

EFFECT OF IRRADIATION WITH  $^{60}\text{Co}$  ON TRANSFER  
AND MOTILITY OF SPERM AND ON MATING  
OF THE TOBACCO BUDWORM<sup>1</sup>

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ABSTRACT

Adult tobacco budworms, *Heliothis virescens* (F.), irradiated as pupae within 24 hr of adult eclosion with 20 or 40 krad gamma irradiation were paired singly in all possible combinations. The effects of treatment on the quantity and motility of sperm were generally related to dose. However, the quantity and motility of sperm and mating frequency of irradiated males were affected by 20 or 40 krad at 5 days after adult eclosion but not after 10 days. The mating frequency of irradiated females was not affected by either dose at either time, but fecundity was affected. A dose of 40 krad completely sterilized both sexes; a dose of 20 krad caused 64 and 98% sterility in males and females, respectively. Both doses of irradiation increased longevity of males, but female longevity was increased with 20 krad.

Field tests involving releases of sterile male tobacco budworms, *Heliothis virescens* (F.), on the Island of St. Croix, U.S. Virgin Islands, were unsuccessful because laboratory-reared males were not sexually competitive with males of the natural population for the native females. Basic information on the overall reproductive capacity of an insect is essential to develop a control method based on the sterile male technique. Some of this information is lacking for the tobacco budworm.

Flint and Kressin (1969) suggested that the presence of normal quantities of sperm in the spermatheca of the tobacco budworm, rather than frequency of mating, is basic to normal oviposition; they also found that sperm from irradiated (I) or unirradiated (U) males was equally effective in stimulating oviposition. The effect of gamma irradiation on the motility of tobacco budworm sperm has not been determined, though it has been for the bollworm, *H. zea* (Boddie) (Snow et al. 1972). A study was therefore conducted to determine the effects of  $^{60}\text{Co}$  irradiation on mating frequency, the amount of sperm transferred and its motility in the spermatheca, and the fecundity, fertility, and longevity of treated tobacco budworm moths.

MATERIALS AND METHODS

The budworms used were taken from our laboratory colony (Guerra et al. 1971). Male or female pupae were irradiated with 20 or 40 krad within 24 hr of adult eclosion at a rate of 815.2 rad/min with SD 8% of mean. These doses were chosen because they are considered to be a substerilizing (Guerra

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et al. 1974) and a sterilizing dose—a 97% reduction in hatch was obtained with 30 krad (Wolfenbarger and Guerra 1971). Only moths that eclosed within 24 hr of irradiation were paired. The females from the single-pair replicates were dissected at 5 or 10 days after pairing or upon death. Each test was replicated 4 times during a 2-month period in 1973, with 40-50 pairs/cross.

Female spermathecae were dissected by holding and applying a little pressure to the female abdomen with the thumb and index fingers of the left hand and pulling out the protruded oviscaptum with a fine pair of forceps until the entire sexual organs were completely removed. These organs were then placed on a glass slide and the spermatheca was separated, placed on another slide in a drop of 0.25 M sucrose, and covered gently with a cover glass for microscopical examination.

The relative amounts and motility of eupyrene sperm in the spermatheca were determined by the rating system developed by Holt and North (1970) and modified by Snow et al. (1972); we used the methods of Snow et al. (1972) to determine motility of eupyrene sperm. Thus when 60% or more of the visible eupyrene sperm were motile in the spermatheca, we assumed that the spermatheca contained a normal quantity of motile sperm and that these females successfully mated. Spermatophores (an indicator of mating frequency) and eggs were counted by the methods of Wolfenbarger and Guerra (1970), and longevity was recorded as described by Guerra et al. (1971). Sterility was based on the observed difference in egg hatch of the I X U moths and the check.

Binomial confidence limit techniques were used to test the differences between the check and irradiation treatments in the percentage of females with no sperm or with normal quantities in the spermathecae. These techniques were also used to compare the differences in sperm motility. All differences were compared at the 5% level of probability.

#### RESULTS AND DISCUSSION

The proportion of females with normal quantities of sperm after 5 days was reduced 37% from the check when males were irradiated with 20 krad and 94% when the males received 40 krad (Table 1); reductions were significant. After 10 days, the quantity was equal among females paired with males irradiated at 20 krad, and that of females paired with males irradiated with 40 krad was reduced 67%, a significant difference. However, 32 and 20% of the check females had less than normal quantities of sperm (between 1 and 59% of eupyrene sperm) after 5 and 10 days, respectively. For the purpose of this work, we reported only females with normal or no sperm at all. We suggest that this difference among the check females in quantity of sperm at 5 and 10 days results because female budworms oviposit, reabsorb, or expel unused sperm. For example, Flint and Kressin (1969) found that 15-20% of their strain of tobacco budworms failed to transfer a normal quantity of sperm. Also, Taylor (1967) and North and Holt (1968) indicated that when sperm was not transferred in normal quantities, the female budworm remated (as determined by spermatophores per female) more often than when she received normal quantities.

When males were irradiated at 20 krad, the number of females with sperm of normal motility was reduced 50%, a significant difference, after 5 days compared with females placed with U males and 100% after 10 days

TABLE 1. EFFECTS OF GAMMA IRRADIATION ON REPRODUCTION OF TOBACCO BUDWORMS IRRADIATED AS PUPAE AND PAIRED 0-24 HR AFTER ECLOSION (40-50 SINGLE PAIR REPLICATES).

Cross* (Female X Male)	Irrad- iation dose (krad)	After 5 days				After 10 days							
		% Females with quantity of eupyrene sperm in spermatheca		% Females with indicated motility of sperm		No. of spermato- phores/Female†		% Females with quantity of eupyrene sperm in spermatheca		% Females with indicated motility of sperm		No. of spermato- phores/Female††	
		None**	Normal†	None**	Normal†	None**	Normal†	None**	Normal†	None**	Normal†		
U X U		4	64	4	60	2.96	abc	32	48	40	28	5.28	a
U X T	20	32	40	39	30	3.32	ab	40	50	100	0	3.24	b
T X U	20	0	72	0	74	2.68	bcd	8	50	13	58	4.32	ab
T X T	20	8	44	21	38	3.52	a	32	32	92	8	3.60	b
U X T	40	44	4	91	0	2.52	cd	64	16	96	0	4.24	ab
T X U	40	0	72	0	64	2.52	cd	8	60	0	54	3.52	b
T X T	40	55	10	80	0	2.44	d	40	30	87	0	3.68	b

\*U = untreated; T = treated.  
 \*\* s indicates percent significantly different from check at 5% probability level based on binomial confidence limits of 0-14% for 4% females in check with condition, 20-47% for 32% females and 25-57% for 40% males.  
 † "Normal" quantity of sperm as described by Flint and Kressin (1969). "Normal" motility of sperm based on numerous observations at this laboratory of sperm transferred by untreated males. s indicates percent significantly different from check at 5% probability level based on binomial confidence limits of 49-77% for 64% females in check with condition, 34-63% for 48% females and 16-42% for 28% females.  
 †† Means followed by same letter are not significantly different at 5% level by analysis of variance and Duncan's multiple range test.

(both were significant); at 40 krad, no motile sperm were present in the females after either period. These results are similar to those reported by Snow et al. (1972) for the bollworm.

When I females were paired with U males, the proportion with normal motility was equal to the check at 5 and 10 days after pairing. This finding is especially important if sterile tobacco budworm females are to be used in a release program. Such females receive motile sperm in their spermathecae, and they also receive normal quantities and mate as often as untreated females. Thus I females of this species are completely "normal" in terms of sperm transfer.

Irradiation with 40 krad completely sterilized both sexes; 20 krad caused 64, 83, and 98% sterility in I male X U female, I female X U male, and I male X I female crosses, respectively (Table 2). These results are similar to those shown by others for the same crosses at ca. the same doses (Flint and Kressin 1967, El Sayed and Graves 1969, Wolfenbarger and Guerra 1970). Likewise, frequency of mating was generally similar to that determined in other tests for the lifetime of the insects, regardless of dose or sex irradiated.

Fecundity was significantly affected whenever one or both sexes were irradiated, regardless of dose. However, it is much more important that the increased motility of sperm and the presence of presumably normal quantities of sperm did not cause normal fecundity in I females. Moreover, since all the U females were equally fecund when equal amounts of sperm were transferred, we conclude that the dose of irradiation affected egg laying and fertility more than it affected any other factor.

Mating frequency (Table 1) during these tests was comparable between treatments (including the check) in the 5-day group (2-3 spermatophores/female). When adult pairs were permitted to stay together 10 days, mating frequency was somewhat greater in the check (5 spermatophores/female),

TABLE 2. EFFECT OF GAMMA IRRADIATION ON REPRODUCTION OF TOBACCO BUDWORMS (IRRADIATED AS PUPAE) WHEN 24-HR-OLD MOTHS WERE PAIRED DURING THEIR LIFE (40-50 SINGLE PAIR REPLICATES).

Cross* (Female X Male)	Irradiation dose (krad)	% sterility**	No./Female†		Longevity †	
			Spermato- phores	Eggs	Female	Male
U X U			4.92 a	796 a	13.0 b	13.2 b
U X T	20	64	2.85 b	466 b	12.7 b	12.1 b
T X U	20	83	3.85 ab	449 b	15.9 a	16.3 a
T X T	20	98	2.85 b	307 bc	12.5 b	12.4 b
U X T	40	100	3.83 b	277 e	11.8 b	11.8 b
T X U	40	100	3.92 ab	108 d	14.8 b	15.5 a
T X T	40	100	3.57 ab	101 d	12.8 b	13.2 b

\*U = untreated; T = treated.

\*\*Based on 61% hatch of the check.

†Means followed by same letter are significantly similar by Duncan's multiple range test at 5% level of probability.

but mating frequency was comparable in the rest of the treatments (3-4 spermatophores/female). Similar results were obtained when pairs were permitted to mate their entire lifespan (Table 2).

Irradiation significantly increased male longevity at both doses of irradiation; female longevity was significantly increased with 20 krad. Also, the percentage of females that mated in all treatments (data not shown) was 92-100% after 5 days and 96-100% after 10 days, therefore it appears that in the laboratory the majority of the matings for the lifespan took place during the 1st 5 days after adult emergence.

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