XA0201539

The effect of substerilizing doses of gamma radiation on the pupae of the carob moth *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae)

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Abstract. We investigated various effects of gamma radiation on the carob moth, *Ectomyelois ceratoniae*, treated with 200–600 Gy at different pupal ages. Irradiation resulted in a decrease of adult emergence. This effect was both dose and age dependent. At 500 and 600 Gy, no pupae developed into normal adults when treated at the age of 4–5 days. Only 6% normal adults emerged when the pupae were treated at the age of 6–7 days with 500 Gy. When 8–9 d old pupae were irradiated with 500 and 600 Gy, 30% and 10% normal adults emerged, respectively. Other emerged moths exhibited various malformations, mostly wing deformities. When pupae were treated with 400 or 500 Gy, fecundity and fertility of both untreated females mated with irradiated males or irradiated females mated with untreated males were drastically reduced. When 9–10 d old pupae were irradiated with 200, 250 and 300 Gy, adult morphology, fecundity, fertility and egg hatch were slightly affected. Mating behaviour of irradiated males also was affected. Competitiveness of males irradiated with sub-sterilizing doses varied depending on irradiation dose and number of insects present in the mating cages. A significant reduction of competitiveness was observed in males treated with ≤300Gy.

1. INTRODUCTION

The carob moth, *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae), is a major fruit pest in the Mediterranean basin and Near East regions, where it attacks citrus, pomegranate, carob, almond, date fruits and stored products such as almonds, nuts, dates and pistachios. Although much work has been done in Tunisia and other Mediterranean countries on biology and control of the carob moth, no effective control has been achieved in the field [1, 2]. Chemical controls have failed in both pomegranate and date orchards mainly due to the biology and dispersion ability of this pest. Carob moth females lay eggs in the pomegranate calyx and larvae develop inside fruits. In dates, only the first two instars develop outside the ripe dates while the later instars penetrate inside the fruits.

Because of the success of the sterile insect technique against the new world screwworm, (*Cochliomyia hominivorax*), radiation-induced sterility and related genetic methods have been suggested for the control of Lepidopteran pests [3–8]. There is an interest to use these genetic methods for the suppression of carob moth populations. The present study deals with improving mass rearing technology and assessing the effects of gamma radiation on carob moth pupae. The irradiation data are used to determine the best radiation dose and age of pupae for inducing full and partial sterility in the carob moth.

2. MATERIALS AND METHODS

2.1. Insect rearing

Carob moths were collected from a large number of dates in oases of Djerid in southern Tunisia. After evaluating several different diets in our laboratory, the following artificial diet was selected for mass rearing of the carob moth: soybean cake (40%), sucrose (40%), nipagin (0.2%), sodium benzoate (4.8%) and distilled water 15%. The soybean cake was ground in a

Culati Grinder, and the other ingredients (sucrose, nipagin, and sodium benzoate) were added and mixed well. Once the diet was prepared it was stored in a cold room.

After adult emergence, the moths of both sexes are placed inside large cloth cages (50 x 70 x 100 cm). After 48 hours, females are removed from the cages and placed individually on filter paper, soaked with 10 % sucrose solution and covered with transparent plastic cups (5 cm high x 8 cm diameter). Egg collections from each female are placed separately on the prepared diet for hatching. In this manner we are able to examine fecundity, fertility and longevity of individual females. Larvae are reared either individually in glass tubes maintained on wood racks (3000 tubes/rack) or in small plastic jars (24 x 18 x 11 cm) containing 40–50 larvae per jar.

2.2. Radiation experiments

Effect of gamma irradiation on pupae of different age and sex. Pupae were sexed and placed in separate groups for irradiation. Both male and female pupae from three age groups (4–5, 6–7 and 8–9 d-old) were treated with three different doses of gamma radiation (400, 500 and 600 Gy) at a dose rate of 140 Gy/min. In each treated group, adult emergence was recorded and compared with the untreated control.

Effect of sub-sterilizing doses on the fertility in the parental generation. For these trials, 9–10 d old pupae (i.e., 24 to 48 hours before adult emergence) were exposed to 200, 250 and 300 Gy. The pupae were held separately in small containers at the following conditions: 28±1°C, 70±5 relative humidity and a photoperiod of 14L:10D. Within a few hours after emergence, irradiated males were mated to untreated females and irradiated females to untreated males. Mated females were removed and placed in oviposition containers. Eggs were collected and counted daily during the lifetime of the females. The eggs laid on filter paper were transferred to the artificial diet. This experiment was repeated three times at different dose rates in two different irradiation centres: Salah Azaeiz Hospital Centre of Tunis and the INRAT Centre.

Moths irradiated with 200, 250 and 300 Gy as 9–10 day old pupae were released into mating cages at the following ratios of treated males to untreated males and untreated females: (0:1:1, 1:1:1, 2:1:1, 3:1:1, 4:1:1 and 1:0:1). After 48 hours in the mating cages, females were removed and placed in glass tubes. Eggs laid by individual females were collected and counted daily throughout the life of the female. Egg hatch was determined after eggs were incubated for 3 days. The competitiveness values of irradiated males or females were calculated according to the competitiveness index of Fried [9].

3. RESULTS AND DISCUSSION

Rearing carob moth larvae on the artificial diet in mass, produced insects of comparable developmental characteristics to those reared individually (Table 1). No cannibalism was observed among larvae reared in groups.

Although a low proportion of females mated successfully in the cages when our experiments began, mating significantly improved when host fruit (dates) was introduced into mating cages [10]. Reduction in egg hatch was considerably higher in the irradiated females than in the untreated females that mated with irradiated males. The mean egg hatch decreased from 95% in the control to 70.8%, 63% and 14.3% for females treated with 200, 250 and 300 Gy, respectively. The mean egg hatch for untreated females mated with irradiated males also decreased with increasing radiation dose, but this decrease was less than for the irradiated females (Table 2).

Table 1. Comparison in development of individually reared or group-reared *Ectomyelois ceratoniae* larvae

Parameter measured	Individually reared (1 egg per tube)	Group reared (~45 eggs per jar)
No. of eggs in each system	436	906
No. of last instars larvae obtained (%)	392 (90)	687 (76)
No. of pupae obtained (%)	354 (90)	605 (88)
Duration of development (days)	41.5±1.6	39.7±1.4

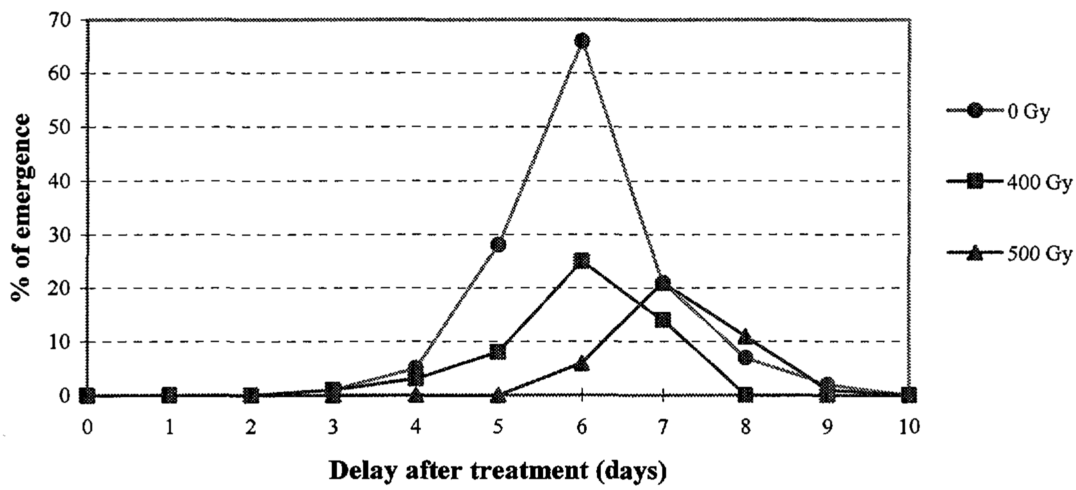

FIG 1 .Effect of irradiation on the duration of pupal development (4- day-old pupae treated) for Ectomyelois ceratoniae.

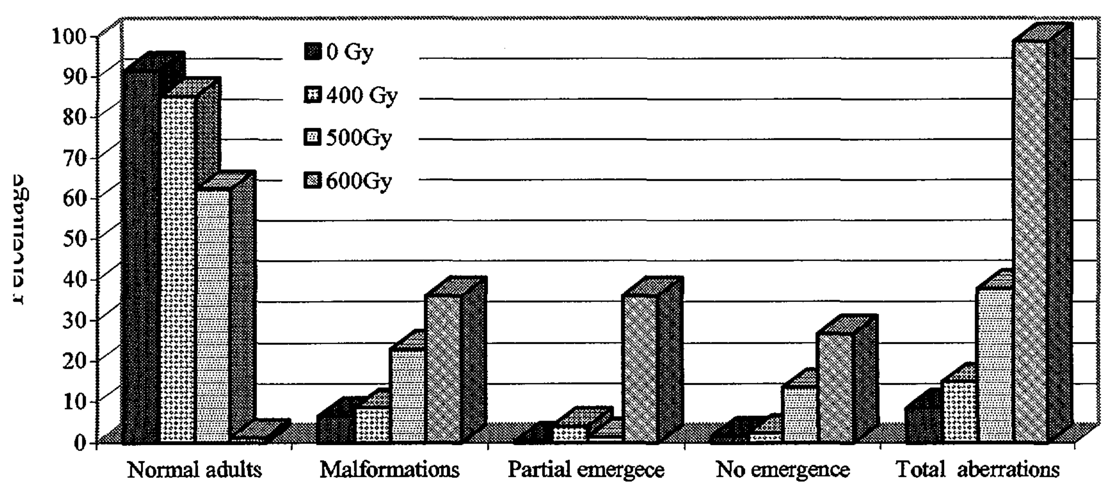

FIG. 2. Effect of irradiation on adult emergence (8–9 d-old pupae treated) for Ectomyelois ceratoniae.

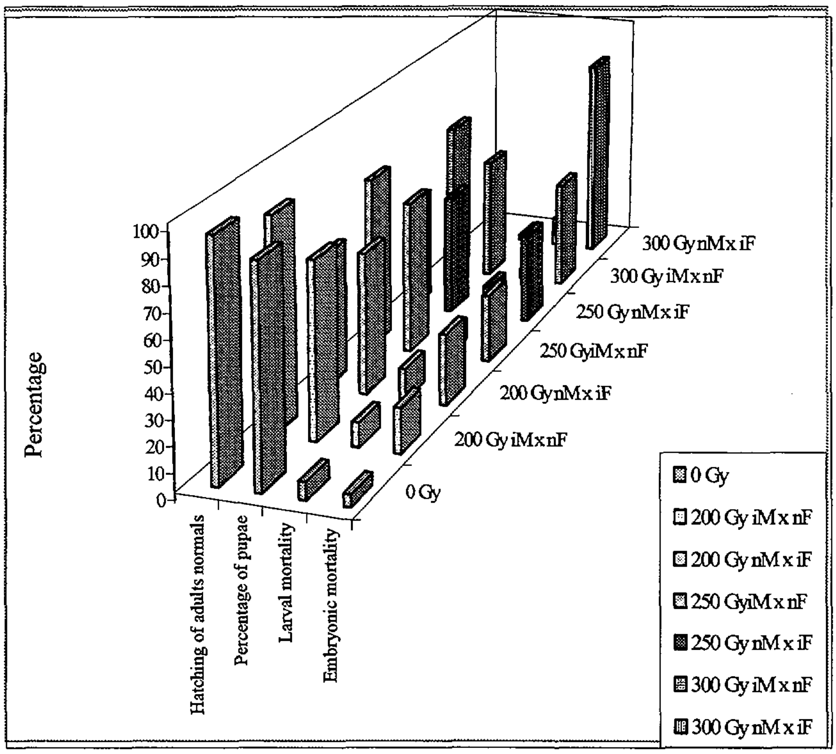

FIG. 3: Irradiation The effect of substerilizing doses of irradiation on F1 progeny (9–10 days old pupae treated).

The incidence of mating decreased with increasing dose of radiation. This decrease was significant in the irradiated female x normal male cross, where the mating percentages were 68.6%, 48%, 40%, and 36.3% for 0, 200, 250 and 300 Gy, respectively. In the reciprocal cross, the mating percentages were 69.2%, 61% and 61.1%, respectively. Sub-sterilizing doses of radiation affected the survival of progeny at different stages during egg, larval and pupal development (Fig. 3). Stage survival decreased with increasing dose of radiation. Irradiation induced embryonic mortality and morphological abnormality in progeny of treated parents. These effects were more pronounced in the progeny of irradiated females than in the progeny of irradiated males.

The dose level and the age of the pupae at the time of irradiation significantly affected the duration of pupal development and the percentage of adult emergence. The 400 Gy treatment did not cause any delay in development of young pupae (4–5 d-old), with 50% adult emergence during the 6th day after treatment (Fig. 1). Peak emergence of adults from pupae irradiated with 500 Gy was delayed for 24 hours. In 6–7 d-old pupae, Dhouibi and Omrane [11] reported that emergence curves of pupae treated with 400 Gy and of untreated pupae were similar, with peak emergence during the 4th day after treatment, while pupae exposed to 500 Gy exhibited peak emergence during the 5th day after irradiation. The emergence of 8–9 d-old pupae treated with 400 Gy were similar to that for control pupae, with peak adult emergence on the second day after treatment. The dose of 500 Gy caused a reduction in the percentage of adult emergence and a delay of 24 hours in the duration of the pupal stage (Fig. 2). Sub-sterilizing doses of radiation (200, 250 and 300 Gy) administered to mature pupae (9–10 d-old) stimulated peak eclosion for both sexes to occur 12 hours after the treatment. Irradiation did not influence the longevity of carob moths irradiated as pupae. Female longevity varied between 6.1 to 7.3 days for both control and irradiated females. Male longevity varied between 5.3 and 6 days for both the control and irradiated males. These doses of radiation had a slight effect on mating activity, mainly when the irradiated females were mated with normal males. In the mating cages, increasing the number of irradiated males improved mating activity, but increasing numbers of irradiated females decreased the mating rate.

The fecundity of *E. ceratoniae* varies greatly depending on the dose of radiation given to the pupae [10]. However, fecundity was only slightly affected by sub-sterilizing doses in the irradiated male by normal female or irradiated female by normal male crosses (Table 2). Fertility decreased inversely with the irradiation dose. Fertility was more affected than fecundity. Egg hatch was 43% in the mated irradiated (300 Gy) females crossed with normal males and 91.4% in the control (Table 2). The irradiation doses necessary to induce sterility in *Ectomyelois ceratoniae* pupae are very high in comparison with other Lepidopteran species. Indeed, Quang [12] obtained full sterility of males and females of *Plutella xylostella* when pupae were treated with 200 Gy. Similar results were obtained by Brower [13] on *Ephestia cautella* and by Ahmed et al. [14] on *Plodia interpunctella.* However, irradiated males emerging from mature pupae treated with 200, 250 and 300 Gy were as competitive as the untreated males (Table 2).

Table 2. Competitiveness of *Ectomyelois ceratoniae* males irradiated with 200, 250 and 300 Gy and confined with untreated insects at the indicated ratios

Ratio iM:nF:nM (# replicates)[1]	Mating rate %	No. of eggs/female	Fertility %	Egg hatch %	Competitiveness Value
		200 Gy			
0:1:1 (30)	68.6	124.3±36.2	91.4	94.9	
1:1:1 (35)	67	118.3±31.2	83.8	84.1	4.38
2:1:1 (31)	68.2	116±29.5	85.4	85.0	1.48
3:1:1 (27)	84	113.4±35.1	85	83.7	1.87
4:1:1 (23)	83	115.4±32.4	84.3	83.4	1.62
1:1:0 (39)	69.2	114.6±31.2	80.8	81.7	
		250 Gy			
0:1:1 (23)	69	119.9±29.8	90.1	93.6	
1:1:1 (32)	65	113.3±28.3	79.7	80.5	1.60
2:1:1 (32)	69	112.8±29.8	76.9	79.2	1.06
3:1:1 (23)	68	111.4±36.4	76.7	77.5	1.86
4:1:1 (25)	82	113±37.5	76.7	75.6	1.62
1:1:0 (41)	61	112.1±36.6	78.4	72.4	
		300 Gy			
0:1:1 (32)	68	120.8±31.8	90.9	94.3	
1:1:1 (38)	67	116.9±28.5	72.7	72.9	1.24
2:1:1 (28)	66	113.2±31.7	74.7	75.2	0.48
3:1:1 (29)	80	105.4±29.6	76.5	70.7	0.52
4:1:1 (28)	81	106.6±32.5	75	67.6	0.55
1:1:0 (36)	61.1	95.7±26.9	72.5	55.6	

[1]im= treated male; nF= untreated female; nM= untreated male.

ACKNOWLEDGEMENT

We thank to the International Atomic Energy Agency for the funding (71/71TV) to support this research and Dr. Maaleg, head of the Children Biomedical Centre of Tunis for his authorization to use the hospital gamma radiation source. We also thank Besbes Mounir for dose calibration and Ben Salah, head of the INRAT Zoology Laboratory.

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