INHERITED STERILITY BY SUBSTERILIZING RADIATION IN *SPODOPTERA LITURA* (LEPIDOPTERA: NOCTUIDAE): BIOEFFICACY AND POTENTIAL FOR PEST SUPPRESSION

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ABSTRACT

Spodoptera litura reared on host plants and on synthetic diet were irradiated with two substerilizing doses of gamma radiation, 100 Gy and 130 Gy, and examined for inherited sterility. Irradiation affected mating success in the parental (P) and F_1 generations. F_1 sterility was higher than P sterility, and F_1 males inherited more sterility than did F_1 females. F_1 progeny developed at a slower rate compared with controls. F_1 survival to adulthood decreased with increasing dose of radiation. Sex ratio in F_1 moths was skewed towards male. Life tables were constructed for *S. litura* reared on host plant and synthetic diets, and the impact of radiation on population characteristics was ascertained. Reproductive rate (R_0) was significantly decreased as a consequence of irradiation, and the effect was more severe in F_1 crosses than in P crosses. There was a negative correlation between the dose of radiation and the percent embryo formation in P crosses. Whereas in F_1 crosses, radiation dose (given to male parents) was positively correlated with the percent unhatched embryonated eggs. Early mortality of eggs prevailed in unhatched eggs derived from P crosses, and late embryonic lethality was the major cause of F_1 sterility. Effects of irradiation are discussed with an emphasis on assessing the potential of the inherited sterility principle for pest control.

Key Words: common cutworm, irradiation, F_1 sterility, synthetic diet, embryonic lethality, population dynamics

RESUMEN

Insectos de la especie *Spodoptera litura* provenientes de colonias mantenidas en plantas huesped o en dieta artificial se irradiaron a dos dosis subesterilizantes de radiacion gamma, 100 Gy y 130 Gy, y fueron examinados para detectar la presencia de esterilidad hereditaria. La irradiacion afecto la habilidad de copula en las generaciones P y F_1. Se encontro que la esterilidad en la generacion F_1 es mas alta que en la generacion P y los machos F_1 heredaron mas esterilidad que las hembras de la misma generacion. Asimismo, se encontro que la velocidad de desarrollo de la generacion F_1 es mas lenta que en el grupo control (no irradiado), y la supervivencia al estado adulto se redujo a medida que los insectos fueron expuestos a dosis de radiacion mas altas. Finalmente, la tasa sexual se vio favoracida hacia el sexo macho en la generacion F_1. Se construyeron tablas de vida para ambas colonias y se investigo el efecto de la radiacion sobre varios parametros de estas poblaciones. La tasa reproductiva (R_0) se redujo significativamente y el efecto fue mas severo en parejas de insectos de la generacion F_1. Asimismo, se encontro una correlacion negativa entre la dosis de radiacion y la formacion de hevecillos embrionados en la generacion P. Esta corralacion fue positiva en la generacion F_1 cuando la radiacion se aplico solamente a los machos. Se detecto mortalidad temprana dentro de los huevecillos no eclosionados provenientes de cruces en la generacion P y se detecto muerte tardia de los huevecillos embrionados en cruces en la generacion F_1. En este articulo discutimos estos efectos con enfasis a la aplicacion de la esterilidad F_1 como metodo de control para esta especie.

Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae), the common cutworm, is an economically serious and polyphagous pest in India. This pest is reported to attack a wide range of food plants (112 cultivated plants belonging to 44 families worldwide and 60 plants in India) (Lefroy 1908; Moussa et al. 1960; Thobbi 1961; Chari & Patel 1983). A multifaceted approach is required for the control of this pest because it has developed resistance against a range of insecticides and because other control measures are inadequate when applied alone (Ramakrishnan et al. 1984; Armes et al. 1997). The sterile insect technique (SIT) has been used for Lepidoptera but insects in this order are radio-resistant, presumably due to their holokinetic chromosomal configuration (Bauer 1967). Therefore, lepidopterans require large doses of radiation for sterilization, leading to somatic damage and reduced competitiveness in the irradiated insect.

A favored alternative to using fully sterile moths in SIT is the use of F_1 sterility. F_1 survivor progeny of sub-sterile parental (P) male moths result when sub-sterilizing doses of radiation are applied to the P males. The resulting F_1 progeny are more sterile than the irradiated parent, and the irradiated moths are more competitive as a result of receiving a lower dose of radiation. Inherited sterility in the progeny of treated males has been shown to have potential in suppressing populations of lepidopteran pests (North & Holt 1969; Knipling 1970; North 1975; LaChance 1985).

Previous studies of substerilizing gamma-radiation doses on the growth, bioenergetics and reproductive behavior of *S. litura* in the F_1 progeny of treated moths indicated the potential of managing this pest by using inherited sterility (Seth & Sehgal 1993). In this study we evaluated the reproductive performance and mating behavior of *S. litura* in response to two substerilizing doses (100 Gy and 130 Gy) when reared on two different diets.

MATERIALS AND METHODS

Insect Rearing

Spodoptera litura was reared on two diets, the natural food, castor leaves (*Ricinus communis*) and a meridic diet containing chickpea seeds and sinigrin as a phagostimulant (Table 1). Insects were held at ambient environmental conditions, 26.8 ± 1°C temperature, 75 ± 5% R.H. and a photoperiod of 12L:12D in the insectary. Larvae developing on the castor leaves were allowed to pupate in moist, loose soil. Larvae developing on the chickpea diet pupated in the diet container.

Irradiation of Insects

Irradiation of 0-24-h old adult males was conducted in the Genetics Division, Indian Agricultural Research Institute, New Delhi, using a Co^{60} source at the dose rate of about 13.5 Gy/min. On the basis of our initial studies (Seth & Sehgal, 1993), two gamma-radiation doses, 100 Gy and 130 Gy, were selected for this study.

TABLE 1. CONSTITUENTS OF THE SEMI-SYNTHETIC DIET PROPOSED FOR REARING OF *SPODOPTERA LITURA*.

Ingredients	Amount
Agar	25 g
Deionized water	750 ml
Casein	44 g
Ground chickpea seeds	93.50 g
Wessons salts	12.50 g
Cholesterol	1.25 g
Yeast (dried, brewer's)	19 g
Methyl-p-hydroxybenzoate	1.25 g
Sugar	39 g
Sorbic acid	2 g
Deionized water	400 ml
4 M KOH	6.25 ml
Corn oil	2.50 ml
Linseed oil	2.50 ml
Formaldehyde (10%)	5.50 ml
Sinigrin (1%)	3.53 ml
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), Evion (vitamin E; 0.2 g; Merck Co.), vitamin mixture (2 g; Roche Co.).

Reproductive Performance and Viability of Irradiated Moths and Their Progeny

Various reproductive parameters were assessed by pairing treated insects (irradiated P males and F_1 moths derived from the treated P males crossed with normal females) with their normal (N) counterparts from the stock culture. Eggs from single-pair matings were counted daily and the number hatching was monitored in 10 replicated samples of 80-100 eggs each (up to first 3 days of egg laying). Corrected sterility and control of reproduction of insect population due to irradiation were determined according to the methods described by Abbott (1925) and Seth & Reynolds (1993).

Experiments on mating success were conducted in laboratory cages (each cage having 10-15 pairs, comprising one replicate). The mating success of moths was assessed by dissection of females immediately after death. The presence of a spermatophore in the bursa copulatrix indicated that the female had mated; the number of spermatophores indicated the mating frequency.

The viability of treated moths, and the survival and developmental pattern of F_1 progeny were determined. Diurnal observations on the insect behavior and the growth index for each treatment was calculated.

Population Characteristics

Various features of population dynamics were studied by constructing life tables for *S. litura* on castor leaves and chickpea diet. The female life tables of P and F_1 generation were constructed as described by Birch (1948), and elaborated by Howe (1953), Morris & Miller (1954), and Atwal & Bains (1989). For ascertaining the life table characteristics, a defined size of population was established in field-cages for a particular cross. Then seven batches of 200-250 eggs collected from each cross were reared to determine age specific mortality in different life stages and successful adult emergence. The life tables gave the probability at birth of a female being alive at age x, designated as l_x (l_0 = 1). The age schedule for female births denoted the mean number of female offspring produced per unit time by a female of age x, designated as m_x. The net reproductive rate (R_0) was calculated from l_x and m_x. Mean length of generation time (T_c), innate capacity for increase in number (rm) and finite rate of increase (λ) were calculated from the data generated in the life tables.

Embryonic Development

The embryonic development in F_1 and F_2 eggs was studied to understand the stage at which irradiation induced lethality was manifested. The developmental state of embryos was assessed in dechorionated eggs. The eggs were treated with 3-5% NaClO for 30-40 min, fixed with 10% formalin for 8-12 h, and stained with lactoacetic-orcein or Feulgen stain. The stained embryos were examined under the microscope. The embryonic development was classified as stage I (cleavage/early development), stage II (germ band stage), and stage III (embryo stage). Some embryos were recorded as abnormal.

Statistical Analysis

The effect of radiation on various parameters in the two diets was subjected to analysis of variance (ANOVA). Data were usually obtained in replicates of ten, unless otherwise specified in the text. Percentage data were transformed using arcsine $\sqrt{x}$ before ANOVA. Means were separated at the 5% significance level by least significant difference (LSD) test (Snedecor & Cochran 1989).

RESULTS

Reproductive Performance and Viability of Irradiated Moths and Their Progeny

For the untreated controls, the pre-oviposition period ranged from 1.65 to 1.72 d on the castor leaf diet and 1.69 to 1.70 d on the chickpea. The dose of radiation did not significantly affect pre-oviposition period for the P and F_1 crosses in either diet. The oviposition period was not affected in P crosses, but was significantly reduced in most F_1 crosses (Table 2). Also, the radiation treatments caused a significant reduction in fecundity of the mated female during P crosses and F_1 crosses, with the greatest reduction in F_1 crosses. Even normal females mated with treated males (P or F_1) showed a reduction in fecundity. For ex-

TABLE 2. EFFECT OF SUBSTERILIZING GAMMA-RADIATION DOSES AND DIET ON THE OVIPOSITIONAL BEHAVIOR AND LONGEVITY OF *SPODOPTERA LITURA* PARENTS AND F_1 PROGENY.

Diet	Dose (Gy)	Cross type[1]	Preoviposition period (days)	Oviposition period (days)	Eggs per female (no.)	Life span (days)	
						Male	Female
			P crosses				
Castor leaf	0	Nm × Nf	1.65 ± 0.07 a	7.23 ± 0.32 a	1893 ± 59 a	10.0 ± 0.4 a	9.4 ± 0.3 a
	100	Pm × Nf	1.68 ± 0.17 a	7.25 ± 0.17 a	1847 ± 68 b	9.4 ± 0.7 a	9.4 ± 0.5 a
	130	Pm × Nf	1.89 ± 0.13 a	7.21 ± 0.25 a	1795 ± 93 b	9.4 ± 0.4 a	9.1 ± 0.5 a
			F_1 crosses				
	0	Nm × Nf	1.72 ± 0.08 a	7.62 ± 0.31 a	1990 ± 49 a	10.3 ± 0.4 a	9.3 ± 0.3 a
	100	F_1m × Nf	1.62 ± 0.14 a	6.71 ± 0.11 b	1659 ± 78 b	9.4 ± 0.6 a	8.1 ± 0.5 b
		Nm × F_1f	1.72 ± 0.19 a	7.01 ± 0.09 a	1693 ± 80 b	9.8 ± 0.2 a	9.3 ± 0.3 a
		F_1m × F_1f	1.60 ± 0.24 a	6.22 ± 0.49 b	1520 ± 56 b	9.6 ± 0.4 a	8.3 ± 0.4 b
	130	F_1m × Nf	1.78 ± 0.16 a	6.22 ± 0.25 b	1464 ± 85 b	9.1 ± 0.4 b	8.9 ± 0.4 ab
		Nm × F_1f	1.73 ± 0.11 a	6.29 ± 0.42 b	1499 ± 69 b	8.8 ± 0.3 b	8.7 ± 0.9 ab
		F_1m × F_1f	1.85 ± 0.24 a	6.22 ± 0.59 b	1219 ± 30 c	8.8 ± 0.3 b	8.4 ± 0.1 b
			P crosses				
Chickpea diet	0	Nm × Nf	1.70 ± 0.09 a	7.40 ± 0.23 a	2011 ± 70 a	10.1 ± 0.4 a	9.5 ± 0.4 a
	100	Pm × Nf	1.80 ± 0.10 a	7.16 ± 0.11 a	1886 ± 84 b	9.3 ± 0.1 a	8.7 ± 0.2 a
	130	Pm × Nf	1.88 ± 0.11 a	6.66 ± 0.42 a	1727 ± 69 b	8.9 ± 0.3 a	8.6 ± 0.2 a
			F_1 crosses				
	0	Nm × Nf	1.69 ± 0.09 a	7.33 ± 0.24 a	2155 ± 74 a	9.8 ± 0.5 a	9.5 ± 0.4 a
	100	F_1m × Nf	1.63 ± 0.17 a	7.09 ± 0.08 a	1649 ± 70 b	8.8 ± 0.4 a	8.5 ± 0.4 b
		Nm × F_1f	1.62 ± 0.16 a	6.90 ± 0.37 ab	1592 ± 84 b	8.6 ± 0.4 a	8.5 ± 0.4 b
		F_1m × F_1f	1.69 ± 0.13 a	6.34 ± 0.10 bc	1492 ± 59 bc	7.2 ± 0.2 c	7.8 ± 0.1 c
	130	F_1m × Nf	1.89 ± 0.17 a	6.40 ± 0.13 bc	1304 ± 58 d	8.6 ± 0.3 b	8.0 ± 0.3 bc
		Nm × F_1f	1.75 ± 0.17 a	5.95 ± 0.26 bc	1363 ± 75 cd	8.5 ± 0.4 b	7.9 ± 0.2 bc
		F_1m × F_1f	1.84 ± 0.20 a	5.83 ± 0.22 c	1099 ± 53 e	7.0 ± 0.3 c	7.1 ± 0.3 c

[1]N, normal; P, treated parent; F_1, progeny of treated males; m, male, f, female. Means ± SE followed by the same letter in a column in each generation in case of each diet are not significantly different at $P < 0.05$ (ANOVA followed by LSD posttest); n = 10.

ample, the reduction in oviposition in P crosses, with respect to untreated crosses, was 6.2% at 100 Gy and 14.1% at 130 Gy on the chickpea diet. This reduction in oviposition was further increased in the F_1 crosses (23-30% at 100 Gy, and 39-49% at 130 Gy on chickpea diet). Both doses significantly reduced F_1 male and female longevity when the chickpea diet was used. Although the same trend was evident when moths were reared on castor leaves, the reduced longevity for males at the 100 Gy dose was not significant.

A significant, dose-dependent effect was observed on the mating success of P and F_1 moths reared on both diets. The treated males were less successful at mating than untreated males (Table 3). For example, at 130 Gy on chickpea diet, the mating success of P males and F_1 males was 76.4% and 72.7%, respectively, as compared with 89.6% in the control. The mating percentage was more adversely affected in F_1 female crosses and F_1 self crosses. However, the radiation did not significantly affect the mating frequency of the moths from either diet. Radiation-induced suppression effects were examined in terms of corrected sterility and control of reproduction (Table 3). At 100 Gy on chickpea diet, the F_1 male × N female cross gave 76.5% of the total eggs laid by controls (see Table 2) and resulted in 28.3% egg hatch as compared with 78.6% hatch in controls. Therefore, this cross exhibited 63.9% sterility, and 72.3% control of reproduction. For both the diets tested, the control of reproduction in F_1 males (mated with normal females) was more than 71% at 100 Gy and about 83-87% at 130 Gy.

Growth and Survival of F_1 Progeny

The developmental time of F_1 eggs, larvae, pupae and adults reared on the two diets were significantly affected by the radiation treatment given to the male parent (crossed with a normal female). Their developmental rate was slower than that of the control progeny (Table 4); the total developmental time between eggs and adults was in-

TABLE 3. EFFECT OF SUBSTERILIZING GAMMA-RADIATION DOSES AND DIET ON MATING BEHAVIOR, FERTILITY AND REPRODUCTIVE SUPPRESSION OF *SPODOPTERA LITURA* PARENTS AND THEIR PROGENY.

Diet	Dose (Gy)	Cross type[1]	Mating frequency (no. ± SE)	Mating success[2] (%)	Fertility[2] (%)	Corrected Sterility (%)	Control of Reproduction (%)
			P crosses				
Castor leaf	0	Nm × Nf	1.7 ± 0.1 a	92.8 ± 2.2 a	89.7 ± 2.1 a	0	0
	100	Pm × Nf	1.9 ± 0.3 a	84.0 ± 3.1 b	52.3 ± 2.0 b	41.1 ± 1.6	48.0 ± 1.9
	130	Pm × Nf	1.9 ± 0.3 a	72.6 ± 3.8 c	44.8 ± 2.5 c	49.5 ± 2.8	56.6 ± 3.2
			F_1 crosses				
	0	Nm × Nf	1.7 ± 0.1 a	94.1 ± 1.9 a	88.9 ± 1.4 a	0	0
	100	F_1m × Nf	1.9 ± 0.2 a	80.9 ± 3.1 bc	31.6 ± 2.1 cd	64.4 ± 4.3	71.7 ± 4.8
		Nm × F_1f	1.8 ± 0.2 a	73.2 ± 2.2 cd	44.5 ± 2.9 b	49.9 ± 3.3	59.4 ± 3.9
		F_1m × F_1f	1.7 ± 0.3 a	71.4 ± 2.9 de	28.3 ± 3.1 d	68.2 ± 7.4	76.8 ± 8.4
	130	F_1m × Nf	2.0 ± 0.2 a	75.6 ± 3.5 bc	21.8 ± 1.8 de	75.5 ± 6.2	82.8 ± 6.8
		Nm × F_1f	1.7 ± 0.3 a	66.0 ± 2.4 ef	35.9 ± 2.2 c	59.6 ± 3.7	71.0 ± 4.4
		F_1m × F_1f	1.9 ± 0.5 a	59.0 ± 4.0 f	20.2 ± 1.1 e	77.3 ± 4.2	86.7 ± 4.7
			P crosses				
Chickpea diet	0	Nm × Nf	1.7 ± 0.1 a	89.2 ± 2.2 a	84.4 ± 2.9 a	0	0
	100	Pm × Nf	1.8 ± 0.3 a	81.5 ± 2.2 b	46.2 ± 4.1 b	41.2 ± 3.7	48.6 ± 4.3
	130	Pm × Nf	1.8 ± 0.2 a	76.4 ± 3.2 b	41.6 ± 2.1 b	47.1 ± 2.4	57.6 ± 2.9
			F_1 crosses				
	0	Nm × Nf	1.7 ± 0.2 a	89.6 ± 2.4 a	78.6 ± 3.0 a	0	0
	100	F_1m × Nf	1.5 ± 0.3 a	84.0 ± 3.1 ab	28.3 ± 2.3 c	63.9 ± 5.4	72.4 ± 6.1
		Nm × F_1f	1.6 ± 0.1 a	75.4 ± 1.5 bc	43.2 ± 2.9 b	45.0 ± 3.0	59.4 ± 4.0
		F_1m × F_1f	1.5 ± 0.1 a	65.9 ± 2.8 de	22.3 ± 2.0 cd	71.6 ± 6.4	80.4 ± 7.1
	130	F_1m × Nf	2.1 ± 0.1 a	72.7 ± 3.1 cd	17.5 ± 1.8 de	77.8 ± 7.9	86.5 ± 8.8
		Nm × F_1f	2.4 ± 0.1 a	60.9 ± 1.3 ef	26.7 ± 2.2 c	66.1 ± 5.4	78.5 ± 6.5
		F_1m × F_1f	2.0 ± 0.2 a	56.1 ± 3.1 f	14.6 ± 2.1 e	81.4 ± 8.7	90.6 ± 8.1

[1]N, normal; P, treated parent; F_1, progeny of treated males; m, male, f, female.
[2]For statistical analysis by ANOVA, the percentage data were transformed using arcsine $\sqrt{x}$. Means ± SE followed by the same letter in a column in each generation in case of each diet are not significantly different at $P < 0.05$ (ANOVA followed by LSD posttest); n = 10.

TABLE 4. DEVELOPMENTAL PROFILE AND SURVIVAL OF F_1 PROGENY OF IRRADIATED *SPODOPTERA LITURA* REARED ON TWO DIETS.

Diet	Dose (Gy)	Developmental period (days)				% pupation[1]	Adult eclosion[1] %	Growth index[2]	Sex ratio (M:F)
		Egg	Larva	Pupa	Total				
Castor leaf	0	3.6 ± 0.1 a	15.8 ± 0.3 a	7.9 ± 0.2 a	27.2 ± 0.4 a	89.3 ± 4.6 a	82.3 ± 2.5 a	3.0 ± 0.1 a	1:1.03 a
	100	3.9 ± 0.1 b	16.7 ± 0.5 ab	8.5 ± 0.2 ab	29.2 ± 0.6 ab	71.2 ± 2.7 b	64.0 ± 2.8 b	2.2 ± 0.1 b	1:0.69 ab
	130	4.2 ± 0.1 b	17.3 ± 0.4 b	8.6 ± 0.2 b	30.1 ± 0.8 b	62.8 ± 2.9 c	56.3 ± 3.4 b	1.8 ± 0.1 c	1:0.64 b
Chickpea diet	0	3.6 ± 0.1 a	16.2 ± 0.4 a	8.4 ± 0.2 a	28.3 ± 0.7 a	90.2 ± 3.3 a	79.2 ± 2.8 a	2.8 ± 0.1 a	1:0.99 a
	100	3.9 ± 0.1 b	17.9 ± 0.4 b	8.5 ± 0.3 ab	30.7 ± 1.0 ab	68.4 ± 4.5 b	60.9 ± 3.1 b	2.0 ± 0.1 b	1:0.71 b
	130	4.0 ± 0.1 b	18.7 ± 0.3 b	9.1 ± 0.3 b	31.5 ± 0.3 b	58.8 ± 2.1 b	51.7 ± 2.2 c	1.6 ± 0.1 c	1:0.65 c

[1]Observed in group of 25 individuals comprising each replicate, n = 7. For statistical analysis by ANOVA, the percentage data were transformed using arcsine $\sqrt{x}$.
[2]Growth index = % adult eclosion/total developmental period.
Means ± SE followed by the same letter in a column within each diet are not significantly different at $P < 0.05$ (ANOVA followed by LSD posttest).

creased by about 7-9% at 100 Gy and 10-13% at 130 Gy. The F_1 insects experienced significantly more larval and pupal mortality than the controls, and also exhibited a higher rate of pupal and adult malformation than the controls. The proportion of F_1 insects surviving to adults decreased with the increasing dose on both diets tested. About 61-64% of the F_1 progeny of 100 Gy treated male parents emerged as adults, whereas 79-82% adults emerged in the controls. The F_1 growth index was significantly decreased according to the dose of radiation administered to the male parent. The sex ratio of the F_1 generation was significantly skewed towards males as a result of the radiation treatments. For example, F_1 females constituted about 41% of the population at 100 Gy and about 50% of the population in the control.

Population Characteristics

Increase in the total developmental period of F_1 progeny delayed the commencement of oviposition with further debilitating effects on adult survival, life expectancy, oviposition rate and female births with respect to the pivotal age group. The reproductive rate (R_0) was significantly decreased as a consequence of radiation treatment, with the effect being more pronounced in F_1 crosses than in P crosses (Table 5). For instance, R_0 for untreated insects was 785.2 on castor and 740.2 on chickpea diet. On chickpea diet, R_0 was decreased by 16.4% at 100 Gy and 36.6% at 130 Gy in P generation. R_0 was further reduced by about 70% at 100 Gy and about 80% at 130 Gy in F_1 crosses, with respect to the control.

The mean generation time (T_c) was 31.3 days on castor and 32.3 days on chickpea diet for untreated insects. In F_1 crosses, T_c was significantly higher, indicating a protraction of 2-4 days as compared with the control. The T_c delay showed a positive correlation with doses of radiation (Table 5). The intrinsic rate of increase (rm) in untreated insects was 0.21 on castor and 0.20 on chickpea diet. The effect of irradiation on the intrinsic rate of increase was not apparent in P crosses, but was significant in F_1 crosses, where there was a reduction in the rm value (23-25% at 100 Gy and 31-36% at 130 Gy). Irradiation also affected the finite rate of increase of this insect population. For example, on chickpea diet at 130 Gy, it was reduced to 1.15 in F_1 male crosses from 1.23 in the control. A similar pattern was observed on castor leaves.

Embryonic Development of F_1 and F_2 Zygotes

In F_1 and F_2 generations, the embryonic development was disrupted at different stages that reflected a specific pattern of radiation-mediated lethality in P and F_1 moths (Table 6). The reduced egg hatch from P and F_1 males crossed with normal females may have been caused by infertility of eggs or embryonic death. Certain fertilized eggs exhibited embryonic development but were incapable of hatching. The development of such zygotes was arrested at different levels of embryogenesis showing partial to complete embryonation. Some of these eggs could reach the "black head" stage wherein, blackish brown colored head cuticle of the pharate 1st instar larva was visible through translucent chorion of the egg. This type of egg was categorized as an unhatched embryonated egg. At 130 Gy on the chickpea diet, early mortality (EM) of F_1 eggs (derived from P crosses) was 36.9% with no detectable embryonic development. Late embryonic lethality (LEL) was 22.4%, mainly due to embryonic stages blocked at cleavage or early development stage. In F_2 eggs (from F_1 crosses) at the same dose, EM constituted 16.8% and LEL constituted 65.8%. Notably, more than 75% of LEL was restricted to germ band and embryo stages (Table 6, Fig. 1). The formation of embryos was significantly reduced in F_1 eggs (100 Gy and 130 Gy), but was not statistically different from the controls in F_2 eggs at both doses. However, the percentage zygotic viability in P and F_1 crosses was significantly reduced by the dose of radiation (Table 6). EM appeared predominantly in F_1 eggs, whereas F_2 eggs showed sterility mainly due to LEL.

Discussion

The phenomenon of F_1 sterility in *S. litura* was examined over a range of substerilizing doses (Seth & Sehgal 1993). As a result of the reproductive performance and somatic damage caused by the radiation, we selected two doses (100 Gy and 130 Gy) for a more in-depth study. The reduced reproductive performance of the treated moths resulted from the combined effects of reduced longevity, fecundity, mating success, and fertility. The mating percentage was more adversely affected in F_1 females crossed with normal males and in F_1 self crosses as compared with the F_1 males paired with normal females. A greater reduction in reproductive performance was observed at the 130 Gy dose than the 100 Gy dose. The effects of radiation dose on reproductive performance were not significantly influenced by diet. Similar debilitating effects of irradiation on the reproduction of moths have been reported by several workers (North & Holt 1968; Proshold & Bartell 1970; Cheng & North 1972; North 1975; LaChance et al. 1976; Carpenter et al. 1983; LaChance 1985; Sallam & Ibrahim 1993; Omar & Mansor 1993; Ismail 1994; Carpenter et al. 1987; Makee & Saour 1997). Poor reproductive performance and low fertility in treated *S. litura* could be due to one or more of the following reasons: (i) poor ability to mate (El-Sayed & Graves 1969), (ii) failure to produce and transfer as many spermatophores as normal males (Rule et al. 1965; Flint & Kressin 1969), (iii) transfer spermato-

TABLE 5. POPULATION CHARACTERISTICS OF IRRADIATED *SPODOPTERA LITURA* AND THEIR F_1 PROGENY REARED ON TWO DIETS.

Diet	Dose (Gy)	Cross type[1]	Net reproductive rate[2] $R_0 = \sum l_x m_x$	$\sum x l_x m_x$	Mean generation length (days) $T_c = \sum x l_x m_x / R_0$	Innate capacity for increase $rm = \log_e R_0 / T_c$	Finite rate of increase $(\lambda) = \text{antilog}_e rm$
Castor leaf	0	Nm × Nf	785.2 ± 27.4 a	24583.0 ± 683.3 a	31.30 a	0.2129 a	1.237 a
	100	Pm × Nf	602.4 ± 25.4 b	18643.5 ± 774.2 b	30.94 a	0.2068 a	1.229 a
		F_1m × Nf	227.4 ± 10.2 c	7489.2 ± 292.0 c	32.92 b	0.1648 bc	1.179 bc
		Nm × F_1f	212.8 ± 10.3 c	7262.3 ± 355.1 c	34.12 c	0.1571 bc	1.170 bc
		F_1m × F_1f	206.5 ± 6.1 c	6975.2 ± 180.5 c	33.76 bc	0.1578 bc	1.170 bc
	130	Pm × Nf	591.4 ± 27.0 b	18279.8 ± 835.0 b	30.90 a	0.2065 a	1.229 a
		F_1m × Nf	167.7 ± 7.6 d	5805.4 ± 150.2 d	34.62 c	0.1479 cd	1.159 cd
		Nm × F_1f	161.8 ± 7.3 d	5626.6 ± 153.1 d	34.76 c	0.1463 cd	1.157 cd
		F_1m × F_1f	111.1 ± 12.1 e	3819.7 ± 110.7 e	34.36 c	0.1371 d	1.146 d
Chickpea diet	0	Nm × Nf	740.2 ± 38.7 a	23913.1 ± 738.8 a	32.30 a	0.2045 a	1.226 a
	100	Pm × Nf	618.1 ± 28.5 b	19898.6 ± 693.4 b	32.19 a	0.1996 ab	1.220 a
		F_1m × Nf	235.6 ± 21.4 d	8207.3 ± 220.7 d	34.82 bc	0.1568 b	1.169 bc
		Nm × F_1f	220.5 ± 19.3 d	7669.1 ± 131.7 de	34.76 bc	0.1552 b	1.167 bc
		F_1m × F_1f	209.1±13.3 d	7321.7 ± 161.0 e	35.01 c	0.1526 b	1.164 bc
	130	Pm × Nf	469.0 ± 36.7 c	14941.7 ± 328.7 c	31.85 a	0.1931 ab	1.213 a
		F_1m × Nf	152.0 ± 13.1 e	5461.3 ± 120.1 f	35.92 c	0.1399 c	1.150 c
		Nm × F_1f	145.0 ± 11.1 e	5226.3 ± 101.9 f	35.97 c	0.1384 c	1.148 c
		F_1m × F_1f	101.9 ± 8.9 f	3602.7 ± 76.4 g	35.34 c	0.1308 c	1.139 d

[1]N, normal; P, treated parent; F_1, progeny of treated males; m, male, f, female.
[2]l_x, female survival; m_x, female offsprings produced per unit time; x, pivotal age.
Means ± SE followed by the same letter in a column within each diet are not significantly different at $P < 0.05$ (ANOVA followed by LSD posttest); n = 7.

TABLE 6. EMBRYONIC DEVELOPMENT AND ZYGOTE VIABILITY IN EGGS OVIPOSITED BY P AND F_1 MOTHS OF *SPODOPTERA LITURA*.

				Eggs showing[3] (%)						
					Embryonic stage of unhatched eggs					
Diet	Dose (Gy)	Cross type[1]	Eggs per female[2] (no.)	No development	Stage I	Stage II	Stage III	Abnormal	Embryo formation[4] (%)	Unhatched embryonated eggs[4] (%)
Castor leaf	0	Nm × Nf	2088 (88.9%)	6.5 ± 0.2	2.8 ± 0.1	0.9 ± 0.0	0.7 ± 0.0	0	93.3 ± 3.0 a	4.7 ± 0.2 a
	100	Pm × Nf	1847 (52.3%)	26.3 ± 1.7 (55.5)	13.4 ± 0.5 (28.0)	2.5 ± 0.1 (5.2)	2.0 ± 0.1 (4.1)	3.3 ± 0.1 (6.9)	73.5 ± 4.7 bc	28.8 ± 1.4 b
		F_1m × Nf	1755 (31.6%)	11.5 ± 0.6 (16.6)	8.4 ± 0.3 (12.2)	7.6 ± 0.4 (11.1)	37.0 ± 2.1 (54.0)	4.0 ± 0.3 (5.8)	88.6 ± 4.2 a	64.3 ± 3.1 c
	130	Pm × Nf	1795 (44.8%)	33.1 ± 1.6 (60.8)	9.4 ± 0.2 (17.0)	6.4 ± 0.4 (11.5)	3.2 ± 0.2 (5.7)	2.3 ± 0.1 (4.1)	66.4 ± 3.2 c	32.0 ± 1.9 b
		F_1m × Nf	1464 (21.8%)	13.2 ± 0.7 (17.9)	7.5 ± 0.2 (9.6)	10.3 ± 0.6 (13.2)	39.4 ± 2.2 (50.2)	7.1 ± 0.3 (9.1)	86.1 ± 4.1 ab	74.6 ± 4.6 c
Chickpea diet	0	Nm × Nf	2155 (78.6%)	11.2 ± 0.7	5.0 ± 0.2	1.6 ± 0.1	2.2 ± 0.1	0.8 ± 0.03	88.2a ± 4.4	10.8 ± 0.7 a
	100	Pm × Nf	1886 (46.2%)	28.9 ± 1.5 (52.9)	14.1 ± 0.7 (26.2)	4.2 ± 0.2 (7.8)	2.8 ± 0.1 (5.2)	4.2 ± 0.2 (7.8)	71.5 ± 3.7 bc	35.8 ± 2.2 b
		F_1m × Nf	1750 (30.7%)	11.6 ± 0.6 (16.5)	7.7 ± 0.4 (11.1)	9.6 ± 0.4 (13.8)	36.5 ± 1.8 (52.6)	3.8 ± 0.2 (5.4)	88.5 ± 4.5 a	65.3 ± 3.1 c
	130	Pm × Nf	1727 (41.6%)	36.9 ± 1.4 (62.3)	14.3 ± 0.8 (24.4)	3.1 ± 0.1 (5.3)	1.5 ± 0.1 (2.5)	3.1 ± 0.2 (5.3)	63.6 ± 2.4 c	34.5 ± 1.9 b
		F_1m × Nf	1394 (17.5%)	16.8 ± 1.0 (20.1)	5.5 ± 0.3 (6.7)	6.5 ± 0.2 (7.9)	47.0 ± 2.3 (57.0)	6.8 ± 0.4 (8.3)	83.5 ± 4.9 ab	78.8 ± 4.4 d

[1]N, normal moth; P, treated parent; F_1, progeny of treated males; m, male; f, female.
[2]Ovipositional data computed out of mated females; data in parentheses represent % egg hatch.
[3]Data represent % inviable eggs in the particular stage out of total eggs laid; data in parentheses represent % inviable eggs in the stage out of total unhatched eggs; Stage I, cleavage or early developmental phase; Stage II, germ band phase; Stage III, embryo phase.
[4]Percentage data were transformed using arcsine $\sqrt{x}$ before ANOVA, but data shown are back transformations; means ± SE followed by the same letter in a column in case of each diet are not significantly different at $P < 0.05$ (ANOVA followed by LSD post-test); n = 7; each replicate comprising of 80-100 eggs.

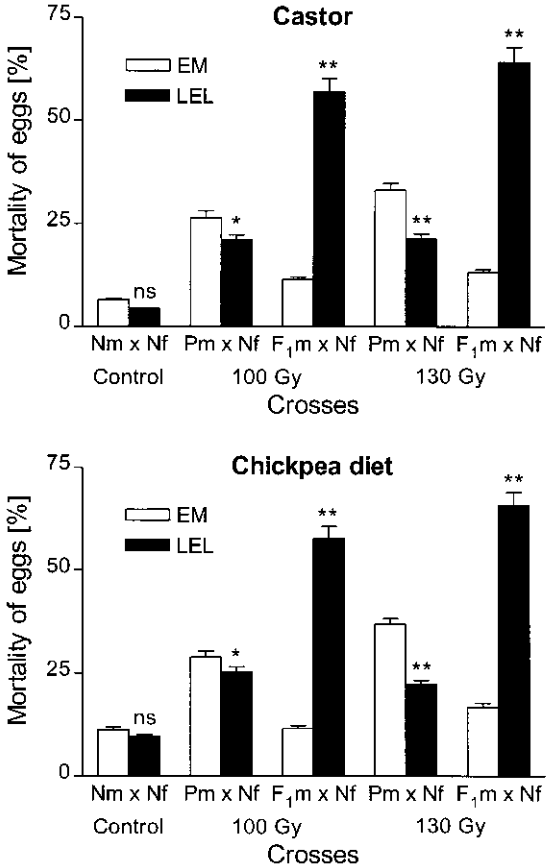

Fig. 1. Effect of substerilizing gamma-radiation doses on the development of eggs derived from the crosses of treated *Spodoptera litura* males and their F_1 progeny, reared on castor leaves and semi-synthetic diet. Bars show mean ± SE; EM, early mortality in eggs; LEL, late embryonic lethality. standard error of the mean (SE). N, normal moth; P, treated parent; F_1, progeny of treated males; m, male; f, female. ns, non-significant at $P < 0.05$; *, significant at $P < 0.05$; **, significant at $P < 0.01$ (t-test conducted between and LEL in each cross).

phores that contain little or no sperm; (iv) abnormal sperm structure, which fails to fertilize the eggs (Ashrafi & Roppel 1973), or (v) inheritance of special chromosome rearrangements (LaChance 1985; Anisimov et al. 1989).

The developmental rate of F_1 larvae originating from the crosses between treated males and normal females of *S. litura* was slower than that of controls, and this delay in development was greater when males were treated with 130 Gy. Because insect development and differentiation are controlled by hormones (Gilbert 1964), the protracted development of F_1 larvae might be due to alteration in hormonal or enzymatic production caused by chromosomal rearrangements, as indicated by Proshold & Bartell (1970, 1972). The F_1 growth index showed a decrease as a consequence of irradiation of male parents. The sex ratio in F_1 generation was skewed towards male as compared with a 1:1 ratio in the control group of insects reared on castor leaves as well as chickpea diet. Sex distortion appears to be general phenomenon occurring in the progeny of irradiated male lepidopterans (Proverbs 1962; Carpenter et al. 1986), probably resulting from the expression of recessive lethal mutations on the single Z chromosome in females but not in ZZ males (Marec 1990).

The effect of irradiation on population characteristics was not observed in the P generation, especially in case of mean generation time, survival and finite rate of increase, because the treatment was given in the adult stage and it could manifest its impact only in the first filial generation. However, irradiation significantly affected population characteristics in F_1 and F_2 generations, particularly the production of females. The production of more males than females, and the reduction in female longevity due to sublethal radiation ultimately reduced the net reproductive rate and the potential rate of increase.

Our data suggest that both inability of irradiated males to fertilize eggs and dominant lethal mutations (DLM) induced in sperm were responsible for reduced egg viability in P crosses. This is because both unhatched embryonated eggs (LEL) and eggs showing no development (EM) were induced by irradiation. The eggs marked in the category of early mortality (EM) could be unfertilized, indicating physiological damage in irradiated males that reduced the ability to transfer sperm. Alternatively, these eggs could have ceased their development at an early stage due to induced DLM (see discussion in Marec et al. 1999). Whereas in F_1 crosses, sterility was largely a result of induced chromosomal aberrations. Therefore, eggs from these crosses showed embryonic development but were unable to hatch, indicating late egg lethality (LEL) as the main cause of egg inviability. Similar findings were made by Proshold & Bartell (1970) in the tobacco budworm, *Heliothis virescens*, and by Bughio (1988) in *Chilo partellus.* LEL was clearly expressed by F_1 males, whereas EM was expressed less, unlike in P males of the codling moths (Anisimov et al. 1989). Similarly, Seth & Reynolds (1993) reported that the main cause of sterility in F_1 generation of *Manduca sexta* was the induction by radiation of lethal mutations that arrested the development in late embryonic life. Also, Marec et al. (1999) observed sterile eggs (eggs with early embryonic lethality as well as unfertilized eggs) and inviable eggs (in which embryos died during different stages of embryogenesis) while studying the gamma radiation induced sterility combined with genetic sexing in *Ephestia kuehniella*.

In view of the overall reproductive performance of P and F_1 moths, and developmental behavior of F_1 insects, our findings suggest the use of 100 Gy as an effective dose for the suppression of *S. litura* populations by the release of partially sterile males. This dose gives better viability of F_1 insects among normal population and thus, a higher inherited sterility effect. Although the higher dose (130 Gy) could be more effective in P crosses, it would less effective in the F_1 crosses. It is worth noting that degree of irradiation impact on the net reproductive rate of P and F_1 crosses observed in their life tables was almost similar to the control of reproductive potential calculated. Proper sperm competition is required for the treated insects to induce reproductive suppression of the feral pest population by using F_1 sterility. Studies on sperm formation, transfer and their competitiveness are in progress (RKS).

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