Adult Emergence and Sterility of Indianmeal Moths (Lepidoptera: Pyralidae) Irradiated as Pupae in Dried Fruits and Nuts

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ABSTRACT Raisins, almonds, and walnuts infested with pupae of the Indianmeal moth, *Plodia interpunctella* (Hübner), were treated with gamma radiation (14.4–92.1 krad). Effects of radiation on adult emergence and reproduction were similar in all commodities. Adult emergence was reduced or eliminated at the two highest doses; adults that did emerge were deformed and unable to mate successfully. Females from pupae irradiated with 26.9–31.9 krad were completely sterile, whereas this dose only partially sterilized males. F_1 progeny of irradiated parents showed a high degree of sterility; female progeny were less affected than males. Presence of partially sterile males after irradiation with 26.9–31.9 krad does not reduce the potential of this dose for eliminating infestations in dried fruits and nuts because irradiated males are unlikely to mate with untreated unmated females within irradiated products.

KEY WORDS *Plodia interpunctella*, gamma radiation, nuts, raisins, sterility

THE FEDERAL DRUG Administration recently amended its regulations to permit the use of ionizing radiation at doses $\leq$100 krad[1] (1 kGy) to inhibit maturation of fresh foods and to eliminate infestations of arthropods in food (Young & Bowen 1986). Current practices rely primarily on chemical fumigation, which may result in excessive residues and damage in some foods. As a result, interest in ionizing radiation as an alternative to fumigation has been generated, particularly for dried fruits and nuts in California.

The Indianmeal moth (IMM), *Plodia interpunctella* (Hübner), is a major pest of dried fruits and nuts. Considerable work has been done on the effect of ionizing radiation on this insect. The possibility of developing a sterile insect release program using radiation-sterilized IMM (Ashrafi et al. 1972a,b, Brower 1975, 1979, Ahmed et al. 1976), along with the use of direct radiation treatments to eliminate infestation in foods (Brower & Tilton 1970, 1971, 1972, Tilton et al. 1978), has been investigated. The effect on all stages of the IMM must be determined when direct radiation treatments are considered, because any stage may be present within the product.

The criteria for control with radiation treatments depend on the stage being treated. Economically practical doses applied to IMM larvae may reduce feeding, delay or halt development, or prevent successful pupation (Ashraf et al. 1971). Comparable doses applied to pupae and adults may not affect longevity or survival of these stages, but may cause partial or complete sterility (Brower 1976, 1981). The objective of our study was to assess the effect of gamma radiation on IMM pupae in foodstuffs, using pupal mortality and subsequent adult fertility, longevity, and mating success to evaluate treatments.

Materials and Methods

Two tests of similar design were done to determine the effect of radiation on IMM pupae. In the first, evaluation was based on pupal mortality and subsequent adult fertility. The second test also used adult longevity, mating success, and progeny sex ratios to evaluate radiation effect.

Test units consisted of glass canning jars (0.48 liter), in which the lid of each jar was replaced with filter paper (9.0 cm) overlying copper screen (40 mesh). All units were steam-sterilized before the addition of 125 g of a selected commodity. Foods tested were shelled 'Nonpareil' almonds, 'Hartley' walnut nutmeats, and raisins.

Mature 12-d-old IMM larvae were obtained from stock cultures maintained continuously on a bran diet (Finney & Brinkman 1967) in our laboratory. Twenty larvae, segregated by sex, were placed in each test unit. Larvae began spinning cocoons within the food layer almost immediately. Five test units of each sex for each of the three foods were used at each of five treatment levels.

[1] We chose to use the older, more familiar rad for expressing the absorbed radiation dose, even though the System Internationale (SI) unit is the Gray (Gy). 1 krad = 10 Gy.

Irradiation was done at the Sandia National Laboratories in Albuquerque, N. Mex. Pupae were irradiated on day 9 after infestation. The day before treatment, paper toweling was loosely packed on top of the food in each jar to prevent shifting and injury to the test insects during transit. Jars were then wrapped in plastic foam sleeves and packed in insulated plastic ice chests for shipment by air freight to Albuquerque. Controls (0 krad) were also shipped, but left untreated.

Applied doses were monitored by dosimeters (TLD-400 [LiF]) added to test units before treatment. After irradiation, the dosimeters were analyzed and the actual dose received by the test units was determined. For the first experiment, average applied doses were 15.7, 31.9, 60.7, and 92.1 krad. Doses of 14.4, 26.9, 59.4, and 82.2 krad were received in the second test.

Immediately after treatment, the units were repacked and returned to Fresno. All units were held at 27°C and newly emerged adults were counted and removed daily. After adult emergence ceased, the material in each jar was carefully examined for dead insects and pupal exuvia.

Fertility Studies: P_1 Generation. When suitable numbers of emerging P_1 adults were available, experiments were done to determine the effect of radiation on reproductive potential. Three different mating combinations were used: irradiated females crossed with irradiated males (I), irradiated females outcrossed with untreated males (N), and irradiated males outcrossed with untreated females. Crosses of untreated insects obtained from control pupae were used as controls.

Each mating pair was placed in a plastic food carton (0.48 liter). A hole (1 cm diameter) was cut in each lid and covered with fine screen to provide ventilation. Moths were allowed to mate and oviposit within the cartons. Most eggs were laid within a circular groove (1 mm deep) on the lid of the carton. Egg viability was estimated by counting the number of hatched and unhatched eggs present on the lid. To facilitate observation of P_1 adults, only a small amount (20 g) of the bran diet medium was initially added to each carton to provide food for neonate F_1 larvae. When both adults were dead, an additional 80 g of diet was added to ensure an adequate food supply for the developing larvae.

The cartons were held at 27°C and checked daily for F_1 adult emergence. Adults were collected each day at 0830 hours and stored frozen for later counting. Single, unmated adults emerging shortly after 0830 hours were used in F_1 generation fertility studies.

In the second experiment, P_1 adult longevity was determined by examining the cartons daily. Dead P_1 adults were removed and stored in 70% EtOH after determination of their sex. Females were later dissected and the presence or absence of spermatophores was determined. The sex of F_1 adults was also determined and sex ratios were calculated.

Table 1. Percentage of adults emerging from IMM pupae irradiated in raisins, almonds, and walnuts

Dose (krad)	Test 1[a]		Dose (krad)	Test 2[a]	
	♀♀	♂♂		♀♀	♂♂
0	93a	94a	0	90a	89a
15.7	93a	93a	14.4	89a	90a
31.9	92a	85b	26.9	84a	86a
60.7	72b	65c	59.4	57b	56b
92.1	0c	0d	82.2	6c	3c

Values given are means of combined data for all commodities (n = 300). Analysis of variance with arcsine transformation.

[a] Column means followed by the same letter are not significantly different ($P > 0.05$; Duncan's [1955] multiple range test).

Fertility Studies: F_1 Generation. Adult progeny from each P_1 mating combination were crossed in three different mating combinations, designated as F_1 female × F_1 male (F_1 intracrosses), F_1 female × N male (outcrossed F_1 females), and N female × F_1 male (outcrossed F_1 males). All procedures were identical to P_1 fertility studies except that egg viability was not determined. Because irradiated P_1 females were sterile at the higher treatment levels and produced few progeny at 15.7–14.4 krad, only progeny from outcrossed P_1 males were used in the first test. In the second test, progeny of P_1 intracrosses and outcrossed P_1 females treated with 15.7–14.4 krad were also used.

Data were subjected to analysis of variance; arcsine transformations were used on percentage of adults emerging from irradiated pupae. Significant ($P < 0.05$) treatment means were separated by Duncan's (1955) multiple range test. A χ^2 test used to evaluate F_1 sex ratios.

Results

Type of food did not significantly affect the response of IMM to radiation. Because no significant differences were found in adult emergence or progeny numbers from walnuts, almonds, or raisins, the data were combined for further analysis.

No adults emerged from pupae treated at the highest dose (92.1 krad; test 1) (Table 1). The few adults that emerged from pupae treated with 82.2 krad (test 2) were deformed and died almost immediately after emergence. Emergence was reduced significantly compared with controls at 60.7–59.4 krad in both tests, and the resulting adults were weak, deformed, and unable to mate. No significant reduction in adult emergence was found at the lowest three doses (14.4, 15.7, and 26.9 krad), whereas emergence from male pupae was reduced at 31.9 krad.

Fertility Studies: P_1 Generation. Reproductive studies were limited to the two lowest treatment levels in both tests because only deformed adults emerged at higher doses. In the second test, suc-

Table 2. Viability of eggs and resulting numbers of adult progeny produced by IMM pupae irradiated in raisins, almonds, and walnuts

Dose (krad) Test 1 (test 2)	Cross	% eggs hatching[a]		Adult progeny[a]			
				Test 1		Test 2	
		Test 1	Test 2	No. progeny	% reduction[b]	No. progeny	% reduction[b]
0	N♀ × N♂	97.2	99.1	241.5a	—	200.3a	—
15.7 (14.4)	I♀ × I♂	0.6	0	0.3c	99.9	1.3d	99.3
	I♀ × N♂	1.7	1.9	0.6c	99.8	1.8d	99.1
	N♀ × I♂	78.8	86.1	197.1b	18.4	156.0b	22.1
31.9 (26.9)	I♀ × I♂	0	0	0c	100	0d	100
	I♀ × N♂	0	0	0c	100	0d	100
	N♀ × I♂	34.1	39.4	22.9c	90.5	48.4c	75.8

Values given are means of combined data for all commodities (test 1, $n = 15$; test 2, $n = 30$).
[a] Column means followed by the same letter are not significantly different ($P > 0.05$; Duncan's [1955] multiple range test).
[b] Percentage reduction compared with control progeny numbers.

cessful mating was indicated by the presence of spermatophores in the females. All control females contained at least one spermatophore. The percentage of females with spermatophores in the 14.4-krad treatment was 93.3% in all crosses. In the 26.9-krad treatment, the percentages of mated females for intracrosses, outcrossed female, and outcrossed male combinations were 64.3, 76.7, and 72.4%, respectively.

The average adult life span of untreated females in the second test was 7.3 ± 1.31 d ($\bar{x} \pm$ SD). Treatment with 14.4 and 26.9 krad significantly extended female life to 12.7 ± 2.73 and 9.7 ± 2.75 d, respectively. Life spans of untreated adult males averaged 9.6 ± 2.02 d. Males irradiated with 14.4 krad lived 9.4 ± 1.53 d. At 26.9 krad, life spans of males were significantly shortened to 6.8 ± 1.38 d.

Both egg viability and numbers of adult progeny were greatly reduced compared with controls in the intracrosses and outcrossed female combinations at 15.7–14.7 krad (Table 2). No eggs hatched in these combinations at 31.9–26.9 krad. A slight reduction in eggs hatching and progeny numbers occurred in the outcrossed male combinations at 15.7–14.4 krad, with a greater decrease at 31.9–26.9 krad.

Sex ratios of adult progeny from controls were nearly 1:1. Progeny numbers from intracrosses and female outcrosses at 14.4 krad were too low to determine sex ratios accurately. Progeny sex ratios from male outcrosses were slightly skewed in favor of males ($\chi^2 = 12.2$); males constituted 52.5% of progeny. At 26.9 krad, male outcrosses produced 65.1% males ($\chi^2 = 132.5$).

Fertility Studies: F_1 Generation. At the lower treatment levels, the highest overall reduction in F_1 reproductive capacity occurred when both parents were irradiated (Table 3). F_1 progeny of outcrossed irradiated males produced fewer offspring than F_1 progeny of outcrossed irradiated females, although the difference was not significant. The reproductive capacity of female F_1 progeny was consistently less affected than that of their male counterparts.

The reproductive capacity of F_1 progeny of outcrossed males was greatly reduced at 31.9–26.9 krad. Again, female progeny were slightly less affected than males, but fertility was reduced by ≥93.5% in all mating combinations.

Mating success followed a pattern similar to that of progeny numbers. All control females and outcrossed F_1 females at the lower treatment levels contained spermatophores (Table 3). At the higher treatment levels, the highest percentage of females containing spermatophores occurred in the outcrossed F_1 females. F_1 intracrosses and outcrossed male combinations showed different percentages of successful mating ranging from 20.0 to 86.7%. Sex ratios of F_1 progeny were very close to 1:1.

Discussion

To obtain adequate control of IMM infesting dried fruits and nuts, reproduction of the treated insects must be stopped. The complete sterility of females after irradiation with ca. 30 krad suggests this dose is an adequate treatment level. Although 31.9–26.9 krad had little or no effect on adult emergence from irradiated pupae, no viable eggs were produced; therefore, the product received no further damage. Our results agree with those of Brower (1976), in which complete sterility of female pupae was obtained at 35 krad.

Males were only partially sterile at 31.9–26.9 krad. Brower (1976) found that 50 krad greatly reduced the number of adult progeny of irradiated males mated with untreated females, but sterility was not complete. However, for control purposes, incomplete male sterility should not be considered a disadvantage; within the treated product, irradiated males presumably will mate with irradiated, sterile females. Treated males could produce progeny only by mating with females invading the

Table 3. Progeny numbers and mating success of F_1 offspring of IMM pupae irradiated in raisins, almonds, and walnuts

Dose (krad) Test 1 (test 2)	Parental cross	F_1 cross	Test 1[a]		Test 2[a]		
			No. progeny	% reduction[b]	No. progeny	% reduction[b]	% spermatophores[c]
0	Control	N♀ × N♂	280.6a	—	332.6a	—	100
15.7 (14.4)	I♀ × I♂	F_1♀ × F_1♂	—	—	1.5b	99.5	33.3
		F_1♀ × N♂	—	—	84.0ab	74.7	100
		N♀ × F_1♂	—	—	22.0b	93.4	20.0
	I♀ × N♂	F_1♀ × F_1♂	—	—	35.3b	89.3	33.3
		F_1♀ × N♂	—	—	176.7ab	46.9	100
		N♀ × F_1♂	—	—	107.5ab	67.8	70.0
	N♀ × I♂	F_1♀ × F_1♂	3.9c	98.6	10.7ab	96.8	66.7
		F_1♀ × N♂	118.3b	57.8	164.7ab	50.5	100
		N♀ × F_1♂	38.8c	86.2	58.0b	82.6	86.7
31.9 (26.9)	N♀ × I♂	F_1♀ × F_1♂	16.8c	94.0	0b	100	46.7
		F_1♀ × N♂	18.2c	93.5	5.0b	98.5	86.7
		N♀ × F_1♂	7.3c	97.4	0b	100	40.0

[a] Column means followed by the same letter are not significantly different ($P > 0.05$; Duncan's [1955] multiple range test).
[b] Percentage of reduction from control progeny numbers.
[c] Percentage of females with one or more spermatophores.

product after irradiation. These invading females probably would be mated already, so the presence of partially sterile males would not contribute significantly to a posttreatment infestation.

Adults from pupae irradiated with 31.9–26.9 krad showed a decrease in the presence of spermatophores compared with controls, indicating a decrease in successful mating. This is probably due to somatic tissue damage resulting in subnormal adults. Ahmed et al. (1976) studied the sexual competitiveness of IMM irradiated as mature pupae and found only a slight decrease in the competitiveness of males irradiated with 50 krad. Females irradiated at that dose mated more often than controls. However, Ahmed et al. (1976) chose mature pupae for irradiation because adult development was essentially complete and the least amount of somatic tissue damage would result. The stage irradiated in our studies was less developed and more susceptible to somatic tissue damage.

The F_1 progeny of males irradiated as pupae with 31.9–26.9 krad showed a high degree of sterility. The reproductive capacity of female F_1 progeny was less affected than that of male F_1 progeny, but still showed a reduction in progeny numbers of >93%. Successful mating of male F_1 progeny was considerably reduced, also indicating a decrease in sexual competitiveness.

Although the presence of partially sterile males in the treated product should not present a threat of reinfestation, these males may actually inhibit posttreatment infestation in the manner of a sterile insect release (Cornwell et al. 1966). However, our results indicate that such males may not be competitive with untreated males and would have little impact on invading populations.

Previous work on the potential of gamma radiation to control IMM populations in dried fruits and nuts used eggs, young larvae, and ovipositing adults as target stages (Brower & Tilton 1970, 1971, 1972). Those studies suggested doses of 20–25 krad to obtain practical levels of control. Our studies support this recommendation because intracrosses of IMM irradiated at 15.7–14.4 krad were very nearly sterile. However, because the life span of female moths irradiated as pupae was extended by >5 d with 15.7–14.4 krad, the use of 30 krad or higher may be necessary to reduce female longevity. Also, Brower & Tilton (1972) showed that a dose of 40 krad would probably be needed to prevent feeding damage in packaged nutmeats. Our additional studies (unpublished data) indicate that even 45 krad will not completely stop feeding damage caused by older larvae, but 30 and 45 krad will reduce damage considerably. Estimates of maximum/minimum dose ratios for most irradiator designs range from 1.5:1 to 3:1, and so 40 or 45 krad may be too high for product safety or to conform with the new regulations. A minimum dose of 30 krad is recommended for practical control.

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