogeneity in flight ability among doses at higher tubes ( $F_{4,25} = 4.55$ ,  $P < 0.01$  for 15 cm,  $F_{4,25} = 8.04$ ,  $P < 0.01$  for 20 cm,  $F_{4,25} = 13.79$ ,  $P < 0.01$  for 25 cm and  $F_{4,25} = 6.10$ ,  $P < 0.01$  for 30 cm). Generally, the percentage of fliers decreased with increasing tube height and with increasing gamma radiation dose at a particular tube

height. Reductions in flight ability of fruit flies due to irradiation were noted by a large number of researchers (e.g. NAKAMORI, SOEMORI 1981; SMITH et al. 1981; RESILVA et al. 2007). The data presented in this study are in accordance with observations of others and confirm the negative effect of irradiation on flying capability at greater tube heights ( $\geq 15$  cm) and greater doses ( $\geq 50$  Gy). Flight ability evaluation is part of routine quality control tests of mass-reared tephritid fruit flies. This parameter is important, since it says about sterile insect capability of dispersal in the field after their release. During standard quality control tests of fruit flies, 10 cm tubes are used and values of minimum percent fliers should range from 60–85%, depending upon fruit fly species (FAO/IAEA/USDA 2003). Flight ability values observed in our experiments were close to the lower limit of that range. The values did not exceed 67.5% for treated flies and 10 cm tube and reached 68.7% in the control and the same tube height. Flight ability of control adults is rather low in our bioassay, which may indicate some problems during rearing process and/or pupae handling. It is known that flight ability of adults is adversely affected during pupae processing at a critical stage of their development – the “droopy wing syndrome” (SHARP et al. 1980; SHARP, LITTLE 1982).

For many fruit fly species, optimal doses of gamma radiation were successfully determined for sterilization (e.g. COLLINS et al. 2008, 2009). However, in choosing an optimal sterilization dose for SIT, a balance needs to be reached between the levels of sterility and mating competitiveness of males (TOLEDO et al. 2004; PARKER, MEHTA 2007). Insects that receive a too low dose are not sufficiently sterile and those that receive a too high dose may be uncompetitive, reducing the effectiveness of SIT as it requires that

a greater number of sterile insects must be released. There appears to be a general consensus that the irradiation process negatively affects the total competitiveness of males (PEREIRA et al. 2007) and that one simple way to lessen this impact, and thereby to increase the effectiveness of SIT, is to reduce the sterilizing dose (SHELLY et al. 2005). In general, data from our laboratory bioassay demonstrate that the effect of irradiation on peach fruit fly is consistent with the results for other tephritid fruit fly species. According to these results, taking into consideration the necessity of compromise between sterility and competitiveness, the best irradiation dose of *B. zonata* pupae treated 48 h before eclosion should be within a range of 70 and 90 Gy. At 70 Gy, sterility induction was greater than 5% in our bioassay, which is higher than the levels of sterility that are suggested for SIT programmes [e.g. > 99.5% sterility is recommended for *C. capitata* (FAO/IAEA/USDA 2003)] and 90 Gy resulted in total sterility of peach fruit flies. A range of 20 Gy is too large to be recommended as an effective dose for SIT programmes, therefore further research should focus on determination of a more precise value.

Since male competitiveness is a complex of many individual factors under natural conditions, such as ability to survive, mating propensity, mating compatibility, post-mating and other factors, which have not been studied here, they remain to be studied under semi-field and field conditions.

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