

## **Flight Activity and Mating Behavior of Irradiated *Spodoptera litura* (Lepidoptera: Noctuidae) Males and Their F1 Progeny for Use of Inherited Sterility in Pest Management Approaches**

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# Flight activity and mating behavior of irradiated *Spodoptera litura* (Lepidoptera: Noctuidae) males and their F<sub>1</sub> progeny for use of inherited sterility in pest management approaches

Rakesh K. Seth\*, Zubeda Khan, Dev K. Rao and Mahtab Zarin

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## Abstract

The dose of radiation applied to the Oriental leafworm (also known as the common cutworm), *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) for use in inherited sterility (IS) sterility programs must not materially reduce the quality and competitiveness of the released males. To assess the quality of sub-sterilized male moths and their F<sub>1</sub> progeny, their flight and mating behaviors were investigated. Thus, parental (P) generation *S. litura* males that had been irradiated with either 100 or 130 Gy and their F<sub>1</sub> male descendants were subjected to flight assay tests to assess flight ability, orientation towards a pheromone source and mating behavior, i.e., mating performance using various sex ratios, sequential matings, and effect of female age. The orientation behavior of either P or F<sub>1</sub> males towards pheromone-baited traps was not different from that of non-irradiated males. Mating abilities of irradiated P generation males and their F<sub>1</sub> male progeny with non-irradiated females were studied using several sex ratios, but large differences were not found even when the sex ratio was increased from 1:1 to 5:1. A study on sperm use patterns revealed the precedence of sperm of the last male to mate, for example, when a non-irradiated female was sequentially mated with a non-irradiated P male, followed by an irradiated P male and finally by an F<sub>1</sub> male, as well as in all other possible sequences of such males. Mating success, remating propensity and fertility were significantly influenced by mating sequences that included irradiated males. Further, the age of the female at the first mating influenced fertility in matings with F<sub>1</sub> males, and mating success and remating propensity were reduced in several of sequences of matings involving non-irradiated and irradiated males and F<sub>1</sub> males, with reductions being more apparent when females were aged 5–6 d. The present study indicates that gamma doses of either 100 or 130 Gy are suitable for the suppression of *S. litura* using an IS approach.

Key Words: irradiation; inherited sterility; mating success; pheromone; sex ratio; sperm precedence

## Resumen

La dosis de radiación aplicada al gusano oriental de la hoja (también conocido como el gusano cortador común), *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) para su uso en programas de esterilidad hereditaria (SH) no debe reducir sustancialmente la calidad y la competitividad de los machos liberados. Para evaluar la calidad de las polillas machos sub-esterilizados y su progenie F<sub>1</sub>, se investigaron su comportamiento de vuelo y de apareamiento. Por lo tanto, los machos de *S. litura* de generación pariente (P) que habían sido irradiados con 100 o 130 Gy y los machos descendientes F<sub>1</sub> fueron sometidos a pruebas de ensayo de vuelo para evaluar la capacidad de vuelo, su orientación hacia un fuente de feromonas y su comportamiento de apareamiento, como su desempeño de apareamiento utilizando diversas ratios sexuales, apareamientos secuenciales y el efecto de la edad del sexo femenino. El comportamiento de orientación de los machos de generación P o F<sub>1</sub> hacia las trampas cebadas con feromonas no fue diferente de los machos no irradiados. Se estudió la habilidad de apareamiento de los machos irradiados de generación P y su progenie machos F<sub>1</sub> con hembras no irradiadas utilizando varios ratios sexuales, pero no se encontraron grandes diferencias, incluso cuando el ratio de los sexos se incrementó de 1:1 a 5:1. Un estudio sobre los patrones de uso de esperma reveló la precedencia de esperma del último macho que se apareó, por ejemplo, cuando una hembra no irradiada fue secuencialmente apareada con un macho P no irradiado, seguido de un macho P irradiado y finalmente por un macho F<sub>1</sub>, así como en todas las otras secuencias posibles de tales machos. El éxito de apareamiento, la propensión de re-aparearse y la fertilidad fueron influenciadas significativamente por las secuencias de apareamiento que incluyeron machos irradiados. Además, la edad de la hembra en el primer apareamiento influyó la fertilidad de los apareamientos con machos F<sub>1</sub>, y el éxito de apareamiento y la propensión de re-aparearse son reducidos en varias de las secuencias de apareamientos que involucran los machos no irradiados y machos irradiados F<sub>1</sub> con reducciones siendo más evidentes cuando las hembras tenían entre 5–6 días de edad. El presente estudio indica que las dosis gamma de 100 ó 130 Gy son adecuadas para la supresión de *S. litura* utilizando el enfoque de SH.

Palabras Clave: irradiación; esterilidad hereditaria; éxito de apareamiento; feromona; ratio de sexos; precedencia de esperma

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The Oriental leafworm or common cutworm, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) is a notorious polyphagous insect pest of more than 100 cultivated species of field, vegetables, fruit and ornamental crops and weeds throughout the tropical and temperate

Asia-Pacific regions (Ahmad et al. 2013). In India, *S. litura* has developed resistance against commonly used insecticides, creating a serious threat to the agricultural industry (Ramakrishnan et al. 1984; Armes et al. 1997; Rame Gowda 1999). In addition, the injudicious use of

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broad-spectrum insecticides has caused major problems in terms of residues in food, environmental contamination, outbreaks of secondary pests, and reductions in populations of beneficial insects. These developments combined with the mounting awareness and concern of the general public have increased demands for pest control methods that are both efficient and friendly to the environment (Vreysen et al. 2006).

The sterile insect technique (SIT) involves the mass-rearing, sterilization using ionizing radiation and the sequential release of the male sex in the target area to introduce an intolerable load of dominant lethal mutations into the zygotes of the target pest population. Lepidopteran insects are much more radio-resistant to induction of dominant lethals than most other orders of insects (e.g., Diptera), but the treatment of moths with a fully sterilizing dose significantly reduces their mating competitiveness in the field. This limitation has resulted in a modified approach called either the  $F_1$  sterility or inherited sterility (IS) technique that uses smaller irradiation doses that result in sub-sterile male parents to be released (Proverbs 1962; North 1975; Proverbs et al. 1978). The lower sub-sterilizing radiation dose used to induce  $F_1$  sterility better maintains the quality and competitiveness of the released insects as evidenced by improved dispersal after release, increased mating ability, and superior sperm competition. The effect of irradiation on behavioral characteristics of various lepidopteran pest species is well documented (Carpenter et al. 1997, 2005). The ability of sterile insects to fly and disperse after their release in the field is an essential attribute, because those insects that cannot fly to shelter or reach a food source, or find mating arenas are unfit for a control program that has an IS component. Reduced flight and dispersal capabilities and increased mortality due to rearing and/or irradiation have been well documented (Dame et al. 1969; Rajagopalan et al. 1973; Sharp 1976; Nelson & Milby 1980; Nakamori & Soemori 1981; Smith et al. 1981). Chemical communication is often involved in mating, feeding, and other key ecological interactions of insects (Matthews & Matthews 1978), and long-range attractants are very useful for assessing the distribution of wild and sterile insects, monitoring over-flooding ratios (Vreysen 2005), and evaluating specific aspects of sterile male quality (Calkins & Parker 2005). The sex pheromone of *S. litura* was found to be 9Z,11E, tetradecadienyl acetate and 9Z,12E, tetradecadienyl acetate, in a ratio of 9:1, and—when formulated as Spodolure™—was found to be a valuable tool in field studies (Nandgopal et al. 2005; Gedia et al. 2007).

Given that population suppression by the SIT/IS is basically a function of matings between sterile or sub-sterile males and wild females (McInnis et al. 1994), the ability of released males to compete for female mates is critical. The mating competitiveness of sterile or sub-sterile males is a function of their mating propensity and mating compatibility. Mating propensity of a male is defined as the tendency to locate a mate, copulate and inseminate, and is primarily of concern as a component of sterile insect quality (Calkins & Parker 2005). Mating propensity of a female is the tendency of the female to mate more than once. Mating compatibility is a relative measure of how readily the males and females of 2 populations of insects from different circumstances intercross with successful amphimixis. Mating compatibility in relation to the SIT/IS most often refers specifically to matings of sterile or sub-sterile males with wild females (FAO/IAEA/USDA 2003). In programs that release sterile or sub-sterile insects it is necessary to ensure that those insects are compatible with the target insect population (FAO 1992; Cayol et al. 2002), and Calkins & Parker (2005) described methods of assessing and quantifying mating compatibility.

Another important component for the SIT/IS is the level of remating of wild females (Lance & McInnis 2005; Whitten & Mahon 2005). Moreover, sperm precedence is considered a crucial parameter for ef-

ficient implementation of the SIT/IS against those lepidopteran species that normally mate more than once. Snow et al. (1970) found incomplete, last male precedence with extensive sperm mixing in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), and Martin et al. (1989) concluded that sperm use patterns are not necessarily fixed within a species. Carpenter (1992) also observed much variation in the sperm use pattern in female corn earworms, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) and suggested that the outcome of sperm competition within an individual female was strongly influenced by competition of all ejaculates involved.

Laboratory methods for assessing insect quality, such as flight ability, longevity, pupal weight, etc., are routinely used in tephritid fruit fly programs that have a SIT component, but the application of such methods has been quite limited in programs against lepidopteran pests (Calkins & Parker 2005). Understanding the expression of moth quality in relation to production and biological parameters in the rearing process in a hierarchy of linked assays, such as flight ability tests, wind tunnel flight performance tests, mating tests in laboratory cages, field cage tests, and release-recapture tests in the field, would lead to greatly improved routine operational use of appropriate assays to assess and manage insect quality. In this context, the present study was conducted to measure several key behavioral and physiological characteristics of *S. litura* irradiated with either 100 or 130 Gy in ways relevant to the IS technique. Thus irradiated P generation and  $F_1$  males were assessed with regard to flight ability, orientation to a sex pheromone source, success in mating with non-irradiated females of several ages, and precedence in sperm use in females mated with irradiated and non-irradiated males in different sequences.

## Materials and Methods

### INSECT REARING

*Spodoptera litura* were collected from fields of the Indian Agricultural Research Institute, New Delhi, and maintained in the laboratory on a chickpea-based semi synthetic diet (Seth & Sharma 2001) at  $27 \pm 1^\circ\text{C}$ ,  $75 \pm 5\%$  RH and a photoperiod of 12:12 h L:D.

### INSECT IRRADIATION

*Spodoptera litura* moths were irradiated using a  $^{60}\text{Co}$  Gamma Cell-5000 (Gamma -5000 irradiator, Board of Radiation and Isotope Technology, Mumbai) that was located in the radiobiological unit of the Institute of Nuclear Medicine and Allied Sciences (INMAS), Ministry of Defense, Delhi-110054. The dose rate of the Gamma Cell ranged between 1.71 and 1.98 KGy/h. Freshly emerged adults (0–1 d old) were selected and exposed to sub-sterilizing doses of either 100 or 130 Gy, i.e., the doses for the  $F_1$  sterility technique against *S. litura* proposed by Seth & Sehgal (1993) and Seth & Sharma (2001). Fricke dosimetry was performed on the Gamma Cell to establish the dose distribution and authenticate the validity of gamma dose administered at a given target dose rate.

### FLIGHT ASSAYS OF *SPODOPTERA LITURA*

The flight ability of  $F_1$  males in the presence of non-irradiated females was tested using 2 different approaches in a flight assay chamber consisting of a  $45 \times 45 \times 60$  cm perspex cage that had a black hollow cylinder (25.4 cm diam, 30.5 cm height) placed inside with its open end oriented upwards. In approach-I, mature male pupae

were placed on the floor inside the black hollow cylinder and female mature pupae were placed on the floor outside the cylinder within the flight assay chamber; whereas in approach-II, female mature pupae were placed inside the black cylinder and male mature pupae were placed outside the cylinder within the flight assay chamber. Male flight ability was tested using a male:female ratio of 2:1, because previously we had shown that with this ratio essentially all *S. litura* females would be mated and produce normal complements of eggs. Each replicate for testing flight capacity consisted of a group of 24  $F_1$  males and 12 non-irradiated (U) females, and each test was replicated 15 times. First, we assessed the emergence of male and female adults from the mature pupae, and then we assessed their flight ability. Those male and female insects that were found sitting on the side walls and ceiling of the chamber 12–14 h after emergence were also recorded as being able to fly. Finally, the flight ability of  $F_1$  male insects was assessed in terms of their successful performance in mating.

#### RESPONSES OF SUB-STERILE P AND $F_1$ MALES TO SEX PHEROMONE-BAITED TRAPS

The responses of sub-sterile P males and their  $F_1$  sons towards sex pheromone traps (Fero-T<sup>TM</sup>) were assessed in field cages (~ 274 × 183 × 213 cm). A cohort (22 to 30) of 0–1 d old non-irradiated male moths, with 50% of the moths marked with fluorescent dye on the thorax, were released in a field cage that contained a pheromone trap obtained from PCI (Pest Control India Pvt Ltd, India). The purpose of dye-marking was to distinguish moths belonging to different treatment cohorts. The pheromone trap consisted of a canopy mounted several cm above a funnel, which emptied into a transparent bag to hold captured moths. The traps were baited with a sex pheromone lure (Spodo-lure<sup>TM</sup>, Pest Control India Ltd.) that was fixed to the canopy over the funnel. The experiments were replicated 24 times. The numbers of males captured in the various replicates were observed and recorded after 48 h. The percent capture of the total number of males, the total males that had been marked and the total unmarked were computed.

#### MATING EFFECTIVENESS OF IRRADIATED P AND $F_1$ MALES AS REFLECTED BY FEMALE MATING PARAMETERS USING SEVERAL SEX RATIOS

Mating effectiveness of P and  $F_1$  males when paired with non-irradiated females in several sex ratios was evaluated in terms of the average mating frequency, mating success and mating propensity of the females. An indication of the inadequacy of some components of the ejaculate of the treated male in meeting the needs of the female is the extent to which mating frequency and percent remating propensity are numerically greater and the extent to which percent mating success is numerically smaller than in control matings. Both irradiated (I) *S. litura* males and their  $F_1$  sons were crossed with non-irradiated females using several male:female ratios (1:1, 2:1 and 5:1), and mating frequencies, mating success, and re-mating tendencies of the females were assessed at each ratio during a period of 72 h. Different sizes of cages were used for the various ratios, i.e., (i) 20 × 20 × 20 cm cage for evaluating 12 to 15 pairs in a 1:1 male:female ratio, (ii) 20 × 20 × 25 cm cage for evaluating 12 to 15 females paired in a 2:1 male:female ratio and (iii) 25 × 25 × 30 cm cage for evaluating 12 to 15 females paired in a 5:1 male:female ratio. The size of the cage was selected to providing at least 200 cm<sup>3</sup> per insect.

Mating effectiveness was studied by pairing 0–1 d old virgin moths in the following combinations: [i] non-irradiated (U) females × non-

irradiated (U) males, [ii] U females × I males, which were irradiated with either 100 or 130 Gy as 0–24 h old adult P moths; likewise [iii]  $F_1$  moths derived from the cross, U females × I males and their progeny were crossed in 3 combinations (a)  $U♀ \times F_1♂$ , (b)  $F_1♀ \times U♂$  and (c)  $F_1♀ \times F_1♂$ . Mating frequency per female was calculated by dividing the total number of spermatophores found in the bursa copulatrix of mated females by the number of mated females. Percent of mating success—i.e., percent of mated females out of the total number of copulating pairs of moths—was calculated by dividing the number of females with one or more spermatophores by the total number of females and multiplying by 100. Percent remating propensity—i.e., the percent of females with a tendency to remate—was calculated in 2 different ways. In the first way we computed the percent that had mated 2 or 3 times—as indicated by the presence in each bursa copulatrix of 2 or more spermatophores—out of the total pool of females, which included some without a spermatophore in the bursa copulatrix. In the second way, we computed the percent that had mated 2 or 3 times out of the total that have mated at least one time, i.e., females without a spermatophore were excluded from the total.

#### SEQUENTIAL MATING FOR EVALUATION OF SPERM USE PATTERN AND REPRODUCTIVE SUCCESS

Multiple mating could affect fertility. Therefore, the process of sequential matings of females with sub-sterilized male moths and non-irradiated moths was carried out to assess its effect on the pattern of sperm utilization. Sequential matings of virgin females with  $U♂$ ,  $I♂$  or  $F_1♂$  males were studied in the following combinations: (i)  $U♀ \times U♂ \times U♂$ , (ii)  $U♀ \times U♂ \times I♂$ , (iii)  $U♀ \times I♂ \times U♂$ , (iv)  $U♀ \times I♂ \times I♂$ , (v)  $U♀ \times U♂ \times F_1♂$ , (vi)  $U♀ \times F_1♂ \times U♂$  and (vii)  $U♀ \times F_1♂ \times F_1♂$ . Zero to 1 d old female moths were used for the first mating, and freshly emerged male moths were offered to the mated females again during 24 to 36 h after the first mating. In each cross reproductive parameters such as mating success, remating propensity, fertility or egg hatch after the first and second mating were assessed. Twelve freshly emerged females and 24 males were used per replicate. The success of the second mating was verified by examining the female moths for the presence of a second spermatophore in the bursa copulatrix.

#### EFFECT OF FEMALE AGE ON MATING AND REMATING SUCCESS WITH IRRADIATED P MALES OR $F_1$ MALES

The effect of female age during the first mating on egg hatch, mating success and remating propensity when paired with either irradiated P males or  $F_1$  males was assessed by allowing females of 3 different age groups (0–1 d, 2–3 d, and 5–6 d old) to mate with male moths for a period of 72 h. For each female age group, 8 freshly emerged male moths were paired with 16 female moths, and mating success and remating propensity were assessed. Each test was replicated 12 to 15 times. The effect of age of the female moths when mated to either irradiated P or  $F_1$  males was assessed with respect to their receptivity (mating success and remating propensity), sperm transfer and fertility (egg hatch).

#### STATISTICAL ANALYSIS

The above experiments were usually replicated 15 times and any variation in replicate number has been specified in the text. The data were subjected to an ANOVA. Percent data was arcsine  $\sqrt{x}$  transformed before being subjected to the ANOVA, but data shown in tables are back transformed.  $P \leq 0.05$  level was considered to be significant. LSD

posttest was used to determine significant differences among the various treatments (Snedecor & Cochran 1989).

## Results

### EFFECT OF IRRADIATION OF THE P GENERATION ON FLIGHT ABILITY OF F<sub>1</sub> MALE MOTHS

Flight Ability of F<sub>1</sub> Males assessed with the Approach-I Assay [♂ In: ♀ Out]

Flight behavior is an important attribute of F<sub>1</sub> male moths in programs that have an IS component. The present study showed that the percent emergence of F<sub>1</sub> males derived from 100 Gy-treated male parents was not significantly different from that of non-irradiated control males. However, emergence of F<sub>1</sub> male moths derived from 130 Gy-treated male parents was reduced by 12% in comparison with non-irradiated control moths (83.6%) ( $F = 3.40$ ;  $df = 2,42$ ;  $P < 0.05$ ) (Table 1). Nevertheless, the percent of emerged F<sub>1</sub> male moths that engaged in flight and that were able to fly out of the black cylinder in the center of the flight assay chamber was not influenced by irradiation ( $F = 3.12$ ;  $df = 2,42$ ;  $P > 0.05$ ). The percent of F<sub>1</sub> male moths landing on the ceiling and side walls of the chamber was similar for the 100 Gy-treated P males and the non-irradiated control group, whereas it was lower for the 130 Gy-treated males in comparison with the non-irradiated control group (82.1%) ( $F = 3.89$ ;  $df = 2,42$ ;  $P < 0.05$ ). Further, the percent non-irradiated female adults landing on the ceiling and side walls was similar for the 100 and 130 Gy-treatment group and the non-irradiated control group (72.7%). The percent mating success of these F<sub>1</sub> male fliers was slightly reduced (4.2%) in the 100 Gy-treatment group (81.6%), followed by a 17.4% reduction in mating success in the 130 Gy-treatment group in comparison with the non-irradiated control moths (85.8%) ( $F = 3.47$ ;  $df = 2,42$ ;  $P < 0.05$ ) (Table 1).

Flight Ability of F<sub>1</sub> Males assessed with the Approach-II Assay [♀ In: ♂ Out]

Percent emergence of F<sub>1</sub> male moths was similar for the 100, 130 Gy and non-irradiated groups (Table 2). Likewise, there was no difference in percent of fliers of F<sub>1</sub> males that were descendants from male parents treated with either 100 or 130 Gy in comparison with the non-irradiated control. A total of 81.7% non-irradiated male moths

and 79.6% female moths showed some ability to fly as indicated by their presence on the side walls and ceiling of the flight assay chamber. Likewise, the percent of F<sub>1</sub> male moths—descendants from male parents treated with either 100 or 130 Gy and non-irradiated female moths—that landed on the side-walls and ceiling of the chamber were similar. The mating success of F<sub>1</sub> male moths, descendants from male parents treated with 100 Gy was similar to that of the non-irradiated control group, but was reduced by 16% in the 130 Gy-treatment group in comparison with the non-irradiated control group (83.4%) ( $F = 3.78$ ;  $df = 2,42$ ;  $P < 0.05$ ) (Table 2).

### RESPONSES OF SUB-STERILE P AND F<sub>1</sub> MALES TO SEX PHEROMONE-BAITED TRAPS

The total percent of non-irradiated male moths trapped was 73.5% with no significant differences between unmarked non-irradiated males and marked non-irradiated males; hence, the fluorescent dye per se did not affect the response to the sex pheromone-baited trap. The percent of total moths (marked and unmarked) collected in the pheromone traps were not significantly different between the 100, 130 Gy and the F<sub>1</sub> (descendants from 100 Gy-treated P males) treatment groups in comparison with the non-irradiated control group. On the other hand, there was a slight but significant reduction in the total number of F<sub>1</sub> (descendants from 130 Gy-treated P males) moths collected in the pheromone traps ( $F = 5.92$ ;  $df = 4,115$ ;  $P < 0.05$ ) (Table 3).

### MATING EFFECTIVENESS OF IRRADIATED P AND F<sub>1</sub> MALES AS REFLECTED BY FEMALE MATING PARAMETERS USING SEVERAL SEX RATIOS

The over-flooding ratio of released sterile to wild fertile insects is considered to be one of the major determinants of adequate infusion of sterility into the wild insect pest population; and such infusion also depends on a high degree of mating effectiveness of released males, i.e., in transferring an adequate complement of sperm and accessory gland secretions. Therefore, the mating effectiveness of irradiated P males and their F<sub>1</sub> male progeny was evaluated based on certain mating parameters of the non-irradiated females in the crosses using 3 male:female ratios (1:1, 2:1 and 5:1). An indication of the inadequacy of some components of the ejaculate of the treated male in meeting the needs of the female is the extent to which mating frequency and

**Table 1.** Emergence and flight ability of F<sub>1</sub> male *Spodoptera litura*<sup>1</sup> and their success in mating with non-irradiated P females based on the approach-I flight ability assay in which mature male pupae were placed on the floor inside of the vertical hollow black cylinder (pipe) within the flight assay chamber and mature female pupae on the floor outside of the pipe, i.e., ♂ in : ♀ out.

Irradiation dose given to fathers of F <sub>1</sub> males	% Emergence of F <sub>1</sub> male moths	% Fliers of F <sub>1</sub> male moths (12 to 14 h after emergence)	% F <sub>1</sub> ♂ & ♀ insects <sup>2</sup> recorded on side-walls and ceiling of the cage		% Mating success <sup>3</sup>
			F <sub>1</sub> Males	U Females	
0 Gy	83.6 ± 4.1a	93.3 ± 3.5a	82.1 ± 4.5a	72.7 ± 3.6a	85.8 ± 4.2a
100 Gy	80.1 ± 3.9ab	88.9 ± 3.8a	77.5 ± 3.5ab	70.7 ± 4.9a	81.6 ± 4.9ab
130 Gy	73.5 ± 3.1b	87.4 ± 4.7a	69.9 ± 2.4b	70.2 ± 3.5a	70.9 ± 3.6b
F-value	$F = 3.40^*$	$F = 3.12$	$F = 3.89^*$	$F = 2.20$	$F = 3.47^*$
	$df = 2,42$	$df = 2,42$	$df = 2,42$	$df = 2,42$	$df = 2,42$

<sup>1</sup>Derived from irradiated male parents; each replicate used a group of 24 F<sub>1</sub> males and 12 non-irradiated (U) females. Flight ability was tested using approach-I assay with male insects kept as mature pupae placed on the floor of a vertical black pipe segment (25.4 cm diam and 30.5 cm height) with an open upper end that was placed in the middle of the 45 × 45 × 60 cm perspex flight assay chamber. Female mature pupae were placed in the chamber outside the black cylinder.

<sup>2</sup>♂ & ♀ insects that had landed on the side walls or ceiling of the cage 12 to 14 h after emergence

<sup>3</sup>Mating success was recorded 36 to 48 h after emergence.

Means ± SE followed by the same letter in a column are not significantly different at  $P < 0.05$  (ANOVA followed by LSD post-test); percent data were arcsine transformed before ANOVA, but data in table are back transformations;  $n = 15$ ; \*Significance at  $P \leq 0.05$ .

**Table 2.** Emergence and flight ability of  $F_1$  male *Spodoptera litura*<sup>1</sup> and their success in mating with non-irradiated P females based on the approach-II flight ability assay with mature female pupae placed on the floor inside of the vertical black pipe segment inside a flight assay chamber and mature male pupae outside the pipe, i.e., ♀ in : ♂ out.

Irradiation dose given to fathers of $F_1$ males	% Emergence of $F_1$ male moths	% Fliers of $F_1$ male moths (12 to 14 h after emergence)	% $F_1$ ♂ & ♀ insects <sup>2</sup> on side-walls and ceiling of the cage		% Mating success <sup>3</sup>
			$F_1$ Males	U Females	
0 Gy	86.7 ± 3.9a	89.7 ± 4.6a	81.7 ± 4.1a	79.6 ± 4.9a	83.4 ± 3.4a
100 Gy	82.9 ± 4.9a	90.7 ± 6.7a	80.7 ± 6.1a	75.3 ± 3.4a	77.1 ± 3.8ab
130 Gy	89.7 ± 5.2a	94.6 ± 4.8a	77.9 ± 5.6a	68.2 ± 3.9a	69.6 ± 2.9b
F-value	F = 2.82	F = 3.19	F = 2.87	F = 3.21	F = 3.78*
	df = 2,42	df = 2,42	df = 2,42	df = 2,42	df = 2,42

<sup>1</sup> Derived from irradiated male parent; each replicate used a group of 24  $F_1$  males and 12 non-irradiated (U) females. Flight ability was tested using approach-II assay (female insects were kept as mature pupae on the floor inside the black cylinder (25.4 cm diam; 30.5 cm height) placed in the middle of the perspex flight assay chamber (45 × 45 × 60 cm), along with male mature pupae placed outside the black cylinder.

<sup>2</sup> ♂ & ♀ insects that had landed on the side walls or ceiling of the cage 12 to 14 h after emergence.

<sup>3</sup> Mating success was recorded 36 to 48 h after emergence.

Means ± SE followed by the same letter in a column are not significantly different at  $P < 0.05$  (ANOVA followed by LSD posttest); percent data were arcsine transformed before ANOVA, but data in table are back transformations;  $n = 15$ ; \*significance at  $P \leq 0.05$

percent remating propensity are numerically greater and the extent to which percent mating success is numerically smaller than in control matings.

#### Mating Effectiveness of Irradiated Males and their $F_1$ Sons in a 1:1 Male:Female Ratio

Using an equal number of male and female moths, the average mating frequency—i.e., number of matings per male of 100 Gy-treated (sub-sterilized) males with non-irradiated P females was  $1.58 \pm 0.06$ , which was not significantly different from the non-irradiated control group in which the males has a mating frequency of  $1.65 \pm 0.04$  (Table 4). The mating frequency was  $1.72 \pm 0.04$  in case of 130 Gy-treated (sub-sterilized) males with non-irradiated P females, which was not significantly different from the control group. The average mating frequency of  $F_1$  males (descendants of P males treated with 100 Gy) with non-irradiated P females and that of  $F_1$  females (descendants from P males irradiated with 100 Gy) and mated with non-irradiated P males were similar to those of the control groups, whereas the mating frequency of the  $F_1$  sons of 130 Gy-treated fathers was increased to  $1.81 \pm 0.04$  in the  $U \text{♀} \times F_1 \text{♂}$  cross, and that of the  $F_1$  daughters of 130 Gy-treated fathers was increased to  $1.74 \pm 0.05$  in the  $F_1 \text{♀} \times U \text{♂}$  cross. Moreover, the mating frequencies of females were significantly enhanced in cases of  $F_1$  self-crosses ( $F_1 \text{♀} \times F_1 \text{♂}$ ) in both the 100 and 130 Gy-treatment groups ( $F = 2.72$ ;  $df = 8,711$ ;  $P < 0.05$ ) (Table 4).

Percent of mating success in crosses involving either the irradiated fathers (P) (or the  $F_1$  sons from 100 and 130 Gy-irradiated P males) was in most cases reduced (sometimes significantly, but sometimes not) with the greatest reduction in the  $F_1$  self-cross of the 130 Gy-treatment group.  $F_1$  male descendants from 100 Gy-treated P males when outcrossed with non-irradiated females showed 78.5% mating success, which was not significantly different from that of the non-irradiated control (81.9%). In the 130 Gy-treatment group, the outcrosses of  $F_1$  males and females with their non-irradiated counterparts showed significant reductions in mating success (74.3 and 68.6%, respectively) in comparison with the non-irradiated control ( $F = 3.21$ ;  $df = 8,126$ ;  $P < 0.05$ ).

The percent remating propensity of non-irradiated females crossed with 100 Gy-irradiated males was not significantly different from the non-irradiated control. However in the corresponding cross with 130 Gy-irradiated males, the percent remating propensity was increased significantly in comparison both with the non-irradiated control and the 100 Gy-treatment group. Likewise in the 100 Gy-treatment group, remating was increased in crosses of non-irradiated females with  $F_1$  males and in the  $F_1$  self-cross in comparison with the non-irradiated control, but the difference was significant only for the self-cross. Similarly in the 130 Gy-treatment group, the percent remating propensity of females crosses involving  $F_1$  individuals was significantly increased in comparison with the non-irradiated control ( $F = 4.45$ ;  $df = 8,126$ ;  $P < 0.05$ ) (Table 4).

**Table 3.** Effect of gamma irradiation on the percent of capture of either *Spodoptera litura* P generation or  $F_1$  males in a sex pheromone-baited trap (Fero-t<sup>TM</sup>) in a field cage. P generation males were either non-irradiated (0 Gy) or had been irradiated with either 100 or 130 Gy.

Nature of released male moths	% Total insects trapped		% Unmarked male moths trapped in pheromone trap		% Marked male moths trapped in pheromone trap	
	Total insects trapped		moths trapped in pheromone trap		moths trapped in pheromone trap	
Unmarked (0 Gy) vs. marked (0 Gy) male moths	73.5 ± 3.5a		50.1 ± 2.54aA		49.9 ± 2.5aA	
Unmarked (0 Gy) vs. marked P (100 Gy) male moths	71.9 ± 3.1ab		49.2 ± 2.6aA		51.3 ± 2.9aA	
Unmarked (0 Gy) vs. marked P (130 Gy) male moths	65.2 ± 2.9 ab		52.8 ± 1.63aA		47.1 ± 1.83aA	
Unmarked (0 Gy) vs. marked $F_1$ (100 Gy) male moths	68.4 ± 3.2ab		52.9 ± 2.9aA		46.7 ± 2.8aA	
Unmarked (0 Gy) vs. marked $F_1$ (130 Gy) male moths	63.3 ± 2.6b		54.6 ± 3.6aA		44.9 ± 1.6aB	
F-value	F = 5.92*		F = 2.24		F = 2.27	
	df = 4,115		df = 4,115		df = 4,115	

Fifty percent of male adults were marked on the thorax with yellow fluorescent dye; 24 to 30 moths were released in a ~ 274 × 183 × 213 cm cage and the number of males captured in a pheromone trap was observed after 48 h. Marked moths were P generation males that were either non-irradiated (0 Gy) or had been irradiated with either 100 or 130 Gy, or the corresponding  $F_1$  males. Means ± SE followed by same small letter within a column are not significantly different at  $P < 0.05$  level (ANOVA followed by LSD posttest). Percent data was arcsine transformed before ANOVA, but data in the table are back transformed. Means ± SE followed by same capital letter between % unmarked moths and % marked moths within each release regimen are not significantly different at  $P < 0.05$  level (Student's t-test);  $n = 24$ ; \*significance at  $P \leq 0.05$ .

**Table 4.** Mating frequency, mating success and remating propensity of non-irradiated (U) female *Spodoptera litura* crossed with irradiated (I) P males treated with either 100 or 130 Gy, and in various crosses involving their F<sub>1</sub> sons and/or daughters. All crosses involved a 1:1 male:female ratio.

Dose of irradiation (Gy) given to P generation males	Mating cross	Average mating frequency of females	% Mating success of females	% Remating propensity <sup>1</sup> of females
0 Gy	U ♀ x U ♂	1.65 ± 0.04ab	81.9 ± 3.1a	43.8 ± 2.6ab (52.0 ± 2.5a)
100 Gy	U ♀ x I ♂	1.58 ± 0.06a	79.7 ± 4.1ab	40.3 ± 2.9a (52.6 ± 2.2a)
130 Gy	U ♀ x I ♂	1.72 ± 0.04bcd	76.7 ± 2.2bcd	51.2 ± 2.6c (66.7 ± 2.7cd)
F <sub>1</sub> 100 Gy	U ♀ x F <sub>1</sub> ♂	1.69 ± 0.05abc	78.5 ± 2.5abc	49.2 ± 3.2bc (62.7 ± 2.9bc)
	F <sub>1</sub> ♀ x U ♂	1.63 ± 0.08ab	75.6 ± 3.1bcd	40.8 ± 2.9a (53.9 ± 2.4ab)
	F <sub>1</sub> ♀ x F <sub>1</sub> ♂	1.80 ± 0.06cd	71.2 ± 2.6cde	51.4 ± 2.6c (72.2 ± 3.2ef)
F <sub>1</sub> 130 Gy	U ♀ x F <sub>1</sub> ♂	1.81 ± 0.04cd	74.3 ± 2.7bcd	53.3 ± 2.9c (71.1 ± 3.4ef)
	F <sub>1</sub> ♀ x U ♂	1.74 ± 0.05bcd	68.6 ± 2.5de	47.3 ± 2.6bc (68.9 ± 3.1de)
	F <sub>1</sub> ♀ x F <sub>1</sub> ♂ ×	1.85 ± 0.04d	64.5 ± 2.6e	49.2 ± 2.2bc (76.6 ± 3.9f)
F value		F = 2.72* df = 8,711	F = 3.21* df = 8,126	F = 2.94* df = 8,126 (F = 4.45* df = 8,126)

Means ± SE followed by same letter within a column are not significantly different at  $P < 0.05$  (ANOVA followed by LSD posttest); percent data were arcsine transformed before ANOVA, but data in table were back transformed. Evaluation of 12 to 15 pairs of freshly emerged adults constituted each replicate for assessing mating success. i.e., percent of females that mated—i.e., based on the presence of 1 or more spermatophores in the bursa copulatrix—out of the total that had copulated.

<sup>1</sup>Percent remating was computed in 2 different ways. In the first way we computed the percent that had mated 2 or 3 times—as indicated by the presence in each bursa copulatrix of 2 or 3 spermatophores—out of the total pool of females; this pool included some that had not mated, i.e., no spermatophore was found in their bursae copulatrix. In the second way—shown in brackets below the values for the first way—we computed the percent that had mated 2 or 3 times out of the total that had mated at least 1 time, i.e., females without at least 1 spermatophore were excluded from the total.

For ascertaining percent mating success,  $n = 15$ , and  $n = 80$  for determining mating frequency; \*significance at  $P \leq 0.05$ .

### Mating Effectiveness of Irradiated Males and their F<sub>1</sub> Sons in a 2:1 Male:Female Ratio

Since the results obtained with the 2:1 sex ratio were either almost identical or only marginally different from those obtained with the 1:1 sex ratio, the data are not shown. However the mating frequency with 100 Gy-treated males was numerically greater with the 2:1 ratio, and both percent mating success and percent mating propensity were marginally greater in almost all cases with the 2:1 ratio than with the 1:1 ratio.

### Mating Effectiveness of Irradiated Males and their F<sub>1</sub> Sons in a 5:1 Male:Female Ratio

The mating frequency of non-irradiated females crossed with 100 Gy-treated sub-sterilized males was not significantly different from that of the non-irradiated control (1.61 ± 0.09 vs 1.71 ± 0.05, respectively), whereas in the corresponding cross with 130 Gy-treated males, the mating frequency of females was significantly increased to 1.81 ± 0.09 in comparison with the 100 Gy-treated males. The mating frequencies of non-irradiated females crossed with the F<sub>1</sub> sons of P males irradiated with either 100 or 130 Gy were very similar to those of females that mated with the corresponding P males, and the results did not differ significantly from the control. In contrast the mating frequencies were significantly greater in the self-cross of the F<sub>1</sub> of the 100 Gy-treatment group and in all self-crosses of the 130 Gy-treatment group than in the non-irradiated control ( $F = 2.13$ ;  $df = 8,711$ ;  $P < 0.05$ ) (Table 5).

Percent of mating success in crosses involving irradiated P males and in crosses involving their F<sub>1</sub> progeny of the 100 and 130 Gy-treatment groups were numerically but not significantly less than in the

control, except in the crosses F<sub>1</sub> ♀ × U ♂ and F<sub>1</sub> ♀ × F<sub>1</sub> ♂ of the 130 Gy-treatment group ( $F = 2.81$ ;  $df = 8,126$ ;  $P < 0.05$ ) (Table 5).

Percent remating propensity of non-irradiated females mated with either 100 Gy-treated P males or their F<sub>1</sub> sons did not appear to be enhanced regardless of the method used in computing it. However when non-mated females were excluded from the computation, matings of non-irradiated females with either 130 Gy-treated P or their F<sub>1</sub> sons showed significantly enhanced percent remating propensities, i.e., 70.8 and 67.8%, respectively. Also when F<sub>1</sub> males in the 100 and 130 Gy-treatment groups were crossed with corresponding F<sub>1</sub> females, remating was significantly increased in comparison with the non-irradiated controls, and this effect was especially great in the 130 Gy F<sub>1</sub> self-cross, i.e., 67.5 and 78.3%, respectively ( $F = 4.45$ ;  $df = 8,126$ ;  $P < 0.05$ ) (Table 5).

### VARIOUS COMBINATIONS OF SEQUENTIAL MATINGS OF NON-IRRADIATED *S. LITURA* FEMALES WITH NON-IRRADIATED AND IRRADIATED P MALES OR THEIR F<sub>1</sub> SONS

The effect of sub-sterilizing radiation on sperm priority and mixing patterns in *S. litura* was studied by mating non-irradiated females with either 100 or 130 Gy-irradiated P males and their F<sub>1</sub> progeny in various sequences with untreated males.

#### Effect of Sequential Mating on Fertility and Sperm Use Pattern

When non-irradiated females each mated 2 times with a non-irradiated male (control sequence) slightly more than 90% of their eggs hatched after either mating. In mating sequences in which matings with 100 Gy sub-sterilized P males was followed by second matings

**Table 5.** Mating frequency, mating success and remating propensity of non-irradiated (U) female *Spodoptera litura* crossed with irradiated (I) P males treated with either 100 or 130 Gy, and in various crosses involving their F<sub>1</sub> sons and/or daughters. All crosses involved a 5:1 male:female ratio.

Dose of irradiation (Gy) given to P generation males	Mating cross	Average mating frequency of females	% Mating success of females	% Remating propensity <sup>1</sup> of females
0 Gy	U ♀ × U ♂	1.71 ± 0.05ab	87.9 ± 3.4a	51.2 ± 3.4ab (61.4 ± 1.5ab)
100 Gy	U ♀ × I ♂	1.61 ± 0.09a	85.9 ± 3.6ab	49.4 ± 3.2a (59.6 ± 3.4a)
130 Gy	U ♀ × I ♂	1.81 ± 0.09b	83.3 ± 4.8abc	58.3 ± 3.1b (70.8 ± 3.6bc)
F <sub>1</sub> 100 Gy	U ♀ × F <sub>1</sub> ♂	1.67 ± 0.10ab	82.3 ± 3.9abc	49.8 ± 4.2ab (62.1 ± 3.7ab)
	F <sub>1</sub> ♀ × U ♂	1.80 ± 0.10ab	79.9 ± 4.8abc	58.4 ± 3.9b (65.6 ± 4.1ab)
	F <sub>1</sub> ♀ × F <sub>1</sub> ♂	1.88 ± 0.09b	79.6 ± 3.6abc	52.1 ± 3.9ab (67.5 ± 5.9bc)
F <sub>1</sub> 130 Gy	U ♀ × F <sub>1</sub> ♂	1.81 ± 0.08b	78.9 ± 4.3abc	56.2 ± 3.9ab (67.8 ± 5.6bc)
	F <sub>1</sub> ♀ × U ♂	1.88 ± 0.10b	77.8 ± 4.8bc	49.9 ± 2.6ab (62.3 ± 3.5ab)
	F <sub>1</sub> ♀ × F <sub>1</sub> ♂	1.89 ± 0.06b	70.8 ± 3.1c	52.3 ± 3.4ab (78.9 ± 2.9c)
F value		F = 2.13* df = 8,711	F = 2.81* df = 8,126	F = 2.37* df = 8,126 (F = 3.69* df = 8,126)

Means ± SE followed by same letter within a column are not significantly different at  $P < 0.05$  (ANOVA followed by LSD posttest); percent data were arcsine transformed before ANOVA, but data in table were back transformed. Evaluation of freshly emerged adults (60 ♂ vs 12 ♀) constituted each replicate for assessing mating success based on the presence of 1 or more spermatophores each in the bursae copulatrices of females that had paired.

<sup>1</sup>Percent remating of females was computed in 2 different ways. In the first way we computed the percent that had mated 2 or 3 times—as indicated by the presence in each bursa copulatrix of 2 or 3 spermatophores—out of the total pool of females; this pool included some that had not mated, i.e., no spermatophore was found in their bursae copulatrices. In the second way—shown in brackets below the values for the first way—we computed the percent that had mated 2 or 3 times out of the total that had mated at least 1 time, i.e., females without at least 1 spermatophore were excluded from the total.

For ascertaining percent mating success,  $n = 15$ , and  $n = 80$  for determining mating frequency; \*significance at  $P \leq 0.05$

with non-irradiated P males, the percent egg hatch was similar to that of the non-irradiated control; but when the second matings were with irradiated males, the percent egg hatch was about the same as after first matings with irradiated males. Therefore in sequential matings of non-irradiated females with non-irradiated and 100 Gy sub-sterilized P males, sperm precedence of the last mating was strongly evident with little evidence of sperm mixing, irrespective whether the last mating was with a non-irradiated or a 100 Gy-irradiated male (Table 6). Similar observations were made with crosses using 130 Gy-irradiated males in the same mating sequences with non-irradiated males (Table 6).

In the mating sequences involving F<sub>1</sub> male descendants of 100 Gy sub-sterilized P males and non-irradiated P males, the percent egg hatch was similar to that of the control when a non-irradiated female mated with a non-irradiated P male even when the female had already been mated with an F<sub>1</sub> male; but when the second mating was with an F<sub>1</sub> male, the average percent egg hatch was about the same as after the first mating with an F<sub>1</sub> male, i.e., 36.9 and 38.3% after the first and second mating, respectively. Sequential matings of non-irradiated females with non-irradiated and F<sub>1</sub> males of the 100 and 130 Gy-treatment groups showed strong levels of sperm precedence with little variability, e.g., first matings of non-irradiated females with non-irradiated P males followed by second matings with F<sub>1</sub> males—descendants of P males treated with 100 Gy—resulted in a reduction in egg hatch from 90.4% after the first matings to 38.9% after the second matings. Conversely, first matings of non-irradiated females with F<sub>1</sub> males—descendants of P males treated with 100 Gy—followed by second matings with non-irradiated males, resulted in increased egg hatch from 35.7% after the first mating to 88.9% after the second mating. Similar results were obtained in mating sequences with the F<sub>1</sub> male descendants of P males irradiated with 130 Gy (Table 6).

### Effect of Sequential Mating on Mating Success

In sequential matings involving 100 Gy-irradiated P male moths, mating success did not differ significantly from that in the non-irradiated control (91.1%). Percent mating success using 130 Gy-irradiated P males was significantly reduced by 11.3 and 16.9% in the mating sequences, U ♀ × U ♂ × I ♂ and U ♀ × I ♂ × I ♂, respectively; and reduced, but not significantly, by 6.9% in the mating combination U ♀ × I ♂ × U ♂ in comparison with the non-irradiated control. Mating successes in the crosses using F<sub>1</sub> males of the 100 Gy-treatment group were similar to that of the non-irradiated control, except in the mating sequence U ♀ × F<sub>1</sub> ♂ × F<sub>1</sub> ♂, where mating success was significantly reduced to 76.1%, i.e., a 16.4% reduction in comparison with the non-irradiated control. Mating successes of the F<sub>1</sub> males of the 130 Gy-treatment group were significantly reduced by 15.9, 13.8 and 19.8%, respectively, in the 3 mating sequences in comparison with the non-irradiated control ( $F = 11.7$  df = 12,182;  $P < 0.05$ ) (Table 6).

### Effect of Sequential Mating on Remating Success

Remating success of non-irradiated females in the non-irradiated control experiment was 52.6%—computed on the basis of the number of females that had mated at least one time—whereas in the sequential matings with 100 or 130 Gy-irradiated, and non-irradiated P males, the remating success of non-irradiated females was significantly increased in all mating combinations (Table 6).

Remating propensities of F<sub>1</sub> progeny were enhanced in all mating sequences, but to a lesser extent than the remating propensities of their respective 100 and 130 Gy-irradiated P ancestors. The ob-

**Table 6.** Fertility, mating success and remating propensity in sequential matings of non-irradiated (U) female *Spodoptera litura* with non-irradiated and irradiated male (I♂) or F<sub>1</sub>♂ (progeny of irradiated male parent).

Dose of irradiation (Gy) given to P generation males	Mating Sequence <sup>1</sup>	% Viability of eggs oviposited after 1st mating	% Viability of eggs oviposited after 2nd mating	% Mating success	% Remating success <sup>2</sup>
0 Gy	U♀ × U♂ × U♂	90.7 ± 2.3a	90.1 ± 2.1a	91.1 ± 2.4a	52.6 ± 3.1a
100 Gy	U♀ × U♂ × I♂	88.9 ± 1.9a	52.2 ± 3.2b	84.6 ± 5.2ab	64.1 ± 2.7bc
	U♀ × I♂ × U♂	49.5 ± 1.3b	82.9 ± 4.8a	87.3 ± 4.9a	69.2 ± 3.7cd
	U♀ × I♂ × I♂	48.6 ± 1.7b	47.2 ± 2.5b	82.9 ± 4.4ab	65.3 ± 2.6c
130 Gy	U♀ × U♂ × I♂	90.5 ± 3.9a	45.9 ± 2.6bc	80.8 ± 4.2bc	69.6 ± 1.5cd
	U♀ × I♂ × U♂	45.9 ± 1.6c	82.1 ± 4.8a	84.8 ± 4.1ab	73.7 ± 2.1d
	U♀ × I♂ × I♂	40.5 ± 1.4c	41.4 ± 2.3c	75.7 ± 3.4bcd	71.5 ± 4.2cd
F <sub>1</sub> 100 Gy	U♀ × U♂ × F <sub>1</sub> ♂	90.4 ± 2.2a	38.9 ± 1.9c	85.5 ± 4.6ab	51.2 ± 2.7a
	U♀ × F <sub>1</sub> ♂ × U♂	35.7 ± 1.5d	88.9 ± 3.5a	82.6 ± 4.4ab	65.6 ± 2.9c
	U♀ × F <sub>1</sub> ♂ × F <sub>1</sub> ♂	36.9 ± 2.2d	38.3 ± 1.4c	76.1 ± 5.4bcd	68.3 ± 4.2cd
F <sub>1</sub> 130 Gy	U♀ × U♂ × F <sub>1</sub> ♂	90.3 ± 2.9a	24.4 ± 1.6d	75.2 ± 3.4cd	56.8 ± 4.8ab
	U♀ × F <sub>1</sub> ♂ × U♂	21.1 ± 1.2e	84.9 ± 3.2a	77.3 ± 3.9bcd	67.2 ± 2.2cd
	U♀ × F <sub>1</sub> ♂ × F <sub>1</sub> ♂	18.4 ± 1.8e	21.5 ± 1.4d	71.7 ± 3.6d	68.9 ± 3.2cd
F value		F = 161.7* df = 12,182	F = 149.3* df = 12,182	F = 11.7* df = 12,182	F = 9.42* df = 12,182

<sup>1</sup>0–1 d old moths were used for the first mating, and a 2nd mating opportunity was offered to the females during 24 to 36 h after the 1st matings by providing freshly emerged males.

<sup>2</sup>Percent remating of females, i.e., the percent that had mated 2 or 3 times out of the total that had mated at least one time based on the number of spermatophores in each bursa copulatrix.

In each replicate for assessing mating success, freshly emerged adults (24 ♂ paired with 12 ♀) were evaluated. Means ± SE followed by same letter within a column are not significantly different at  $P < 0.05$  level (ANOVA followed by LSD posttest). Percent data were arcsine transformed before ANOVA, but data in table are back transformations;  $n = 15$ ; \*significance at  $P \leq 0.05$

served dose-dependent decrease in mating success correlated with an increase in remating propensity when non-irradiated females were paired with sub-sterilized P males or their F<sub>1</sub> progeny ( $F = 9.42$ ;  $df = 12,182$ ;  $P < 0.05$ ) (Table 6).

#### EFFECT OF FEMALE AGE ON EGG HATCH, MATING SUCCESS AND REMATING PROPENSITY WITH IRRADIATED MALE P AND F<sub>1</sub> MALES

Age of female moths at the time of mating with either partially sterile P males or their F<sub>1</sub> sons may influence their mating parameters, and, hence, may influence efficacy of the IS technique.

Fertility (percent egg hatch) was not affected by the age—i.e., 0–1 d, 2–3 d and 5–6 d—of non-irradiated female moths when mated with either non-irradiated or sub-sterilized (100 or 130 Gy) males (Table 7). However, the percent egg hatch of 5–6 d old females was reduced slightly but significantly when mated with the F<sub>1</sub> sons of P males irradiated with 100 Gy, and reduced more substantially when mated with the F<sub>1</sub> sons of P males irradiated with 130 Gy. Percent mating success was not affected by the age—i.e., 0–1 d, 2–3 d and 5–6 d—of non-irradiated female moths when paired with non-irradiated males (control). However, the percent mating success of 5–6 d old females was reduced slightly but significantly when mated with the P males irradiated with 100 Gy, and reduced more substantially when mated with P males irradiated with 130 Gy. On the other hand, the percent mating success of 5–6 d old females was not reduced significantly when mated with the F<sub>1</sub> sons of P males irradiated with 100 Gy, nor when mated with the F<sub>1</sub> sons of P males irradiated with 130 Gy.

Percent remating—computed on the basis of the number of females that had mated at least one time—of 5–6 d old females relative to that of younger females was slightly, but significantly reduced when mated with non-irradiated males, and reduced substantially and significantly when mated with P males irradiated with either 100 or 130 Gy. Percent remating of 5–6 d old females relative

to that of younger females was significantly reduced when mated with the F<sub>1</sub> sons of P males irradiated with 100 Gy; whereas when the F<sub>1</sub> sons of P males irradiated with 130 Gy were used, percent remating of 2–3 d and 5–6 d of non-irradiated females was significantly reduced (Table 7).

## Discussion

Programs that release sterile or partially sterile moths can be strongly influenced by a variety of ecological and behavioral factors intrinsic to a species' basic biology, and which can or cannot make that pest species amenable to the implementation of the IS technique for lepidopteran pest control (Carpenter et al. 2005; Lance & McInnis 2005). Among such crucial behavioral factors are flight ability, mating propensity and sperm competence of partially sterile male moths and their F<sub>1</sub> male progeny.

#### IMPACT OF IRRADIATION ON FLIGHT ABILITY

The present study revealed no difference between the flight abilities of F<sub>1</sub> male descendants of 100 Gy-irradiated P males and the flight abilities of non-irradiated P males. However F<sub>1</sub> male descendants of 130 Gy-irradiated P males showed a 12–16% reduction in flight ability in comparison with the non-irradiated control. In the 100 Gy-treatment group, the percent of F<sub>1</sub> males that emerged was not significantly affected by irradiation, whereas in the 130 Gy-treatment group, there was a 12.1% reduction in emergence in comparison with the non-irradiated control. The percent adult emergence and the propensity to fly or escape from a vertical flight cylinder (flight ability) are important components of the mating behavior of irradiated moths (Suckling et al. 2004a b; Calkins & Parker 2005; Carpenter et al. 2007).

In the flight assay using approach-I (♂ in: ♀ out), the proportions of emerged moths capable of flying out of the flight cylinder and to orient toward a source of the sex pheromone—versus females placed outside the cylinder in the flight assay chamber—were not affected

**Table 7.** Effect of age of non-irradiated (U) female *Spodoptera litura* on egg hatch, mating success and remating propensity when paired with gamma irradiated (I) males and their F<sub>1</sub> sons.

Dose (Gy) administered to male parent	Ages of females and males in cross <sup>1</sup>	% Fertility	% Mating success	% Remating <sup>2</sup>
0 Gy	0–1 d U ♀ × 0–1 d U ♂	97.2 ± 2.9a	88.1 ± 4.4a	55.6 ± 2.9a
	2–3 d U ♀ × 0–1 d U ♂	95.7 ± 1.5a	79.2 ± 4.2a	56.5 ± 2.8a
	5–6 d U ♀ × 0–1 d U ♂	87.4 ± 2.2a	80.1 ± 3.8a	48.1 ± 2.4b
F value		F = 1.74 df = 2,42	F = 2.45 df = 2,42	F = 3.65 df = 2,42*
100 Gy	0–1 d U ♀ × 0–1 d I ♂	49.1 ± 1.2a	85.2 ± 4.2a	60.5 ± 3.1a
	2–3 d U ♀ × 0–1 d I ♂	48.3 ± 1.4a	76.5 ± 3.8ab	61.4 ± 3.4a
	5–6 d U ♀ × 0–1 d I ♂	44.3 ± 1.8a	74.4 ± 3.7b	41.3 ± 1.9b
F value		F = 1.99 df = 2,42	F = 3.82* df = 2,42	F = 4.17* df = 2,42
130 Gy	0–1 d U ♀ × 0–1 d I ♂	42.8 ± 0.6a	79.9 ± 3.9a	60.8 ± 4.2a
	2–3 d U ♀ × 0–1 d I ♂	40.9 ± 1.5a	74.3 ± 3.6ab	52.2 ± 2.6ab
	5–6 d U ♀ × 0–1 d I ♂	40.3 ± 0.9a	67.7 ± 3.3b	45.8 ± 2.2b
F value		F = 0.87 df = 2,42	F = 3.96* df = 2,42	F = 4.83* df = 2,42
F <sub>1</sub> 100 Gy	0–1 d U ♀ × 0–1 d F <sub>1</sub> ♂	35.3 ± 0.99a	81.3 ± 4.1a	64.6 ± 3.2a
	2–3 d U ♀ × 0–1 d F <sub>1</sub> ♂	32.9 ± 1.4ab	72.5 ± 5.6a	57.5 ± 2.8a
	5–6 d U ♀ × 0–1 d F <sub>1</sub> ♂	31.6 ± 1.3b	71.5 ± 3.9a	32.3 ± 1.6b
F value		F = 3.79* df = 2,42	F = 3.12 df = 2,42	F = 6.78* df = 2,42
F <sub>1</sub> 130 Gy	0–1 d U ♀ × 0–1 d F <sub>1</sub> ♂	27.5 ± 1.1a	75.9 ± 3.9a	64.3 ± 3.0a
	2–3 d U ♀ × 0–1 d F <sub>1</sub> ♂	26.4 ± 0.6a	72.9 ± 3.6a	35.6 ± 1.6b
	5–6 d U ♀ × 0–1 d F <sub>1</sub> ♂	19.9 ± 1.5b	67.9 ± 3.5a	32.9 ± 1.9b
F value		F = 8.72* df = 2,42	F = 3.04 df = 2,42	F = 10.84* df = 2,42

<sup>1</sup>I ♂: irradiated male P moth; F<sub>1</sub> ♂: F<sub>1</sub> male progeny of irradiated male parent male; U: non-irradiated (non-irradiated) moth. Evaluation of freshly emerged male moths and female moths of different age groups (16 ♂ vs 8 ♀) constituted each replicate for assessing mating success and remating propensity.

<sup>2</sup>Percent remating of females, i.e., the percent that had mated 2 or 3 times out of the total that had mated at least one time based on the number of spermatophores in each bursa copulatrix; n = 12 to 15; \*Significance at P ≤ 0.05.

Means ± SE followed by same letter within a regimen in a column are not significantly different at P < 0.05 level (ANOVA followed by LSD posttest); percent data were arcsine transformed before ANOVA, but data in table are back transformations; n = 12.

by irradiation treatments of either 100 or 130 Gy. In a study on the painted apple moth, *Teia anartoides* Walker (Lepidoptera: Lymantriidae), Stephens et al. (2006) reported that the percent of irradiated moths reaching a female placed in a wind tunnel was not significantly different in comparison with the non-irradiated control. These authors considered flight ability as the most important quality parameter—as it was a measure directly related to the ability of irradiated moths to successfully locate wild females—that could lead to increased effectiveness of a SIT/IS program (Stephens et al. 2006). Moreover the above pattern of flight activity reported by Stephens et al. (2006) was consistent with the findings reported in a study with male *T. anartoides* where quality of the moths was assessed with a radiation dose of 160 Gy (Suckling et al. 2002). Carpenter et al. (2007, 2012) evaluated the ability of codling moths to fly out of cylinders of different heights and diameters over a period ranging from 24 to 88 h, and concluded that the importance of this characteristic was comparable to other quality parameters (e.g., longevity and ability and propensity to mate). Their results were fairly similar to our findings.

In the flight assay using approach-II (♀ in: ♂ out), the numbers of F<sub>1</sub> moths recorded on the ceiling and side walls of the flight assay chamber were similar for the 100 and 130 Gy-treatment groups in comparison with the non-irradiated control. Suckling et al. (2007) reported that both irradiated and non-irradiated control *T. anartoides* males showed similar behavioral responses to calling females. Thus responding males first activated their wings, then took-off and flew upwind via zig-zag anemotaxis toward the females. Suckling et al. (2004b) reported that despite reduced arrival success of irradiated males to tethered females

in the field; their ability to mate was not affected. However, some degree of reduced competitiveness of males has to be accepted in return for the associated inherited sterility (Suckling et al. 2007).

In the present study, the successful flight ability of the F<sub>1</sub> male descendants of 100 Gy-treated males was correlated with their mating success, which was not significantly different from the non-irradiated control moths. However, but such a correlation in percent mating success did not exist with the F<sub>1</sub> male descendants of 130 Gy-treated males.

#### RESPONSES OF IRRADIATED MALE MOTHS AND THEIR F<sub>1</sub> PROPENSITY TO THE SEX PHEROMONE

Activation followed by pheromone-oriented flight is the initial response of a receptive male in a chain of epigamic events leading to mating (Giebultowicz & Zdarek 1996). The number of male moths captured in pheromone-baited traps or virgin female-baited traps was considered a good measure of male competitiveness (Carpenter et al. 1989; Bloem et al. 1999). Long-range attractants are very useful for assessing the distribution of wild and sterile insects, monitoring over-flooding ratios (Vreysen 2005), and evaluating specific aspects of sterile male quality (Calkins & Parker 2005).

Our present findings suggested that the responses of F<sub>1</sub> male descendants of male parents irradiated with either 100 or 130 Gy towards pheromone traps in field cages were not different from non-irradiated control moths. Significantly more 100 Gy-treated males of the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) were recaptured

in female-baited traps than were males treated with 200 and 250 Gy (Bloem et al. 1999). In another study conducted on *H. zea*, the number of matings, observed single tethered females or males captured in pheromone traps were considered measures of male competitiveness, and no significant differences were found between non-irradiated and 100 Gy-treated males (Carpenter et al. 1989). Adequate responses of irradiated male P moths and their  $F_1$  descendants towards pheromone-baited traps is one of the key parameters that provide indications for successful mating, and our data showed that the responses of treated *S. litura* males and their  $F_1$  male descendants were similar to those of the non-irradiated control.

#### EFFECT OF VARIOUS SEX RATIOS ON MATING SUCCESS

Knowledge of the threshold ratio of released sterile or sub-sterile males versus wild females or males required to cause a downward trend in the density of the pest population is a prerequisite for successful and efficient implementation of pest programs that have a SIT or IS component. Our studies on the mating behavior of P and  $F_1$  male moths in various cross combinations and male:female ratios (1:1, 2:1, and 5:1) showed that percent mating success was affected only slightly by treatments with 100 and 130 Gy in comparison with the non-irradiated control. In support of such a finding, Ocampo (2001) reported that the mean number of mating pairs involving 100 Gy-treated and non-irradiated males of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) was not significantly different when using a 1:1 or a 5:1 male:female ratio. In our study, the percent remating propensity of mated non-irradiated females was significantly greater in crosses with sub-sterilized P and  $F_1$  males in comparison with non-irradiated males, which was also consistent with the findings of Ocampo (2001).

When using a 2 ♂:1 ♀ sex ratio, the mating effectiveness of irradiated male *S. litura* and their  $F_1$  sons was better in some cases or equivalent when compared to mating parameters of moths crossed in sex ratio of 1 ♂:1 ♀; but the pattern of mating effectiveness did not differ between the 2 sex ratios, 1:1 and 2:1. It was reported in *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae), in view of SIT application, that these relatively small male weevils, attempted to prolong the pre-copulatory guarding (give-up time) to enhance their mating opportunities with females under the male-biased condition (sex ratio of 2 ♂:1 ♀) in comparison with the equal sex-ratio condition (sex ratio of 1 ♂:1 ♀) (Kumano et al. 2010). When 5 male moths were kept with 1 female, there appeared to be a slightly enhanced effect on mating frequency in crosses with P and  $F_1$  (descendants of 100 and 130 Gy-treated males) males in comparison with the mating frequencies involving sex ratios of 2 ♂:1 ♀ (data not shown) and 1 ♂:1 ♀.

The remating propensity of non-irradiated females with  $F_1$  males was significantly greater than their remating propensity with non-irradiated males when the male:female ratio was 1:1 but not significantly greater when the ratio was 5:1. The  $F_1$  females were in general less successful in mating with non-irradiated males than the non-irradiated control. The increase in remating propensity of non-irradiated females with irradiated P or  $F_1$  moths appeared to be correlated with a decrease in mating success.

#### IRRADIATION IMPACT ON SPERM USE PATTERN DURING MULTIPLE MATING

Multiple mating is a widespread phenomenon in insects, especially Lepidoptera, where both males and females are capable of mating more than once (Drum-Mond 1984). In such species, progeny may be fathered exclusively by the first male, by several males, or by the most

recent male, indicating either complete sperm displacement or mixing of sperm after each mating (McCaughey & Reilly 1984; Smith 1984). In our study on sperm prioritization by female *S. litura*, non-irradiated females were sequentially mated to non-irradiated males and sub-sterilized P males or their  $F_1$  male progeny in various mating sequences, and the results indicated sperm precedence of the last mating with evidence of little sperm mixing. In an earlier study, complete sperm precedence was observed during multiple matings involving non-irradiated male *S. litura* and males that were treated with the sterilizing gamma dose of 250 Gy (Seth et al. 2002). Carpenter et al. (1997) reported that in the fall armyworm, *S. frugiperda*, the sperm complements from males irradiated with 100 Gy were successfully competitive with sperm complements from non-irradiated males, but sperm competitiveness was reduced in males irradiated with 150 Gy.

The  $F_1$  sterile progeny of sub-sterilized males may also transfer less than a full complement of sperm (Carpenter et al. 1987; Proshold et al. 1993; Carpenter et al. 2005). Sterilization-related reductions in the amount of sperm transferred to females can reduce sterile male competitiveness by increasing the incidence of remating among females that mate with both sterile and wild males (Haynes & Mitchell 1977; Carpenter et al. 1987). When females mate with both sterile and wild males, the proportion of eggs fertilized by the sperm of sterile males can be influenced by the species' patterns of sperm precedence and/or the competitiveness of the male ejaculates. In many species, sperm from the most recent mating takes precedence over sperm from an earlier mating (Brower 1975; Etman & Hooper 1979; Saul & McCombs 1993), although some species show first-mating (El Agoze et al. 1995) or variable sperm precedence (Conner 1995; LaMunyon & Huffman 2001) and specialized mechanisms exist in some species to expel or otherwise inactivate sperm from previous matings (Waage 1979). Often sperm from different matings mix to various degrees, and in such cases the proportion of offspring a male sires would depend at least in part on the competitiveness of his ejaculate. The degree of sperm precedence and its effect on the inherited sterility technique must be considered to predict the success of this radio-genetic control method.

#### EFFECT OF FEMALE AGE ON REPRODUCTIVE PERFORMANCE OF SUBSTERILIZED MALE MOTHS AND THEIR $F_1$ PROGENY

It is known that the age of the female at her first mating can affect the reproductive potential of lepidopteran insects (Roger & Marti 1996; Ellis & Steele 1982). Therefore, the effect of female age on egg hatch, mating success and remating propensity when the females are paired with sub-sterilized male moths and their  $F_1$  sons needs to be assessed to better understand the mating potential of released sterile moths in relation to the efficacy of the SIT/IS technique.

In our study, there was no effect of the female's age on mating success when she mated with a non-irradiated *S. litura* male. The remating propensity of non-irradiated *S. litura* females was not affected during their first 3 d, but was reduced when the females were 5–6 d old. A study conducted on an insect of the same genus, the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) showed that when females first mated during 1–2 d they received significantly more spermatophores than when females were older at the first mating (Roger & Marti 1996). Likewise, in the African cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), both fecundity (number of eggs oviposited) and fertility (egg hatch) were reduced when mating was delayed beyond a critical period (> 2 d delay) (Ellis & Steele 1982). Our study showed that the mating success of 5–6 d old non-irradiated females paired with irradiated P males was significantly less than that of 0–1 d old females, whereas the mating success of non-irradiated females paired with  $F_1$  males was independent

of the age of the females. Differential responses in relation to the age of females mated to irradiated males were manifested to both mating success and remating success. Remating propensity of 0–1 d old non-irradiated females mated with irradiated P and  $F_1$  males was greater than the remating propensity of older non-irradiated females (i.e., 5–6 d old). Importantly the matings involving  $F_1$  males and 5–6 d old non-irradiated females resulted in reduced egg hatch, leading to increased sterility (i.e., 77.2%) in comparison with the sterility in matings of  $F_1$  males and 0–1 d old females (i.e., 71.7% sterility).

Unsuccessful matings (evidenced by lack of transfer of viable sperm) and low fertility among lepidopteran species are not uncommon, especially if the initial mating after emergence is delayed by 3 or more d (Ellis & Steele 1982; Proshold et al. 1982; Lingren et al. 1988; Unnithan & Paye 1991; Proshold 1996), and the negative influence of female age on successful matings and rematings with either irradiated P or  $F_1$  males might have a bearing on the operational success of  $F_1$  sterility technique.

The present study evaluated the flight ability of *S. litura* males, orientation of males toward the female sex pheromone, mating behavior of non-irradiated females in various ratios with either irradiated P or  $F_1$  males—i.e., the females' mating frequency, mating success, and remating propensity—sperm use patterns in females multiple-mated with males irradiated with sub-sterilizing doses (either 100 or 130 Gy) and corresponding  $F_1$  males. Securing wild mates by released males and their field-raised  $F_1$  sons is of prime importance for programs that have an IS component, and adequate flight and mating behavior of the released insects are prerequisites in this respect. Our data indicated that many of the parameters examined were not degraded or only slightly so by the sub-sterilizing doses administered. Information on these parameters would be very useful for simulation modeling and for predicting the operational success of the IS technique as part of an integrated approach for the management of major pests including *S. litura*.

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