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Evaluation of Lepidoptera population suppression by radiation induced sterility

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FOREWORD

This publication results from the second FAO/IAEA Research Co-ordination Project (CRP) on Inherited Sterility in Lepidoptera (caterpillars of moths). The present CRP and a previous one entitled "Radiation Induced F_1 Sterility in Lepidoptera for Area-Wide Control" were initiated in response to requests from Member States for the development of environment friendly alternatives to current control of moth pests.

The first five-year CRP (1987–1991) dealt primarily with aspects such as determining the effects of various radiation dose levels on the resulting sterility in the treated parents and their F_1 progeny in different Lepidoptera species. In addition, models were developed on the suppressive effects of F_1 sterility on field populations, and some studies were conducted in laboratory or field cages to assess the impact of inherited sterility on pest suppression. The research results were published in 1993 in the IAEA Panel Proceedings Series.

This follow-up CRP (1994–1998) has built on the results of the first CRP and has focused on addressing a more challenging phase, consisting of rearing key pest moths and evaluating their application for pest control purposes. The specific objective of the CRP was therefore to assess the potential of suppressing populations of caterpillar pests in the field by inherited sterility methods, i.e. by rearing and releasing irradiated moths and/or their progeny in combination with other biological control methods. The ultimate goal is to have alternative environment-friendly control methods available to be able to reduce the vast quantities of insecticide that are used in agriculture to combat Lepidoptera pests and that adversely affect the trade balance of developing countries because they must use hard currency to import them.

The two FAO/IAEA sponsored Lepidoptera CRPs have resulted in expanded research and implementation programmes on F_1 sterility in combination with natural enemies. Such programmes are under way in Tunisia for suppression of the carob moth, *Ectomyelois ceratoniae*, and on the island of Mauritius for control of the diamondback moth, *Plutella xylostella*. F_1 sterility programmes for other Lepidopteran pest species also are being considered in other countries.

This TECDOC was prepared by S. Bloem, USA, with assistance from J.E. Carpenter, USA. The IAEA officer responsible for this publication was J. Hendrichs of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

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SUMMARY

Lepidopteran species are the most important pests of major annual and perennial crops, forests, and stored products throughout the world. More than 25% of the species that appear on a list of the 300 most important exotic insects that threaten the USA are in the order Lepidoptera. In a supplement to that list, where the 30 most serious threats to agriculture are named, 50% of the species are lepidopterans. Unfortunately, control of Lepidopteran pests worldwide is achieved almost entirely through the use of synthetic insecticides. This dependence on insecticides has contributed to the development of insecticide resistance in many of the most serious pests. Relevant examples include the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae) and the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae), where resistance has developed even against the microbial insecticide *Bacillus thuringiensis*. Heavy reliance and frequent indiscriminate use of pesticides has also resulted in pesticide residues in food and has had a significant negative impact on the environment. Of particular importance to agriculture is the destruction of crop pollinators and other of beneficial insects — parasites and predators that maintain secondary pests under control. Development of alternative tactics to the use of insecticides alone is therefore a major emphasis of most local, national and international research organizations concerned with pest control.

Genetic pest suppression is unique among biological methods in that it involves the release of genetically modified insects to control the same species. Sterile insect technique (SIT) programmes have been successful against a number of pest Diptera (including the screwworm fly, *Cochliomyia hominivorax*, and the Mediterranean fruit fly, *Ceratitis capitata*), and numerous mass rearing facilities have been constructed worldwide to support these programmes. However, compared to dipterans, lepidopterans generally are more expensive to rear and have a propensity to fly greater distances. Additionally, lepidopterans are more radio-resistant than dipterans. As a consequence, the larger dose of radiation required to completely sterilize lepidopterans reduces their competitiveness and performance in the field. Nevertheless, two SIT programmes are currently operating against pest Lepidoptera, namely the pink bollworm programme in the USA, and the codling moth programme in Canada, and both of these programmes have been very successful.

One approach to reduce the negative effects of radio-resistance in Lepidoptera has been the use of inherited or F_1 sterility. F_1 sterility was first documented in studies on the codling moth. Subsequently, investigators have reported F_1 sterility in many Lepidopteran species of economic importance. Like SIT, F_1 sterility involves the mass rearing and release of genetically altered insects to insure that when matings occur in the field, a significant proportion of matings involve a treated, released insect. However, F_1 sterility takes advantage of two unique genetic phenomena in Lepidoptera. First, Lepidopteran females generally are much more sensitive to radiation than are males of the same species. This allows the dose of radiation to be adjusted so that treated females are completely sterile and males are partially sterile. Second, when these partially sterile males mate with fertile females the radiation-induced deleterious effects are inherited by the F_1 generation. As a result, egg hatch is reduced and the resulting (F_1) offspring are both highly sterile and predominately male. The lower dose of radiation used in F_1 sterility increases the quality and competitiveness of the released insects. In addition, because F_1 sterile progeny are produced in the field, the release of partially sterile insects offers greater suppressive potential than the release of fully sterile insects and is more compatible with other pest control mechanisms or strategies.

Table 1. Listing of the species of Lepidoptera investigated by participants of the FAO/IAEA Co-ordinated Research Project on evaluation of population suppression by irradiated Lepidoptera and their progeny

Family	Species	Common name	Crop
Noctuidae	*Spodoptera litura*	Common cutworm or Tobacco caterpillar	Soybeans, sorghum, corn, vegetables
	Spodoptera exigua	Beet armyworm	Cotton, vegetables
	Spodoptera frugiperda	Fall armyworm	Forage grass, corn
	Helicoverpa armigera	Corn earworm or Cotton bollworm (Old World)	Corn, cotton, tomato
	Helicoverpa zea	Corn earworm	Corn, cotton, tomato
Gelechiidae	*Pectinophora gossypiella*	Pink bollworm	Cotton
	Diatraea saccharalis	Sugarcane borer	Sugarcane
Tortricidae	*Cydia pomonella*	Codling moth	Pome fruit, walnut
	Cydia molesta	Oriental fruit moth	Stone & pome fruit
Pyralidae	*Ectomyelois ceratoniae*	Carob or date moth	Date, carob, pomegranate
	Ephestia kuehniella	Mediterranean flour moth	Stored grain
	Crocidolomia binotalis	Cabbage webworm	Crucifer vegetables
	Chilo suppressalis	Asian rice stem borer	Rice
Crambidae	*Ostrinia furnacalis*	Asian corn borer	Corn
	Ostrinia nubilalis	European corn borer	Corn
Plutellidae	*Plutella xylostella*	Diamondback moth	Crucifer vegetables
Arctiidae	*Spilosoma obliqua*	Hairy jute caterpillar	Jute

E. F. Knipling explored the theoretical application of F_1 sterility for control of Lepidopteran pests. Using mathematical models, he suggested that when releasing partially sterile insects, the sterile-to-wild overflooding ratio could be as low as ¼ of what is normally required for fully sterile insects. Population models developed by other researchers using data collected from several pest species corroborate Knipling's findings.

Field releases of partially sterile insects have demonstrated the potential of using F_1 sterility to control many Lepidopterans, including the cabbage looper, *Trichoplusia ni*, the corn earworm, *Helicoverpa zea*, the gypsy moth, *Lymantria dispar* and the codling moth, *Cydia pomonella*. In addition, many studies have shown that F_1 sterility can be effectively combined with other biological controls such as pheromone mating disruption, entomopathogens, host plant resistance and natural enemies. As a result of these many studies, F_1 sterility is regarded as the most favourable genetic method for most applications against Lepidoptera.

The FAO/IAEA Sponsored Co-ordinated Research Projects (CRPs)

The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture promotes agricultural development through the peaceful use of atomic energy. This mission is accomplished through Technical Cooperation Projects, Co-ordinated Research Projects, publications, meetings and training courses. In response to the recommendations of a group of

Table 2. Listing of the type of studies conducted and species investigated by participants during the FAO/IAEA Co-ordinated Research Project on "Evaluation of population suppression by irradiated Lepidoptera and their progeny"

Type of Study	Species
Diet Development and Insect Rearing	*Chilo suppressalis* *Cydia molesta* *Helicoverpa armigera* *Pectinophora gossypiella* *Spilosoma obliqua* *Spodoptera litura*
Radiation Biology	*Cydia pomonella* *Diatraea saccharalis* *Ectomyelois ceratoniae* *Helicoverpa armigera* *Ostrinia nubilalis* *Pectinophora gossypiella* *Plutella xylostella* *Spilosoma obliqua* *Spodoptera frugiperda* *Spodoptera litura*
Genetics and Genetic Sexing	*Ephestia kuehniella*
Effects of Radiation on Sperm Development and Sperm Competitiveness	*Helicoverpa armigera* *Ostrinia furnacalis* *Spodoptera frugiperda*
Mating Competitiveness of Irradiated Insects and Their Progeny	*Cydia pomonella* *Cydia molesta* *Crocidolomia binotalis* *Diatraea saccharalis* *Helicoverpa armigera* *Ostrinia furnacalis* *Plutella xylostella*
Compatibility of F_1 Sterility with Other Control Strategies	*Cydia pomonella* *Helicoverpa zea* *Pectinophora gossypiella* *Plutella xylostella* *Ostrinia furnacalis* *Ostrinia nubilalis* *Spodoptera exigua* *Spodoptera frugiperda*
Use of F_1 Sterility to Enhance Parasitoids	*Helicoverpa zea* *Plutella xylostella* *Spodoptera frugiperda*
Field Releases for Suppression	*Cydia molesta* *Cydia pomonella* *Ostrinia furnacalis* *Ostrinia nubilalis* *Plutella xylostella*
Population Models	*Cydia pomonella* *Plutella xylostella*

consultants that met at the IAEA in Vienna in 1984, the Insect and Pest Control sub-programme of the Joint FAO/IAEA Division designed and initiated the first five-year (1987–1991) Co-ordinated Research Project (CRP) on Radiation Induced F_1 Sterility in Lepidoptera for Area-Wide Control. Research by CRP participating scientists focused largely on modelling the effects of releasing partially sterile moths on the field dynamics of feral

populations, conducting laboratory studies to evaluate the relationship between radiation dose and sterility and conducting selected field-cage evaluations. Scientists from ten countries participated in this CRP, and the research results were published by the IAEA in 1993. As a result of the research progress during the CRP, the participants recommended to the Joint Division that a second Co-ordinated Research Project should be considered which would emphasize field applications of inherited or F_1 sterility for Lepidopteran pests. A second CRP entitled "Evaluation of Population Suppression by Irradiated Lepidoptera and Their Progeny" was therefore initiated in 1995, with the objective of assessing the potential for controlling populations of pest Lepidoptera by releasing irradiated moths and/or their progeny in combination with other biological control methods.

Significant contributions were made by the present CRP in addressing this more applied phase of Lepidoptera inherited sterility research and development. Many components were addressed, such as mastering the mass rearing of different Lepidopteran pests and irradiating them at the correct dose to achieve almost complete sterility in females with only partial sterility in males. In addition, this new phase has required the development of rearing procedures and techniques for parasitoids associated with these Lepidopteran pests. It has also involved collecting baseline data on field populations, including the density of the pest and natural enemy populations and their rate of increase at the time that partially sterile moth releases are initiated. Furthermore, it has involved assessment of the behaviour of irradiated moths and their progeny in field cages and in the field in relation to pheromone calling and response, capacity to mate, and ability to form spermatophores and to transfer competitive sperm to the spermatheca of wild females. For these studies, special techniques such as morphological mutant strains, mating tables involving female moths with clipped wings or on tethers, as well as analysis of spermatogenesis, sperm precedence, and chromosomes were used. Finally, a number of CRP participants have started to assess the suppression of wild populations, the interactions and synergism of inherited sterility with other biological methods, and the overall economics of the approach. Several of the research teams presented data on more efficient and economical semi-synthetic diets to rear their respective insects. Laboratory data on mating competitiveness of partially sterile insects, field evaluation of performance by substerile F_1 progeny either alone or in combination with parasitoids is also presented in the research reports.

These inherited sterility studies have covered some of the most important Lepidopteran pests worldwide of annual and perennial crops and stored-product pests (see Tables 1 and 2). They included diamondback moth of vegetables, pink and the cotton bollworm, sugarcane borer, date or carob moth, rice stem borer, Asian and European corn borers, codling moth, stored grain moths, peach borer, tobacco caterpillar, jute hairy caterpillar, and others. Mass rearing has been one of the main hurdles to overcome due to microbial diseases in the laboratory colonies and other related rearing problems. A one-week workshop on Lepidopteran rearing was successfully held in Jakarta, in conjunction with the first research coordination meeting, to address these difficulties and to advise CRP participants on good rearing practices. As a result, many participants were able to improve the quality and capacity of their insect rearing which, in turn, had a favourable impact on the conduct of their research.

This CRP concluded in 1998. During this time period, three Research Co-ordination Meetings (RCMs) were held to allow participants to discuss initial results and share ideas. The first RCM was held in Jakarta, Indonesia (24–28 April 1995), the second meeting was held in Vienna, Austria (2–6 September 1996), and the final meeting was held in Penang, Malaysia (28 May–2 June1998) in conjunction with the FAO/IAEA International Conference on Area-

Wide Control of Insect Pests Integrating The Sterile Insect and Related Nuclear and Other Techniques.

Twenty-five scientific teams from twenty-two different countries (Bangladesh, China, Pakistan, Myanmar, Syria, India, Java, Philippines, Mauritius, Viet Nam, Tunisia, Bulgaria, Romania, Czech Republic, Russian Federation, Ukraine, Iran, Austria, United States of America, Brazil, Cuba and Canada) participated in this second CRP. The research findings are published in three separate venues: as refereed publications in various scientific journals (see Appendix 1), as a block of four manuscripts in the Florida Entomologist, volume 84 part 2, and in this TECDOC. The papers in the Florida Entomologist report important research findings from four different countries and on four different species; the effects of a substerilizing dose of gamma radiation (100 Gy) on the mating competitiveness and mating propensity of the Old World cotton bollworm, *Helicoverpa armigera*, in the Philippines; the effects of different doses of radiation on the common cutworm, *Spodoptera litura* reared on two different diets in India; the competitiveness of mutant and irradiated males of the Mediterranean flour moth, *Ephestia kuehniella*, in the laboratory by counting eupyrene (fertile) and apyrene (non-fertile) sperm transferred to the female during copulation; and the potential of combining F_1 sterility and the parasitoid *Cotesia plutellae* in a system to manage the diamondback moth, *Plutella xylostella*, in Viet Nam.

Major Findings and Impact of the Research Conducted During the F_1 Sterility CRP

The research conducted during this CRP revealed principles that were common to all species studied. These can be summarized into two major points:

(1) The use of F_1 sterility is an effective and environmentally safe method for Lepidopteran pest suppression that is useful under a variety of environments and crop production strategies.

(2) F_1 sterility is compatible with all pest control tactics. The combination of F_1 sterility with pheromones, natural enemies, host plant resistance, entomopathogens and insecticides results in synergistic pest population suppression.

This CRP also highlighted several important points and areas that would benefit from further research and development to increase the economic viability of F_1 sterility programmes.

(1) The inclusion of F_1 sterility or of alternative genetic control methods within conventional Integrated Pest Management (IPM) technology, including other biological methods, should be promoted.

(2) Pilot tests should be conducted in areas sufficiently isolated so that reliable data can be obtained. The flight range of normal and treated moths should be assessed.

(3) In economically important Lepidoptera, research should focus on improving rearing technologies, searching for morphological sex-linked markers and developing genetic sexing techniques.

   (a) Development of diets using locally available ingredients would reduce rearing costs, especially in locations with developing economies.

   (b) Improvements in mass rearing are needed to take advantage of the economy of scale as evidenced in dipteran SIT programmes.

(4) Development of genetic sexing techniques, especially those that would eliminate females at the egg or early larval stage, would reduce rearing costs, would increase the efficiency of rearing, irradiation and release by 100% and would eliminate assortative mating of released moths in the field.

(5) F_1 sterility should be combined with alternative genetic and biological methods in order to magnify the effect of population reduction.

(6) Only moths that were irradiated as late pupae or pharate adults, that is, pupae less than 48 hours before adult emergence, or in the early adult stage should be released.

(7) Insects with synchronized emergence can be stockpiled by storing pupae at proper low temperatures for several weeks in order to have sufficient insects for pilot field releases. An optimal temperature and maximal storage period may be species-dependant and should be tested on each species before using this approach.

The research from the F_1 sterility CRP has contributed to the development of a new Co-ordinated Research Project on "Evaluating the Use of Nuclear Techniques for the Colonization and Production of Natural Enemies". This new CRP was initiated in October 1999 and is planned to run until 2004, with scientific teams from fourteen countries. As one of the research objectives in this new CRP, F_1 sterility is being developed as a method to study possible Lepidopteran biocontrol agents for invasive noxious weeds. The Joint Division is also starting a CRP on "Improvement of codling moth SIT to facilitate expansion of field application" which is planned to run from 2002–2006.

The FAO/IAEA sponsored CRPs have had major impacts in the direction of future research for the control of Lepidopteran pests. For example, expanded research and implementation programmes on F_1 sterility in combination with natural enemies are underway in Tunisia for suppression of the carob moth, *Ectomyelois ceratoniae*, and on the island of Mauritius for control of the diamondback moth, *Plutella xylostella*. F_1 sterility programmes for other Lepidopteran pest species also are being considered.

Observations on the growth parameters of *Spilosoma obliqua* (Lepidoptera: Arctiidae) reared on artificial diets and reproductive competence of this irradiated pest and its progeny

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Abstract. Ten trials were conducted to standardize an artificial diet for *Spilosoma oblique*. The main ingredients included agar, mulberry leaves, yeast, casein, cellulose, sucrose, glucose, ascorbic acid, sorbic acid, antibiotics, vitamin C, Wesson´s salt, and vitamin B complex in variable proportions. A diet formulation with increased amounts of Wesson´s salt, choline chloride and iron supplement was found to be most suitable when growth parameters were measured. The deleterious effects when 6-day old pupae were treated with 100 and 150 Gy of gamma radiation were increased in the F_1 generation as compared to the parental generation. Various combinations of crosses between treated and untreated moths indicated that females were more sensitive to gamma radiation when compared to males. F_1 sterility was attained when male moths treated with 100 Gy were allowed to mate with untreated females. Two species of hymenopteran parasitoids of the genus *Glyptapanteles* and *Meteorus* were found to infest *Spilosoma obliqua*. These parasitoids may serve as an effective addition to an integrated pest management program for this pest in Bangladesh.

1. INTRODUCTION

Jute (*Corchorus capsularis* and *Corchorus olitorius*) is one of the prime cash crops of Bangladesh, but this valuable agricultural commodity suffers great losses due to the severe attack by the jute hairy caterpillar, *Spilosoma oblique* during jute growing season [1]. This pest also attacks sunflower, pulse, groundnut, radish, and soybean [2]. The application of SIT-F_1 sterility is gaining popularity in the U.S.A [3], Canada [4] and in some other countries as a potential method of pest control that reduces the use of harmful insecticides. Laboratory cultures and mass rearing of the target insect species are prerequisites for the effective use of this nuclear technology.

Accordingly, we concentrated on developing artificial diets that would allow for the successful rearing of the jute hairy caterpillar under laboratory conditions. Diet development was an essential step for the continuous supply of good quality insects for a sterile insect release programs. At the same time, proper attention was given to the improvement of general laboratory conditions. Our initial efforts to develop suitable artificial diets for these caterpillars were unsuccessful due to an epidemic nuclear polyhedrosis virus and the presence of a hymenopteran parasitoid in our colony material. Recently, we have been successful in producing a suitable diet for large scale rearing of *Spilosoma obliqua*. In this report we present results on the performance of *Spilosoma obliqua* on artificial diet, the response of *Spilosoma obliqua* to sterilizing and substerilizing doses of radiation [5], and the mating performance and sperm transfer of adults irradiated as pupae.

2. MATERIALS AND METHODS

2.1. Improvement of artificial diets and laboratory rearing

Five artificial diets consisting of agar, mulberry leaves, yeast, casein, cellulose, sucrose, glucose, ascorbic acid, sorbic acid, tetracycline, Vanderzandt's, modified Wesson's salt

mixture, vitamin B complex, propionic acid and distilled water were prepared for rearing the larvae of *Spilosoma obliqua* (Table 1, A-E). The ingredients choline chloride and iron are referred to as a new group V and were added along with the other ingredients in some of the diets. We also reared the caterpillar on fresh host food (mulberry leaves) as a comparison. Temperature, relative humidity and photoperiod in the rearing room were 32±1°C, 80±5% and 12L:12D. The laboratory colony was initiated with eggs collected from the field.

Collected eggs were washed with 1% sodium hypochlorite solution for 5 minutes and rinsed with distilled water for 10 minutes. Eggs were dried at room temperature for 15–30 minutes and 100 eggs were placed on 20 ml of artificial diet in glass beakers (250 ml). Larvae were apportioned with fresh diet and moved to separate containers as they developed. At 10 days, larvae (3^{rd} instar) were thinned to 12 larvae in each beaker and provided with fresh diet. Fourth instars were fed and thinned to 4 larvae in each beaker. Diet was added at the end of each instar until pupation. The pupae were collected and sexed and kept in separate jars until adult emergence. Pairs of newly emerged adults were placed together in an oviposition jar lined with blotting paper. The jar bottom was covered with round filter paper. A 10% sugar solution placed on a cotton pad was provided as food. Egg clusters were laid on the blotting paper in 1–2 days, and eggs were collected after this period.

A laboratory colony was reared on fresh mulberry leaves and held under the same conditions as the larvae reared on the artificial diets. The larvae were supplied with fresh leaves three times daily. Biological parameters were recorded for insects reared on both artificial and natural diets. These parameters included duration of different developmental stages, percent pupation, pupal weight, percent adult emergence, sex ratio, longevity of male and female moths, fecundity and fertility.

2.2. Radiation biology

Six day old male and female pupae were irradiated separately at 0 (untreated), 100 and 150 Gy of gamma radiation. A Co^{60} source (88 Gy/minute) was used to treat the pupae. Irradiated male and female pupae of each treatment were placed in separate cages for adult emergence. After emergence, treated females (TF), treated males (TM), untreated females (UF), and untreated males (UM) were confined in the mating and oviposition cages in the following combinations: (i) UF x UM, (ii) TM x UF), and (iii) TF x UM. Data on mating, female fecundity, egg hatch, and longevity of both male and female moths were recorded.

After collecting F_1 females (F_1F) and F_1 males (F_1M) from P crosses at each dose, further crosses with new virgin insects of the same age and generation were made in the following combinations: (i) UF x UM, (ii) F_1M x UF, and (iii) F_1F x UM. The moths obtained from F_1 crosses (F_2 generation) of respective dose were further crossed with untreated insects as follows: (i) UF x UM, (ii) F_2M x UF, and (iii) F_2F x UM. In F_1 and F_2 crosses, similar data were recorded as in the P crosses.

2.3. Parasitoids

Two hymenopteran parasitoids of *Spilosoma obliqua* were found in the course of our studies. These were collected and sent to the USDA, Systematic Entomology Laboratory, Beltsville, MD, USA for taxonomic identification. These parasitoids might be integrated into an integrated management program for this pest in Bangladesh.

3. RESULTS AND DISCUSSION

3.1. Improvement of artificial diet and laboratory rearing

The composition of diets A, B, C, D, and the modified diet E are compared in Table 1. Studies using the laboratory cultures of *Spilosoma obliqua* showed that the larvae adapted successfully to the modified diet E compared with the other diets on the basis of their post-embryonic growth and developmental profile. Caterpillars placed on diet A consumed a negligible amount of food without any sign of post-embryonic development and ultimately died. On the other hand, caterpillars reared on diets B and C consumed more food, but the insects on diet B did not grow after the 2nd moult (3rd instar). Caterpillars on the diet C reached penultimate instar but failed to form pupae. On artificial diet D, the larval and pupal period increased whereas pupal weight, adult emergence, and egg hatch decreased in comparison with the natural diet. Addition of choline chloride and iron into diet E was quite effective in producing better growth than on any of the diets tested.

Comparisons of *Spilosoma obliqua* reared on diet E and natural diet revealed that the duration of larval (from 4th to 6th instar) and pupal periods increased on diet E (Table 2), whereas oviposition, egg hatch, pupal production, pupal weight, and adult emergence decreased (Table 3). Longevity of adults (Table 2) and sex ratio did not differ on either of the larval diets (artificial or natural).

Table 1. Artificial diets used to rear *Spilosoma obliqua* in the laboratory

Group	Ingredients	Diets				
		A	B	C	D	E
I	Agar (g)	30.00	18.00	18.00	18.00	18.00
	Distilled water (ml)	750	950	950	950	950
II	Jute leaf powder (g)	75.00	-	-	-	-
	Mulberry leaf powder (g)	-	80.00	80.00	80.00	80.00
	Torula yeast(g)	10.00	25.00	26.00	30.00	30.00
	Casein (g)	12.00	20.00	22.00	22.00	24.00
	Cellulose (CMc) (g)	4.20	6.00	6.50	6.50	6.50
	Sucrose (g)	12.00	10.00	10.00	10.00	10.00
	Glucose (g)	6.00	5.00	6.00	6.00	6.00
III	Ascorbic acid (g)	2.00	3.60	3.70	3.70	3.70
	Sorbic acid (g)	2.00	1.00	1.00	1.00	1.00
	Sodium benzoate (g)	2.00	-	-	-	-
	Sodium acetate (g)	1.00	-	-	-	-
	Vanderzant's (g)	2.50	3.00	5.00	5.00	6.00
	Wesson's salt mix(g)	4.00	1.50	0.75	0.75	1.50
	Tetracycline (g)	8.00	0.30	0.30	0.30	0.30
	Ampicillin (g)	4.00	-	-	-	-
	Vitamic B complex (g)	-	-	-	1.0	1.0
IV	Propionic acid (ml)	-	1.00	-	0.75	1.00
V	Choline chloride (g)	-	-	-	-	2.00
	Iron (g)	-	-	-	-	0.20

Table 2. Duration of different developmental stages of *Spilosoma obliqua* on artificial diet E and on natural diet

Diet[1]	Time (days) to complete each developmental stage									
	L1[2]	L2	L3	L4	L5	L6	L1-6	Pupae	Males	Females
E	3.5 ±0.5	3.5 ±0.5	3.4 ±0.6	3.7 ±0.4	4.0 ±0.8	3.2 ±0.9	21.3 ±3.7	12.5 ±2.0	4.0 ±0.5	5.5 ±0.5
Natural	3.5 ±0.5	3.5 ±0.5	3.3 ±0.7	3.4 ±0.6	3.3 ±0.4	2.9 ±0.8	19.9 ±3.5	8.6 ±0.4	4.0 ±0.5	5.5 ±0.5

[1] 100 larvae were tested in each diet.
[2] L= larval instar.

Table 3. Fecundity, egg hatch, pupae production, and adult emergence[1] of *Spilosoma obliqua* reared on artificial diet e and on natural diet

Diet	Fecundity (±SD)	% Egg Hatch (±SD)	% Pupation (±SD)	Male Pupal Weight	Female Pupal Weight	% Adult Emergence (±SD)
E	485.6± 39.8	87±6.5	79±7.1	0.22±0.01	0.39±0.02	90±3.0
Natural	546.6± 55.3	91±4.8	85±7.5	0.25±0.01	0.45±0.01	94±2.3

[1] Each data point represents mean of 10 replicates; each replicate contains 10 pairs of insects.

3.2. Radiation biology

The larval developmental period was significantly ($P<0.05$) prolonged in all crosses with increasing dose of radiation when compared with the untreated control (Table 4). It was observed that the larvae of the F_1 generation were profoundly affected by radiation at both doses (100 and 150 Gy). At the same time, larval developmental time was significantly reduced in TM x UF crosses. Also, some deleterious morphological effects were observed including impairment of melanization and tanning of pupae (15–18% at 100 Gy and 20–25% at 150 Gy), and delayed pigmentation of adults.

Mating behaviour, fecundity, egg hatch, and longevity of adults of both sexes when 6 day-old pupae were irradiated are shown in Table 5. The mating frequency was markedly reduced at 150 Gy, whereas no difference from the control was observed at 100 Gy. Fecundity decreased as the radiation dose increased. Significantly ($P<0.05$) fewer eggs were laid from the crosses of TF x UM in all test doses which suggests that females were more susceptible to gamma radiation than are males. Our results correspond with the findings of Graham et al. [6] and Begum et al. [7]. The percentage of egg that hatched decreased with the increase of radiation dose indicating that egg hatch was dose dependent. Irradiation at both 100 and 150 Gy significantly reduced longevity of adults, particularly of males.

Mating frequency, fecundity and egg hatch in F_1 crosses decreased markedly with the increasing radiation dose. At both doses, reproductive parameters were higher in F_1F x UM crosses than in the reciprocal crosses (Table 6). This trend was also observed in F_2 crosses

Table 4. Effects on larvae (parental and f_1 generation) from irradiated pupae of *Spilosoma obliqua*

Mean duration of the different larval instars (days ± SD)								
Dose	Crosses	L1	L2	L3	L4	L5	L6	L1-L6
Parental Crosses								
0	UM x UF	3.3±0.3a	3.5±0.5a	3.4±0.2c	3.5±0.4c	3.6±0.4d	2.8±0.0c	20.1±2.6f
100	TM x UF	3.3±0.4a	3.6±0.7a	3.4±0.8c	4.1±0.6bc	4.5±0.5bcd	3.9±0.6b	22.8±3.6e
	UM x TF	3.4±0.6a	3.5±0.4a	3.5±0.5c	4.3±0.3bc	4.7±0.6bc	3.8±0.4b	23.2±2.8de
150	TM x UF	3.2±0.4a	3.5±0.5a	3.7±0.6c	4.6±0.8ab	4.9±0.7abc	4.4±0.5ab	24.3±3.4cd
	UM x TF	3.5±0.5a	3.7±0.3a	3.5±0.4c	4.8±0.8ab	5.1±0.7abc	4.2±0.4ab	24.8±3.1cd
F_1 Crosses								
0	UM x UF	3.2±0.2a	3.5±0.5a	3.4±0.3c	3.6±0.4c	3.5±0.3d	2.7±0.4c	19.9±2.1f
100	F_1M x UF	3.6±0.6a	3.7±0.7a	3.9±0.7bc	4.5±0.5abc	4.3±0.4cd	4.1±0.6ab	24.1±3.5cde
	UM x F_1F	3.5±0.5a	3.6±0.4a	4.3±0.3abc	4.8±0.6ab	5.2±0.8abc	4.5±0.7ab	25.9±3.3bc
150	F_1M x UF	3.4±0.4a	3.5±0.6a	4.7±0.8ab	5.0±1.0ab	5.5±0.9ab	4.9±0.8a	27.0±4.5b
	UM x F_1F	3.6±0.7a	4.1±0.8a	5.1±1.2a	5.4±1.4a	5.9±1.7a	4.7±1.1ab	28.8±6.9a

[1] Each data point represents mean of 5 replicates; each replicate contains 20 larvae. In a column, means followed by the same letter are not significantly different at 5% level by DMRT (Duncan´s Multiple Range Test).

Table 5. Mating success, fecundity, egg hatch, and longevity[1] of *Spilosoma obliqua* irradiated as 6 day-old pupae with different doses of gamma radiation

Dose (Gy)	Crosses	Mated (%)	Eggs/female (Mean ± SD)	Egg hatch (%)	Longevity (Mean ± SD)	
					Male	Female
0	UM x UF	100a	583.4±55.0a	99a	4.0±0.5a	5.5±0.8a
100	TM x UF	100a	374.2±60.0b	56b	2.5±0.3b	5.5±0.6a
	UM x TF	100a	317.6±34.4c	47b	4.0±0.5a	4.5±0.5b
150	TM x UF	75b	267.2±42.3d	27c	2.5±0.4b	5.5±0.5a
	UM x TF	71b	209.0±28.3e	22c	3.9±0.7a	5.1±0.6ab

[1] Each data point represents mean of 5 replicates (5 pairs per replicate). In a column, means followed by the same letter are not significantly different at 5% level by DMRT.

(Table 7). Similar to the parental generation, longevity of males was more affected at both doses in the crosses of TM x UF than longevity of females in the TF x UM crosses.

Irradiation of the parental generation with substerilizing doses resulted in marked reduction of egg hatch in both F_1 and F_2 progeny. These results are typical for radiation-induced inherited sterility [8]. Levels of sterility in the parental and F_1 crosses were very similar to those reported by Arthur et al. [9, 10] for the sugarcane borer and the fall armyworm. Based on the results of this study, and with respect to the decrease in the number of eggs laid and the percentage of egg hatch in the TM x UF crosses, 100 Gy seems to be a sufficient dose to induce an appropriate level of F_1 sterility in adult moths.

3.3. Incorporation of parasitoids with F_1 sterility programs

During our laboratory and field studies, we observed several parasitoids and predators attacking *Spilosoma obliqua*, especially from the order Hymenoptera. Taxonomists identified two new hymenopteran parasitoids of the genus *Glyptapanteles* and *Meteorus* collected at our

Table 6. Mating success, fecundity, egg hatch, and longevity[1] of f_1 *Spilosoma obliqua* moths whose parents were irradiated as pupae with different doses of gamma radiation

Dose (Gy)	Crosses	Mated (%)	Eggs/female (Mean ± SD)	Egg hatch (%)	Longevity (Mean ± SD)	
					Male	Female
0	UM x UF	100	602.7±42.9a	97	4.0±0.5a	5.5±0.8a
100	F_1M x UF	79	246.3±38.4b	21	2.4±0.7b	5.5±0.5a
	UM x F_1F	84	291.6±47.4b	28	3.9±0.6a	4.7±0.7a
150	F_1M x UF	51	108.0±29.8c	9	2.1±0.6b	5.4±0.4a
	UM x F_1F	63	139.4±39.6c	14	4.0±0.5a	5.0±0.5a

[1] Each data point represents mean of 5 replicates (5 pairs/replicate). In a column, means followed by the same letter are not significantly different at 5% level by DMRT.

Table 7. Mating success, fecundity, egg hatch, and longevity[1] of F_2 *Spilosoma obliqua* moths (P moths were irradiated as pupae)

Dose (Gy)	Crosses	Mated (%)	Eggs/female (Mean ± SD)	Egg hatch (%)	Longevity (Mean ± SD)	
					Male	Female
0	UM x UF	100	547.5±62.6a	97	4.2±0.7a	5.4±0.3a
100	F_2M x UF	82	313.3±57.1b	39	2.4±0.6b	5.5±0.5a
	UM x F_2F	89	353.7±41.3b	43	4.0±0.5a	5.3±0.7a
150	F_2M x UF	61	145.4±28.4c	16	2.6±0.4b	5.4±0.3a
	UM x F_2F	68	188.6±36.7c	19	4.0±0.5a	5.2±0.8a

[1] Each data point represents mean of 5 replicates (5 pairs/replicate). In a column, means followed by the same letter are not significantly different at 5% level by DMRT.

rearing facility. We established a laboratory culture of *Spilosoma obliqua* to be used for rearing natural enemies. This is a prerequisite for planning any biological control program. The use of natural enemies should be a promising addition to nuclear techniques, such as SIT, F_1 sterility technique, for the control of jute hairy caterpillars.

4. CONCLUSIONS

In conclusion, we were successful in developing an artificial diet for rearing *Spilosoma obliqua*, but further research is required to make the diet more suitable for all larval stages and to increase the rate of pupal production. Currently, the diet is prepared using many imported, expensive ingredients. Therefore, attempts are being made to increase cost-effectiveness by substituting local materials.

Irradiation doses of 100 and 150 Gy were used to study the level of induced sterility in *Spilosoma obliqua* treated as 6 day-old pupae. With these doses, treated pupae produced viable, competitive males. Further evaluation is required to evaluate these substerilizing doses in the field. As a preliminary observation, incorporation of hymenopteran parasitoids (i.e., *Glyptapanteles* sp. and *Meteorus* sp.) may prove to be an additional, effective biological agent for controlling the target jute pest in the field.

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Growth, development, reproductive competence and adult behaviour of *Spodoptera litura* (Lepidoptera: Noctuidae) reared on different diets

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Abstract. *Spodoptera litura* was reared on natural food (castor leaves, *Ricinus communis*) and on a several semi-synthetic diets using quasi mass rearing techniques. The effect of the different diets and rearing regimes on *S. litura* growth, development, reproductive competence and adult behaviour was measured. *Spodoptera litura* reared from a modified chickpea-based diet provided the greatest growth index and index of adequacy. These studies were conducted as a prerequisite for the evaluation of F_1 sterility technique.

1. INTRODUCTION

*Spodoptera litura (*Lepidoptera: Noctuidae), the common cutworm, is an economically serious and polyphagous pest in India. This pest attacks a wide range of food plants belonging to diverse botanical origins (112 cultivated food plants belonging to 44 families all over the world; 60 plants known from India) [1–4]. A multifaceted approach is required for the control of this pest because it has developed resistance against a range of insecticides and because of limitations in other control strategies when applied as a single tactic [5, 6]. The sterile insect technique (SIT), including F_1 sterility, can be used for Lepidoptera (group to which *S. litura* belongs). In a preliminary study, the effect of substerilizing doses of gamma radiation on the growth, development and reproductive behaviour of *S. litura* in F_1 progeny of treated moths suggested this pest might be managed by the F_1 sterility technique [7]. As a pre-requisite to in-depth evaluations of the reproductive performance and behaviour of *S. litura* in response to two substerilizing doses (100 Gy and 130 Gy), we developed quasi mass rearing techniques and evaluated several semi-synthetic diets.

2. MATERIALS AND METHODS

Quasi mass rearing technology was evaluated using the natural food (castor leaves) and semi-synthetic diets for the ability to produce high quality *Spodoptera litura* required for radiation biology experiments. Environmental conditions in the insectary were 26.8±1^{o}C, 75±5% R.H. and 12L:12D photoperiod.

2.1. Rearing on natural food

The eggs laid by mated females were incubated at high relative humidity (about 80%) and maintained in containers with castor leaves (*Ricinus communis*) to provide the 1st instar neonates immediate access to food. First instars were placed in groups of 100 each in a 500 ml container. From the 4th instar onwards, larvae were reared in groups of 12–15 in 1 litre containers on castor leaves. Larvae were allowed to pupate in moist, loose soil. To avoid any mechanical injury, the pupae were sexed on the 3rd or 4th day after the sclerotization and hardening of pupal integument. Adult moths eclosed in 7–8 days. Moths, generally 10–12 pairs, were held for mating and oviposition in cages (20 x 20 x 20 cm) with 15–20% honey solution as food. Castor leaf was provided as an ovipositional substrate. After 8–10

generations adult moths were collected from agricultural fields and mixed with the laboratory colony so that vigour could be maintained and genetic deterioration caused by extended laboratory culture could be avoided.

2.2. Rearing on semi-synthetic diet

In order to develop a suitable semi-synthetic diet for mass rearing, various combinations of ingredients were evaluated for optimal growth and development of this moth. Recipes were modified from a variety of different diets used for different species of *Spodoptera* [8–11]. The proposed semi-synthetic diet consisted mainly of a ground dry seed source (chickpea, wheat, wheat germ or soybean) mixed with yeast and synthetic additives in an agar base. A chickpea-based (CpN), in which chickpea was used as a main carbohydrate complement, was reasonably satisfactory in preliminary experiments. Therefore, two more chickpea-based, semi-synthetic diets (with little modification) were prepared for evaluation: CpCs (chickpea based semi-synthetic diet with castor leaf powder) and CpSn (chickpea-based, semi-synthetic diet with sinigrin) (Table 1).

Agar was added to water and autoclaved. All ingredients of parts B and C were mixed thoroughly and added to the dissolved agar. Finally, the antibiotic and vitamin mix (part D)

Table 1. Constituents of the semi-synthetic diet proposed for rearing *Spodoptera litura*.

Ingredients	Amount
PART A	
Agar	25.00 g
Deionized water	750.00 ml
PART B	
Casein	44.00 g
Ground chickpea seeds	93.50 g
Wesson's salts	12.50 g
Cholesterol	1.25 g
Brewer's yeast	19.00 g
Methyl-p-hydroxybenzoate	1.25 g
Sugar	39.00 g
Sorbic acid	2.00 g
Deionized water	400.00 ml
4 M KOH solution	6.25 ml
PART C	
Corn oil	2.50 ml
Linseed oil	2.50 ml
Formaldehyde 10% solution	5.50 ml
Sinigrin (1%)	3.53 ml
PART D	
Antibiotic and vitamin mixture[1]	7.50 g
Choline chloride	1.25 g

[1] Composition: chloramphenicol (2 g), streptomycin (4 g), tetracycline (36 g), ascorbic acid (80 g), vitamin E (Evion, 0.2 g; Merck Co.), vitamin mixture (2 g; Roche Co.).

was added when the mixture cooled to about 70°C. When the diet cooled completely it was covered and stored at 4°C. Neonates were placed in a plastic chamber (8 cm diam x 8 cm) containing a strip of diet. About 100 larvae were placed in each chamber and allowed to feed and grow in a gregarious manner. After 4–5 days, 3rd instars were placed individually with diet in glass specimen tubes (2.5 cm diam. x 10 cm) or plastic containers (6 x 6 x 6 cm). Fresh diet was replaced after 72–96 h. Larvae pupated inside the diet. Pupae were collected after 48–72 and were allowed to eclose in mating/oviposition cages.

2.3. Handling techniques to control microbial contamination

Various protocols were adopted to prevent microbial infection during the rearing of *S. litura*. Examples of these protocols included:

(i) formaldehyde fumigation to disinfect the insectary before introduction of insects,

(ii) washing glassware and plastic containers with detergent, 5% formalin and oven drying at about 70–80°C,

(iii) washing castor leaves with water and 0.001% $KMnO_4$,

(iv) surface sterilization of eggs for 3–5 seconds with 0.2% sodium hypochlorite or 2% formalin,

(v) surface sterilization of pupae for 10 s with 1% sodium hypochlorite or 4–5% formalin, and

(vi) adding diet to insect containers under aseptic conditions in a laminar flow hood.

2.4. Diet suitability for insect maintenance

Suitability of the various diets was determined using an index incorporating growth, development, fecundity (indicated in terms of female pupal weight) and survival into one empirical factor, similar to the index described by Raulston [12]. The suitability of the diets was also evaluated in terms of reproductive behaviour of the reared insects. Experiments on mating success were conducted in cages (each cage having 10–15 pairs, comprising one replicate). The mating success of moths was assessed by dissection of females immediately after the death. The presence of a spermatophore in the bursa copulatrix indicated that the female had mated; the number of spermatophores indicated the number of matings.

3. RESULTS

Growth indices revealed that the chickpea-based diets were better than the other semi-synthetic diets. Of the three types of chickpea diets evaluated, the CpSn diet (Table 1) was more suitable for insect growth. Insects that fed on CpSn diet had a growth index of 2.61 and an index of adequacy of 1.54. These values were similar to those calculated for insects reared on castor leaves (Table 2). The index of adequacy for larvae that developed on the Soybean-based diet was about 20% lower than that for larvae that developed on the CpSn diet.

Table 2. Growth and development of *Spodoptera litura* on different diets.

Nature of Food	% Pupation[1]	Larval Period	Female Pupal Weight (g)	Developmental Period (days)	% Adult Emergence[1]	Growth Index[2]	Sex Ratio M:F	Index of Adequacy[3]
Castor leaf	88.4a	16.2±0.7a	0.339±0.007a	27.9±0.3a	83.6±3.3a	2.99a	1:0.98	1.748a
Wheat germ diet	57.3c	17.5±0.8ab	0.320±0.012ab	1.4±0.9bc	52.3±2.6d	1.66c	1:1.01	0.955d
Wheat diet	71.8b	18.1±0.5b	0.313±0.010b	31.9±0.7c	64.3±2.4c	2.01bc	1:1.10	1.109cd
Soybean diet	76.7b	17.1±0.4a	0.310±0.008b	29.5±0.8b	67.8±3.3bc	2.29bc	1:1.02	1.233c
Chickpea diet CpCs[4]	78.4b	16.8±0.5a	0.314±0.002b	29.8±0.7b	69.5±2.9b	2.33b	1:0.95	1.304bc
Chickpea diet CpN[5]	79.1b	16.8±0.4a	0.344±0.008a	29.7±0.8b	71.8±2.5b	2.41b	1:0.94	1.470b
Chickpea diet CpSn[6]	81.4ab	16.5±0.4a	0.339±0.009a	28.6±0.6ab	74.9±2.9ab	2.61ab	1:0.96	1.540ab

[1] Observed in groups of 25 larvae = 1 replicate (analyzed with ANOVA; data transformed using arcsine square root).
[2] Growth index = % adult formation / developmental period.
[3] Index of adequacy = (female pupal weight / larval period) x % adult formation.
[4] Chickpea based diet + yeast + synthetic constituents + castor leaf powder (3.5 g/litre).
[5] Chickpea based diet + yeast + synthetic constituents.
[6] Chickpea based diet + yeast + synthetic constituents + sinigrin.

Means ± SE followed by the same letter in a column are not significantly different at $P < 0.05$ (calculated using ANOVA followed by LSD post test); n = 10.

Table 3. Reproductive and mating parameters of *Spodoptera litura* reared on different diets.

Nature of Food	Preoviposition Period (d)	Oviposition Period (d)	Eggs per Female	Mating Frequency	Mating Success[1] (%)	Fertility[1] (%)	Longevity (days)	
							Male	Female
Castor leaf	1.72±0.08a	7.62±0.31a	2088±49a	1.7±0.1a	94.1±1.9a	88.9±1.4a	10.3±0.4a	9.3±0.3a
Chickpea diet (CpSn)	1.69±0.09a	7.33±0.24a	2155±74a	1.7±0.2a	89.6±2.4ab	78.6±3.1b	9.8±0.5ab	9.5±0.2a
Soybean diet	1.75±0.09a	7.25±0.31a	1750±105b	1.6±0.2a	87.5±2.9ab	72.5±3.5b	9.5±0.6ab	9.0±0.3a
Wheat diet	1.85±0.08a	5.65±0.35b	985±125c	1.5±0.3a	86.6±4.3ab	53.4±4.2c	7.8±0.9b	7.3±0.6b
Wheat germ diet	1.92±0.12a	5.35±0.59b	746±172c	1.4±0.6a	77.0±6.9b	50.3±3.5c	7.1±0.7c	6.9±0.5b

[1] For statistical analysis by ANOVA, the percentage data were transformed using arcsine square root.
Means ± SE followed by the same letter in a column are not significantly different at $P < 0.05$ (calculated using ANOVA followed by LSD post test); n = 10.

Because the adult's behavioural competence is a crucial parameter in deciding the success of the F_1 sterility technique, assessment of adult behaviour was also examined on the semi-synthetic diets. Average longevity of mated males and females was not significantly different for castor leaves, the CpSn diet and the soybean diet, however, longevity decreased on the wheat germ and wheat-based diets. Mean oviposition was highest for females that developed on the CpSn diet (Table 3). On soybean diet, the mean number of eggs laid per mated female was 1750. Oviposition was reduced by 54.2% on wheat-based diet and by 65.3% on wheat germ diet compared to that on CpSn diet. Egg viability was greatest on castor leaves (88.9%), followed by on CpSn diet (78.6%), soybean diet (72.5%), wheat-based diet (53.4%) and wheat germ diet (50.3%). Insects reared on CpSn diet exhibited better fertility in comparison with the insects reared on other semi-synthetic diets (Table 3). Among all the semi-synthetic diets evaluated, CpSn diet was found to be most suitable for *Spodoptera litura* rearing. This diet showed an increased growth index due to the incorporation of sinigrin as a phagostimulant. This addition caused enhanced survival and higher fecundity. Therefore, the improved chickpea based diet (CpSn) along with the natural food were selected as the diets to use for rearing *S. litura* for further studies.

4. DISCUSSION

The operation of a mass rearing facility is a basic pre-requisite for conducting evaluations on sterile insect technique (SIT) and F_1 sterility principle. Castor leaves were used to culture *S. litura* given that growth and reproduction of the insects reared on this host was comparable with that of wild insects. In addition, the quality of insects could be maintained for a long time (R. K. Seth, unpublished). Moreover, the use of castor leaves economical compared with the cost of semi-synthetic diet. Therefore, besides developing semi-synthetic diet, a culture of *S. litura* also was maintained on castor leaves for evaluation of F_1 sterility.

The main carbohydrate component in the different semi-synthetic diet was supplied as ground, dry seeds (i.e., chickpea, wheat, wheat germ, or soybean). Satisfactory growth of *Spodoptera* species has been reported from soybean-based [9,10] and wheat germ-based diets [11]. However, in this study the best index of adequacy for growth of *S. litura* was recorded when insects were reared on the chickpea-based diet. The diet's major component was carbohydrates, of which sucrose provided 6.6% of the total. Microbial inhibitors (formaldehyde, methyl-p-hydroxybenzoate, sorbic acid, and antibiotic mix) comprised about 1.14% of the diet. Brewer's yeast was added as a source of vitamin B complex. Although brewer's yeast contains choline, the amount has been reported to be insufficient [13]. Therefore, multivitamin tablets (Roche Co.) and choline chloride were added. Vitamin C has been shown to be an essential requirement for a limited number of herbivores [14]. Our diet contained 0.33% of L-ascorbic acid and provided satisfactory growth of *S. litura*. These results agree with the findings reported by Levinson and Navon [9]. Vitamin E was added to improve adult survival and reproduction.

Most insects require sterols in their diet. As such, we added cholesterol to the diets tested. In addition, a dietary requirement for polyethenoid fatty acids has been demonstrated for several lepidopterans [15]. Unsuccessful moulting, eclosion and reproduction are common symptoms of linoleic or linolenic acid deficiency. David et al. [11], working with *Spodoptera exempta*, showed that diets containing corn and linseed oil yielded more adults than diets containing cholesterol. As such, corn oil and linseed oil were added to the chickpea diet. A sinigrin solution (0.0025%) was added as a phagostimulant which resulted in better growth and increased the index of adequacy for this diet.

In our experiments the fecundity of *S. litura* was slightly enhanced in moths reared on chickpea diet (up to 2,155 eggs per mated female) compared to moths reared on castor leaves. Similarly, Lukefahr and Martin [16], Tamhankar et al. [17] and Tamhankar and Dongre [18] reported that synthetic diet-reared lepidopterans had better fecundity than those reared on natural food. We conducted an assessment of the quality of the reared insects. This included calculation of the growth index, and evaluation of adult behaviour and mating competence of moths. We found that pupal characteristics were important and useful in assessing the quality and vigour of *S. litura* [19]. Threshold values for adult survival, sperm transfer and mating competitiveness also should be established for assessing the use of F_1 sterility as a control strategy.

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Cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae): large scale rearing and the effect of gamma radiation on selected life history parameters of this pest in China

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Abstract. Effective large scale rearing of the cotton bollworm, *Helicoverpa armigera* (Hübner), has been developed in China. A "celled unit" system was developed to replace the traditional test tube for cotton bollworm laboratory rearing. Larvae are reared at 26.5ºC, ≈70% RH, and a long day photoperiod of 14L:10D. Pupae are harvested at about day 20. Percent adult emergence is between 89–93%, and adult females lay an average of 768 eggs. Under this rearing system one generation is completed in 40–42 days and percent pupation is about 66–71%. Mature *Helicoverpa armigera* female and male pupae were treated with different doses of gamma radiation and out-crossed with untreated mates. Mating ability of both sexes was not affected by radiation. Treated females were highly sterile and laid significantly fewer eggs than untreated controls. Females treated with 300 Gy were completely sterile, while females treated with 250 Gy and 200 Gy still had minimal residual fertility.

1. INTRODUCTION

The cotton bollworm, *Helicoverpa armigera* (Hübner), is considered to be one of the most important lepidopteran pests attacking cotton (*Gossypium hirsutum*) in China. The Department of Pest Control of the Institute for Application of Atomic Energy (CAAS) conducts research with the objective of finding more effective ways of controlling or suppressing populations of *Helicoverpa armigera*. In particular, our focus has been on using the Sterile Insect Technique through the application of F_1 Sterility for cotton bollworm control. The preliminary results of this work, primarily the improvements we have made in large scale rearing and the effect of irradiation on selected life history parameters of *Helicoverpa armigera*, are reported here.

2. MATERIALS AND METHODS

2.1. Rearing of *Helicoverpa armigera*

The ingredients used in the artificial diet for *Helicoverpa armigera* are listed in Table 1. The diet recipe was provided to us by Institute of Plant Protection, CAAS, and was modified by our laboratory. Diet preparation is as follows: after the ingredients are weighed, the agar, sucrose, sorbic acid and nipagin are added to 700ml water and the mixture is heated until boiling while stirring constantly. In a separate container, the sterilized cornmeal, soybean meal and brewers yeast are mixed with 600ml water, and then added to the agar solution. The mixture is thoroughly mixed and allowed to cool to 75ºC. The vitamins, formaldehyde and cotton seed oil are added.

Two rearing methodologies for *Helicoverpa armigera* were compared in our laboratory. In the glass test tube individual rearing system, the diet is prepared as above and dispensed into a porcelain box (23 x 15 x 4 cm) and allowed to cool completely. When the diet has gelled it is cut into 1.3–1.5 cm^2 portions. One diet recipe yields about 180–200 diet squares. The glass test tubes (2.5 cm diameter x 8.0 cm high) are sterilized and each tube receives one diet square and sealed with a sterilized cotton plug.

Table 1. Diet ingredients in the artificial diet for *Helicoverpa armigera*

Ingredients	Quantity
Soybean Meal	100.00 g
Cornmeal	200.00 g
Brewer's Yeast	90.00 g
Sucrose	40.00 g
Agar (C.P.)	13.00 g
Sorbic Acid (C.P.)	2.00 g
Nipagin (C.P.)	12.00 g
Formaldehyde (37–40%)	2.00 ml
Vitamin C (C.P.)	6.00 g
Vitamin B Complex [1]	20 tablets
Cotton Seed Oil	4.00 ml
Water	1300.00 ml

[1] Each tablet contains 3 mg B_1, 1.5 mg B_2, 10 mg Niacinamide, 1 mg Pantothenic Acid

The cell-unit rearing system is as follows: The prepared diet is dispensed into the bottom of a 3-layered cell unit (Fig. 1), which is made of 128 small individual cells (2 x 2 x 1.3 cm). A Plexiglas plate is placed at the bottom of each cell-unit. The cell-unit has a cover made of fine copper mesh, which provides ventilation while preventing the larvae from escaping the cells. The middle layer of the unit provides free moving space for the larvae. One diet recipe is enough to fill three cell-units. In each system, newly hatched larvae were transferred to the diet for rearing.

Cotton bollworm adults are kept inside metal cages (20 x 20 x 30 cm), covered on the top with blue cotton cloth. Adults mate and females lay eggs on the cotton cloth. Eggs are removed from the cloth and incubated in sealed glass jars until they hatch. Newly hatched larvae are transferred to the diet using a small sheep's hairbrush. The brush is sterilized with 0.16% Bestaquam-s solution. Two neonates are placed per tube or per cell. Larval rearing takes about 19 days at 26.5°C, 70% RH, and a photoperiod of 14L:10D. Larvae pupate inside the diet. Pupae are harvested beginning at day 20. Pupal collection is faster in the cell-unit system because diet does not need to be extracted from individual tubes. Pupae are kept at 26.5°C and 40–50% RH and ambient photoperiod, and 100 pupae are placed inside the metal cages (covered with sawdust) prior to emergence. Emerging moths are collected and sexed daily, then placed into other cages for mating and egg laying. Twenty-five to thirty pairs are used per cage. The adult room is maintained at 26.5°C, 75–85% RH and a photoperiod of 14L:10D. A 10% sucrose solution is supplied for adult nutrition. Oviposition cloths and sucrose solution are changed daily. Collected eggs are maintained at 4–6°C. At this temperature eggs can be held for about 7–10 days. Eggs are incubated at 26.5°C for hatching.

Quality control parameters including percent pupation, pupal weight, percent adult emergence and adult longevity were measured for both the test tube and the cell-unit rearing systems. Percent pupation, which gives an indication of rearing efficiency, and can be affected by the size of the rearing container. Pupae were sexed after harvest and 30 males and 30 females are individually weighed and a mean weight is calculated for each sex. Pupal weight can be affected by many factors. In our laboratory we have observed that pupal weight can be significantly affected by the size of the rearing container, with smaller pupae being produced in smaller containers.

We also recorded the number of days that females laid eggs. We have discovered that when cotton bollworm are reared in the laboratory for more than two years without the introduction of new genetic material, the total egg laying period is reduced from the normal 7–8 days to 3–4 days and the average number of eggs laid per day, especially the first day after mating, is significantly increased. Nonetheless, the total number of eggs laid in their lifetime is not different between wild and laboratory adapted strains. During field-cage experiments conducted by our laboratory in 1998–1999, we found that the mating ability of the colony that showed shorter egg laying periods was significantly reduced. It appears that egg laying period is an important indicator of cotton bollworm quality. Other parameters, such as total number of eggs, percent hatch and sperm quality are routinely checked when a new colony is established from field-collected material.

2.2. Radiation Biology Studies

The *Helicoverpa armigera* used in this study came from our laboratory colony. Founder material used to initiate this colony was from Hennan Province, China. The colony has been in culture less than two years, and 9–10 generations are reared per year. A $Cobalt^{60}$ source with a dose rate of 3 Gy/min was used to irradiate the bollworm pupae. Female and male pupae were sexed and irradiated 1–2 days before adult emergence with 200, 250 and 300 Gy of gamma radiation. Emerging adults were individually paired inside glass jars and allowed to mate and lay eggs. For each dose tested the following crosses were made: Normal (untreated) females crossed with normal males, normal females crossed with irradiated males and irradiated females crossed with normal males. Insects were held at 26±1°C, 85±10% R.H., and a photoperiod of 14L:10D (06:00–20:00). A 10% sucrose solution was supplied for adult nutrition and changed daily. Insects were allowed to mate and lay eggs until female death. Test jars were covered with a blue cotton cloth that served as an ovipositional substrate. Cloths were changed daily. Adult longevity, mating ratio (= percent mated adults), mating frequency per pair (= number of matings by each pair in their lifetime), total number of eggs, and number of hatched eggs were recorded for each treatment. The data were analyzed using the SPSS (Statistics Package for Social Science) software package.

3. RESULTS AND DISCUSSION

3.1. Rearing of *Helicoverpa armigera*

Results comparing the two rearing systems for *Helicoverpa armigera* are shown in Table 2. Percent pupation in the cell-unit system was significantly lower than in the glass test tube rearing system. All other parameters measured were almost identical between the two systems. The cell-unit system is not as tightly sealed as the test tubes and, as such, more larvae escaped from the cell-units prior to pupation. Larval escape was the main reason for the lower percent pupation, however, more pupae were collected from the cell-unit system per unit time. Insect quality in the two different rearing systems was almost the same.

In the glass test tube rearing system, one diet recipe is sufficient to prepare about 200 test tubes, which will yield no more than 180 pupae. In contrast, in the cell-unit system, one diet recipe can fill three units, giving a total of 384 cells, which will yield 260–270 pupae. As such, the cell-unit system produces almost twice the number of pupae per diet recipe. Furthermore, the glass test tube system is more difficult to handle and the glass tubes occupy more space than the cell-units. During larval rearing the test tubes are handled individually such as for apportioning the diet, transferring the larvae, sealing the tube, etc.

Table 2. Comparison of two different rearing systems for *Helicoverpa armigera*

Parameter		Glass test tube		Cell-unit	
		No. examined	Results	No. examined	Results
Percent pupation		3482	85–90	6912	66–71
Mean pupal weight (mg)	F	180	317.2 ± 56.9	180	321.4 ± 61.6
	M	180	324.4 ± 72.9	180	329.4 ± 87.1
Percent adult emergence		1800	88–94	1800	89–93
Adult longevity (days)	F	180	16.9 ± 4.1	225	17.1 ± 3.9
	M	180	14.1 ± 3.0	180	13.7 ± 3.1
Egg laying period (days)		51	5–7	57	5–7

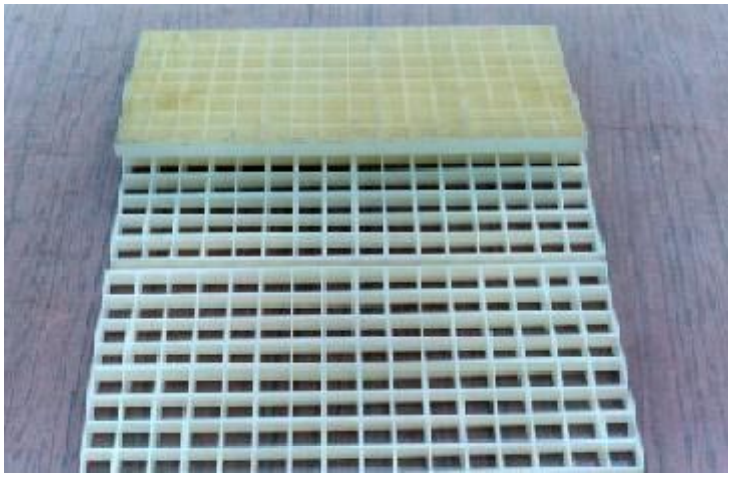

FIG. 1. Cell-unit used for large scale rearing of Helicoverpa armigera.

This is not the case with cell-units. Furthermore, newly hatched larvae can be sprinkled on top of the diet filled units which increases the speed with which diet can be seeded and decreases the adverse effects of handling the larvae. The cell-unit system saves on labour, time and money. Most laboratory colonies of *Helicoverpa armigera* in China are maintained using the glass test tube rearing system that is not well suited for large scale rearing. We encourage our colleagues to switch to the cell-unit system. Our current level of production is 40,000 pupae per week using the cell-unit system.

3.2. Radiation Biology Studies

Results of the effects of three doses of gamma radiation on some life history parameters of *Helicoverpa armigera* are shown in Tables 3 and 4. When normal (untreated) females were mated with irradiated males treated with different doses of gamma radiation, no significant difference in female fecundity was observed when results were compared to the controls.

However, percent egg hatch was significantly affected (Table 4). When irradiated females mated with normal (untreated) males, fecundity and fertility were significantly affected. Liu Xiaohui [1] showed that when cotton bollworms are treated with doses as high as 400 Gy, irradiation does not affect their mating ability under laboratory environments. However, 400 Gy significantly affects percent egg hatch as well as sperm transfer, sperm quantity and sperm activity. On the other hand, a dose of 250 Gy does not affect sperm transfer and sperm activity in cotton bollworms.

Table 3. Irradiation effects on mating and adult longevity of *Helicoverpa armigera*

Dose (Gy)		No. observed	Mating ratio (%)	Mating degree (mean ± SD)	Adult Longevity in days (mean ± SD)	
					Females	Males
NFxNM		225	88	1.96 ± 1.07a	17.1±3.9 a	13.7±3.1 a
NFxTM	200	109	93	2.43 ± 1.31 a	-	15.8±3.7 a
	250	123	83	2.22 ± 1.21 a	-	16.6±4.2 a
	300	128	87	2.19 ± 1.14 a	-	13.9±3.8 a
NMxTF	200	66	87	2.1 ± 1.11 a	16.9±3.4 a	-
	250	70	85	2.5 ± 1.32 a	19.7±4.5 a	-
	300	68	85	1.7 ± 0.98 a	16.4±4.3 a	-

Means followed by different letters in the same column are significantly different (Duncan's multiple range tests; with significance level 0.05)

Table 4. Irradiation effects on fecundity and fertility of *Helicoverpa armigera*

Dose (Gy)		No. observed	Eggs per Female (mean ± SD)	Percent egg hatch (mean ± SD)
0		52	768 ± 528 a	71.06 ± 19.38 a
NFxTM	200	41	754 ± 445 a	37.14 ± 19.46 b
	250	51	641 ± 384 a	33.03 ± 19.08 c
	300	36	678 ± 397 a	25.00 ± 15.02 d
NM×TF	200	28	535 ± 375 b	1.06 ± 2.98 e
	250	32	475 ± 384 b	0.36 ± 1.64 e
	300	28	362 ± 314 b	0^{e}

Means followed by different letters in the same column are significantly different (Duncan's multiple range tests; with significance level 0.05)

These results suggest that 200 Gy and 250 Gy might be suitable doses to employ in a sterility program against cotton bollworm. Our results indicate that irradiation does not affect the mating ability of treated cotton bollworms when measured in the laboratory environment. However, much research remains to be done including studies on mating ability, mating competitiveness, and flight ability of treated bollworms in the field. It is clear from our results that the higher the dose of radiation, the lower the quality and the competitiveness of irradiated moths. Our laboratory intends to assess whether we can rear a male-only colony and we will also study the effects of F_1 sterility for *Helicoverpa armigera* in the field.

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The effects of radiation on the biology and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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Abstract. The effect of irradiating male *Helicoverpa armigera* with a substerilizing dose (100 Gy) of gamma radiation on the growth, development and reproduction of subsequent generations was studied in the laboratory. This dose of gamma radiation had no significant detrimental effects on larval and pupal weights or on the duration of the pupal period in the F_1 progeny. However, it lengthened the duration of the larval period by two days. In the F_2 generation, the progeny of the Tf_1F x Tf_1M cross had significantly lighter pupae. The effects of this substerilizing dose of radiation and of the resulting inherited sterility on the reproduction of *Helicoverpa armigera* were similar to those described for other species of Lepidoptera. No detrimental effects on P_1 and F_1 female fecundity were recorded. Crosses involving Tf_1 females laid only about one half the number of eggs laid by the controls, however the range in the number of eggs laid by these females fell within the normal range for *Helicoverpa armigera*. Fertility of crosses involving P_1 males was greatly affected; fertility in these females was only 61% of that exhibited by the controls. This deleterious effect was inherited in the F_1 and F_2 generations and was maximally expressed when F_1 progeny of the NF x TM cross were inbred. Egg hatch was almost completely inhibited in sibling crosses while outcrosses of the F_1 progeny showed a 64–70% reduction in egg hatch when compared to controls.

1. INTRODUCTION

The corn earworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae), is one of the most destructive pests of agricultural crops in the Philippines. It is a highly polyphagous insect feeding on 83 different plant species [1]. Recommended control measures against *Helicoverpa armigera* include planting of resistant varieties, the use of synthetic and microbial insecticides, and the release of egg parasitoids, *Trichogramma* spp. (Hymenoptera: Trichogrammatoidea). Another control strategy that can be used for *Helicoverpa armigera* is the use of irradiated males that are capable of transferring sterility to the next generation, which is termed F_1 or inherited sterility. This strategy is compatible with conventional control methods and potentially can be integrated as a major component of area-wide management of *Helicoverpa armigera*. The major advantages of releasing partially sterile males over that of releasing fully sterilized males is (1) releasing a more competitive male with less radiation-induced somatic damage and (2) the subsequent introduction of built-in sterility into the native population [2].

North and Holt [3] induced inherited sterility in *Helicoverpa zea* by treating males with 200 Gy and mating them with normal females. The fertility from this cross was 36% compared with 78% from an untreated cross. Moreover, a higher incidence of sterility was observed when F_1 progeny from the irradiated males were out-crossed to untreated moths. However, fecundity in female progeny from crosses between untreated females and F_1 males or from crosses between F_1 females and untreated males did not differ from the controls. The authors concluded that *H. zea* was an ideal candidate for the application of inherited sterility as a new method of pest control and other authors have agreed [4, 5] with this conclusion.

Carpenter et al. [6] studied inherited sterility in *H. zea*. They report that a dose of 100 Gy induced deleterious effects that were inherited through the F_2 generation. Reduced fecundity

and fertility and increased larval and adult mortality in treated corn earworm were reported by these authors. Carpenter and Gross [7] conducted field studies to investigate the efficacy of using inherited sterility to suppress seasonal populations of *H. zea*. They demonstrated that seasonal increases of wild *H. zea* were significantly delayed or reduced (or both) in areas where irradiated, substerile males were released. The incidence of larvae with chromosomal aberrations collected from the test sites indicated that irradiated males were competitive with wild males in mating with wild females, and were successful in producing F_1 offspring that further impacted the wild population. The potential of combining inherited sterility with releases of the parasitoid *Archytas marmoratus* (Diptera: Tachinidae) to manage *H. zea* was recently demonstrated in the laboratory and in the field [8].

Helicoverpa zea is closely related of *Helicoverpa armigera*. Laster and Hardee [9] studied the inter-mating compatibility between *H. zea* and *Helicoverpa armigera*. Although no induction of backcross sterility was detected, the high percentage of female mating indicated a high degree of genetic compatibility between the two species. As such, studies on the radiation biology of *H. zea* should be relevant to *Helicoverpa armigera*. However, to better determine the potential of delayed sterility in a control program for *Helicoverpa armigera*, detailed information is needed about the biology and reproduction of irradiated males and their progenies. Therefore, the current study was undertaken to document the effect of the substerilizing dose of radiation used on corn earworm (100 Gy) on the biology, fertility and survival of F_1 and F_2 adults of *Helicoverpa armigera*.

2. MATERIALS AND METHODS

The *Helicoverpa armigera* used in this study were taken from the laboratory colony maintained at the Toxicology Laboratory, Department of Entomology, UP Los Baños. The colony has been in culture for 32+ generations. All larvae were reared at ambient conditions (27±2ºC) in plastic cups (50 ml) containing a soybean-corn diet [10]. A dose of 100 Gy of gamma radiation was applied to mature pupae (pharate males) using a Co^{60} Gammacell 220 delivering ca. 3.45 Gy/min. Emerging males (T) were paired with normal (N) females. N x N crosses served as controls. The progeny resulting from the N x N and N x T crosses were used in the F_1 crosses (see below). Adult F_1 moths from the N x T crosses were inbred and outcrossed with moths from the N x N crosses. Nf_1 moths were inbred to serve as controls and as source of Nf_2 moths for F_2 crosses. All possible crosses of F_2 moths were made depending on the number of surviving adults in each line. Mating scheme for all crosses preformed is shown in Table 1.

Table 1. Mating crosses

Generation	Type of Mating (F x M)	Progeny Designation
P_1	N x T	Tf_1
	N x N	Nf_1
F_1	Tf_1 x Tf_1	A
	Nf_1 x Tf_1	B
	Tf_1 x Nf_1	C
	Nf_1 x Nf_1	D
F_2	A x A, A x B, A x C, A x D	
	B x A, B x B, B x C, B x D	
	C x A, C x B, C x C, C x D	
	D x A, D x B, D x C, D x D	

Each individual cross was placed inside pint-sized cardboard containers to mate and lay eggs. A cotton ball soaked in 30% honey solution was provided as a food source. The top of each container was covered with nylon cloth that served as an ovipositional substrate. Moths were allowed to mate and lay eggs for 7 days after which the females were dissected and the number of spermatophores in the bursa copulatrix was counted to determine mating status. Ovipositional cloths were changed daily and eggs were counted to assess female fecundity from the different crosses. Egg cloths were incubated for three days and the number of eggs that hatched was counted. First instar larvae from each cross were reared on soybean-corn diet and their growth and development were monitored. Larvae were weighed after each moult and the duration of each stadium (larval and pupal) was noted. Adult emergence and sex ratio were recorded. Only individuals that completed development (egg to adult) were considered for analysis. In all cases, treatments were subjected to analysis of variance and multiple mean separation tests were conducted using Waller-Duncan's K-ratio t test [11].

3. RESULTS

When P_1 males of *Helicoverpa armigera* were treated with 100 Gy, the larval weights of the F_1 4th instar larvae were significantly affected (Table 2). Larval weights averaged 28.1 mg as compared to 36.4 g for the untreated controls (F = 8.85, d.f. = 80). The remaining larval and pupal weights of the progeny from both treated and untreated male parents were not significantly different. In the F_2 generation, larval weights were not significantly affected by treatment. However, the pupae from the Nf_1F x Tf_1M cross were significantly lighter when compared to the control (F = 5.83, d.f. = 72). The lighter pupal weights recorded from the Nf_1F x Tf_1M cross were similar to the F_1 pupal weights recorded earlier. Very few offspring were produced from the Tf_1F x Tf_1M cross (A) and no individual was able to complete development in this particular treatment (n = 10; survival = 0).

Table 2. Larval and pupal weights of f_1 and f_2 *Helicoverpa armigera* when the parent was treated with 100 gy of gamma radiation [1]

Cross	No. of Larvae	Mean Larval Weight of Each Instar (mg) (±SD)					Mean Pupal Weight (mg) (±SD)
F_1 progeny		2nd	3rd	4th	5th	6th [2]	
NF x TM (Tf_1)	50	1.2 ± 0.6 a	7.8 ± 3.0 a	28.1 ± 10.0 b	126.5 ± 63.0 a	267.4 ± 49.0 a	398.3 ± 50.0 a
NF x NM (Nf_1)	50	1.3 ± 0.6 a	8.1± 3.0 a	36.4 ± 16.0 a	152.0 ± 64.0 a	266.1 ± 47.0 a	410.1 ± 44.0 a
F_2 progeny							
Tf_1F x Tf_1M (A)	10	-	-	-	-	-	-
Nf_1F x Tf_1M (B)	50	1.2 ± 0.5 a	6.6 ± 3.0 a	36.9 ± 23.0 a	194.5 ± 133.0 a	257.3 ± 74.0 a	407.1 ± 48.0 b
Tf_1F x Nf_1M (C)	50	1.6 ± 0.6 a	9.8 ± 5.0 a	49.5 ± 22.0 a	231.5 ± 78.0 a	363.2 ± 75.0 a	452.8 ± 48.0 a
Nf_1F x Nf_1M (D)	50	1.6 ± 0.4 a	10.6 ± 5.0 a	56.3 ± 24.0 a	263.1 ± 73.0 a	357.2 ± 94.0 a	447.4 ± 37.0 ab

[1] Means (±SD) on the same column, with the same letter are not significantly different (K ratio = 500). N = untreated; T = treated.
[2] Means based on few individuals, majority of the insects exhibited only five instars.

Treatment of the parental males with 100 Gy significantly prolonged the developmental period of the F_1 larvae (F = 14.92, d.f. = 80), while F_1 pupal duration was not significantly affected (F = 1.72, d.f. = 80) (Table 2). Duration of larval development in untreated crosses was about 18 days while larval duration in the progeny of irradiated male parents was about 20 days. The duration of larval and pupal development in the F_2 progeny was not significantly different among treatments. Larval development took about 14–15 days (F =1.68, d.f. = 72) while the length of pupation was about 9 days (F = 1.50, d.f. = 72) in all crosses.

In general, larvae in the F_1 generation took longer to develop than larvae of the F_2 generation. Even larvae from untreated P_1 crosses (N x N) developed slower that those from untreated F_1 crosses (Nf_1 x Nf_1). An explanation for this might be that the temperature in the rearing room during the F_1 generation rearing was lower (24.36ºC) than that during the F_2 generation (28.22ºC). The cooler temperature experienced by the F_1 larvae prolonged their developmental period.

Gamma radiation (100 Gy) caused <10% reduction in the percentage of larvae surviving to adulthood in the F_1 generation (Table 3). However, the percentage of F_2 progeny surviving to the adult stage was greatly reduced as compared to the controls. When one of the parents of the F_2 progeny was a Tf_1, (either B or C), very few larvae survived to adulthood (<35%) compared with 90% survival in the controls. Finally, this dose of radiation did not produce a significant shift in the sex ratio of *Helicoverpa armigera*. The normal female : male ratio in our *Helicoverpa armigera* colony is 1.0F:1.4M

The effects of radiation and inherited sterility on the reproductive potential of *Helicoverpa armigera* are similar to those described for other species of Lepidoptera, including those for the closely related species *H. zea* and *H. virescens* [6, 12]. When the treated *Helicoverpa armigera* males were outcrossed to normal females, the components of reproduction (i.e., eggs per female and egg hatch) were affected differently by radiation treatment (Table 4). This dose (100 Gy) did not have a detrimental effect on P_1 female fecundity, and females mated with treated males laid nearly as many eggs as did the controls (F =0.26, d.f. = 99). In the F_1, generation, low fecundity was recorded from the cross of Tf_1 females mated with Tf_1 males; however, the differences were not significant (Table 4) (F =3.91, d.f. =60). Fecundity in all F_1 crosses fell within the normal range.

Table 3. Effect on some life history parameters of *Helicoverpa armigera* when the parent was treated with 100 gy of gamma radiation [1]

Cross	Mean Larval Period (days) (± SD) [1]	Mean Pupal Period (days) (± SD) [1]	% Larvae Surviving to Adulthood	Sex Ratio (F:M)
F_1 progeny of				
NF x TM (Tf_1)	19.90 ± 1.85 a	11.15 ± 0.62 a	78.00	1.0:1.3
NF x NM (Nf_1)	18.07 ± 1.40 b	11.40 ±0.68 a	86.00	1.0:1.4
F_2 progeny of				
Tf_1F x Tf_1M(A)	-	-	-	-
Nf_1F x Tf_1M(B)	15.28 ±2.55 a	9.36± 0.78 a	28.00	1.0:1.4
Tf_1F x Nf_1M(C)	15.06 ± 1.07 a	8.88 ± 0.67 a	32.00	1.0:0.5
Nf_1F x Nf_1M(D)	14.40 ± 1.01 a	9.16 ± 0.49 a	90.00	1.0:1.4

[1] Means in the same column, followed by the same letter are not significantly different (K ratio = 500). N = untreated; T = treated.

When evaluating the effect of 100 Gy treated P_1 males on the reproduction of the F_2 progeny, 16 total crosses were possible. However, only seven crosses were made (A x A, B x B, B x D, D x D, D x A, D x B and A x D), based on the availability of surviving adults from the F_1 generation. Of these, only the first four crosses produced mated females (ascertained by the presence of spermatophore in the bursa copulatrix), however, the percentage of these matings that produced eggs was greatly reduced. Mated females from the A x A and B x B crosses laid as many eggs as did the control (D x D). The number of eggs per female was greatly reduced in the B x D cross. Because only very few successful mating pairs per treatment were recovered, we did not subject the data to statistical analysis.

For all females that mated, mean number of spermatophores did not differ significantly among treatments. In the P_1 matings, females crossed with irradiated males mated 1.5 times while females crossed with nonirradiated males mated 1.3 times in seven days (F value = 2.96, d.f. = 99). In the F_1 matings, the number of matings in treatment groups ranged from 1.4–1.6 times while matings among control insects averaged 1.8 times (F value = 0.81, d.f. = 60). Tf_1 females mated as frequently as Nf_1 females (i.e., spermatophore counts were not significantly different among treatments).

In the P_1 generation, female fertility when females were mated to treated males was only 64% of the control (F value = 46.93, d.f. = 99). The inherited deleterious effects were fully expressed when the F_1 progeny of NF x TM were inbred (Table 4). In particular, crosses between siblings exhibited 92% reduction in fertility when compared with the control, while the outcrosses of the F_1 progeny had about 68–71% reduction in egg hatch compared with the control (F =84.69, d.f. = 60).

A frequency distribution of the percentage egg hatch in all untreated females crossed with P_1 males as well as of the various crosses among the F_1 progeny showed a shift toward sterility.

Table 4. The effect of 100 gy of gamma radiation applied to pharate adults of *Helicoverpa armigera* on the reproductive parameters of p_I, f_1, and f_2 adults[1]

Mating Type (F x M)	No. of Pairs	No. of Mated Females	Progeny	Eggs per Mated Female in 7 days	Percent Egg Hatch	Spermatophores per Mated Female
				P_I		
N x T	110	45	Tf_1	1001.7 ± 431.9 a	45.9 ± 19.8 b	1.3 ±0.6 a
N x N	110	51	Nf_1	1046.4 ± 445.6 a	71.6 ± 17.7 a	1.5 ± 0.6 a
				F_1		
Tf_1 x Tf_1	65	16	A	786.6 ± 539.2 a	5.5 ± 6.3 c	1.4 ± 0.6 a
Nf_1 x Tf_1	65	11	B	937.3 ± 592.6 a	23.0 ± 16.6 b	1.5 ± 0.5 a
Tf_1 x Nf_1	65	17	C	1102.9 ± 500.5 a	21.0 ± 16.9 bc	1.6 ± 0.8 a
Nf_1 x Nf_1	65	20	D	1374.1 ± 522.5 a	71.8 ± 12.3 a	1.8 ± 0.9 a
				F_2		
A x A	7	1		923.00	0.00	1.0 ± 0.0
B x B	15	1		991.00	18.8 ± 23.9	1.0 ± 0.0
B x D	16	4		366.5 ± 340.6	26.7 ± 1.3	1.3 ± 0.5
D x D	15	5		980.4 ± 504.8	64.2 ± 9.9	1.6 ± 0.9

[1] Means (±SD) on the same column (per generation), followed by the same letter are not significantly different (K ratio=500). N = untreated; T = treated.

In the P_1 generation, only 24% of the females mated to irradiated males had $\geq$60.1% egg hatch, while 74% of the untreated controls had $\geq$60.1% egg hatch. A similar frequency distribution was observed in the fertility of eggs laid by the progeny of P_1 treated males. In Nf_1 females crossed with F_1 male progeny from P_1 irradiated males (Tf_1M), only 27% had a fertility higher than 40%, while in the Tf_1F crossed with Nf_1M, only 18% showed fertility higher than 40%. Reduction in egg hatch had its greatest expression when F_1 progeny of the NF x TM cross was inbred. One hundred percent of the Tf_1F crossed with Tf_1M were less than 20% fertile, while 100% of the controls had egg hatch of $\geq$60.1%.

4. DISCUSSION

Several parameters of growth and reproduction of *Helicoverpa armigera* were measured to evaluate the effect of a substerilizing dose of gamma radiation (100 Gy) on this particular species. Our results showed that the weight of F_1 4th instar larvae, the duration of F_1 larval development, the F_2 pupal weights, the F_2 larval survival and the fertility in the P_1, F_1, and F_2 were significantly affected. Proshold and Bartell [12] proposed that the simplest explanation for delayed development in progeny of treated *H. virescens* would be an alteration in hormonal and enzymatic production caused by inherited chromosomal rearrangements. In our study, larval development in F_1 *Helicoverpa armigera* larvae was significantly delayed, while development of F_2 larvae was not affected. No significant effect on pupal development time for either generation was observed. Carpenter [13] concluded that chromosomal aberrations in *H. zea* occur less frequently in the F_2 than in the F_1 generations. Our results suggest the possibility that the F_2 generation of *Helicoverpa armigera* is beginning to recover from the chromosomal aberrations caused by irradiation of the parental males. This situation is advantageous in the application of F_1 sterility because it suggests that field development of progeny from released substerilized males might be in synchrony with that of the wild population.

Carpenter et al. [6] suggest that the best indicator of the amount of radiation-induced deleterious effects in the P_1 progeny is the number of larvae surviving to the adult stage. In our experiments, larval survival was only reduced by <10% in the F_1 generation while larval survival in the F_2 generation was greatly reduced. The F_2 progeny from Tf_1 parents had problems with moulting and resulted in a high incidence of deformed larvae and pupae and pupal death. Nonetheless, individuals that did survive had similar developmental period to the controls. This developmental synchrony is expected to occur even under field conditions.

Fecundity in the P_1 and F_1 generations was not affected by treatment while fertility was greatly affected (Table 3). The inherited deleterious effects in the F_1 generation caused a significant reduction in egg hatch. In addition, the moths in each of the F_1 crosses exhibited lower mating than those of the parental crosses, which is to be expected [5]. Similar findings were reported for *H. zea* [6] and for *H. virescens* [14].

Inherited sterility in the progeny of irradiated Lepidoptera has been shown to be the result of reciprocal chromosomal translocations [2, 6]. Our findings with *Helicoverpa armigera* agree with these reports. In related research [15], we observed that at 100 Gy, 86% of the *Helicoverpa armigera* sperm contained several chromosomal rearrangements in the form of chains and rings. At 150 Gy, only 1.7% of the spermatocytes examined appeared normal [15]. In general, 4 to 38 chromosomes were involved exhibiting from 1 to 6 translocations. It has been stated [12] that a single translocation per irradiated sperm would render the F_1 progeny at least 50% sterile, and more complex translocations would increase the level of sterility.

Proshold and Bartell [12] suggested that the sterility of the male could arise from any one of three causes: (1) lack of mating, (2) lack of sperm transfer, and (3) reduced hatch of fertilized eggs. In our study, females crossed with irradiated males had 41% successful matings and the data presented in Table 3 was collected from these mated females. This would suggest that lack of mating is not the main cause of sterility in *Helicoverpa armigera*. The data on fertility demonstrates that reduced hatch of fertilized eggs could be the major cause of sterility. Lack of sperm transfer was not examined in our study.

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Radiation-induced substerility of *Ostrinia furnacalis* (Lepidoptera: Pyralidae) integrated with the release of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) for area-wide control

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Abstract. The mating competitiveness of *Ostrinia furnacalis* F_1 male moths (progeny of male parents irradiated with 200 Gy) was compared with the mating competitiveness of untreated moths. These studies revealed that F_1 male moths were involved in more than 50% of the matings with normal females. The flight ability and response towards sex pheromone was similar for F_1 and untreated moths, although the number of F_1 moths captured was slightly less than the number of untreated moths captured. The number of eupyrene sperm in the testes of P_1 moths treated with 200 Gy was similar to the number of eupyrene sperm in the testes of normal moths. However, the number of sperm bundles was significantly reduced in the testes of 200 Gy F_1 moths. Compared to normal moths, daily sperm descent into the duplex ejaculatorius was affected only at day 3 after eclosion of F_1 moths. Sperm transfer to spermatheca by 200 Gy F_1 male moths was less than that of their irradiated (200 Gy) parents and of normal moths. Successive releases of *Trichogramma ostriniae* in the egg stage of first and second generation *Ostrinia furnacalis* were combined with the release of F_1 moths from male parents treated with 200 Gy. The combination of the F_1 sterility technique with augmentative biological control suppressed the wild population of this pest in 500 hectares of field corn.

1. INTRODUCTION

As expected for most lepidopteran insects, the Asian corn borer, *Ostrinia furnacalis* (Guenee), is quite radio-resistant. The doses of radiation required to induce full sterility are sufficiently high to significantly reduce the viability of the moths. Nevertheless, because of the unique nature of lepidopteran chromosomes, a sub-sterilizing dose of radiation induces inherited sterility or F_1 sterility, which has many advantages over the sterile insect technique (SIT) [1, 2]. Previous studies on the Asian corn borer showed that the percentage egg hatch of normal females mated with males irradiated with 200 Gy and 250 Gy was 28 or 27% compared with 43 or 42% for females mated with males irradiated with 100 Gy and 150 Gy. The sterility of the F_1 progeny from male moths treated with 200 Gy was 3–14%. Therefore, 200 Gy is an acceptable dose for an inherited sterility program in which both irradiated males and females of *Ostrinia furnacalis* are released [2].

The development of inherited sterility as an effective control strategy requires cost-effective mass rearing of quality insects, and careful evaluation of this technique in the field. In the present study, we evaluated the mating behaviour, sperm movement and transfer, and the ability of F_1 male moths to disperse in the field. Also, we evaluated the integration of releases of *Trichogramma ostriniae*, a biological control agent, with F_1 sterility as an effective tactic for the management of *Ostrinia furnacalis*.

2. MATERIALS AND METHODS

The insects used in our experiments were taken from the Asian corn borer colony kept at the Institute for Application of Atomic Energy, CAAS, Beijing. The laboratory colony is

reared on a modified larval diet in which sawdust and de-fatted soybeans replace agar and soybeans, respectively. All parameters evaluated including pupal eclosion, pupal weight, adult emergence, mating ability, number of egg masses laid per female and percentage egg hatch, indicated that this modified diet was acceptable. The cost of ingredients for the modified larval diet was reduced by 41.6%, and the handling procedures were simplified.

To measure mating competitiveness of the F_1 progeny, male pupae were irradiated with 200 Gy. The radiation source was a Co^{60} irradiator with a dose rate of 200–203 Gy/min. The pupae were placed in polystyrene boxes and irradiated 2 days before adult emergence. After emergence the males were paired with normal females and their progeny were reared in the laboratory until the emergence of F_1 adults. The wings of male and female F_1 moths were marked with fluorescent powder mixed with alcohol. Different ratios of F_1 and normal moths were placed in small cages and the incidence of mating was checked every 15 min. Data recorded for each female moth included the number of egg masses laid, percentage egg hatch and the presence or absence of a spermatophore. The number of eupyrene sperm bundles in the testes and duplex ejaculatorius of 200 Gy males, 200 Gy F_1 males and normal males was counted at 1, 2 and 3 days after emergence. A dissecting and a phase contrast microscope were used to observe the formed spermatophores and the eupyrene sperm in the spermatheca.

We conducted field studies to evaluate the ability of F_1 males to disperse from a release site. In 1996, normal males (n = 9,101) and 150 Gy F_1 males (n = 10,740), marked with Calco Red and Sudan Blue II, respectively, and released into a cornfield. Traps baited with sex pheromone were set along the southeast radius at 100, 330 and 550m from the release site. In 1997, normal males (n = 9,203) and 200 Gy F_1 males (n = 11,200) marked as above, were released into the field, and dispersal was evaluated in the same way as in 1996. Data from this mark/recapture study was analyzed with the test of percentage comparison for two samples [3].

Trichogramma ostriniae (Hymenoptera: Trichogrammatidae) were released into 500 ha of corn each year (from 1994 to 1997) during the egg stage of the first generation of *Ostrinia furnacalis*. On June 14, 17 and 20, about 150,000 *T. ostriniae* were released per hectare. Each hectare had 15 release sites from which 10,000 *T. ostriniae* were released (n = 75 million total). In 1997, additional *T. ostriniae* were released during the egg stage of the second generation of *Ostrinia furnacalis*. These additional parasitoids were released in three groups of 25 million on July 12, 15 and 18 (n = 75 million total). Also during 1995–1997, about 2 million irradiated (200 Gy) *Ostrinia furnacalis* adults (both male and female) were released per year during the first *Ostrinia furnacalis* generation into the same area that received releases of *T. ostriniae*.

3. RESULTS AND DISCUSSION

The F_1 male adults from 200 Gy irradiated male parents were competitive with normal males in mating with females in laboratory cages. Out of the total matings observed, the F_1 males were involved in more than 50% of all matings.

In the field, the number of males captured at 550 m from the release point divided by the total number released was not significantly different (u = 2.326 at P = 0.01) between normal males and 150 Gy F_1 males (u = 0.2740) in 1996 (Table 1). Similar results were obtained in 1997 when normal males and 200 Gy F_1 males (u = 0.8377) were used (Table 2). However, the ratio of total number of males captured in all traps divided by total number released was significantly different between normal males and 150 Gy F_1 males (u = 4.184) and between normal males and 200 Gy F_1 males (u = 4.731). Apparently, some of the released F_1 males had

a reduced ability to disperse or to respond to sex pheromone in the field. In general, the irradiated 150 Gy F_1 males demonstrated a greater ability to disperse and/or respond to sex pheromone in the field than did the 200 Gy F_1 males.

Table 1. Number of 150 Gy F_1 *Ostrinia furnacalis* males and normal males captured in sex pheromone traps in the field in 1996

	Trap distance from release point (m)					
	100[a]		330[b]		550[b]	
	NM	150 Gy F_1M	NM	150 Gy F_1M	NM	150 Gy F_1M
No. Captured	47	21	85	75	30	20

[a] 3 traps; [b] 6 traps; NM = normal males.

Table 2. Number of 200 Gy F_1 *Ostrinia furnacalis* males and normal males captured in sex pheromone traps in the field in 1997

	Trap distance from release point (m)					
	100[a]		330[b]		550[b]	
	NM	200 Gy F_1M	NM	200 Gy F_1M	NM	200 Gy F_1M
No. Captured	35	19	102	83	41	24

[a] 3 traps; [b] 6 traps; NM = normal males.

Compared with normal males, the number of eupyrene sperm bundles was not reduced in the testes and in the duplex ejaculatorius of 200 Gy irradiated males. However, the number of eupyrene sperm bundles in the testes of 200 Gy F_1 males was significantly reduced, and the number of eupyrene sperm bundles in the duplex ejaculatorius of 200 Gy F_1 males 3 days after emergence was significantly less than that in unirradiated males (Table 3). Spermatophores formed by 200 Gy F_1 males during mating appeared to be normal. We did not count the number of eupyrene sperm in the spermatophore and in the spermatheca. However, we did observe that the volume of the sperm component from the F_1 males was less than that from normal males.

Results of releasing irradiated moths and the egg parasitoid *T. ostriniae* to control *Ostrinia furnacalis* in the field are presented in Table 4. The parasitism rate of *T. ostriniae* in the release area was about 50% on first generation eggs of *Ostrinia furnacalis* compared with 5–8% in the control field. The parasitism rate was 82% in the field where *T. ostriniae* was released against the second generation of *Ostrinia furnacalis* compared with 27% parasitism observed in the control field.

Irradiated (200 Gy) male and female *Ostrinia furnacalis* moths were released into the same field with the parasitoid from 1995 to 1997. The release ratio of irradiated males to wild males increased from 2.2:1 in 1995 to 6:1 in 1997 (Table 4). The number of larvae per 100 corn plants and the percentage of damaged corn plants in the treated area declined each year, however, a corresponding decline did not occur in the control (CK) field. In 1997, the combined release of irradiated moths and *T. ostriniae* resulted in 81.8% parasitism and 10.2% undeveloped embryos in *Ostrinia furnacalis* eggs during the second generation. Therefore, the percentage of egg mortality reached 92% (Table 4).

Table 3. Sperm production in testes and ejaculatorius duplex of *Ostrinia furnacalis* as a consequence of irradiation

Treatment	Age (days)	No. of moths examined	Eupyrene sperm bundles in testes*	Eupyrene sperm bundles in duplex ejaculatorius*
normal	1	16	262.34±50.53a	22.88±12.36cd
male	2	14	198.71±39.50bc	45.36±21.59b
	3	13	184.81±42.08cd	75.38±24.58a
200 Gy	1	8	231.98±21.22ab	27.38±16.23cd
treated	2	7	188.87±34.30bcd	34.57±14.02bc
males	3	7	188.87±39.84bcd	62.57±10.11a
200 Gy F_1	1	8	169.80±69.45cde	7.00±8.83d
male	2	8	140.25±33.48de	30.25±8.83bcd
	3	8	127.95±23.60e	34.75±12.50bc

* Means in a column followed by the same letter are not significantly different at P > 0.05 according to LSM multiple range test.

Table 4. The effect of releasing *Trichogramma ostriniae* and irradiated (200 gy) male and female *Ostrinia furnacalis* moths to control feral populations of *Ostrinia furnacalis* in a 500 ha corn field

Year & treatment[b]	No. of parasitoids /ha	% parasitism	No. of parasitoids /ha	% parasitism	No. 200 Gy/ha	Release ratio[a]	No. of larvae /100 plants	% damaged corn plants
	1st generation		2nd generation					
1994 T	150000	58.20	-	-	4000	-	9.3	6.1
1994 CK	-	5.17	-	-	-	-	38.5	23.8
1995 T	150000	48.27	-	-	4000	2.2:1	7.0	3.5
1995 CK	-	7.32	-	-	-	-	41.2	35.2
1996 T	150000	52.98	-	-	4000	4:1	5.2	3.1
1996 CK	-	6.81	-	-	-	-	53.1	28.5
1997 T	150000	50.10	150000	81.8	4000	6:1	3.1	1.9
1997 CK	-	7.83	-	27.10	-	-	52.8	25.3

[a]ratio of irradiated to wild moths
[b]T = treatment; CK = check.

4. CONCLUSIONS

The mating competitiveness of *Ostrinia furnacalis* F_1 male moths (progeny of male parents irradiated with 200 Gy) was compared with the mating competitiveness of normal moths. These studies revealed that F_1 male moths were involved in more than 50% of the matings with normal females. The flight ability and response to sex pheromone was similar for F_1 and normal male moths, although the number of F_1 moths captured was slightly less than the number of normal moths captured. The number of eupyrene sperm in the testes of P_1 moths treated with 200 Gy was similar to the number of eupyrene sperm in the testes of normal moths. However, the number of sperm bundles was reduced in the testes of 200 Gy F_1 moths. Compared to normal moths, daily sperm descent into the duplex ejaculatorius was

affected only at day 3 after eclosion of F_1 moths. Sperm transfer to the spermatheca by 200 Gy F_1 male moths was less than for their irradiated (200 Gy) parents and for normal moths. Successive releases of *Trichogramma ostriniae* in the egg stage of first and second generation of *Ostrinia furnacalis* were combined with the release of 200 Gy treated moths in the adult stage of the first generation. The combination of F_1 sterility with augmentative biological control suppressed the wild population of this pest in 500 hectares of field corn.

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

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

1. INTRODUCTION

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

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

2. MATERIALS AND METHODS

2.1. Insect rearing

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

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

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

2.2. Radiation experiments

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

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

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

3. RESULTS AND DISCUSSION

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

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

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

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

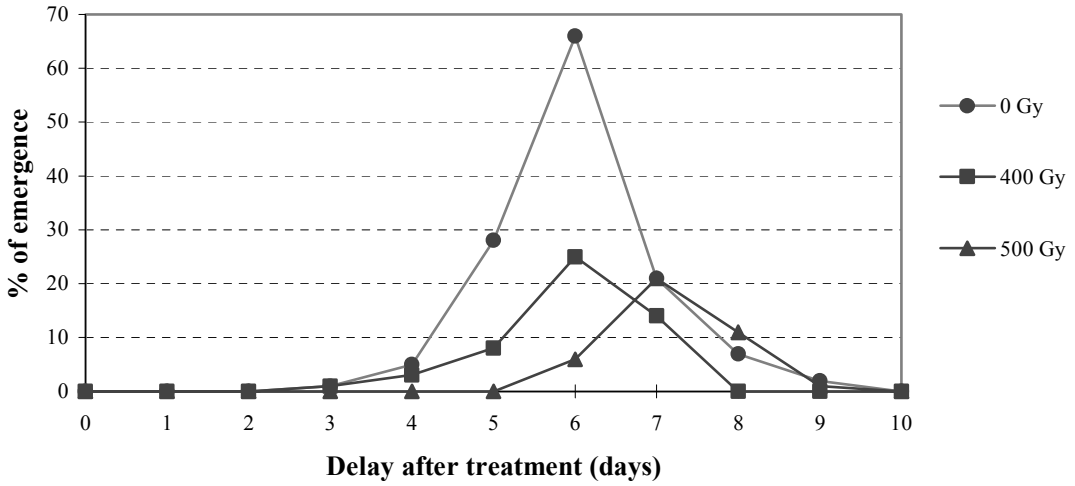

FIG 1 .Effect of irradiation on the duration of pupal development (4- day-old pupae treated) for Ectomyelois ceratoniae.

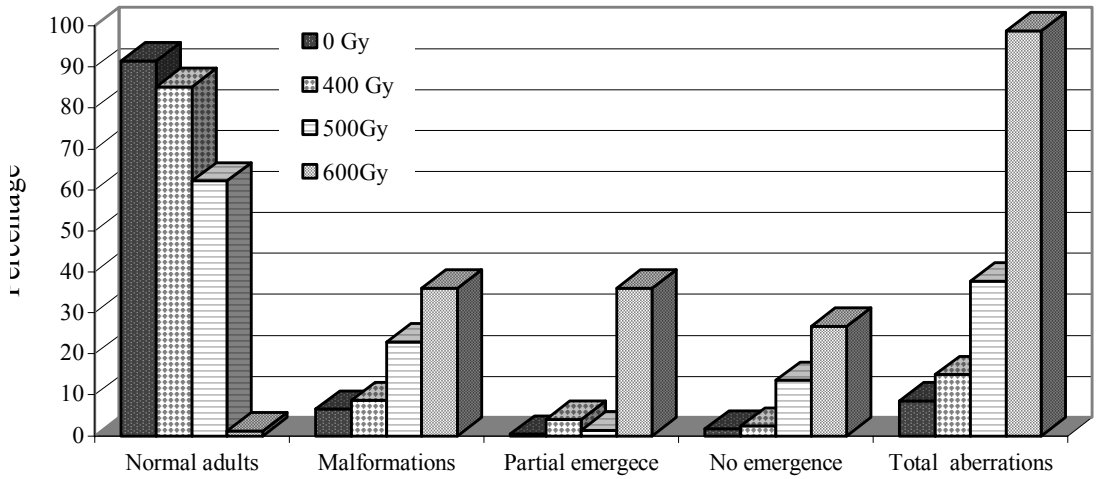

FIG. 2. Effect of irradiation on adult emergence (8–9 d-old pupae treated) for Ectomyelois ceratoniae.

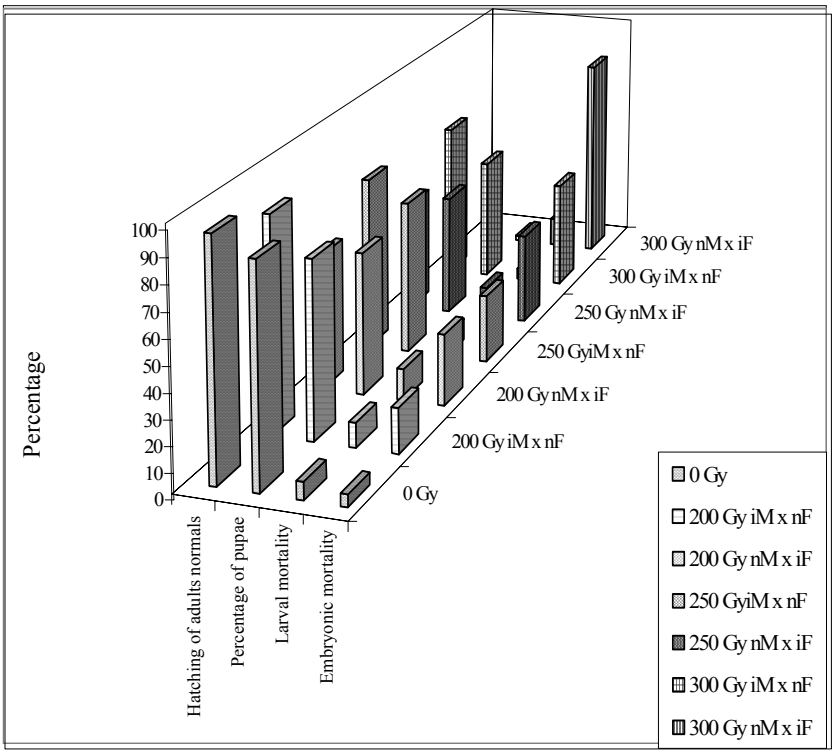

FIG. 3: Irradiation The effect of substerilizing doses of irradiation on F1 progeny (9–10 days old pupae treated).

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

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

doses of radiation had a slight effect on mating activity, mainly when the irradiated females were mated with normal males. In the mating cages, increasing the number of irradiated males improved mating activity, but increasing numbers of irradiated females decreased the mating rate.

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

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

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

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

ACKNOWLEDGEMENT

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Suppression of oriental fruit moth (*Grapholita molesta*, Lepidoptera: Tortricidae) populations using the sterile insect technique

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Abstract. The Oriental fruit moth (OFM) is a major insect pest of peaches in Bulgaria. Its control usually requires several insecticide treatments per season. This, however, gives rise to serious toxic residue problems. A program for suppression of OFM populations involving the use of sterile-insect technique (SIT) has been developed as an alternative to the chemical methods for OFM. Relevant information regarding laboratory rearing, radiation and basic biology are presented here. Expected effects of some release programs are modelled using appropriate mathematical simulations. Results obtained in a small field experiment showed high efficacy of a program integrating F_1 male sterility technique and classic SIT.

1. INTRODUCTION

The Oriental fruit moth (OFM), *Grapholita molesta* Busck (Lepidoptera*:* Tortricidae), is an economically important pest in Bulgaria. The insect occurs throughout the country but develops more numerous populations in the southern regions, where most commercial orchards are located. Peaches are the most important host of OFM in Bulgaria, while apples, quince and plum are secondary hosts. OFM larvae cause stem wilting by penetrating the tips of young twigs and burrowing downward. In addition, their feeding on green and ripening fruit results in damaged fruit with holes often filled with viscous sap and excrement. Feeding injuries cause premature fruit drop, decreased fruit marketability and compromise fruit exports. Larvae of the summer generations may cause serious damage on fruits of the middle-early and late peach varieties.

At present, control of OFM is usually achieved by several insecticide treatments directed mainly against the late spring and summer generation larvae. This practice, however, often results in toxic pesticide residues on fruits. Pesticide residue is a major concern because peaches are largely used for production of baby food on which no toxic contamination is allowed. The negative consequences of the intensive application of insecticides against OFM necessitated a re-evaluation of such control programs. Accordingly, efforts were turned to search for alternative methods which do not cause negative side-effects. So far, however, no effective biological control agents have been established and used successfully in OFM control programs. For this reason, the sterile insect technique (SIT), as a genetic approach to OFM population suppression, was investigated as an alternative to the chemical methods. SIT is based on the idea that successive releases of fully sterilized moths (classical SIT) during the overwintering generation (OWG) of OFM and continuing through the next spring generation (NSG) may reduce the density of the summer population to an acceptable (low) level. As a consequence, insecticide treatments could reduced or eliminated. The F_1 sterility technique, involving releases of partially sterilized male moths, is expected to produce a similar effect after one application against the OWG of OFM, resulting in inherited male sterility in the successive filial generations.

2. MATERIALS AND METHODS

2.1. Mass rearing of OFM

A semi-synthetic diet had been previously evaluated and found to be effective for rearing OFM (Table 1, Diet 1) [1]. This diet was slightly modified and tested on our colony (Table 1, Diet 2). Vicomplex (Pharmachim, Bulgaria), which contains vitamins A, B, C, D_2 and NPP was used in Diet 1, while Vitamino (Virbac Laboratories, France), a multivitamin mixture containing 9 vitamins, amino acids and minerals was used in Diet 2. Phytin (Pharmachim, Bulgaria) a calcium-magnesium salt of inosite-hexaphosphoric acid containing 22% organically bound phosphorus was used. It is considered a tonic that may have an invigorative effect on the organism. Nipagin (methyl-p-hydroxybenzoate) and benzoic acid were used as fungicides. Tetracycline dissolved in distilled water (1 g in 50 ml) was used as a bactericide. Diet 2 was found to be more effective and is less expensive to rear OFM. As such, it was selected for routine OFM rearing.

Table 1. Semi-synthetic larval diets for rearing *Grapholita molesta*

Ingredients	Amount	
	Diet 1	Diet 2
Agar	30.00 g	25.00 g
Brewer's yeast	45.00 g	15.00 g
Wheat germ	55.00g	65.00 g
Corn flour	48.00 g	70.00 g
Peach puree	150.00 g	-
Apple puree	-	150.00 g
Sucrose	10.00 g	20.00 g
Corn oil	2.0 ml	2.00 ml
Vitamin C	4.5 g	5.00 g
Vitamin E	0.05 g	0.04 g
Vitamin mixture	0.20 g	0.15 g
Phytin	0.25 g	0.25 g
Nipagin	1.50 g	1.50 g
Benzoic acid	1.50 g	1.50 g
Tetracycline solution	4.50 ml	4.50 ml
Distilled water	650.00 ml	650.00 ml

Diet spheres, made of approximately 38 g medium (~3 cm in diameter) are most suitable for larval development. They provided appropriate moisture and good texture and remain suitable for a sufficiently long period of time. Five larvae can normally develop in a single sphere. Strips of corrugated paper (width 10–15 mm) were placed in the rearing containers as pupation sites for mature larvae. Moth emergence, mating, oviposition and egg hatch take place inside glass jars. Cotton pads soaked in 2% sucrose solution provide adult nutrition. A ratio of 1 male:3 females is optimal for effective laboratory mating [2]. Infestation of the nutritive spheres is achieved by placing the spheres inside the jars during egg hatch for 24 h. A constant temperature of 26°C is optimal for larval development and pupation. A photoperiod of 16L:8D prevents diapause induction [3]. Temperatures between 23–25°C are required for moth eclosion, oviposition and egg hatch.

All corrugated paper strips were fumigated with formaldehyde to prevent mould contamination of the diet. We isolated a granulosis virus, belonging to the family

Baculoviridae subgroup B from our OFM colony [4]. Virus management requires frequent total disinfection of the rearing room, rearing boxes and other equipment with 1% KOH solution.

2.2. Radiation biology

Irradiation was conducted in a Co^{60} irradiator and the insects were treated with doses ranging from 50 to 500 Gy [5].

2.3. Field experiments

A typical peach-growing region in South Bulgaria was used for this experiment. Mature pupae were treated with partially or completely sterilizing doses of radiation and allowed to emerge from strips of corrugated paper in the field. The following treatments were released into different 1 ha blocks: (1) F_1 Sterility + SIT: successive releases of partially and fully sterile males and completely sterile females were made into populations of the OWG and the NSG of OFM; (2) Chemical control: 3 insecticide treatments were applied against populations of OFM in the OWG, NSG and first summer generation; (3) Untreated control: the area was left untreated for OFM. All blocks contained early, middle early and late peach varieties. Fruit injury by OFM larvae was recorded from samples of $\leq$1000 fruits for each group of varieties in each treatment.

2.4. Field biology and population dynamics

OFM pheromone traps (Pherocon-OFM), C^{14}-labeled male OFM moths [6] and the release-recapture method [7] were used to determine the dispersal of released insects and to evaluate trap efficiency. All irradiated moths needed for the field trial were additionally marked with Calco-Red dye added to the larval diet.

2.5. Simulations

Effects of different SIT programs were evaluated by modelling several scenarios using mathematical models [8].

3. RESULTS

3.1. Mass rearing of OFM

Some biological characteristics of OFM reared on immature apples and Diet 1 are summarized in Table 2 [1]. These parameters were used to develop quality control standards for OFM laboratory populations.

Data collected from 5 selected (of 39 total consecutive) generations of OFM reared on Diet 1 are shown in Table 3. Data for 5 selected (of 13 consecutive) generations of OFM reared on Diet 2 are summarized in Table 4. There is a slight increase in both male and female pupal weights over successive generations. Significant increases in egg production over time were also evident. In general, all data indicates that long term rearing on a suitable diet and under favourable laboratory conditions does not have a negative effect on the reproductive and developmental biology of OFM.

Table 2. Some biological characteristics of *Grapholita molesta* reared on immature apples and on diet 1.

Parameters[1]	Sex	Green apples	Diet 1	QC Standard
Fully developed larvae (%)		70.4±2.0	68.5±1.9	≥65.0
Development time larva-imago (days)	M	24.8±0.8	27.3±0.4	
	F	24.8±1.0	27.5±0.2	
Pupal weight (mg)	M	9.1±0.1	9.2±0.2	≥8.5
	F	11.6±0.3	11.3±0.4	≥10.5
Moths eclosion (% of developed larvae)		94.6±0.6	92.4±0.8	≥90.0
Sex ratio (M:F)		1.0±0.1	1.1±0.1	0.8–1.2
Adult longevity (days)	M	18.7±0.5	17.6±0.5	≥17.0
	F	19.1±0.5	20.0±0.5	≥19.0
Fecundity (mean # of eggs per female)		30±2	27±2	≥25
Fertility (% egg hatch)		88.1±3.0	76.2±3.0	≥70.0

[1] Average for the first five generations.

Table 3. Developmental and reproductive parameters of five selected generations of *Grapholita molesta* reared on diet 1.

Parameters	Sex	QC	Generation #				
		Parameters	1	8	28	31	39
Fully developed larvae (%)		≥65	59.9	72.7	69.4	68.4	70.8
Pupal weight (mg)	M	≥8.5	8.2	10.2	9.0	8.8	9.2
	F	≥10.5	10.7	12.2	11.5	12.0	12.6
% Moth emergence		≥90.0	91.4	94.2	92.3	91.0	92.5
Sex ratio (M:F)		0.8–1.2	0.97	0.98	0.90	1.02	1.10
Adult longevity (days)	M	≥17.0	17.9	17.1	17.5	18.0	17.2
	F	≥19.0	20.7	21.8	20.2	19.6	21.5
Fecundity (mean no. of eggs/female)		≥25	29	34	32	39	44
Fertility (% egg hatch)		≥70.0	70.9	75.0	72.6	74.0	77.5

Table 4. Developmental and reproductive parameters of five selected generations of *Grapholita molesta* reared on diet 2.

Parameters	Sex	QC	Generations				
		Parameters	1	3	6	10	13
Fully developed larvae (%)		≥65	70.4	74.0	71.6	74.5	74.0
Pupal weight (mg)	M	≥8.5	9.4	9.9	9.4	9.3	10.1
	F	≥10.5	13.4	12.5	11.6	12.6	13.5
% Moth emergence		≥90.0	90.0	92.5	96.3	94.5	96.0
Sex ratio (M:F)		0.8–1.2	0.8	1.2	1.0	0.9	1.1
Adult longevity (days)	M	≥17.0	18.6	16.8	17.2	17.0	19.2
	F	≥19.0	21.0	21.4	20.6	23.0	22.2
Fecundity (mean no. of eggs/female)		≥25	38	41	48	54	50
Fertility (% egg hatch)		≥70.0	79.9	77.1	73.9	80.8	80.0

Table 5. Some developmental parameters of *Grapholita molesta* after pupae were stored at 4–5°c for different periods of time

Parameters	Sex	QC Parameters	Storage period (days)						
			30	45	60	90	120	150	180
% Emergence		≥90	83.3	89.5	87.6	69.9	18.8	20.1	5.4
Sex ratio (M:F)		0.8–1.2	1.12	1.04	0.96	1.10	1.46	1.68	4.9
Adult Longevity (days)	M	≥17	16.7	17.2	16.9	14.0	10.0	11.0	4.8
	F	≥19	18.7	17.0	18.6	18.2	13.4	10.8	8.3
Fecundity (# eggs/female)		≥25	26	27	25	26	22	18	17
% Egg hatch		≥70	76.8	86.6	77.3	78.6	75.7	87.0	78.0

Table 6. Radiation induced sterility in *Grapholita molesta*.

Dose (Gy)	F_1 embryonic mortality (% ± SE)			
	P crosses[1]: IM x NF		P crosses[1]: IF x NM	
	A	B	A	B
0[2]	15.4±2.8	14.3±1.6	17.0±2.0	13.8±1.9
50			79.8±3.1	77.5±2.8
100	37.5±2.2	34.8±1.8	98.5±2.3	99.0±0.6
150	42.8±3.1	36.9±2.6	100	100
400	92.5±2.1	94.1±2.0		
450	94.8±1.2	95.6±1.9		
500	96.2±1.6	97.0±1.5		

[1] I, irradiated; N, non-irradiated; A, pupae irradiated; B, adults irradiated.
[2] Control crosses: NM x NF.

Mature pupae collected in the laboratory were kept at 4–5°C for different periods of time ranging from 30 to 180 days. The results of routine quality control tests on the stored pupae showed that under such conditions pupae could survive successfully for 60 days without any deleterious effects on the emerging adults (Table 5). However, when pupae were stored for more than 60 days, progressive reduction in moth emergence, longevity and fecundity were recorded. Sex ratio was also skewed in favour of the males. Only egg hatch remained unaffected.

3.2. Radiation biology

Data related to the development of SIT are given in Table 6 (Genchev and Gencheva [5]). For males and females, irradiated as mature pupae or as adults, the sterilizing effects are expressed as dominant lethal mutations in spermatozoa or in oocytes. A dose of gamma between 400–500 Gy, administered to pupae or adults resulted in almost complete male sterility (93–97%), but had less of an effect on adult longevity (Table 7, after [5]). In general, these results suggest that a dose of 400–500 Gy induces a level of sterility that satisfies the requirements of SIT. The dose that causes complete female sterility is 150 Gy. This dose is much lower than the dose needed for male sterilization, and provides a good opportunity for releasing both sexes without any risk that the irradiated females will contribute larvae to the population density.

Table 7. Longevity of *Grapholita molesta* males exposed to various radiation doses.

Dose (Gy)	Mean adult longevity ±SE (days)	
	Irradiated pupae	Irradiated imago
0[1]	21.5±1.2	20.0±1.0
100	20.8±1.6	19.0±0.8
150	21.2±1.2	21.8±1.8
400	20.1±1.0	18.5±1.1
450	18.5±1.5	17.5±0.8
500	18.3±1.0	14.8±0.9

[1] Untreated control.

Table 8. Embryonic mortality and sex ratio in f_1, f_2 and f_3 generations of *Grapholita molesta* when male pupae were irradiated with 125 gy

Parental cross[1]	Embryonic mortality (%)	Sex ratio (M:F)
UM x UF	11.5	0.96:1
IM x UF	40.9	1.10:1
UM x IF	>99.0	-
UM x UIF	9.7	1.00:1
F_1M (IM x UF) x UF	85.1	2.84:1
UM x UF	10.8	0.93:1
F_2M (F_1M x UF) x UF	69.1	2.70:1

[1]U= untreated; I= irradiated; M=male; F= female

Doses between 100 and 150 Gy, causing partial sterility in male OFM (37–43%), are considered as appropriate to be tested for induction of F_1 sterility. These doses are currently being tested. However, 125 Gy (Table 8) is being used in an experimental release program.

Results from laboratory experiments with fully sterile moths (pupae treated with 450 Gy) are presented in Table 9. All the release ratios provided high mortality of the F_1 eggs and may be suitable in a release program.

3.3. Basic biology

OFM completes 3 full and 1 partial generation each year. The insect hibernates as a mature larva. Pupation in the overwintered generation usually occurs in April, and about 30 days are required for the development of a generation in the field. Table 10 presents data for moth population dynamics, summarized from historical observations and from using pheromone traps at regional stations in North and South Bulgaria. This information is essential for scheduling the timing of the OFM release program. If the intended targets are the populations of OWG and NSG in South Bulgaria, then releases of sterilized moths should be made between 5–20 April and 25 May–5 June, respectively. As such, the sterile insects needed should be available no later than the beginning of April or the middle of May, respectively.

Studies where released radio-labelled male moths were used indicated that the maximum distance where OFM was trapped was 80 m. As such, the distance between traps should be less than 160 m. The average trap efficiency recorded in our studies was 6.1%. This value was derived from data of 12 field tests where traps were placed in a circle with radius of 50 m

Table 9. Mating competitiveness of sterile *Grapholita molesta* males irradiated as pupae with 450 Gy.

Only males irradiated		Both sexes irradiated	
Parental Ratio IM:NM:NF[1]	F_1 egg mortality (% ± SE)	Parental Ratio IM:IF:NM:NF[1]	F_1 egg mortality (% ± SE)
0:1:1[2]	15.4±2.8	0:0:1:1[1]	13.8±2.0
10:1:1	88.5±4.5	10:10:1:1	89.2±6.0
12:1:1	90.0±4.2	12:12:1:1	91.3±5.4
15:1:1	92.6±4.5	15:15:1:1	91.9±4.0

[1] I= irradiated; N= non-irradiated; M= male; F= female.
[2] Control cross of non-irradiated moths.

Table 10. Population dynamics of *Grapholita molesta* in Bulgaria.

Generation	Region	A	B	C	D
OWG	South	10–15 March	5–20 April	15 April–5 May	8–12
	North	15–25 March	5–25 April	20 April–10 May	9–13
I	South	10–15 May	25 May–5 June	15–30 June	6–10
	North	15–20 May	28 May–10 June	17–30 June	6–10
II	South	20–22 June	1–5 July	15–25 July	5–8
	North	20–25 June	1–10 July	15–25 July	6–10
III	South	20–25 July	28 July–5 Aug.	10–20 Aug.	5–9
	North	26–30 July	1–15 Aug.	5–21 Aug.	5–9
IV	South	20–25 Aug.	1–10 Sept.	5–15 Sept.	9–13
	North	20–25 Aug.	1–10 Sept.	10–20 Sept.	10–14

OWG, overwintered generation.
A, the date of earliest beginning of the flight.
B, the date of most frequent beginning of the flight.
C, the date of most frequent flight peaks.
D, time interval (days) from the beginning of the flight and the flight peak.

around the release site. Confirming tests carried out in 1997 showed that trap efficiency varied between 6.4 and 5.7%. We used this data to calculate the population density and we found that it ranges between 400 and 600 moths per hectare (males + females, sex ratio of 1:1). Because these data indicate a relatively low population density, the OWG should be the most appropriate target for initiating the sterile moth release program.

3.4. Simulations of SIT programs for OFM population suppression

The effects of two successive releases of fully sterile moths into wild populations of different sizes were modelled using the following model equations: N = mc for the size of free developing population (f x f = 1) and $N(F_1) = mc/(a + 1)$ for the size of F_1 population after SIT application (s x f = 0). In these equations N = number of insects in the population (both males and females); m = number of females in the population (m = N/2); n = number of males in the population (n = N/2); b = sex ratio of males to females (b = n/m); a = ratio of sterile males to fertile males; and c = fecundity (number of eggs per female). The following postulates were followed: b = 1 (constant); c = 25 (constant); a = 15 : 1 (= 15) or 20 : 1 (= 20), respectively (variable); N for initial population = 2000, 1600, 1200, 800 and 400 moths per

Table 11. Simulated population changes in three *Grapholita molesta* generations after application of SIT[1].

Population Treatment	Initial ratio[2] IM:NM:NF	Population size (no. of moths per generation)			
		P	F_1	F_2	F_3
Non-treated	0:1:1	2000	17500	153125	1339844
Treated	15:1:1	2000	1094	598	5233
Treated	20:1:1	2000	833	347	3038
Non-treated	0:1:1	1600	14000	122500	1071875
Treated	15:1:1	1600	875	479	4191
Treated	20:1:1	1600	667	278	2430
Non-treated	0:1:1	1200	10500	91875	803906
Treated	15:1:1	1200	656	359	3141
Treated	20:1:1	1200	500	208	1823
Non-treated	0:1:1	800	7000	61250	535938
Treated	15:1:1	800	438	240	2100
Treated	20:1:1	800	333	139	1216
Non-treated	0:1:1	400	3500	30625	267969
Treated	15:1:1	400	219	120	1050
Treated	20:1:1	400	167	70	613

[1] Two successive releases of fully sterile moths in the P and F_1 generations.
[2] Initial ratio of released irradiated (I) males to non-irradiated (N) moths of the wild population.

hectare; f x f = 1 for the cross of fertile male with fertile female producing normal offspring; s x f = 0 for the cross of sterile male with fertile female producing no offspring; a total correction of 30% for natural embryonic, larval and pupal mortality was introduced for every simulated population. The postulate for variable value of the ratio of released sterile males to fertile wild males was accepted in order to simulate the interactions between the moths depending on the ratio in the population. The constants, i.e. sex ratio, fecundity and total correction for natural mortality, were supported by data obtained in laboratory experiments.

The expected population reduction after the application of SIT is summarized in Table 11. Although proposed situations are simulated and the postulate s x f = 0 (i.e., an absolute male sterility) is fixed, the data clearly indicate the downward trend in the OFM population occurring when fully sterilized moths are introduced. Because the number of sterilized individuals for each release is the same, the second treatment in an already reduced population will result in a significantly increased ratio sterile to fertile males (27:1 and 48:1 from an initial ratio of 15:1 and 20:1, respectively). This increase in ratios is a very important factor governing the process of OFM population suppression by SIT. Population reductions predicted by the model for two successive releases in populations of 800 and 400 moths per hectare are of special interest because these are similar to the densities found for the OWG generation in Bulgaria.

When classic SIT is used initially and is followed by F_1 sterility, the suppressive effects will result from the following consequences. 1) Loading the parental (P) population with dominant lethal mutations; 2) introduction of various chromosomal aberrations in the already lowered F_1 population which further impacts the F_2 population and gives rise to high level of inherited male sterility; 3) a corresponding reduction in the F_3 generation accompanied with inheritance of male sterility, but at a lower rate; and 4) slow population increase in the F_4 generation.

Table 12. Simulated population changes in four *Grapholita molesta* generations after application of F_1 sterility and sterile-insect techniques consecutively.

Release program[1]	Population size (no. of moths per generation)				
	P	F_1	F_2	F_3	F_4
Free reproduction	800	7000	61250	535938	4689458
(no release)	400	3500	30625	267969	2344729
F_1 sterility method[2]	800	4200	2450	1225	21437
(one release)	400	2100	1225	612	10718
SIT + F_1 sterility[2]	800	438	766	448	3920
(2 successive releases)	400	219	383	224	1960
F_1 sterility[2] + SIT	800	4200	44	51	893
(2 successive releases)	400	2100	22	26	446

[1] Treated generations: P (1st release) and F_1 (2nd release); both sexes were released and the initial ratio of released to wild moths were a = 15:1; data were calculated for two initial population sizes, 800 and 400 wild moths.
[2] F_1 male sterility = 80%, F_2 male sterility = 60%, and sex ratio b = 3:1 (i.e., threefold reduction of m) in both F_1 and F_2 generations were used in calculations.

Table 13. Results of a small-scale field trial for evaluating the efficacy of an integrated sterile-insect release program for *Grapholita molesta* control.

Treatment	% Injured Fruits		
	Early Varieties	Middle-early Varieties	Late Varieties
F_1 sterility + SIT[1]	0	0.8	0.5
Insecticides[2]	0	1.0	3.5
Untreated control	~ 0.1	19.5	35.0

[1] Successive releases in overwintered generation (OWG) and next spring generation (NSG).
[2] Used against the populations of OWG, NSG and the summer generation I.

When F_1 sterility is used first and is followed by SIT the expected suppressive effects will result from: 1) introduction of chromosomal aberrations in the P generation, causing a reduction in the F_1 generation and a high level of inherited male sterility; 2) loading of F_1 individuals with dominant lethal mutations through the release of fully sterile moths, resulting in suppressed reproduction because of simultaneous effect of dominant lethals and chromosomal aberrations; 3) strong reduction of the F_2 generation and secondary inheritance of male sterility; and 4) corresponding reduction of the F_3 and a slow increase in the F_4.

Results of simulated single application of F_1 sterility and simulations of both programs is presented in Table 12. The data suggest that the most effective program for OFM would integrate consecutive releases of F_1 sterile OFM followed by the classic SIT. Undoubtedly, the use of SIT plus F_1 sterility and even F_1 sterility by itself could also be applied, but to populations of lower initial densities.

3.5. Small scale field trial

Based on results from the model simulations, the integrated release program involving F_1 sterility against the OWG generation plus SIT against the NSG generation was evaluated under field conditions. We assumed the following to be true: Sex ratio in OFM is 1:1; the pre-release population density was estimated to be 600 (300M + 300F) moths per hectare; the OFM do not fly long distances and are generally distributed uniformly in the peach orchard.

The release of partially sterile males for the OWG started on 29 April, 1997. In order to achieve the 20:1 sterile to wild male ratio, 12,000 irradiated pupae were dispersed in the field three times at intervals of 10–11 days. The release of fully sterilized moths against the NSG generation began on 10 June, 1997. A total of 11,900 irradiated pupae were introduced into the orchard at the same time intervals. Flight records showed a steady flooding of the OWG population with partially sterile male OFM. In most cases, the number of trapped marked males was greater than the number of wild male moths. A similar situation was observed after introduction of completely sterile individuals into the population of NSG but without predominance of the sterile males. A probable reason for this might be the increased number of wild males because of the inherited F_1 sterility effects (see sex ratio in Table 8).

Percentages of fruit damage from the different experimental treatments are shown in Table 13. These data unequivocally indicate a very high effectiveness of the program for genetic control of OFM. Integrating F_1 sterility and classic SIT resulted in a strong population reduction, followed by a reduced increase in the OFM population density. As a consequence, additional insecticide treatments were not needed to control this pest.

4. CONCLUSIONS

General conclusions from our experiments are summarized below:

(1) The technology developed for mass rearing OFM is efficient and technically feasible.

(2) Appropriate radiation doses for complete and partial sterilization for OFM pupae and adults have been determined. The level of mating competitiveness of the sterilized male moths is adequate.

(3) The basic biology and radiation biology make OFM suitable for development and application of SIT for population suppression.

(4) Information on flight behaviour and population dynamics is essential for calculating the timing and number of OFM to be released in an SIT program.

(5) The overwintering generation is the most suitable as a target for sterile moth releases because of the relatively low population level.

(6) Data obtained from modelling simulation suggest a downward trend in OFM populations when sterile moths are introduced.

(7) Field experiment suggested high effectiveness of a program integrating F_1 sterility and classic SIT.

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

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

1. INTRODUCTION

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

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

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

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

2. MATERIALS AND METHODS

2.1. Insects

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

2.2. Irradiation

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

2.3. Effects of gamma radiation on fecundity and fertility

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

2.4. Effect of gamma radiation on male longevity

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

2.5. Effect of gamma radiation on male mating competitiveness

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

2.6. Effect of gamma radiation on male mating capacity

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

2.7. Data Analysis

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

3. RESULTS

3.1. Effects of gamma radiation on female fecundity and fertility

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

3.2. Effects of gamma radiation on male fecundity and fertility

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

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

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

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

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

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

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

3.3. Male Longevity

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

3.4. Male Mating Competitiveness

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

3.5. Male Mating Capacity

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

4. DISCUSSION

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

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

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

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

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

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

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

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

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

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

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Feasibility of integrating radiation-induced F_1 sterility and biological control for population suppression of the pink bollworm, *Pectinophora gossypiella*, in Pakistan

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Abstract. Substitution of casein and wheat germ with locally available ingredients (chickpea flour, soybean flour, wheat husk and sawdust) in the specified casein-wheat germ diet affected various biological parameters of pink bollworm (PBW), *Pectinophora gossypiella*. The diet containing chickpea flour performed significantly better and is more economical than the other diets tested. The highest PBW field populations were recorded in the month of October when large numbers of fruiting bodies were present in the cotton. Field behavioural observations revealed that mating and other sexual activities of treated and native moths varied significantly with time of night and peak activity was during 03:00–04:00 hours. Male moths treated with 100 Gy as mature pupae responded well to gossyplure baited traps. The attraction of male moths to irradiated virgin females decreased significantly with increasing doses of radiation. Male moths responded more readily to virgin untreated females than to irradiated females. Field-cage studies demonstrated that irradiated moths (100 Gy) released at a 50:1 treated to normal ratio at three week intervals reduced larval infestations inside the cages to subeconomic level. Studies suggested that there is a great potential for integrating the egg parasitoid, *Trichogramma chilonis*, with the sterile insect technique to control cotton bollworms.

1. INTRODUCTION

Cotton, *Gossypium hirsutum* is considered the backbone of the agricultural economy of Pakistan and accounts for one third of the country's foreign export earnings. It also supports many agri-based industries particularly textile mills. Within Asia, Pakistan ranks third in acreage and fifth in cotton production [1]. Unfortunately, seed cotton yield is very low in Pakistan as compared with other cotton growing countries of the world. Among the various factors responsible for the low yield include poor crop husbandry, low soil fertility and the ravages caused by insect pests.

Bollworms cause major losses to the cotton crop in Pakistan [2]. Pink bollworm, PBW, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) is the most injurious of the cotton bollworms present. The number of insecticide applications used to control PBW per season ranges between 10–17 [3] which creates problems such as the development of resistance, disturbance in biological equilibrium and environmental pollution. Biological methods to control PBW have been suggested for use in Pakistan due to their lower cost and as alternatives to manage the development of resistance [4]. Among these biological methods, the genetic control technique that utilizes ionizing radiation to induce sterility is one of the potential components of integrated management of PBW.

Releases of partially sterile male moths are more effective in population suppression than the use of fully sterile males [5]. Henneberry and Clayton [6] observed that reproduction of F_1 irradiated PBW male and female moths was reduced by at least 88% when they were crossed with untreated males or females. Flint et al. [7, 8] reported that PBW treated with 100 Gy were more effective at suppressing PBW infestations inside cages than were moths treated with higher doses. The theoretical advantages of releasing partially sterile insects appear promising, but the contribution of the F_1 generation in suppressing field populations has not

been completely documented. A radiation dose of 100 Gy applied to mature pupae or 150 Gy applied to adults (<24h) is suitable for inducing F_1 sterility in pink PBW [6, 9].

The impact of releasing sterile moths may be influenced by possible adverse behavioural changes in the treated insects. Graham [10] observed that mass-reared and irradiated PBW males were compatible with native males in mating with native females and produced equal number of spermatophores. However, mass reared males have been reported not to mate competitively with native females in field-cages [11] or as frequently with native females in the laboratory [7, 12, 13].

The full potential of SIT/F_1 sterility as an area-wide control strategy for PBW may only be achieved when it is integrated with other management techniques, especially where more than one species of bollworm exist. The introduction and conservation of parasitoids in cotton fields is a good base for any sustainable integrated pest management program. Generalist egg parasitoids belonging to the genus *Trichogramma* have contributed significantly to the control of many lepidopterous pests [14]. The objective of our studies was to examine the suitability of integrating F_1 sterility with parasitoids and nematodes for the control of PBW.

2. MATERIALS AND METHODS

2.1. Artificial diet for PBW

PBW can be successfully reared on casein-wheat germ diet, however, this diet is expensive because of the high cost of the casein and wheat germ. We tested five different diets where we replaced casein or wheat germ with locally available ingredients in order to reduce the cost of rearing PBW (Table 1). Eggs were collected from females that had been reared on the casein-wheat germ diet. One hundred neonate larvae were transferred to the test diet media and the experiment was replicated four times. Insects were reared at 27±2°C, a relative humidity of 70±5% and a 14L:10D photoperiod. Several biological parameters were measured for insects reared on the different test diets.

2.2. Population survey in the target area

In order to achieve effective control of PBW it is important to have information on its incidence and field phenology. We conducted a population survey using Delta traps baited with the pheromone gossyplure (a 50:50 mixture of Cis-Cis and Cis-trans isomers of 7,11-hexadecadienyl acetate). The gossyplure strip was placed inside the trap bottom. Ten traps were hung in the cotton field within the top 15 cm of the cotton foliage. The sticky surface of each trap was renewed periodically and the traps were re baited every 2 weeks for the entire season to maintain them at full catching efficiency. Traps were examined periodically for moth catches. Other bollworms caught in the traps were also counted.

2.3. Behaviour of treated PBW in the field: Mating table studies

Laboratory-reared PBW were marked with fluorescent dye and treated with 100 Gy of gamma radiation as mature pupae. Treated males were released in the cotton field at a ratio of 5:1, treated to native. The number and source (treated or native) of PBW males caught at different hours of the night was assessed using gossyplure-baited traps. Mating tables as described by Snow et al. [15] were placed in a cotton field. Twenty clipped-wing virgin female PBW, ten each from treated (100 Gy) or native (collected from cotton bolls) sources were placed into the mating tables. In another experiment, females from both sources were

Table 1. Diet ingredients used in the different artificial diets tested for *Pectinophora gossypiella*

Diet Ingredients (sufficient to prepare 1L of diet)	Casein-Wheat Germ	Chickpea Wheat Germ	Soy Wheat Germ	Casein Wheat Husk	Casein Sawdust
Agar	20.00 g	20.00 g	20.00 g	20.00 g	20.00 g
Casein	40.60 g	-	-	40.60 g	40.60 g
Wheat germ	34.80 g	34.80 g	34.80 g	-	-
Soy Flour	-	-	34.80 g	-	-
Chickpea Flour	-	34.80 g	-	-	-
Wheat Husk	-	-	-	34.80 g	-
Sawdust	-	-	-	-	34.80 g
Sugar	40.60 g	40.60 g	40.60 g	40.60 g	40.60 g
Alphacel	5.80 g	5.80 g	5.80 g	5.80 g	5.80 g
Wesson's Salts	11.60 g	11.60 g	11.60 g	11.60 g	11.60 g
Methyl-P	11.90 g	11.90 g	11.90 g	11.90 g	11.90 g
Water	774 ml	774 ml	774 ml	774 ml	774 ml
Choline Chloride 10%	11.60 ml	11.60 ml	11.60 ml	11.60 ml	11.60 ml
Formaldehyde 10%	4.80 ml	4.80 ml	4.80 ml	4.80 ml	4.80 ml
KOH 22%	5.80 ml	5.80 ml	5.80 ml	5.80 ml	5.80 ml
Vitamin Solution [1]	3.90 ml	3.90 ml	3.90 ml	3.90 ml	3.90 ml

[1]Vitamin Solution contains: 24 g D-pantothenic acid, 12 g nicotinic acid amide,6 g folic acid; 6 g riboflavin, 3 g thiamin hydrochloride, 3 g pyridoxin, 0.24 g biotin; 0.012 g B-12, in distilled water to make one liter.

placed together in the mating tables. Experiments were replicated five times. Pairs found in copula were collected at hourly intervals from 23:00 to 05:00 hours. Females were brought to the laboratory and confined in oviposition cages to collect eggs. The eggs collected were incubated and percent hatch was recorded.

2.4. Behaviour of treated PBW in the field: Trapping studies

The ability of irradiated females to attract males may play an important role in population suppression especially when both sexes are irradiated and released. As such, this ability was examined in our studies. Mature female PBW pupae were irradiated at 0, 50, 100, 150 and 200 Gy and kept separated by dose. Newly emerged females from each dose were used to bait Delta traps. Five virgin females were confined inside small wire and gauze cages (5 x 3.5 cm) and suspended inside the traps. The females were provided with a 10% sugar solution on a cotton wick. Traps were hung at the level of plant canopy and placed in the field in the evening. Male moths captured in each trap were counted and removed daily. The effect of female age on their attractiveness to male moths also was determined.

2.5. Evaluation of F_1 sterility in field-cages

Field-cage studies were conducted to evaluate the effect of F_1 sterility for the control of PBW. Six field-cages (1.8 x 1.8 x 1.8 m) were placed in a cotton field at the seedling stage. The native population of PBW remaining inside the field-cages (mainly from diapausing larvae) was monitored using gossyplure-baited traps and inspection of cotton fruiting bodies on a weekly basis. After confirming there was no infestation inside the cages, five pairs of laboratory-reared, untreated adult moths were introduced into the cages during the first week

Table 2. Development data for various life stages of *Pectinophora gossypiella* on different artificial diet

Biological Parameters	Casein Wheat Germ	Chickpea Wheat Germ	Soy Wheat Germ	Casein Wheat Husk	Casein Sawdust
Length of larval period (d)	17.90 [d]	20.55 [c]	24.58 [b]	24.27 [b]	30.44 [a]
Adult emergence (%)	74.23 [a]	61.02 [b]	48.00 [c]	55.81 [b]	17.11 [d]
Larval weight (mg)	18.82 [a]	17.64 [b]	14.85 [c]	18.45 [a]	9.87 [d]
Pupal weight (mg)	12.17 [a]	11.87 [a]	9.42 [b]	7.26 [c]	7.68 [d]
Pupal recovery (%)	72.75 [a]	61.50 [b]	44.50 [c]	49.75 [c]	13.50 [c]
Eggs per female	148.47 [a]	103.80 [b]	69.60 [d]	92.86 [c]	8.83 [c]
Percent Hatch	68.12 [a]	62.75 [b]	55.90 [c]	69.38 [a]	13.38 [d]
Longevity male (d)	13.20 [a]	9.93 [b]	8.33 [c]	10.38 [b]	4.38 [d]
Longevity female (d)	12.47 [a]	10.00 [b]	9.46 [b]	9.63 [b]	4.80 [c]

Means sharing the same letters are not significantly different ($P < 0.05$).

Table 3. Population fluctuation of pink, spotted and spiny bollworms during cotton season

Months	Adult moths captured per trap per week		
	PBW	Spotted BW	Spiny BW
June	0.0	1.5	0.5
July	0.5	4.0	0.5
August	2.5	17.5	2.0
September	10.25	11.0	5.0
October	60.0	5.0	11.0
November	11.5	6.5	5.0
December	12.0	9.0	6.0

of August. In addition, 100 pairs of treated (100 Gy) adults were released into two of the cages (20:1 irradiated to normal ratio) and 250 pairs into two other filed-cages (50:1 irradiated to normal ratio). Two cages were kept as control and received only the untreated PBW. Releases at these ratios were made into the cages at three week intervals; three total releases in each cage. Rosette blooms and infestation in green bolls were recorded at weekly intervals.

2.6. Potential of *Trichogramma chilonis* to parasitize the eggs of cotton bollworms

Trichogramma chilonis was reared on eggs of the Angoumois grain moth, *Sitotroga cerealella*. The parasitized eggs were placed individually in small transparent plastic ampoules (0.5 x 2.5 cm sealed at both ends). Upon emergence, the parasitoid were sexed and a single pair was released into vials containing fresh eggs of pink, spotted (*Aerias vittella*; Lepidoptera: Noctuidae) and spiny (*Aerias* sp.) bollworms glued on paper cards. Parasitism on each card was recorded. The effect of host egg age and parasitoid age on parasitism of different bollworms eggs was recorded.

2.7. Evaluation of the entomopathogenic nematode, *Steinernema riobravis*, for control of PBW

Steinernema riobravis was imported from Phoenix, AZ. A soil preserved nematode solution (10%) was prepared and applied in 5, 10, 15, 20 and 25 ml quantities to blotting paper in petri dishes. Five diapausing larvae of PBW were exposed to the different nematode doses in each petri dish. The experiment was replicated four times. The treated larvae were reared in the laboratory at 27±2°C, 70±5% R.H and 14L:10D photoperiod Pupation and mortality of PBW larvae was recorded for each treatment.

3. RESULTS

3.1. Artificial diet for PBW

A comparison of the biological parameters measured for PBW reared on the different test diets is summarized in Table 2. Diet composition had a significant impact on various biological parameters. The replacement of casein with chickpea or soybean flour and the replacement of wheat germ with wheat husk or sawdust resulted in delayed pupation and reduced pupal recovery. Larvae reared on the casein-wheat germ diet produced significantly ($P > 0.05$) heavier pupae, higher pupal recovery and shorter larval and pupal developmental times. The fecundity and fertility of the adults emerged reared on the casein, wheat germ diet was also higher. The chickpea wheat germ diet was the most promising of the diets tested. PBW reared on this diet performed significantly better than those reared on the other diets tested. The other test diets did not prove effective for rearing PBW.

3.2. Population survey in the target area

Results of the PBW population survey using pheromone traps are presented in Table 3. The moth population was very low during the hot months of summer i.e. May to July. The population started to increase in July and continued growing through August and September. Peak moth populations were recorded in October, after which the population started to decline. Populations of spotted bollworm peaked in August, while populations of spiny bollworm followed the same trends for PBW.

3.3. Behaviour of treated PBW in the field: Mating table studies

The number and source (treated or native) of PBW males caught at different hours of the night in gossyplure-baited traps are presented in Table 4. For both groups, males were first captured between 22:00–23:00, and peak captures occurred between 02:00–04:00 hours.

The number of mating pairs collected from mating tables containing treated, native or mixed source clipped-wing females revealed that mating activity for both treated and native PBW moths began at 23:00 hours. Peak sexual activity was recorded during the early morning (02:00–03:00) when a total of 99 pairs were collected. The treated males responded well to the calling behaviour of the native females. Furthermore, the lower number of treated females found copulating with native males suggests that irradiation affected the sexual activity of the treated females. However, calling behaviour occurred at similar times during the night in both treated and native females.

The fecundity and fertility of clipped-wing PBW females collected in copula in mating tables are presented in Table 5. Fecundity and fertility varied significantly for mating pairs collected during different hours of the night. Significantly more eggs were laid by females collected

Table 4. mean number of *pectinophora gossypiella* moths captured per trap baited with gossyplure at different hours of the night

Collection hours	Mean number males captured	
	Treated	Native
20:00–21:00	0.0 g	0.0 c
21:00–22:00	0.0 g	0.0 e
22:00–23:00	1.0 f	1.5 d
23:00–24:00	1.75 e	1.5 d
24:00–01:00	1.5 ef	1.7 d
01:00–02:00	2.2 de	2.35 c
02:00–03:00	10.75 b	11.68 b
03:00–04:00	18.2 a	19.85 a
04:00–05:00	3.75 c	2.95 c
05:00–06:00	2.8 d	2.7 c

Means sharing the same letters are not significant ($P < 0.05$).

Table 5. Mean number of eggs per female laid by *Pectinophora gossypiella* collected during different hours of the night

Hour of Observation	Mean number of eggs per female			Percent Hatch		
	IM x NF	NM x IF	NM x NF	IM x NF	NM x IF	NM x NF
23:00–24:00	26.5 e	18.8 b	24.2 d	2.5 c	7.1 d	66.7 c
24:00–01:00	25.7 cd	16.5 b	27.8 c	4.5 b	10.6 b	56.9 d
01:00–02:00	35.9 b	17.86 b	36.96 b	3.9 b	8.9 c	70.8 b
02:00–03:00	37.8 ab	21.6 a	41.8 a	6.8 a	12.6 a	86.7 a
03:00–04:00	39.5 a	18.5 b	37.5 b	2.9 c	8.9 c	71.1 b
04:00–05:00	21.9 d	15.9 b	29.8 c	1.6 d	5.2 e	60.5 d

Means sharing similar letters are not significant ($P < 0.05$).
NM = Native male; NF = Native female; IM = Irradiated male; IF = Irradiated female

during early the morning (02:00–04:00) in all types of pairings. Untreated females mated to untreated males had the highest fecundity, followed by untreated females mated to irradiated males. Treated females mated to irradiated males failed to lay eggs. Fertility was significantly higher for normal male x normal female crosses followed by normal male x irradiated female and irradiated male x normal female.

3.4. Behaviour of treated PBW in the field: Trapping studies

The mean number of PBW males captured in traps baited with females irradiated at different doses is shown in Table 6. Our results show that male moths responded to all female treatments irrespective of irradiation dose. However, female attractiveness was significantly reduced with increasing doses of gamma radiation. The response of males to traps baited with untreated females was significantly higher than for traps baited with irradiated females. Female age affected male moth capture. Captures of male moths was drastically reduced when calling females were older that 2 days.

Table 6. Mean number of *Pectinophora gossypiella* male moths captured in traps baited with females irradiated at different doses of gamma radiation

Age of females (days)	Number of moths captured in female baited traps* when females were treated with				
	0 Gy	50 Gy	100 Gy	150 Gy	200 Gy
1	A 17.75 b	B 11.75 b	B 10.25 b	C 7.00 b	D 3.50 bc
2	A 37.25 a	B 24.75 a	C 18.00 a	C 15.00 a	D 8.50 a
3	A 10.00 c	B 6.75 c	B 5.75 c	B 4.50 bc	B 4.25 b
4	A 4.75 d	B 3.00 d	B 1.75 d	B 2.00 cd	B 1.50 bc
5	A 1.00 e	A 0.25 d	A 0.50 d	A 0.50 d	B 0.0 c

*Each trap baited with five virgin females.
Letters on the left and right sides of the mean values show intra and inter column variations. Means sharing similar letters are not significant ($P < 0.05$).

Table 7. Mean percent larval infestation of *Pectinophora gossypiella* in the flowers and green bolls of cotton in field cages

Release Ratio Irradiated : Normal	Percent larval infestation in	
	Flowers	Green bolls
50:1	4.93 b	5.24 b
20:1	6.71 b	8.57 b
control	21.87 a	21.35 a

Means sharing the same letters in each column are not significant ($P < 0.05$).

3.5. Evaluation of F_1 sterility in field-cages

The data collected from cotton flowers and green bolls (Table 7) indicated that cages where moths were released at a ratio of 50:1 had lower infestations (4.93% for flowers; 5.24% for bolls) when compared to cages where moths were released at a 20:1 ratio (6.71% for flowers; 8.57% for bolls). PBW infestation was significantly higher in the flowers and green bolls of the control cage.

3.6. Potential of *Trichogramma chilonis* to parasitize the eggs of cotton bollworms

Parasitism by *T. chilonis* of eggs from different cotton bollworms is shown in Table 8. Parasitism was much higher in PBW eggs than in spotted or spiny bollworms. The parasitic potential of a single *T. chilonis* female for PBW was 21.7 eggs per female as compared to 2.98 and 2.76 in spotted and spiny bollworms eggs, respectively. Furthermore, the parasitic potential of *T. chilonis* decreased as the age of host eggs increased.

Parasitoid age also affected the parasitoid potential of *T. chilonis*. Freshly emerged parasitoids parasitized significantly more eggs when compared to one and two-day old parasitoids (Table 9). In the case of spotted and spiny bollworms, freshly emerged (0–1 day) *T. chilonis* parasitized only fresh bollworm eggs, whereas they successfully parasitized PBW eggs of up to 3 days in age (Table 8).

Table 8. Parasitic potential of *Trichogramma chilonis* as influenced by the age of the host eggs

Age of host eggs (days)	Potential parasitism in eggs of		
	PBW	Spotted Bollworm	Spiny Bollworm
0–1	21.7 a	2.98	2.76
1–2	16.0 b	0.0	0.0
2–3	4.6 c	0.0	0.0
3–4	0.7 d	0.0	0.0

Means sharing the same letters are not significant ($P < 0.05$).

Table 9. Effect of *Trichogramma chilonis* age on parasitization in eggs of different bollworm species

Age of Parasite (days)	PBW	Spotted Bollworm	Spiny Bollworm
0–1	25.5 a	3.1	3.3
1–2	4.9 b	0.0	0.0
2–3	0.7 c	0.0	0.0

Means sharing the same letters are not significant ($P < 0.05$).

Table 10. Mortality percentage in diapausing larvae of *Pectinophora gossypiella* treated with nematodes

Quantity of Nematodes (ml)	Mean mortality (%)
5	46.67 c
10	60.00 bc
15	53.33 c
20	73.33 ab
25	86.66 a
control	0.0

Means sharing the same letters are not significant ($P < 0.05$).

3.7. Evaluation of the entomopathogenic nematode, *Steinernema riobravis*, for control of PBW

The highest larval mortality was recorded in PBW treated with 25 ml of the 10% soil preserved nematode solution (Table 10). No larval mortality was recorded in the control. Percent pupation of larvae in the control was higher than in the treated larvae.

4. DISCUSSION

Economical production of laboratory insects is a major prerequisite for the successful application of a sterile or F_1 sterility release program. We report the substitution or replacement of diet ingredients with locally available materials in order to reduce the cost of rearing. In our studies casein was substituted with chickpea flour or soybean flour, and wheat germ was replaced by wheat husk or sawdust. Pupal recovery, fecundity and fertility were reduced in the chickpea flour diet when compared to the normal diet, however, the cost of the chickpea diet is much more economical. Shaver and Raulston [16] substituted casein with

soybean meal in the diet for tobacco budworm and reported that the cost of rearing was drastically reduced. Raulston and Shaver [17] reported that agar and casein in the diet of tobacco budworm contributed 54% of the total cost and that a low agar-casein diet reduced the cost of rearing by 25%. Our results indicate that although the chickpea, wheat germ diet was not as effective as the normal diet for rearing of PBW, this diet has some potential because it performed well concerning larval development, fecundity and fertility of PBW. The nutritional quality of this diet needs to be examined in the future.

Field surveys of PBW indicated that peak populations occur in the month of October. This might be due to the presence of large number of available fruiting bodies in the cotton field. A similar phenological pattern has been reported by other authors [18, 19]. Most of the behavioural studies on PBW have been conducted in the laboratory or in field-cages. The impetus to study the effect of gamma radiation on mating behaviour in PBW was heightened following the discovery of inherited sterility in the progeny of partially moths [5]. Our field behavioural studies on irradiated PBW indicated that males responded well to gossyplure-baited traps when they had been treated with 100 Gy. However, others [13] have reported that laboratory reared irradiated PBW males failed to mate as frequently (with native or laboratory-reared females) as did the native or laboratory-reared, untreated males.

In our mating table studies, the highest number of males were attracted to and mated with clipped-wing irradiated virgin females between 02:00 and 03:00 hours. However, Henneberry and Keaveny [20] collected the highest number of mating native male and female PBW between 19:00–22:00 and 01:00–05:00 hours. Female calling behaviour and male response are primarily controlled by day length, temperature and the interaction of these two factors [21]. Short days and cool temperatures result in earlier mating or trap response. Trap response by PBW changed from early to late as the season progressed [20, 21, 22].

Our studies suggest that the ability of gamma irradiated female PBW to attract native males was dose dependent, and attractiveness was significantly higher when females were treated with 100 Gy. Releases of partially sterilized PBW at three week intervals at a ratio of 50:1 treated to normal, reduced the larval infestation to sub-economic levels inside field-cages. These experiments showed that the number of progeny produced by the partially sterile moths was limited and caused no significant damage to the crop. These results agree with those of Bariola et al. [23] and Flint et al. [8]. The reduced larval infestation in cotton fruiting bodies suggested an additive effect of induced and inherent sterility in PBW moths. As such, we would suggest that releases of irradiated moths should be carried out more frequently than three-week intervals to insure that sexually active, sterile or partially sterile males are always present to mate with the native females.

Our studies showed *T. chilonis* preferred PBW eggs when compared to spotted and spiny bollworms eggs. This preference might be due to the colour of the host eggs. Vinson [24] reports that host egg colour plays a key role in the recognition process of parasitoids. Ashraf et al. [25] observed that *T. chilonis* preferred green, yellow and white ovipositional substrates when compared to blue, black, red and pink substrates. The colour of PBW eggs is light yellow while spotted and spiny bollworm eggs are blackish gray. We suggest that *T. chilonis* can be integrated with genetic methods for the control of PBW.

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Integration of pheromones and biological control for the management of cotton bollworms in Pakistan

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Abstract. The management of cotton bollworms in a semi-isolated area through the use of inundative releases of the egg parasitoid *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae) in conjunction with pheromones suppressed populations of the pink and spotted bollworms to sub-economic levels. The parasitoid was more effective against pink bollworm than spotted bollworm. Applications of either pheromones or parasitoids by themselves were less effective when compared to the combined treatment. The level of parasitism in the cotton field was comparatively low in June and July but gradually increased during August and September. Maximum parasitism was recorded in November. Studies indicated that temperature affected the establishment of the parasitoid, and populations increased significantly when favourable conditions prevailed in the cotton field.

1. INRODUCTION

The pink bollworm, PBW, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) is a pest of great economic importance in many cotton-growing countries. The control of this pest depends largely on the application of pesticides, which has precipitated the development of resistance. As a result, in order to achieve effective control, more chemical applications per season are needed [1]. Furthermore, control of this pest using insecticides becomes ineffective due to the concealed feeding habits of the larvae inside the cotton bolls [2]. The continued application of insecticide to manage this pest also can lead to serious outbreaks of secondary pests in cotton [3, 4].

Alternative control strategies, such as mating disruption with synthetic pheromone [5, 6] and conservation of natural enemies [7], are being studied for their potential role in an integrated pest management program for PBW. Although effective control of PBW using mating disruption has been reported in Pakistan [5, 6], secondary infestations by the spotted bollworm, *Aerias vittella* (Lepidoptera: Noctuidae), require additional insecticide applications for their control. As such, the full potential of mating disruption may only be achieved if it is integrated with other environmentally friendly techniques that can control the other bollworm species. The egg parasitoid, *Trichogramma chilonis* has the potential to control all three species of cotton bollworms and would integrate well with mating disruption on an area-wide basis.

2. MATERIALS AND METHODS

Studies were conducted in a semi-isolated area (202.35 ha) planted with cotton. The area was divided into five blocks that received one the following treatments. The first block was treated with *Trichogramma chilonis* which had been reared on eggs of the Angoumois grain moth (*Sitotroga cerealella*) in the laboratory. The parasitoid colony was maintained at 25±2ºC and 60±70% relative humidity. To prepare the parasitoids for release, eggs of the Angoumois grain moth were glued to white paper cards and exposed to adult parasitoids for 24 h. The parasitoids were released in the field by attaching the cards with parasitized eggs to cotton leaves at 14 d intervals at the rate of 20,000 parasitoids per hectare for the duration of the season. The second block was treated with commercially available PBW and spotted bollworm pheromones at the prescribed rates [8, 9]. The third block received a combination of

parasitoids and pheromones. The fourth block was treated with conventional insecticides, receiving a total of six sprays during the season. The fifth block was untreated and served as a control.

The presence and establishment of the parasitoids in the cotton fields was ascertained using sentinel cards of Angoumois grain moth eggs prepared as described above. These cards were left in the field for 24 h and then brought back to the laboratory. The assessment was repeated every 15 days. In addition, field infestations of pink and spotted bollworms were recorded at weekly intervals. The data were analyzed statistically using the DMR test.

3. RESULTS AND DISCUSSION

Pink and spotted bollworms were effectively controlled in blocks receiving the combination of pheromones and egg parasitoids (Table 1). Infestations of both species in the insecticide treated blocks were comparable to those found in the pheromone plus parasitoid treatments. However, infestations detected in the block that received only parasitoids or only pheromones was much higher than in the other treatments. In the case of PBW on flowers, this difference was significant. Spotted bollworm infestations were higher than PBW in all the treatments. This might be due the possibility that the spotted bollworm pheromone became less effective over time because of degradation and isomerization of active ingredients by ultraviolet light [9].

Table 1. Mean percent infestation by pink bollworm, *Pectinophora gossypiella*, and spotted bollworms, *Aerias vittella*, in the different experimental blocks

Treatments	Percentage Infestation by			
	PBW		Spotted bollworm	
	Flowers	Green Bolls	Flowers	Green Bolls
Pheromones + Parasitoids	5.91 d	4.52d	9.11 c	8.21 c
Pheromones	8.90 c	8.03 c	11.32 c	14.12 b
Parasitoids	12.54 b	11.39 b	14.64 b	13.89 bc
Insecticides	6.88 d	6.97 c	10.50 c	9.80 c
Untreated Control	22.14 a	16.25 a	31.19 a	23.93 a

Means followed by the same letters are not significant ($P < 0.05$).

Pink and spotted bollworm infestations were significantly lower in all treatments as compared to the untreated controls suggesting that all treatments had some degree of field effectiveness. However, parasitoid establishment in the field was low during the warmest moths and gradually increased as the temperature and relative humidity became more favourable (Table 2). Parasitoid establishment started increasing in August and maximum parasitism rates were recorded during November. Our results suggest that temperature in the field plays an important role in the establishment and field persistence of the parasitoids.

Our results suggest that pheromones by themselves did not control the infestation of both species of bollworms as effectively as the use of combined treatments (parasitoids plus pheromones). However, the true treatment effects may have been obscured because the distance between our different blocks may have been too small (~50m). Henneberry et al. [10] suppressed PBW with pheromones to a significant degree when compared with results

Table 2. Mean percent parasitism by *Trichogramma chilonis* after releases into cotton fields

Month	Percent parasitization after releases		
	1 day after release	7 days after release	14 days after release
June	E 0.5 a	0.0	0.0
July	E 0.26 a	0.0	0.0
August	D 1.13 a	D 0.44 b	0.0
September	C 8.44 a	C 1.57 b	C 0.88 b
October	B 15.37 a	B 5.19 b	B 2.04 c
November	A 22.51 a	A 8.88 b	A 7.12 b

Letters on the left and right sides of the mean values show intra and inter column variations.
Means followed by the same letters are not significant ($P < 0.05$).

recorded in insecticide treated blocks. However, their experiments were conducted in large isolated areas. Parasitoids have long been recognized as an important insect suppressive tool and in many cases they have controlled the target pest to a degree where no further control treatments have been necessary. Nonetheless, biological control has not always provided adequate control [11]. Our preliminary results suggest that the application of parasitoids in combination with pheromones might be appropriate for the control of the lepidopteran pest complex in cotton in Pakistan. Studies in large isolated blocks should be conducted to assess the full potential of combining these environmentally friendly tactics.

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The use of gamma radiation to control two serious pests of Brazilian agriculture

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Abstract. This paper reports on the application of nuclear techniques to control two of the most important Lepidopteran insects pests in Brazil: *Diatraea saccharalis*, the sugarcane borer, and *Spodoptera frugiperda*, the fall armyworm. All experiments had the objective of finding the dose of gamma radiation capable of causing sterility in the first and second generations by irradiating the parental generation. For *D. saccharalis*, five day-old pupae were irradiated with doses of 100, 125 and 150 Gy. Fertility was reduced to 15% when moths were treated with 100 Gy and no egg hatch was recorded at 125 and 150 Gy. Fertility was 4.3% and 10.9% in the F_1 generation and was 9.5% and 25.5% in the F_2 generation, when treated males were mated to normal females and treated females were mated to normal males, respectively. The results of our research suggest a possible alternative tactic to control or even eradicate sugarcane borer from Brazil. For *S. frugiperda*, five day-old pupae were treated with doses of 50, 100, 125, 150 and 175Gy. Moths of the F_1 and F_2 generations were obtained only from parents treated at 50Gy. When higher doses were used, only the crosses where irradiated males were mated to normal (untreated) females produced moths of the F_1 and F_2 generations. Irradiation of the parental generation induced different sterility levels in the offspring. Female fall armyworm were more radiosensitive than males, and substerilizing doses of gamma radiation did not affect the life cycle of the first and second filial generations in this species. The level of sterility in the F_1 and F_2 generations was higher than the sterility of the parents irradiated at the same dose. These results are encouraging and indicate that inherited sterility might be used for control of this insect in Brazil. Large field experiments should be conducted to confirm the laboratory findings.

1. INTRODUCTION

The production of sugar and alcohol from sugarcane in Brazil is seriously hampered by the presence of the sugarcane borer *Diatraea saccharalis* (Lepidoptera: Gelechiidae). In general it could be stated that for each 1% of attacked internodes in a sugarcane stalk one can expect a reduction of 0.75% in sugar yields. Damage by this pest has been estimated to be between 5–15% of internodes in the field, which translates into a 4–10% loss in the total sugar production in Brazil. Worldwide, there are basically two different control methods for the sugarcane borer. In the United States, sugarcane borer control mainly relies on pesticides, while in countries like Brazil the use of biological control agents is more common. The use of pesticides is expensive, both in economic and environmental terms and there is always the threat of resistance development. Biological control against the sugarcane borer does not provide sufficient control to be a stand-alone tactic.

The species *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is originally from the tropical zones of the American continent. This insect causes heavy damage to many species of pasture grasses and to maize, sorghum, rice, wheat, barley, and other species of Graminae. It has been reported [1] that losses in maize of up to 34.0% in Brazil (county of Piracicaba) are caused by the fall armyworm. Losses caused by *S. frugiperda* in Canada and the United States reached around 300 million dollars per year during 1976–1977 [2].

Because of heavy reliance on the application of pesticides for pest control throughout the world, many pest species have developed resistance, including a number of lepidopterans. Cross-resistance also has been documented in many of these species. As a result, we are constantly in search of innovative ways to control insect pests. One of the promising methods is the use of gamma radiation to affect the reproductive ability of pest insects. Radiation induces changes in the insect chromosomes that affect reproductive capability. However, many other undesirable effects also can manifest themselves in the irradiated insects. Thus, finding the optimal dose of radiation that will maximize sterility while minimizing the undesirable effects on insect quality is the goal of many researchers. One way in which this combination might be achieved is through the use of F_1 or inherited sterility, where a low dose of radiation is applied to the parental generation and higher sterility is expressed in the offspring. The present paper reports on the application of nuclear techniques to control two of the most important Lepidopteran insects pests in Brazil: *Diatraea saccharalis*, the sugarcane borer, and *Spodoptera frugiperda*, the fall armyworm.

The induction of F_1 sterility was first documented in codling moth, *Cydia pomonella* [3]. The offspring from crosses between treated males and untreated females expressed a high level of sterility in both F_1 males and females. The theoretical basis of F_1 sterility was discussed by Knipling [4,5]. Ouye et al. [6] examined the effect of irradiation on pink bollworm pupae of different ages. They found that susceptibility to radiation decreased with increasing age (i.e., 3 day-old pupae treated with 100 Gy resulted in sterile adults, while 5 to 7 day-old pupae needed 220 and 300 Gy, respectively, to be fully sterile). Flint and Kressin [7] treated tobacco budworm pupae and adults with 350 Gy of gamma radiation, and found that at this dose both sexes reached 99% sterility. LaChance et al. [8] working with pink bollworm found that when males were treated with doses higher than 75 Gy and out-crossed to fertile females, fewer eggs were laid by the females. Carpenter et al. [9] showed that fall armyworm males that had been treated with 100 Gy had a suppressive effect on the number of progeny up to the third generation. LaChance [10] stated that F_1 sterility has many advantages over the sterile male technique, including higher competitiveness and the requirement of lower radiation doses to achieve the same results. Carpenter et al. [11] studied the effects of induced inherited sterility on the corn earworm. They showed that males irradiated with a dose of 100 Gy were as competitive as non-irradiated insects. Henneberry and Clayton [12] irradiated pupae of the pink bollworm with doses of 50, 100, and 150 Gy. They found that females were more radiosensitive than males.

Arthur et al. [13] irradiated 6 day-old pupae of the sugarcane borer with doses from 50 to 500 Gy to induce sterility in the F_1 generation. They showed that a dose of 400 Gy induced total sterility, and a dose of 100 Gy reduced the viability of the eggs laid by the females of the F_1 generation by 59% when compared to the parental generation. Arthur et al. [14] irradiated 5 day-old fall armyworm pupae with 50, 75, 100, and 125 Gy of gamma radiation. They showed that 125 Gy induced sterility in 96% of the males and 90% of the females in the F_1 generation.

2. MATERIALS AND METHODS

2.1. *Diatraea saccharalis*

The insects used these experiments came from a colony maintained at the Copersucar Experimental Station, close to Piracicaba, São Paulo, Brazil. All experiments were carried out in the Entomology Section of the Center of Nuclear Energy for Agriculture, of the University

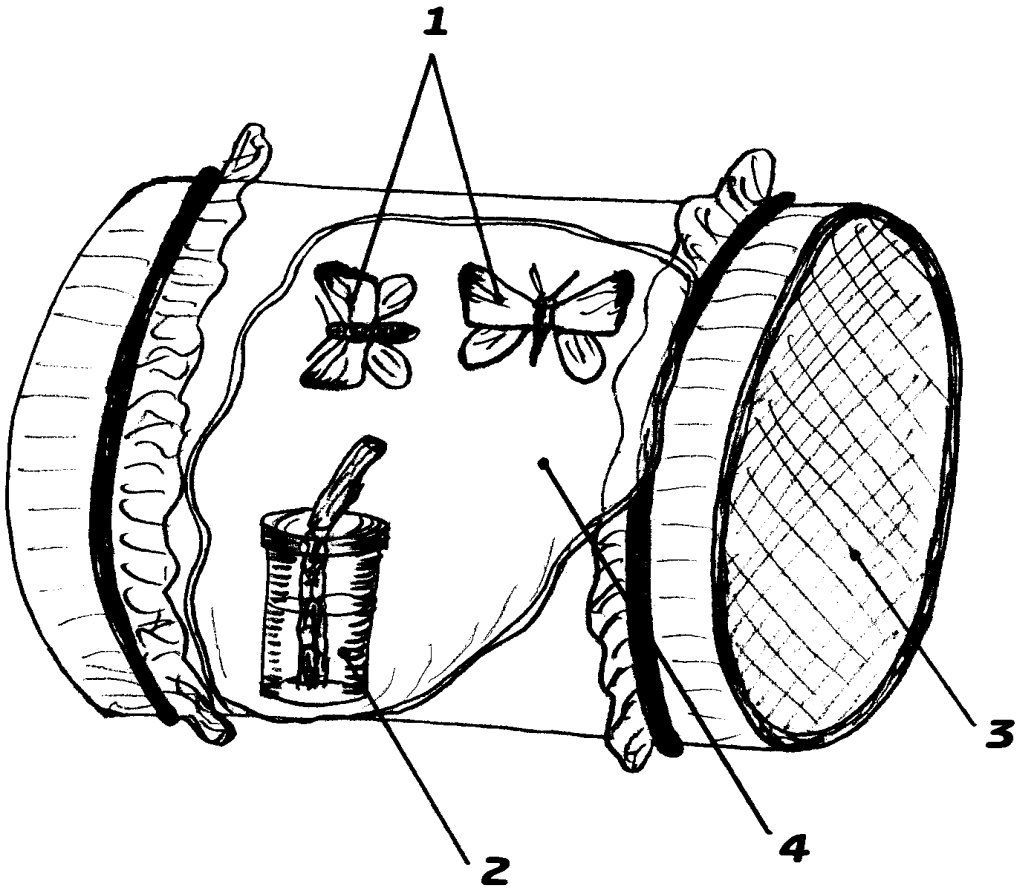

FIG. 1. Mating and oviposition cages used in the fall armyworm experiments. the cages (10 cm diam x 20 cm length) hold one pair of moths (1), which are provided with white paper as an ovipositional substrate (4) and a 10% honey solution for adult feeding (2). ventilation is through the fine plastic screen (3) held in place by a rubber band.

of São Paulo. After sexing, sugarcane borer pupae were irradiated with doses of 0, 100, 125, and 150 Gy at a dose rate of 3.0 kGy per hour using a Co^{60} source. Pupae were placed inside small plastic bags (250 ml) and emerging adults were collected. The adults were paired with virgin adults of the same age but of the opposite sex on a daily basis. Vials were checked each day and dead males and females were discarded. The number of fertile and non-fertile eggs was recorded daily and the newly hatched larvae were transferred to a vial containing artificial diet. After the life cycle was completed, five of each F_1 or F_2 males and F_1 or F_2 females were crossed with new virgin untreated insects of the same age and generation. The viability of the eggs produced from these crosses was used to determine the sterility of the F_1 and F_2 generations. All experiments were carried out in a chamber maintained at 21–25°C, 65±10% relative humidity, and a photoperiod of 14L:10D.

2.2. *Spodoptera frugiperda*

The colony of fall armyworm was started from infested maize collected in fields in Piracicaba, State of São Paulo, Brazil. In the laboratory insects were reared on artificial diet slightly modified from the recipe by Perkins et al. [15]. About 12–15 ml of diet were placed in each (50 ml) glass vial and sealed with a cotton ball. The diet contains the following ingredients: 165g beans (dry weight), 79.2g wheat germ, 50.2g Brewers yeast, 5.1g Vitamin

Table 1. Effect of selected doses of gamma radiation applied to pupae of *Diatraea saccharalis* on longevity, fecundity and fertility of the same generation [a]

Dose (Gy)	Cross	Longevity M (days)	Longevity F (days)	Eggs per female	Percent egg hatch
0	NM x NF	11.0	12.0	180.0	80.3
100	IM x NF	11.6	11.6	91.2	15.0
	NMx IF	11.0	10.0	29.5	0.0
125	IM x NF	9.0	9.5	52.6	0.0
	NM x IF	10.2	11.2	0.0	0.0
150	IM x NF	10.0	9.0	103.0	0.0
	NM x IF	11.4	12.0	56.0	0.0

[a] Five replicates per treatment. N = untreated (normal); I = irradiated.

Table 2. Effect of selected doses of gamma radiation applied to pupae of the parental generation of *Diatraea saccharalis* on longevity, fecundity and fertility of the F_1 generation [a]

Dose (Gy)	Cross	Longevity M (days)	Longevity F (days)	Eggs per female	Percent egg hatch	Percent sterility
0	NM x NF	10.5	11.0	202	81.2	18.8
100	IM x NF	9.5	10.0	201	4.3	95.7
	NM x IF	10.0	11.0	251	10.9	89.1

[a] N = untreated (normal); I = irradiated.

Table 3. Effect of selected doses of gamma radiation applied to pupae of the parental generation of *Diatraea saccharalis* on longevity, fecundity and fertility of the F_2 generation [a]

Dose (Gy)	Cross	Longevity M (days)	Longevity F (days)	Eggs per female	Percent egg hatch	Percent sterility
0	NMx NF	9.5	10.0	230	81.2	18.8
100	IMx NF	10.5	11.0	215	9.5	90.5
	NM x IF	12.0	10.0	242	25.5	74.5

[a] N = untreated (normal); I = irradiated.

C, 1.65g Sorbic acid, 3.15g Methyl-p-hydroxibenzoate, 12.5g formaldehyde, 20.5g agar and 1195ml distilled water. This amount of diet is sufficient to prepare 100 rearing vials. Each vial received one newly hatched larva. Vials were kept in a room at 23–27°C, 65±10% relative humidity, and a photoperiod of 14L:10D.

The pupae collected from the vials were separated by sex and weighed. Pupae were irradiated at day 5 with doses of: 0 (control), 50, 100, 125, 150, 175, and 200 Gy of gamma radiation from a Co^{60} source, at a dose rate of 2.15 kGy per hour. Parental, F_1 and F_2 crosses to untreated counterparts were made for both treated males and treated females at all doses that produced viable offspring.

Table 4. Effect of selected doses of gamma radiation applied to pupae of the parental generation of *Spodoptera frugiperda* on larval longevity, and survival in the F_1 and the F_2 generations [a].

P generation		F_1 generation		F_2 generation	
Dose (Gy)	Cross	Length of larval development (days)	Larval survival (%)	Length of larval development (days)	Larval survival (%)
0	NM x NF	20.5 e	94.0	20.8 bc	98.0
50	IM x NF	24.8 bc	74.0	27.8 a	72.0
	NM x IF	24.1 cd	84.0	23.4 ab	94.0
100	IM x NF	26.7 ab	94.0	23.8 ab	64.0
	NM x IF	0.0	0.0	0.0	0.0
125	IM x NF	26.2 ab	58.0	0.0	0.0
	NM x IF	0.0	0.0	0.0	0.0
150	IM x NF	22.8 d	46.0	28.4 a	60.0
	NM x IF	0.0	0.0	0.0	0.0
175	IM x NF	24.9 abc	50.0	27.2 a	70.0
	NM x IF	0.0	0.0	0.0	0.0
200	IM x NF	0.0	0.0	0.0	0.0
	NMx IF	0.0	0.0	0.0	0.0

[a] Data followed by the same letter are statistically similar at the 95% confidence level of 95% — Waller-Duncan test. N = untreated (normal); I = irradiated.

For all crosses, fecundity and fertility data were collected. Five replicates were completed with P generation moths. Crosses were placed inside cylindrical shaped cages (Figure 1) (10 cm diam x 20 cm high) with fine plastic screen covering the ends and with white paper lining the inside of the cylinder to serve as an ovipositional substrate. The adults were provided a 10% honey solution through a cotton wick inserted into a small glass vial. Eggs were collected daily, and 100 eggs per day were used for evaluation of fertility. The following biological parameters were recorded: a) larval and pupal survival and longevity, b) mean pupal weight, c) adult survival, sex ratio and total number of eggs. Waller-Duncan tests at 95% confidence level were used to ascertain statistical significance of the data.

3. RESULTS AND DISCUSSION

3.1. *Diatraea saccharalis*

The effect of selected doses of radiation on the longevity, fecundity and fertility of the parental generation is shown in Table 1. When males treated with 100 Gy were crossed with untreated females, percent egg hatch was reduced to 15%. When irradiated females (100 Gy or above) were crossed with normal males, percent egg hatch was zero. When adult moths of the resulting F_1 and F_2 generations were outcrossed to normal (untreated) counterparts, the percent egg hatch was 4.3% for crosses between irradiated males and untreated females and 10.9% when crosses involved an untreated male and a treated female of the F_1 generation. When the crosses were between F_2 generation adults the percent egg hatch was 9.5% and 25.5% for the same crosses (Tables 2 and 3). In the parental generation, fecundity and fertility were reduced with increased doses of radiation.

Table 5. Effect of selected doses of gamma radiation applied to pupae of the parental generation of *Spodoptera frugiperda* on fecundity and fertility of adults of the P, F_1, and F_2 generations [a]

		P generation		F_1 generation		F_2 generation	
Dose (Gy)	Cross	Eggs per female	Percent egg hatch	Eggs per female	Percent egg hatch	Eggs per female	Percent egg hatch
0	NMx NF	4148	97.0	4983	85.0	5109	87.2
50	IM x NF	6076	86.0	3324	81.5	5996	90.7
	NM x IF	5458	77.5	6256	89.5	6530	87.5
100	IM x NF	4378	72.0	4712	34.6	4876	49.8
	NM x IF	2610	4.9	0.0	0.0	0.0	0.0
125	IM x NF	5026	7.0	3520	2.7	0.0.	0.0
	NM x IF	894	0.0	0.0	0.0	0.0	0.0
150	IM x NF	4676	10.4	2439	2.4	985	13.2
	NM x IF	1084	0.0	0.0	0.0	0.0	0.0
175	IM x NF	3252	6.0	4530	3.0	843	6.2
	NM x IF	0.0	0.0	0.0	0.0	0.0	0.0
200	IM x NF	30.46	0.0	0.0	0.0	0.0	0.0
	NM x IF	0.0	0.0	0.0	0.0	0.0	0.0

[a] Data followed by the same letter are statistically similar at the 95% confidence level of 95% — Waller-Duncan test. N = untreated (normal); I = irradiated.

Our results show that females are more susceptible than males to the effects of gamma radiation.

Sub-sterilizing doses of radiation applied to the parental generation resulted in significant reductions in egg hatching in the F_1 and F_2 generations. Our results are similar to those reported earlier for sugarcane borer and fall armyworm [13, 14]. When male pupae were treated with 100 Gy the percentage of eggs that hatched was higher in the parental generation than when female pupae were irradiated at the same dose (Table 1); however, in the resulting F_1 and F_2 generations, the sterility of male progeny was higher that the sterility of female progeny (Tables 2 and 3).

3.2. *Spodoptera frugiperda*

Results for *S. frugiperda* are shown in Tables 4 and 5. The duration of larval development in the progeny of irradiated parents was significantly longer (Table 4) in both F_1 and F_2 generations. In addition, fewer of the offspring of irradiated parents survived when compared to the larval survival in the untreated controls. Our results are similar to those obtained for many other species of Lepidoptera [16, 17, 18, 19, 20]. In our experiments, offspring were only produced from females treated with 50 Gy. However, males treated at 50, 100, 125, 150 and 175 Gy and crossed to untreated females produced F_1 generation larvae (Table 4).

The effect of radiation on the number of eggs laid by untreated females mated to irradiated males was greatest at doses above 150 Gy (Table 5). Females treated with 50 Gy were able to lay a similar number of eggs as untreated females. However, females treated with higher doses showed a marked decrease in the number of eggs they laid when compared to controls. At doses of 175 and 200 Gy, no eggs were laid by the treated females. Percent fertility in

males irradiated with doses between 50 and 175 Gy decreased gradually and reached zero at 200 Gy. These results agree with those reported by other authors working with fall armyworm [21, 22].

We showed that deleterious effects, including sterility induced by gamma radiation applied to the parental generation, are transferred to the first and second filial generations in *S. frugiperda*. However, it is worth noting that the sterility in the F_2 generation was lower (higher fertility) than in the F_1, suggesting that deleterious effects are lost during successive generations. Our results are similar to those reported by other authors working on fall armyworm and other lepidopteran species [i.e., 2, 3, 9, 10, 13, 19, 20, 22, 23, 24, 25].

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The use of F_1 sterility and parasitoids for population suppression of lepidopteran pests of crucifers in Indonesia

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Abstract. We report on the population suppression of diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and cabbage webworm (CWW) *Crocidolomia binotalis* Z. (Lepidoptera: Pyralidae) using releases of irradiated (200 Gy) substerile moths. The impact of substerile DBM was studied in field-cages and moths were released at a 9:1 treated : untreated ratio. Our results show that releasing F_1 substerile male and female DBM resulted in a high level of sterility (73.03% and 73.30% in the F_1 and F_2 generations, respectively) in the untreated population, while the release of only F_1 males induced a lower level of sterility (55.40% and 56.44% in the F_1 and F_2 generations, respectively). When substerile moths were released once per generation, the level of sterility was 44.78% in the F_1 and 68.01% in the F_2 generations. The effect of releasing substerile males only, females only, and substerile male and female CWW on the untreated population were studied in the laboratory. Percent egg hatch was 22.17% for male only releases. For female and mixed sex releases these percentages were 28.50% and 24.75%, respectively. For DBM, some studies combined releases of substerile DBM with releases of the parasitoid *Diadegma semiclausum* (H) (Hymenoptera: Ichneumonidae) in field-plots. Pupal viability in the F_1 generation in the area that received both parasitoids and substerile DBM was 32.5%. The effect on pupal viability when only a single tactic was used was lower than when both tactics were combined. The release of substerile males only gave a pupal viability of 57.5% and releases of the parasitoid *D. semiclausum* resulted in 81% pupal viability. When substerile DBM were released into a small isolated forested area in Malang, East Java, the average number of moths caught per week at the release area from June to October 1996 was about 89.42% of that found in the untreated control area. When population fluctuations of wild DBM were followed for 12 months, the lowest population level was found to occur in April, and the highest population was recorded in August. The highest level of parasitization by *D. semiclausum* in the laboratory was on second instar DBM larvae.

1. INTRODUCTION

The diamondback moth (DBM, *Plutella xylostella*) and the cabbage webworm (CWW, *Crocidolomia binotalis*) are the most important pests of cabbage or other cruciferous plants in Indonesia [1]. DBM larvae primarily attack young cabbage plants, while CWW larvae attack all development stages of the plants. The use of insecticides to control these insects is inadequate. It was reported that a strain of DBM in West Java was resistant to pyrethroid insecticides [2]. The parasitoid *Diadegma semiclausum* has been used in Indonesia as an alternative means to control DBM. The use of this parasitoid is intended as one of the components in integrated pest management of cabbage in Indonesia. The level of parasitization in several cabbage production areas in West Java (Lembang and Pangalengan) varies between 6% and 76% [3].

The approach for control of DBM and CWW using the sterile insect technique (SIT) is being explored in several countries in the Asia Pacific Region because both pests are very important, and present control measures are inadequate [4, 5]. As stated by Knipling [6], the use of insects to control populations of their own kind through the transfer of damaged genetic material at mating represents an approach that could prove to be useful for the control of a number of major insect pests throughout the world. In DBM, a dose of 300 Gy induces

about 90% sterility, while a dose of 200 Gy induces about 73.2% sterility in irradiated DBM males and 56.81% sterility in their F_1 progeny [7].

The strategy of integrated pest management (IPM) in cabbage in Indonesia does not intend to eradicate the target pest species, but seeks to maintain the pest populations below the economic injury level. The Indonesian Department of Agriculture has determined that this level is five larvae per ten cabbage plants for DBM (*P. xylostella*), and three eggs clusters per ten cabbage plants for CWW (*C. binotalis*) [8]. If the level of parasitization of field collected DBM is less than 75%, it becomes necessary to control this pest with insecticides.

Our experiments examined the effect of combining SIT and the release of *D. semiclausum* on populations of DBM in small field plots. We also report on experiments that examined the potential of SIT to control DBM in field-cages and in a small isolated. Finally, progress on experiments examining the effect of releasing irradiated males, irradiated females or irradiated unsexed CWW on CWW populations under laboratory conditions are also reported.

MATERIALS AND METHODS

Unsexed, 3 to 4 day-old DBM pupae were irradiated with 200 Gy in a Co^{60} Gammacell irradiator in Jakarta. This substerilizing dose (200 Gy) resulted in 73.2% sterility in the P generation males and 56.8% sterility in F_1 males. Field-cage experiments to examine the effect of releasing irradiated, partially sterile P and F_1 DBM were conducted at a cabbage production area in Cipanas, West Java during 1997.

Five field-cages (2.5 x 1.5 x 1 m) were placed over rows of insecticide free cabbage plants. Fifty pairs of untreated DBM moths were introduced into the first cage that served as a control. Fifty pairs of untreated DBM moths plus 450 irradiated (200 Gy) male moths (a 1:9, untreated to treated ratio) were introduced into the second cage. The third cage received 50 pairs of untreated DBM plus 450 F_1 DBM males (the offspring of irradiated males mated to normal females). The fourth cage was similar to the third, but the number of F_1 progeny included both male and female (450 pairs) moths. The fifth cage was similar to the second cage, but, at the time that the next generation of DBM were expected, a second release of irradiated DBM males was made into the cage. The parameters measured in each cage included: percent egg hatch, number of pupae, and number of DBM moths emerging in the first and second generations.

Egg hatch was assessed by removing a sample of 250 eggs from each field-cage. Using a fine-tipped brush, the DBM eggs were transferred from the cabbage leaves to the sticky surface of masking tape, and were observed for hatch after 7 days using a dissecting microscope. The number of pupae developing in each cage was determined by collecting and counting the pupae from the cabbage plants. Pupae were placed in small cages inside the field-cages to observe adult emergence. Adults were released back into the field-cages.

Data for CWW were collected in the laboratory. For this experiment, six-day old CWW pupae were treated with 250 Gy. This dose caused 63.95 % sterility in male CWW [4]. The effects of releasing treated male and treated mixed sex populations of CWW were assessed in laboratory cages. The first cage received 60 pairs (males and females) of treated and 20 pairs of untreated CWW (3:1 treated:untreated ratio). The second and third cages received 60 treated males (cage 2) or 60 treated female (cage 3) CWW and 20 pairs of untreated CWW (both cages). Cages 4 through 7 were used to ascertain the sterility level between the treated and untreated moths when released at different ratios (Table 2).

Table 1. The effect of releasing 200 Gy treated *Plutella xylostella* and their F_1 progeny on the untreated population inside field-cages

Treatment	% Sterility [1]		No. of Pupae [1] (% of control)		No. of Moths [1] (% of control)	
	P	F_1	F_1	F_2	F_1	F_2
50 UM+ 50 UF Control	5.20	3.47	829 (100)	1706 (100)	772 (100)	1566 (100)
50 UM+ 50 UF + 450 IM	44.95	44.36	477 (57.5)	963 (56.4)	282 (36.5)	520 (33.2)
50 UM+ 50 UF + 450 F_1M	55.4	56.44	350 (42.2)	697 (40.8)	163 (21.1)	309 (19.7)
50 UM+ 50 UF + 450 F_1M+F	73.03	73.30	128 (15.4)	261 (15.2)	31 (4)	62 (3.9)
50 UM+ 50 UF + 450 IM + 450 IF	44.78	68.01	471 (56.9)	549 (32.1)	261 (33.8)	181 (11.5)
LSD 5%	7.49	10.79	66.85	103.82	54.53	78.19
LSD 1%	10.73	15.36	95.10	147.67	77.56	111.21
CV	9.22	12.08	8.14	6.83	9.89	8.15

U = Untreated
I = DBM 3 to 4 day-old pupae treated with 200 Gy
F_1 = Progeny from a cross between I male x U female
[1]= average of 3 replications

Table 2. Effect of releasing irradiated male or female and unsexed adults of *Crocidolomia binotalis* (CWW) on egg hatchability under laboratory conditions

Treatment IM : IF : UM : UF	No. of eggs	No. of hatched eggs	Percent egg hatch
3 : 3 : 1 : 1	503	124	24.75
3 : 0 : 1 : 1	523	116	22.17
0 : 3 : 1 : 1	507	145	28.50
0 : 0 : 4 : 1	524	501	92.43
0 : 0 : 1 : 4	645	600	93.02
1 : 0 : 0 : 1	476	0	0
0 : 1 : 1 : 0	510	0	0
LSD 5%			0.22
LSD 1%	-	-	0.32
CV (%)			2.36

U = Untreated CWW I = Irradiated CWW 3 to 4 day-old pupae with a dose of 250 Gy

Experiments to assess the impact of combining irradiated DBM with releases of the parasitoid *D. semiclausum* were conducted in the field. The study site was an isolated area (1,000 m^2) located in a forested area in Cangar, Malang, East Java. The area is about 2 km away from cabbage production fields. We assumed that this distance would prevent reinfestation to occur from outside the study area. Approximately 3,000 male DBM pupae treated with 200 Gy were used in these experiments.

Baseline data on population dynamics of DBM were obtained during 1991 and 1992 by trapping the moths with pheromone traps and by direct counting of pupae at random (5

plants/day) in the cabbage production areas at Ciloto, Cileutik and Citere, in West Java Province. Pheromone (Delta type) traps baited with pheromone (SJ, Takeda Chemical Industries, Japan) were used in this study.

Observations were made on parasitism rates by *D. semiclausum* on DBM in order to find an efficient and effective rearing methodology for this parasitoid in the laboratory. The level of parasitism in mixed-age larvae was assessed by introducing 10 pairs of *D. semiclausum* into a cage containing 1,000 DBM larvae of each instar.

2. RESULTS AND DISCUSSION

The impact of releasing 200 Gy treated DBM at a ratio of 9:1 treated : untreated inside field-cages resulted in 44.95% and 44.3% sterility in the P and F_1 generations (Table 1). The release of substerile F_1 males, derived from crosses between treated males (I) and untreated females (U), resulted sterility levels for the P and F_1 generations to be 55.4% and 56.44%, respectively. When releases were repeated once per generation, the sterility in the F_1 generation was 44.78% and it increased to 68.01% in the F_2 generation. The sterility observed in this experiment was highest (73.03% and 73.3% for the F_1 and F_2 generations, respectively) when unsexed F_1 progeny were released into the field-cages. However, the release of F_1 progeny has some disadvantages because it requires additional rearing costs before field releases are made. Therefore, continuous releases of treated P generation DBM males should be considered for a program against DBM in Indonesia because the level of sterility in the F_2 generation was high (68.01%) and might reduce the wild population over time.

For CWW, the effect of releasing treated males, treated females and treated males and females on the egg hatchability of untreated CWW is shown in Table 2. Percent fertility was significantly lower as compared to the control (LSD 1% = 0.32) when irradiated males (24.75%), females (22.17%), and both sexes were released together (28.50%) into the laboratory cages. Percent egg hatch was lowest when only treated males were released. We therefore suggest that this method should be considered in the implementation of SIT for CWW in the field.

Table 3. The effect of combining substerile *Plutella xylostella* and *D. semiclausum* to control *P. xylostella* in small field plots

		First Generation			Second Generation		
No.	Treatment	% Sterility	% Pupal Mortality		Unhatched Eggs (% Sterility)	Parasitoid (No.)	DBM Pupal Viability (%)
			DBM	Parasitoid			
1.	50 UF+50 UM	5.2	0 (U)	-	5.47	-	100
2.	50 UF+50 UM + 450 IF+M	44.9	0 (U) 14.6 (I)	-	54.3	-	57.5
3.	50 UF+50 UM + 25 PF+M	4.9	0 (U)	0	4.47	19	81
4.	50 UF+50 UM + 450 IF+M + 25 PF+M	43.7	0 (U) 14.6 (I)	0	52.9	35	32.5

U=Untreated DBM
I =Irradiated DBM pupae 3 to 4-d -old with a dose of 200 Gy
P = *Diadegma semiclausum*

The effect of combining releases of partially sterile DBM with those of the parasitoid *D. semiclausum* in small field plots is shown in Table 3. Pupal viability in the F_1 generation in the area that received parasitoids and substerile DBM was 32.5%. The effect on pupal viability by each single tactic was lower than when combined releases were made. The release of substerile males only gave a pupal viability of 57.5% and releases of the parasitoid *D. semiclausum* resulted in 81% pupal viability. It appears that combining the two tactics is the most effective approach.

Results of the small field experiment where substerile DBM were released is shown in Table 4. The average number of DBM moths caught in pheromone traps per month from June to October 1996 ranged from 74.2 to 149.6; these captures were always lower than those recorded from the untreated area. The average level of fertility recorded at both sites is shown in Table 4. The values for the treated area (75.6%-79.3%) were always lower than those recorded for the untreated area.

Table 4. Effect of releasing 200 Gy treated male *Plutella xylostella* on population reduction in the small isolated area of Cangar

Month in 1996	Mean number of moths captured week[1]		Average level of fertility	
	Control Area	Release Area	Control Area	Release Area
June	85.7	74.2	95.4	79.1
July	107.4	96.3	94.7	75.6
August	117.7	103.2	95.7	77.9
September	124	114.5	95.1	79.3
October	164.2	149.6	93.4	75.7

[1] Average of 10 pheromone traps

Table 5. Comparison of the number of *Plutella xylostella* captured in pheromone traps, number of pupae and rainfall at cabbage production areas, in West Java Province

Month	No. of moths [1]	No. of pupae [2]	Rainfall (mm) [3]
November, 1991	8565	531	574.5
December, 1991	8146	485	334.4
January, 1992	6331	456	329.1
February, 1992	6572	262	341.9
March, 1992	5206	327	513.5
April, 1992	3344	116	454.7
May, 1992	4773	277	148.1
June, 1992	7057	462	102.9
July, 1992	8908	1131	124.1
August, 1992	11617	1536	191.3
September, 1992	9150	1003	357.2
October 1992	7111	405	533.1

[1] No. of DBM caught in 15 pheromone traps at Ciloto, Cinere and Cileutik
[2] No. of DBM pupae collected from 150 cabbage plants each at Ciloto, Citere and Cileutik
[3] At Ciloto

The yearly pattern of population fluctuation for DBM was similar at the three different cabbage production areas (Ciloto, Cileutik, and Citere) in West Java Province (Table 5). The lowest population level was found in April, and the highest population was present in August. No correlation with rainfall is evident from this data. We would suggest that the best time to initiate an SIT program would be in April because an SIT program is most effective when the target population is low.

As shown in Table 6, the level of parasitization by *D. semiclausum* was highest on second instar DBM larvae followed by parasitization of third instar larvae. Our results suggest that *D. semiclausum* does not attack DBM larvae that are in the first or the fourth instar of larval development.

Table 6. Parasitism by *Diadegma semiclausum* on *Plutella xylostella* (DBM) larvae of different instars

DBM larval instar [1]	Parasitism by *D. semiclausum* [2]	
	Total	Percentage
First	0	0
Second	634	63.4
Third	307	30.7
Fourth	0	0

[1] Ten pairs of *D. semiclausum* were released with 1000 DBM larvae of each instar and left together for 12 hours (= 1 replicate)
[2] Average of four replicates.

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Field trials in South China to control the diamondback moth, *Plutella xylostella* (Lepidoptera*:* Plutellidae) using radiation-induced sterility

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Abstract. This paper discusses the control of the diamondback moth, *Plutella xylostella* by the sterile insect technique (SIT). Our studies included mating characteristics, sterility of the F_1 generation, dispersal and recapture of irradiated moths, as well as control of DBM using SIT and F_1 sterility, and an economic evaluation of F_1 sterility to protect cabbage. Male DBM mated an average of 16 times and female DBM mated an average of 4 times. However, irradiated male DBM only mated an average of 7.2 times. Seventy percent of matings occurred from 18:00–24:00 h with an average duration of 80 min. Irradiated male moths and untreated male moths exhibited the same attraction to female moths. After 10 days, most (94.2%) of the released, sterile DBM were recaptured within 40 m of the release site. Only one DBM was recaptured at 120 m from the release site. The area of dispersal was calculated to be 696 m^2 during the first three days. In a field study to control DBM by releases of irradiated insects, the ratio of sterile to wild DBM was 4.7:1. During this study, the egg sterility in the F_1 and F_2 generations was 79.0% and 81.7%, respectively. The developmental times for the F_1 and F_2 generations were 4 and 12 days longer, respectively, than for DBM in the control area. Thus, the number of DBM generations was reduced in the treated field. With successive releases over two generations, the control effectiveness was 80.8% in the F_1 generation and 79.1% in the F_2 generation. The cost of using F_1 sterility to control DBM in a small field was similar to the cost of using pesticides. Therefore, the use of F_1 sterility should be an economically viable control strategy for DBM that also would help protect the environment from the overuse of pesticides.

1. INTRODUCTION

The sterile insect technique (SIT) and radiation-induced inherited sterility (F_1 sterility) have been considered as potential methods for controlling several lepidopteran pests. The diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Plutellidae), is one of the most important of all lepidopteran pests and causes great economic loss in south China. DBM can reduce production of all mustard family vegetables by 30–50% in some years, and has become resistant to many pesticides. Because of the need to increase the effectiveness of pest control and reduce environmental pollution, we have studied the control of DBM by the SIT and F_1 sterility for many years. These studies have included mating characteristics, sterility of the F_1 generation, release-recapture of sterile DBM, control of DBM by the SIT and F_1 sterility in the field, and an economic evaluation of F_1 sterility to protect cabbage.

2. MATERIALS AND METHODS

2.1. Mating characteristics of DBM

DBM were collected from a suburb of Hangzhou and reared on artificial diet. Mature pupae were irradiated with gamma rays with 250 Gy (dose rate 1.62 Gy/min). The DBM were paired into the following combinations, UF x UM and UF x IM (U= untreated moths; I= irradiated moths), and placed in separate glass containers. Mated female moths were placed in separate containers for oviposition. Data were recorded for the time of day that mating occurred,

mating frequency, duration of copula, time interval between successive matings, fecundity and egg hatch.

2.2. Release-recapture of DBM

The study was conducted in a 12 x 240 m cabbage field that was bordered on one side by a wall. The release site for the DBM was positioned near the centre of the wall at the edge of the field. Traps were positioned in semicircles around the release site. Radii for the semicircles were 10, 25, 40, 60, 80, 100 and 120 m. There were 4, 6, 9, 14, 16, 17 and 4 traps placed in each semicircle, respectively (70 traps total). For each trap, a female DBM was confined in a small cloth bag and positioned above a container holding water and detergent (as a wetting agent). Sterile male moths (2,758) marked with crystal violet were released, and the number of trapped marked males was recorded for 10 days.

Table 1. Fecundity and fertility of 30 female *Plutella xylostella* mated sequentially with the same unirradiated (normal) male moth

Mating Order	No. Eggs Laid	% Egg Hatch
1	105	79.1
2	105	73.3
3	114	86.8
4	111	83.8
5	107	74.8
6	99	70.7
7	62	79.0
8	81	80.2
9	50	78.0
10	46	82.6
11	65	73.9
12	52	90.4
13	20	85.0
14	53	77.3
15	21	71.4
16	58	81.0
17	93	82.8
18	24	83.3
19	1	0
20	21	81.0
21	50	80.0
22	130	77.7
23	23	82.6
24	0	0
25	87	74.7
26	0	0
27	35	85.7
28	48	89.6
29	0	0
30	0	0

Table 2. fecundity and fertility of 14 female *plutella xylostella* mated sequentially with the same irradiated male moth

Mating Order	No. Eggs Laid	% Egg Hatch
1	101	29.0
2	159	33.3
3	106	30.2
4	84	27.4
5	78	32.1
6	112	27.7
7	147	36.1
8	90	21.1
9	80	27.6
10	153	23.5
11	95	29.5
12	78	9.0
13	0	0
14	0	0

2.3. Suppression of wild DBM by releasing irradiated moths

This study was conducted in two isolated cabbage fields: a release field of 0.8 ha, and a control field of 0.067 ha. The densities of DBM in the two fields were similar, reaching 30,000 per ha. Twenty release racks were distributed in the release area, and sterile pupae in mesh-covered plates were positioned on these racks. The plates were positioned 20 cm above the cabbage plants. Ten releases of sterile moths (n = 351,630) were made during two consecutive generations of DBM. The mean ratio of sterile DBM to wild DBM was 4.7:1. The population of DBM was estimated once every 4–5 days by random sampling. Six hundred heads of cabbage were inspected in the release area and 200 heads were inspected in the control area on each sampling date. Samples of DBM eggs were collected from the release and control areas and brought back to the laboratory.

2.4. Economic evaluation of F_1 sterility to protect cabbage production

Two isolated cabbage areas were selected for this study. The sterile DBM were released in a 1 ha area that was not protected with insecticide treatments. The control area was 1 ha and was treated with insecticide for DBM control. Thirty release racks were distributed in the release area allowing for the release of 572,670 sterile moths in the spring and 600,350 sterile moths in the fall. Two crops of cabbage were produced during the year. The commercial value of F_1 sterility to protect the vegetable crop was calculated.

3. RESULTS

3.1. Mating characteristics of DBM

We observed a total of 267 DBM matings. All matings occurred between 18:00 and 06:00 h. Seventy percent of the matings occurred during the period from 18:00 to 24:00 h. Mating duration ranged between 20–205 min. (80 min average). The time between consecutive matings for the same male moth ranged from 5–9,880 min (60 min average).

Table 3. Effects of distance from the release site and time after release on the number of *Plutella xylostella* recaptured

Date	Number of moths captured								% Moths	% Recapture
(day/month)	10m	25m	40m	60m	80m	100m	120 m	Total	Captured	
19/5	19	0	2	1	0	0	0	22	5.8	0.80
20/5	64	6	4	0	0	0	0	74	19.5	2.68
21/5	71	2	3	1	1	2	0	80	21.1	2.90
22/5	14	2	0	4	0	1	0	21	5.5	0.76
23/5	46	19	13	1	0	0	0	79	20.8	2.86
24/5	28	16	5	4	2	1	1	57	15.0	2.07
25/5	9	18	12	0	1	1	0	41	10.8	1.49
28/5	1	1	3	1	0	0	0	6	1.5	0.22
Total	252	64	42	12	4	5	1	380		
% of Total	66.3	16.8	11.1	3.2	1.1	1.3	0.2		100.0	
% Recapture	9.14	2.32	1.52	0.44	0.15	0.18	0.03			13.78

Untreated female moths mated a maximum of 8 times (4 times average). Untreated male moths mated a maximum of 30 times (16 times average), in contrast to irradiated male moths which mated a maximum of 14 times (7.2 times average).

The number of eggs laid by 30 female moths that mated sequentially with the same male moth was similar for the first 6 females (average 107 eggs). However, the number of eggs decreased to an average of 51 for females that were 7 to 12 in the mating order. The fertility of females generally was not affected by the mating sequence (Table 1). Fecundity and fertility of female moths that mated with the same irradiated male were not affected by the sequence of mating (Table 2). Egg hatch ranged from 9.0–36.1%. The sterility of irradiated male moths persisted for 14 matings.

3.2. Release-recapture of DBM

The results showed that 380 marked sterile DBM were recaptured during the first 10 days following release (Table 3). The recapture rate was 13.78%. At a distance of 10 m from the release site 66.3% DBM were recaptured. Only one marked male was trapped 120 m from release site. At a distance of 40 m from the release site 94.2% of the DBM were recaptured during the 10 days following the release, while 92.7% of the recaptured DBM were trapped during the first six days after release. Only 1.5% of the recaptured DBM were trapped during the last 3 days. The dispersion area was calculated to be 216 m^2 on day 5 and 1256 m^2 on day 6.

3.3. Suppression of wild DBM by releasing irradiated moths

A total of 2,502 eggs were collected during the F_1 generation from the release area, and 96 eggs from control area. Nineteen percent of eggs from the release area hatched in the laboratory. The corrected sterile rate of eggs from the release area was 79.0%. During the F_2 generation 1,497 eggs were collected from release area, and 187 eggs were collected from the control area. Only 16.0% of eggs collected from the release area hatched. The corrected sterile rate of eggs from the release area was 81.7% (Table 4).

The highest density of early instar F_1 DBM was 1.17 per m^2 in the release area and 6.10 per m^2 in the control area. As a consequence, the density of early instar DBM in the release area

Table 4. Number of eggs and percent egg hatch for *Plutella xylostella* during two generations following the release of irradiated moths

Treatment	Parameter Measured	F_1	F_2
Release Area	Number of eggs	2502	1497
	Number of Hatched eggs	476	249
	% Egg hatch	19.0	16.0
	Corrected % egg hatch	21.0	18.3
	Corrected sterile rate (%)	79.0	81.7
Control Area	Number of eggs	96	187
	Number of hatched eggs	87	170
	% Egg hatch	90.60	90.90
	Corrected % egg hatch	100.00	100.00
	Corrected sterile rate (%)	0	0

Table 5. Comparison between conventional and F_1 sterility control tactics for protecting cabbage against *Plutella xylostella*

Control method	Cost of pesticide and labor[1]	Cost of irradiated DBM	Cabbage production (kg)	Cabbage value (USD)	Profit (USD)
F_1 sterility	126.50	2891.57	60,000–67,500	6,409	3,158
Pesticide	469.88	-	60,000–67,500	4,337	3,939

[1]The cost of manpower was 3.62 US$/day.

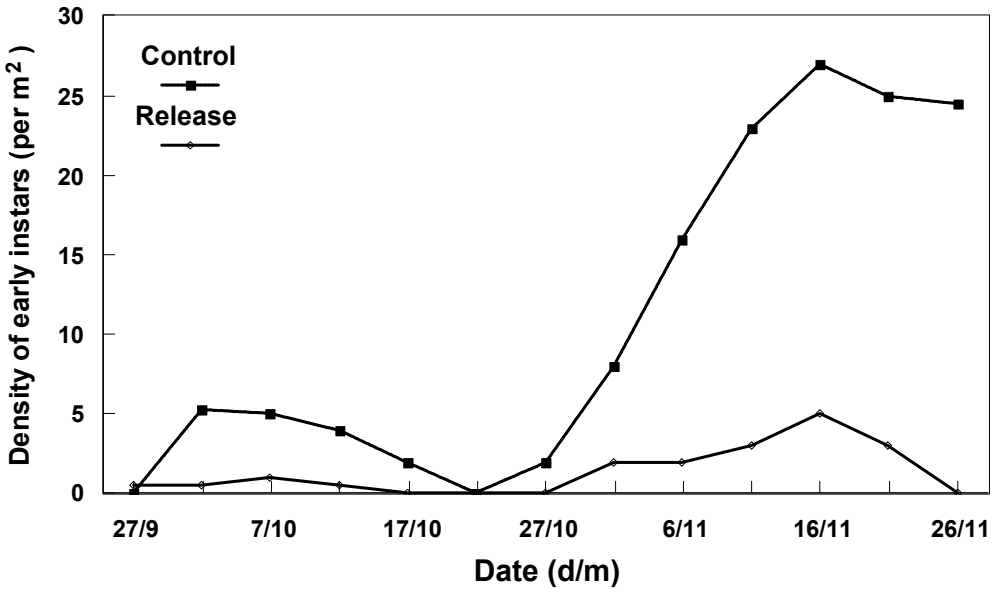

FIG. 1. Effect of sterile Plutella xylostella on density of early instars for two generations following the release of irradiated (250 Gy) moths in a cabbage field.

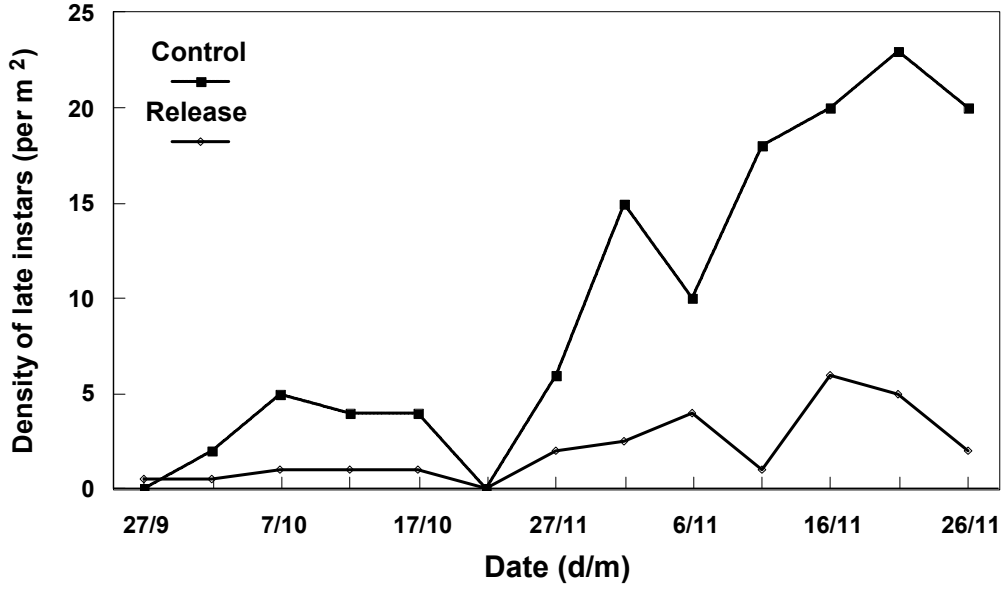

FIG. 2. Effect of sterile Plutella xylostella on density of late instars for two generations following the release of irradiated (250 Gy) moths in a cabbage field.

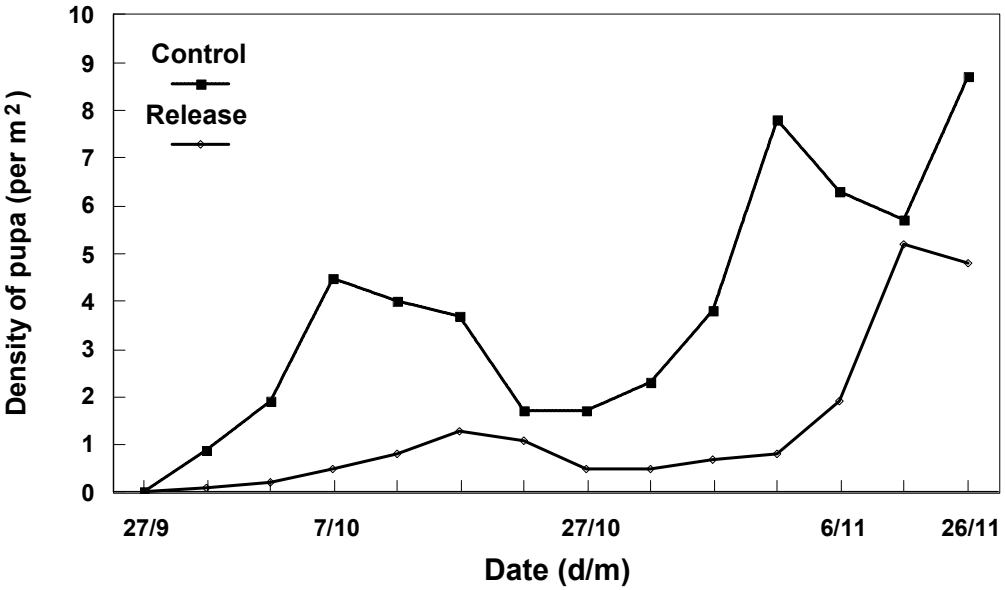

FIG. 3. Effect of sterile Plutella xylostella on density of pupae for two generations following the release of irradiated (250 Gy) moths in a cabbage field.

was only 19.18% of the density of early instars in the control area. Effectiveness of control was calculated at 80.8% during the F_1 generation. The highest density of early instar F_2 DBM larvae was 5.54 per m^2 in release area and 26.50 per m^2 in control area. Therefore, the density of early instars in release area was only 20.91% of the density in the control area. Effectiveness of control was calculated to be 79.1% in the F_2 generation (Figure 1). The

density of late instar DBM was greater in the control area than in the release area. The effectiveness of control based upon late instar DBM densities was calculated at 80.7% (Figure 2). The cabbage in the release area was of marketable quality, but the cabbage in the control area could not be marketed. Peak densities of DBM pupae for F_1 and F_2 generations indicate that the generation time was shorter for the DBM in the control area than for the release area. Also, peak densities of pupae for each generation were lower in the release area than in the control area (Figure 3).

3.4. Economic evaluation of F_1 sterility to protect cabbage production

We compared the calculated commercial value of using F_1 sterility and pesticides to protect cabbage from DBM damage. The cost of using F_1 sterility was about $3,018.00 USD per year and the cost for using pesticides was about $470.00 USD per year. The cabbage yield was 60,000–67,500 kg/ha per year. The value of the cabbage protected with pesticides was about $4,337.00 USD ($0.07 USD/kg), and the value of the cabbage protected with F_1 sterility was $6,409.00 USD ($0.11 USD)(Table 5).

4. DISCUSSION

A dose of 250 Gy was a suitable sterilizing dose for the control of the DBM. The mating behaviour and mating ability of sterile male moths irradiated with dose of 250 Gy was the same as that of untreated male moths. In our field studies, the effectiveness of control for DBM using F_1 sterility was 80.7% in the first generation and 79.1% in the second generation. The developmental time of each DBM generation was longer in the release area than the control area. Thus, the number of generations would be reduced and, therefore, should add to the control effectiveness of releasing sterile moths. Our results showed that the commercial value of using F_1 sterility to control DBM in a small field was similar to using pesticides. Therefore, the use of F_1 sterility should be an economically viable control strategy for DBM that also would help protect the environment from the overuse of pesticides.

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Potential use of F_1 sterility and the parasitoid, *Cotesia plutellae*, to control diamondback moth, *Plutella xylostella*, in Myanmar

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Abstract. Diamondback moth (DBM), *Plutella xylostella* males irradiated with 100 Gy and larval parasitoids (*Cotesia plutellae*) were studied for their potential to control DBM in cabbage fields of Nyaung-Le-Bin Township, Bago Division. The following treatments were evaluated as control tactics: release of irradiated male DBM, augmentative release of parasitoids, and combined release of irradiated male DBM and parasitoids. These treatments reduced the larval population of feral DBM.

1. INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella*, is a serious pest of cabbage and other cultivated crucifers in Myanmar. Because the sterile insect technique (SIT) has been an effective method for the control of other lepidopteran pests, Myanmar scientists have studied the potential use of SIT for control of DBM. These studies have included population dynamics of DBM and its natural enemies, radiation biology of DBM, rearing technology for DBM and its parasitoids, and effectiveness of larval and pupal parasitoids of DBM. The overall objectives of these studies have been the development of SIT for population reduction of DBM, the reduction in the application of insecticides and the conservation of natural enemy populations in the field. In this report we describe a study designed to evaluate releases of irradiated male DBM, a larval parasitoid, and a combination of irradiated male DBM and parasitoids as control tactics for DBM.

2. MATERIALS AND METHODS

Cabbage is usually grown in the winter season (December–March) in fields of Nyaung-Le-Bin Township, Bago Division. Populations of DBM adults reach their peak during the first two weeks of February. We designed an experiment to evaluate the ability of SIT and augmentative biological control to suppress the seasonal population increase of DBM. The experiment was conducted in a cabbage field as a randomized complete block design. There were 3 experimental treatments and each treatment was replicated 4 times. Experimental treatments were (1) release of irradiated male DBM, (2) augmentative release of the larval parasitoid, *Cotesia plutellae*, and (3) combined release of irradiated male DBM and *C. plutellae*. Control plots were in a separate location. Each field plot was 4.6 x 1.2 m (5.5 m^2). To ascertain the treatment effects, the number of larvae per 10 plants was recorded each week from each treatment.

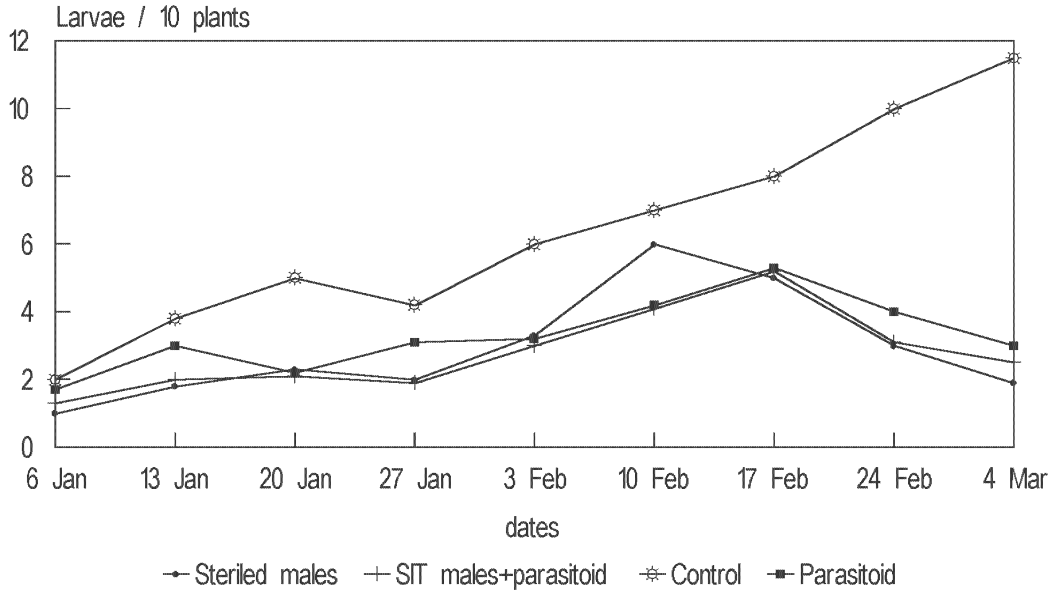

FIG.1. Effect of releasing irradiated diamondback moth (DBM), Plutella xylostella, males and the parasitoid, Cotesia plutellae on larval populations of feral DBM in cabbage.

3. RESULTS

The mean number of DBM larvae per 10 plants for each treatment is presented in Figure 1. The DBM larval population was higher in the control plots than in any of the treatment plots. Although the DBM larval populations were similar for all treatments and the control at the beginning of the experiment (January 6), the population in the control plots increased at a greater rate throughout the duration of the experiment. The differences between DBM larval populations of the control and the other treatments were greatest at the end of the experiment (March 4). The declining DBM larval population in the treatment plots beginning on February 17 may have been influenced by the in-field production of parasitoids (progeny of released parasitoids) and sterile F_1 DBM (progeny of irradiated, released DBM). No differences were observed between the different treatments. It is possible that the small size of the treatment plots allowed released moths and parasitoids to disperse from their release plot into other treatment plots. Nevertheless, these data indicate that the release of irradiated male DBM and the release of the parasitoid, *C. plutellae*, can reduce the seasonal increase of DBM populations in cabbage in Myanmar.

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APPENDIX 1

SCIENTIFIC PUBLICATIONS FROM RCM PARTICIPANTS RESULTING FROM WORK ASSOCIATED WITH THEIR RESEARCH PROJECT

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