

Radiation Induced F₁ Sterility in Lepidoptera for Area-Wide Control

PROCEEDINGS OF THE FINAL RESEARCH CO-ORDINATION MEETING
PHOENIX, ARIZONA, 9-13 SEPTEMBER 1991
ORGANIZED BY THE JOINT FAO/IAEA DIVISION
OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE



The cover picture shows a mating pair of *Helicoverpa zea*, corn earworm.
By courtesy of United States Department of Agriculture.

PANEL PROCEEDINGS SERIES

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IN LEPIDOPTERA
FOR AREA-WIDE CONTROL**

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AND HELD IN PHOENIX, ARIZONA, 9-13 SEPTEMBER 1991

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FOREWORD

Caterpillars or larvae belonging to the order Lepidoptera are among the most damaging pests of food crops, forests and stored products throughout the world. Currently, most of these species are controlled largely by applying chemical insecticides. In many instances this practice has induced insecticide resistance. In addition, the frequent use of chemicals often destroys both natural enemies that keep a variety of pests in check and pollinating insects and other beneficial organisms.

Non-chemical methods of controlling lepidopterous pests are being developed and, in some instances, are being widely used. These alternatives include resistant crop varieties, insect pathogens, predators, parasites, use of pheromones or other semiochemicals for mating disruption and trapping, use of trap crops and toxic baits, and cultural methods.

Genetic control of lepidopterous pests includes the use of the sterile insect technique. This technique has been used since 1968 to protect 0.4 million hectares of cotton in the San Joaquin Valley of California, United States of America, from the pink bollworm. Another form of genetic control in Lepidoptera is the use of inherited sterility, which is especially pronounced in the first filial (F_1) generation following the exposure of the parents to substerilizing doses of ionizing radiation. Lepidoptera are unusual among the insects in that very high doses (300 to 500 Gy) of radiation are required to induce sexual sterility. These high doses impair mating behaviour and reproductive physiology. However, at lower rates, normally 80 to 150 Gy, the irradiated insects are partially sterile; their F_1 progeny are almost completely sterile although still competitive.

In addition, the F_1 generation can be reared in the field. This may be important because the rearing of most lepidopterous species is costly. On the other hand, certain other species can be mass reared at almost any time of year in factories, stockpiled in diapause, irradiated and activated for release in synchrony with the wild population.

It is likely that substantial cost reductions may be gained by combining the use of F_1 sterility with various methods of control. Resistant crop cultivars should be planted if available. In addition, high level populations of lepidopterous pests may first be reduced by using pathogens, parasites or predators, and then prevented from rebuilding to damaging levels by means of F_1 sterility.

A co-ordinated research programme on Radiation Induced F_1 Sterility in Lepidoptera for Area-Wide Control was organized in accordance with the recommendations of a consultants group that met in Vienna in November 1984. Research Co-ordination Meetings have been held at Otis, Massachusetts, USA, Beijing, China, and finally in Phoenix, Arizona. Special thanks are in order for D.A. Lindquist, B.A. Butt and L.E. LaChance for organizing this programme and for leading the meetings.

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INTRODUCTION

Very high doses of ionizing radiation are required to induce full sexual sterility in Lepidoptera. Fully sterilizing doses are detrimental to the behaviour and reproductive physiology of Lepidoptera. Such damage greatly diminishes the ability of irradiated males to compete for mates with wild males. The undesirable effects of such high doses of radiation include failure of irradiated males:

- (a) To disperse strongly, to seek out appropriate niches or to behave in synchrony with wild males,
- (b) To respond to calling females and/or to mate,
- (c) To form the spermatophore, to include both eupyrene and apyrene sperm in the spermatophore or to position the spermatophore stalk at the opening of the seminal duct,
- (d) To transfer eupyrene sperm in sufficient quantity via the seminal duct to the spermatheca.

It appears that inherited sterility can be induced in all species of Lepidoptera by means of doses of ionizing radiation that induce only low levels of sterility in irradiated individuals. Moreover, inherited sterility appears to be manifested in a similar pattern throughout the Lepidoptera family as follows:

- (1) The level of sterility is much higher in the F_1 generation than in the treated parent;
- (2) The level of sterility in the F_2 generation is lower than that in the F_1 ;
- (3) The number of male progeny in the F_1 generation is normal or nearly normal, whereas the number of female progeny is very low or absent depending on the dose.

Since relatively low doses of radiation are sufficient to induce inherited sterility, many of the detrimental effects of fully sterilizing doses can be partially or completely avoided, so that the irradiated males and their partially sterile descendants are able to compete with wild males. In addition, the detrimental effects of irradiation are least when the dose is delivered to pupae just prior to the emergence of the adults.

An important question is the selection of the dose to induce inherited sterility. A number of factors must be considered in selecting the dose. If a high dose is selected to induce a correspondingly high level of sterility in the F_1 generation, then the released and F_1 individuals will have a very strong suppressive effect on the wild population (provided that their competitiveness is not badly impaired). However, in this case the suppressive effect of F_2 descendants will be greatly dampened, and that of subsequent generations will be insignificant. On the other hand, if the rate of increase of the pest population is low so that sufficient suppression can be exerted by F_1 individuals that have a moderate level of fertility (e.g. 15 or 20%), then a relatively low dose may be used and significant suppressive effects will persist in the F_2 generation and perhaps even in the F_3 generation.

EFFECT OF SUBSTERILIZATION DOSES OF RADIATION ON THE BIOLOGY OF DIAMONDBACK MOTH

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Abstract

EFFECT OF SUBSTERILIZATION DOSES OF RADIATION ON THE BIOLOGY OF DIAMONDBACK MOTH.

The pupae of the diamondback moth, *Plutella xylostella*, were exposed to four substerilizing doses (100, 150, 200 and 250 Gy) of gamma radiation. The fecundity, sterility and progeny development of parental crosses and certain F_1 backcrosses (progeny of irradiated males) were studied in the laboratory. All doses caused sterility in the parental crosses and F_1 backcrosses. Doses above 20 Gy greatly affected the development of larvae in parental crosses of irradiated females with normal males and of irradiated males with irradiated females, as no pupation was observed. The study indicated that a dose between 150 and 200 Gy would be suitable for inherited sterility of the diamondback moth. However, the backcross of progeny from irradiated males showed no significant increase in inheritance of deleterious effects.

1. INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella*, is one of the most important pests of cruciferous crops in Malaysia. The insect was first recorded in Fraser's Hill, Pahang, in 1925 and became a major pest of cabbage in the Cameron Highlands in 1934 [1]. Since then, it has been widely recognized as a serious pest of crucifers in both highland and lowland areas. Without chemical control, damage caused by the DBM on cruciferous crops could reach a level of 70-90% destruction.

Insecticides have become the main method of control for this insect. The heavy reliance on, and indiscriminate use of, insecticides have caused the DBM to become

resistant to most of the insecticides available on the market [2, 3]. The need for other control methods has risen following the failure of insecticides to control this pest. Among other control measures, biological control looks very promising [4]. In the development of pest management for DBM, other possible control methods need to be explored, including the sterile or partially sterile insect technique.

The technique of partial sterility or inherited sterility is preferred for the control of lepidopterous pests as complete sterilization tends to induce physiological disturbances such as reduction of mating competitiveness, lack of sperm transfer, etc. The progeny of the irradiated parents at substerilizing doses have also been shown to be more sterile than the parents. The potential of inherited sterility in pest control was demonstrated by Knipling [5] through a mathematical model. Numerous studies have been conducted on lepidopterous pests such as *Helicoverpa zea*, *Spodoptera frugiperda* and *Trichoplusia ni*. Inherited sterility was reviewed extensively by LaChance [6]. The DBM has been suggested as a potential candidate for inherited sterility [7]. Earlier work indicated that 300 Gy of gamma irradiation is a sterilizing dose for DBM, causing more than 90% sterility of both P₁ male and female adults [8]. The objective of the following study was to evaluate the effect of doses of gamma irradiation below 300 Gy on the development of DBM from various possible parental crosses and selected F₁ crosses.

2. MATERIALS AND METHODS

Diamondback moths were collected from Serdang in Selangor and cultured on sawi leaves in the laboratory at $27 \pm 2^\circ\text{C}$ and $80 \pm 15\%$ relative humidity. The larvae were given fresh leaves daily while adults were fed with 5% honey solution soaked into cotton wool pads. The pupae were collected and irradiated for 1 d before emergence by using a gamma cell (⁶⁰Co) irradiator that had a dose rate of about 60 Gy/min. The pupae were irradiated at 100, 150, 200 and 250 Gy. The males were then separated from the females. The emerging males (m) and females (f) were paired for the following parental crosses (I, irradiated; N, normal; A, progeny of the cross I_m × I_f):

$$\begin{aligned} & I_m \times I_f \\ & I_m \times N_f(A) \\ & N_m \times I_f \\ & N_m \times N_f \end{aligned}$$

The male and female adults emerging from the parental cross of

$$I_m \times N_f(A)$$

were then paired with normal female and male adults, respectively, for F_1 back-crosses and designated as follows:

$$A_m \times N_f$$

$$A_f \times N_m$$

and the development of the F_2 generation was then studied.

Five pairs of DBMs were placed in a 2.5 L plastic cylinder for mating and oviposition. The adults were provided with 5% honey solution. A sawi leaf was placed in the plastic cylinder for oviposition. After 2 d, the leaf was removed and replaced with another leaf for a further 2 d. The total number of eggs deposited on both leaves was counted to assess the relative fecundity of females from different crosses. The number of eggs hatched was recorded and fresh leaves were supplied daily to the larvae. The larvae were then allowed to complete their development. The number of larvae used in this study is given in Table I. The numbers of fourth instar larvae and pupae were recorded. The percentages of fourth instar larvae and pupae obtained from the eggs hatched were used for a statistical analysis. The treatments were subjected to an analysis of variance and the means were compared using the Duncan multiple range test (SAS Institute test, 1982).

3. RESULTS AND DISCUSSION

The development of DBMs obtained from the parental crosses was found to be dependent on dose. A dose above 200 Gy greatly affected oviposition, egg hatch and development of the larvae (Tables II, III and IV). At doses of 200 and 250 Gy,

TABLE I. NUMBER OF LARVAE IN PARENTAL AND F_1 CROSSES USED TO STUDY THE NUMBER OF LARVAE THAT SURVIVED TO THE PUPAL STAGE

Dose (Gy)	Treatment				
	$I_m \times I_f$	$N_m \times I_f$	$I_m \times N_f$	$A_f \times N_m$	$A_m \times N_f$
100	58	572	1057	359	870
150	21	207	1696	580	431
200	—	105	288	797	719
250	—	160	346	—	175

TABLE II. EFFECT OF GAMMA IRRADIATION OF DBM PUPAE ON REPRODUCTION OF P_1 ADULTS ($I_m \times I_f$)

Radiation dose (Gy)*	Total number of eggs**	Egg hatch (%)	Fourth instar larvae (%)	Pupae (%)
Control (11)	404 ^a	79.4 ^a	70.0 ^a	47.7 ^a
100 (4)	231 ^b	6.3 ^b	18.0 ^b	3.1 ^b
150 (4)	221 ^b	2.6 ^{bc}	0.0 ^c	0.0 ^b
200 (4)	47 ^c	0.0 ^c	0.0 ^c	0.0 ^b
250 (4)	53 ^c	0.0	0.0 ^c	0.0 ^b

* Values in brackets are the number of replicates.

** Means followed by the same letter are not significantly different ($p = 0.05$).

TABLE III. EFFECT OF GAMMA IRRADIATION OF DBM PUPAE ON REPRODUCTION OF P_1 ADULTS ($N_m \times I_f$)

Radiation dose (Gy)*	Total number of eggs**	Egg hatch (%)	Fourth instar larvae (%)	Pupae (%)
Control (11)	404 ^a	79.4 ^a	70.0 ^a	47.7 ^a
100 (8)	350 ^a	20.3 ^b	40.0 ^b	18.5 ^b
150 (5)	388 ^a	10.5 ^c	70.9 ^a	34.7 ^a
200 (4)	26 ^b	0.0 ^d	0.0 ^c	0.0 ^c
250 (4)	40 ^b	0.0 ^d	0.0 ^c	0.0 ^c

* Values in brackets are the number of replicates.

** Means followed by the same letter are not significantly different ($p = 0.05$).

parental crosses of $I_m \times I_f$ and $N_m \times I_f$ failed to produce pupae (Tables II and III). In those crosses the number of eggs deposited was also greatly reduced compared with the lower doses and the control ($p = 0.05$), indicating that a dose above 200 Gy reduced female fecundity. However, the numbers of eggs deposited after doses of 100 and 150 Gy were not significantly different when compared with the control $N_m \times I_f$ crosses (Table III). The proportion of the eggs hatched was significantly reduced, indicating that these doses caused female sterility.

Parental crosses of irradiated males with normal females showed no apparent loss of fecundity of females but significantly reduced the egg hatch (Table IV). However, the percentages of eggs hatched and larvae surviving to pupae from these crosses appeared to be higher compared with the other parental crosses. The F_1 backcross of either male or female from $I_m \times N_f$ showed no significant decrease in fecundity but significant reductions in egg hatch and development of F_2 generation larvae and pupae compared with control crosses ($p = 0.05$) (Tables V and VI).

TABLE IV. EFFECT OF GAMMA IRRADIATION OF DBM PUPAE ON REPRODUCTION OF F_1 ADULTS ($I_m \times N_f$)

Radiation dose (Gy)*	Total number of eggs**	Egg hatch (%)	Fourth instar larvae (%)	Pupae (%)
Control (11)	404 ^{ab}	79.4 ^a	70.0 ^a	47.7 ^a
100 (6)	301 ^b	58.9 ^b	47.4 ^{bc}	26.8 ^b
150 (6)	571 ^a	49.7 ^c	53.6 ^b	42.1 ^a
200 (6)	469 ^{ab}	39.6 ^d	25.1 ^d	9.5 ^c
250 (2)	475 ^a	40.3 ^d	41.0 ^c	26.7 ^b

* Values in brackets are the number of replicates.

** Means followed by the same letter are not significantly different ($p = 0.05$).

TABLE V. EFFECT OF GAMMA IRRADIATION ON REPRODUCTION OF F_1 ADULTS ($A_f \times N_m$)

Radiation dose (Gy)*	Total number of eggs**	Egg hatch (%)	Fourth instar larvae (%)	Pupae (%)
Control (11)	404 ^a	79.4 ^a	70.0 ^a	47.7 ^a
100 (3)	340 ^{ab}	35.9 ^c	55.5 ^{ab}	25.4 ^{bc}
150 (3)	444 ^{ab}	46.7 ^{bc}	43.5 ^{bc}	19.8 ^c
200 (3)	567 ^a	49.7 ^b	34.4 ^c	15.8 ^c

* Values in brackets are the number of replicates.

** Means followed by the same letter are not significantly different ($p = 0.05$).

TABLE VI. EFFECT OF GAMMA IRRADIATION ON REPRODUCTION OF F₁ ADULTS (A_m × N_f)

Radiation dose (Gy)*	Total number of eggs**	Egg hatch (%)	Fourth instar larvae (%)	Pupae (%)
Control (12)	404 ^a	79.4 ^a	70.0 ^a	47.7 ^a
100 (4)	436 ^a	51.7 ^b	73.4 ^a	41.3 ^a
150 (3)	378 ^a	37.0 ^c	49.3 ^{bc}	19.6 ^b
200 (4)	359 ^a	50.9 ^b	30.6 ^c	8.9 ^b
250 (2)	253 ^a	35.2 ^c	28.3 ^c	9.2 ^b

* Values in brackets are the number of replicates.

** Means followed by the same letter are not significantly different ($p = 0.05$).

TABLE VII. ADULT EMERGENCE FROM IRRADIATED PUPAE OF DBM

Dose (Gy)	Number of pupae (not separated)	Normal (%)	Deformed (%)	Mortality (%)
Control	—	80	6	14
100	220	75	10	15
150	200	65	16	19
200	170	62	18	20
250	190	55	30	15

It appears from the results of parental and F₁ crosses that the dose of 100 Gy may not be suitable for inherited sterility because of the significantly lower sterility demonstrated. Preliminary results on the female progeny from A_m × N_f backcrossed with normal males showed almost no difference in the percentage of eggs hatched ($\approx 67\%$) compared with the control. However, the F₂ male progeny from the same cross backcrossed with normal females still exhibited sterility (hatchability of about 45%).

We propose that a dose of between 150 and 200 Gy should be used for further study in the development of the DBM inherited sterility programme. A dose of 250 Gy may not be suitable, since it caused deformities in 30% of the emerged adults (Table VII). It should be noted that at a radiation dose of either 150 or 200 Gy,

no increase in sterility in the backcross generation was observed. The dose of 100 Gy, however, has been shown to have greater inherited deleterious effects for the progeny of irradiated males of fall army worms [9]. A similar dose was also used to sterilize corn earworms partially [10]. It could be that the DBM may require a higher dose of radiation for inherited sterility as it has a relatively short lifecycle in the tropics and has developed a resistance to almost all the insecticides available on the market. Further studies are required to investigate the inheritance of deleterious effects caused by radiation doses of between 150 and 200 Gy to the DBM.

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REFERENCES

- [1] CORBETT, G.H., PAGDEN, H.T., A review of some recent entomological investigations and observations, *Malays. Agric. J.* **29** (1941) 347.
- [2] SYED, A.R., in *Management of Diamondback Moth and other Crucifer Pests* (Proc. 2nd Int. Workshop, Taiwan) (TALEKAR, N.S., Ed.), Asian Vegetable Research and Development Center, Taiwan (1990) 437.
- [3] FAUZIAH, I., DZOLKHIFLI, O., WRIGHT, D.J., *ibid*, p. 391.
- [4] SOON, L.G., *Diamondback Moth Management* (Proc. 1st Int. Workshop), Asian Vegetable Research and Development Center, Taipei (1986) 159.
- [5] KNIPLING, E.F., *Suppression of pest Lepidoptera by releasing partially sterile males: A theoretical appraisal*, *Bioscience* **20** (1970) 465.
- [6] LaCHANCE, L.E., *Genetic Methods for the Control of Lepidopteran Species: Status and Potential*, Rep. ARS-28, US Department of Agriculture, Washington, DC (1985).
- [7] BUTTS, B.A., personal communication.
- [8] HOEDAYA, M.S., SURTADJI, D., RAHAYU, A., personal communication.
- [9] CARPENTER, J.E., YOUNG, J.R., SPARKS, A.N., *Fall armyworm (Lepidoptera: Noctuidae): Comparison of inherited deleterious effects in progeny from irradiated males and females*, *J. Econ. Entomol.* **79** (1986) 46.
- [10] CARPENTER, J.E., YOUNG, J.R., SPARKS, A.N., CROMROY, H.L., CHOWDHURY, M.A., *Corn earworm (Lepidoptera: Noctuidae): Effects of substerilizing doses of radiation and inherited sterility on reproduction*, *J. Econ. Entomol.* **80** (1987) 483.

STUDIES ON F₁ RADIATION STERILIZATION OF DIAMONDBACK MOTH AND MULBERRY WILD SILKWORM

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Abstract

STUDIES ON F₁ RADIATION STERILIZATION OF DIAMONDBACK MOTH AND MULBERRY WILD SILKWORM.

The study began in 1988 under the aegis of the FAO/IAEA co-ordinated research programme on Radiation Induced F₁ Sterility in Lepidoptera for Area-Wide Control. During the following four years the control of the mulberry wild silkworm (*Bombyx mandarina* Moore) and the diamondback moth (*Plutella xylostella* L.) by means of radiation induced sterility was studied.

1. CONTROL OF THE MULBERRY WILD SILKWORM BY THE STERILE IRRADIATION TECHNIQUE

The mulberry wild silkworm (MWS) (*Bombyx mandarina* Moore) is one of the main phyllophagous pests of mulberry trees. This species occurs in the mulberry growing regions throughout China and causes severe damage. The MWS eats mulberry leaves at the same time that farmers collect leaves to feed domestic silkworms (*Bombyx mori* L.). The damage cannot be effectively controlled by using chemical pesticides. The MWS has the characteristics of weak flight and a single host. Mulberry gardens are usually well isolated. All these factors favour the application of the sterile insect technique (SIT) to control MWSs. From 1984 to 1988, we conducted studies on the effects of radiation on the MWS, field studies and studies on artificial diet rearing of the MWS. The principal results were:

- (a) *Studies on the radiation dose required to sterilize the MWS.* The mature pupae are treated with ⁶⁰Co gamma rays. The emergence rate (Y) of the adults decreases with increasing dose (x). The correlation formula is

$$Y = 87.26688e^{-0.0844x}$$

TABLE I. EFFICIENCY OF F₁ STERILITY INHERITANCE OF WILD SILK-WORM INDUCED BY RADIATION

Parental + F ₁ crosses	Pairs	Eggs plated	Hatched eggs	Hatching rate (%)	Sterile rate (%)	Corrected sterile rate (%)
200 Gy to P ₁ ♂ × N ♀						
F ₁ ♀ × N ♂	1	96	0	0	100	100
N ♀ × F ₁ ♂	1	81	7	8.6	91.4	89.9
250 Gy to P ₁ ♂ × N ♀						
F ₁ ♀ × N ♂	2	241	0	0	100	100
N ♀ × F ₁ ♂	4	228	0	0	100	100
Control N ♀ × N ♂	5	353	320	85.6	14.4	0

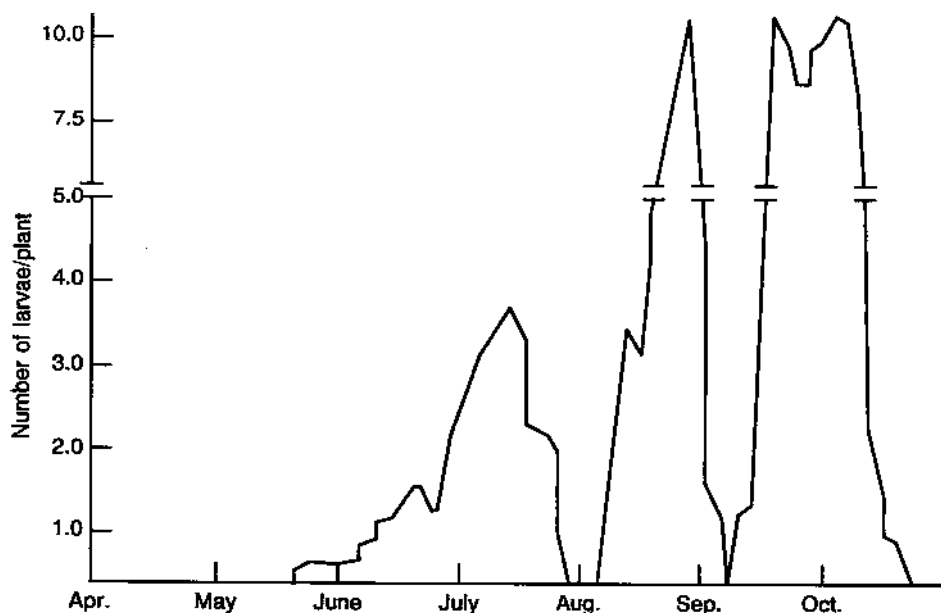


FIG. 1. Curves of the third generation larvae in Zhejiang Province.

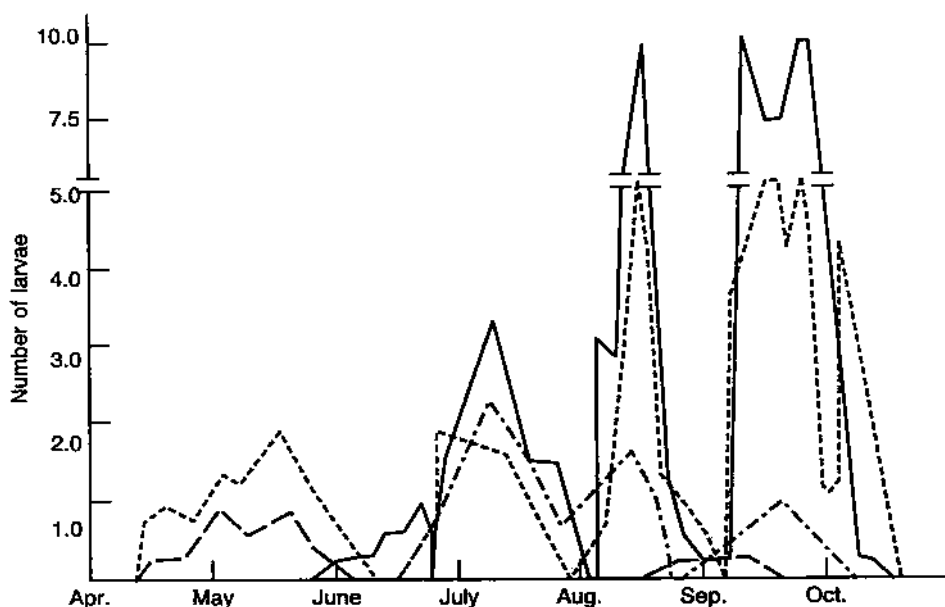


FIG. 2. Curves of phylophagous insect pests on mulberry trees during 1988: —, *B. mandarina* Moore; - - -, *Hemerophila atrilineata*; - · - ·, *Euproctis similis*; - · - ·, *Diaphania pyloalis*.

The morphological deformity rate increases with increasing dose, following the formula

$$Y = 1.6921e^{-0.7809x}$$

The egg sterility increases with increasing dosage, following the formula

$$Y = 93.0068e^{-1.0588x}$$

The study on the effect of sterile inheritance of F_1 treated by radiation indicated that the appropriate substerile dosage for the MWS was 250 Gy (Table I).

- (b) *Studies on the biology and ecology of the MWS.* Wild silkworms have three or four generations per year and the eggs overwinter in diapause. The fourth generation is the most damaging. The larvae population in 1988 was as high as 10.2/plant. The moths of wild silkworms emerged during daytime and became active at night, but flew only a short distance to find mating partners. The trend of a natural MWS population is shown in Fig. 1.

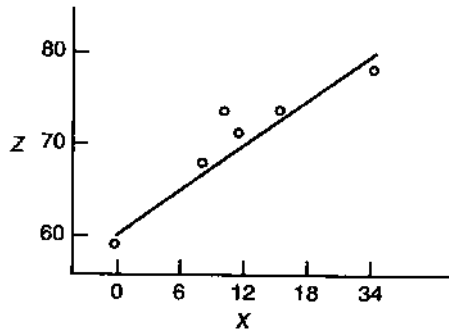


FIG. 3. Relationship between photoperiod and mating rate in the wild silkworm, $Y = 0.61625x + 65.165$.

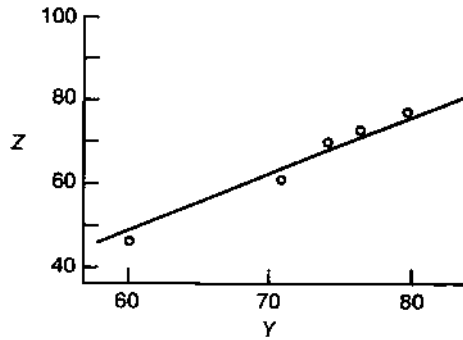


FIG. 4. Relationship between the mating rate and hatching rate in the wild silkworm, $Z = 1.05865Y - 18.136$.

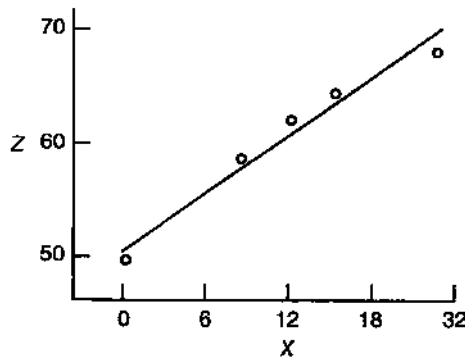


FIG. 5. Relationship between the hatching rate and photoperiod in the wild silkworm, $Z = 0.65625x + 50.805$.

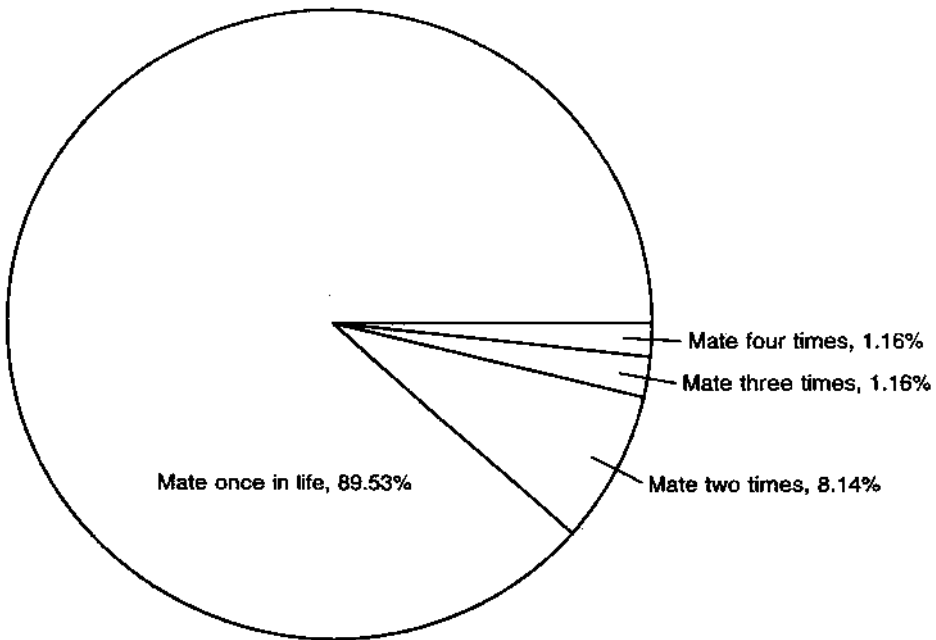


FIG. 6. Mating times of the wild silkworm.

- (c) *The possibility of using the SIT to control wild silkworms.* The wild silkworm is the most important insect pest on mulberry trees. The larval population reaches as high as 12.2/plant with 114 750/ha at the time of heavy infestation. It is difficult to use conventional methods to control the MSW. This is because the natural infestation usually coincides with the time of artificial silkworm rearing. The isolated or separated mulberry fields, and inactive migration and oligophagous habits of the insect, indicate that the application of SIT may serve as a new approach (Fig. 2).
- (d) *Effect of temperature and photoperiod on mating and oviposition of the wild silkworm.* The mating rate of wild silkworms is 78.4%, with an average oviposition of 179.3 eggs per female. The hatching rate is 61.3% under the suboptimum temperature, 25°C, with five different photoperiod treatments. The mating and hatching rates increase with the increasing illumination time. Their correlations are indicated by the formulas

$$Y = 0.61625x + 65.165$$

and

$$Z = 0.65625x + 50.805.$$

The statistical test showed that the correlation coefficients were highly significant (Figs 3-5).

- (e) *Mating habits and competitive ability of sterile wild silkworms.* The mating time of the wild silkworm is from 08.00 to 05.00 the next day during which the mating is at its peak from 13.00 to 23.00. It was found that 89.53% of mated wild silkworms mated once and the rest (10.4%) two to four times (Fig. 6). Compared with the natural wild silkworm, there was no difference in mating competitive ability after being treated by 250-500 Gy of radiation.

2. CONTROL OF THE DIAMONDBACK MOTH BY F₁ STERILITY

The diamondback moth (DBM) is one of the main pests of cruciferous vegetables. It causes the most severe damage in the southern Chang Jiang River Valley of China. After a Research Co-ordination Meeting on Radiation Induced F₁ Sterility in Lepidoptera for Area-wide Control was held in Beijing in May 1989, the research object of the co-ordination research programme was changed from the MWS to the DBM. Since 1989 studies on controlling the DBM by using the SIT have been proceeding, including DBM artificial mass rearing, quality control, sterile DBM marking and field cage release tests as well as observations on the parasitization and population changes of DBMs at different times in Hangzhou over one year. The main results are as follows.

2.1. System for mass rearing the DBM

- (a) An amount of 13.89 kg of No. 83-28 semisynthetic diet was divided into 120 rearing boxes maintained at a temperature of $23 \pm 1^\circ\text{C}$ and a relative humidity of about 70%. A total of 130 000 normal DBM larvae were successfully reared. On average 9.33 larvae were reared on 1 g of diet. Three generations were reared continuously with this diet (Tables II, III).
- (b) Comparison among different rearing systems. Five methods using three rearing systems for the DBM reported in recent years were compared. The three rearing systems are:
- (i) Germinating vegetable seeds [1, 2],
 - (ii) Artificial synthetic diet [3, 4],
 - (iii) Semisynthetic diet made by the present authors.

The comparison of results shows that, although the system of rape seedling rearing is simple, with no mould fungus appearing during the process of rearing, it is not convenient to renew the diet. The system of radish seedling

TABLE II. MASS REARED DBM

Quantity of semisynthetic diet (kg)	Rearing boxes ^a	Eggs put on diet	Pupae obtained	Pupae obtained per gram of diet
13.89	120	300 000	129 600	9.33

^a Plastic boxes were used for rearing (22 cm × 15 cm × 5 cm), with covers that had 13 holes (with diameters of 1 cm).

TABLE III. CONTINUOUS REARING OF DBM FOR THREE GENERATIONS WITH No. 83-28 DIET PRESCRIPTION

Rearing generation	Larval developing period (d)	Pupal weight (mg)	Adult longevity (d)		Ovipositions (eggs/♀)
			♀	♂	
1	10.2	5.1	15.0	21.5	104.0
2	11.6	4.8	21.3	20.4	88.1
3	11.3	5.2	16.8	25.1	157.2

TABLE IV. STERILE EFFECT OF TWO GROUPS OF DBMs PAIRED IN DIFFERENT CROSSES

Pupal length (mm)	Pupal weight (mg)	Sterile DBM (I) × normal DBM (U) (% egg hatch)				
		U♀ × U♂	U♀ × I♂	I♀ × I♂	I♀ × U♂	I♂ × U♀ × U♂
5.0-5.4	4.30	96.0	22.3	0	0	83.7
5.5-5.9	5.30	98.4	31.4	0.6	6.1	87.5

TABLE V. EFFECTS OF SUDAN BLUE II DYESTUFF FED TO DBM IN ARTIFICIAL DIET

Concn of Sudan Blue II dyestuff (%)	Larval development period (d)	Pupal weight (mg)	Pupae produced (%)	Number examined	Number marked	Resolving rate (%)
0 (control)	11.3	4.6	18.6	31	0	0
0.017	10.8	5.2	33.7	21	0	0
0.044	11.6	4.7	22.4	21	17	81
0.051	13.2	4.9	14.1	20	20	100
0.058	15.0	5.0	6.3	13	13	100

rearing used by Ke et al. [1] can meet the needs of the larvae throughout all their stages, but the diet moulds easily and is time and labour intensive, and the water content in the diet is also difficult to control. The effects of the three artificial diets were observed. Under the same feeding conditions, with no diet being renewed, the number of moths using the Biever and Hsiao diet [3, 4] is fewer than the number reared by the semisynthetic diet prepared by us.

2.2. Quality control of sterile DBMs

DBM pupae were graded according to the pupae length and then irradiated. The biological data, mating competitiveness and flying ability were determined after the pupae of each grade had emerged as adult moths. All of these factors are involved in Table IV.

2.3. Marking methods for the DBM

- (a) *Dye marking method.* The marking dyestuff, made from an emulsification mixture of Sudan Blue II and double Tween-40, was mixed homogeneously in the agar of the semisynthetic diet for the DBM. The larvae were marked after eating the diet. The test results are as follows:
- (i) The marking has relations with dyestuff concentration. Table V shows that the weight of pupae was increased to different degrees after the Sudan Blue dyestuff was added to the diet.
 - (ii) The resolving power of marked insects increased with the increase of dyestuff concentration. As the concentration reached 0.051 and 0.058%, the resolving power was up to 100%, while the larvae growth period was prolonged and the pupae rate was decreased.

- (b) *Radioactive isotope ^{32}P method for marking the insects.* A colourless and transparent solution of $\text{NaH}_2^{32}\text{PO}_4$ was added to the semisynthetic diet. The ^{32}P radioactivities in the diet were 7.4, 17.76 and 25.16 kBq/g. DBMs were labelled after they ate the diet. Table VI indicates that when the radioactivity was 7.4 kBq/g in the diet, the marked insects could live 12.5 d; meanwhile, the radioactivity in each adult insect was 148.8 counts/min. Therefore, a 7.4 kBq/g diet reached the targeted aim for permanent insect marking. The radioactivity of ^{32}P , 7.4 kBq/g, has no obvious influence on the longevity of the marked DBM.

2.4. Experiments on radiation sterilized DBMs in field cages

In order to compare the effects of 150 and 350 Gy radiation doses for DBMs, net cage release tests of the two dosages were done in 1990 and 1991. The tests were performed in nine net cages, each cage having an area of $2 \times 4 \text{ m}^2$. There were three treatments, 350, 150 Gy and 0 (control) and three repetitions with a release ratio of sterile moths to normal ones of 8:1 (9:1 in 1990). Population trends were studied for two generations.

The test results show that the population density of DBMs treated with 350 and 150 Gy for the first generation is 28.8 and 82.2%, respectively, compared with the control, and the level of control is 71.2 and 17.8%, respectively, which is similar to the result achieved from the test in a 300 m^2 cage. It shows that the treated sterile DBMs have a mating capacity similar to normal ones, and that the controlling effect of 350 Gy for the first generation is better than that of 150 Gy. For the second generation, the controlling effect of 150 Gy increases up to 60.8%, close to the value of

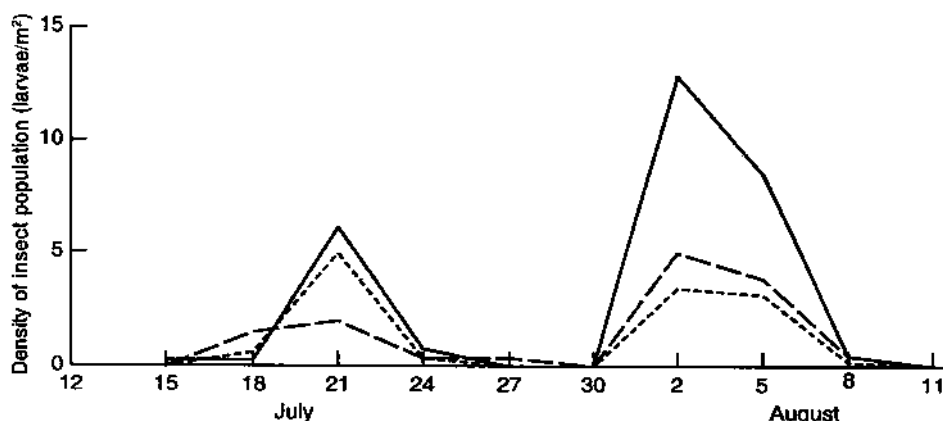


FIG. 7. Growth and decline curves of DBM larvae in different cages: — 0 Gy (CK); - - - 150 Gy; 350 Gy.

TABLE VI. THE RADIOACTIVITY OF DBM PUPAE AND ADULTS WITH ^{32}P IN DIFFERENT CONCENTRATIONS

Radioactivity of synthetic diet (kBq/g)	Pupae number of determination	Radioactivity (counts \cdot min $^{-1}$ \cdot head $^{-1}$)		Adult insects		Mean radioactivity (counts \cdot min $^{-1}$ \cdot head $^{-1}$)
		Mean	Range	No. of determination	Mean longevity (d)	
7.4	30	396.7	297.7-571.7	10	12.5	148.8
17.76	30	1089.1	668.0-1526	10	9.3	418.8
25.16	30	1314.9	797.0-1985.3	10	9.7	456.6

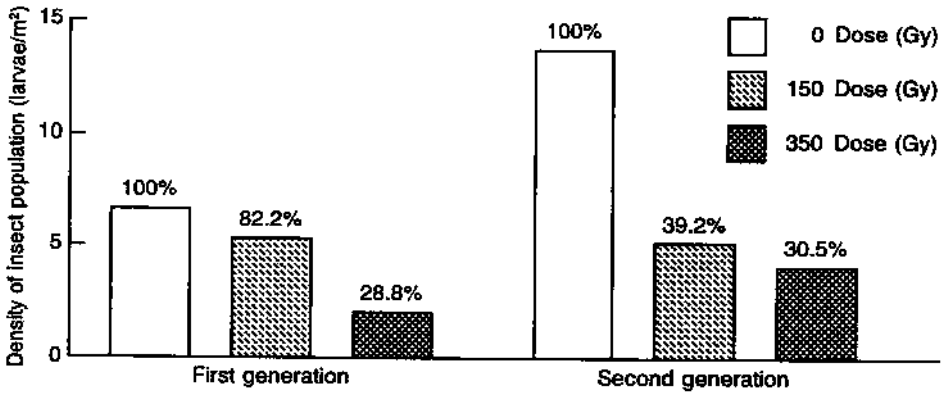


FIG. 8. Comparison of the highest densities of insect populations after different treatments.

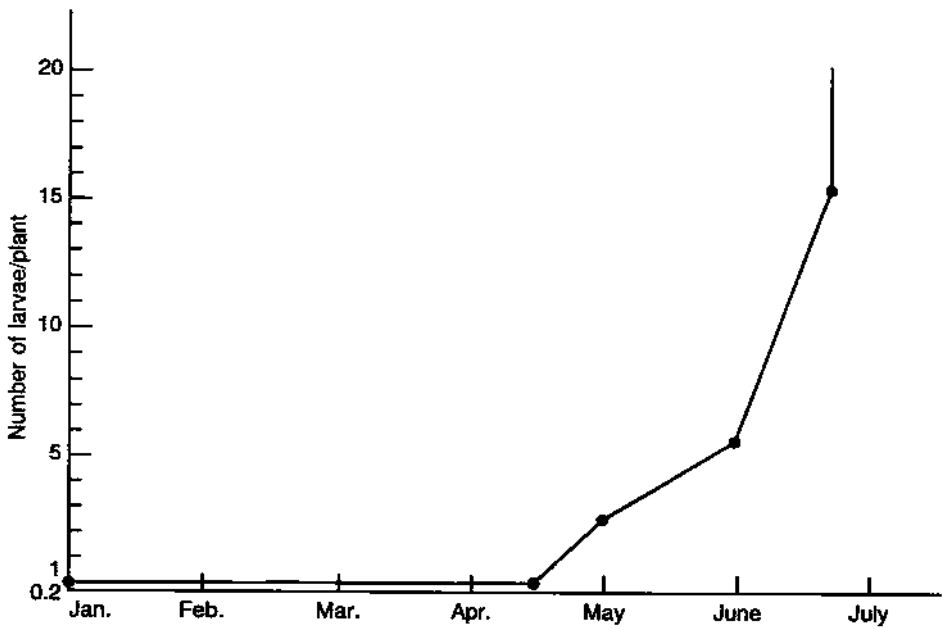


FIG. 9. The curves of the DBM larvae in the wild during 1991.

69.5% for 350 Gy, which indicates that the 150 Gy treatment has an obvious inherited sterility effect. Tests are still in progress: present indications are that they will yield results very similar to those found in previous years (Figs 7, 8).

2.5. Field studies on the DBM

The field studies on the DBM began in January 1991 using the fixed point and random sampling methods. Observations were made every three days and will be finished by the end of 1991. The results that have been achieved are as follows:

The percentage of wild silkworms that mate once in their lifetime is 89.53, the percentage that mate twice is 8.14 and the percentages that mate two or three times are both 1.16.

- (a) *DBM incidence in the field.* In the past, DBMs in the vegetable fields of Hangzhou have had 9–14 generations per year for many years, with an insect density peak usually in May. However, the density peak was delayed until June in 1991. The studies show that the incidence of the DBM from January to April is very low, but the insect density rises during the middle of April, and reaches its peak at the end of June. Cold, wet and stormy weather has influenced the DBM incidence (Fig. 9).
- (b) *Parasitic natural enemies of DBM and other vegetable pests.* Through the observations and calculation on the DBM pupae collected in the field and their emergence indoors, the DBM in the Hangzhou vegetable fields have a parasitization ratio of 15.46% in late spring and early summer. The main parasites are wasps, mainly braconids, ichneumonids and chalcids. In addition to the DBM, the other crucifer vegetable pests in Hangzhou — cabbage aphids, cabbage butterflies and the owl moth species — are most harmful to vegetable production. Aphids cause severe harm and are very numerous. May is the month of peak incidence for cabbage butterflies, while the larvae of the owl moth species appear in June and increase in numbers very rapidly.

REFERENCES

- [1] KE, Lidao, et al., Study of mass-rearing of the diamondback moth on radish germ, *Insect Know.* 18 5 (1981) 233.
- [2] LIM, Chan, Effect of utilization of rape-seedlings on rearing the diamondback moth, *Insect Know.* 3 4 (1986) 168.
- [3] BIEVER, K.D., BOLDT, P.E., Continuous laboratory rearing of the diamondback moth and related biological data, *Ann. Entomol. Soc. Am.* 64 (1971) 651.
- [4] HSIAO, J.H., HOU, R.F., Artificial rearing of the diamondback moth, *Plutella xylostella* L., on a semisynthetic diet, *Bull. Inst. Zool., Acad. Sci.* 17 (1978) 97.

RADIATION INDUCED F₁ STERILITY IN DIAMONDBACK MOTH, *Plutella xylostella* L., AND TROPICAL ARMY WORM, *Spodoptera litura* F.

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Abstract

RADIATION INDUCED F₁ STERILITY IN DIAMONDBACK MOTH, *Plutella xylostella* L., and TROPICAL ARMY WORM, *Spodoptera litura* F.

A 300 Gy dose of gamma irradiation can be used as the sterilizing dose for the diamondback moth (DBM). This caused 90% sterility of the moths. Releases of irradiated moths into the normal populations (nine irradiated to one normal) in laboratory cages, field cages and small field plots reduced the F₁ population by 61, 55 and 42%, respectively. The effects of substerilizing doses of 175 and 200 Gy to the irradiated parents caused 26 and 36% sterilities, respectively. The dose of 175 Gy caused the levels of sterility of the male and female F₁ progeny to be 54 and 74%, respectively, while the dose of 200 Gy caused 56% sterility of the F₁ male and 71% of the F₁ female. The sterility of the F₂ population was nearly the same as that of the irradiated parents. At a radiation dose of 175 Gy the sterility of F₂ males was about 11% and of females 37%. At a dose of 200 Gy the levels of sterility of the males and females were 9 and 14%, respectively. Gamma irradiation did not affect the fecundity of the irradiated parents or of the F₁ and F₂ progeny. The average number of eggs produced by one pair of DBM moths was 180. The viabilities of unirradiated pupae, irradiated parents, F₁ and F₂ at a dose of 200 Gy were 94, 89, 54 and 65%, respectively. The effect of releasing F₁ sterile moths into untreated populations at a ratio of 45:1 could be a reduction in the population by 22%. Delta traps baited with the sex pheromone S_j showed the highest trapping efficiency. The highest population of DBMs in the dry season is in May and the lowest point is in July. The longevity of the tropical army worm was slightly affected by gamma irradiation. The dose of irradiation did not affect the viability of the pupae. A substerilizing dose of 100 Gy caused 43% parental sterility, 76% F₁ sterility and 54% F₂ sterility.

1. INTRODUCTION

The diamondback moth (DBM), one of the most important pests of cruciferous crops [1, 2], attacks many varieties of cabbage. The other important insect pests of cabbage are cabbage webworm, *Crocidolomia binotalis* (Zell), and cabbage looper, *Diachrysis (Plusia) orichalcea* L. [3]. The economic loss of cabbage crops caused

by DBMs occurs especially in the dry season when control measures using insecticides are not very effective [4]. The use of insecticides as a control measure has caused the development of resistance in several species of insects. Soekarna et al. [5] proved that the Lembang strain of DBM has developed up to an 11-fold resistance to permethrin.

The tropical army worm (TAW) *Spodoptera litura* F. is a polyphagous insect known to attack at least 19 species in Indonesia [1]. It is a common pest of tobacco both in nurseries and in the field. If adequate control measures are not maintained throughout the season, serious damage can be inflicted on the important tobacco crop cultivated for cigar wrappers in Sumatra and Java. Damage can also be incurred after harvest when the tobacco leaves are dried in barns for several weeks. Some studies on the radiation effects on the sterilities of DBM and TAW have been reported in Refs [6] and [7], respectively.

In developing the sterile insect technique to control DBM and TAW, experiments have been carried out to determine the sterilizing and substerilizing doses of gamma radiation. The preliminary experiments on the use of various kinds of traps and pheromones to study the DBM populations in commercial cabbage fields were also carried out. This paper reviews the results previously obtained and describes the progress of current activities.

2. MATERIALS AND METHODS

2.1. Diamondback moths

The insects used in this study were obtained from a laboratory colony that had been maintained on cabbage plants.¹

Doses ranging from 0 to 500 Gy were used to study the effects of radiation on the sterility of male moths. A dose of 300 Gy was then selected for the study of the way in which the F_1 population reduction was affected by the release of irradiated moths. Irradiated moths were released into the normal populations at a ratio of 9 irradiated to 1 normal under laboratory, field cage and field conditions. The degree of population reduction was calculated from the total F_1 population found in release cages and plots as compared with that found in cages and plots without sterile insect release.

Substerilizing doses of 0 to 200 Gy were used to study the sterility of irradiated pupae and of their F_1 and F_2 progeny. Observations were also made on other biological parameters such as the viabilities of pupae and adults, fecundity and adult malformations.

¹ Sexed 3- to 4-day-old pupae were exposed to gamma radiation from a ^{60}Co source.

A preliminary study on population fluctuations of DBMs during the dry season was conducted by using various kinds of traps and pheromone baits. This experiment was conducted at a commercial cabbage field at Cipanas, west Java, about 200 km southwest of Jakarta. Three kinds of traps (delta type, wing type and cup type) were compared in this experiment. The pheromone baits Px and Sj produced by Takeda Chemical Industries, Ltd were tested. Both baits have the same chemical composition, the only difference is the technique of preparation. Pheromone baits were loaded with (z)-11-hexadecenyl acetate, (z)-11-hexadecenal and (z)-11-hexadecenol.

2.2. Tropical army worms

The insects used in this test were obtained from a laboratory colony. The larvae of TAWs were reared on soybean leaves to develop the laboratory colony. Adult moths were fed with a 10% honey-water solution.

In an attempt to determine the substerilizing dose of the radiation for TAWs, 6-day-old male pupae were irradiated in a Gamma Cell 220 Irradiator at doses of 0, 50, 75, 100, 125 and 150 Gy. Fifty pupae were exposed to each dose to study the effects of gamma radiation on the malformations of the emerging moths and on their longevity. The number of insects used to study the sterility of irradiated moths and of subsequent generations were five pairs of crosses between irradiated moths, or their progeny, with unirradiated moths of the opposite sex. Two hundred first instar larvae from each treatment were reared to study the effects of irradiation on the viability of larvae and pupae.

3. RESULTS AND DISCUSSION

3.1. Diamondback moths

In general, increasing levels of gamma irradiation applied to DBM pupae resulted in increased levels of sterility (Table I). The fecundity of irradiated moths was slightly affected by doses of 400 and 500 Gy. The number of eggs produced between moths treated with high doses of radiation was less than in control matings. A dose of 300 Gy caused 90% sterility while nearly complete sterility was achieved with a dose of 500 Gy.

The results of releasing sterile insects into normal populations are presented in Table II. The release of 450 irradiated moths into a laboratory cage containing 50 unirradiated moths reduced the F_1 population by 61.1%. In the field cage test the F_1 population decreased from 378 moths in the unreleased cage to 180 moths in the released cage. This indicated a reduction of 55.6%. In the field test the ratio of irradiated to unirradiated moths of 14:1 reduced the F_1 population by 42.4%.

TABLE I. STERILITY OF MALE DBM IRRADIATED AS PUPAE

Radiation dose (Gy)	Number of eggs collected ^a	Male sterility ^a (%)
0	365 ± 77	21.1 ± 8.3
100	320 ± 93	47.6 ± 1.1
200	331 ± 152	73.2 ± 4.2
300	276 ± 49	90.0 ± 1.8
400	118 ± 97	94.9 ± 3.6
500	86 ± 47	98.9 ± 1.1

^a The average ± standard deviation of three replications produced by five pairs of moths.

TABLE II. REDUCTION OF F₁ POPULATION OF DBM AS AFFECTED BY RELEASE OF RADIOSTERILIZED MOTHS (*pupae irradiated with a dose of 330 Gy*)

Type of test	Parental (P) population (moths) ^a		F ₁ population (moths)		Reduction (%)
	Unreleased	Released	Unreleased	Released	
Laboratory ^b	50U	50U + 450I	1141	430	61.1
Field, cage ^c	50U	50U + 450I	378	180	55.6
Field, plot	50U	338U + 4827I	809 ^d	466 ^d	42.4

^a U, unirradiated; I, irradiated.

^b Average of five tests.

^c Average of three tests.

^d First instar larvae.

3.1.1. The effect of substerility doses on moth malformation, fecundity and sterility of male pupae of DBMs

A smaller proportion of male pupae exposed to gamma irradiation eclosed. In the central group 94% of the pupae developed to normal moths and 2% were malformed. However, at a dose of 200 Gy the percentage of normal moths emerging

TABLE III. MALFORMATION, FECUNDITY AND STERILITY OF IRRADIATED MALE PUPAE OF DBM
(number of pupae at each radiation dose)

Radiation dose (Gy)	Emerged moths (%)		Number of eggs	Sterility (%) ^a
	Malformation	Normal		
0	2	94	1984	3.89
100	4.66	91.33	1467	11.93
125	6	90	1834	15.29
150	6.66	89.33	2161	19.19
175	3.33	90	1936	26.35
200	4.66	89.33	1918	36.02

^a Produced by 10 pairs of moths, the average of 3 replications.

TABLE IV. VIABILITY, FECUNDITY AND STERILITY OF F₁ IRRADIATED DBM MALE PUPAE

Radiation dose (Gy)	Viability ^a (%)		Fecundity ^b		Sterility ^c (%)	
	Pupae	Moths	Male	Female	Male	Female
0	74.33	71.66	1826.00	1826.00	12.21	12.21
100	58.66	57.66	1614.66	1954.00	41.52	42.86
125	46.66	41.00	1638.66	2083.66	55.01	56.18
150	54.66	52.66	1541.00	1781.33	55.19	53.58
175	46.00	38.66	1534.66	1404.00	54.55	74.09
200	54.66	49.66	1852.00	1656.00	56.81	71.74

^a The average percentage of 100 neonatal larvae, 3 replications.

^b The average number of eggs produced by 10 pairs of each sex, 3 replications.

^c The average of 10 pairs of moths of each sex, 3 replications.

TABLE V. VIABILITY, FECUNDITY AND STERILITY OF F₂ IRRADIATED DBM MALE PUPAE

Radiation dose (Gy)	Viability ^a (%)				Fecundity ^b				Sterility ^c												
	F ₁ ♂ × U♀ ^d		F ₁ ♀ × U♂		F ₁ ♂ × U♀		F ₁ ♀ × U♂		F ₁ ♂ × U♀		F ₁ ♀ × U♂										
	Pupae	Moths	Pupae	Moths	Male	Female	Male	Female	Male	Female	Male	Female									
0	74.33		69.66		1395		1395		1395		1395		4.32		4.32		4.32		4.32		
100	76.66	75.00	63.66	58.00	1186	1683	951	1072	9.47	11.92	10.79	7.51	19.56	9.88	15.55	19.36	8.90	17.06	33.65	15.55	41.85
125	61.00	51.00	56.00	50.66	1382	1113	1096	1222	7.59	15.93	13.26	8.90	17.06	33.65	15.55	41.85	8.82	13.18	9.33	14.69	
150	69.66	65.00	47.66	43.33	1108	1147	977	1328	8.82	13.18	9.33	14.69	17.06	33.65	15.55	41.85	8.82	13.18	9.33	14.69	
175	37.00	29.33	19.66	17.00	1139	1074	1015	696	8.82	13.18	9.33	14.69	17.06	33.65	15.55	41.85	8.82	13.18	9.33	14.69	
200	62.33	56.33	68.00	60.66	1465	1119	1223	1376	8.82	13.18	9.33	14.69	17.06	33.65	15.55	41.85	8.82	13.18	9.33	14.69	

^a The average percentage of 100 neonatal larvae, 3 replications.

^b Number of eggs produced by 10 pairs of moths of each sex, 2 replications.

^c Ten pairs of moths, 2 replications.

^d U, unirradiated

TABLE VI. PERCENTAGE^a OF POPULATION REDUCTION OF DBM AS AFFECTED BY RELEASE OF F₁ MOTHS OF IRRADIATED MALE PUPAE

Treatment (number of released moths), pairs	Replication				Total	Average
	1	2	3	4		
5U ^b	—	—	—	—	—	—
5U + 45(I-1) ^c	8.93	2.37	22.16	5.22	38.68	9.67
5U + 45(I-2) ^d	14.43	16.91	17.66	28.73	77.73	19.43
5U + 45(I-3) ^e	25.43	20.47	33.53	12.31	91.74	22.93

^a Calculated value, $[(u-R)/u] \times 100\%$, where u is the offspring population (moths) in the unreleased cage/control and R is the offspring population (moths) in the released cage.

^b U, unirradiated.

^c I-1, irradiated with a dose of 100 Gy.

^d I-2, irradiated with a dose of 150 Gy.

^e I-3, irradiated with a dose of 200 Gy.

was still 89.3% and the percentage of malformation was 4.6%. The fecundity was not affected by gamma irradiation. The higher the dose pupae were exposed to, the higher the sterility of the emerging moths. Comparison of the results of earlier tests (Table I) with the current results (Table III) shows differences in the percentage of sterility induced. The dose of 200 Gy produced 73 and 36% sterility in the earlier and current studies, respectively. The difference might be due to strains and radiation dose rates. However, control matings in the first test produced high degrees of sterility (21%). When these values are compared relative to control mating, the results are in closer agreement.

3.1.2. Viability, fecundity and sterility of F₁ irradiated DBM male pupae

The viability of irradiated pupae and moths was much different from that of the unirradiated ones (Table IV). The viability of unirradiated pupae was 74% as compared with 55% for ones irradiated with 200 Gy. Similar results were also found in moth emergence. Fecundity was not affected by the doses of radiation. The sterility of F₁ moths was affected by irradiation. Increasing doses of irradiation slightly increase the male sterility. The average sterility of the F₁ male and female moths was about 64% at 175 Gy and 63% at 200 Gy, respectively. These two substerilizing doses can be considered for the irradiation of DBMs.

TABLE VII. USE OF VARIOUS KINDS OF TRAPS AND PHEROMONES FOR TRAPPING DBMs IN THE CIPANAS AREA, WEST JAVA

Trap-bait	Number of DBM caught in traps								Total ^a
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	
Delta-Sj	192	241	177	119	63	36	243	188	1259
Delta-Px	163	250	197	117	35	27	195	103	1087
Delta-Vf	76	114	88	34	46	27	108	81	574
Wing-Sj	160	167	120	83	42	32	166	94	864
Wing-Px	189	174	152	91	27	20	178	57	888
Wing-Vf	157	169	108	115	68	39	115	164	935
Cup-Sj	22	79	45	27	14	10	47	58	302
Cup-Px	41	40	41	13	25	9	45	58	272
Cup-Vf	50	64	27	24	22	11	38	50	286

^a Average of three replications

Least significant difference between:	p=0.05	p=0.01
factor traps	100.51	138.49
factor baits	100.51	138.49
factor traps and baits	174.09	239.86

3.1.3. Viability, fecundity and sterility of F_2 irradiated DBM male pupae

The viability of the F_2 progeny of irradiated male pupae was slightly lower than that for control matings (Table V). The average viability of F_2 s (all doses) and unirradiated pupae was 61 and 71%, respectively. The fecundity of F_2 moths was not affected by the gamma irradiation doses applied. The effect of the gamma irradiation on the sterility of the F_2 generations was reflected in the declining level of sterility of the F_1 . The level of sterility in the F_2 generation was close to that of the irradiated pupae (P). The highest sterility obtained in the F_2 generation was 26% at 175 Gy. At the highest dose tested, 200 Gy, it was 11%.

3.1.4. Impacts of releases of F_1 sterile moths on population reduction

The releases of F_1 moths, the progeny of pupae that were irradiated with doses of 100, 150 and 200 Gy, into field cages showed various levels of population

reduction (Table VI). F_1 progeny from a dose of 200 Gy reduced the population by about 23%. The doses of 100 and 150 Gy only caused reductions of about 10 and 19%, respectively. The results of releasing F_1 progeny of either 175 or 200 Gy are similar (Table IV). Either dose could be considered for programme use.

3.1.5. Efficiency of various kinds of traps and pheromone baits and preliminary study of population fluctuations of DBMs in dry season

The combination of delta traps and S_j pheromone baits was significantly ($p < 0.01$) more efficient than other trap-bait combinations (Table VII). The number of moths caught during eight weeks for delta traps baited with S_j dispensers was 1259. The delta trap was significantly different ($p < 0.01$) from the wing trap or

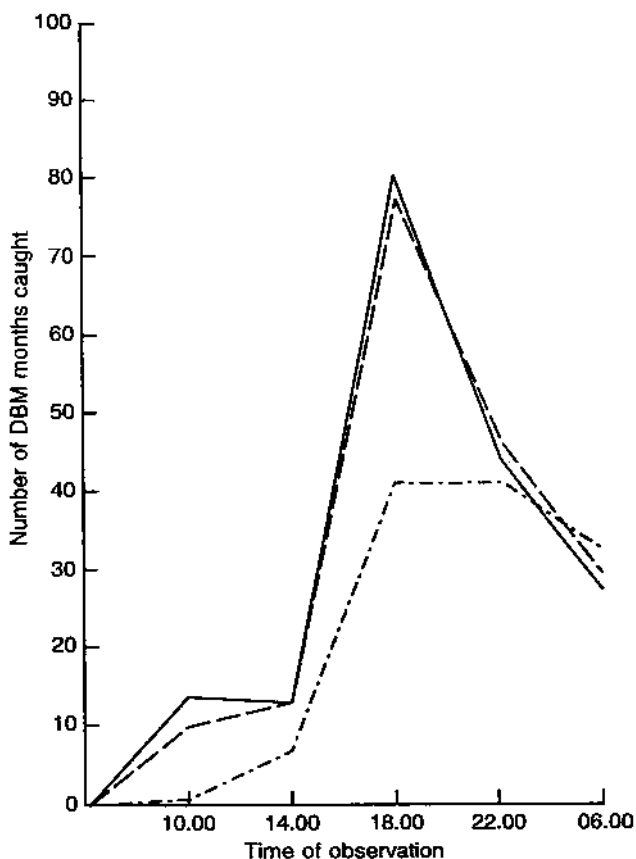


FIG. 1. Effects of baits on DBM caught at various times of the day and night: — Px, --- S_j, virgin female.

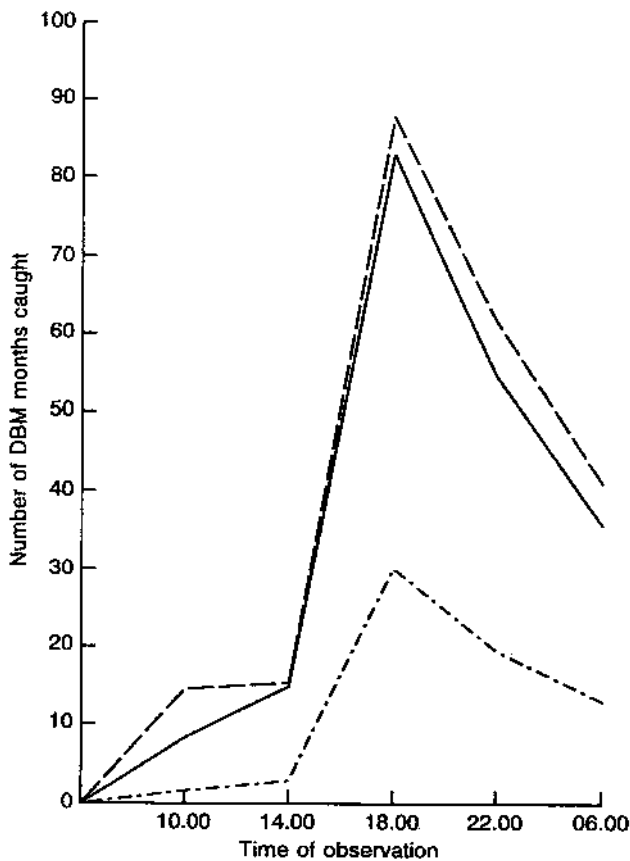


FIG. 2. Effects of traps on DBM caught at various times of the day and night: — wing, --- delta, - · - · - cup.

cup trap when the traps were baited with Sj and Px baits. There was no significant difference between the trap designs when they were baited with virgin female moths (Vf). The Sj bait only showed a significant difference compared with other baits when these were combined with the delta trap.

The same test was also conducted at various times during the day. Every 4 h the moths in the traps were observed. Similar results were obtained in this experiment. The delta trap and Sj pheromone caught significantly more insects than the other treatments. The delta trap baited with Sj and Px formulations caught 80 and 77 moths, respectively (Fig. 1). The number of moths caught in the delta trap, wing trap and cap trap were 87, 81 and 29, respectively (Fig. 2).

The preliminary experiment to study population fluctuations of DBMs was conducted in a commercial cabbage field, Cipanas, west Java. This experiment was

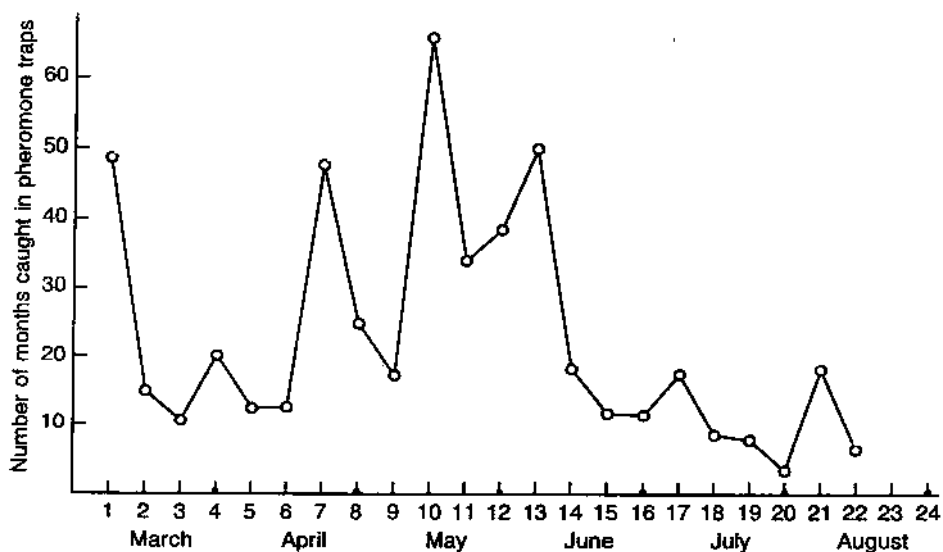


FIG. 3. Number of DBM caught every week during dry season at Cipanas, west Java, Indonesia.

conducted for six months during the dry season. The number of DBMs caught on the first week of March was 49, in the second week of March, 145, in the third week of April, 47, in the second week of May, 62, and in the first week of June, 51 (Fig. 3). These were the highest points of DBM capture during the dry season. The lowest point of moth capture was in the fourth week of July, when the number of DBM trapped was only three.

3.2. Effects of gamma irradiation on sterility of irradiated TAW moths and the subsequent F_1 and F_2 generations

The sterility of unirradiated moths and 50 and 150 Gy irradiated male moths was 27, 33 and 60%, respectively (Table VIII). A radiation dose of 150 Gy reduced the longevity of the emerged moths. The maximum age of unirradiated moths was 10 d, while that of 150 Gy irradiated moths was 8 d. The pupal viability of the irradiated group was not affected by gamma irradiation. The moth emergence of unirradiated moths was 82% as compared with 83% for the 150 Gy irradiated pupae. The viability of F_1 larvae slightly decreased with the increasing dose of radiation. The viability of F_1 larvae was 37, 22 and 20%, respectively, for the unirradiated and 50 and 150 Gy irradiated pupae. The higher the doses applied, the higher the sterility of the irradiated pupae. The sterility of unirradiated and 50 and 150 Gy irradiated

TABLE VIII. EFFECTS OF GAMMA IRRADIATION ON THE STERILITY OF EMERGED MALE (P), F₁ AND F₂ GENERATIONS OF TAW

Radiation dose ^a (Gy)	Emerged males (P)					F ₁			F ₂	
	Normal pupae (%)	Malformed pupae (%)	Sterility (%)	Longevity (d)	Larva viability (%)	Sterility (%)		Larva viability (%)	Male ^b	Female ^c
						Male	Female			
0	82.3	3.7	27.54	3-10	37.00	32.95	32.95	35.00	30.82	30.82
50	76.7	2.7	33.40	2-10	22.50	40.86	38.20	30.00	35.02	39.09
75	78.0	7.3	32.41	1-9	29.50	76.91	54.49	34.50	44.80	45.55
100	77.3	6.0	43.62	1-9	33.50	96.71	56.70	17.25	53.36	56.58
125	82.3	3.0	51.33	1-9	20.75	99.09	88.46	12.25	—	—
150	83.3	5.7	60.04	1-8	20.25	100	97.66	—	—	—

^a Exposed to six-day-old male pupae.

^b The average of male progeny of both F₁ males and females, three replications.

^c The average of female progeny of both F₁ males and females, three replications.

males was, respectively, 27, 33 and 60%. The F_1 sterilities of both males and females, however, were greater than those of the unirradiated ones. A radiation dose of 100 Gy caused F_1 male and female sterilities of about 96 and 56%, respectively. The sterility was nearly 33% for both unirradiated control male and female treatments. Gamma irradiation of male pupae also reduced the viability of their F_2 larval progeny. In the control (unirradiated) about 35% of larvae developed to moths. At a dose of 125 Gy only 12% of F_2 larvae developed to adult moths. The sterility of the F_2 generation tends to be correlated positively with that of the parental generation. Doses of 100 to 150 Gy should be considered as the best irradiation doses because there is nearly 100% sterility in the F_1 generation.

4. CONCLUSIONS

A sterilizing dose of 300 Gy in these studies does not show promise for use in developing the sterile insect technique (SIT) for DBM control. The reduction of F_1 populations by releases of irradiated moths was quite low, particularly in the test field. Lower substerilizing doses are, however, promising for developing the SIT for DBM control. Doses of 175 and 200 Gy result, respectively, in 64 and 63% DBM sterility. However, the reduction of populations as affected by the release of F_1 sterile moths was still low (about 22%). Fluctuations of DBM populations can be monitored by using delta traps baited with sex pheromones.

The 100 Gy dose can be recommended as a substerilizing dose for TAWs. This dose produces 76% sterility in the F_1 generation. The higher, 125 Gy dose, causes higher sterility in the F_1 TAW generation but reduces larvae viability and longevity.

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REFERENCES

- [1] KALSHOVEN, L.G.E., *Pests of Crops in Indonesia*, P.T. Ichtiar Baru Van Houve, Jakarta, Indonesia (1981).
- [2] SASTRODIHARDJO, S., "Diamondback moth in Indonesia", *Diamondback Moth Management (Proc. 1st Int. Workshop Tainan, 1985)*, The Asian Vegetable Research and Development Center, Shanhua, Taiwan, China (1986).

- [3] SASTROSISWOJO, S., SUKARNA, D., OMOY, T.R., "The effect of several kinds of pyrethroid synthetic insecticide on caterpillar cabbage leaf feeder and parasitoid of *Diadegma encerothaga* on cabbage fields", paper presented at 2nd Entomological Congr., Jakarta, 1983.
- [4] SUDARWOHADI, S., The relationship between planting time of cabbage and the population dynamic of *Plutella maculipennis* Curtis and *Crocidolomia binotalis* Zeller, Bull. Penel. Hort. 33 (1974) 3-14.
- [5] SOEKARNA, D., KILLIN, D., SUDARWOHADI, S., "Status of resistancy of diamondback moth caterpillar *Plutella maculipennis* Curt to pyrethroid insecticide", paper presented at Symp. on Entomology, Bandung, 1982.
- [6] HOEDAYA, M.S., SUTRISNO, S., The reduction of population of cabbage pests *Plutella maculipennis* Curtis and *Crocidolomia binotalis* Zeller as affected by the release of radiosterilized moths, J. BATAN 16 3 (1983) 18-24.
- [7] MOCHIDA, O., MIYAHARA, Y., Effect of gamma radiation on male pupae and adults of *Spodoptera litura* (F) (Lepidoptera: Noetuidae) following pupae exposure, Appl. Entomol. Zool. 9 (1974) 41-47.

RADIATION INDUCED F₁ STERILITY IN *Helicoverpa zea* (BODDIE): POTENTIAL FOR AREA-WIDE CONTROL

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Abstract

RADIATION INDUCED F₁ STERILITY IN *Helicoverpa zea* (BODDIE): POTENTIAL FOR AREA-WIDE CONTROL.

The potential of using F₁ sterility as a management strategy for the corn earworm (CEW), *Helicoverpa zea* (Boddie), has been revealed through laboratory and field investigations. A 100 Gy dose of radiation induced deleterious effects in CEWs, which were inherited through several generations. Irradiated, laboratory reared CEWs were competitive with non-irradiated, laboratory reared CEWs in attracting and securing mates under field conditions. Females that mated to non-irradiated males and males irradiated with 100 Gy had the same mating propensity and the same intermating interval. Although there was a difference in mortality between larvae from irradiated and non-irradiated parents when reared in the laboratory, this mortality differential was reduced when larvae were reared under natural conditions in the field. Other studies revealed that there was no interaction between inherited sterility and diapause in CEWs when depth of pupation, initiation and termination of diapause, egg hatch, fecundity or survival were investigated. A pilot test that investigated the efficacy of using inherited sterility for suppressing seasonal population increases of CEWs was conducted from 1988 to 1990. Results of the pilot test revealed that irradiated (100 Gy) males released in mountain valleys were competitive with wild males in infusing their genes into the wild population. Analyses of seasonal population curves of wild CEW males calculated from mark-recapture data suggested that seasonal increases in wild CEW males were delayed or reduced in valleys where irradiated males were released.

1. INTRODUCTION

The corn earworm (CEW), *Helicoverpa zea* (Boddie), is one of the most destructive pests of field crops in the United States of America. The preferred host of the CEW is maize [1-4]; however, in the USA the greatest economic damage is sustained when the CEW feeds on cotton. Annual agricultural losses and control costs can exceed US \$100 million in the southeastern USA alone [5]. Currently, control of the CEW is achieved almost entirely through the use of synthetic organic insecticides on a crop-to-crop basis. Such a management strategy is costly, non-selective, and only effective at the target site for a short period of time. Although

the most successful control measure has been the use of insecticides directed at the larval stage, the CEW has developed relatively high levels of resistance to all generic insecticides available for its control except the pyrethroids [6-8].

Insecticide resistance, the mounting concern over pesticide pollution, and the desire to manage the CEW effectively both within fields and on an area-wide basis have encouraged scientists to identify and develop new methods of control for the CEW. One such method has been the use of inherited sterility. North and Holt [9] induced inherited sterility in the CEW by irradiating males with a 200 Gy dose of radiation and mating them with normal females. They also found that all males that mated transferred a normal ratio of eupyrene to apyrene sperm. North and Holt concluded that the CEW is an ideal candidate for the use of inherited sterility for population suppression. Knipling [10] and LaChance [11] also cited the CEW as a potential candidate for the use of inherited sterility. North and Holt [9] reported that the largest single problem with the use of irradiated male moths in suppressing the natural population is their lack of competitiveness, primarily due to the inability of irradiated males to transfer sperm successfully. Carpenter et al. [12] stated that the field survival and competitiveness of larvae from the irradiated parents are important considerations when studying inherited sterility because most of the radiation induced deleterious effects are manifested in the F_1 generation. Other aspects of competitiveness, such as mating, ability to diapause and ability to resist insecticides as well as normal CEWs, are also important.

2. EFFECTS OF F_1 STERILITY

2.1. Reproduction

Carpenter et al. [13] demonstrated the effects of substerilizing doses of radiation and inherited sterility on CEW reproduction. They found that inherited deleterious effects resulting from irradiation of P_1 males existed for several generations. A reduced fecundity and egg hatch, together with an increased incidence of larval and adult mortality, were observed. A 100 Gy dose of radiation induced deleterious effects that were inherited through the F_1 generation. These radiation induced deleterious effects were similar to those reported in other species of Lepidoptera. Results and analysis of this study indicated that the use of substerilizing doses of radiation and inherited sterility has a much greater potential as a selective management strategy for CEWs than does the conventional 100% sterilizing dosage.

2.2. Survival of F_1 larvae

Carpenter et al. [14] compared CEW larvae from selected crosses of irradiated (100 Gy) and normal parents for their ability to survive in the field on whorl stage

sweet corn and in the laboratory on a meridic diet. Survival rates in the field and the laboratory were highest for larvae from normal parents (inbred) and lowest for larvae from irradiated parents (inbred). The mortality for all larvae was greater in the field than in the laboratory. The mortality differential between CEW larvae from irradiated and normal parents was greater in the laboratory than in the field.

2.3. Mating competitiveness

The mating competitiveness of irradiated CEW moths was investigated by Carpenter et al. [15]. They reported that laboratory reared moths irradiated with 100 Gy and released in field cages were competitive with non-irradiated laboratory reared moths. The competitiveness of irradiated moths was not altered by the time interval (5 and 30 h) between irradiation and release, the mating status of the male, or by the time interval (24 and 48 h) between mating and release. Normal and irradiated, laboratory reared, males released in the field in either the presence or absence of laboratory reared females were not significantly different in their nocturnal behaviour and mating competitiveness.

2.4. Influence of irradiation and mating history on mating propensity of female CEWs

Laboratory strain CEW females that were mated with untreated males, irradiated (100 Gy) males, or male progeny from irradiated (100 Gy) males and then held for 24 or 48 h were compared for their ability to lure males into the traps or to attract and mate with a male [16]. The mating propensity of females that had mated with either untreated or irradiated males and held for 24 h was less than the mating propensity of virgin females. Females of the same group that were held for 24 h after mating were as attractive to males as virgin females. The mating propensity of females mated with the male progeny of irradiated males was not significantly different from the mating propensity of virgin females. The percentage of eupyrene sperm found in the spermatheca of females that had mated with the male progeny of irradiated males was significantly lower than the percentage eupyrene sperm found in the spermatheca of females that had mated with the male progeny of irradiated males was significantly lower than the percentage eupyrene sperm found in the spermatheca of females that had mated with untreated or irradiated males. These data suggest that sperm from irradiated (100 Gy) males was competitive with normal sperm, but sperm from F_1 males was less competitive than normal sperm because F_1 male sperm would be displaced more quickly by sperm from a subsequent mating owing to the shorter intermating interval.

2.5. Diapause

The interaction of inherited sterility and diapause in the CEW was investigated by Carpenter and Gross [17]. Laboratory strain females were crossed with irradiated (100 Gy) and non-irradiated males. The progeny of laboratory crosses were studied to determine the effects of inherited sterility on the ability of CEWs to initiate, maintain and break diapause. The effects of diapause on the fecundity and fertility of emerging adults from different genetic stock were also evaluated. There was no interaction between inherited sterility and diapause when depth of pupation, initiation or termination of diapause, egg hatch, fecundity or survival were investigated. Diapause had no significant effect on the egg hatch; however, the paired moths that emerged from diapaused pupae produced significantly fewer eggs.

2.6. Chromosomal aberrations

The testes from the progeny of irradiated male CEWs were dissected and examined cytologically to identify the larval stage or age that would ensure the presence of spermatocytes (I) undergoing meiotic division and to determine the lowest dose of radiation necessary to induce visible chromosomal aberrations in 100% of the larvae [18]. The results from this study revealed that cytological examination of chromosomal aberrations could be accomplished best when the larvae were 3- or 4-day old fifth instars. The percentages of F_1 and F_2 males with visible chromosomal aberrations was dependent upon the dose of radiation administered to the P_1 male. A 60 Gy dose was sufficient to induce visible aberrations in all F_1 larvae. Chromosomal aberrations were observed less frequently in F_2 larvae than in F_1 larvae.

2.7. Sperm precedence

Sperm priority patterns in *H. zea* were studied by sequentially mating females to substerilized (100 Gy) and to normal males [19]. The progeny of substerilized males were identified by the presence of visible chromosomal aberrations in the testes. The sperm from irradiated males was competitive with the sperm from normal males when the intermating interval of the female was 48 h, but the sperm competitiveness of the irradiated male was reduced when the intermating interval of the female was 24 h. The sperm use by twice mated females did not change significantly over time after the second mating. *H. zea* demonstrated incomplete last male precedence with extensive sperm mixing. The variability in the degree of sperm precedence was high, suggesting that the outcome of sperm competition within each female depends upon the relative competitiveness of all the ejaculates involved.

3. PILOT TEST STUDIES

A pilot test entitled "Efficacy of Using Inherited Sterility for Suppressing Seasonal Population Increases of *Heliothis zea* (Boddie)" was conducted to assess the impact of inherited sterility on seasonal variations of populations of CEWs in small mountain valleys in western North Carolina, USA, and to determine the effects of inherited sterility as a function of distance from the release sites of irradiated male CEWs. Preliminary studies were conducted in 1985 and 1986 prior to the pilot test to gain information about the seasonal profiles of adult populations of CEWs and the fall army worm, *Spodoptera frugiperda* (J.E. Smith), on larval infestations of both species of early season annual hosts and corn, and on the relationship between corn phenology and levels of larval infestation by CEWs. Because *S. frugiperda* does not overwinter in North Carolina, the presence of this species in the test area indicated that meteorological and climatic conditions were favourable for transporting migrant insects from the south and that these migrating insects (especially CEWs and *S. frugiperda*) were attracted to the test sites. Primary findings revealed that peak populations of CEW moths were in synchrony with maturing sweet and field corn,

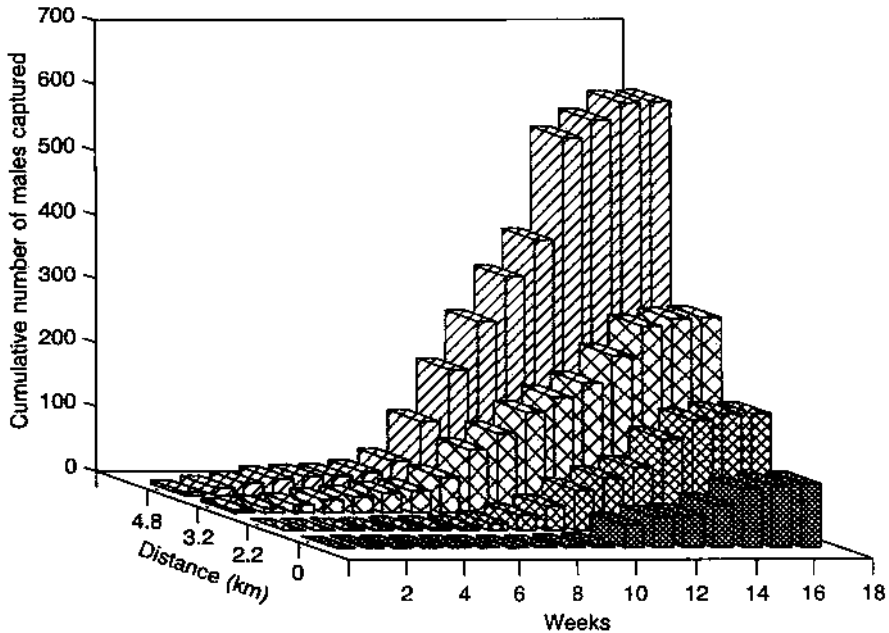


FIG. 1. Influence of the distance from the release site of irradiated (100 Gy) *H. zea* males on the number of wild *H. zea* males captured in pheromone traps.

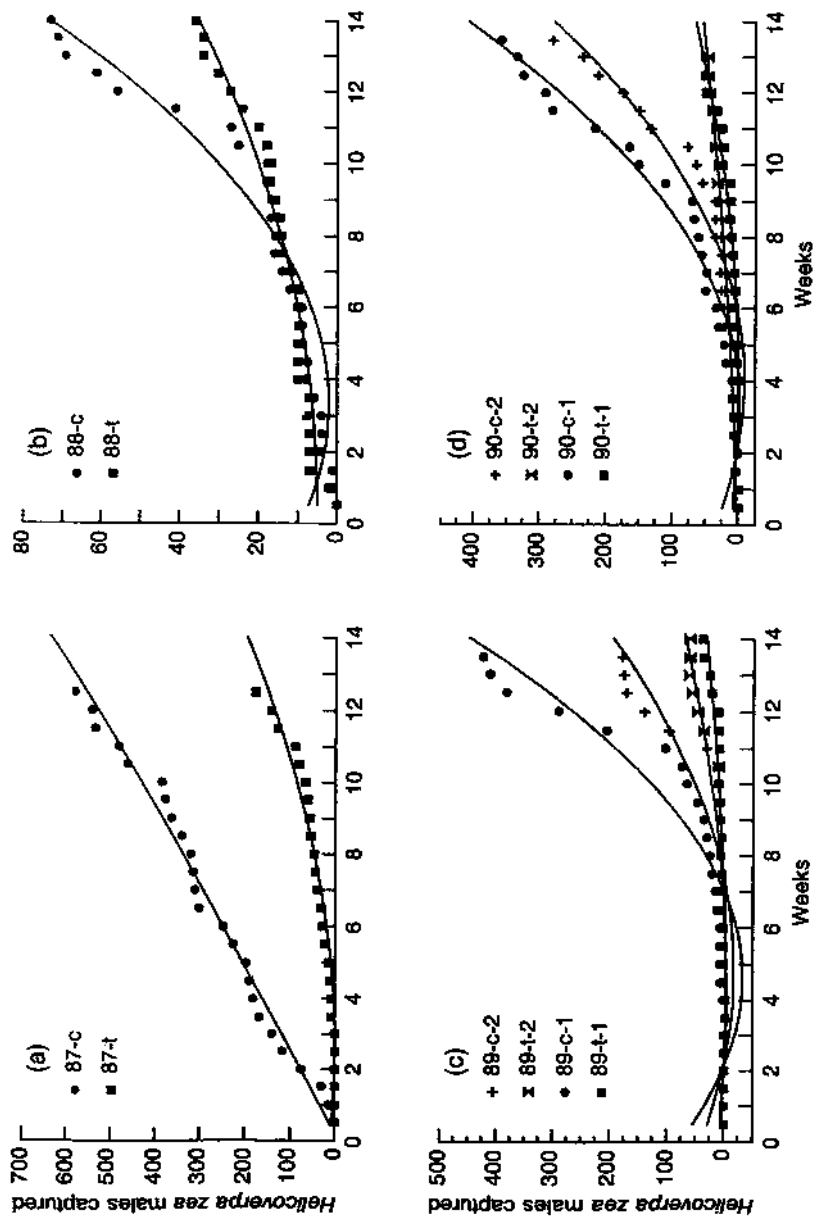


FIG. 2. Regression lines for cumulative wild *H. zea* males captured plotted against time (weeks). Regression lines fit the data significantly ($p = 0.05$).

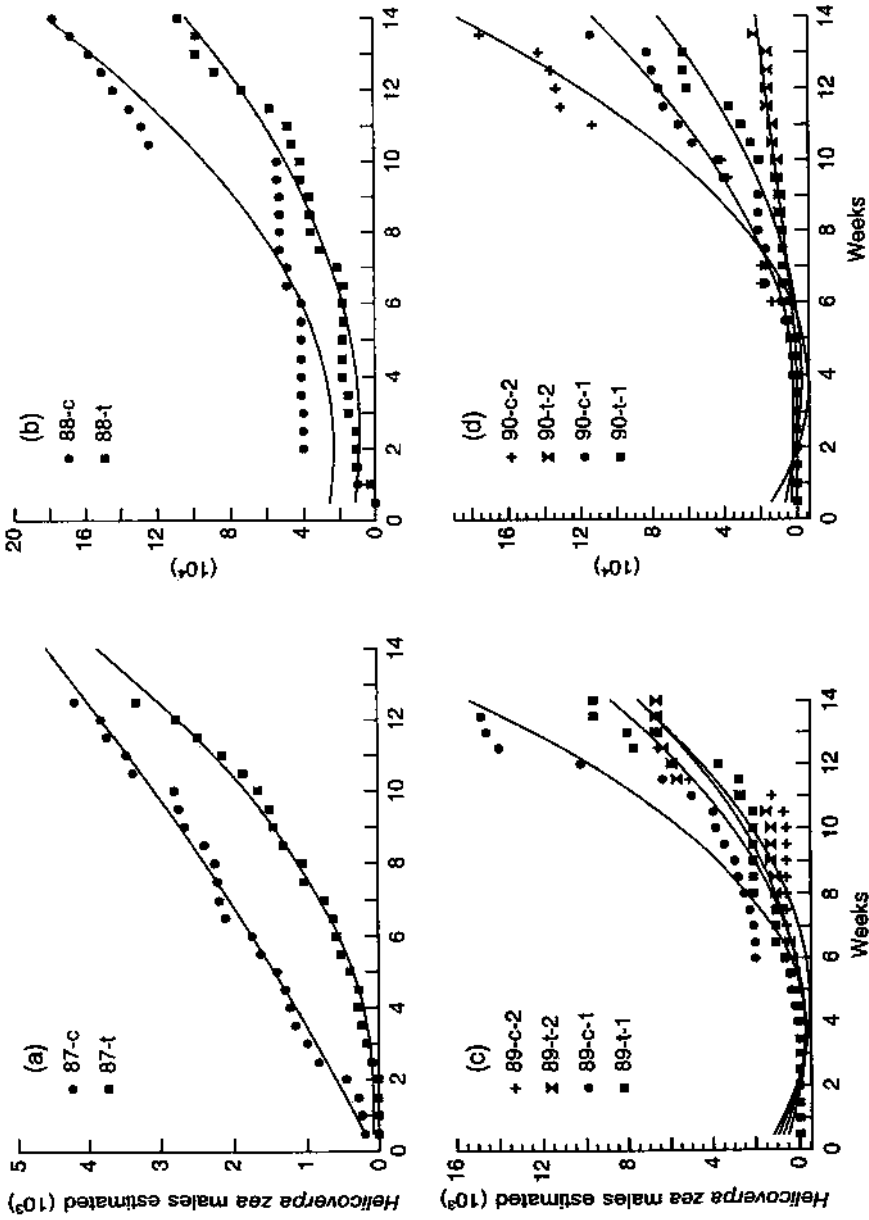


FIG. 3. Regression lines for the cumulative estimates of wild *H. zea* populations plotted against time (weeks). Regression lines fit the data significantly ($p = 0.05$).

suggesting that distinct generations of CEW occurred as if from endemic populations rather than from migrants. Collections of diapausing pupae of CEWs during October 1985 reinforced the idea that spring populations are probably supplied from the local overwintering generation. In addition, a small number of irradiated (100 Gy) males were released in one of the mountain valleys. Traps baited with pheromone lures were positioned at the release site and 2.2, 3.2 and 4.8 km from the release site. A ratio of approximately 3.5:1 substerile:wild males was maintained throughout the growing season. The relationship between the distance from the release site of 100 Gy males and the number of wild males captured (Fig. 1) suggested that as the distance from the release site is increased, the number of wild males captured also increased ($r = 0.965$).

The relationship between the number of larvae collected with chromosomal aberrations and the number of wild CEW males suggested that when the incidence of chromosomal aberrations was high, the number of wild CEW males captured was low, and when the number of wild CEW males was high, the incidence of chromosomal aberrations was low ($\chi^2 = 4.44$, d.f. (degrees of freedom) = 1, $p < 0.05$).

Although these strong correlations suggest that the released 100 Gy males produced a treatment effect, this study lacked a control and was not replicated. The pilot test studies (1987–1990), however, were designed with controls and were replicated over time. Irradiated males were marked and released in one valley (t, treated) and non-irradiated males were marked and released in another valley (c, control). In 1989 and 1990, two pairs of locations (t-1, c-1 and t-2, c-2) were used. The control and treated locations were reversed each year to eliminate any bias due to location. Both marked males and wild males were captured throughout the growing seasons. The mark-recapture data were used to estimate the wild male population at each location. The ratio of irradiated males to wild males, based upon trap capture data, varied throughout the season of each year but usually averaged about 5:1.

Regression analyses of the cumulative wild males captured at the treated and control locations revealed that the population curves were significantly different in slope and curvature (Fig. 2). Regression analyses of the cumulative estimates of wild males from treated and control locations also revealed that the population curves were significantly different (Fig. 3). Only the c-2 and t-2 locations deviated from this trend in 1989.

The treatment efficacy in the pilot test studies was also evaluated by the incidence of chromosomal aberrations in larvae collected from the test locations. Regression analyses of the seasonal incidence of chromosomal aberrations in collected larvae were compared with regression analyses of the irradiated:wild male ratio (1988–1990). These analyses revealed that the percentage of larvae with chromosomal aberrations was significantly different from the irradiated:wild male ratio.

The data obtained from the pilot test provide the information needed to assess the usefulness of inherited sterility for suppressing population increases of CEW. Analyses of seasonal population curves of wild CEW males calculated from mark-recapture data suggested that seasonal increases of wild CEW males were delayed or reduced in valleys where irradiated (100 Gy) males were released. The incidence of chromosomal aberrations in larvae collected from the test sites during the growing seasons indicated that the irradiated males were very competitive in infusing their genes into the wild population. In some cases, it appeared as if the irradiated males were more competitive than the wild males. This apparent increase in competition by the irradiated males is probably a result of wild females emerging as adults one day before wild males emerge as adults, thereby giving any irradiated males present an advantage. The high incidence of chromosomal aberrations in larvae collected in the test sites compared with the irradiated:wild male ratio could also be explained, in part, as a result of a substantial F_1 male moth population. Because F_1 male moths caught in traps were unmarked, the number of wild (normal) male moths could have been overestimated. Also, because the progeny of F_1 males might have visible chromosomal aberrations, the F_1 larval population could have been overestimated.

4. CONCLUSIONS

The desire to manage the CEW effectively on an area-wide basis continues as a primary concern for many scientists. The importance of the sterile insect technique (SIT) in the elimination of incipient invaders, as well as established populations of important pest species, is well documented. More recently, however, laboratory models developed for the CEW suggest that the release of radiation induced sub-sterile moths into endemic populations could be eight to nine times more efficient in its negative impact on populations than the SIT because it promotes the inheritance of detrimentally altered genetic material. These model projections are corroborated in part by the results from laboratory and field data and from a recently completed pilot test which revealed that the seasonal increases of wild CEW males were delayed or reduced in locations where irradiated (100 Gy) males were released, and that irradiated males were competitive in infusing their genes into the wild population.

The full potential of F_1 sterility as an area-wide control strategy for the CEW will be realized only when F_1 sterility is combined with other suppression methods. Unilateral applications of non-insecticidal methods for area-wide control have had limited success in managing the CEW. Therefore, the most desirable management approach for area-wide suppression would necessitate the integration of various control strategies. The demonstrated potential of F_1 sterility to reduce the reproductive ability of the CEW and the compatibility of F_1 sterility with other control strategies suggest that F_1 sterility should be a major component of such an integrated

approach. Future research should fully investigate the potential for integrating F_1 sterility as a primary component with biological control and host plant resistance for managing the CEW.

REFERENCES

- [1] QUAINANCE, A.L., BRUES, C.T., The cotton bollworm, USDA Bur. Entomol. Bull. **50** (1905) 112 pp.
- [2] ISLEY, D., Relation of hosts to abundance of cotton bollworm, Arkansas Agric. Exp. Stn. Bull. **320** (1935) 1-30.
- [3] LINCOLN, C., ISELY, D., Corn as a trap crop for the cotton bollworm, J. Econ. Entomol. **40** (1947) 437-438.
- [4] HARDWICK, D.F., The corn earworm complex, Mem. Entomol. Soc. Can. **40** (1965) 247 pp.
- [5] STERLING, S., Insect Detection, Evaluation, and Prediction Committee: Report of the Southeastern Branch of the Entomological Society of America, Department of Entomology, North Carolina State University, Raleigh, NC (1983) 35 pp.
- [6] HARRIS, R.A., GRAVES, J.B., NEMEC, W.J., VINSON, S.B., WOLFENBARGER, D.A., "Insecticide resistance", distribution, abundance, and control of *Heliothis* species in cotton and other host plants, Southern Coop. Ser. Bull. **169** (1972).
- [7] SPARKS, T.C., Development of insecticide resistance in *Heliothis zea* and *Heliothis virescens* in North America, Bull. Entomol. Soc. Am. **27** (1981) 186-192.
- [8] WOLFENBARGER, D.A., BODEGAS, P.R., FLORES, R.G., Development of resistance in *Heliothis* spp. in the Americas, Australia, Africa, and Asia, Bull. Entomol. Soc. Am. **27** (1981) 181-185.
- [9] NORTH, D.T., HOLT, G.G., Population control of Lepidoptera: the genetic and physiological basis, Man. Entomol. **4** (1970) 53-69.
- [10] KNIPLING, E.F., The Basic Principles of Insect Population Suppression and Management, Agricultural Handbook 512, US Department of Agriculture, Washington, DC (1979) 659 pp.
- [11] LaCHANCE, L.E., Genetic Methods for the Control of Lepidopteran Species: Status and Potential, ARS-28, US Department of Agriculture, Washington, DC (1985).
- [12] CARPENTER, J.E., YOUNG, J.R., SPARKS, A.N., Fall armyworm (Lepidoptera: Noctuidae): field survival of F_1 larvae from partially sterile parents, Fla. Entomol. **68** (1985) 290-296.
- [13] CARPENTER, J.E., YOUNG, J.R., SPARKS, A.N., CROMROY, H.L., CHOWDHURY, M.A., Corn earworm (Lepidoptera: Noctuidae): effects of substerilizing doses of radiation and inherited sterility on reproduction, J. Econ. Entomol. **80** (1987) 483-489.
- [14] CARPENTER, J.E., YOUNG, J.R., CROMROY, H.L., SPARKS, A.N., Corn earworm (Lepidoptera: Noctuidae): comparison of field survival of larvae from normal and irradiated parents, J. Econ. Entomol. **80** (1987) 883-886.

- [15] CARPENTER, J.E., SPARKS, A.N., PAIR, S.D., CROMROY, H.L., *Heliothis zea* (Lepidoptera: Noctuidae): effects of radiation and inherited sterility on mating competitiveness, J. Econ. Entomol. **82** (1989) 109-113.
- [16] CARPENTER, J.E., SPARKS, A.N., CROMROY, H.L., Corn earworm (Lepidoptera: Noctuidae): influence of irradiation and mating history on the mating propensity of females, J. Econ. Entomol. **80** (1987) 1233-1237.
- [17] CARPENTER, J.E., GROSS, H.R., Interaction of inherited sterility and diapause in the corn earworm (Lepidoptera: Noctuidae), J. Econ. Entomol. **82** (1989) 1354-1357.
- [18] CARPENTER, J.E., Effect of radiation dose on the incidence of visible chromosomal aberrations in *Helicoverpa zea* (Lepidoptera: Noctuidae) Environ. Entomol. (in press).
- [19] CARPENTER, J.E., Sperm precedence in *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), J. Econ. Entomol. **85** (1992) 779-782.

COMPUTER MODEL FOR PREDICTING THE EFFECT OF INHERITED STERILITY ON POPULATION GROWTH

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Abstract

COMPUTER MODEL FOR PREDICTING THE EFFECT OF INHERITED STERILITY ON POPULATION GROWTH.

A Fortran based computer program was developed to facilitate modelling different inherited sterility data sets under various paradigms. The model was designed to allow variable input for several different parameters, such as rate of increase per generation, release ratio and initial population levels, reproductive rates and sex ratios resulting from different matings, and the number of nights a female is active in mating and oviposition. The model and computer program should be valuable tools for recognizing areas in which information is lacking and for identifying the effect that different parameters can have on the efficacy of the inherited sterility method.

1. INTRODUCTION

The induction of inherited sterility by using substerilizing doses of radiation produces progeny with varying degrees of sterility at each generation. Therefore, it is difficult to visualize the potential effect of persistent genetic suppressive action through several successive generations unless appropriate calculations are made based on the number and reproductive success of the various types of progeny.

Knipling [1] first used population models to describe suppression of reproduction in insects by the release of sterile insects. Later, Knipling [2] demonstrated the potential advantage of inherited sterility over the sterile insect technique (SIT) through the use of population models. The basic rationale and methods that Knipling used to develop his models guided other researchers to model data obtained from

studies on inherited sterility [3-6]. LaChance [7] found that all theoretical models comparing inherited sterility with the SIT have demonstrated that inherited sterility is more effective in suppressing native populations than an equal number of fully sterile insects.

The population models developed by Knipling were generally based upon the following assumptions:

- (a) A normal population increases fivefold each generation,
- (b) This is a closed population,
- (c) Mortality is not density dependent,
- (d) All irradiated insects and their progeny are fully competitive with normal insects in mating and sperm transfer.

Studies by Carpenter et al. [8] revealed that the mating propensity of females mated with the male progeny of irradiated (100 Gy) males was not significantly different from the mating propensity of virgin females. Therefore, the Knipling model was modified to include the effect of the sperm competitiveness of F_1 males on the female mating propensity. The formulas for the Knipling model and the modified model are presented in Carpenter et al. [8].

The modified model uses calculations that require input data for rate of increase per generation, reproductive rate per mating type, sex ratio of progeny from each mating type, and release ratio of irradiated:wild. These calculations are both time consuming and tedious. Therefore, a Fortran based computer program was developed to facilitate modelling different inherited sterility data sets under various paradigms.

2. MODEL PARAMETERS

Many of the model parameters require an input of data from the user. A description of the various parameters within a data file is given as follows.

2.1. Beginning population

The user must designate the beginning population. It is recommended that the normal male (NM) and the normal female (NF) populations begin at about 1000 each to give the model more precision. The amount designated for the treated male (TM) and the treated female (TF) will dictate the release ratio.

2.2. Generation number

The generation number is listed prior to the data for that generation. Generation 01 is the generation in which irradiated insects are released.

2.3. Number of nights active

The number of active nights refers to the number of nights a female is available for oviposition and mating. This model allows up to 20 active nights.

2.4. Number of levels

The number of levels is equal to the number of different types of matings that are possible. For example, if a population is comprised of normal males, normal females and irradiated males, then only two mating types are possible (normal \times normal and normal \times irradiated).

2.5. Fold

This number refers to the rate of increase for the designated generation.

2.6. Per cent virgin

This value is the percentage of females that will remate after 24 h. Two values per generation can be listed in a data file. The first value refers to females that have mated with normal males. The second value refers to females that have mated with irradiated males or their progeny. The 'per cent virgin' values are considered only when the 'number of nights active' is higher than one.

2.7. Sex ratio for each level

The sex ratio is expressed as the percentage of progeny from each type of mating that are females.

2.8. Reproductive rate for each level

The reproductive rate is expressed as a percentage of the reproduction of normal insects. For example, if a normal female that mated with a normal male produced 1000 progeny, then the reproductive rate would be 100%. If an irradiated female that mated with an irradiated male produced 100 progeny, then the reproductive rate would be 10%.

3. MODEL PERFORMANCE

The effectiveness of inherited sterility in reducing pest population levels can be affected by many different parameters. The computer model was employed

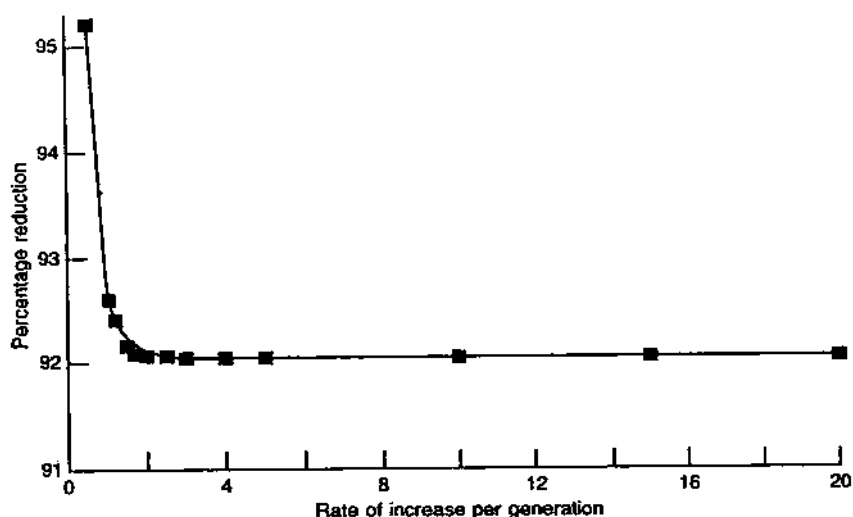


FIG. 1. Relationship between rate of increase per generation and the percentage reduction in normal population growth when *Helicoverpa zea* males are irradiated with 100 Gy and released at an irradiated to wild ratio of 9:1.

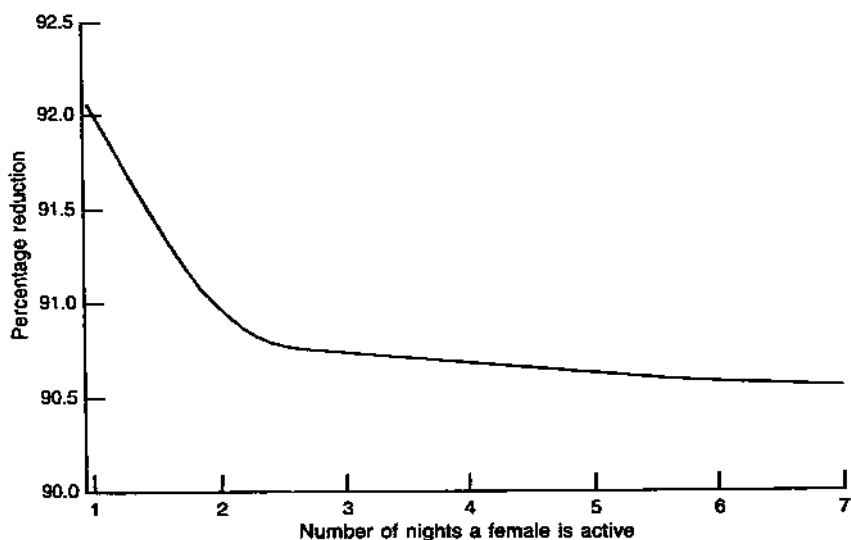


FIG. 2. Relationship between the number of nights a female is active and the percentage reduction in normal population growth when *H. zea* males are irradiated with 100 Gy and released at an irradiated to wild ratio of 9:1. The rate of increase for a normal population is fivefold per generation, and 34% of the females mated with normal males and 76% of the females mated with F_1 males from irradiated fathers will mate again after 24 h.

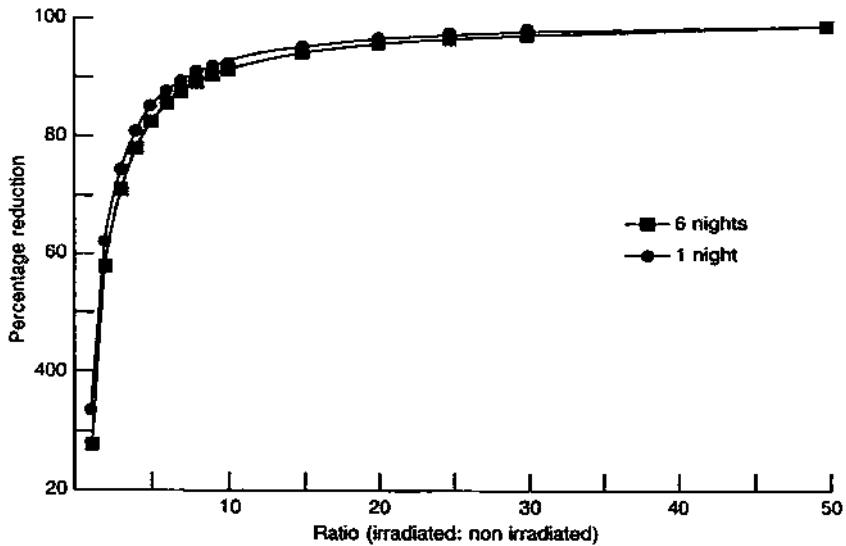


FIG. 3. Relationship between the release ratio, the number of nights a female is active (●, one night; ■, six nights) and the percentage reduction in normal population growth when *H. zea* males are irradiated with 100 Gy and the rate of increase for a normal population is fivefold per generation.

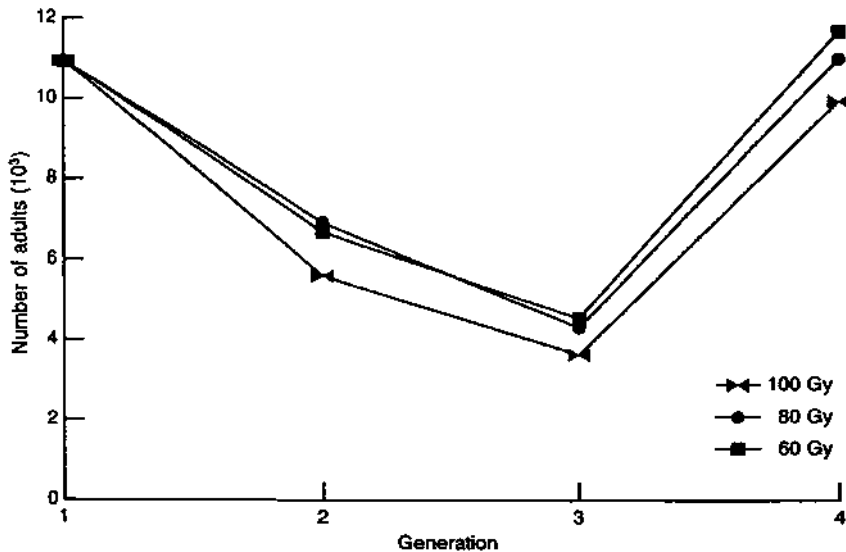


FIG. 4. Effect of radiation dose (■, 60 Gy; ●, 80 Gy; ▲, 100 Gy) on a hypothetical *S. frugiperda* population when irradiated males are released at an irradiated to wild ratio of 9:1 and the rate of increase for a normal population is ninefold per generation.

to quantify the influence of several different parameters on the predicted efficacy of the inherited sterility method. The data for the model were taken from Carpenter et al. [8] unless stated otherwise.

The relationship between the percentage reduction in normal population growth and the rate of increase per generation suggests that the efficacy of inherited sterility is almost constant when the rate of increase per generation is twofold or higher (Fig. 1). Even when there is no population growth (onefold per generation), the increase in the percentage reduction of normal population growth is less than 1%. A substantial increase in treatment efficacy (3%) is observed only when the normal population is in decline (0.5-fold per generation).

The relationship between the percentage reduction in normal population growth and the number of nights a female moth is active was calculated with the following parameters: the normal rate of increase per generation was fivefold; the release ratio of irradiated (100 Gy) males to wild males was 9:1 (Fig. 2). A slight decrease in the efficacy of inherited sterility occurs when the number of nights a female is active is increased from one night to two nights. The treatment efficacy is almost constant from two to seven nights. The number of nights a female is active exerts the greatest influence on the treatment efficacy when the release ratio is low (1:1) and the least influence on the treatment efficacy when the release ratio is high (50:1) (Fig. 3).

An increase in the release ratio has its greatest effect on the percentage reduction in normal population growth when the release ratio is less than 10:1 (Fig. 3). Increasing the release ratio above 10:1 will have little effect on the treatment efficacy.

Data from Carpenter et al. [6] were used to calculate the effect of radiation dose on population suppression (Fig. 4). All three doses were efficacious, but the 100 Gy dose provided the greatest degree of suppression. A wider range of doses must be studied, however, before the model can predict the optimum dose.

4. CONCLUSIONS

Population models are valuable tools for predicting the efficacy of the inherited sterility method. Models aid in recognizing areas in which information is lacking and assist in identifying the effect that different parameters can have on treatment efficacy. As knowledge of inherited sterility and pest biology and ecology becomes more comprehensive, population models should be modified and expanded to accommodate all available information.

The computer model presented here has been designed to allow variable inputs for several different parameters. The use of this model to analyse existing inherited sterility data on *Helicoverpa zea* (Boddie) and *Spodoptera frugiperda* (J.E. Smith) has revealed that the efficacy of inherited sterility is almost constant when the rate

of increase per generation is twofold or higher. A sharp increase in treatment efficacy resulted when the normal population was in decline. The treatment efficacy was also affected by radiation dose, release ratio and sperm competitiveness.

REFERENCES

- [1] KNIPLING, E.F., Possibilities of insect control or eradication through the use of sexually sterile males, *J. Econ. Entomol.* **48** (1955) 459-462.
- [2] KNIPLING, E.F., Suppression of pest Lepidoptera by releasing partially sterile males: a theoretical appraisal, *Bioscience* **20** (1970) 465-470.
- [3] NORTH, D.T., HOLT, G.G., Population suppression by transmission of inherited sterility to progeny of irradiated cabbage looper, *Trichoplusia ni*, *Can. Entomol.* **101** (1969) 513-520.
- [4] NIELSON, R.A., BRISTER, C.D., Induced genetic load in descendants of irradiated greater wax moths, *Ann. Entomol. Soc. Am.* **73** (1980) 460-467.
- [5] BROWER, J.H., Inheritance of partial sterility in progeny of irradiated males of *Ephesia caustella* (Lepidoptera: Pyralidae) and its effect on theoretical population suppression, *Can. Entomol.* **112** 2 (1980) 131-140.
- [6] CARPENTER, J.E., YOUNG, J.R., KNIPLING, E.F., SPARKS, A.N., Fall armyworm (Lepidoptera: Noctuidae): inheritance of gamma-induced deleterious effects and potential for pest control, *J. Econ. Entomol.* **76** (1983) 378-382.
- [7] LaCHANCE, L.E., Genetic Methods for the Control of Lepidopteran Species: Status and Potential, ARS-28, US Department of Agriculture, Washington, DC (1985).
- [8] CARPENTER, J.E., SPARKS, A.N., CROMROY, H.L., Corn earworm (Lepidoptera: Noctuidae): influence of irradiation and mating history on the mating propensity of females, *J. Econ. Entomol.* **80** (1987) 1233-1237.

REARING AND GAMMA RADIATION EFFECTS ON MATURE PUPAE OF PINK BOLLWORM AND THEIR F₁ PROGENY

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Abstract

REARING AND GAMMA RADIATION EFFECTS ON MATURE PUPAE OF PINK BOLLWORM AND THEIR F₁ PROGENY.

Pink bollworm larvae were successfully reared in captivity on a casein wheat germ diet. The substitution of casein with soyflour, corn-cob grit and wheat germ, and casein for peanut flour, resulted in delayed development, reduced pupal recovery and fecundity of the adult moths. This reduction was more drastic in corn-cob grit and peanut flour diets. The irradiation of mature pupae at 50–200 Gy resulted in decreased adult emergence with increased gamma radiation doses, and more deformed moths were recorded at a dose of 200 Gy. Adults following irradiation of mature pupae when crossed with untreated males or females or treated individuals crossed to treated exhibited reduced fecundity and fertility with the increasing doses. This reduction was more pronounced when treated males were crossed with treated females. Females were relatively more sensitive to gamma radiation, as a reduced number of eggs was obtained when treated females were crossed with untreated males. At 200 Gy, no F₁ progeny were obtained from any cross involving treated parents. The fecundity and fertility were reduced significantly when F₁ males or F₁ females from male parents irradiated as mature pupae were mated with untreated insects at both 100 and 150 Gy. However, inherited sterility was more pronounced when F₁ males were crossed with untreated females than when F₁ females were crossed with untreated males. Similarly reduced fecundity and fertility in F₁ progeny from female parents irradiated as mature pupae, both at 100 and 150 Gy, were also recorded in crosses as described for male F₁ progeny. The fecundity and fertility were the lowest in F₁ progeny of both male and female parents irradiated as mature pupae when compared with the F₁ progeny of male or female irradiated parents separately.

1. INTRODUCTION

Pink bollworm, *Pectinophora gossypiella* (Saunders), is a serious insect pest of cultivated cotton (*Gossypium* spp.) in Pakistan and other countries. Insecticide use for the control of pink bollworm currently ranges from 10 to 17 applications per season [1], which creates problems such as insect resistance to insecticides,

disturbance in biological equilibrium and environmental pollution. Biological (non-insecticidal) methods of controlling the pink bollworm have been emphasized in recent years because of insecticide costs and the pink bollworm's resistance to insecticides [2].

Amongst the biological methods, the genetic control method utilizing ionizing radiation is one of the potential components of an integrated control strategy that is receiving renewed interest. The establishment of laboratory rearing is one of the foremost prerequisites for the application of sterile insect release methods (SIRMs) to control target insects. Pink bollworm laboratory rearing on an artificial diet was originally accomplished in individual containers with a single insect per container [3-5]. The most successful diets are modifications of a medium based on wheat germ [6]. Mangum et al. [7] modified and expanded pink bollworm rearing to produce millions of insects. The successful use of radiation induced sterility to eradicate the screwworm fly from the southeastern part of the United States of America [8] led to the initiation of radiation studies on the pink bollworm. Knippling [9] reported a theoretical model which indicated that releases of partially sterile male lepidoptera would be more effective in population suppression than releases of completely sterile males. In this case, lower doses of radiation are proposed because partially sterile released males and females will have a minimum of somatic and morphological damage, enhancing their ability to compete with native populations for mating. Released males interbreeding with the native population results in reduced egg hatching and sterile F_1 progeny.

Ouye et al. [10] reported that treatment of 5- to 7-day old pink bollworm pupae with 300-600 Gy of gamma radiation induced almost complete sterility in adults but caused reduced male longevity. Graham et al. [11] reported that newly emerged pink bollworm moths exposed to 250 Gy of gamma radiation or more and crossed with untreated insects produced no fertile adult progeny. Cheng and North [12] and LaChance et al. [13] reported similar results. Therefore, it is possible that the production of large numbers of sterile F_1 male progeny by males exposed to 50-100 Gy which mated with native females could contribute to the efficiency of a sterile release programme.

The theoretical advantages of releasing partially sterile insects appear promising in this case and others; however, the contribution of F_1 sterile pink bollworm progeny in suppressing populations in the field has not been completely documented. Flint et al. [14, 15] indicated that pink bollworm moths treated with 100 Gy and released in field cages were more effective than moths exposed to higher doses of irradiation. Bartlett and Butler [16] found that the few F_1 female progeny of irradiated (150 and 200 Gy) females paired with untreated males were more than 90% sterile. The releases of sterile males only, sterile females only (100, 150 or 200 Gy) or mixed sexes (100 Gy) reduced pink bollworm populations by 82.6 to 99.9% over two generations. Henneberry and Clayton [17] found that the number of progeny produced by laboratory reared males exposed to 150 Gy or higher and paired with

untreated females was reduced by over 80% as compared with those produced by untreated pairs. The reproduction of pink bollworm male and female moths from 100 or 150 Gy irradiated pupae mated to untreated females or untreated males was reduced by 88% or more [18].

Pink bollworms irradiated as adults and released at St Croix, USA, were not competitive, and high ratios of sterile to native moths were required to achieve suppression of the population [19]. Bartlett [20] stated that the adult irradiation has two undesirable features, the first is the lack of feeding in the first hours of adult life and the second is the loss of scales, which handicaps the sustained flight and survival capabilities under environmental stresses. However, moths from 5-7 day old pupae exposed to radiation had minimum adult deformities [18]. Furthermore, irradiated pupae are easier to handle for transport for release because there are no problems about mobility, anaesthetization or cooling for treatment. The objective of the following studies was to determine the effects of gamma radiation on mating and reproduction of laboratory reared mature pink bollworm pupae and the inherited effects on mating and reproduction of their F_1 progeny.

2. MATERIALS AND METHODS

2.1. Laboratory rearing on artificial diet

Four different diets were tried for pink bollworm rearing in the laboratory and the composition of each diet is given in Table I. The culture was started with larvae collected from a cotton field that were allowed to pupate in flowers and bolls. The adults emerging from pupae were paired in oviposition cages (circular plastic containers of 0.5 L capacity having a plastic lid fitted with a screen cover). A 10% sucrose solution was provided as food. Paper towelling (two thirds the area of screen cover) was placed over the cover as an oviposition substrate. The towelling was held in place with small metal washers and a piece of cardboard. Eggs were removed daily and kept in conical flasks plugged with cotton until hatching occurred. The neonate larvae after hatching were reared on the respective diet media according to the procedure described by Martin [21]. The observations on various biological parameters were recorded from laboratory rearing at $27 \pm 2^\circ\text{C}$ and $70 \pm 5\%$ relative humidity (RH) and 14:10 (light:dark) photoperiod.

2.2. Radiation effects on mature pupae

Six 7-day old pupae from the laboratory culture were irradiated with 50, 100, 150 and 200 Gy at a dose rate of 4.35 Gy/min in a ^{60}Co gamma irradiator. A similar group of pupae was held as a control. Treated and untreated pupae were held in the same conditions previously described. Data on adult emergence and deformed moths were recorded.

TABLE I. COMPOSITION OF DIFFERENT ARTIFICIAL DIETS USED FOR PINK BOLLWORM REARING

Diet ingredients	Composition of diet ingredients			
	Casein wheat germ	Soyflour wheat germ	Wheat germ corn-cob grit	Peanut flour
Agar agar (g/L)	20.0	24.2	4.41	20.0
Casein (g/L)	40.6	—	—	—
Wheat germ (g/L)	34.8	34.8	31.7	—
Soyflour (g/L)	—	80.0	—	—
Peanut flour (g/L)	—	—	—	140.0
Corn-cob grit (g/L)	—	—	73.5	—
Sucrose (g/L)	40.6	17.6	10.59	—
Brewer's yeast (g/L)	—	—	—	20.0
Dextrose (g/L)	—	—	—	20.0
Alphacel (g/L)	5.8	3.0	—	10.0
Wesson's salt (g/L)	11.6	11.6	12.9	2.88
Methyl- <i>p</i> -hydroxybenzoate (g/L)	1.9	1.9	1.59	—
Water (mL/L)	774	774	882	750
Sodium benzoate (g/L)	—	—	—	1.9
Acetic acid (25%) (mL/L)	11.6	16.4	11.7	—
Choline chloride (10%) (mL/L)	11.6	11.6	7.5	—
Formaldehyde (10%) (mL/L)	4.8	4.8	4.4	—
KOH (22%) (mL/L)	5.8	5.8	5.3	—
Cotton seed oil (mL/L)	—	—	—	40
Vitamin mixture (mL/L) ^a	3.9	3.9	3.5	—
Sorbic acid (g/L)	1.9	—	0.95	—

^a Composition of vitamin mixture (stock solution): D-pantothenic acid, 24.0 g; nicotinic acid amide, 12.0 g; folic acid, 6.0 g; riboflavin, 6.0 g; thiamin hydrochloride, 3.0 g; pyridoxin, 3.0 g; biotin, 0.24 g; B₁₂, 0.012 g; water to make one litre.

2.2.1. Production of P_1 progeny

The fecundity, fertility and number of matings per female of P_1 moths were recorded for the following crosses for each radiation dose:

- (a) UTM \times UTF (control)
- (b) TM \times UTF
- (c) UTM \times TF
- (d) TM \times TF

where UT stands for untreated, T for treated, M for male and F for female.

Each treatment was replicated four times using 500 mL oviposition cages with five pairs of moths per cage per replicate. All females were dissected after death and examined for the presence of spermatophores to determine the mating occurrence.

2.2.2. Production of F_1 progeny

The moths used in this experiment were obtained from the P_1 crosses of the respective radiation doses, and the F_1 moths of each dose were crossed separately in the following combinations to record their mating frequency, fecundity and fertility:

- (a) UTF \times UTM (control)
- (b) UTF \times F_1 M
- (c) F_1 F \times F_1 M
- (d) F_1 F \times UTM

where F_1 F stands for females obtained from P_1 irradiated as mature pupae and F_1 M for males obtained from P_1 irradiated as mature pupae.

The procedures adopted to record mating frequency, fecundity and fertility were similar to those described earlier for P_1 progeny. The data were subjected to analysis of the significance of variations according to Duncan's multiple range test at $p = 0.05$.

3. RESULTS

3.1. Laboratory rearing

The developmental data for various life stages of pink bollworm reared on a casein wheat germ diet, a soybean wheat germ diet, a corn-cob grit wheat germ diet and a peanut flour diet are presented in Table II. The diet composition had a significant impact on the various biological aspects measured. The replacement of casein with soybean flour and corn-cob grits, and the replacement of casein and wheat germ

TABLE II. DEVELOPMENTAL DATA FOR VARIOUS LIFE STAGES OF PINK BOLLWORMS ON DIFFERENT DIETS*

Biological aspects	Casein wheat germ	Soyflour wheat germ	Corn-cob grit wheat germ	Peanut flour
Larval period (d)	19.66 ^c	23.51 ^b	33.99 ^a	34.15 ^a
Larval weight (mg)	18.55 ^a	14.25 ^b	12.75 ^c	10.00 ^c
Pupal recovery (%)	80.00 ^a	65.00 ^b	20.00 ^c	15.00 ^c
Pupal life (d)	9.64 ^b	10.36 ^b	12.37 ^a	12.50 ^a
Pupal weight (mg)	12.84 ^a	11.37 ^b	10.50 ^c	9.62 ^c
Adult life male (d)	11.75 ^a	9.85 ^b	8.97 ^{b, c}	7.55
Adult life female (d)	15.75 ^a	11.75 ^b	11.32 ^b	10.67 ^b
Number of eggs/female	154.55 ^a	94.75 ^b	36.90 ^c	16.37 ^d
Hatch percentage	77.58 ^a	65.27 ^b	21.18 ^c	6.06 ^d
Incubation period (d)	4.65 ^b	5.50 ^{a, b}	6.25 ^a	6.50 ^a
Adult emergence (%)	73.92 ^a	57.71 ^b	43.33 ^c	35.41 ^c

* Each figure is an average of twenty insects with four replications. Means within a row followed by the same letter are not significantly different; Duncan's multiple range (DMR) test, $p = 0.05$.

with peanut flour resulted in delayed pupation. The larvae reared on a casein wheat germ diet yielded significantly heavier pupae (12.84 mg) when compared with soyflour wheat germ (11.37 mg), corn-cob grit wheat germ (10.50 mg) and peanut flour diets (9.62 mg).

The pupal recovery was also significantly higher for a casein wheat germ diet (80%) than in a soyflour wheat germ (65%), corn-cob grit wheat germ (20%) and peanut flour diet (15%). The pupal development time was longer in a soyflour wheat germ diet (10.36 d), corn-cob grit diet (12.37 d) and peanut flour diet (12.50 d) as compared with a casein wheat germ diet (9.64 d).

Adults from the larvae reared on a casein wheat germ diet lived significantly longer with a significantly higher fecundity and fertility than the adults from other artificial diets. Although the incubation period was shorter for eggs from the wheat germ casein diet, it was not significantly less than for the eggs from other diets. The results indicated that the substitution of casein and wheat germ with different ingredients resulted in the reduced fecundity and fertility of the adults: however, diets in

which soyflour was substituted for casein were comparatively more economical than the casein wheat germ diet. Efforts are in progress to improve the diet further while keeping in view the economics of rearing.

3.2. Radiation effect on mature pupae

3.2.1. Moth emergence

Moth emergence following irradiation of mature pupae varied significantly with the irradiation doses and a negative correlation existed between adult emergence and radiation doses. Adult emergence was reduced significantly with increased gamma radiation doses. The number of deformed moths was comparatively higher when the pupae were irradiated at 200 Gy (Table III).

3.2.2. Fecundity and fertility of P_2 adults

The fecundity of P_1 moths, following irradiation of mature pupae, decreased with increasing radiation doses (Table IV). Reduced numbers of eggs were produced for all crosses involving treated females (UTM \times TF). This demonstrates that pink bollworm females were comparatively more sensitive to gamma radiation than males. However, the number of eggs per female was significantly greater in TM \times UTF crosses at 50, 100, 150 and 200 Gy doses. The fertility (per cent hatch) of P_1 moths was also significantly higher in TM \times UTF crosses at all doses when compared with UTM \times TF crosses. The per cent egg hatch was significantly

TABLE III. EFFECTS OF GAMMA RADIATION ON MOTH EMERGENCE OF PINK BOLLWORMS FOLLOWING IRRADIATION OF MATURE PUPAE (50 'males' and 50 'females')

Dose (Gy)	Moth emergence (%)		Moths deformed (%)	
	Male	Female	Male	Female
0	88	90	6	8
50	74	82	4	6
100	84	76	8	12
150	80	72	10	14
200	64	60	12	18

TABLE IV. MATING, FECUNDITY, FERTILITY AND LONGEVITY OF PINK BOLLWORM ADULTS FOLLOWING IRRADIATION OF MATURE PUPAE*

Dose (Gy)	Mated (%)	Spermatophore/ female**	Eggs/ female**	Hatch (%)	Longevity (d)	
					Male	Female
UM × UF						
0	92.0 ^a	1.18 ^a	104.85 ^a	79.48 ^a	13.18 ^a	12.14 ^{a, b}
TM × UF						
50	57.41 ^c	0.71 ^b	53.71 ^b	53.32 ^b	10.00 ^b	14.71 ^a
100	34.16 ^c	0.34 ^c	47.99 ^b	48.22 ^b	11.43 ^b	12.02 ^{a, b}
150	31.66 ^c	0.32 ^c	28.76 ^c	36.80 ^c	10.61 ^b	11.55 ^b
200	33.33 ^c	0.33 ^c	6.67 ^{c, f}	10.00 ^c	9.33 ^c	11.00 ^b
UM × TF						
50	66.67 ^b	0.66 ^b	22.83 ^{c, d}	29.19 ^d	14.50 ^a	14.83 ^a
100	33.33 ^c	0.33 ^c	10.44 ^e	22.61 ^d	12.44 ^{a, b}	10.78 ^b
150	41.67 ^d	0.42 ^c	14.69 ^d	12.68 ^c	8.77 ^c	9.34 ^c
200	33.33 ^c	0.33 ^c	4.67 ^f	7.14 ^f	9.00 ^c	12.33 ^{a, b}
TM × TF						
50	71.43 ^b	0.71 ^b	25.00 ^e	36.57 ^c	14.57 ^a	12.57 ^{a, b}
100	40.00 ^d	0.40 ^c	5.50 ^f	17.63 ^{d, e}	8.90 ^c	9.40 ^c
150	32.29 ^{c, f}	0.32 ^c	2.39 ^f	1.66 ^g	8.90 ^c	8.79 ^c
200	25.00 ^f	0.25 ^c	0.00 ^g	0.00 ^h	8.00 ^c	10.50 ^b

* Means followed by the same letter in the same column are not significantly different; DMR test, $p = 0.05$.

** Each figure is an average of three replicates with five pairs/replicate.

reduced when either parent was irradiated with any dose. The egg hatch was dose dependent and at 200 Gy all eggs produced by TF \times TM crosses failed to hatch. The F_1 larval survival also decreased as the irradiation dose increased in the P_1 generation. The longevity of the P_1 adult moth was variable. All the crosses involving irradiated moths mated significantly less frequently than the control moths.

3.2.3. Production of F_1 progeny

The results on mating, fecundity, fertility and longevity of the F_1 progeny of pink bollworms obtained from male parents irradiated as mature pupae (Table V) indicated significant variations among various crosses of different radiation doses. The frequency of F_1 matings as measured by the number of spermatophores per female was not affected by the gamma irradiation of mature pupae. The fecundity and fertility was reduced significantly in all the crosses with increasing radiation doses. The number of eggs per female and the percentage egg hatch was significantly lower in $F_1M \times UTF$ crosses of all the doses tested when compared with the control (check). However, the hatch percentage in $F_1M \times UTF$ crosses was significantly lower at 100 Gy (12.57%) and 150 Gy (1.50%) when compared with the crosses, $F_1F \times UTF$ at corresponding doses of 100 Gy (4.204%) and 150 Gy (9.12%) of gamma radiation. The fecundity was significantly higher in $F_1M \times UTF$ crosses than in $F_1F \times UTM$ crosses at all the radiation doses tested. Small numbers of eggs were laid in crosses $F_1F \times F_1M$ (7.41) at 100 Gy and 8.05 eggs/female at a dose of 150 Gy. The hatch of eggs from $F_1F \times F_1M$ crosses was very low (4.48%) at 100 Gy and all eggs from this cross failed to hatch with the 150 Gy treatment.

The results on mating, fecundity, fertility and longevity of F_1 progeny of pink bollworms obtained from female parents irradiated as mature pupae (Table VI) indicated that the mating frequency was reduced in $F_1F \times F_1M$ crosses at all doses tested. The numbers of spermatophores found per female involved in $F_1F \times F_1M$ matings were significantly different from all the other crosses at all the doses tested. The fecundity and fertility of all the crosses were significantly less than the control. The mean number of eggs per female and the hatch percentage were comparatively lower in $F_1M \times UTF$ crosses than in $F_1F \times UTM$ crosses. All eggs failed to hatch in crosses $F_1M \times F_1F$ at doses of both 100 and 150 Gy.

Incidences of mating frequency were not affected at 50 Gy; however, the numbers of spermatophores per female were significantly reduced at 100 Gy in $F_1F \times F_1M$ and $F_1M \times UTF$ crosses. The fecundity and fertility of the F_1 progeny of crosses made with both irradiated parents (Table VII) was significantly lower than the crosses of F_1 progeny when only one parent was irradiated. The longevity of male and female moths from irradiated pupae was in some cases less than the longevity of untreated moths.

TABLE V. MATING, FECUNDITY, FERTILITY AND LONGEVITY OF F₁ PROGENY OF PINK BOLLWORMS OBTAINED FROM MALE PARENTS IRRADIATED AS MATURE PUPAE*

Dose (Gy)	Crosses	Mated (%)	Spermatophore/ female**	Eggs/ female**	Hatch (%)	Longevity (d)	
						Male	Female
0	UTF × UTM	85.40 ^a	0.75 ^a	94.60 ^a	66.01 ^a	13.77 ^a	11.20 ^a
50	UTF × F ₁ M	70.00 ^{b,c}	0.70 ^a	92.10 ^d	47.56 ^b	13.30 ^a	10.50 ^a
	F ₁ F × F ₁ M	75.00 ^{a,b}	0.75 ^a	66.12 ^c	11.15 ^d	15.37 ^a	10.50 ^a
	F ₁ F × UTM	70.00 ^{b,c}	0.80 ^a	81.20 ^b	37.44 ^c	14.50 ^a	11.40 ^a
100	UTF × F ₁ M	60.00 ^c	0.60 ^a	62.06 ^{c,d}	12.57 ^d	13.06 ^a	10.17 ^a
	F ₁ F × F ₁ M	70.83 ^{b,c}	0.71 ^a	7.41 ^e	4.48 ^e	11.62 ^{b,c}	9.08 ^{a,b}
	F ₁ F × UTM	70.00 ^{b,c}	0.70 ^a	51.70 ^d	42.04 ^b	12.13 ^{a,b}	10.40 ^a
150	UTF × F ₁ M	60.00 ^c	0.60 ^a	57.30 ^d	1.50 ^f	11.33 ^{b,c}	9.63 ^{a,b}
	F ₁ F × F ₁ M	60.00 ^c	0.60 ^a	8.05 ^e	0.00 ^g	8.55 ^c	9.55 ^{a,b}
	F ₁ F × UTM	48.33 ^d	0.48 ^b	6.75 ^e	9.12 ^d	9.00 ^c	7.93 ^c

* Means followed by the same letter in the same column are not significantly different; DMR test, $p = 0.05$.

** Each figure is an average of three replicates with five pairs/replicate.

TABLE VI. MATING, FECUNDITY, FERTILITY AND LONGEVITY OF F₁ PROGENY OF PINK BOLLWORMS OBTAINED FROM FEMALE PARENTS IRRADIATED AS MATURE PUPAE*

Dose (Gy)	Crosses	Mated (%)	Spermatophore/female**	Eggs/female**	Hatch (%)	Longevity (d)	
						Male	Female
0	UTF × UTM	85.40 ^a	0.75 ^a	94.60 ^a	66.01 ^a	13.77 ^a	11.20 ^a
	UTF × F ₁ M	75.00 ^c	0.75 ^a	35.50 ^c	35.21 ^c	13.50 ^a	13.75 ^a
	F ₁ F × F ₁ M	10.00 ^f	0.10 ^c	69.00 ^c	48.21 ^b	14.00 ^a	14.33 ^a
	F ₁ F × UTM	80.00 ^b	0.80 ^a	54.60 ^d	52.38 ^b	14.60 ^a	14.00 ^a
100	UTF × F ₁ M	75.00 ^c	0.75 ^a	53.08 ^d	18.59 ^d	11.00 ^a	8.62 ^b
	F ₁ F × F ₁ M	10.00 ^f	0.10 ^c	6.60 ^f	0.00 ^f	9.70 ^{a,b}	7.70 ^b
	F ₁ F × UTM	45.00 ^d	0.45 ^b	72.90 ^b	47.79 ^b	10.80 ^a	7.80 ^b
150	UTF × F ₁ M	87.00 ^a	0.87 ^a	51.67 ^d	11.75 ^c	10.87 ^a	10.25 ^b
	F ₁ F × F ₁ M	15.00 ^e	0.25 ^b	4.25 ^f	0.00 ^f	7.75 ^c	9.00 ^b
	F ₁ F × UTM	12.00 ^f	0.12 ^c	2.00 ^f	0.00 ^f	6.87 ^b	5.25 ^b

* Means followed by the same letter in the same column are not significantly different; DMR test, $p = 0.05$.

** Each figure is an average of three replicates with five pairs/replicate.

TABLE VII. MATING, FECUNDITY, FERTILITY AND LONGEVITY OF F₁ PROGENY OF PINK BOLLWORMS OBTAINED FROM MALE AND FEMALE PARENTS IRRADIATED AS MATURE PUPAE*

Dose (Gy)	Crosses	Mated (%)	Spermatophore/ female**	Eggs/ female**	Hatch (%)	Longevity (d)	
						Male	Female
0	UTF × UTM	85.40 ^a	0.75 ^a	94.60 ^a	66.01 ^a	13.77 ^a	11.20 ^a
	UTF × F ₁ M	60.00 ^a	0.60 ^a	21.80 ^c	22.02 ^b	14.00 ^a	10.60 ^a
	F ₁ F × F ₁ M	75.00 ^a	0.75 ^a	21.25 ^c	16.47 ^c	10.25 ^b	10.00 ^a
	F ₁ F × UTM	80.00 ^a	0.80 ^a	25.60 ^b	17.47 ^{b,c}	14.60 ^a	10.80 ^a
100	UTF × F ₁ M	60.00 ^a	0.60 ^a	8.10 ^d	5.55 ^d	11.80 ^{a,b}	11.00 ^a
	F ₁ F × F ₁ M	25.00 ^b	0.25 ^b	1.12 ^e	0.00 ^e	4.25 ^c	5.00 ^b
	F ₁ F × UTM	20.00 ^b	0.20 ^b	1.90 ^e	2.80 ^d	10.80 ^b	6.30 ^b

* Means followed by the same letter in the same column are not significantly different; DMR test, $p = 0.05$.

** Each figure is an average of three replicates with five pairs/replicate.

4. DISCUSSION

The development of laboratory rearing is one of the major prerequisites leading to the application of sterile insect release methods for the control of target insect species. A major breakthrough was the development of a technique using physical barriers between layers of diet [22]. Pink bollworm rearing methods have been continually improved and modified according to the conditions prevailing in a specific agroecosystem. In the present studies, the pink bollworm was successfully reared on a casein wheat germ diet. The substitution of casein with soyflour, corn-cob grit and wheat germ, and casein for peanut flour, was made to reduce the cost of laboratory rearing. Out of these diets, the soyflour wheat germ diet was better but the pupal recovery, fecundity and fertility were reduced from those found in the casein wheat germ diet. However, the cost of rearing was reduced 50% using the soyflour wheat germ diet compared with the casein wheat germ diet. These results are in close agreement with Shaver and Raulston [23]. The corn-cob grit, wheat germ and peanut diets were found to be unsuitable because the pupal recovery and adult fecundity and fertility were drastically reduced.

Pupal irradiation showed a higher incidence of adult deformity at higher doses (150, 200 Gy) when compared with the control. Graham et al. [11] and Flint et al. [24] reported similar results when both parents were treated with doses ranging from 50 to 170 Gy. In the present studies no adult F_1 progeny was obtained when both parents were irradiated and crossed at 200 Gy. These results are in close conformity with those of Ouye et al. [10], who demonstrated that pink bollworm pupae exposed to 250 Gy or more of gamma radiation produced sterile adult moths. The fertility from matings of treated males (50–200 Gy) with untreated females was considerably lower than that from the reciprocal matings. The percentage reduction in the F_1 progeny also followed a similar trend when the P_1 parents were treated with 100–150 Gy. Henneberry and Clayton [18] reported that the fecundity and fertility of untreated females paired with F_1 male moths from 100 or 150 Gy irradiated male parents were reduced as compared with untreated pairs; however, the F_1 female progeny were less affected than the F_1 male progeny.

The radiation induced sterility of moths that emerged from irradiated pupae was comparable with the F_1 sterility [11, 12, 13, 17]. The males from irradiated pupae elicited lower oviposition responses when paired with untreated females. This difference could be attributable to the lack of eupyrene sperm transferred by irradiated males during mating. However, LaChance et al. [25] reported such results in the case of adult radiation. In other lepidoptera, females lacking eupyrene sperm after mating tend to be more receptive to a second mating [26–28]. Therefore, additional studies need to be conducted to determine the relative competitiveness of moths from irradiated pupae and adults in relation to laboratory and native strains. Encouraged by the sterility induced at 100 Gy, field cage studies are in progress to evaluate the feasibility of F_1 sterility for the suppression and control of the pink bollworm.

REFERENCES

- [1] NATWICK, E.T. (Ed.), Cotton Insect and Production, Colorado River Cotton Growers Association, El Centro, CA (1987) 3-4.
- [2] BARIOLA, L., "Evidence of resistance to synthetic pyrethroids in field populations of pink bollworms in southern California", Beltwide Cotton Production (Proc. Res. Conf., 1985), National Cotton Council, Memphis, TN (1985) 138.
- [3] BECKMAN, H.F., BRUCKART, S.M., REISER, R., Laboratory culture of the pink bollworm on chemically defined media, *J. Econ. Entomol.* **46** (1953) 627.
- [4] VANDERZANT, E.S., REISER, R., Aseptic rearing of the pink bollworm on synthetic media, *J. Econ. Entomol.* **49** (1956) 7.
- [5] VANDERZANT, E.S., Growth and reproduction of the pink bollworm on an amino acid medium, *J. Econ. Entomol.* **50** (1957) 219.
- [6] ADKISSON, P.L., VANDERZANT, E.S., BULL, D.L., ALLISON, W.E., A wheat germ medium for rearing the pink bollworm, *J. Econ. Entomol.* **53** (1960) 759.
- [7] MANGUM, C.L., RIDGWAY, W.O., BRAZZEL, J.R., Large-Scale Laboratory Production of the Pink Bollworm for Sterilization Programmes, ARS 18-35, US Department of Agriculture, Washington, DC (1969) 7 pp.
- [8] BUSHLAND, R.C., "Sterility principle for insect control: Historical development and recent innovations", *Sterility Principle for Insect Control or Eradication* (Proc. Symp. Athens, 1970), IAEA, Vienna (1971) 3-14.
- [9] KNIPLING, E.F., Suppression of pest lepidoptera by releasing partially sterile males. A theoretical appraisal, *Bioscience* **20** (1970) 465.
- [10] OUYE, M.T., GARCIA, R.S., MARTIN, D.F., Determination of the optimum sterilizing dosage for pink bollworms treated as pupae with gamma radiation, *J. Econ. Entomol.* **57** (1964) 387.
- [11] GRAHAM, H.M., OUYE, M.T., GARCIA, R.D., DELAROSA, H.H., Dosages of gamma irradiation for full and inherited sterility in adult pink bollworms, *J. Econ. Entomol.* **65** (1972) 645.
- [12] CHENG, W., NORTH, D.R., Inherited sterility in the F₁ progeny of irradiated pink bollworms, *J. Econ. Entomol.* **65** (1972) 1272.
- [13] LaCHANCE, L.E., BELL, R.A., RICHARD, R.D., Effect of low doses of gamma irradiation on reproduction of male pink bollworms and their F₁ progeny, *J. Environ. Entomol.* **2** 4 (1973) 653.
- [14] FLINT, H.M., PALMER, D.L., BARIOLA, L.A., HORN, B., Suppression of populations of native pink bollworm in field cages by the release of irradiated moths, *J. Econ. Entomol.* **67** (1974) 55.
- [15] FLINT, H.M., WRIGHT, G., SALLAM, H., HORN, B., A comparison of irradiated or chemosterilized pink bollworm moths for suppressing native populations in field cages, *Can. Entomol.* **107** (1975) 1069.
- [16] BARTLETT, A.C., BUTLER, G.D., Jr., A model of pink bollworm populations and its use for evaluating the effects of radiation, *Southwest. Entomol.* **4** (1979) 216.
- [17] HENNEBERRY, T.J., CLAYTON, T.E., Effects on reproduction of gamma irradiated, laboratory reared pink bollworms and their F₁ progeny after matings with untreated laboratory reared or native insects, *J. Econ. Entomol.* **74** (1981) 19.

- [18] HENNEBERRY, T.J., CLAYTON, T.E., Effects of gamma radiation on pink bollworm (Lepidoptera: Gelechiidae) pupae: Adult emergence, reproduction, mating and longevity of emerged adults and their F_1 progeny, *J. Econ. Entomol.* **81** (1988) 322.
- [19] HENNEBERRY, T.J., KEAVENY, D.F., Suppression of Pink Bollworm by Sterile Moth Releases, ARS-32, US Department of Agriculture, Washington, DC (1985).
- [20] BARTLETT, A.C., Radiation Induced Sterility in the Pink Bollworm, ARM-W-1, US Department of Agriculture, Washington, DC (1978) 25 pp.
- [21] MARTIN, D.F. (Ed.), Pink Bollworms: Insect Colonization and Mass Production, Academic Press, New York (1966) 355.
- [22] RICHMOND, C.A., IGNOFFO, C., Mass rearing pink bollworms, *J. Econ. Entomol.* **57** (1964) 503.
- [23] SHAVER, T.N., RAULSTON, J.R., A soybean wheat germ diet for rearing the tobacco budworm, *Ann. Entomol. Soc. Am.* **64** (1971) 1077.
- [24] FLINT, H.M., STATEN, R.T., WRIGHT, B., Irradiation of pink bollworms with sterilizing doses: Production of F_1 progeny, *Southwest. Entomol.* **2** (1977) 16.
- [25] LACHANCE, L.E., RICHARD, R.D., PROSHOLD, F.I., Radiation response in the pink bollworm: A comparative study of sperm bundle production, sperm transfer and oviposition response elicited by native and laboratory reared males, *Environ. Entomol.* **4** (1975) 321.
- [26] NORTH, D.T., HOLT, G.G., "Radiation studies of sperm transfer in relation to competitiveness and oviposition in the cabbage looper and corn earworm", Application of Induced Sterility for Control of Lepidopterous Populations (Proc. Panel, Vienna, 1970), IAEA Vienna (1971) 87.
- [27] SNOW, J.W., JONES, R.L., NORTH, D.T., HOLT, G.G., Effects of irradiation on ability of adult male corn earworms to transfer sperm, and field attractiveness of females mated to irradiated males, *J. Econ. Entomol.* **65** (1972) 906.
- [28] RAULSTON, J.R., SNOW, J.W., GRAHAM, H.M., LINGREN, P.D., Tobacco budworm: Effect of prior mating behaviour and sperm content on the mating behaviour of females, *Ann. Entomol. Soc. Am.* **68** (1975) 701.

EFFECT OF GAMMA RADIATION AT PUPAL STAGE ON FALL ARMY WORM PARENT AND F₁ GENERATION REPRODUCTION

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Abstract

EFFECT OF GAMMA RADIATION AT PUPAL STAGE ON FALL ARMY WORM PARENT AND F₁ GENERATION REPRODUCTION.

To induce sterility in the F₁ generation, pupae of the fall army worm, *Spodoptera frugiperda* (J.E. Smith), were irradiated at the age of five days. The radiation source was a ⁶⁰Co panoramic irradiator. The pupae were irradiated at the dose rate of 2.60 kGy/h with doses of 0 (control), 50, 75, 100 and 125 Gy. The percentage hatch of eggs laid by adults that originated from pupae irradiated with 125 Gy was 15.0 and 10.0% for males and females, respectively. By crossing this irradiated parent generation, it was found that the egg hatch in the F₁ generation was 4% for descendants of treated males and 10% for descendants of treated females.

1. INTRODUCTION

The fall army worm, *Spodoptera frugiperda* (J.E. Smith), is indigenous to tropical and subtropical regions of the American continents. Its economic importance derives from its preference for many graminaceous plants, including maize, sorghum, wheat, rice and pasture grasses. In Brazil, Cruz and Turpin [1] calculated that the losses caused by the fall army worm to maize at the six week stage reached a value of 18.7%.

Genetic methods of insect population suppression, after more than forty years of study, appear to be gaining acceptance. In addition, the method of using ionizing radiation to induce sterility in the F₁ generation by irradiating the parents is now a possible alternative. The greatest advantages of using substerilizing doses of radiation are increased competitiveness of the released insects and possible integration with other non-polluting methods to control insect pests in agriculture.

The main objective of the present research was the establishment of procedures to induce sterility into the F₁ generation of the fall army worm *S. frugiperda* (J.E. Smith) through gamma irradiation of the parent generation with substerilizing doses.

2. LITERATURE REVIEW

In 1964, Ouye et al. [2] published a paper relating the results of irradiation of pupae of the pink bollworm at the ages of 1, 2, 3, 5 and 7 d with a dose of 100 Gy. The authors reported that the susceptibility to radiation decreased with the increase of age, and irradiation of pupae at ages of up to 3 d resulted in sterile adults. In addition, the paper indicates that pupae of a similar condition irradiated at ages of 5 and 7 d reached sterility only with doses of 220 and 300 Gy, respectively.

Also, Flint and Kressin [3] presented the results of irradiating pupae of the tobacco budworm at the age of 36 h before adult emergence. Furthermore, they irradiated adults at the age of 36 h. With a dose of 350 Gy of gamma radiation, both sexes reached 99% sterility, and only 450 Gy induced total sterility.

LaChance et al. [4] irradiated adults of the pink bollworm at ages of up to 24 h with doses of 25, 50, 75, 100 and 125 Gy and crossed irradiated males with virgin females. They observed that at doses of more than 75 Gy a smaller number of eggs was induced. In addition, the radiation induced low reproduction in the F_1 generation with doses higher than 75 Gy.

Carpenter et al. [5] irradiated adults of the fall army worm at ages of 1 to 12 h after emergence with doses of 60, 80 and 100 Gy. After the irradiation they proceeded to cross treated males with normal females, which showed that males irradiated with 100 Gy had a suppressive effect on the number of progeny up to the third generation.

LaChance [6] stated that, in considering the use of inherited sterility, one had to consider all the biological characteristics of the species. Further, inherited sterility has many advantages over the sterile male technique, including higher competitiveness and lower radiation doses to induce sterility.

Carpenter et al. [7] irradiated females of the fall army worm with a dose of 100 Gy and crossed these insects with normal males. They showed that only 58% of the eggs laid by the treated females hatched.

In 1987 Carpenter et al. [8] studied the effects of induced inherited sterility and substerilizing doses of gamma radiation on adults of the corn earworm. They concluded that males irradiated with a dose of 100 Gy were as competitive as normal insects.

Henneberry and Clayton [9] irradiated pupae of the pink bollworm at ages of 2 to 6 d, with doses of 50, 100 and 150 Gy. The authors found that females were more susceptible to the effects of radiation than males, and that radiation induced sterility in the F_1 generations.

Arthur et al. [10] irradiated pupae of the sugarcane borer at the age of 6 d with gamma radiation at doses from 50 to 500 Gy to induce sterility in the F_1 generation. They concluded that a dose of 400 Gy induced total sterility into the resulting adults, and a dose of 100 Gy reduced the viability of the eggs laid by the females of the F_1 generation by up to 54.9%.

Sallam [11] irradiated pupae of the cotton leafworm, *S. litoralis* (Boisd.), close to the emergence of the adults with doses of 75, 100, 125 and 150 Gy of gamma radiation. The treatment induced the production of non-viable eggs by females of the F₁ generation as radiation doses increased.

Hoedaya [12] irradiated 6 d old pupae of the tropical army worm, *S. litura* (Fabricius), with doses of 50, 75, 100, 125 and 150 Gy. The experiment showed that a dose of 50 Gy induced sterility in 40.86% of males and 38.2% of females in the F₁ generation.

3. METHODOLOGY

The insects utilized in this experiment came from the rearing facility of the Entomology Laboratory of the Nuclear Energy Centre for Agriculture, University of São Paulo, Piracicaba.

After sexing and individual introduction into glass vials 8 cm in height and 2.5 cm in diameter, 24-hour-old pupae were left under controlled environmental conditions of $25 \pm 1^\circ\text{C}$ and $70 \pm 5\%$ RH until they were 5 d old. At this age, they were irradiated in a ⁶⁰Co source with an activity of 1.62×10^{14} Bq at a dose rate of 2600 Gy/h. The radiation doses were 0 (control), 50, 75, 100 and 125 Gy. After irradiation, the emerged adults were crossed with normal adults of the same age. Every treatment (dose) had five pairs of insects. The five pairs of insects were maintained in cages made of PVC tubes 20 cm in height and 10 cm in diameter. The inner sides of the cages were covered with a normal sheet of white paper, and the cages set in Petri dishes lined with paper at the bottom. The cage tops were covered with a small mesh screen, held in place with a rubber cord. A solution of 10% bee honey served as food. The laid eggs were collected every two days. After counting, 100 eggs were separated to determine viability. After eclosion, 50 larvae from each treatment were individually held in glass vials and fed with maize leaves until pupation. The pupae were weighed and sexed. After emergence of the F₁ adults, they were crossed with normal untreated adults of the same age.

All experiments were conducted in a climatic chamber at a temperature of 24 to 26°C, RH of 65 to 75%, and a photoperiod of 10 h light and 14 h dark.

4. RESULTS

The effect on egg viability when untreated females were crossed with treated males is shown in Table I. A dose of 125 Gy induced the highest level of genetic damage. The viability of the eggs decreased to 15% when female moths from irradiated pupae were crossed with normal male moths. Similar results occurred when irradiated males were crossed with normal females, resulting in an egg

TABLE I. TOTAL NUMBER OF EGGS LAID AND VIABILITY AFTER DIFFERENT MOTH PAIRINGS OF *S. frugiperda*: PARENT GENERATION (five crossed pairs at each dose)

Dose (Gy)	Crosses ^a	Mean number of eggs laid	Percentage of viable eggs	Percentage of non-viable eggs
0	$F_N \times M_N$	6142	91.0	9.0
50	$F_I \times M_N$	4743	81.0	19.0
50	$M_I \times F_N$	6050	77.3	22.7
75	$F_I \times M_N$	4431	75.0	25.0
75	$M_I \times F_N$	4160	64.0	36.0
100	$F_I \times M_N$	3642	53.4	46.6
100	$M_I \times F_N$	3156	47.0	53.0
125	$F_I \times M_N$	2967	15.0	85.0
125	$M_I \times F_N$	2554	10.0	90.0

^a F_I , irradiated females; F_N , non-irradiated females; M_I , irradiated males; M_N , non-irradiated males.

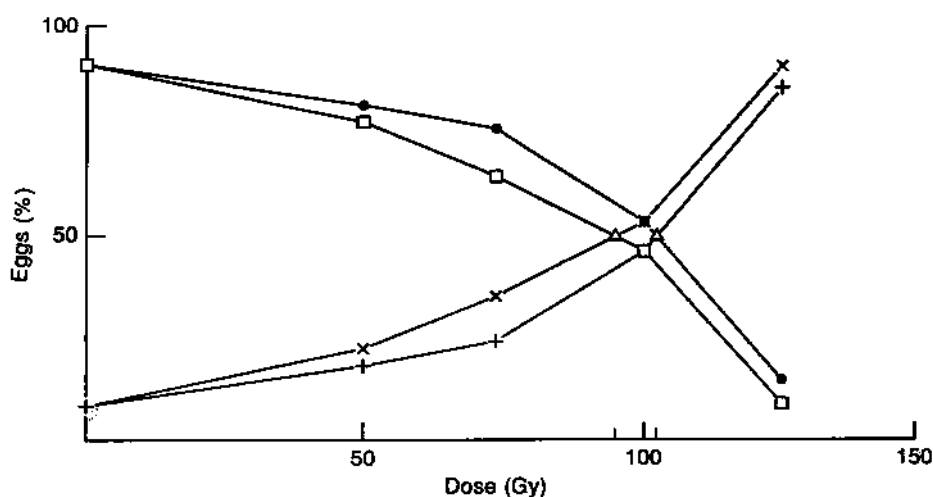


FIG. 1. Percentage of viable and non-viable eggs laid by parent generation irradiated at the pupal stage (irradiated males crossed with non-irradiated females and non-irradiated males crossed with irradiated females): ●, F_I viable; +, F_I non-viable; ×, M_I viable; □, M_I non-viable.

TABLE II. TOTAL NUMBER OF EGGS LAID AND VIABILITY AFTER DIFFERENT MOTH PAIRINGS OF *S. frugiperda*: F₁ GENERATION (five crossed pairs at each dose)

Dose (Gy)	Crosses ^a	Mean number of eggs laid	Percentage of viable eggs	Percentage of non-viable eggs
0	F _N × M _N	6142	91.0	9.0
50	F _I × M _N	2017	64.0	36.0
50	M _I × F _N	1647	58.0	42.0
75	F _I × M _N	1240	46.0	54.0
75	M _I × F _N	1961	32.0	68.0
100	F _I × M _N	753	17.0	83.0
100	M _I × F _N	637	8.0	92.0
125	F _I × M _N	604	10.0	90.0
125	M _I × F _N	517	4.0	96.0

^a F_I, irradiated females; F_N, non-irradiated females; M_I, irradiated males; M_N, non-irradiated males.

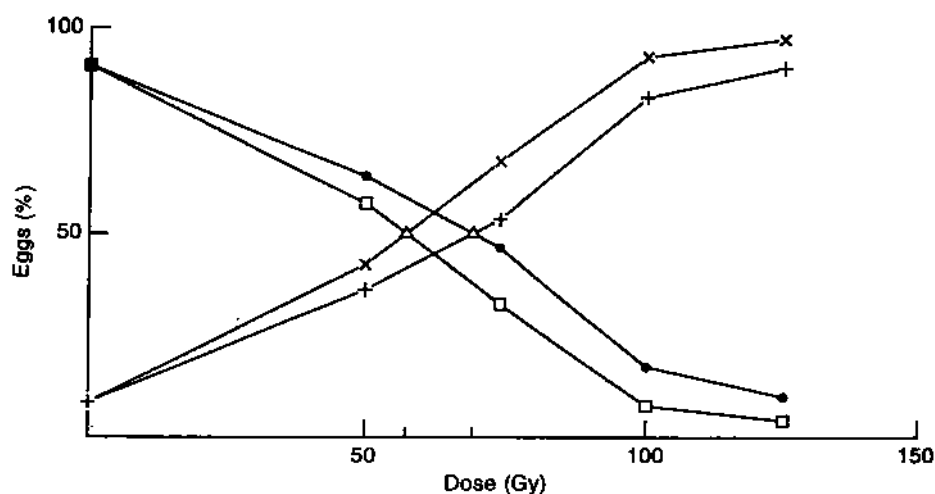


FIG. 2. Percentage of viable and non-viable eggs laid by F₁ generation (males from irradiated pupae crossed with females from untreated pupae and males from untreated pupae crossed with females from irradiated pupae): ●, F_I viable; +, F_I non-viable; □, M_I viable; × M_I non-viable.

viability of 10%. At a dose of 100 Gy, viability was approximately 50% when either sex was treated.

The results compiled are plotted in Fig. 1, which shows the LD₅₀ dose to be 90 Gy when male parent pupae were irradiated. When females were irradiated, the LD₅₀ dose was calculated to be 105 Gy.

In Table II, we show the egg viability of F₁ females. The crossing of irradiated male parents resulted in an LD₅₀ dose in the F₁ generation of 60 Gy. For egg viability when female parents were irradiated, the LD₅₀ dose for the eggs laid by the F₁ generation was 70 Gy (Fig. 2).

In all cases, parent generation or F₁ generation, an increase in the radiation dose resulted in an increase in the number of non-viable eggs. Furthermore, the results showed that males were more susceptible than females to gamma radiation.

5. CONCLUSIONS

The results obtained from this research work showed that the fall army worm moths from pupae exposed to gamma radiation were sterilized. The level of sterility increased with the radiation dose.

Substerilizing radiation doses in the parent generation resulted in a significant reduction in egg hatching in the F₁ generation. These results were very close to those obtained by LaChance [6] for the pink bollworm. Also, we can observe that irradiation of males at the pupal stage resulted in a lower percentage of eggs hatching than irradiation of females in the parent generation (number of F₁ insects produced), or in the number of eggs laid by moths of the F₁ generation (F₂ eggs).

REFERENCES

- [1] CRUZ, I., TURPIN, F.T., Efeito da *Spodoptera frugiperda* em diferentes estádios de crescimento da cultura de milho. Rev. Pes. Agro., Brasil. 17 (1982) 355-359.
- [2] OUYE, M.T., GARCIA, R.S., MARTIN, D.F., Determination of the optimum sterilizing dosage for pink bollworms, treated as pupae with gamma radiation, J. Econ. Entomol. 57 (1964) 387-390.
- [3] FLINT, M., KRESSIN E.L., Gamma irradiation of the tobacco budworm: sterilization, competitiveness, and observations on reproductive biology, J. Econ. Entomol. 61 (1968) 477-483.
- [4] LaCHANCE, L.E., BELL, R.A., RICHARD, R.D., Effect of low doses of gamma irradiation on reproduction of male pink bollworms and their F₁ progeny, J. Environ. Entomol. 2 (1973) 653-658.
- [5] CARPENTER, J.E., YOUNG, J.R., SPARKS, A.N., Fall armyworm (*Lep. noctuidae*): comparison of inherited deleterious effects in progeny from irradiated males and females, J. Econ. Entomol. 79 (1986) 46-49.

- [6] LaCHANCE, L.E., Genetic methods for the control of Lepidopteran species: status and potential, ARS-28, US Department of Agriculture, Washington, DC (1985) 29.
- [7] CARPENTER, J.E., YOUNG, J.R., KNIPLING E.F., SPARKS, A.N., Fall army-worm (*Lep. noctuidae*): inheritance of gamma-induced deleterious effects and potential for pest control, *J. Econ. Entomol.* **76** (1983) 378-382.
- [8] CARPENTER, J.E., YOUNG, J.R., CROMROY, H.L., Corn earworm: comparison of field survival of larvae from normal and irradiated parents, *J. Econ. Entomol.* **80** (1987) 883-886.
- [9] HENNEBERRY, T.J., CLAYTON, T.C., Effects of gamma radiation on pink boll-worm (*Lep. gelechidae*) pupae: Adult emergence, reproduction, mating and longevity of emerged adults and their F₁ progeny, *J. Econ. Entomol.* **81** (1988) 322-326.
- [10] ARTHUR, V., WALDER, J.M.M., WIENDL, F.M., Sugar cane borer control through F₁ sterilization. *Acta Agric. Nucl., Sin.* **4** (1990) 57-63.
- [11] SALLAM, H.A., Atomic Energy Authority, Cairo, personal communication, 1989.
- [12] HOEDAYA, M.S., personal communication, 1989.

INHERITED STERILITY IN PROGENY OF GAMMA IRRADIATED MALE COTTON LEAFWORM, *Spodoptera littoralis* (Boisd.)

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Abstract

INHERITED STERILITY IN PROGENY OF GAMMA IRRADIATED MALE COTTON LEAFWORM, *Spodoptera littoralis* (Boisd.).

The fecundity, egg hatch and mating of normal females of *Spodoptera littoralis* (Boisd.) crossed with males irradiated as 7-day-old pupae with doses of 50, 75, 100, 125, 150 and 200 Gy of gamma radiation were studied. The inherited effects of irradiation were studied until the F₃ generation. Also, the progeny of males treated with 100 Gy were outbred or inbred with untreated individuals for two successive generations and the progeny were observed for any inherited deleterious effects on reproduction. The fecundity of females was significantly reduced by increasing the dosage applied to P₁ males. The egg hatch of P₁ females was significantly reduced at 125, 150 and 200 Gy treatments as compared with the untreated control. The F₁ generation was significantly more sterile than the irradiated parents. The irradiation of P₁ males did not affect their mating, sperm transfer or mating frequency nor were these impaired in the successive generations of the different crosses. However, a detectable reduction in the percentage of mated females with sperm and a significant increase in number of spermatophores per mated female were observed among the F₁ generation at a dose level of 200 Gy. The larval and pupal mortality in the F₁ and F₂ generations were high and dose dependent. The average developmental time from egg hatch to adult emergence was not affected and also the sex ratio of resulting progeny was about normal. Males irradiated with 100 Gy of gamma radiation and released into field cages caused significant reductions in the populations. The population of the cages containing partially sterile males was reduced by about 43% in comparison with control cages. Laboratory mating competitiveness indicated that males irradiated with 100 or 150 Gy and their F₁ sons were fully competitive against untreated males in mating with untreated females.

1. INTRODUCTION

The release of sterile males for the control of natural insect populations has not been fully successful with species of Lepidoptera, because they are highly resistant to irradiation [1-3]. Generally, males require at least 300 to 450 Gy to induce full sterility, which causes severe physiological and somatic damage. On the other hand,

Proverbs et al. [4] and Carpenter et al. [5] have reported success in the use of lower substerilizing doses of gamma radiation to control populations of *Laspeyresia pomonella* and *Heliothis zea*, respectively.

Wakid and Hayo [6] and Souka [7] conducted radiation studies with *Spodoptera littoralis* (Boisd.) and have shown that the progeny of adults treated with substerilizing doses exhibited various degrees of sterility, including a reduction in fecundity and fertility and alterations in sex ratios.

In the present study, the effects of low doses of gamma radiation (ranging between 50 and 200 Gy) on the reproduction of P₁ males and their F₁ progeny were studied. In addition, field cage studies were conducted to determine the impact of release of irradiated (100 Gy) cotton leafworm males. Additional laboratory tests were carried out on the mating competitiveness of irradiated males and their F₁ male progeny.

2. STUDY 1

The objective of the following study was to determine the effects of low doses of gamma radiation (ranging between 50 and 200 Gy) on the reproduction of P₁ males and the inherited effects on their F₁, F₂ and F₃ progeny.

2.1. Materials and methods

The test individuals used in this study were obtained from a stock culture of the cotton leafworm maintained for thirty generations in the laboratory of the Radiobiology Department of the Atomic Energy Authority at Inshas. Full grown larvae, obtained from the stock culture were fed on fresh castor oil plant leaves. Mature male pupae (1–2 d before emergence) were irradiated with a gamma cell (⁶⁰Co source) that had a dose rate of approximately 6.50 Gy/min. The irradiation dosages used were 50, 100, 150 and 200 Gy. Male moths within one day of emergence were paired with unirradiated virgin females; 20 pairs were used for each treatment. Two litre ice cream containers fitted with a vial containing a cotton wick and provisioned with a 10% sugar solution served as mating chambers. The containers were lined with a sheet of white paper to serve as an oviposition substrate and the top was covered with cloth fixed in place with a rubber band. Five pairs of moths were placed in each container. This trial was replicated four times. Eggs from each mating container were collected daily for 7 d after crossing and held until hatch. After 7 d, each female was dissected to determine the number of spermatophores in the bursa copulatrix and the presence of sperm in the spermatheca. The F₁ progeny were reared using the method previously described. F₁ males were mated with normal females and the resulting F₂ males were again mated to normal females.

The holding conditions for all the life stages in this study were 25–32°C and 60–70% relative humidity (RH). The data were analysed using the analysis of variance (ANOVA) technique and the means were separated using Duncan's multiple range test.

2.2. Results

The average number of eggs deposited by females was not significantly affected when they had been mated to males irradiated with 50 and 100 Gy. Crosses with males irradiated with higher doses resulted in significantly fewer eggs. The fecundity was significantly reduced when F_1 male progeny, whose male parents had been exposed to any dose, were mated with normal females. The trend of decreasing fecundity with increasing dose was still apparent when F_2 males were mated to normal females; however, only treatments of 100 Gy and greater produced significantly fewer eggs. The oviposition of normal females was not significantly reduced when they were crossed with male F_1 , F_2 or F_3 progeny of any treatment.

2.3. Hatchability of eggs

The proportion of eggs that hatched was significantly lower when P_1 males were treated with 150 and 200 Gy (Table I). F_1 males were more sterile than their parents and significant reductions in egg hatch were found for all irradiation treatments (50 Gy). F_2 males crossed with normal females resulted in greater proportions of eggs hatching than F_1 matings. However, only treatments of 100 Gy or greater produced significantly lower hatch rates. Crosses of F_3 males from all treatments produced normal egg hatch rates. These data indicate that chromosome aberrations present in the F_1 generation produced lower egg hatching rates. Some of these lethal changes were lost and F_2 males were more fertile than their male parents. By the F_3 generation the male fertility was normal and all the inherited sterility affects were apparently lost.

2.4. Mating frequency and sperm transfer

Irradiation of males did not significantly affect the percentage of mated females. Also, there was no substantial difference in the percentage mated females among F_1 , F_2 and F_3 progeny.

Sperm transfer by irradiated males and their progeny was high (Table II). Only one treatment, females mated with F_1 males from the 200 Gy group, showed a significant reduction in sperm present in the spermatheca.

In general, the average number of spermatophores per mated female increased with increasing doses for irradiated males and for their F_1 and F_2 progeny. The spermatheca of females mated to P_1 males treated with 150 or 200 Gy contained a

TABLE I. TREATMENT OF MALE *S. littoralis* (Boisd.) WITH SUBSTERILIZING DOSES OF GAMMA RADIATION, AND EFFECT ON FECUNDITY AND HATCHABILITY OF PARENTS (P₁) AND THEIR F₁, F₂ AND F₃ PROGENY (20 pairs were used in each treatment)

Dose to (P ₁) male (Gy)	Average number of eggs/female*			Hatchability* (%)			
	P ₁	F ₁	F ₂	F ₃	F ₁	F ₂	F ₃
0	1402 ^a	1585 ^a	1342 ^a	1373 ^a	85.52 ^a	87.35 ^a	80.98 ^a
50	1437 ^a	1253 ^b	1298 ^a	1269 ^a	79.88 ^a	66.20 ^b	72.35 ^a
100	1299 ^a	968 ^c	1013 ^b	1305 ^a	73.24 ^b	51.23 ^c	60.65 ^b
150	985 ^b	651 ^c	1062 ^b	1176 ^a	57.54 ^b	38.64 ^c	52.81 ^b
200	924 ^b	566 ^c	937 ^b	1217 ^a	48.16 ^b	33.92 ^d	45.73 ^b

* Means followed by the same letter in the same column are not significantly different at $p = 0.05$.

TABLE II. TREATMENT OF MALE *S. littoralis* (Boisd.) WITH SUBSTERILIZING DOSES OF GAMMA RADIATION, AND EFFECT ON MATING OF PARENTS (P₁) AND THEIR F₁, F₂ AND F₃ PROGENY (20 pairs were used in each treatment)

Dose to (P ₁) male (Gy)	Mating			Mated females with sperm* (%)			Average number of spermatophores/mated female*					
	P ₁	F ₁	F ₂	F ₃	P ₁	F ₁	F ₂	F ₃	P ₁	F ₁	F ₂	F ₃
0	100	95	94.73	100.00	100.00 ^a	100.00 ^a	94.44 ^a	100.00 ^a	2.05 ^a	2.21 ^a	2.36 ^a	2.10 ^a
50	100	100	100.00	100.00	95.00 ^a	95.00 ^a	95.00 ^a	95.00 ^a	2.10 ^a	2.50 ^a	1.95 ^a	2.47 ^a
100	95	95	100.00	100.00	94.73 ^a	94.73 ^{ab}	95.00 ^a	100.00 ^a	2.00 ^a	2.73 ^{ab}	2.15 ^a	2.15 ^a
150	100	90	90.00	94.73	100.00 ^a	88.88 ^{ab}	94.44 ^b	94.44 ^a	2.32 ^a	3.15 ^b	2.22 ^a	2.26 ^a
200	95	90	89.47	90.00	89.47 ^a	81.25 ^a	88.23 ^a	100.00 ^a	2.66 ^a	2.88 ^b	2.58 ^a	2.73 ^a

* Means followed by the same letter in the same column are not significantly different at $p = 0.05$.

TABLE III. TREATMENT OF MALE *S. littoralis* (Boisd.) WITH SUBSTERILIZING DOSES OF GAMMA RADIATION, AND EFFECT ON SURVIVAL, DEVELOPMENTAL TIME AND SEX RATIO OF F₁, F₂ AND F₃ PROGENY

(200 larvae were tested in each treatment)

Dose (Gy)	Survived to adult stage* (%)	Development time (d)	Sex ratio	
			Male	Female
<i>F₁ generation</i>				
0	85.5 ^a	28.78	0.99	1
50	41.5 ^b	28.84	0.86	1
100	44.5 ^b	29.85	1.17	1
150	24.0 ^{bc}	31.44	1.04	1
200	35.5 ^{bc}	30.28	1.95	1
<i>F₂ generation</i>				
0	84.0 ^a	30.10	1.04	1
50	66.5 ^b	31.40	0.95	1
100	73.0 ^b	31.47	1.08	1
150	44.0 ^c	31.46	0.93	1
200	51.0 ^c	30.47	1.12	1
<i>F₃ generation</i>				
0	83.0 ^a	26.22	1.05	1
50	75.5 ^{ab}	26.94	0.97	1
100	72.0 ^b	25.63	0.93	1
150	59.0 ^c	26.18	0.87	1
200	70.0 ^b	27.50	1.00	1

* Means followed by the same letter in the same column in each generation are not significantly different at $p = 0.05$.

larger number of spermatheca than those of the controls (not significantly). Significantly greater numbers of spermatophores were found in females paired to male F₁ progeny of 150 and 200 Gy treatments. The mating frequency has not shown any significant effect among F₂ or F₃ treatments.

2.5. Larval survival

Survival of F_1 larvae to the adult stage was significantly lower for all irradiation treatments (Table III). The number of surviving larvae was dose dependent and mortality increased as the dose applied to P_1 males was increased. Among the F_2 , the reduction in survival was reduced for all treatments and was significantly lower in the 150 and 200 Gy treatments. The mortality of F_3 larvae was reduced compared with that of the F_1 and F_2 , but was still significantly greater than the controls at the three highest irradiation treatments.

2.6. Developmental time

The average developmental time required from egg hatch to adult emergence seemed to be about normal for the progeny of all treatments (doses and generations).

2.7. Sex ratio

Table III also shows that the sex ratio among the progeny of irradiated males seemed about that found in the control group. Generally, as dose increased, the sex ratio in the F_1 shifted in favour of the males and at the highest dose (200 Gy) the male to female ratio was nearly 2:1.

3. STUDY 2

The objective of this second study was to characterize the effects of low doses of gamma radiation (ranging between 75 and 150 Gy) on reproduction of P_1 males and the inherited effects on the progeny resulting from outcrosses or inbreds.

3.1. Materials and methods

Full grown larvae, obtained from the stock culture, were fed on a broad bean diet, prepared as described by Shorey and Hale [8]. Twenty larvae were reared in 1 L plastic containers, containing 200 mL of diet. Mature male pupae (within a day of emergence) were irradiated using a gamma cell (^{60}Co source) that had a dose rate of approximately 4.5 Gy/min. The irradiation dosages used were 75, 100, 125 and 150 Gy. Other experimental techniques used were the same as those described in the first study.

Two sets of tests were carried out. In the first, newly emerged treated (T) males were paired with normal (N) females. Males of the F_1 generation were paired with newly emerged normal females, and this was done again in F_2 and F_3 . In the second set of tests, the F_1 and F_2 progeny of males treated with only 100 Gy were

TABLE IV. TREATMENT OF MALE *S. littoralis* (Boisd.) WITH SUBSTERILIZING DOSES OF GAMMA RADIATION, AND EFFECT ON FECUNDITY, HATCHABILITY AND MATING OF PARENTS (P₁) AND THEIR F₁, F₂ AND F₃ PROGENY (20 pairs were used in each treatment)

Generation	Dose to P ₁ males (Gy)	Eggs/female*	Hatchability* (%)	Mating* (%)	Mated females with sperm* (%)	Spermatophores/mated females*
P ₁	0 (Control)	1655 ^a	84.3 ^a	100.0 ^a	100.0 ^a	2.3 ^a
	75	1610 ^a	77.5 ^a	95.0 ^a	94.7 ^a	2.1 ^a
	100	1562 ^a	68.7 ^{ab}	100.0 ^a	100.0 ^a	2.3 ^a
	125	1487 ^a	63.5 ^b	100.0 ^a	90.0 ^a	2.0 ^a
	150	1619 ^a	60.8 ^b	49.4 ^a	100.0 ^a	2.5 ^a
F ₁	0 (Control)	1571 ^a	86.5 ^a	100.0 ^a	100.0 ^a	2.1 ^a
	75	1546 ^a	64.8 ^a	100.0 ^a	100.0 ^a	2.1 ^a
	100	1408 ^{ab}	55.1 ^{bc}	94.4 ^a	100.0 ^a	1.8 ^a
	125	1515 ^{ab}	48.5 ^{bc}	100.0 ^a	95.0 ^a	1.9 ^a
	150	1397 ^b	45.7 ^c	94.4 ^a	100.0 ^a	1.8 ^a
F ₂	0 (Control)	1722 ^a	82.7 ^a	95.0 ^a	100.0 ^a	2.5 ^a
	75	1587 ^{ab}	66.5 ^{ab}	100.0 ^a	95.0 ^a	2.5 ^a
	100	1561 ^{ab}	63.8 ^b	95.0 ^a	94.7 ^a	2.1 ^a
	125	1463 ^b	56.5 ^b	94.7 ^a	100.0 ^a	2.6 ^a
	150	1608 ^{ab}	55.7 ^b	100.0 ^a	95.0 ^a	2.2 ^a

F ₃	0 (Control)	1497 ^a	85.4 ^a	100.0 ^a	95.0 ^a	2.6 ^a
	75	1389 ^a	79.5 ^a	94.1 ^{ab}	100.0 ^a	2.2 ^a
	100	1475 ^a	71.3 ^a	95.0 ^{ab}	100.0 ^a	2.2 ^a
	125	1417 ^a	75.6 ^a	100.0 ^a	95.0 ^a	2.5 ^a
	150	1385 ^a	72.9 ^a	89.5 ^b	94.4 ^a	2.1 ^a

* Means followed by the same letter in the same column for each generation are not significantly different at $p = 0.05$.

TABLE V. EFFECTS OF IRRADIATION OF MALE *S. littoralis* (Boisd.) WITH A SUBSTERILIZING DOSE (100 Gy) ON THE FECUNDITY, HATCHABILITY AND MATING OF FEMALES AMONG THE TREATED PARENTS (P₁) AND F₁ AND F₂ PROGENY RESULTING FROM ALL POSSIBLE CROSSES
(20 pairs were used in each treatment)

Generation	Crosses*†		Eggs/ female ⁺	Hatchability ⁺ (%)	Mating ⁺ (%)	Mated female with sperm ⁺ (%)	Spermatophores/ mated females ⁺
	Male	Female					
P ₁	N	N	1460 ^a	85.2 ^a	99.2 ^a	98 ^a	2.1 ^a
	T	N	1485 ^a	64.1 ^b	100.0 ^a	99 ^a	2.1 ^a
F ₁	1	1	1608 ^a	86.6 ^a	100.0 ^a	100 ^a	2.5 ^a
	2	1	1526 ^{ab}	50.8 ^b	99.5 ^a	100 ^a	2.4 ^a
	1	2	1495 ^{ab}	58.7 ^b	100.0 ^a	96 ^a	2.0 ^a
	2	2	1410 ^b	25.0 ^c	94.2 ^a	95 ^a	2.1 ^a
F ₂	A	A	1575 ^a	83.5 ^a	97.1 ^a	97 ^a	2.2 ^a
	A	B	1611 ^a	64.8 ^b	100.0 ^a	100 ^a	1.9 ^a
	A	C	1550 ^a	65.4 ^b	95.0 ^a	93 ^a	1.8 ^a
	B	A	1506 ^a	54.7 ^b	100.0 ^a	98 ^a	2.3 ^a
	B	B	1442 ^a	52.5 ^b	96.7 ^a	99 ^a	1.8 ^a
	B	C	1435 ^a	57.8 ^b	92.5 ^a	92 ^a	2.2 ^a
C	A	A	1498 ^a	59.7 ^b	95.8 ^a	94 ^a	2.3 ^a
	C	B	1515 ^a	55.9 ^b	93.5 ^a	100 ^a	1.9 ^a
	C	C	1480 ^a	51.2 ^b	96.7 ^a	97 ^a	2.0 ^a

* N, normal, T, treated; 1, progeny from P₁N × N; 2, progeny from F₁T × N; A, progeny from F₁1 × 1; B, progeny from F₁1 × 2; C, progeny from F₁2 × 1.

⁺ Means followed by the same letter in the same column in each generation are not significantly different at $p = 0.05$.

paired with normal females ($T_M \times N_F$). The progeny from these matings were inbred or outbred with normal individuals, using all possible crosses between them.

In both studies, twenty pairs were used for each treatment. The biological aspects studied were the number of eggs/female, egg hatch, mating and sperm transfer. Larval survival, developmental time and sex ratio were recorded for the progeny of all mating types. Data were analysed using ANOVA and Duncan's multiple range test ($p = 0.05$).

3.2. Results

The average number of eggs deposited per female was not significantly affected by any P_1 male treatment (Table IV). The fecundity of females mated with males from the highest treatment group (150 Gy) was significantly lower than that for the control matings. Although the fecundity of F_2 matings was lower than that of the controls for all treatments only the fecundity of F_2 males from a 125 Gy treatment was significantly lower than the control. Among the F_3 generation, there were no substantial differences in egg production.

Table V shows that the egg production among the F_1 generation was significantly affected when $P_1 T \times N$ progeny were inbred (2×2), while the fecundity among other crosses of P_1 , F_1 and F_2 was not affected.

3.3. Hatchability of eggs

The hatchability was reduced by increasing the dose to the male parent (Table IV). This effect was more pronounced in the F_1 generation. The F_2 generation was more fertile than the F_1 . Doses of 100 Gy or greater produced significantly lower rates of egg hatching than the control. The results on the hatchability of the F_3 generation indicate that an obvious increase in egg hatching occurred at all doses tested when compared with F_1 and F_2 . These results indicate that the detrimental effects of irradiation continue in the population through F_1 and to a lesser extent through F_2 and F_3 .

The reduction in egg hatch in F_1 was significantly reduced for outcrosses of $P_1 T \times N$ progeny (treatments 2×1 and 1×2) (Table V). When $P_1 T \times N$ progeny were inbred (2×2), the egg hatch was significantly lower than that in the controls. Among F_2 , significant reductions in egg hatch were recorded in all crosses, indicating that deleterious factors were still present in the population.

3.4. Mating frequency and sperm transfer

The results indicate that irradiation of males did not significantly affect the percentage of mated females in either test (Tables IV and V) (doses, generations and crosses). Only the matings of F_3 were significantly decreased at 150 Gy.

TABLE VI. EFFECT OF TREATMENT OF MALE *S. littoralis* (Boisd.) WITH SUBSTERILIZING DOSES OF GAMMA RADIATION ON SURVIVAL, DEVELOPMENTAL TIME AND SEX RATIO OF F₁, F₂ AND F₃ PROGENY

Dose (Gy)	Survivals* (%) (100 larvae/pupal mortality)	Development time* (d)	Sex ratio	
			Male	Female
<i>F₁ generation</i>				
0	88 ^a	29.6 ^a	0.98	1
75	70 ^b	30.2 ^a	1.10	1
100	53 ^c	30.7 ^a	1.05	1
125	43 ^c	31.1 ^a	1.16	1
150	41 ^c	31.2 ^a	1.14	1
<i>F₂ generation</i>				
0	84 ^a	31.5 ^a	1.12	1
75	80 ^a	32.0 ^a	1.12	1
100	73 ^{ab}	31.8 ^a	1.20	1
125	68 ^{bc}	32.6 ^a	1.08	1
150	59 ^c	32.7 ^a	1.21	1
<i>F₃ generation</i>				
0	85 ^a	30.2 ^a	1.00	1
75	84 ^a	30.9 ^a	0.94	1
100	79 ^a	31.3 ^a	0.96	1
125	78 ^a	32.1 ^a	1.11	1
150	76 ^a	31.4 ^a	0.95	1

* Means followed by the same letter in the same column in each generation are not significantly different at $p = 0.05$.

TABLE VII. EFFECT OF TREATMENT OF MALE *S. littoralis* (Boisd.) WITH A SUBSTERILIZING DOSE OF 100 Gy GAMMA RADIATION ON SURVIVAL, DEVELOPMENTAL TIME AND SEX RATIO OF P₁ AND ALL POSSIBLE CROSSES OF F₁ AND F₂ PROGENY (200 larvae were tested in each treatment)

Crosses*		Survivals [†] (%) (100 larvae/ pupal mortality)	Development time [†]	Sex ratio [†]	
Male	Female			Male	Female
<i>P₁ progeny</i>					
N	N	91 ^a	29.2 ^a	1.08 ^a	1
T	N	70 ^b	29.8 ^a	1.27 ^a	1
<i>F₁ progeny</i>					
1	1	85 ^a	28.5 ^a	0.95 ^a	1
2	1	43 ^b	29.7 ^a	1.00 ^a	1
1	2	41 ^b	29.2 ^a	1.10 ^a	1
2	2	18 ^c	31.4 ^a	1.60 ^b	1
<i>F₂ progeny</i>					
A	A	87 ^a	30.4 ^a	1.14 ^a	1
A	B	76 ^{ab}	31.1 ^a	1.11 ^a	1
A	C	71 ^b	31.7 ^a	1.25 ^a	1
B	A	80 ^{ab}	29.9 ^a	1.13 ^a	1
B	B	66 ^{bc}	32.2 ^a	1.18 ^a	1
B	C	75 ^{ab}	30.7 ^a	1.28 ^a	1
C	A	74 ^{ab}	31.5 ^a	1.05 ^a	1
C	B	75 ^{ab}	30.1 ^a	1.13 ^a	1
C	C	54 ^c	30.6 ^a	1.02 ^a	1

* N, normal; T, treated; 1, progeny from P₁N × N; 2, progeny from P₁T × N; A, progeny from F₁1 × 1; B, progeny from F₁1 × 2; C, progeny from F₁2 × 1.

[†] Means followed by the same letter in the same column in each generation are not significantly different at *p* = 0.05.

The ability of sperm to reach the spermathecae (percentage of mated females with sperm) indicates that there was no obvious effect among the females of P_1 , F_1 , F_2 and F_3 at all tested doses (Table IV). The same results were obtained among the females of P_1 , F_1 and F_2 at all crosses (Table V).

3.5. Larval survival

Table VI shows the numbers of F_1 , F_2 and F_3 larvae that reached the adult stage. Among F_1 treatments, larval survival was significantly lower at all doses tested compared with the control. Among F_2 , reductions in survival were obvious for all treatments. However, they were only significantly lower than the controls at 125 and 150 Gy. The least mortality was observed among the progeny of the F_3 matings.

Table VII shows that the mortality of the progeny of P_1 matings increased significantly in comparison with the control group. The inherited deleterious effects in the progeny of F_1 matings were more obvious when $P_1T_M \times N_F$ progeny were inbred (2×2) than when they were outcrossed, i.e. $P_1T_M \times N$ progeny (2×1 and 1×2). Among the progeny of F_2 matings the reduction in survival was obvious at all crosses. A significant reduction in larval/pupal survival was recorded in the outcross ($A \times C$) and in the inbred crosses ($B \times B$ and $C \times C$).

3.6. Developmental time

The average developmental periods (from egg hatch to adult emergence) seemed to be about normal in the progeny of all treatments (doses, generations and crosses).

3.7. Sex ratio

The sex ratios among all treatments studied did not differ from the normal 1:1 observed in the control treatment (Table VI).

Similar results were obtained among the different generations and crosses in Table VII, with the exception of the progeny of F_1 inbred (2×2) matings. In this case the sex ratio was significantly altered in favour of males.

4. STUDY 3

The objective of this study was to determine the suppressive effects on native populations of releasing irradiated (100 Gy) males in field cages. Further, a laboratory test was carried out on the mating competitiveness of irradiated parental males and their F_1 males.

4.1. Materials and methods

During the first week of August 1989, maize was planted inside six field cages 7 m in diameter and 3.5 m high, constructed on an experimental farm near Cairo. The maize received regular irrigation, but no other cultural or insecticidal treatments.

Cage populations were initiated in all six cages by the release of four pairs (one day old) of untreated moths/cage on 5 September. Untreated moths were obtained as egg masses collected from an infested cotton field and reared in the laboratory on fresh castor oil plant leaves for one generation. Maize plants were about 60 cm high when infested, and each cage contained approximately 200 maize plants. At the same time, laboratory reared males (irradiated as full grown pupae with 100 Gy gamma rays at a dose rate of about 3 Gy/min) were released in three of the cages at a ratio of ten treated males for one normal pair (40 treated males/cage). The other three cages served as controls. Laboratory reared moths were fed on a kidney bean diet [9].

Samples of irradiated males were retained in the laboratory and crossed with normal females for egg hatch determination. The average percentage hatch was found to be about 60% (range 32–75%). These findings show that released irradiated P_1 males were partially sterile and there was considerable variation in sterility induced by irradiation. All six cages were checked every two days and the egg

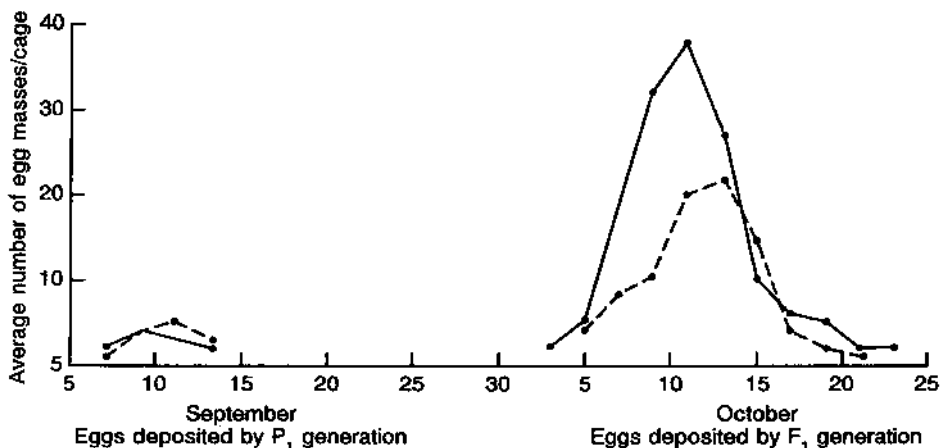


FIG. 1. Populations of two cotton leafworm treatments as indicated by egg masses (average of 3 cages/treatment): —, control ($4N_M + 4N_F$); ---, treatment ($4N_M + 4N_F + 40$ irradiated σ).

masses deposited on plant leaves were marked and counted. Cage population densities were determined by recording the egg masses for two months (September and October).

4.2. Results

On 7 September we started to mark and count the egg masses deposited on the plant leaves in all cages. The average number of egg masses in the control treatment was 11 egg masses/cage (8, 12 and 13), while treated cages contained 12.3 egg masses/cage (10, 13 and 14) (Fig. 1). At the beginning of October, females of the next generation started to lay eggs. The average number of egg masses was 148 egg masses/cage (88, 161 and 195) for the control group and 84 egg masses/cage (61, 92 and 99) for the treated population.

Populations in the control group increased 13.5-fold during September. Populations in the cages treated with sterile males only increased 6.8-fold. The average number of egg masses deposited by the F_1 generation in control treated cages was significantly higher than those deposited by the treated population. The population in the treated cages was reduced by about 43% in comparison with the control treatment.

The experiment was terminated owing to the lack of green maize leaves and the short duration of the maize plant (110–120 d).

5. FURTHER LABORATORY STUDY: MATING COMPETITIVENESS OF IRRADIATED MALES AND THEIR F_1 MALE PROGENY

The objective of the following study was to determine the numbers of viable eggs laid by normal females after they had been caged either with males irradiated with 100 or 150 kGy plus normal males, or with F_1 males (i.e. progeny of males that had been treated with 100 or 150 kGy) plus normal males. In the various cases the ratios of irradiated or F_1 males:normal males:normal females were 0:1:1, 1:0:1, 1:1:1 and 5:1:1, respectively.

F_1 males were the descendants of irradiated male parents (100 or 150 Gy) and normal females. Each cage was provisioned with a 10% sugar solution and was also supplied with fresh leaves of the ornamental plant, *Nerium oleander*, to serve as an oviposition site for the females. The egg masses were collected daily and the number of hatched eggs in each population was recorded.

The cotton leafworms used in this study were reared as larvae on a kidney bean diet [9]. The experimental conditions throughout the study were 25–32°C and 60–70% RH.

The competitiveness value and expected egg hatch rates were computed as described by Fried [10]. A competitiveness value (CV) of 1.0 indicates full competitiveness and a CV of between 0.75 and 1.0 indicates good competitiveness.

5.1. Statistical analysis

The Student F test and the Duncan multiple range test were used in the statistical analysis of the data [11].

TABLE VIII. MATING COMPETITIVENESS OF MALES IRRADIATED WITH 100 OR 150 Gy AND THEIR F_1 MALES (AVERAGE OF 4-5 REPLICATES)

Cross ratio $T\sigma:N\sigma:N\phi$	Dose (Gy)	Egg hatch (%)		CV
		Observed	Expected	
P_1				
0:1:1	Control (0)	82.3	—	—
1:0:1	100	65.8*	—	—
1:1:1	100	71.5	74.05	1.04
5:1:1	100	69.2	68.55	0.99
1:0:1	150	58.5*	—	—
1:1:1	150	73.3	70.40	0.96
5:1:1	150	57.3*	62.47	1.09
F_1				
0:1:1	Control (0)	84.1	—	—
1:0:1	100	55.2*	—	—
1:1:1	100	60.6*	69.65	1.15
5:1:1	100	56.6*	60.02	1.06
1:0:1	150	43.7**	—	—
1:1:1	150	50.8*	63.90	1.26
5:1:1	150	44.5**	50.43	1.13

* Significantly different from the untreated control ($p = 0.05$).

** Significantly different from the untreated control ($p = 0.01$).

5.2. Results

The results of the mating competitiveness of irradiated parental males and their F_1 males are presented in Table VIII. In cages with males irradiated with 100 or 150 Gy within the two tested ratios, lower egg hatches occurred compared with the untreated control. However, the reduction was significant only at the 5:1 ratio when P_1 males were irradiated with 150 Gy.

The average egg hatch in cages with F_1 males was significantly reduced for all treatments (doses and ratios) when compared with the untreated control (84.1%). The comparison of treatment effects indicates that the ratio of 5 treated:1 untreated consistently gave a greater reduction in the egg hatch than the 1:1 ratio.

The computed CVs were not different for P_1 or F_1 males, for the irradiation treatment or for the ratios of treated:untreated males tested. Thus, it can be concluded that under laboratory conditions, the cotton leafworm males irradiated by low doses of 100 or 150 Gy were fully competitive, and the same was true for their F_1 male progeny.

6. DISCUSSION

In lepidopterous species, the irradiation of males with substerilizing doses is associated with high levels of sterility in their F_1 progeny. Generally, the use of substerilizing doses of irradiation is advised to achieve an effective combination of induced partial sterility and inherited chromosome aberrations to suppress natural populations [3]. Inherited sterility in the F_1 progeny of an irradiated male *S. littoralis* was reported by Wakid and Hayo [6], Souka [7] and Sallam and Ibrahim [12].

Most of the results from past studies were confirmed by the present studies. No apparent effect on the fecundity of females when crossed with irradiated males was found. However, the fecundity of females crossed with F_1 and F_2 males was reduced, particularly when higher irradiation dosages were administered to F_1 males. The F_1 was more sterile than its irradiated parent. A reduction in egg hatch was recorded at all treatments involving the F_2 progeny of irradiated males, indicating that deleterious factors were still present in the population. Similar results were reported by Souka [7] and El-Naggar et al. [13] on *Agrotis ipsilon* and Carpenter et al. [5] on *Heliothis zea*.

Sperm transfer was normal for F_1 , F_2 and F_3 progeny of irradiated males. The number of spermatophores per mated female did not exhibit a dose response and was not significantly different from the control group. Similar results were obtained by El-Naggar et al. [13] and Carpenter et al. [5].

The mortality of the larvae and pupae progeny of mating F_1 and F_2 males with normal females was high and dose dependent. However, the average developmental

time of F_1 , F_2 and F_3 progeny was about normal. Also, the sex ratio was slightly altered in favour of males. This change in sex ratio was only significant among F_1 progeny when $P_1T \times N$ progeny were inbred (2×2) (Table VII). Similar results were obtained by El-Naggar et al. [13] and Carpenter et al. [5].

Males irradiated with 100 Gy and released into field cages caused significant reductions in population. Laboratory mating competitiveness indicated that males irradiated with 100 or 150 Gy and their F_1 male progeny were fully competitive with untreated males in mating with untreated females.

It could be concluded from the results that inherited sterility is more effective than the sterile insect technique for population suppression and might be useful in an integrated control programme against the cotton leafworm, *S. littoralis*, in Egypt. Certainly, more studies need to be conducted before making recommendations.

REFERENCES

- [1] NORTH, D.T., HOLT, G.G., "Genetic and cytogenetic basis of radiation-induced sterility in the adult male cabbage looper, *Trichoplusia ni*", Isotopes and Radiation in Entomology (Proc. Symp. Vienna, 1967), IAEA, Vienna (1968).
- [2] NORTH, D.T., HOLT, G.G., Inherited sterility in progeny of irradiated male cabbage looper, *J. Econ. Entomol.* **61** (1968) 928-931.
- [3] LaCHANCE, L.E., BELL, R.A., RICHARD, R.D., The effect of low doses of gamma irradiation on reproduction of male pink bollworm and their F_1 progeny, *Environ. Entomol.* **2** (1973) 653-658.
- [4] PROVERBS, M.D., NEWTON, J.R., LOGAN, D.M., BRINTON, F.E., Codling moth control by release of radiation sterilized moths in a pome fruit orchard and observations of other pests, *J. Econ. Entomol.* **68** (1975) 555-560.
- [5] CARPENTER, J.E., YOUNG, J.R., SPARKS, A.N., CROMROY, H.L., CHOWDHURY, M.A., Corn earworm (Lepidoptera: Noctuidae): Effects of substerilizing doses of radiation and inherited sterility on reproduction, *J. Econ. Entomol.* **80** (1987) 483-489.
- [6] WAKID, A.M., HAYO, J.A., Inherited sterility in progeny of irradiated male cotton leafworm, *Spodoptera littoralis* (Boisd.), *Z. Angew. Entomol.* **76** (1974) 331-335.
- [7] SOUKA, S.R., Effects of irradiation by sterilizing and substerilizing doses on parents and F_1 of the cotton leafworm, *Spodoptera littoralis* (Boisd.), *Bull. Soc. Entomol. Egypt* **63** (1981) 19-27.
- [8] SHOREY, H.H., HALE, R.L., Mass-rearing of the larvae of nine Noctuid species on a simple artificial medium, *J. Econ. Entomol.* **58** (1965) 522-524.
- [9] RASHAD, A.M., AMMAR, E.D., Mass rearing of the spiny bollworm, *Erias insulana* (Boisd.) on semi-artificial diet, *Bull. Soc. Entomol. Egypt* **65** (1984-1985) 239-244.
- [10] FRIED, M., Determination of sterile insect competitiveness, *J. Econ. Entomol.* **64** (1971) 869-872.
- [11] STEEL, R.G.D., TORRIE, J.H., Principles and Procedures of Statistics. A Biometrical Approach, 2nd edn, McGraw-Hill, New York (1980).

- [12] SALLAM, H.A., IBRAHIM, S.M., "Inherited sterility among irradiated male cotton leafworm, *Spodoptera littoralis* (Boisd.)", Nuclear Science and Applications (Proc. 4th Conf.), Atomic Energy Authority of Egypt, Cairo (1988) 422-430..
- [13] EL-NAGGAR, S., MEGAHED, M.M., SALLAM, H.A., IBRAHIM, S.M., Inherited sterility among *Agrotis ipsilon* laboratory population exposed to gamma irradiation, Insect Sci. Appl. 5 6 (1984) 501-503.

POSSIBILITIES OF USING RADIATION INDUCED F₁ STERILITY FOR CONTROL OF EUROPEAN CORN BORER IN ROMANIA

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Abstract

POSSIBILITIES OF USING RADIATION INDUCED F₁ STERILITY FOR CONTROL OF EUROPEAN CORN BORER IN ROMANIA.

Investigations were undertaken to develop the foundation for control in the future of the European corn borer, *Ostrinia nubilalis* (Hübner), with a pest management system based on sterility expressed to the greatest extent during the F₁ generation of progeny of moths irradiated with gamma rays. As a basis for the mass rearing of the pest, a diet was developed from locally available ingredients. The ingredients are bean meal, wheat bran, brewer's yeast, milk powder substitute for calves, salt mixture used in poultry production, sugar, ascorbic acid, sorbic acid, glacial acetic acid, formaldehyde, agar and water. Using this diet, 1000 moths can be reared for as little as one US dollar. Complete sterility induced by exposure to gamma rays occurs at a lower dose in females than in males. When males that are exposed as six-day-old pupae to 150 Gy are mated to untreated females, 67.5% of the eggs hatch. Further, when the sons of treated males are mated to untreated females, 42.8% of the eggs hatch, when daughters of treated males are mated to untreated males, 40.7% of the eggs hatch, and when sons and daughters of treated males are mated to each other, 9.1% of the eggs hatch. The amount of mortality following egg hatch was not recorded. However, in field cage experiments, F₁ larvae damaged 4, 8 and 0% of corn stalks for these respective crosses compared with the 76% damage by larvae from untreated parents. The corresponding yield of kernels of corn in grammes per plant was 57, 42, 46 and 27. In order to mark moths for field studies they were reared on diet containing Calco red dye. Traps baited with the various enantiomers of the sex pheromone were used to study the dispersal of released moths and the dates of adult moth emergence in various regions of Romania.

1. INTRODUCTION

The European corn borer (ECB), *Ostrinia nubilalis* (Hübner), is one of the most damaging maize pests. It covers a huge area in almost all worked zones, especially in the Northern Hemisphere, since it thrives in a very wide range of climates, from the Equator to temperate zones. Its economic significance has led to intensive research on this insect, especially in North America.

In Romania the ECB becomes the most harmful pest after panicle emergence, and is found in all maize culture zones. Losses induced reached up to 40% of grain yield [1]. Multiannual data indicated averages of 44% plants attacked, 1.1 larvae per plant, 23 180 larvae per hectare and 550 kg/ha yield loss or 7.5% [2].

Under the ecological conditions prevailing in this country, the insect develops one generation per year, except for zones in the south, where a partial second generation occurs. The population of this second generation is less than 20% of the previous generation. The mass flight of the moths of the overwintering generation commonly takes place a few days before panicle emergence. The main attack sites are the larval tunnels inside the stems. The attack on maize is economically important. However, the pest also feeds on hemp and sorghum crops as well as on various species of wild flora.

Owing to the special significance of ECB in maize, a series of investigations was started in Romania, intended to prevent outbreaks of this pest through chemical [3] and biological means [4-8] and by resistant hybrids [9-15].

During recent years, particular attention was paid to the study of synthetic sex pheromones [16-18] and since 1988 to the investigations on male sterilization by radiation [19, 20].

2. MASS REARING OF EUROPEAN CORN BORER

Because all investigations designed to prevent ECB attack are limited by the availability of large numbers of insects, the mass rearing of this insect was essential. Consequently, a series of experiments was performed that established that all diets that had bean meal as a basic ingredient were satisfactory for the growth of the ECB. Further, the development of the insect was not negatively influenced by any of the ingredients. The diet composed of the ingredients shown in Table I was very practical and was used in all investigations on male sterilization. These ingredients are readily available and inexpensive. Depending on the degree of use of the rearing facilities, the cost of 1000 pupae ranged from US \$1 to US \$5.

When evaluating the performance of *O. nubilalis* moths reared on an artificial diet for various numbers of generations, it was found that fecundity and longevity values did not exhibit significant differences between generations. Data obtained in an experiment with the 81st generation and in another with the 106th generation were similar to those achieved in the 8th and 9th generations, respectively [15].

It is worth mentioning that the ECB has been mass reared for 152 successive generations, and this is still in progress. Thus, a mass rearing technique has been developed, fitting the Romanian conditions. On the basis of this technique, using a diet composed essentially of indigenous ingredients, the insect can be reared continuously.

With a continuous supply of ECBs it was possible to perform experiments on sterilization by irradiation.

TABLE I. INGREDIENTS USED IN THE DIET FOR *O. nubilalis* (Hübner)
(1 batch = 4.500 kg)

Ingredient	Amount	(%)
Bean meal	372.0 g	8.20
Wheat bran	160.0 g	3.53
Brewer's yeast	136.0 g	3.00
Milk powder substitute for calves	106.0 g	2.34
Salt mixture for poultry	40.0 g	0.88
Sugar	133.0 g	2.93
Ascorbic acid	13.6 g	0.30
Sorbic acid	10.0 g	0.22
Glacial acetic acid	14.8 mL	0.33
Formaldehyde	8.4 mL	0.19
Agar	40.0 g	0.88
Water	3500.0 mL	77.20

3. USE OF IONIZING RADIATION FOR PARTIAL STERILIZATION OF ECB

In order to develop the fundamentals of genetic control through F_1 sterility as a component of an integrated control programme for the ECB, investigations were conducted at the Research Institute for Cereals and Industrial Crops at Fundulea. Our work, related to the technique of sterile insect release, has been conducted under the FAO/IAEA co-ordinated research programme Radiation Induced F_1 Sterility in Lepidoptera for Area-Wide Control.

Application of this technique required knowledge on methodology, dose of radiation and biological effects on the insects exposed to radiation, which would ensure the required degree of substerility with minimum detrimental effects to the irradiated insects.

Treatments were performed at the Institute of Nuclear Physics and Engineering in Magurele, using a ^{60}Co source, delivering 18.05 Gy/h, in 23 mm diameter and 125 mm high containers, located 15 cm from the cobalt source. These containers held 300 pupae. Insects used in experiments were taken from a laboratory strain that had been reared according to techniques developed at Fundulea.

The preliminary trials involved studies on the role of pupal age and radiation dose on the emergence rate of moths. Irradiation was applied to one to six-day-old

TABLE II. EMERGENCE PERCENTAGE OF IRRADIATED PUPAE

Age of pupae (d)	Control	Radiation dose (Gy)				
		100	200	300	400	500
1	96.50	5.50	1.00	0.00	0.00	0.00
2	95.25	12.50	9.25	0.50	0.00	0.00
3	91.50	65.75	55.50	41.75	30.25	10.50
4	93.25	93.00	90.25	88.50	74.50	68.75
5	94.00	92.75	91.50	90.25	86.75	84.50
6	96.25	94.50	94.00	94.90	90.75	86.25

TABLE III. EFFECT OF VARIOUS GAMMA RADIATION DOSES ON ECB EGG FERTILITY

Dose (Gy)	Cross variant ^a		Egg fertility (%)
	Male	Female	
Control	N	N	91.70
100	I	N	68.20
	N	I	8.40
200	I	N	30.80
	N	I	2.01
	I	I	1.35
250	I	N	19.10
	N	I	1.25
	I	I	0.30
300	I	N	13.50
	N	I	0.00
	I	I	0.00
400	I	N	0.06
	N	I	0.00
500	I	N	0.00
	N	I	0.00

^a I, irradiated; N, normal.

TABLE IV. STERILITY INHERITANCE OF ECB IN F₁ GENERATION COMPARED WITH P₁ GENERATION

Dose (Gy)	Egg fertility (%)	
	P ₁ ^a	F ₁ ^b
200	30.8	0.4
250	19.1	0.2
300	13.5	0.0
Control (N × N) ^c	91.7	89.2

^a P₁, irradiated male crossed with normal female (I × N).

^b F₁, male descended from irradiated male and normal female (I × N) crossed with normal female.

^c I, irradiated; N, normal.

pupae that were held at 28–30°C and 60–80% RH. Adults emerged from the treated pupae on the seventh to ninth day after pupation.

The experiment with irradiated pupae revealed that emergence depended on their age at the time of treatment and the radiation dose. The older the pupae, the less affected was their emergence, compared with the unirradiated check pupae (Table II). Adult emergence was negatively influenced directly in proportion to the radiation dose.

As a result of these preliminary trials, subsequent investigations used only six-day-old pupae, from which adults emerged within the following 48 h.

An experiment was conducted to establish the degree of sterility induced by different radiation doses. Analysis of the data in Table III shows that the percentage of sterility rose in proportion to the increased radiation dose. The two sexes presented different levels of sterility at the same radiation dose; males were more resistant to radiation than females. Complete sterility of females was obtained with 300 Gy, whereas the males were only 86.5% sterile at this dose.

Because pupal sexing is particularly difficult, it is necessary to irradiate both male and female pupae for sterile insect releases. It is required that the partially sterile males be as competitive as the normal ones. The females are completely sterile and unable to generate larvae that would subsequently attack corn plants.

In order to demonstrate the inheritance of sterility of the ECB in the F₁ generation, the following experiment was carried out. The normal females were crossed with males treated with 200, 250 and 300 Gy to produce the F₁ generation. The F₁ males were crossed with normal females, egg hatch being 0.4% at 200 Gy, 0.2% at 250 Gy and 0.0% at 300 Gy (Table IV).

TABLE V. TREATMENT OF *O. nubilalis* (Hübner) MALE WITH SUBSTERILIZING DOSE OF 100 Gy AND EFFECT ON LONGEVITY, FECUNDITY, HATCHABILITY OF P₁ AND POSSIBLE CROSSES OF F₁ AND F₂ PROGENY

Generation	Cross variant ^a		Egg masses/ female	Hatchability (%)	Longevity of adults (d)		Sterile pairs (%)
	Male	Female			Male	Female	
P ₁	N	N (Co)	7.6	90.5	8.9	7.7	4.2
	I	N	7.7	67.5	7.5	6.7	6.7
F ₁	N	N (Co)	7.4	88.0	6.9	7.0	5.7
	A	N	7.6	53.6	8.1	8.3	8.5
	N	A	7.2	59.2	7.2	7.8	3.2
	A	A	6.0	25.4	6.1	6.8	8.2
F ₂	N	N (Co)	6.8	85.3	8.7	7.9	6.8
	N	(A × N)	7.2	70.1	8.2	8.1	8.1
	N	(N × A)	6.9	80.1	8.3	7.8	8.4
	(A × N)	N	6.7	64.0	7.9	8.0	8.9
	(A × N)	(N × A)	7.1	45.2	8.1	7.6	7.1
	(A × N)	(A × N)	6.5	38.3	7.8	7.9	12.8
	(N × A)	N	7.3	68.4	8.5	8.1	7.5
	(N × A)	(A × N)	7.0	49.3	8.7	8.2	8.6
	(N × A)	(N × A)	6.7	32.8	8.1	8.2	14.3

^a N, normal; I, irradiated; A, progeny from irradiated male and normal female (I × N); Co, control.

These data revealed that in the F_1 generation male sterility is complete at 300 Gy and almost complete at 200 and 250 Gy. Compared with the sterility in the P_1 generation (69.2% at 200 Gy; 80.9% at 250 Gy and 86.5% at 300 Gy), the sterility of the F_1 generation was much higher.

P_1 pupae were also irradiated as six-day-old pupae with 100 or 150 Gy. The emerging males were crossed with normal females. The offspring of these crossings were inbred or backcrossed. Data for F_1 and F_2 crosses were recorded for number of egg masses/female, percentage of egg hatch, adult life span and percentage of sterile pairs.

The data in Tables V and VI show no significant lowering of the number of egg masses laid per female in the P_1 , F_1 or F_2 generations, after irradiation with 100 and 150 Gy, respectively. In general, the number of egg masses was a little lower when adults descended from the P_1 cross of $I \times N$ were inbred.

We failed to obtain a sufficient population to test in the F_2 population descended from $I \times I F_1$. Although the number of eggs hatching reached 25.4% for 100 Gy and 0.1% for 150 Gy, the larvae obtained did not reach the adult stage.

In all combinations tested in F_1 , hatching was reduced by increasing the radiation dose. The hatching percentage in F_1 was lower in the three groups descending from $P_1 I \times N$, when compared with the parent generation. Egg viability in the F_1 generation was obviously reduced, in comparison with the control, for both doses applied, indicating the existence of a recessive lethal effect within these populations. In the F_2 generation, a decrease of egg hatching percentage was also noted in all combinations tested, indicating the inheritance of harmful effects induced by males being irradiated in the parent generation. It is remarkable that groups analysed in the F_2 generation, though more fertile than those in the F_1 , still exhibited a noticeable effect of lethal genes. No significant reduction of adult life span was noted in the P_1 , F_1 or F_2 generations.

The percentage of sterile couples usually increased in the progeny of irradiated males, reaching the highest values in inbred groups. This experiment showed that the F_1 sterility obtained at 100 Gy was generally less than at 150 Gy. Consequently, a radiation dose of 150 Gy was used for field cage releases.

Experiments performed in 1990, and ongoing at the time of writing (1991) in $3 \text{ m} \times 2 \text{ m} \times 2 \text{ m}$ field cages, demonstrated a theoretical possibility of controlling the pest by releases of insects having inherited F_1 sterility (Table VII).

The data in this table demonstrate no significant reductions in the number of egg masses laid by irradiated females, except for group 4. The percentage hatching was lower in all groups using F_1 moths and this effect still persisted in the F_2 generation but disappeared in the F_4 generation.

The percentage of stems attacked and the number of overwintering larvae per stem were correlated with the hatching percentage.

Releases of ECBs in the F_1 generation in field cages demonstrated that F_1 sterility can be used as a part of an integrated control system along with other means of control.

TABLE VI. TREATMENT OF *O. nubilalis* (Hübner) MALE WITH SUBSTERILIZING DOSE OF 150 Gy AND EFFECT ON LONGEVITY, FECUNDITY, HATCHABILITY OF P₁ AND POSSIBLE CROSSES OF F₁ AND F₂ PROGENY

Generation	Cross variant ^a		Egg masses/ female	Hatchability (%)	Longevity of adults (d)		Sterile pairs (%)
	Male	Female			Male	Female	
P ₁	N	N (Co)	7.6	90.5	8.9	7.7	4.2
	I	N	8.1	58.3	6.9	7.5	10.2
F ₁	N	N (Co)	7.4	88.0	7.5	7.0	5.7
	A	N	7.5	42.8	7.2	7.5	18.7
	N	A	8.0	40.7	8.2	6.7	15.2
	A	A	7.1	9.1	6.0	5.9	22.5
F ₂	N	N (Co)	7.7	88.7	7.4	7.2	4.3
	N	(A × N)	6.9	60.3	8.5	7.9	10.5
	N	(N × A)	7.3	64.7	7.7	7.8	11.2
	(A × N)	N	8.0	55.9	8.2	8.0	10.1
	(A × N)	(N × A)	7.5	59.2	8.1	8.0	12.3
	(A × N)	(A × N)	7.8	33.5	7.9	7.3	18.7
	(N × A)	N	6.9	60.8	8.9	7.9	8.0
	(N × A)	(A × N)	8.7	53.2	7.5	7.8	10.8
	(N × A)	(N × A)	7.3	29.9	7.8	7.3	21.1

^a N, normal; I, irradiated; A, progeny from irradiated male and normal female (I × N); Co, control.

TABLE VII. RESULTS OBTAINED AT FUNDULEA IN FIELD CAGE RELEASE TESTS IN 1990

Number of variant	Cross variant		Egg masses/ female	Egg hatchability (%)	Stems damaged (%)	Number of hibernating larvae/stem	Kernel yield/ plant (g)
	Male	Female					
1	Isolated control		2.5	42.5	76	1.92	27
2	F ₁ ^a	N ^b	2.7	20.3	4	0.04	57
3	N	F ₁ ^a	2.6	19.5	8	0.08	42
4	F ₁ ^a	F ₁ ^a	1.9	3.7	0	0.00	46
5	F ₂ ^a	N	2.8	25.7	36	0.60	40
6	F ₂ ^a	F ₂ ^a	2.3	14.2	16	0.28	36
7	F ₄ ^a	F ₄ ^a	2.5	40.3	80	1.80	31
8	Isolated control		—	33.3	8	0.08	58

^a Progeny from P males irradiated with dose of 150 Gy and normal female. N, normal (unirradiated).

4. USE OF SYNTHETIC SEX PHEROMONES IN BIOLOGICAL AND ECOLOGICAL RESEARCH ON ECB

Taking into account the possibility of using F_1 sterility as a component of an integrated control system for the ECB, great attention was paid to elucidating its flight dynamics in the field by means of pheromone traps.

TABLE VIII. NUMBER OF *O. nubilalis* (Hübner) MALES CAUGHT/TRAPPED IN 1987

Locality	Period	Type of pheromone	
		E5 ^a	I ^b
Lovrin	13 May - 8 Sep.	12.25	30.50
Fundulea	4 June - 17 Sep.	30.00	21.25
Valu Traian	4 June - 17 Sep.	28.75	23.00
Podu Iloaiei	24 June - 3 Aug.	10.00	0.50
Turda	1 June - 25 Sep.	4.00	—

^a Average value, 17.00.

^b Average value, 15.05.

TABLE IX. NUMBER OF *O. nubilalis* (Hübner) MALES CAUGHT/TRAPPED IN 1988

Locality	Period	Type of pheromone	
		E5 ^a	I ^b
Lovrin	6 May - 2 Sep.	15.35	3.00
Fundulea	6 June - 6 Sep.	28.00	3.50
Valu Traian	2 June - 28 Sep.	57.00	8.33
Oradea	2 June - 25 Aug.	36.00	37.66
Podu Iloaiei	8 June - 15 Aug.	20.66	0.66
Turda	20 May - 29 Aug.	5.66	0.66
Suceava	16 June - 1 Sep.	7.66	0.66

^a Average value, 24.33.

^b Average value, 7.78.

TABLE X. NUMBER OF *O. nubilalis* (Hübner) MALES CAUGHT/TRAPPED IN 1989

Locality	Period	Type of pheromone	
		E5 ^a	I ^b
Lovrin	13 June – 12 Sep.	32.50	—
Fundulea	24 May – 14 Sep.	50.75	5.25
Valu Traian	17 June – 13 Sep.	37.75	12.50
Oradea	13 June – 31 Aug.	27.00	11.50
Podu Iloaiei	1 June – 17 Aug.	42.50	—
Turda	22 May – 11 Sep.	101.66	4.00
Suceava	15 June – 31 Aug.	11.25	0.00

^a Average value, 43.34.

^b Average value, 6.65.

TABLE XI. NUMBER OF *O. nubilalis* (Hübner) MALES CAUGHT/TRAPPED FROM 1987 TO 1989

Locality	Type of pheromone	
	E5	I
Lovrin	20.02	16.75 ^a
Fundulea	36.25	10.00
Valu Traian	41.16	12.19
Oradea	31.50 ^a	25.50 ^a
Podu Iloaiei	34.39	0.58 ^a
Turda	37.10	1.55
Suceava	9.45 ^a	0.33 ^a

^a Two year results.

The results of Tables VIII–XI showed the relatively high number of ECB males captured throughout the experimental period, this varying with locality and pheromone variant used. In Romania, both *cis* and *trans* phenotypes of this species are present. The latter seems to be missing in the central and northeastern zones (Podu Iloaiei, Turda, Suceava). The *trans* phenotype was generally less numerous.

Even though the synthetic sex pheromone developed in Romania is less efficient than a sample of pheromone obtained from the United States of America, it can be used to record the flight of ECB males, thus providing flight curves for the first and second generations.

We stress that in Romania, the first generation, occurring in the second half of June, is particularly important. The second generation, specific to the southern part of the country, appears in August, and is practically devoid of damaging potential. This generation is significant only for the successive corn crops on which numerous pest populations congregate.

Sex pheromones are now used in ecological studies, mainly regarding the migration and evaluation of pest populations.

The development of a technique for sterile insect releases assumes that there are some practical means to evaluate insect populations existing in a certain area. Our results with pheromone traps for *O. nubilalis* show this is possible.

5. LABELLING ADULTS FOR RELEASE AND RECAPTURE

Attempts to label adults with fuchsine or fluorescent dyes were successful in cages but failed in the field. This was due to the trauma to which insects were exposed, and to the relatively tedious technique required to visualize the marker.

At present, Calco red dye is used in investigations on the pest dynamics and estimation of the natural population. This marker worked very well in our trials in labelling adults obtained from larval rearing on the regular diet, to which the dye had been added.

TABLE XII. EFFECT OF CALCO RED DYE ADDED TO THE DIET OF THE ECB

Dye concentration ^a	Number of pupae/ rearing box ^b	Adult emergence (%)	Egg masses/ female	Egg fertility (%)
0.00	105.25	82.50	3.7	90.30
0.01	98.00	78.25	2.8	91.50
0.02	99.75	78.00	3.1	85.70

^a Dye was diluted in sunflower oil at a rate of 10 mL oil for 1 g of dye.

^b Fifteen egg masses per rearing box.

No significant differences were noted in preliminary tests between the control group and groups with various amounts of dye (0.01 and 0.02 g/mL) in terms of the number of pupae obtained per rearing box, percentage of moths emerged, number of egg masses per female and egg fertility (Table XII).

6. CONCLUSIONS

In order to study the possibility of using radiation induced F_1 sterility for the control of the ECB, the establishment of mass rearing on an artificial diet under laboratory conditions became imperative. As a result of a large range of experiments, a convenient mass rearing technique and an artificial diet, composed essentially of indigenous ingredients, have been developed. When evaluating the performance of *O. nubilalis* moths reared for various numbers of generations on an artificial diet, we found that the fecundity and longevity values did not exhibit significant differences between generations.

Investigations regarding the effect of various gamma radiation doses on ECB pupae for F_1 sterility indicated that emergence depended on their age at treatment, and on the radiation dose. In older pupae emergence was greatest at the lower dose. The degree of sterility rose in proportion to the increased radiation dose. The two sexes of ECB presented different levels of sterility at the same radiation dose; males were more resistant to radiation than females. Complete sterility of females was obtained with 300 Gy. Males had partial sterility (86.5%) at the same dose.

Experiments on sterility inheritance of ECB irradiated at substerilizing doses revealed that the F_1 generation sterility was higher than the P_1 generation sterility. In the F_2 generation, inherited sterility was also noted, but at a lower rate than in the F_1 generation.

Data obtained in field cage releases, using a radiation dose of 150 Gy, demonstrated the possibility of controlling the pest by releases of insects having inherited F_1 sterility.

In order to develop a sterile insect release technique, attention was paid to the investigations on the ECB dynamics and the estimation of the natural population by means of pheromone traps and markers. It was found that the synthetic sex pheromone developed in Romania can be used to monitor male flight. Likewise, Calco red dye worked very well in labelling adults obtained from larval rearing on a regular diet, to which dye was added.

7. PROSPECTS

Judging from the above mentioned facts, it seems that the inherited F_1 sterility of the ECB could be a component of an integrated control system along

with biological control agents (*Bacillus thuringiensis*, *Trichogramma* spp.) and resistant corn hybrids.

For that purpose, the following detailed investigations are needed:

- (a) Mark and recapture of males in order to establish the dispersal capacity and level of population in certain corn growing areas;
- (b) Field cage releases including interaction of different factors (the inherited F_1 sterility, *B. thuringiensis*, *Trichogramma* spp., tolerant corn hybrids);
- (c) Determination of the most efficient ratio between wild and F_1 males in field cage and large scale releases;
- (d) Assessment of the impact of inherited sterility on seasonal populations of ECB in a small corn growing area encircled by forest;
- (e) Determination of the effects of inherited sterility as a function of distance from release sites;
- (f) Determination of the effects of inherited sterility on the ability of *O. nubilalis* to overwinter;
- (g) Studies on inheritance of radiation induced chromosomal aberrations;
- (h) Research on hybridization of the ECB (*O. nubilalis*) and the Asian corn borer (*O. furnacalis*);
- (i) Investigate genetic sex linked markers for ECBs.

REFERENCES

- [1] PAULIAN, F., BARBULESCU, A., MUSTEA, D., BELU, V., PEIU, M., A contribution to the knowledge of the biology and control of maize borer (*Pyrausta nubilalis* Hb.), RPR, An. ICCA, Ser. B 29 (1961) 397-420.
- [2] PAULIAN, F., et al., The evolution of European Corn Borer (*Ostrinia nubilalis* Hb.) and damaging potential recorded between 1971-1975, Romania, Probl. Prot. Plant. 6 1 (1976) 23-48.
- [3] VOINESCU, I., BARBULESCU, A., Efficacy of granular insecticides in control of corn borer (*Ostrinia nubilalis* Hb.), An. ICCPT 53 (1986) 383-386.
- [4] GALANI, C., VOINESCU, I., BARBULESCU, A., Efficacy of some microbiological products based on *Bacillus thuringiensis* Berliner in control of corn borer (*Ostrinia nubilalis* Hb.), An. ICCP 16 (1979) 235-241.
- [5] ROSCA, I., BARBULESCU, A., Numerical limiting by biologic factors of corn borer (*Ostrinia nubilalis* Hb.), Romania, St. Cerc. Biol. Seria Biol. Anim. 35 (1983) 32-35.
- [6] ROSCA, I., BARBULESCU, A., Attempts on biological testing of *Bacillus thuringiensis* products with *Ostrinia nubilalis* Hb. as a test species, Probl. Prot. Plant. 14 (1986) 133-140.
- [7] ROSCA, I., BARBULESCU, A., VONICA, L., Preliminary data regarding role of biological factors in reducing populations of *Ostrinia nubilalis* Hb., Plant Protection (Proc. 8th Natl Conf. Iasi, 1983), Academia de Ştiinţe Agricole Şi Silvici, Fundulea (1983) 293-310.

- [8] ROSCA, I., BARBULESCU, A., PISICA, C., VONICA, I., Spreading in Romania and role of species *Sinophorus crassifemur* Thoms. and *Lydella thomsoni* Hert., parasite on grubs of *Ostrinia nubilalis* Hb. St. Cerc., Biol. Seria Biol. Anim. 36 (1984) 92-95.
- [9] BARBULESCU, A., Studies conducted at Fundulea on maize resistance to *Ostrinia nubilalis* Hb., Probl. Prot. Plant. 9 (1981) 373-380.
- [10] LESCUS, A., SARCA, T., The behaviour of sweet corn lines and hybrids to the attack of *Ostrinia nubilalis* Hb., Probl. Prot. Plant. 8 (1980) 151-157.
- [11] BARBULESCU, A., SARCA, T., Testing of some hybrid combinations between maize lines tolerant to the European Corn Borer (*Ostrinia nubilalis* Hb.), Probl. Prot. Plant. 8 (1983) 5-9.
- [12] BARBULESCU, A., COSMIN, O., SARCA, T., BICA, N., NEGUT, C., Testing for resistance to *Ostrinia nubilalis* Hb. of some maize lines (1975-1978), Probl. Prot. Plant. 9 (1981) 36-38.
- [13] BARBULESCU, A., COSMIN, O., SARCA, T., BICA, N., NEGUT, C., Testing for resistance to *Ostrinia nubilalis* Hb. of some maize lines (1979-1981), Probl. Prot. Plant. 10 (1982) 93-97.
- [14] BARBULESCU, A., COSMIN, O., New data regarding behaviour of corn lines and hybrids to the attacks of the borer (*Ostrinia nubilalis* Hb.), An. ICCPT 46 (1980) 341-346.
- [15] BARBULESCU, A., COSMIN, O., Maize inbred lines with some degree of resistance to *Ostrinia nubilalis* Hb., Probl. Prot. Plant. 15 4 (1987) 301-306.
- [16] ROSCA, I., "Researches regarding using of gamma rays as genetical control of European Corn Borer (*Ostrinia nubilalis* Hb.)", Environmental Protection (Proc. 8th Natl Symp. Constanta, 1991), Academia de Ştiinţe Agricole Şi Silvice, Fundulea (1991) 125-131.
- [17] ROSCA, I., et al., "Possibilities of using synthetic sexual pheromones in protection of cereal and technical crop cultures", Plant Protection (Proc 9th Natl Conf. Bucharest, 1985) Vol. 2, Academia de Ştiinţe Agricole Şi Silvice, Fundulea (1985) 1-13.
- [18] ROSCA, I., et al., personal communication, 1989.
- [19] BARBULESCU, A., ROSCA, I., "Results regarding sterilization through irradiation of male *Ostrinia nubilalis* Hb., important pest of corn in Romania", Radiation-induced F₁ Sterility in Lepidoptera for Area-wide Control (Proc. Mtg Beijing, 1989), FAO/IAEA, Vienna (1989) 7 pp.
- [20] ROSCA, I., "Researches regarding sexual synthesis pheromones of moths injurious of field crops", Environmental Protection (Proc. 8th Natl Symp. Constanta, 1991), Academia de Ştiinţe Agricole Şi Silvice, Fundulea (1991) 142-150.

STUDIES ON CHROMOSOMAL ABERRATIONS AND INHERITED STERILITY IN ASIAN CORN BORER, *Ostrinia furnacalis* (Guenee)

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Abstract

STUDIES ON CHROMOSOMAL ABERRATIONS AND INHERITED STERILITY IN ASIAN CORN BORER, *Ostrinia furnacalis* (Guenee).

F₁ sterility and chromosomal aberrations in the Asian corn borer, *Ostrinia furnacalis* (Guenee), were induced by different doses of gamma radiation. The chromosome number of the Asian corn borer is $n = 31$ pairs. The results showed that chromosomal aberrations in spermatocytes of the F₁ generation were directly related to high F₁ sterility; however, the sterility was observed for only one generation and fertility was recovered in the next generation.

1. INTRODUCTION

The Asian corn borer (ACB), *Ostrinia furnacalis* (Guenee), is a destructive insect pest in maize production. Many studies have been conducted to find ways of controlling it. One of these studies, inherited sterility (F₁ sterility), is a promising management system and has shown its advantage compared with other conventional methods. Similar to other lepidopterous insects, ACB is radioresistant, so that higher radiation doses are needed to induce sterility. However, research has shown that high radiation doses significantly reduce the longevity, flight ability and mating competition of irradiated insects. It has been shown that the lower the radiation dose used, the higher the competitive ability of the treated insects. However, by reducing the dose in order to alleviate somatic damage, sterility was also reduced, which poses a dilemma. Radiation injures not only the somatic cells of the insect but the chromosomes in the germ-cells as well. It is well known that the chromosome contains the genetic material, so the severity and quantity of damage to germ-cells would directly affect the fertility of irradiated insects.

Preliminary studies relating to different radiation doses, to induced sterility and to chromosomal aberrations in ACB are reported.

2. MATERIALS AND METHODS

The ACB were reared in a laboratory using an artificial diet at room temperature and 50–70 RH. Pupae were irradiated with gamma radiation at doses of 100, 150, 200 and 250 Gy, one or two days before adult emergence. Adults were immediately paired with normal individuals of the opposite sex. Eggs were collected daily and then cages of ACB with different treatments were reared and the fertility was determined. The rates of hatching, pupation and emergence were calculated in each generation.

The chromosome slides were prepared as described in Sections 2.1 and 2.2.

2.1. Air dried method

The larval testes of third to fifth instars from 35–60 individuals were dissected in insect-Ringer's solution, transferred into 0.7% hypotonic sodium citrate solution and left for 10 min and then divided into pieces. The germ-cells were sedimented by centrifugation for 10 min at 800–1000 rev./min and were fixed in Carnoy solution (with a ratio of absolute ethyl alcohol to glacial acetic acid of 3:1) for 10 min and sedimented again. Then they were fixed again in Carnoy solution and sedimented once more. The air dried preparations were made from the final fixed suspension. Some of the suspension was taken with a small pipette and dropped on a cold slide that was chilled in ice-water. The preparations were dried at room temperature and stained with 10% Giemsa in Sorensen's phosphate buffer (pH6.8) for 20–30 min. Then they were examined and photographed.

2.2. Cell suspension method

Another chromosome preparation method was also tried because it required fewer larvae compared with the method mentioned above. The larval testes from third to fifth instars — 10–20 individuals for each treatment — were dissected in insect-Ringer's solution, transferred into Carnoy solution and fixed for about 10 min. Then one to two droplets of 45% glacial acetic acid were dropped on the slide, which was preheated to about 40°C. Four to six fixed testes were placed into acetic acid, and the testes squashed to release the germ-cells. Finally, some Carnoy solution was dropped on the slide. The slides were stained with 10% Giemsa in Sorensen's phosphate buffer (pH6.8) for 20–30 min, after which they were dried at room temperature. They were then examined and photographed.

3. RESULTS AND DISCUSSION

3.1. Chromosome analysis

Chromosomes of ACB, like many other species of Lepidoptera, are either polycentric or possess diffuse centromeres. The analysis of chromosomes showed that the ACB has $n = 31$ pairs of chromosomes (Fig. 1). Among the individual

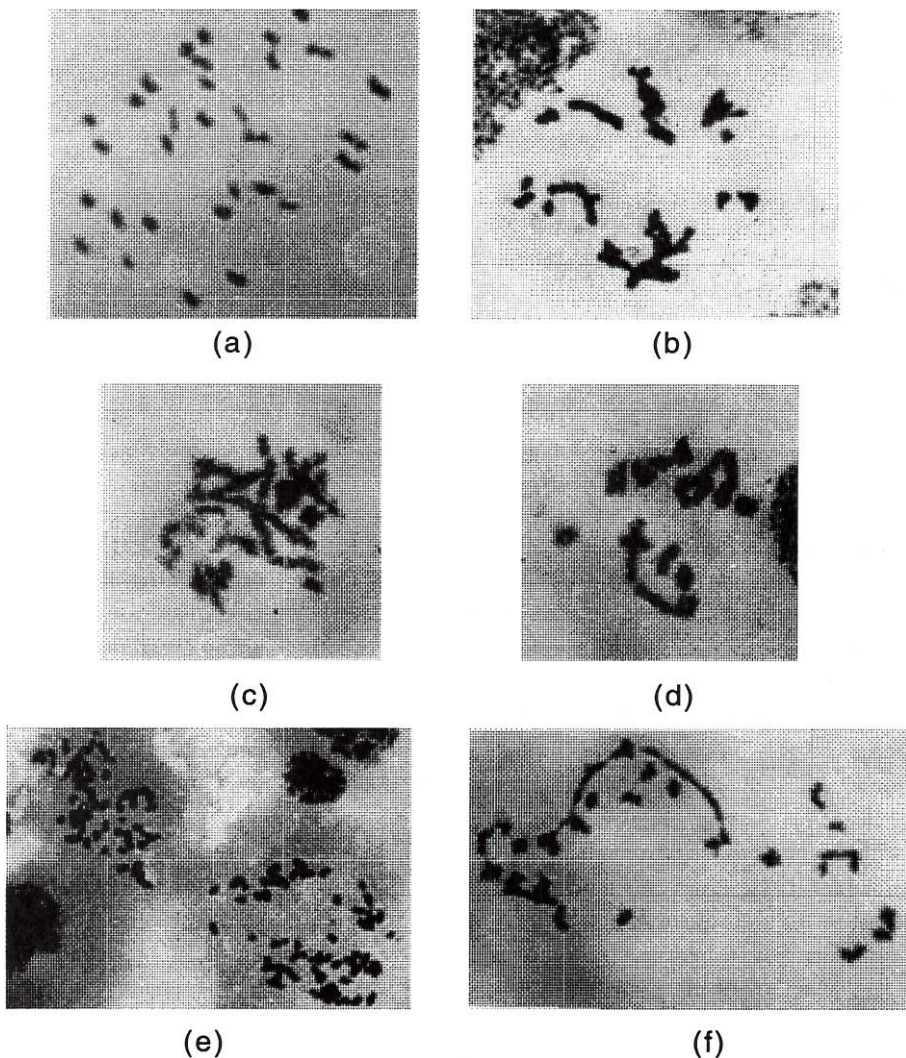


FIG. 1. Meiotic metaphase chromosomes of the ACB: (a) normal chromosomes, (b)–(f) abnormal chromosomes (e.g. chains in (b), (c), (d), (f) and fragments in (e)).

TABLE I. COMPARISON OF CHROMOSOMAL ABERRATIONS IN F₁ AND F₂ MALES WITH DIFFERENT TREATMENTS

(♂ was the P₁ sex irradiated and the metaphase rate was about 0.04% of examined cells)

Dose (Gy) to parent ^a	Number of metaphases examined	Number of abnormal metaphases	Number of normal metaphases	Abnormality (%)
<i>F₁ generation</i>				
100	17	13	4	76.5
150	41	32	9	78
200	23	19	4	82.6
250	20	17	3	85
<i>F₂ generation</i>				
100	53	12	41	22.7
150	57	15	42	26.3
200	—	—	—	—
250	—	—	—	—

^a No data were obtained with 200♂F₂ and 250♂F₂ because of F₁ sterility.

chromosomes of ACB there were no significant differences in length, and they were dot-like chromosomes. It is difficult to measure the length of the chromosome with conventional chromosome analysis methods because they are so small that the deviations of the chromosome in different cells may be as long as the length of the chromosome, or even longer. This polycentric type of chromosome structure could explain the radioresistance of the ACB. In the common chromosome structure, each chromosome has only one centromere. Radiation induced chromosome breaks in sperm with monocentric chromosomes result in chromosomal fragments without a centromere that are lost during cell division and lead to dominant lethal mutations. However, with the polycentric chromosome, which has more than one region for spindle fibre attachment, the fragments induced by radiation will very probably have at least one centromere and so can function as a normal chromosome, so fewer dominant lethal mutations would be expected.

Many types of chromosomal aberrations were found in the progeny produced by irradiated parents, such as fragments, chains and rings. The study showed that there were significant differences between the F₁ and F₂ generations in the number of chromosomal aberrations. About 80% of the metaphases examined were abnormal or had chromosomal aberrations in the F₁ generation, while only about 20% were

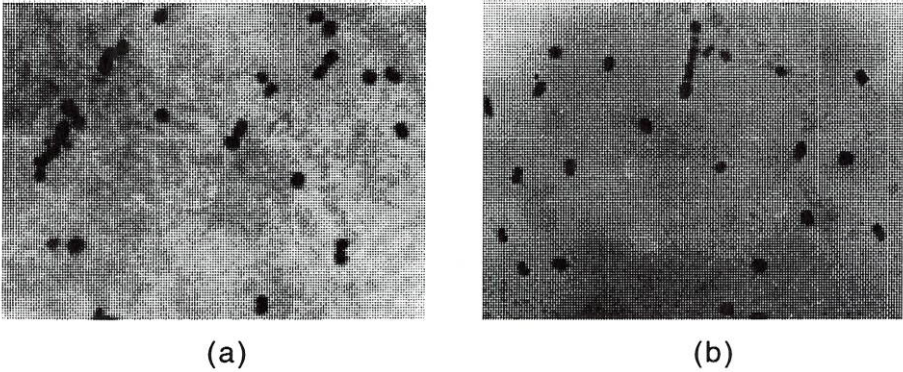


FIG. 2. Abnormal chromosomes in the F_2 generation: long chromosome chains are visible in both (a) and (b).

in the F_2 generation (Table I). In addition, more severe abnormalities in chromosomes were found in the F_1 generation than in the F_2 generation. For example, in the F_1 generation a chain could involve more than seven individual chromosomes but only two to three chromosomes in the F_2 generation (Fig. 2). Table I also shows that there were no significant differences between the different treatments. Table I shows only percentage abnormal metaphases among the total number of metaphase cells examined. No attempt was made to quantify the degree of abnormality in individual cells.

Broken chromosomes can reunite in many ways. A typical chromosomal rearrangement is the reciprocal translocation in which parts of two different broken chromosomes have rejoined. In general, most such reciprocal translocations can be transmitted to the offspring because each of them has a centromere in them. However, some chromosomal rearrangements may occur with one or two acentric fragments (without any centromeres) and dicentric chromosomes (one chromosome with two centromeres). Such dicentric chromosomes can produce a bridge during division which, when broken, may result in deficiency or duplication of genetic material in daughter cells. The cells will die. That is the usual action of dominant lethal mutations with the monocentric chromosome structure. However, it is not necessarily a problem with polycentric chromosome structures in mitotic divisions when such a chromosome rearrangement occurs because of its specific chromosome structure. However, it is fatal to a progeny in meiosis because this type of rearrangement produces gametes with deficient or duplicated genetic material that are contained in the germ-cells and this condition is lethal to embryos. This phenomenon is the main cause of F_1 sterility.

3.2. Inherited sterility in F₁ and F₂ generations of ACB

Table II shows that the sterility of the P generation became higher as the dose increased while 200 and 250 Gy treated males showed no significant difference. However, the sterility for all the different treatments increased sharply from the P generation to the F₁ generation, even though lower irradiation doses were used in the parents, such as 100 and 150 Gy (Table III). This showed that the above assertion that chromosome aberrations are the major cause of F₁ sterility was the best explanation of such results. Thus, F₁ sterility is related to chromosome aberrations in F₁,

TABLE II. COMPARISON OF EGG HATCH WHEN ACB MALES ARE TREATED WITH VARIOUS DOSES OF GAMMA RADIATION

(♂ was the P₁ sex irradiated)

Dose (Gy) to parent	Number of eggs	Number of eggs hatched	Eggs hatched (%)
Control	3210	3107	96.8
100	2578	1115	43.3
150	3556	1505	42.3
200	3238	902	27.9
250	3158	860	27.2

TABLE III. INHERITED STERILITY IN THE F₁ ACB FROM PARENTS WITH DIFFERENT TREATMENTS

(♂ was the P₁ sex irradiated)

Dose (Gy) to parent	F ₁ sex outcrossed	Number of eggs	Number of eggs hatched	Eggs hatched (%)
Control	—	2794	2655	95
100	♀	1615	450	27.9
100	♂	1260	309	24.5
150	♀	1180	91	7.7
150	♂	1183	297	25.1
200	♀	1444	203	14.1
200	♂	823	27	3.3
250	♀	735	7	0.95
250	♂	1041	3	0.3

TABLE IV. INHERITED STERILITY^a IN THE F₂ ACB FROM PARENTS OF F₁*(♂ was the P₁ sex irradiated)*

Dose (Gy) to parent ^a	F ₁ sex outcrossed	F ₂ sex outcrossed	Number of eggs	Number of eggs hatched	Eggs hatched (%)
Control	—	—	4638	4310	93
100	♀	♀	1424	575	40.4
100	♀	♂	5359	4958	93
100	♂	♀	586	511	94
100	♂	♂	4788	3751	78.3
150	♀	♂	197	176	89.3
150	♂	♀	1651	1217	93.7
150	♂	♂	3457	3159	91.4

^a No data on the F₃ generation with 200♂ F₁♂ × N♀ and 250♂ F₁♂ × N♀ treatments were obtained because of F₁ sterility. No F₃ progeny were produced in lines where the male parent had received 200 or 250 Gy.

either to the quantity of germ-cells with chromosome aberrations or to the abnormal degree of chromosomes, in other words, the frequency of chromosomal rearrangements induced by radiation. The results in Table IV show that the fertility of the F₂ generation recovered greatly regardless of the dose used in the P generations. The egg hatching rate of the F₃ generation from different treatments was similar. It was inferred that few chromosome rearrangements were transmitted in F₂ adult progeny because most of the F₂ generation died as a result of duplications or deficiencies of genetic material caused by chromosomal rearrangements.

BIBLIOGRAPHY

LaCHANCE, L.E., Genetic Methods for the Control of Lepidopteran Species: Status and Potential, Rep. ARS-28, US Department of Agriculture, Washington, DC (1985).

LaCHANCE, L.E., et al., Cytogenetics of inherited partial sterility in three generations of the large milkweed bug as related to holokinetic chromosomes, *Chromosoma* 29 (1970) 20-41.

LI, Y.Y., KANG, W., Cytogenetic study on the sterility of peach fruit borer *Carposina nipponensis*, Wals. Acta Agric. Nucl. Sin. 2 4 (1988) 207-213.

NORTH, D.T., HOLT, G.G., Inherited sterility in progeny of irradiated cabbage loopers, J. Econ. Entomol. 61 (1968) 928-931.

SNIEDER, D., TER VELDE, H.J., Inherited partial sterility and chromosomal rearrangements in succeeding generations after irradiation of *Adoxophyes orana* (Lepidoptera Tortricidae) with substerilizing doses of X-rays and fast neutrons, *Entomol. Exp. Appl.* **18** (1975) 321-328.

ZHANG, H.Q., et al., Possibility of using the sterile-insect technique to control the corn borer, *At. Energy Sci. Technol.* **6** (1965) 548-556.

GYPSY MOTH F₁ STERILITY PROGRAMME: CURRENT STATUS

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Abstract

GYPSY MOTH F₁ STERILITY PROGRAMME: CURRENT STATUS.

In the United States of America the gypsy moth, *Lymantria dispar* (L.), is progressively invading the country from its original site of introduction in Massachusetts. The use of F₁ sterility is being developed to eradicate the isolated infestations that occur sporadically far beyond the generally infested zone. F₁ sterility is also being developed to retard the rate of expansion of the generally infested zone. The optimal dose to induce F₁ sterility appears to be 80 Gy of gamma rays. Pilot trials are being carried out in which treated male pupae are distributed into the wild, or in which the F₁ progeny of treated males and normal females are distributed during the egg stage. The latter approach permits mass rearing during most of the year and the stockpiling of diapausing eggs, as well as simplified shipment and distribution. In the past, mass rearing proved to be somewhat unreliable because of the occurrence of 'abnormal performance syndrome' or APS. This syndrome is characterized by a poor hatch, high mortality of neonates and retarded larval development. The syndrome is diet related, and can be avoided by fastidious attention to meeting the nutritional requirements of the rearing stocks. A special container was designed for the shipment and release of pupae. Also, a special formulation of the pheromone was developed that appears to permit the correlation of trap catches with local egg mass densities. Released males are marked internally with Calco red dye or externally with a fluorescent dye. Also, a morphometric method has been developed to distinguish between wild and released moths. F₁ males mate readily, but the frequency of sperm transfer is low. Nevertheless, wild females mated to F₁ males appear to behave as though they had been mated to normal males.

The gypsy moth, *Lymantria dispar* (L.), was introduced into the United States of America in Medford, Massachusetts, in 1969. Since that time it has greatly expanded its range at the rate of 16–24 km a year through natural spread. Currently it is one of the most important forest pests in the USA. During the last major outbreak in 1981 this pest defoliated over 5 million hectares. Within the generally infested area, emphasis is placed on reducing the impact of defoliation. Along the leading edge of the infested area, current thinking is that a programme should be in

place to slow or contain the natural spread. An additional problem presented by the gypsy moth is that populations are often transported from the generally infested areas artificially by human activities. Most often these transported populations are associated with the movement of household articles and are found in residential areas. Annually, to detect these transported populations 300 000 pheromone baited traps are deployed. Current thinking is that two types of populations are good targets for control using the sterile insect technique: isolated populations in residential areas and sparse populations along the leading edge of the generally infested area.

Broadcast applications of pesticides in residential areas are becoming less acceptable; the development of alternative target specific tactics such as the sterile insect technique have become a high priority for the United States Department of Agriculture. In addition, in discussions about a proposed containment programme for the leading edge of the generally infested area, it becomes apparent that target specific, environmentally sensitive and socially acceptable control tactics will be a necessary component of any integrated programme.

Basic biology

A brief description of the insect biology of the gypsy moth will illustrate why the F_1 sterility programme has evolved in the way it has.

The gypsy moth is univoltine and overwinters as diapausing eggs. Hatching occurs in early spring about the time of bud breaking of some of its *Quercus* hosts. Male larvae generally have five instars and females six. Later, instars of both sexes normally feed at night and migrate to resting locations on tree boles or on the ground for the day. Insects normally pupate near larval resting sites. In sparse populations these pupation sites are usually hidden locations. The pupal period lasts for 12–14 d. Adults do not feed and are active during the day. Males, which normally only live for 2–3 d, may mate several times on any given day. Females, which normally mate only once, begin oviposition within a half-hour of completing mating and normally only oviposit one egg mass containing from 200 to 1600 eggs. Sperm transport from the bursa to the spermatheca is rapid and occurs within minutes of mating.

Radiation biology

Basically, 150 Gy administered to late stage male pupae is completely sterilizing. To induce nearly total sterility in the F_1 generation, 100 Gy administered to males is adequate. Lowering the dose to 80 Gy produces a nearly totally sterile F_1 generation but has the advantage of producing a higher number of more competitive insects.

Currently we are evaluating the F_1 sterility technique for use in these two settings; again for eradication of isolated populations and for suppression of popula-

tions along the leading edge of the generally infested area. Two different application techniques have been used on a pilot scale:

- (a) Deployment of male pupae treated with a substerilizing dose of radiation;
- (b) Broadcasting F_1 progeny (egg stage) of irradiated males and normal females.

In the past both techniques have been used successfully to eradicate isolated infestations. In the summer of 1991 direct comparisons were to be made between these two release techniques in replicated blocks in sparse populations along the leading edge of the generally infested area.

Experience has shown that both techniques have advantages and disadvantages. Releases of irradiated pupae are easily timed to coincide with native male flight and theoretically offer a greater suppressive effect for dollars spent in production. In addition, a non-feeding stage of the insects is being released and, therefore, damage is not inflicted on the forest resource. Male gypsy moths, however, are short lived and must be released continuously over the flight period of approximately four weeks. Also, the production of insects is confined to a very narrow time window and, therefore, the rearing facility is being underutilized. The logistics of shipment and field release are also difficult. The release of F_1 eggs has the advantage of a single release in the spring prior to the wild egg hatch. In addition, the release of eggs permits a wider production window because diapausing eggs can be stored. Finally, the logistics of shipment and release are simplified with egg releases. The timing of release, however, is difficult, a potentially damaging stage is being released and the suppressive effects for dollars spent on production are theoretically less.

Currently we are looking into a number of areas to improve the use of techniques or to ease the evaluation burden.

Rearing

In the past we have had a major problem with our rearing effort to produce an effective competitive insect at a reasonable cost. The term we are currently using to describe this problem is abnormal performance syndrome (APS). APS is characterized by failure of eggs to hatch, neonate mortality and retarded larval development. This problem has hindered the rearing programme in the past to the point of delaying further sterile F_1 development work. An interagency team was formed to solve this perplexing problem. Progress has been made in isolating the cause(s) of this intermittent rearing problem and all experimental results to date indicate that it is diet related. The importance of these findings is paramount to the success of the F_1 sterile programme because it has been shown that the nutrition of the parental generation directly affects the performance of their F_1 progeny. In general terms, the importance of the rearing component of any sterility programme cannot be overstressed. In addition, different laboratory production strains of gypsy moth are being developed and evaluated for their competitiveness.

Release technology

To address our problems with synchronizing the F_1 egg hatch with the wild hatch, we are exploring several options. Currently we are storing F_1 eggs on the site to be treated the following year. The results of past experimental work using this technique are promising. A wide variety of field egg placement dates resulted in a spring egg hatch synchronous with the wild egg hatch. In addition, we are experimenting with various laboratory holding conditions that will shorten or prolong the holding period and still provide a synchronous hatch and competitive insects. Basic studies of gypsy moth diapause are also being conducted to understand better the mechanisms involved so that the wild hatch can be better predicted.

In 1991 a new release container was designed for pupal release. This container is used for both shipment and release, and its use has eased the burden on both operations.

Population characterization

The characterization of the distribution and density of low level populations is difficult. This information is important so that adequate numbers of sterile insects can be released to achieve the desired overflooding ratios without wasting valuable resources. In isolated populations, pheromone trapping of males has proven valuable for describing the population distribution and estimating the egg mass density. In the generally infested area, however, a relationship between males in traps and egg mass density has eluded investigators. Recently, a new low release pheromone dispenser has been developed. Captures in traps baited with this dispenser in 1990 field trials were highly correlated with local egg mass density when the traps were deployed in sparse populations along the leading edge of the generally infested area. These findings may allow more precise targeting of these populations at less expense than egg mass surveys.

Evaluation of treatment

Currently a variety of techniques are used to evaluate the impacts of releases. When irradiated male pupae are released the relative mating success or response to a pheromone can be measured by either observing males visiting monitor females (or the resulting egg masses) or by examining males in traps. Males are easily marked, either internally by mixing Calco oil red in the larval diet or externally by using fluorescent dust. Evaluation of the impact of the sterile F_1 generation, however, is more cumbersome when either the egg release or the pupal release strategies are used. Currently we are using a variety of techniques to evaluate the relative survival and competitiveness of the F_1 generation. These include male chromosome analysis, mating (egg mass) evaluation and male trapping.

We have been investigating morphometric techniques to identify the males captured in traps, and the results, although showing some promise, have not conclusively demonstrated the utility of this technique. A pure strain has been selected from laboratory cultures to determine the relative abundance of marked individuals.

We are also closely scrutinizing sperm transfer by normal and sterile laboratory males (irradiated and F_1 progeny). To date, we have found a higher incidence of sperm fragmentation in laboratory reared males and low amounts of sperm transfer by F_1 males. Ongoing mating studies have not demonstrated that F_1 males mate less often or do not elicit the mated response from females. We plan to continue our development activities. In addition, we shall treat approximately 200 hectares using the pupal release technique. We also hope that target isolated populations can be identified that we can treat with the F_1 egg release technique. We envisage the future of the sterile insect technique in gypsy moth control as being one tool, among others, that will offer the land manager advantages over pesticides in particular situations.

CROSSING EXPERIMENTS BETWEEN EUROPEAN CORN BORER, *Ostrinia nubilalis* (Hübner), AND ASIAN CORN BORER, *Ostrinia furnacalis* (Guenee), TO EXPLORE THE POSSIBILITY OF HYBRID STERILITY

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Abstract

CROSSING EXPERIMENTS BETWEEN EUROPEAN CORN BORER, *Ostrinia nubilalis* (Hübner), AND ASIAN CORN BORER, *Ostrinia furnacalis* (Guenee), TO EXPLORE THE POSSIBILITY OF HYBRID STERILITY.

In individual pair tests it was possible to obtain progeny from all possible combinations of males and females of the European corn borer, *Ostrinia nubilalis* (Hübner), and the Asian corn borer, *Ostrinia furnacalis* (Guenee). In most instances, the sex ratio of the progeny was skewed in favour of the males. In all crosses, the egg hatch was in the normal range. No evidence was found for the existence of hybrid sterility in the F_1 progeny of these crosses.

1. PRELIMINARY CROSSING EXPERIMENTS BETWEEN EUROPEAN CORN BORER AND ASIAN CORN BORER

One virgin female and one male European corn borer (ECB), collected from Yining City, Xinjian Province in northwestern China and Hebei Province in northern China, were each crossed with an Asian corn borer (ACB) placed in a cage that was enclosed in a mesh (7 cm × 7 cm × 10 cm), given only water and kept at 28°C in an 8D:16L (D, dark; L, light) photoperiod at 80% RH. Several single pair cages were set up (Table I). The number of eggs laid was recorded for 6–7 d and dead females were dissected to check for spermatophores. The eggs were incubated at 28°C for 3 d, then seeded in larva rearing boxes that contained an artificial larva diet. Pupation started after 18–20 d at 28°C and 80% RH. The sex ratio of the F_1 progeny was observed in approximate terms. The results are shown in Table I.

The results indicated that only a few pairs mated and laid eggs. It was very interesting to note that the sex ratio of the F_1 progeny from the European females crossed with the Asian males was 1:99 in favour of the male.

TABLE I. PRELIMINARY CROSSING EXPERIMENTS BETWEEN THE ACB AND THE ECB IN 1989

Cross		Number of pairs not laying eggs	Number of pairs laying eggs	Rate of mating (%)	Eggs laid per mated female	Hatching rate (%)
♀	♂					
ECB	× ACB	17	5	22.70	135.6	85.55
ACB	× ECB	23	5	17.86	245.4	76.70
ACB	× ACB	0	8	100	447.3	92.10
ECB	× ECB	1	7	87.5	217.4	95.70

$$\chi^2 = 25.25, \chi^2_{0.05,3} = 7.81, \chi^2_{0.01,3} = 11.34, \chi^2 > \chi^2_{0.01,3}.$$

2. CROSSING EXPERIMENTS BETWEEN ECB AND ACB IN 1991

European corn borers were collected from Jinhe county, Xinjiang Province, in northwestern China adjacent to the border with the former USSR. Asian corn borers were collected from Hebei Province in northern China. The handling procedures were the same as with the preliminary tests carried out in 1989. The results (Table II) indicated that there was very little reproductive isolation between the ECB and the ACB (see Table II). The sex ratio of the F_1 progeny was skewed: 1.35♂:1♀ for F_1 (♀ ECB × ♂ ACB), 1.46♂:1♀ for F_1 (♀ ACB × ♂ ECB) and 0.93♂:1♀ for the control (♀ ACB × ♂ ACB). The backcross test of the F_1 progeny with the ACB adults showed that the hatching rates in all four mating groups were very high — 82.37% for ♂ of F_1 (♀ ACB × ♂ ECB) × ♀ ACB, 93.06% for

TABLE II. CROSSING TEST OF THE ACB AND THE ECB IN 1991

Cross		Number of pairs not laying eggs	Number of pairs laying eggs	Rate of mating (%)	Eggs laid per mated female	Hatching rate (%)
♀	♂					
ECB	× ACB	37	34	47.88	354.2	91.44
ACB	× ECB	40	14	25.93	514.0	95.58
ACB	× ACB	1	9	90.00	643.9	96.82
ECB	× ECB	7	15	68.18	268.7	95.19

$$\chi^2 = 21.00, \chi^2_{0.05,3} = 7.81, \chi^2_{0.01,3} = 11.34, \chi^2 > \chi^2_{0.01,3}.$$

♀ of F_1 (♀ ACB × ♂ ECB) × ♂ ACB, 96.72% for ♂ of F_1 (♀ ECB × ♂ ACB) × ♀ ACB and 99.57% for ♀ of F_1 (♀ ECB × ♂ ACB) × ♂ ACB. Therefore there was no evidence of hybrid sterility in the F_1 progeny between the ACB collected from Hebei Province and the ECB collected from Jinhe county of Xinjiang Province.

There was a difference in the F_1 sex ratios for the ECBs collected from Yining City and Jinhe county. If the ECB were collected from Europe or North America, new results in hybrid sterility may be obtained since these are further away from central China than Yining City is from Jinhe county.

STUDY OF THE MECHANISM AND POSSIBILITIES OF USING F₁ STERILITY FOR GENETIC CONTROL OF CODLING MOTH

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Abstract

STUDY OF THE MECHANISM AND POSSIBILITIES OF USING F₁ STERILITY FOR GENETIC CONTROL OF CODLING MOTH.

A computer based model was developed to simulate the suppression through three filial generations of field populations of codling moths, *Laspeyresia pomonella* (L.), that have been exposed to the release of codling moths exposed to doses of gamma rays ranging from 1 to 500 Gy. The main purpose of the model is to select the optimal dose of radiation. The model runs on an IBM compatible computer. Numerous experiments were conducted to provide the experimental data required for the model. The model takes into account the fact that individual moths are affected in different ways by exposure to gamma rays. Some irradiated males mate and form spermatophores, while others fail to mate. Some males that form spermatophores fail to produce eupyrene sperm. These undesirable effects occur with increasing frequencies as the dose increases. Indeed, at high doses of radiation these negative effects are so great that the treated moths are totally non-competitive with the untreated moths. The release of F₁ individuals has a number of advantages for the control of the codling moth. This approach involves the mass rearing and stockpiling of diapausing F₁ larvae during the winter months. It facilitates the synchronization of the emergence and flight of genetically impaired individuals with the emergence and flight of the wild population. In addition, this approach facilitates the separation of the sexes and the release of only F₁ males. The optimum dose for this approach was found to be 100 Gy.

1. DESCRIPTION OF THE RESEARCH

The main aim of the project described in this paper was, on the basis of experimental data obtained by the All-Union Scientific Research Institute for Plant Protection (VIZR), to develop a computerized model simulating releases of codling moths that have been partially or completely sterilized by irradiation. The model was based on the principles used by Knipling and Klassen [1] for the comparative evaluation of the prospects of different types of mutation and induced sterility for the genetic control of arthropod pests. However, when modelling the possible use of F₁

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sterility, they confined themselves solely to indices reflecting the sterility levels of irradiated males and F_1 individuals (assuming the latter to be 100% sterile). Theoretical considerations regarding the mechanisms governing the inheritance of sterility, confirmed by the results of experiments carried out by our group on the codling moth, indicate that F_1 individuals and their progeny are not uniform with respect to inheritance of sterility and changes in their genetic apparatus. Moreover, the type of 'inherited sterility' (F_1 sterility) in Lepidoptera is the result of a whole range of irradiation effects, which can be both positive or negative from the point of view of the efficiency of genetic control and which manifest themselves in irradiated insects in the F_1 and subsequent generations. It is therefore extremely difficult even to select the optimum irradiation dose without having an appropriate model. The model developed by us is designed to take account of these characteristics in the irradiated insects (adult males and females), their F_1 progeny (all stages of development), the F_2 generation (all stages of development) and the F_3 generation (egg stage and emergence of larvae).

These effects were evaluated quantitatively in previous studies carried out by use on the codling moth for a wide range of gamma radiation doses (from 30 to 500 Gy). The results of these studies show that the ratio of positive to negative effects in all generations depends largely on the dose administered and on the sex of the irradiated insects. The results have been partially published [2]. However, for the sake of completeness of the model, information had to be obtained on the competitiveness of males having different types of sterility. For this purpose, special experiments were carried out to make a parallel evaluation of the ratio of the different groups of F_1 males obtained at doses of 100 Gy and of their competitiveness in field cages (for the method see Ref. [2]). In addition, the experiments were repeated to determine more accurately the dependence on the dose of the ratio of F_1 insects having different types of sterility.

An IBM compatible Samsung SD700 computer was used to create a model (Appendix) simulating the release of insects subjected to various irradiation doses, taking into account the characteristics of inherited sterility in Lepidoptera. Making allowance for the corrections introduced and additional information on the changes in the reproductive potential of the insects, we have simulated the reduction in the number of pests (codling moths) in the F_1 , F_2 and F_3 generations for different irradiation doses and different ratios of normal to sterile insects.

2. RESULTS

2.1. Improved determination of the dependence on dose

The deviation of a few points on the dose-effect curve from the law of systematic increase made it necessary for us to carry out these experiments. This

deviation related primarily to the frequency of completely sterile (mating, sp+, with spermatophores present and non-mating, sp-, with spermatophores absent) F₁ males and, in particular, females [2]. The results of the two replications, in which males were irradiated at doses of 60 and 100 Gy and of simultaneous tests subsequently carried out on the F₁ progeny irradiated at different doses, showed that the distribution of the points of the dose-effect curve referred to above does not follow a pattern. Among F₁ males and F₁ females at a dose of 100 Gy, the frequency of sp+ and sp- pairs and consequently of all completely sterile pairs (where all oviposited eggs were non-viable and showed no signs of development) was higher than at a dose of 60 Gy in both replications.

2.2. Evaluation of competitiveness of males

It is logical to assume that the radiation induced hereditary changes revealed in laboratory tests in the form of increased frequency of non-mating males (sp-) would lead to a total loss of their competitiveness under natural conditions as well. This is not so obvious in the case of the other group of males, namely those that mate and form spermatophores but are completely sterile (sp+), although it is perfectly possible since a significant proportion of them should be males that manifest the impaired formation and transfer of eupyrene sperm characteristic of F₁ sterility in Lepidoptera. Our first laboratory experiments to study this problem using a dose of 300 Gy, Ref. [2], demonstrated that almost all sp+ males were totally uncompetitive.

In order to determine more accurately the competitiveness coefficients for different groups of males required for the model, two replications of experiments were carried out from May to August 1990 in the Crimea to determine simultaneously the competitiveness of F₁ males (dose 100 Gy) in field cages and their distribution in groups with different types of sterility in laboratory tests. The results showed that the proportion of competitive F₁ males in field cages corresponds most closely to the frequencies found in laboratory tests of males in whose progeny embryos develop and die. If full competitiveness of sp+ males (fully sterile males that form spermatophores) is assumed, then the competitiveness coefficients expected will always be much higher than the values obtained in the experiment. It would be desirable to obtain more extensive experimental data in order to obtain definite proof of the total loss of competitiveness of sp+ males or to determine the degree of the reduction of competitiveness in the case of partial loss. However, we believe that, for the purposes of modelling releases of irradiated insects and for determining the efficiency of the F₁ sterility method on the basis of laboratory experiments, the assumption that sp+ males are totally uncompetitive at high radiation doses is perfectly satisfactory.

2.3. Development of the model

The release of irradiated insects is modelled with a computer program, which can be used to generate the results of crossing between females and males of different groups. In the parent generation these groups consist of normal females, normal males, irradiated females and irradiated males. In the F_1 and F_2 generations the insects are made up of normal females and normal males in six groups of females and six groups of males. These groups consist of individuals with a level of sterility ranging from 0 to 20% (these are subsequently combined with the normal ones), from 20 to 40%, from 40 to 70% and from 70 to 100%; and also fully sterile males that form spermatophores (sp+) and fully sterile males that do not form spermatophores (sp-). The ratio between irradiated and normal insects in the different groups depends on the irradiation dose and the ratio of normal and released insects in the parent generation. In determining the number and ratio of the different F_1 , F_2 and F_3 groups, changes in the following characteristics of the irradiated insects and their progeny are taken into account:

- (a) The competitiveness of the males,
- (b) The sexual activity of the females,
- (c) The non-viability of the eggs (sterility),
- (d) The non-viability in post-embryonic stages of development,
- (e) The ratio of the sexes.

Changes in fertility are not taken into account. Fertility is taken to be constant. Information on changes in the reproductive potential indices are taken from tables compiled from calculated coefficients reflecting the deviation of each index from the norm for each irradiation dose. Each group of F_1 and F_2 females and males has its own table of coefficients of the changes in reproductive indices and of the frequencies of their distribution in the groups. The modelling is confined to the indices of the number of larvae hatching in the F_3 generation.

2.4. Modelling the release of partially and completely sterilized insects using the codling moth as an example

The program developed was used to model the release of irradiated male codling moths and irradiated insects not divided by sex for radiation doses of 60, 80, 100, 300 and 500 Gy. Assuming that the population increases geometrically from one generation to the next under normal conditions, the efficiency of a single release of irradiated insects in a ratio to normal insects ranging from 1:1 to 30:1 (in steps of one) in the three generations following release is calculated from the reduction in the number of first instar larvae.

2.4.1. First generation

The results of the modelling showed that, in the first generation after the release (Fig. 1(a)), the number of first instar larvae fell the most for all ratios in the case of complete sterility of males (dose of 500 Gy), despite the somewhat lower competitiveness coefficient of these males in comparison with those receiving a substerilizing dose of 300 Gy. Doses of 60, 80 and 100 Gy resulted in substantially lower efficiencies, i.e. percentage reductions of first instar larvae from mating involving a treated parent relative to larvae produced by untreated parents.

The release of irradiated insects that were not separated by sex did not affect the efficiency of the 300 and 500 Gy doses, since these doses cause complete sterility in the females. As a result of the partial sterility of females, a minimum positive efficiency is observed for the release of males and females irradiated with partially sterilizing doses, but only at 100 Gy and for the ratios 1:1 and 2:1. At ratios of irradiated to normal insects of 4:1 and higher, there is an increase in the number of larvae compared with the norm. At a dose of 60 Gy a negative efficiency is observed already at a ratio of 1:1, and at 7:1 the number of larvae is twice as high as when there is no release (no pest control).

2.4.2. Second generation

It is evident that the release of completely sterile insects should not affect the reproductive potential of the second generation after release, but the total number of larvae will be significantly lower as a result of the effect of the release on the first generation. In contrast, the release of partially sterilized insects has a greater effect on the second generation, owing to the increased sterility of F_1 males. The comparison of different doses in the second and third generations was therefore performed on the basis of the relative number of first instar larvae (relative to the number produced when no treated moths are released).

Modelling showed that a dose of 300 Gy is virtually equivalent to complete sterilization, since the very small number of F_1 females obtained after this dose in the progeny of irradiated males is completely sterile and the F_1 males (80% sp^- , the remainder sp^+) are totally uncompetitive (Fig. 2). It is interesting that in the case of the release solely of males irradiated at 60 Gy the number of larvae is lower than for a release of completely sterile males, although this is true only for the ratio 1:1 (at 80 Gy for the ratios 1:1 and 2:1). For other ratios, the efficiency of releases of completely sterile males is higher even in terms of the number of F_2 larvae. At the same time, on the basis of this evaluation the release of completely sterile males is less efficient than the release of males irradiated at 100 Gy for all the ratios modelled. The ratio of released to normal males in this case also affects the efficiency. The difference is greatest for the ratios 6:1 and 7:1, is approximately two times lower for the ratio 1:1, gradually decreases from the maximum at larger ratios (8:1, 9:1,

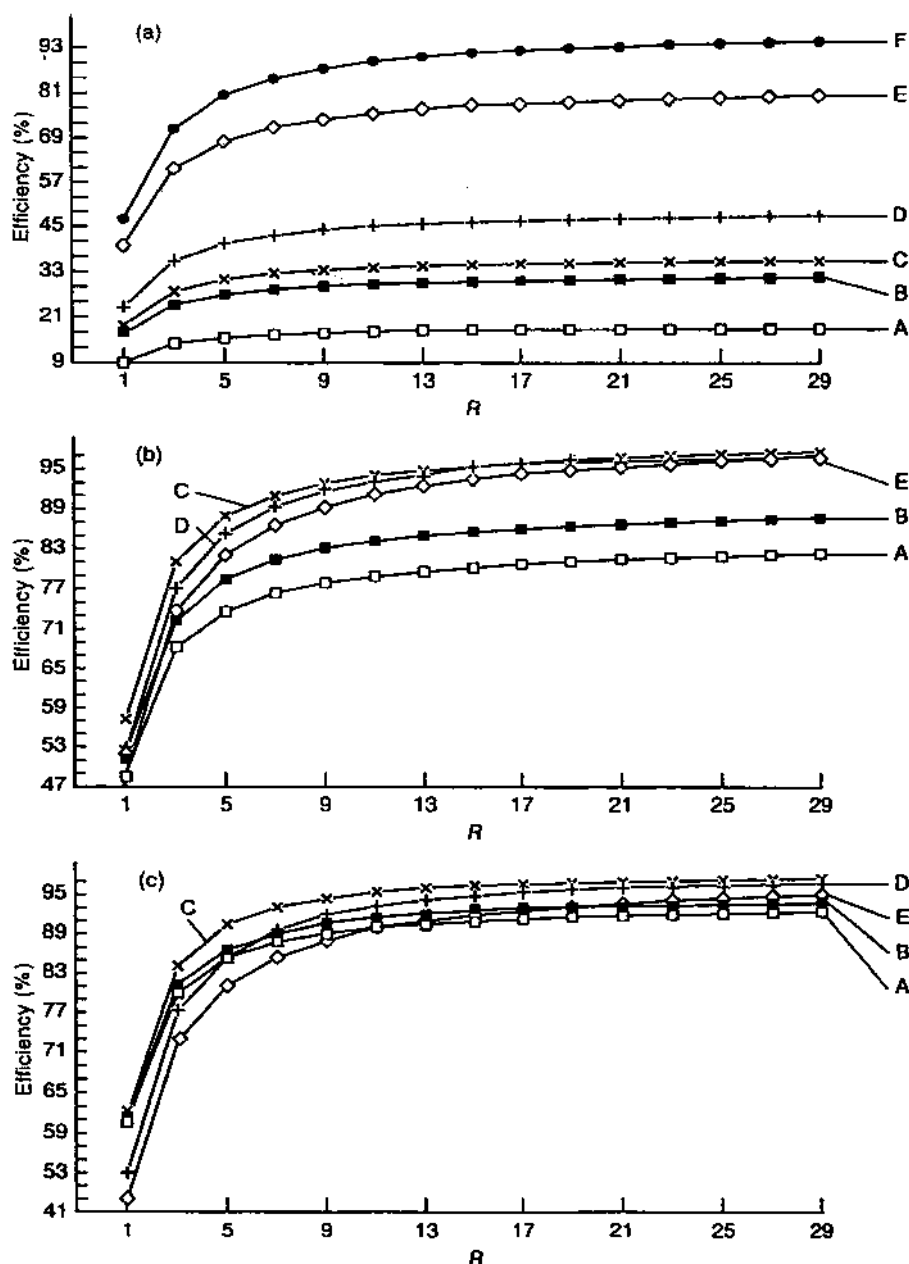


FIG. 1. F_1 generation: dependence of the efficiency (percentage reduction in the number of first instar larvae in comparison with the option of no pest control) of releases of codling moth males irradiated at different doses (in Gy: A, 60; B, 80; C, 100; D, 200; E, 300; F, 500) on their ratio (R) to natural males. (a) First generation after release, (b) second generation after release, (c) third generation after release.

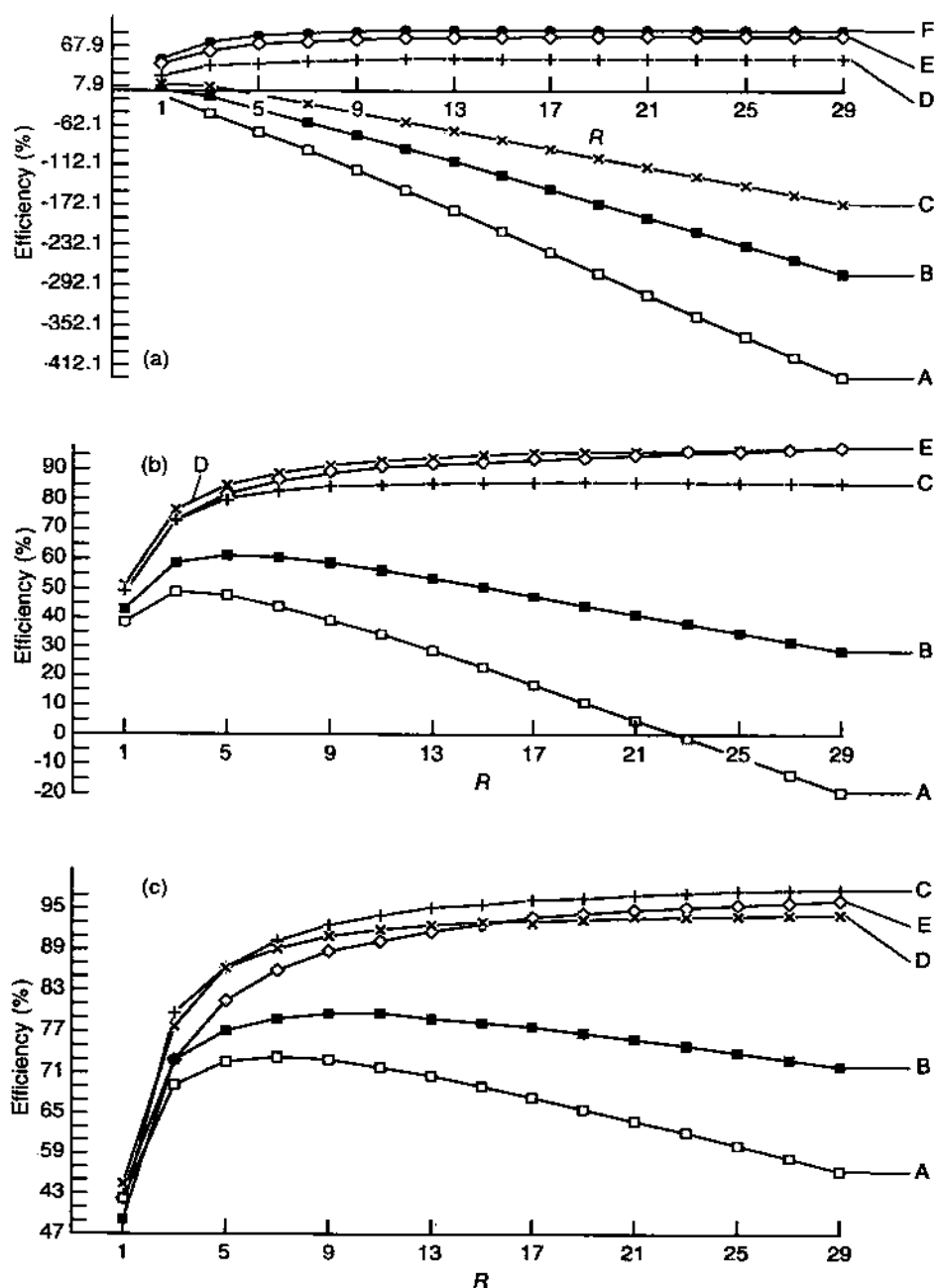


FIG. 2. F_2 generation: dependence of the efficiency of releases of codling moth males irradiated at different doses (in Gy: A, 60; B, 80; C, 100; D, 200; E, 300; F, 500) on their ratio to natural males. (a) First generation after release, (b) second generation after release, (c) third generation after release.

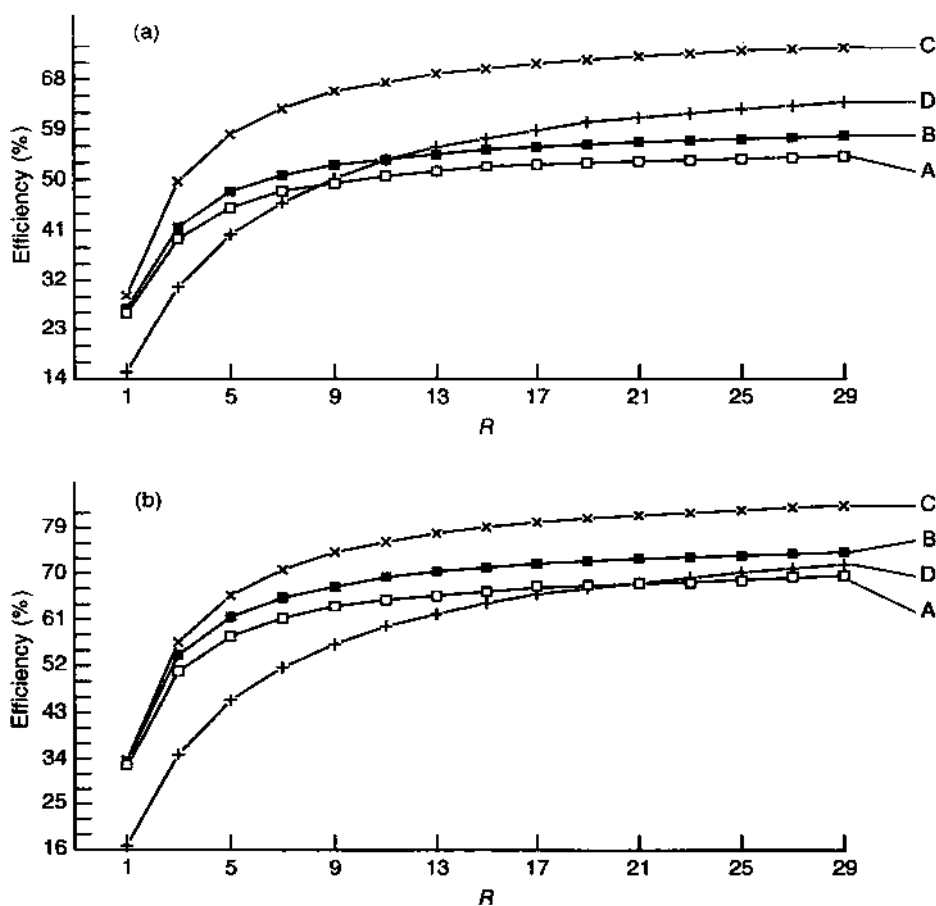


FIG. 3. F_2 generation: dependence of the efficiency of releases of male and female codling moths irradiated at different doses (in Gy: A, 60; B, 80; C, 100; D, 200) on their ratio to natural insects. (a) First generation after release, (b) second generation after release.

etc.) and virtually disappears at 30:1. At this point, the reduction in the number of larvae compared with the variant without release is 96.4%.

Releasing partially sterilized insects without separation by sex reduces efficiency. However, unlike the first generation, the reduction in the number of larvae is observed at all doses and for all the ratios modelled. The only exception is 60 Gy for ratios of released to normal insects higher than 23:1. The dependence on the ratios in these cases is described by dome shaped curves, the point of inflection and the steepness of the 'shoulders' of the curves being a function of the dose (Fig. 3).

For example, at a dose of 100 Gy, the maximum efficiency is 85.8%, which was observed for the ratio 16:1. It is interesting that for ratios from 9:1 to 30:1 the efficiency changes very slightly, falling to 84.6 and to 84.2% respectively. At doses of 80 and 60 Gy, the 'shoulders' are steeper and maximum efficiencies are observed for the ratios 5:1 and 4:1, respectively. On the whole, the reduction in the second generation in the case of releases of insects irradiated at 60 and 80 Gy without separation of the sexes is lower than for releases of completely sterilized (500 Gy) and sub-sterilized (300 Gy) insects. A dose of 100 Gy is evidently better since, for ratios up to 4:1, the positive effects of inherited sterility offset and to some extent even outweigh the negative effects of releasing males that are not completely sterile.

2.4.3. *Third generation*

Most of the inherited radiation induced damage in the spermatozoa of Lepidoptera takes the form of F_1 sterility. However, some inherited changes are preserved and result in the non-viability of the progeny of the F_3 and subsequent generations. The results of our experiments showed that, although on average the level of sterility in the F_2 generation is considerably lower than in the F_1 generation, the contribution of inherited damage to the reduction in the number of F_3 larvae may be significant in a number of cases. (The main reason for this is that, unlike F_1 insects that inherit sterility only from irradiated males, F_2 individuals inherit it from both F_1 females and males.) In the case of releases of males alone, this contribution is sufficient to increase the advantage of 100 Gy irradiation over complete sterilization significantly for all ratios of released to normal insects (Fig. 4). The advantage over total sterilization is also apparent at doses of 60 and 80 Gy, although not for all ratios. For example, in order to obtain 90% efficiency when releasing completely sterile insects, the ratio of released to normal insects should be greater than 11:1. For releases of partially sterile males irradiated at 60, 80 or 100 Gy, this efficiency already increases in the third generation at ratios of 9:1, 7:1 and 5:1, respectively.

In the case of releases without separation of the sexes, the general nature of the change in efficiency observed for the F_2 generation seems to be accentuated. A positive efficiency is observed for all doses and ratios modelled and it is always higher than 50%. Maximum efficiency is reached at ratios of 7:1, 9:1 and higher than 30:1 at doses of 60, 80 and 100 Gy, respectively. However, an efficiency of more than 90% is seen only at 10 Gy and for ratios of 8:1 and higher.

2.4.4. *F_1 release*

Releasing F_1 individuals, which are usually more sterile than the irradiated parents (males), is considered to be one of the most promising methods of using inherited sterility for the genetic control of Lepidoptera pests. In the case of the

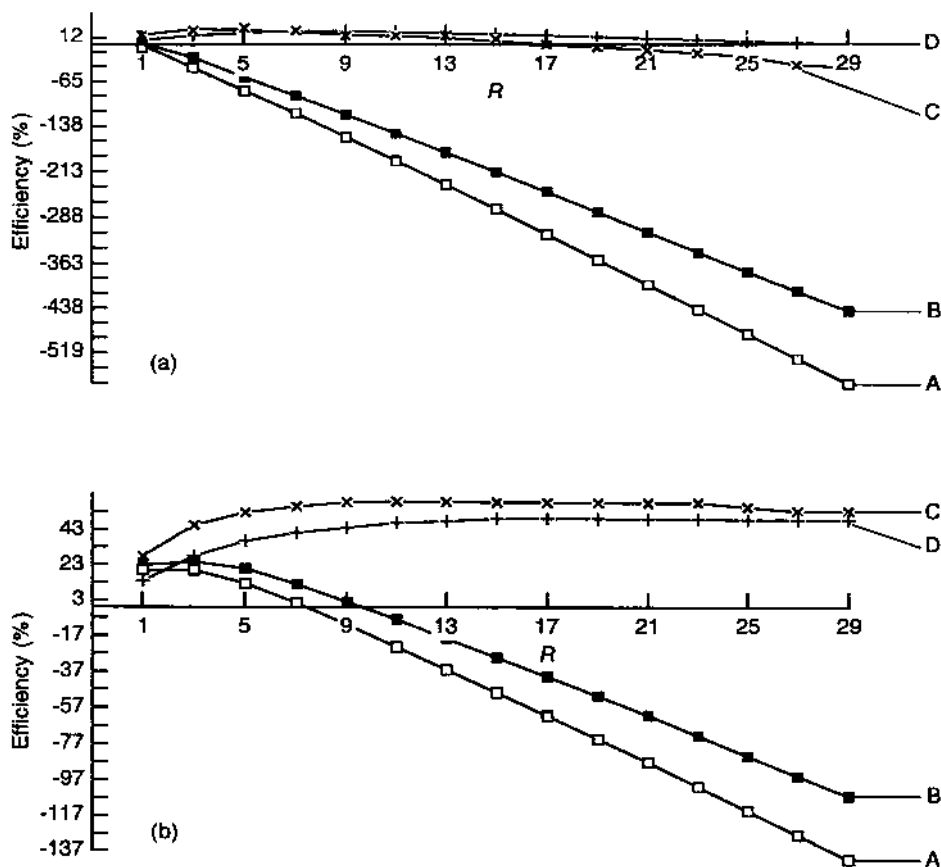


FIG. 4. F_3 generation: dependence of the efficiency of releases of codling moth males irradiated at different doses (in Gy: A, 60; B, 80; C, 100; D, 200) on their ratio to natural males. (a) First generation after release, (b) second generation after release.

codling moth, for example, this method opens up considerable possibilities for the mass rearing of F_1 diapausing larvae and their use to solve the problem of synchronizing the release and flight of the insects under natural conditions [3, 4]. Furthermore, this approach offers much greater technical scope for separating the sexes and releasing only F_1 males and for producing them and building up colonies during the period from autumn to winter. However, the selection of the optimum dose is perhaps even more important in this case than when releasing irradiated insects.

Our model enables us to obtain indices of the efficiency of F_1 larvae releases, although only by using additional calculations. It is obvious that with such releases

a reduction in the number of larvae in the natural population is observed only over two generations. Assuming that the codling moth release takes place at the diapausing larva stage, and taking into account the lower sterility of F_1 females and the possibility of separating codling moths by sex at this stage, the release of F_1 males alone was modelled. It should be noted that, in addition to the index for the reduction in numbers, the variation in the breeding coefficient following the irradiation of males at a given dose has also to be taken into account, since it significantly affects the efficiency of the mass production of insects for release. Clearly, a dose of 500 Gy

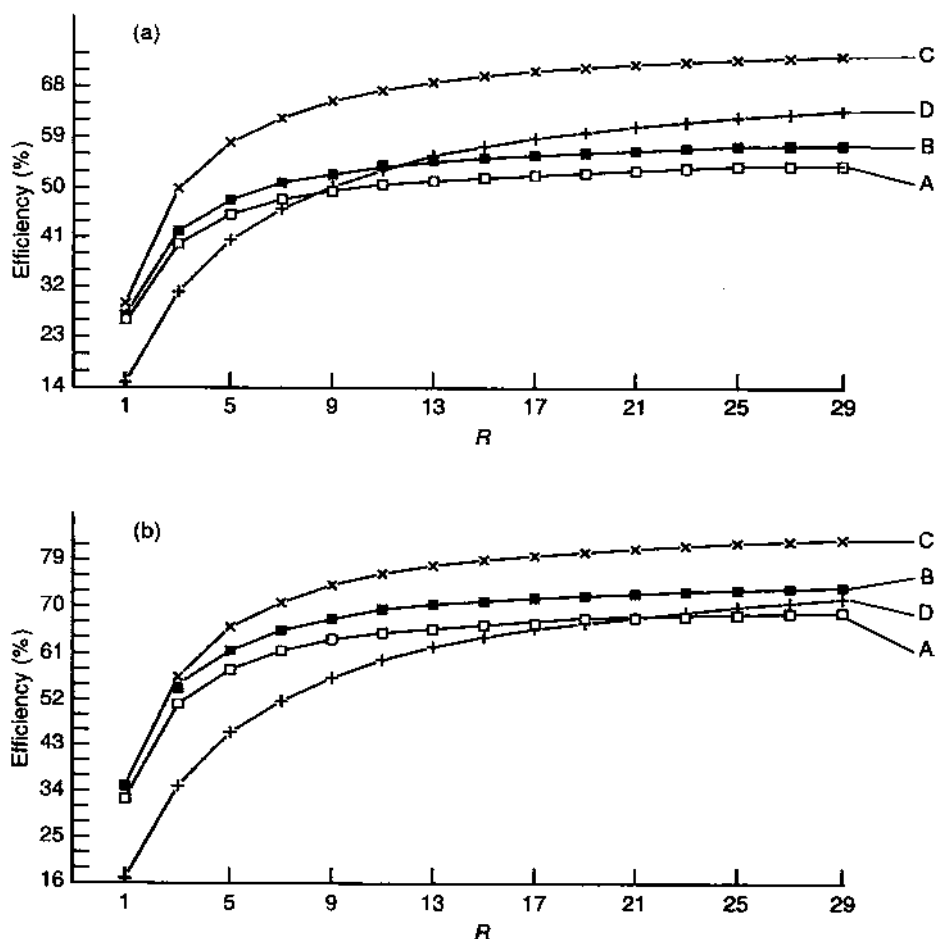


FIG. 5. Dependence of the effectiveness of released F_1 males irradiated at different doses (in Gy: A, 60; B, 80; C, 100; D, 200) on their ratio to wild males. (a) First generation after release, (b) second generation after release.

does not yield the desired effect. At lower doses, it is possible to produce a certain number of diapausing F_1 males. For the five experimental doses, namely 60, 80, 100, 200 and 300 Gy, the numbers produced were 94.6, 83.2, 82.2, 75.2 and 26.6%, respectively, of the number bred without irradiation (in calculating these indices, the non-viability of embryos and the shift in the sex ratio was taken into account). It is evident that although a dose of 300 Gy results in the strongest shift to males in the sex ratio, it clearly has a worse effect than the other doses.

Modelling the release of these males begins by taking into account their non-viability at the post-embryonic stages of development. (It is assumed that this non-viability generally occurs during the period of metamorphosis. In making this assumption, we are probably somewhat underestimating the efficiency of lower doses compared with higher ones, but we do not yet have any separate experimental data on the non-viability of developing larvae from the first to fifth instar as distinct from data on the non-viability of diapausing larvae and pupae.) This index is again highest at 300 Gy, but the overriding factor that makes this dose totally unsuitable for the release of F_1 males is their complete lack of competitiveness and their consequent ineffectiveness.

The results of modelling the release of F_1 males at the diapausing larva stage using other irradiation doses for males in the parent generation are shown in Fig. 5. It can be seen that, of the doses tested, the best dose for all ratios of released to natural males is 100 Gy, both in terms of the reduction in the number of larvae in the generation following release (Fig. 5(a)) and in the second generation (Fig. 5(b)). In comparison with doses of 60 and 80 Gy, the advantage of 100 Gy lies in the greater level of sterility of competitive F_1 males, and in comparison with 200 Gy, there is a higher proportion of competitive F_1 males. It is evident that the optimum dose lies in the 100 to 200 Gy range, and on the basis of data on other species [5] probably in the 100 to 150 Gy range.

3. CONCLUSIONS

The model of the results of releasing partially and completely sterilized insects showed that the computer program takes into account the positive and negative effects that inherited sterility in Lepidoptera have on the reduction in the pest population. It reveals their complex interdependence and the general rules governing changes in the efficiency of the method, which are difficult to appreciate with individual indices. The model can be used for any type of Lepidoptera if the necessary information is available.

The main aim of the computer model is to select the optimum irradiation dose for the release of both irradiated insects and the F_1 generation. The program makes it possible to do this using the results of laboratory experiments. It can also carry out various other tasks of practical importance, for example, evaluating the variation

in efficiency with different ratios of insects released to nature and assessing whether it is useful to divide the sexes.

There are still many areas in which the model can be improved and the initial data refined. In particular, it would be desirable to have more accurate indices of the variation of the competitiveness of males irradiated at different doses. As a consequence of this, the relative efficiency of releasing completely sterile insects (in the above calculations we assumed the reduction to be minimal) may change significantly. The general principles governing the variation in the efficiency of the method will be much more visible if the initial data used in the model are presented in the form not of tabular data but of equations that quantitatively reflect the dependences of the positive and negative effects on the dose. In order to determine these, it would be desirable to have quantitative data on all the parameters used in the model for a further two or three doses in the 100 to 200 Gy range. It would be desirable to be able to model the releases over several generations and to study their efficiency over a larger number of generations. We plan to carry out this work in the future.

Our model allows the efficiency of different irradiation doses and the ratios of released to natural insects to be compared only in the same generation. It is not possible to obtain an overall efficiency index for several generations using this model (although the reduction in the size of the previous generation affects the calculation of the size of the subsequent generation). It is also impossible to compare the release of sterilized adults (or pupae) and F_1 diapausing larvae since the differences between the dynamics of releases and of the flight of natural insects may strongly affect efficiency. In order to solve these and a number of other problems, it would be desirable to establish a model that simulates the releases of irradiated and F_1 insects and is capable of taking into account the dynamics of the size of the natural pest population and the final product yield for a given crop. Such a model could be verified through experiments on releases in a natural population. We plan to carry out such work in the near future on the Crimean population of codling moths.

A wide range of research can be carried out to find ways of improving the ratio of positive to negative effects. In this connection, factors such as dose rate, type of ionizing radiation, nature of the radiation source and so on may be very significant. Solving these problems would also be of practical importance.

4. SUMMARY OF CONCLUSIONS

- (1) The dependence on dose of the frequency of F_1 individuals with impaired reproductive physiology (loss of sexual activity, impairment of gametogenesis, etc.) increases uniformly.
- (2) When F_1 codling moth males are confined individually with virgin females, and when these females lay eggs that show no signs of development after they

- are one week old, then such F_1 males will usually be non-competitive under field conditions.
- (3) A computer program was developed to model the change in the size of the population over three generations after the release of insects irradiated at various doses. The model took into account the positive and negative effects that inherited sterility in Lepidoptera has on the reduction in the pest population and the complex interaction between these effects. An index based on these factors was obtained and an understanding gained of the general principles governing the changes in the efficiency of this genetic control method, which is difficult to achieve on the basis of individual effects.
 - (4) On the basis of the experimental results, releases of codling moths subjected to different doses of gamma radiation were modelled. This exercise demonstrated that the computer program can provide an adequate solution to the problems. It can be used to identify the optimum radiation dose (for the release of both irradiated insects and the F_1 generation), to evaluate the efficiency of releases with different ratios of released to natural insects and to determine the advantages of releasing only males.
 - (5) Research into F_1 sterility mechanisms and efforts to improve the computer model of the use of this mechanism in genetic control programmes should be continued in the following areas:
 - (a) Evaluation of the influence of irradiation conditions, the type of radiation, the radiation source, the dose rate and other factors on the effects that occur in the generations that follow the irradiation of the moths;
 - (b) Development of programs to model multiple releases of irradiated insects and programs that take account of the dynamics of the number of insects during the season;
 - (c) Determination of quantitative indices of the dependence on dose of the variation in the competitiveness of irradiated insects;
 - (d) Experimental determination and mathematical description of the dose-effect relationship for all the parameters considered in the model.

Appendix

Possibilities and Limitations of the Program

In general, this variant of the model has been developed for the optimum dose separation for the use of F_1 sterility effects for the genetic control of Lepidopteran pests. We consider that this variant of the program gives the best possibility for solving this problem. In addition, it is possible to select the optimal ratio of irradiated insects to natural ones if the economic indices for loss of agricultural products and the cost of insect rearing and release are taken into account.

The model is not intended for the prognosis of specific types of release in specific conditions for any particular species. However, if all the indispensable coefficients for simulation have been estimated for the natural conditions and the indices of mass reared insect quality for a particular biofactory can be taken into account, the present variant of the program can be useful.

Nevertheless we consider that for the purpose of prognosis of the results of a genetic control procedure, a special model must be developed that can take into account the dynamics of insect numbers in the natural population and the changes in quality of particular batches of released insects. We plan to develop such a model especially for the Crimean region.

To reduce the size of the algorithm and also the probability of errors in the first variant of the program and to make the simulation of codling moth releases easier, all the coefficients (which are calculated as if the contributions of both parents to the reproductive indices were additive) for F_2 and F_b insects have been made constants in the algorithm. Therefore, the present variant of the model is not universal and cannot be used directly for the simulation of releases in all Lepidopteran species to the third generation. For other species there may be other or different coefficients of reproductive indices in F_2 and F_b progeny.

For irradiated and F_1 insects of another Lepidopteran species such coefficients may be not the same as in the codling moth. However, the present variant of the program already gives the possibility of taking into account the specific reactions of any Lepidopteran pest to radiation and of producing an adequate result for the simulation release in the first and the second generations after the release of irradiated insects and only in the first generation after releases of F_1 insects.

We have already started work on the development of a more universal variant of the program. In particular, we have developed a universal system for designating the abbreviations of the coefficients. This system makes it possible to identify for which reproductive index, for which generation, for which sex, and for which group of insects it applies simply by the short abbreviation.

The first token (letter) marks the reproductive index:

n	Number of individuals,
f	Fecundity,
h	Hatchability of larvae,
e	Ecdysis of adults (viability in post-embryonic stages),
s	Sex ratio (part of females)
p	Part of the concrete group of insects.

The second token (number) marks the generation after irradiation to which the previous index applies to:

0	Generation of releases,
1	First generation after releases,
2	Second generation after releases,
3	Third generation after releases.

The third token (letter) marks the sex of the insects for which the reproductive index (including the viability of offspring in post-embryonic stages of development) was calculated:

m	Males,
f	Females.

The fourth token (letter or number) marks the group of insects:

F_b	Backcross,
r	Released insects,
w	Wild (natural) insects,
1	F_1 or F_b insects with sterility levels of 0–19.9%,
2	F_1 or F_b insects with sterility levels of 20–39.9%,
3	F_1 or F_b insects with sterility levels of 40–69.9%,
4	F_1 or F_b insects with sterility levels of 70–100%.

With the help of these markers all the coefficients that must be brought into the computer before simulation, were designated:

n0mw	Number of natural males,
n0fw	Number of natural females,
n0mr	Number of released males,
n0fr	Number of released females,
f0fw	Fecundity of natural females,
f0fr	Fecundity of irradiated females,
f0mr	Coefficient of irradiated male fecundity,
c0mr	Coefficient of irradiated male competitiveness,
h1mr	Coefficient of irradiated male fertility,
h1fr	Coefficient of irradiated female fertility,
e1mr	Coefficient of irradiated male F_1 offspring viability in post-embryonic stages of development,
e1fr	Coefficient of irradiated female F_1 offspring viability in post-embryonic stages of development,
s1mr	Sex ratio (proportion of females) in progeny of irradiated males,
s1fr	Sex ratio (proportion of females) in progeny of irradiated females,
c1m1	F_1 (here and below in progeny of irradiated males) male competitiveness coefficient (c),
p1m1	Proportion of F_1 males with 0–19.9% sterility levels,
p1m2	Proportion of F_1 males with 20–39.9% sterility levels,
p1m3	Proportion of F_1 males with 40–69.9% sterility levels,
p1m4	Proportion of F_1 males with 70–100% sterility levels,
p1f1	Proportion of F_1 females with 0–19.9% sterility levels,
p1f2	Proportion of F_1 females with 20–39.9% sterility levels,
p1f3	Proportion of F_1 females with 40–69.9% sterility levels,
p1f4	Proportion of F_1 females with 70–100% sterility levels,
f1m1	Coefficient of F_1 male fecundity with 0–19.9% sterility levels,
f1m2	Coefficient of F_1 male fecundity with 20–39.9% sterility levels,
f1m3	Coefficient of F_1 male fecundity with 40–69.9% sterility levels,
f1m4	Coefficient of F_1 male fecundity with 70–100% sterility levels,
f1f1	Fecundity of F_1 females with 0–19.9% sterility levels,
f1f2	Fecundity of F_1 females with 20–39.9% sterility levels,
f1f3	Fecundity of F_1 females with 40–69.9% sterility levels,
f1f4	Fecundity of F_1 females with 70–100% sterility levels,

h2m2	Weighted average fertility coefficient of F_1 males with 20–39.9% sterility levels,
h2m3	Weighted average fertility coefficient of F_1 males with 40–69.9% fertility levels,
h2m4	Weighted average fertility coefficient of F_1 males with 70–100% fertility levels,
h2f2	Weighted average fertility coefficient of F_1 females with 20–39.9% sterility levels,
h2f3	Weighted average fertility coefficient of F_1 females with 40–69.9% sterility levels,
h2f4	Weighted average fertility coefficient of F_1 females with 70–100% sterility levels.

Algorithm of the RESTECOM Program

$i1 = n0mw * n0fw * f0fw / (n0mw + n0mr * c0mr)$, Number of first instar larvae that are produced from the crosses of natural insects between themselves;

$i2 = n0mr * c0mr * n0fw * h1mr * f0fw * f0mr / (n0mw + n0mr * c0mr)$, Number of first instar larvae that are produced from the crosses of natural females with irradiated males;

$i3 = n0mw * n0fr * h1fr * f0fr / (n0mw + n0mr * c0mr)$, Number of first instar larvae that are produced from the crosses of natural males with irradiated females;

$i4 = n0mr * c0mr * n0fr * h1mr * h1fr * f0fr * f0mr / (n0mw + n0mr * c0mr)$, Number of first instar larvae that are produced from the crosses of irradiated insects among themselves;

$i5 = i1 + i2 + i3 + i4$, General number of first instar larvae in the first generation (here and below after releases);

$i6 = i1 * 0.5 + i3 * e1fr * s1fr$, Number of females in the first generation that are produced from the crosses of irradiated females with natural males and also from natural insects crossed together;

$i7 = i2 * e1mr * s1mr + i4 * e1fr * e1mrs1fr * s1mr / 0.5$, Number of F_1 females in the first generation;

$i8 = i6 + i7$, General number of females in the first generation;

$i9 = i1 * 0.5 + i3 * e1fr * (1 - s1fr)$, Number of males in the first generation that are produced from the crosses of irradiated females with natural males and from crosses of natural insects among themselves;

$i10 = [i2 * e1mr * (1 - s1mr) + i4 * e1fr * e1mr * (1 - s1fr * s1mr / 0.5)] * c1$, Number of competitive F_1 males in the first generation;

$i11 = i9 + i10$, General number of competitive males in the first generation;

$i12 = i11 + i8$, General number of adult insects in the first generation;

$i13 = i10 * p1m1 * f1m1 / i11$,

$i14 = i10 * p1m2 * f1m2 * h2m2 / i11$,

$i15 = i10 * p1m3 * f1m3 * h2m3 / i11$,

$i16 = i10 * p1m4 * f1m4 * h2m4 / i11$,

($i13$ – $i16$), The relative fertility coefficients of competitive F_1 males with sterility levels from 0 to 19.9% ($i13$), from 20 to 39.9% ($i14$), from 40 to 69.9% ($i15$) and from 70 to 100% ($i16$). Coefficients take into account the proportion of specific groups of competi-

tive F_1 males between all males in the first generation, their weighted average fecundity and fertility;

$i17 = i9/i11$, Proportion of the males in the first generation that are produced from the crosses of irradiated females with natural males and natural insects among themselves;

$$i18 = i7 * p1f1 * f1f1,$$

$$i19 = i7 * p1f2 * f1f2 * h2f2,$$

$$i20 = i7 * p1f3 * f1f3 * h2f3,$$

$$i21 = i7 * p1f4 * f1f4 * h2f4,$$

($i18-i21$), Number of F_1 females with sterility levels from 0 to 19.9% ($i18$), from 20 to 39.9% ($i19$), from 40 to 69.9% ($i20$) and from 70 to 100% ($i21$). Value takes into account the number of specific groups of coupled F_1 females in the first generation, their weighted average fecundity and fertility;

$i22 = i6 * f0fw$, Number of females in the first generation that are produced from the crosses of irradiated females with natural males and natural insects among themselves;

$i23 = (i13 + i14 + i15 + i16 + i17) * (i18 + i19 + i20 + i21 + i22)$, Number of the first instar larvae in the second generation;

$$i24 = i14 * 0.884,$$

$$i25 = i15 * 0.699,$$

$$i26 = i16 * 0.474,$$

($i24-i26$), Relative breeding coefficients of competitive F_1 males with sterility levels from 20 to 39.9% ($i24$), from 40 to 69.9% ($i25$) and from 70 to 100% ($i26$). Coefficient takes into account all reproductive indices such as $i13-i16$ plus viability in post-embryonic stages of development;

$$i27 = i19 * 0.881,$$

$$i28 = i20 * 0.551,$$

$$i29 = i21 * 0.394,$$

($i27-i29$), Number of adults in the second generation that are produced by F_1 females with sterility levels from 20 to 39.9% ($i27$), from 40 to 69.9% ($i28$) and from 70 to 100% ($i29$). Value takes into account all reproductive indices such as $i18-i21$ plus viability in post-embryonic stages of development;

$i30 = (i13 + i17) * (i22 + i18) * 0.5$, Number of normally fertile females in the second generation;

$$i31 = i13 * i27 * 0.500,$$

$$i32 = i13 * i28 * 0.500,$$

$$i33 = i13 * i29 * 0.500,$$

$$i34 = i24 * i18 * 0.420,$$

$$i35 = i24 * i27 * 0.420,$$

$$i36 = i24 * i28 * 0.420,$$

$$i37 = i24 * i29 * 0.420,$$

$$i38 = i24 * i22 * 0.420,$$

$$i39 = i25 * i18 * 0.440,$$

$$i40 = i25 * i27 * 0.440,$$

$$\begin{aligned}
 i_{41} &= i_{25} * i_{28} * 0.440, \\
 i_{42} &= i_{25} * i_{29} * 0.440, \\
 i_{43} &= i_{25} * i_{22} * 0.440, \\
 i_{44} &= i_{26} * i_{18} * 0.361, \\
 i_{45} &= i_{26} * i_{27} * 0.361, \\
 i_{46} &= i_{26} * i_{28} * 0.361, \\
 i_{47} &= i_{26} * i_{29} * 0.361, \\
 i_{48} &= i_{26} * i_{22} * 0.361, \\
 i_{49} &= i_{17} * i_{27} * 0.500, \\
 i_{50} &= i_{17} * i_{28} * 0.500, \\
 i_{51} &= i_{17} * i_{29} * 0.500,
 \end{aligned}$$

(i₃₁–i₅₁), Numbers of F₂ and F_b females in the second generation that are produced from the crosses of different groups of F₁ males or females with normally fertile insects and among themselves. For example, $i_{37} = i_{24} * i_{29} * 0.42$, number of females in the second generation that are produced from the crosses of F₁ males (with sterility levels from 20 to 39.9%) with F₁ females (with sterility levels from 70 to 100%) calculated by multiplication of i_{29} , number of adult F_b moths that are produced by F₁ females with sterility levels from 70 to 100%, i_{24} , relative breeding coefficient of competitive F₁ males with sterility levels from 20 to 39.9%, and the constant 0.42. The last constant reflects changes in the F₂ sex ratio after crosses of males and females from corresponding groups and is calculated by a special formula;

$$i_{52} = i_{31} + i_{32} + i_{33} + i_{34} + i_{35} + i_{36} + i_{37} + i_{38} + i_{39} + i_{40} + i_{41} + i_{42} + i_{43} + i_{44} + i_{45} + i_{46} + i_{47} + i_{48} + i_{49} + i_{50} + i_{51}, \text{ General number of F}_2 \text{ and F}_b \text{ females in the second generation;}$$

$$\begin{aligned}
 i_{53} &= 0.804 * (i_{13} + i_{49}) + 0.771 * (i_{32} + i_{50}) + 0.494 * (i_{33} + i_{51}) + 0.734 * i_{35} + 0.599 * i_{40} + 0.495 \\
 &* i_{45} + 0.704 * i_{36} + 0.574 * i_{41} + 0.475 * i_{46} + 0.451 * i_{37} + 0.368 * i_{42} + 0.304 * i_{47} + 0.913 * \\
 &(i_{34} + i_{38}) + 0.745 * (i_{39} + i_{43}) + 0.616 * (i_{44} + i_{48}), \text{ General fertility coefficient of F}_2 \\
 &\text{and F}_b \text{ females in the second generation. Coefficient takes into account and summer-} \\
 &\text{izes the number of each group of coupled females and their weighted average fertility} \\
 &\text{(fecundity is not considered at this point);}
 \end{aligned}$$

$$i_{54} = i_{30} + (i_{24} * 0.58 + i_{25} * 0.56) * (i_{18} + i_{22}), \text{ Number of males with normal fertility levels in the second generation;}$$

$$\begin{aligned}
 i_{55} &= i_{24} * i_{27} * 0.472, \\
 i_{56} &= i_{24} * i_{28} * 0.465, \\
 i_{57} &= i_{24} * i_{29} * 0.368, \\
 i_{58} &= i_{25} * i_{27} * 0.456, \\
 i_{59} &= i_{25} * i_{28} * 0.449, \\
 i_{60} &= i_{25} * i_{29} * 0.355, \\
 i_{61} &= i_{26} * i_{18} * 0.410, \\
 i_{62} &= i_{26} * i_{27} * 0.333, \\
 i_{63} &= i_{26} * i_{28} * 0.328, \\
 i_{64} &= i_{26} * i_{29} * 0.259, \\
 i_{65} &= i_{26} * i_{22} * 0.410, \\
 i_{66} &= i_{31} * 0.814,
 \end{aligned}$$

$$i67 = i32 * 0.802,$$

$$i68 = i33 * 0.634,$$

$$i69 = i49 * 0.814,$$

$$i70 = i50 * 0.802,$$

$$i71 = i51 * 0.634,$$

($i55-i71$), Numbers of competitive F_2 and F_b males in the second generation that are produced from the crosses of different groups of F_1 males or females with normally fertile insects and among themselves. For example, $i64 = i26 * i29 * 0.259$, Number of competitive F_2 males in the second generation that are produced from the crosses of F_1 males (with sterility levels from 70 to 100%) with F_1 females (with sterility levels from 70 to 100%) also. It is calculated by multiplication of $i29$, number of adult F_b moths which are produced by F_1 females with sterility levels from 70 to 100%, $i26$, relative breeding coefficient of competitive F_1 males with sterility levels from 70 to 100%, and 0.259, the constant specific for crosses between these groups of F_1 males and females. This constant was calculated by the following steps. From the beginning by a special formula the proportion of males in progeny after such crosses $(1 - 0.361 * 0.500) / 0.500 = 0.639$ was calculated. Then the proportion of males was multiplied by the competitiveness coefficients of F_b males corresponding to specific groups of F_1 males (0.641) and females (0.634). The result was $0.639 * 0.641 * 0.634 = 0.259$;

$$i72 = i55 + i56 + i57 + i58 + i59 + i60 + i61 + i62 + i63 + i64 + i65 + i66 + i67 + i68 + i69 + i70 + i71, \text{ General number of } F_2 \text{ and } F_b \text{ competitive males in the second generation};$$

$$i73 = 0.56 * (i68 + i57 + i60 + i71) + 0.733(i66 + i55 + i58 + i69) + 0.587 * (i67 + i56 + i59 + i70) + 0.823 * (i61 + i65) + i62 * 0.603 + i63 * 0.483 + i64 * 0.425, \text{ General fertility coefficient of competitive } F_2 \text{ and } F_b \text{ males in the second generation which are produced from the crosses of different groups of } F_1 \text{ males or females with normally fertile insects and among themselves};$$

$$i74 = i72 + i54, \text{ General number of all the males in the second generation};$$

$$i75 = (i30 + i53) * (i54 + i73) * 140 / i74, \text{ Number of first instar larvae in the third generation (it is proposed that the fecundity of all groups of insects in the second generation is the same)};$$

$$i76 = i30 + i52, \text{ General number of all the females in the second generation};$$

$$i77 = i76 + i74, \text{ General number of all adult insects in the second generation.}$$

The computer now writes the results of the release simulation:

Number of larvae in the first generation,

Number of adults in the first generation,

Number of all the males in the first generation,

Number of competitive F_1 males in the first generation,

Number of all the females in the first generation,

Number of F_1 females in the first generation,

Number of larvae in the second generation,

Number of adults in the second generation,
Number of all the males in the second generation,
Number of competitive F_b and F_2 males in the second generation,
Number of all the females in the second generation,
Number of F_b and F_2 females in the second generation,
Number of larvae in the third generation,

and asks:

Do you want to work with program again? (Y/N)

REFERENCES

- [1] KNIPLING, E.F., KLASSEN, W., Relative Efficiency of Various Genetic Mechanisms for Suppression of Insect Populations, Technical Bulletin 1533, US Department of Agriculture, Washington, DC (1976).
- [2] ANISIMOV, A.I., LAZURKINA, N.V., SHVEDOV, A.N., Influence of radiation-induced genetic damage on the suppressive effect of inherited sterility in the codling moth (Lepidoptera: Tortricidae), Ann. Entomol. Soc. Am. **82** 6 (1989) 769-777.
- [3] CHARMILLOT, P.J., STAHL, J., ROSSET, S., Autocidal control of the codling moth (*Laspeyresia pomonella* L.). Results of two years of control by depositing sterile diapausing larvae in orchards, Bull. Soc. Entomol. Switzerland **49** (1976) 173-184 (in French).
- [4] AZIZYAN, A.A., AKOPYAN, A.S., "Industrial testing of the genetic method for controlling the codling moth", Genetic and Physical Methods of Controlling Arthropod Pests (Proc. Symp. Ceské Budějovice, 1988), Czechoslovakian Academy of Sciences, Prague (1988) 18-26 (in Russian).
- [5] SALLAM, H.A., "Inherited sterility in progeny of irradiated male cotton leafworm (*Spodoptera littoralis* (Boisd.))", paper presented at IAEA Research Co-ordination Mtg on Radiation-Induced F_1 Sterility in Lepidoptera for Area-Wide Control, Beijing, 1989.

CONCLUSIONS AND RECOMMENDATIONS

(a) Computer simulation models are very helpful in projecting the suppressive action of inherited sterility on pest populations that have been overflowed with irradiated moths. Such projections are complicated because:

- (1) The level of sterility induced by a given radiation dose differs widely from one generation to the next.
- (2) The detrimental effects of irradiation tend to increase uniformly with dose, but the thresholds for various detrimental effects differ widely.
- (3) The rates of increase of some wild populations may be high (e.g. tenfold or more) and therefore require that the F_1 descendants of released moths possess a fairly high level of sterility in order to prevent the wild populations from building to densities that cause economically significant damage; but lower levels of sterility may suffice against other populations whose intrinsic rates of increase are low (e.g. fivefold or less).
- (4) If fairly low doses of radiation are used then F_1 females may be partially fecund and fertile.
- (5) The economic threshold may be so low that a significant increase in population density cannot be tolerated.
- (6) The combined effects of, for example, inherited sterility, releases of predators, parasites or pathogens, or cultural practices may have to be computed.

Two computer models that are able to take most of these considerations into account are presented. Additional efforts are needed to improve the utility, user friendliness and speed of this software.

(b) A computer model was used to reach the conclusion that the optimal dose for inducing inherited sterility in the management of the codling moth, *Cydia pomonella*, is about 100 Gy. This conclusion needs to be validated with field tests or in an operational programme.

(c) F_1 males of the European corn borer, *Ostrinia nubilalis*, are fully sterile if their fathers had received 300 Gy. A dose of about 150 Gy appears to be appropriate for future experiments to suppress this pest in the field. Appropriate field trials should be conducted, to include the combined effects of inherited sterility, and the use of biocontrol agents and of resistant corn hybrids. In addition, an inexpensive diet for mass rearing based on ingredients readily available in eastern Europe has been developed. This diet should be evaluated by workers throughout the geographical range of the pest in order to see if further improvements can be made.

(d) In the Asian corn borer, *Ostrinia furnacalis*, the optimal dose range for inducing useful inherited sterility appears to be 150–200 Gy. The incidence of visible

chromosomal aberrations in metaphase cells of the testis is slightly more than threefold greater in the F_1 generation than in the F_2 generation and this correlates closely with the levels of sterility in the two generations. There appear to be no major technical barriers to the suppression of the Asian corn borer on an operational scale. A large scale pilot test should be conducted to obtain data on the economic returns of this approach and to improve further the technology for mass rearing, distribution and field releases.

(e) In the pink bollworm, *Pectinophora gossypiella*, the optimal dose for the use of F_1 sterility to manage populations appears to lie between 150 and 200 Gy. At these doses the moths are significantly less impaired than at 200 Gy, the dose used in the large, successful, programme to protect cotton in California. Thus it appears that the robustness of this technology can be upgraded. This should be evaluated in a large scale trial, and additional consideration should be given to the diet used for mass rearing. It was found that larvae develop more rapidly, and yield a much higher percentage of pupae that are considerably larger and that develop into much longer lived adults when reared on a casein and wheat germ diet than when reared on diets based on soybean flour and wheat germ, corn-cob grits and wheat germ or peanut flour. Thus the benefits of using a casein based diet need to be weighed against the increased cost.

(f) The optimal dose for inducing a useful level of inherited sterility in the tropical army worm, *Spodoptera litura*, appears to lie in the range of 100 to 150 Gy. Large scale field trials should be undertaken to develop the use of inherited sterility against *S. litura*. In field cage studies to suppress the cotton leafworm, *Spodoptera littoralis*, by releasing moths treated with 100 Gy, it was not completely possible to prevent the population from increasing. Thus, field trials should be conducted with *S. littoralis* moths treated with as much as 200 Gy. The fall army worm, *Spodoptera frugiperda*, can be suppressed by releasing moths treated with 125 Gy. However, the optimal dose appears to be higher. Efforts should be made to assess the technical and economic feasibilities of using inherited sterility to suppress *S. frugiperda* in its overwintering refugia. These refugia should not be allowed to produce significant numbers of migrants that establish highly damaging populations in temperate latitudes where the pest cannot overwinter.

(g) In the diamondback moth, *Plutella xylostella*, very useful levels of inherited sterility can be induced with 150 to 200 Gy. Since populations of this insecticide resistant species can also be suppressed by means of the *Bacillus thuringiensis* endotoxin and by overflooding with several species of parasites, field trials should be undertaken to develop a pest management system involving the use of these biocontrol agents and the use of inherited sterility. The purpose of such trials would be to improve significantly the mass rearing and release technologies both for irradiated moths and for parasites, and to measure the economic returns of this integrated approach against that of using only one of these measures.

(h) In the wild mulberry silkworm, *Bombyx mandarina*, a useful level of inherited sterility can be induced with 200 Gy. However, the data suggest that the optimal dose may be somewhat lower. Additional laboratory studies should be undertaken to establish the optimal dose. Subsequently field trials on the use of inherited sterility against this pest should be undertaken in order to determine whether its selective suppression would be profitable. Since the mulberry silkworm is also attacked by several defoliating insects, it may be necessary to devise an integrated strategy to suppress the entire complex.

(i) Major disruptions in mass rearing of the gypsy moth, *Lymantria dispar*, are caused by the 'abnormal performance syndrome' in which a high percentage of the larvae develop very slowly or die. This syndrome was found to be caused by deficiencies in the diet and can be avoided. Two options are available for infusing inherited sterility into the wild population, i.e. distribution of irradiated pupae or of F₂ eggs. The latter has the great theoretical advantage that the mass rearing and stockpiling of eggs of this univoltine species would be possible on a year round basis. Additional field trials should be conducted to evaluate the technical and economic feasibilities of the infusion of inherited sterility into wild populations by distributing F₁ eggs.

(j) In the corn earworm, *Helicoverpa zea*, a pilot field test showed that the release of males irradiated with 100 Gy in small mountain valleys was effective in infusing inherited sterility into the wild populations, and that this retarded and reduced their seasonal population buildup. In order to evaluate the potential economic returns of this technology, a field trial on an operational scale should be conducted in which the use of inherited sterility is combined with the use of resistant crop cultivars and parasite releases.

(k) A new co-ordinated research programme on the use of radiation induced inherited sterility should be undertaken. This programme should stress field aspects rather than laboratory and field cage studies. Thus the main objective should be to evaluate the potential of suppressing populations of pest Lepidoptera in the field. This will require the acquisition of data on the ecology, dispersal and seasonal buildup of pest populations. In addition, observations will have to be made on the field behaviour of irradiated and wild moths, e.g. times of onset and cessation of calling, attractancy, mating success, etc. Mated females will have to be captured and examined for the presence of eupyrene sperm in spermathecae, and for the levels of sterility induced in them and in their descendants.

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