# Effects of gamma radiation on codling moth (*Cydia pomonella*, Lepidoptera: Tortricidae) fertility and reproductive behaviour

**M. Mansour**
Department of Agriculture,
Syrian Atomic Energy Commission,
Damascus, Syrian Arab Republic

**Abstract.** Studies were conducted with codling moth, *Cydia pomonella* (L.), to examine the effects of gamma radiation on fertility and reproductive behaviour. Data accumulated during these studies showed that egg production and hatch decreased with increasing radiation dose. Females were more sensitive to radiation treatment than were males. A dose of 150 Gy caused 100% sterility in females and significantly reduced fecundity, and a dose of 350 Gy reduced male fertility to less than 1%. Radiation dosages up to 400 Gy had no adverse effect on male longevity or competitiveness in cages using laboratory reared moths. However, males exposed to a dose of 350 or 400 Gy mated fewer times than unirradiated males.

## 1. INTRODUCTION

The codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), is the key pest of more than 40,000 ha of apple orchards in Syria. It is also a major pest of pears, quince and walnuts, and causes tens of millions of dollars in losses to the fruit industry in the country every year. Although the first record of this species as a pest of apple was published in 1635 [1], it was first reported in Syria less than 50 years ago [2]. However, no doubt it existed in the country long before that. In Syria, the infestation rate in neglected apple orchards can be as high as 80–100% [3], making it impossible to grow apples commercially without effective control measures.

To control this pest in Syria, usually 6–8 chemical treatments are applied every year. Chemical control of this insect has many drawbacks. The use of azinophos-methyl (Guthion), an organophosphorous compound which is currently the most widely applied chemical for codling moth control, faces serious problems. Some of these are insecticide resistance [4, 5] and disruption of beneficial species [6]. In addition, high insecticide residues on fruit due to intensive spray programs used for codling moth control in Syria have caused difficulties in exporting the country's surplus of apples [7]. An alternative method to suppress codling moths using A sex pheromone-based mating disruption system, has been developed [8]. However, the success of this system for codling moth control is limited by a number of factors. Some of these include high population density, moth immigration from neighbouring orchards and physical features of the orchard (orchards with steep slopes and large numbers of missing trees have been problematic) [9]. Consequently, supplemental chemical control of codling moth is often required.

The heavy losses to apple production caused by codling moth infestation, and difficulties in exporting fresh fruit due to high insecticide residues, has led to consideration of a new strategy for controlling this pest in Syria. The new policy aims to reduce farmer's reliance on pesticides as the primary means for crop protection by considering the use of the sterile insect technique (SIT) to control or perhaps eradicate this pest from Syrian apple producing regions.

One of the most important prerequisites for the success of SIT is to have the ability to sterilize both sexes without seriously affecting male behaviour, particularly competitiveness and longevity [10]. Studies on the codling moth have shown that it can be sterilized using gamma irradiation [11, 12, 13]. However, geographical strains may differ in their sensitivity to ionizing radiation [14] making it important to assess the radio-sensitivity of widely separated geographical strains. In this article, the radio-sensitivity of the Syrian codling moth strain is assessed and the effects of gamma radiation on the reproductive behaviour of males and females are measured.

## 2. MATERIALS AND METHODS

### 2.1. Insects

Moths used in these experiments were reared in the laboratory on a local diet similar to that reported by Brinton et al. [15]. Rearing rooms were maintained at 28±2°C, 50±10% R.H. and a photoperiod of 16L:8D. Following pupation, the diet was carefully broken and pupae were collected and separated by sex. Male and female pupae were placed into separate plastic containers to continue their development to the adult stage; virgin adult moths were thus obtained.

### 2.2. Irradiation

Moths were irradiated when less than 24 h old. Two sources of gamma irradiation (old and new) were used in this study. The average dose rate of the old source, a Cesium$^{137}$ Gammator, K. S. E/ RIS, was 7.92 Gy/minute, while that of the second source, a Co$^{60}$ Gammacell, Techsnabexport Co. LTD, RUS, was 83.33 Gy/minute. Tests on the effects of gamma radiation on the radio-sensitivity of the Syrian codling moth strain were done using the old source, and those on the effects of gamma radiation on reproductive behaviour were done using the new source. To keep the moths inactive during transportation and irradiation, they were chilled at 0°C for 15 minutes and placed in an insulated container provided with ice bags. Moths were transported to and from the laboratory in this container.

### 2.3. Effects of gamma radiation on fecundity and fertility

Chilled adult moths were placed in petri dishes (9 cm diameter) and treated with the following doses of gamma radiation: 0, 150, 200, 250, 300, 350 and 400 Gy. Treated adults were mated to untreated adults of the same age by confining them inside cylindrical transparent plastic cages (8.5 x 10.5 cm). Ten irradiated moths of one sex were placed in a cage, and the same number of unirradiated moths of the opposite sex were added. Moths were provided with water on moistened cotton balls. Cages were placed randomly on shelves in the rearing room under the same conditions of light and temperature (see above). Moths were allowed to mate and lay eggs on waxed paper liners fitted against the inside of each cage until all moths died. Three days after the cages were set-up the paper liners were collected and replaced. Egg papers were incubated at 28±2°C for five days and then examined for egg hatch under a dissecting microscope. The total number of eggs laid and the number of hatched eggs was recorded, and this procedure was repeated 48 hours later. Each treatment was replicated four times.

### 2.4. Effect of gamma radiation on male longevity

Data on longevity was recorded for male moths within each treatment; dead moths were removed daily from the experimental cages described above.

### 2.5. Effect of gamma radiation on male mating competitiveness

Males that received 350 or 400 Gy were confined with untreated males and untreated virgin females of the same age in different ratios (0:1:1, 1:0:1, 1:1:1, 9:1:1, treated male: untreated male: untreated female, respectively); each ratio at each dose was replicated three times. Cylindrical transparent plastic cages (10.5 x 11.0 cm) with waxed paper fitted against the inside of the cage were used. Five females were placed in each cage and the appropriate number of treated and untreated males was added (e.g., for 9:1:1 ratio, each experimental cage contained 45 treated males, 5 untreated males and 5 untreated females). Three days later, the paper liners with eggs were collected from each cage, incubated at 28±2°C for five days, and fecundity and fertility were recorded. Fried's [16] formula was used to calculate the expected egg hatch for each ratio. Analysis of proportion was used to determine the difference between the observed and expected egg hatch at each tested ratio and dose.

### 2.6. Effect of gamma radiation on male mating capacity

Codling moth males were treated with 0, 250 and 350 Gy of gamma radiation and confined individually in cylindrical transparent plastic cages (3.2 x 6 cm). One virgin female was introduced into each container, left for 24 h, then removed and dissected to ascertain the presence of a spermatophore. Females were added and removed daily for five successive days after which the males were discarded. Thirty males were tested at each dose level. The presence of a spermatophore was used to determine the mating ability of the males [17].

### 2.7. Data Analysis

Data were subjected to analysis of variance. Means were separated by Fisher's protected LSD test [18]. A simple linear regression analysis was performed to examine the relationship between radiation dose and female fecundity.

## 3. RESULTS

### 3.1. Effects of gamma radiation on female fecundity and fertility

Increased radiation dose significantly and consistently decreased fecundity (Table 1). Codling moth females were very sensitive to gamma radiation. A dose of 150 Gy caused complete female sterility and significantly reduced fecundity ($F = 1108.47$; d.f. = 6, 18; $P < 0.05$). A regression line was fitted to show the relationship between radiation dose and female fecundity. The equation for the fitted line ($y = 74.29 - 0.119x$) indicates a very strong linear relationship. The strength of this relationship is confirmed by a high correlation coefficient ($r^2 = 0.96$, $P < 0.0001$).

### 3.2. Effects of gamma radiation on male fecundity and fertility

When treated males were mated to untreated females, egg hatch decreased significantly with increasing dose of radiation applied to the males. At 350 Gy egg hatch in females mated to treated males was reduced to less than 1% (Table 2). However, complete sterility was not observed even at a dose of 400 Gy. Dose of radiation given to males did not reduce female fecundity at 250 Gy or less. However, this effect was significant at dose of 300 Gy or more ($F = 34.57$; d.f. = 6, 18; $P < 0.05$). The equation for the fitted line that represents the relationship between radiation dose and female fecundity ($y = 72.03 - 0.037x$, $r^2 = 0.67$, $P < 0.0001$) indicates a linear relationship.

Table 1. Fertility and fecundity of *Cydia pomonella* when irradiated females were mated to untreated males

| Dose (Gy) | Egg hatch (%) | Mean number of eggs/female |
|---|---|---|
| 0 | 82.9 | 72.9 a |
| 150 | 0 | 53.4 b |
| 200 | 0 | 53.8 b |
| 250 | 0 | 48.8 c |
| 300 | 0 | 39.7 d |
| 350 | 0 | 30.0 e |
| 400 | 0 | 24.7 f |

Means followed by the same letter are not significantly different at $P < 0.05$ (Fisher's LSD test).

Table 2. Fertility and fecundity of *Cydia pomonella* when untreated females were mated to irradiated males

| Dose (Gy) | Egg hatch (%) | Mean number of eggs/female |
|---|---|---|
| 0 | 82.6 a | 68.3 a |
| 150 | 14.7 b | 70.7 a |
| 200 | 10.6 c | 68.2 a |
| 250 | 04.5 d | 68.2 a |
| 300 | 03.7 d | 59.3 b |
| 350 | 0.3 d | 56.6 bc |
| 400 | 0.1 d | 55.9 c |

Means in a column followed by the same letter are not significantly different at $P < 0.05$ (Fisher's LSD test).

### 3.3. Male Longevity

Radiation doses up to 400 Gy had no significant adverse effect on male survival ($P > 0.05$). In fact, increased radiation dosages caused a slight, but insignificant, increase in male longevity.

### 3.4. Male Mating Competitiveness

Irradiated males were equal in competitiveness to normal males under laboratory conditions at all tested ratios (Table 3). Analysis of proportion showed that the observed number of hatched eggs was not different from the expected value ($P > 0.05$).

### 3.5. Male Mating Capacity

Radiation significantly reduced the mean number of matings for irradiated males ($F = 3.31$; d.f. = 3, 57; $P < 0.05$). The mean number of matings for untreated males was 3.2±1.03, while the mean number of matings for males treated with 250 and 350 Gy was 2.7±0.84 and 2.5±0.90, respectively.

## 4. DISCUSSION

The use of ionizing radiation for insect pest management has been discussed since the early 20$^{th}$ century [19]. Recent successful applications of the SIT to the Mediterranean fruit fly, *Ceratitis capitata*, in Chile [20], screwworm fly, *Cochliomyia hominivorax*, in North Africa

Table 3. Effect of gamma radiation on *Cydia pomonella* male mating competitiveness

| Ratio tested[1] | Dose (Gy) | No. Eggs laid | Egg hatch | | P value |
|---|---|---|---|---|---|
| | | | Observed (%) | Expected[2] (%) | |
| 0:5:5 | 0 | 245 | 84.9 | | |
| 5:0:5 | 350 | 250 | 01.2 | | |
| 5:5:5 | 350 | 268 | 39.9 | 43 | > 0.05 |
| 45:5:5 | 350 | 252 | 10.3 | 9.6 | > 0.05 |
| 0:5:5 | 0 | 257 | 77.8 | | |
| 5:0:5 | 400 | 230 | 0.00 | | |
| 5:5:5 | 400 | 243 | 37.9 | 38.9 | > 0.05 |
| 45:5:5 | 400 | 247 | 09.3 | 7.8 | > 0.05 |

[1] Irradiated male: Untreated male: Untreated female. Ratios reflect exact number of moths per cage.
[2] Expected in the hypothesis of equal competitiveness, calculated according to Fried's (1971) formula.

[21], tsetse fly, *Glossina austeni*, in Zanzibar [22] and encouraging results on codling moth eradication from Canada [23] reaffirm the method's efficacy.

Studies on the effects of gamma radiation on the Syrian strain of *C. pomonella* revealed that exposure of females less than 24 h old to a dose of a 150 Gy caused complete sterility and reduced egg production. Exposure of males to radiation dosages of 350 and 400 Gy caused >99% sterility, evidently without undesirable effects on mating competitiveness and adult longevity. These experiments were, however, conducted in small laboratory cages with laboratory-reared moths, and it is recognized that reared sterilized males might not compete efficiently with wild males for wild females under field conditions [24, 25].

The number of matings per treated male was reduced slightly and treated males recovered a small amount of fertility over time. Recovery of fertility in codling moth males exposed to gamma irradiation has been reported earlier [26]. The debilitating effect of gamma irradiation on male mating capacity is in general agreement with data reported by others [17]. However, it differs from that reported by White et al. [27], who showed that irradiated males mate more times than unirradiated males. The average number of eggs deposited by treated or untreated females mated to irradiated males was also reduced. Reduction in the number of eggs deposited by untreated females mated to treated males could be due to a decrease in male mating ability [28].

In general, our results agree with those reported earlier by several other authors [11, 12, 13, 26, 29]. However, there were some differences. For instance, Chernyi et al. [13] found that doses higher than 300 Gy were required to sterilize codling moth females; similar results were also suggested in Proverbs et al. [30]. Our results showed that females were always completely sterile when treated with 150 Gy. In addition, Proverbs et al. [31] and White and Hutt [29] report a significant increase in the longevity of codling moth males exposed to gamma radiation; this is slightly different from our results. Furthermore, and contrary to our findings, White et al. [27] showed that irradiated males mated more times than unirradiated males. Differences in experimental techniques and dose rates could account for much of the variations. Pristavko and Orghel [32] found that exposure of codling moth male pupae to 30 krad (300 Gy) at dose rates of 44 and 308 rad/sec. induced 87 and 99% sterility, respectively. Genetic variability among different geographical strains and differences between laboratory

colonies were found to cause differences in radio-sensitivity as well [14, 33]. In addition, daily rhythm in codling moth radio-sensitivity has also been reported [34].

The sterile insect technique is a promising method for control or eradication of the codling moth from Syria. A sexing system to eliminate sterile females may not be necessary since females can be sterilized at a lower dose than that required to sterilize males. In fact, sterile females can play a positive role. Field trials in several countries have assessed the potential of different species of *Trichogramma* (Hymenoptera: Trichogrammatidae) wasps to parasitize codling moth eggs, and many of them have proved to be effective [35, 36]. In a sterile insect release area, fertile eggs laid by wild codling moth females will be present. However, sterile eggs deposited by released females will represent the overwhelming majority. These eggs provide a suitable substrate for *Trichogramma* species to reproduce and parasitism of fertile eggs should decrease the wild codling moth population faster than when influenced only by the release of sterile moths. In fact, as these parasites prefer fertile eggs [37], the rate of decrease of the wild population could be faster than expected.

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