Mass-Rearing and Field Performance of Irradiated Carob Moth *Ectomyelois ceratoniae* in Tunisia

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ABSTRACT An artificial diet for rearing the carob moth *Ectomyelois ceratoniae* Zeller was developed composed of wheat bran, yeast, sucrose, salt mixture, vitamin C, aureomycine, methyl paraben, lysine, glycerine and distilled water. Carob moths, mass-reared on this artificial diet showed a similar performance to moths reared on this diet as single pairs with respect to larval developmental time, percent adult emergence, adult weight, longevity, percentage egg hatch and sex ratio. However, fecundity and fertility of adults reared on the artificial diet was significantly lower than single-pair reared moths. In addition, an assessment was made of the propensity of male carob moths to respond to the female pheromone during field cage studies using non-irradiated and irradiated males in the presence of virgin non-irradiated females. The data indicated that irradiated males responded equally well to the virgin females as untreated males, irrespective of the male ratio. The dispersal of partially sterile carob moth males was assessed in a pomegranate orchard using mark-release-recapture tests. A similar dispersal between irradiated and normal males was observed for distances between 40 to 80 metres from the release point. Significant differences between dispersal of irradiated versus untreated males were obtained for distances exceeding 100 metres from the release point.

KEY WORDS sterile insect technique, artificial diet, rearing, inherited sterility, dispersal, mark-release-recapture, pomegranate, *Ectomyelois ceratoniae,* carob moth, Tunisia

1. Introduction

The carob moth *Ectomyelois ceratoniae* Zeller is a major insect pest of dates, pomegranate and several other host plants in Tunisia (Dhouibi 1989). Larvae are polyphagous and attack both stored products and field crops in the Mediterranean basin and countries in the Near East region (Gothilf 1969). This pest causes great economic losses and yearly infestation rates range from 20% in dates to 80% in pomegranate in Tunisia (Dhouibi 1982, 1992) (Fig. 1a).

Many control methods have been used to keep populations below economic threshold levels. Controlling the carob moth with insecticides is not efficient because larvae feed and develop inside the fruit, where they are protected (Dhouibi 1989). Moreover, the harmful effect of broad-spectrum insecticides on the environment and the risk of developing insecticide resistance further restrict the use of this control method. Furthermore, oases are fragile ecosystems and since the 1970's, the use of broad-spectrum insecticides has been banned by the Government of Tunisia for dates intended for export. There is therefore a great need to develop alternative control methods, which are both effective and friendly to the environment. These include sanitation, bagging, spraying with *Bacillus thuringiensis* (Berliner) (*Bt*) and the release of natural ene-

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mies. Dhouibi (1992) showed that in an oasis ecosystem, *Bt* spraying could decrease the level of carob moth infestation by 60% when applied against first instar larvae, i.e. before the moth larvae migrate into the dates. Bagging of date clusters decreased carob moth infestations in both oases and pomegranate orchards by 6.0 and 4.5%, respectively (Dhouibi 1982). The success of this control method depends on the timing of bagging and the material used, i.e. to be efficient, bagging should be done in July before the third annual generation of the insect (Dhouibi 1982), and Kraft paper, mosquito netting or plastic films should be used. However, bagging is expensive and therefore unsuitable for large areas. It also requires that infested and fallen fruit are discarded to avoid new infestations the following year (Gothilf 1970, 1984). Finally, biological control using natural enemies provides good control of the pest. The experimental use of two parasites *Habrobracon hebetor* (Say) in pomegranate orchards and *Phanerotoma flavitestacea* (Kohl) in date oases lead to a high level of parasitism (Jemmazi 1994, Charni 1995) and a 40% reduction in infestation level.

In 1999, research was initiated to develop the sterile insect technique (SIT) as a component of area-wide integrated pest management (AW-IPM) for the control of carob moth in Tunisia (Dhouibi and Abderahmane 2001, Abderahmane 2002, Mediouni 2005). The research programme initially focused on improving the mass-rearing of the carob moth, with special emphasis on the development of an artificial diet. In this respect, rearing techniques that had been developed in Canada for the codling moth *Cydia pomonella* (L.) (Proverbs and Logan 1970) were adapted for carob moth rearing. To improve the quality and production of mass-reared insects, various modifications were made to the mass-rearing system and to the artificial diet. An experimental population was established in the laboratory of entomology at the Institut National Agronomique de la Tunisie in Tunis in 1999 from infested field-collected dates.

Studies were initiated to assess the field performance of irradiated male moths. Using field cages, the response of irradiated and non-irradiated carob moth males to the female pheromone was assessed (Robinson and Proverbs 1975, Abderahmane 2002). In addition, the dispersal of released irradiated males was studied using mark-release-recapture tests (Weissling and Knight 1994, Bloem et al. 1998). These field assessments were done over a period of three years (Mediouni 2005). This paper reports the progress made in Tunisia during the last two years with mass-rearing of the carob moth and in assessing the performance of irradiated substerile males in the field.

Table 1. *Composition of carob moth diet: quantities per 1000 grams of diet.*

Ingredient	Weight (grams)
Wheat bran	600.0
Sucrose	120.0
Yeast	23.0
Salt mixture	20.0
Vitamin C	6.7
Aureomycin	6.7
Methyl paraben	1.3
Lysine	3.0
Glycerine	150 millilitres
Distilled water	250 millilitres
Calco Red dye	41 millilitres

2. Mass-Rearing the Carob Moth

2.1. Diet Composition

Research on the development of an artificial diet for rearing the carob moth was initiated many years ago (Gothilf 1969, Dhouibi 1989, Abderahmane 1997). Several formulae based on leguminous seeds such as soybeans, beans, pods of carob and acacia, or on cereals like wheat germ or bran, have been tested. These diets were abandoned because they were costly and prone to bacterial and fungal attacks.

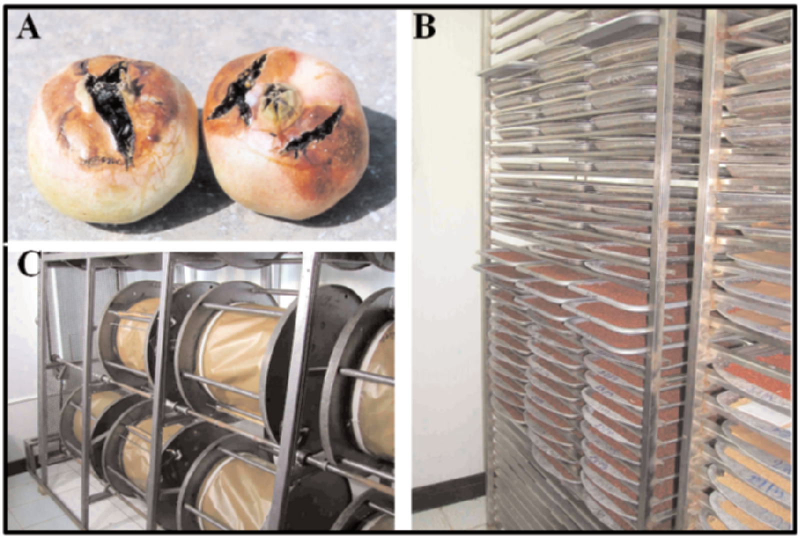

Figure 1. *(a) Damage in pomegranate caused by larvae of the carob moth, (b) larval diet trays stacked in trolleys, and (c) round, slowly rotating oviposition cages (Photos b and c from M.J.B. Vreysen, reproduced with permission).*

Therefore, using the work of Fenny and Brinkman (1967) and Abderahmane (2002), a new artificial diet was developed for mass-rearing the carob moth with wheat bran, sucrose, glycerine and water as major components (Table 1).

2.2. Diet Preparation

The wheat bran was first sterilized at 120°C for two hours. After that, all solid ingredients (Table 1) were weighed and mixed until a homogenous consistency was reached. Glycerine and water were then added followed by Calco Red dye to stain the digestive tract of the larvae and thereby distinguish between wild and released insects. The Calco Red dye was prepared by dissolving 5 grams of Calco Red powder in 80 millilitres of oil and heated to boiling point under continuous agitation. The resulting red suspension was then mixed with the diet. To avoid bacterial and fungal contamination, methyl paraben and aureomycin were added to the diet. Five kilograms of the artificial diet were prepared each day to seed eggs.

2.3. Rearing Procedures

The experimental rearing unit consisted of four rooms each of 9 square metres, i.e. two larval holding rooms, one oviposition room and one adult collection room. In the first step of the production process, virgin female moths were placed together with fertile male moths in locally constructed oviposition cages (dimensions 25 (diameter) x 65 (height) centimetres) for four days (Fig. 1c). The wall of the cylindrical oviposition cages consisted of removable paper sheets (Kraft paper), which were highly suitable for the deposition of eggs. The moths were provided with a sugar solution on moistened rolls of cottonwool. A series of these cages was kept in an acclimatized room (temperature of 28 ± 1°C, a photoperiod of 14:9 (L:D) and 45 ± 5% relative humidity (RH)), where they were slowly rotated on rails to ensure an equal light distribution. The system was highly efficient as shown by the good fecundity of the female moths and the random distribution of the eggs on the paper sheets. The approach is a modification of the system that was developed for codling moth in Canada (Proverbs and Logan 1970).

After several days, when the female moths had deposited sufficient eggs, the paper sheets of the oviposition cages were removed. The sheets were then cut into equal-sized pieces, which were deposited on the trays with the larval diet at a density of 1500-2000 fertile eggs per one kilogram of diet. Up to 85 of these larval trays could be stacked in locally constructed trolleys (2 x 1.5 metres) (Fig. 1b). With an average emergence rate of 80%, it is estimated that between 160 000 and 170 000 adult moths emerged from the pupae held in each trolley. The larvae were reared under the following conditions: a temperature of 28 ± 1°C, photoperiod of 15:9 (L:D) and 75 ± 5% RH.

Dishes with pupae were moved into the collection room, which was kept at 30°C, continuous low light and without any humidity control. The room had a capacity of 819 dishes placed into seven cabinets. Emerging moths were attracted by a light source in the ceiling of the collection room and sucked into a duct system leading to a conical collector in an adjacent room, kept at 0°C. The low temperature immobilized the moths making the collection each morning easy. The collected insects were transferred to the oviposition cages at a density of 30 grams of adult moths per cage, corresponding to approximately 1500 adults per cage.

3. Performance of Carob Moths Reared on Artificial Diet

An assessment was made of various biological performance indicators of carob moths maintained under mass-rearing conditions and single-pair cultures (Tables 2 and 3). The larvae of both experimental groups were reared on the new artificial diet. For all experiments, the data were subjected to Duncan's test at $P < 0.05$ to assess significant differences between the parameters using the statistical package SPSS 10.0 for Windows.

The total development time of moths maintained under mass-rearing conditions (33.6 days) was slightly longer than that of moths maintained under single-pair cultures (34.2 days) (Table 2). These differences could mainly be attributed to the longer development time of the egg (3.1 days versus 3.0 days) and pupal stages (7.3 days versus 7.0 days) (Table 2). The first instar stage (L_1) showed the longest development time and the highest mortality rate (12.4 and 5.9% for the mass-reared and single-pair reared moths, respectively). The development time of the L_2, L_3,

Table 2. *Duration of the different development stages of* Ectomyelois ceratoniae *reared on the artificial diet under single-pair and mass-rearing conditions.*

Developmental stage	Mean duration (days): mass-rearing	Mean duration (days): single-pair rearing
Eggs	3.14 ± 0.2 b (325)	3.01 ± 0.1 a (300)
L1	5.01 ± 0.1 a (275)	4.99 ± 0.3 a (255)
L2	4.58 ± 0.8 a (241)	4.55 ± 0.9 a (240)
L3	4.56 ± 0.5 a (213)	4.54 ± 0.7 a (235)
L4	4.67 ± 0.6 a (208)	4.63 ± 0.8 a (235)
L5	4.89 ± 0.1 a (208)	4.85 ± 0.4 a (235)
Pupae	7.33 ± 0.9 b (203)	7.01 ± 0.2 a (235)
Duration	34.18 days	33.58 days

Values in parenthesis indicate the number of insects used. For the same stage, means followed by the same letter are not significantly different (Duncan's Test at $P < 0.05$).

Table 3. *Biological performance indicators of carob moth maintained under mass-rearing and single-pair rearing conditions on artificial diet.*

Biological parameter	Number of insects used	Mass-rearing	Single-pair rearing
Male pupae weight (milligrams)	1000	35.7 a	36.1 a
Female pupae weight (milligrams)	1000	41.3 a	42.2 a
Adult male weight (milligrams)	1000	16.1 a	17.2 a
Adult female weight (milligrams)	1000	24.3 a	25.2 a
Percentage of egg hatching (%)	1000	93.0 a	95.0 a
Percentage of adult emergence (%)	1000	85.0 a	85.21 a
Male longevity (days)	500	5.8 a	6.2 a
Female longevity (days)	500	8.8 a	8.6 a
Fecundity (number of eggs per female)	1000	115.6 b	182.5 a
Fertility (number of fertile eggs per female)	1000	95.9 b	140.0 a
Sex ratio	1000	1:1 a	1:1 a

Comparison is made for each biological parameter in mass-rearing and single-pair cultures. Means followed by the same letter are not statistically different by Duncan's test.

L_4 and L_5 stages were similar for the mass-reared and single-pair reared moths. The observed duration to complete the life cycle under mass-rearing and single-pair cultures resulted in a total of ten generations per year.

Results presented in Table 3 also indicate that all biological parameters of performance, with the exception of fecundity and fertility, of moths maintained under mass-rearing conditions were similar to those from single-pair cultures. Females kept under mass-rearing conditions produced significantly fewer eggs and these had a lower hatch rate as compared to single-pair rearing. The lower fertility level was most likely correlated to the lack of space necessary for the copulation process. Indeed, Dhouibi (1989) observed that carob moth copulation required adequate space for males to be able to fertilize females. In addition, differences were observed between the biological performance indicators of the two sexes, i.e. the weights of female pupae and adults were higher than those of males and females survived longer than males. However, the sex ratios of moths reared under the different rearing systems was similar.

These initial data on the performance of carob moths reared under mass-rearing conditions and maintained on the wheat bran-based artificial diet are encouraging, with most of the performances similar to those of insects reared as single-pairs. Nevertheless, more research is needed to further increase the fertility and fecundity of the moths to make the mass-rearing of carob moth more efficient.

4. Field Performance of Substerile Carob Moth Males

Male field dispersal was studied in a five hectare pomegranate field using 48 Delta traps (Biological Control System Ltd. Treforest, Mid Glamogan. CF37 5SU U.K.), baited with the synthetic female carob moth pheromone (Dhouibi 1989) and deployed in trees at a height of about 1.5 metres. Dispersal trial releases were undertaken from July to September in 2001, 2003 and 2004 using approximately 1000 irradiated and non-irradiated males per hectare per week. Male moths were treated with a gamma radiation dose of 400 Gy, which is the minimum required to induce complete sterility in female and substerility in male carob moths. The radiation

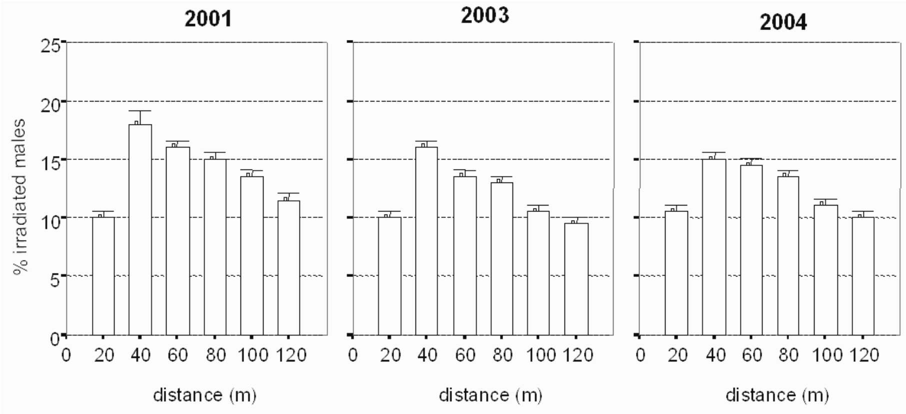

Figure 2. *Field dispersal of irradiated carob moth males in an pomegranate orchard in 2001, 2003 and 2004.*

treatment was given in a ^{60}Co irradiator at a dose rate of 46 Gy per minute.

From a release point chosen in the pomegranate field, six distances (20, 40, 60, 80, 100 and 120 metres from the release point), were selected for male capture using eight traps at each distance in a cross layout. All traps were checked once a week and total numbers of irradiated and non-irradiated moths were counted. Released insects were distinguished from wild ones by their red-marked digestive tract. The dispersal experiments using irradiated and non-irradiated males were conduced in the same area but at different times. The dispersal of irradiated and non-irradiated males was compared at each distance using the SPSS program (version 10.0) (Duncan's Test at $P < 0.05$).

In addition, two large field cages (3 metres (diameter) x 2.5 metres (height)) covered with a mosquito net were placed over two medium-sized trees to study male field competitiveness. In this experiment, the propensity of males to respond to female pheromone (expressed by the number of males caught) was evaluated. In each cage, a Delta trap baited with two virgin non-irradiated females was deployed. Four ratios of irradiated to untreated males were investigated: 1:1, 2.5:1, 5:1 and 10:1. Experiments were carried out from April to July each year and each ratio was replicated five times. For each replicate, the number of captured males was counted three days after the release of irradiated and non-irradiated males into the cages. Comparisons were made between the percentages of captures using the SPSS programme (version 10.0) (Duncan's Test at $P < 0.05$).

4.1. Male Dispersal

The data in Fig. 2 showed that the substerilizing dose of 400 Gy did not affect the ability of males to disperse under field conditions. Indeed, irradiated males were able to reach traps placed at 120 metres from the release point. The percentage of captured males (irradiated and non-irradiated) depended on the distance from the release point, maximum captures being obtained between 40 and 80 metres for the three years (Fig. 2 and Fig. 3). Statistical analyses showed no significant differences in captures of irradiated and non-irradiated males at distances 40, 60 and 80 metres. However, for distances of 20, 100 and 120 metres from the release point, significant differences were recorded in captures of non-irradiated and irradiated males (Duncan's Test at $P < 0.05$). These results were similar for the three years of observations (2001, 2003 and 2004).

Numerous researchers have studied the impact of gamma radiation on the dispersal of

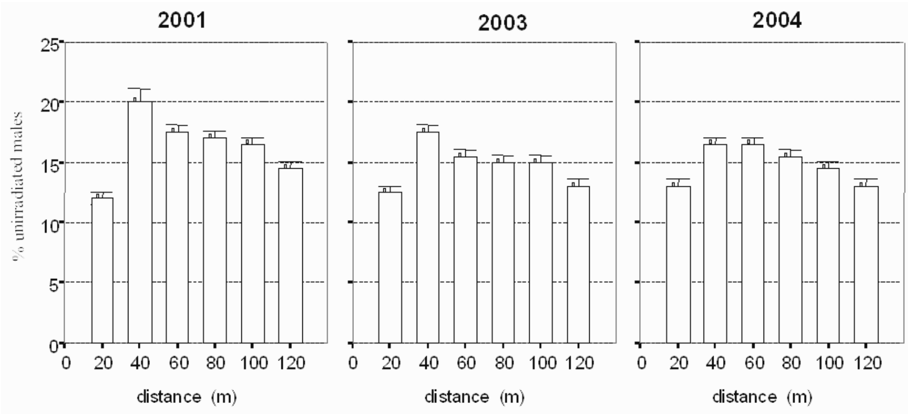

Figure 3. *Field dispersal of unirradiated carob moth males in an pomegranate orchard in 2001, 2003 and 2004.*

irradiated Lepidoptera. Qureshi et al. (1993) indicated a similar dispersal between irradiated and non-irradiated males of the pink bollworm *Pectinophora gossypiella* (Saunders). In addition, Carpenter and Gross (1993) indicated that the number of irradiated released and recaptured males of the corn earworm *Helicoverpa zea* (Boddie) was negatively correlated with the distance between the release point and the trap.

4.2. Propensity of Substerile Males to Respond to Female Pheromone in the Field

For AW-IPM programmes that integrate the SIT, assessing the field competitiveness of irradiated males and the optimal release ratio is essential to ensure that the sterile insects can compete with wild males under natural conditions and are released in appropriate numbers. The results obtained here indicate that when the ratio of irradiated to non-irradiated males increased in the cage, the irradiated to non-irradiated male ratio of the catch in the traps likewise increased (Table 4). During 2001, 24 and 26 irradiated and untreated males were recaptured, respectively at a 1:1 irradiated to non-irradiated male ratio. At this ratio, the difference between the number of captured males was not statistically significant. At other ratios, the captures of irradiated males were higher than those of non-irradiated males, due to increases in the number of irradiated males in the cage. During 2003 and 2004, similar results were obtained. Consequently, after irradiation at the substerile dose of 400 Gy, males of *E. ceratoniae* remain attracted by virgin females and were able to respond to the female pheromone.

5. Conclusions

Dates are an important and valuable export commodity for Tunisia but high infestation rates with the carob moth are causing significant economic losses. Current available control methods are either banned (broad-spectrum insecticides), being phased out (methyl bromide for postharvest fumigation) or have serious limitations (e.g. bagging of the clusters, *Bt* spraying, sanitation, etc.). The SIT could offer a viable, efficient and economic additional component of an integrated approach.

As reported in this paper, important progress has been made with the rearing of carob moth. The development of a wheat bran-based artificial larval diet, of an efficient adult oviposition cages and of an automatic adult moth collection system were critical steps in progressing towards an efficient

Table 4. *Number of irradiated and non-irradiated males of* Ectomyelois ceratoniae *responding to female pheromone under field cage conditions during 2001, 2003 and 2004.*

Ratio IM:NM	Insects per cage	2001	2003	2004
1:1 (5x)[1]	10:10	24/26 a	26/24 a	23/27 a
2.5:1 (5x)	25:10	88/37 b	90/35 b	92/33 b
5:1 (5x)	50:10	221/29 b	218/32 b	225/25 b
10:1 (5x)	100:10	490/10 b	486/14 b	491/9 b

IM: Irradiated males, NM: Non-irradiated males

[1]Values in parenthesis are the number of replicates

Catch ratios of irradiated to non-irradiated males are shown for each year. Comparisons were made between these ratios. Ratios in the same row accompanied by the same letter are not statistically different by Duncan's Test.

mass-rearing system. The fecundity and fertility of the moths maintained under mass-rearing conditions is still inferior to moths reared as single-pair cultures and therefore more work is needed to further improve the performance of moths maintained under a mass-rearing system.

A dose of 400 Gy was needed to obtain full female sterility and substerile male moths. This dose is indicative of the high level of resistance of the carob moth to radiation. However, experiments have shown that despite the high dose, irradiated carob moths responded well to the female sex pheromone, dispersing well up to 120 metres from the release point and showing a similar dispersal pattern to that of non-irradiated moths. Further research will focus on assessing the competitiveness of the irradiated moths under field conditions.

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