INFLUENCE OF PUPAL AGE ON THE RESPONSE OF THE ALMOND MOTH, CADRA CAUTELLA (WALKER), TO GAMMA RADIATION

by

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INTRODUCTION

Of the pests that infest dried cocoa beans in storage, Cadra cautella (Walker) is the most serious Lepidoptera species in Ghana, the world's leading producer of cocoa. C. cautella is called the almond moth in North America, cocoa moth and tropical warehouse moth in Ghana, and is known as the fig moth in other parts of the world. Damage to the dried cocoa beans is caused by the larvae feeding on the germs of the beans in the loose upper layers (Rawnsley 1959), and by the production of silk webbing on the stacks.

Pupation generally takes place along the outer layer of the bags, with a few larvae pupating within the stacked bags (Rawnsley 1958, 1959). When adult moths emerge, they normally leave the cocoa stacks to mate in unoccupied sections of the warehouse, but where movement to the outside is restricted, mating is prevented due to inability of adults to come together and undergo courtship and mating (Amuh and Amoako-Atta 1973).

Rawnsley (1958) described the limitations of insecticides for controlling the almond moth in Ghana. Amuh (1971) suggested the use of gamma irradiation as an alternate measure of control. Currently, the Ghana Atomic Energy Commission, with the assistance of the International Atomic Energy Agency, is studying that possibility.

The sterile-male technique, successfully used to eradicate the screw-worm fly, Cochliomyia hominivorax (Cqrl.) from the island of Curacao (Baumhover et al. 1955, Lindquist 1955) has not met with equal

success on some lepidopterous pests. Many investigators have reported the high radioresistance of Lepidoptera species as compared with other insect orders (Pendlebury et al. 1966, Watters 1968, La Chance et al. 1967, Tilton and Brower 1973, Knipling 1970, La Chance and Riemann 1973) thus requiring higher dosages to completely sterilize the males.

Radiation studies on the almond moth date from 1952 (Dennis 1961) and has been investigated by Pendlebury et al. (1966), Ahmed et al. (1971), Calderon and Gonen (1971), Gonen and Calderon (1971), Cogburn et al. (1973). Calderon and Gonen (1971) determined the effects of radiation on the adult moths, and its effects on the different morphological stages were studied by Cogburn et al. (1973).

If radiation is to be successfully utilized to control the almond moth on cocoa, the effects of gamma radiation on different morphological stages of the insect must be studied. Laudani et al. (1965), Ouye et al. (1964), and Tilton et al. (1966) have shown that the response of an insect to radiation changes during its morphological development. However, detailed information on the response of almond moth pupae of different ages to gamma radiation is lacking.

The purpose of this study was to investigate the effects different radiation dosages applied to pupae of different ages have on adult emergence, adult deformities, mortality of adult moths after emergence, and female fecundity and sterility of irradiated moths.

MATERIALS AND METHODS

C. cautella was obtained from the laboratory stock maintained in the Stored-Product Insect Laboratory, Department of Entomology, Kansas

State University. Procedures used to maintain almond moth cultures were similar to those described by Strong et al. (1968) with the exception of the culture media, which consisted of wheatbran, ground maize, and glycerine in the ratio 8:8:1 (wt/wt). Cultures and all experiments were maintained in a room held at 27 ± 1°C, and 65 ± 5% R.H., with a 12:12 hr photoperiod.

Preliminary investigations showed that moth emergence occurred 32 ± 1 days after cultures were initially established, with peak pupal formation occurring on the 27th day. Thus, cultures 26 days old were used to obtain last instar larvae. Matured larvae ready to pupate normally crawl out of the food, wander around the inner walls of the culture jars before entering the pupation rolls (rolls of corrugated cardboard 1-in. wide by ca. 3-in. diam) placed on the surface of the media. In order to obtain pupae of a known age the pupation rolls initially placed on the culture media were removed from cultures 26 days old. Fresh pupation rolls were placed in the cultures for 8-12 hr, then removed. They were then unfolded and the last instar larvae that had entered to pupate were collected and separated according to sex, distinguished by the male larvae having dark pigmentation (developing testes) on the mid-dorsum of the 5th abdominal segment.

After the larvae had been sexed, they were placed on pupation rolls composed of corrugated cardboard 1-in. thick and 2-in. diam (Plate 1, Fig. 1). Thirty larvae of the same sex were placed on each pupation roll. Pupation generally occurred between 12-24 hr after the last

instar larvae had been reintroduced into the pupation rolls. Thus, pupae were 1 day old a day after the larvae had been reintroduced to the pupation rolls. The pupation rolls were held in the culture room until the pupae reached the desired age.

The pupae were irradiated when 2, 4, 6, and 8 days old at dosages of 0, 10, 20, or 30 krad in a Co-60 gammacell 220 source, located in the Nuclear Engineering Department, Kansas State University. The Co-60 had dosage rates ranging from 1810 rad/min to 1710 rad/min, during the experimental period. Four replications of 30 pupae each were used for each age group and sex at each dosage level. Plastic containers 9-cm in diam and 7-cm deep with an open top (Plate 1, Fig. 2) were used to hold two pupation rolls during radiation. Male and female pupae for each age group and dosage level were held in the same container. After irradiation the pupation rolls were transferred from the plastic containers and placed separately in mason pint jars. These were returned to the culture room for adult emergence. The numbers of normal and deformed adults emerging from the pupation rolls were recorded. Adult moths were considered as deformed when any of the following four criteria were present: (1) twisted wing; (2) extended abdominal segments; (3) external genitalia protruding from the last abdominal segment; and (4) incomplete emergence, with part of the body trapped within the pupation roll.

Fertility and fecundity experiments were conducted on active adult moths that emerged from irradiated 6- and 8-day-old pupae. Irradiated females (I♀) were mated with normal males (N♂), and irradiated males (I♂) with normal females (N♀). Newly emerged moths, 0-8 hr old were

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PLATE I

Fig. 1. Pupation roll composed of corrugated cardboard 1 in. thick and 2 in. in diam. Each roll contained 30 developing pupae of the same age and sex.

Fig. 2. Plastic container for holding the pupation rolls during radiation. Two pupation rolls, one containing male pupae and the other females, were separated by a cotton pad (1 cm thick).

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PLATE I

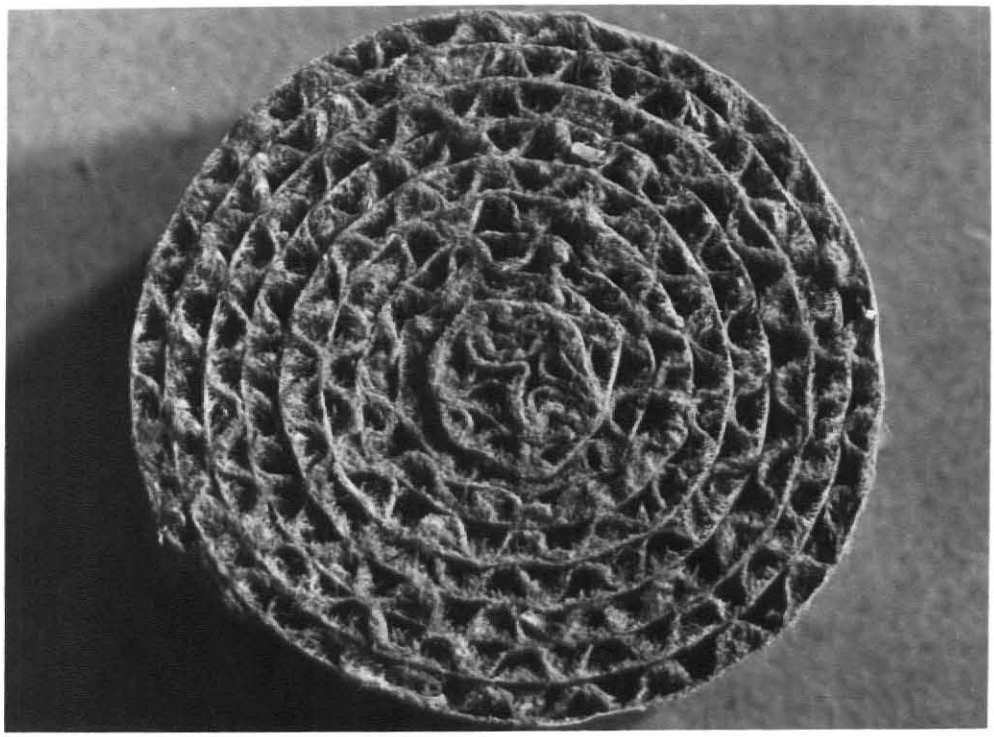

Fig. 1

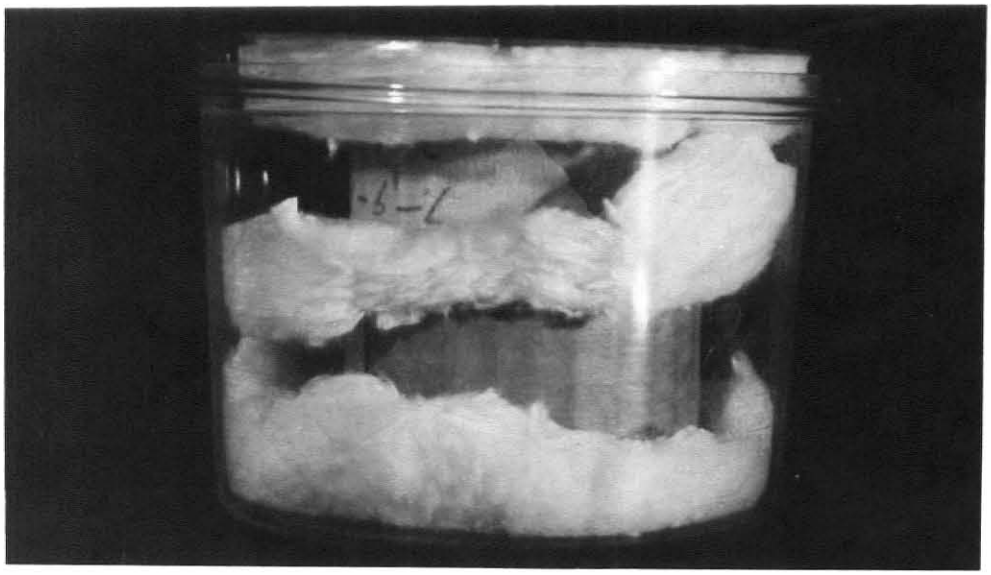

Fig. 2

used in the crosses. To determine oviposition, single pairs were mated in 2-oz glass vials; cotton was used as a stopper. Mated female moths laid eggs loosely in the glass vials. The eggs were collected daily for 8 days by removing the mated pairs from the vials with a suction aspirator, turning vials mouth side down into glass petri dishes (5-cm diam) to collect the eggs, then the moths were reintroduced into their respective vials for further oviposition. The number of eggs laid by each pair and number of ovipositing pairs were recorded. The eggs were incubated in the rearing room. The percent egg hatch in each treatment was obtained by checking the eggs every 8 hr until hatching was completed. Mortality of the mated pairs was recorded.

Data were analyzed using Duncan's multiple range test and the analysis of variance with least significant difference at the 5% level, to evaluate the differences between means and to determine the radiation effect on the degree of sterility of mated pairs.

RESULTS

Effects of Radiation on Adult Emergence and Level of Adult Deformity

Age of the pupae at the time of irradiation significantly affected moth emergence. Irradiated 2-day-old pupae were the most susceptible with no emergence at any treatment level. Pupal development ceased at the black-eye stage and wing formation did not occur (Plate II, Fig. 1). As shown in Fig. 1, 60 and 30% of the female and male adults, respectively, emerged from 4-day-old pupae exposed to 10 krad. All of

PLATE II

Fig. 1. Pupae irradiated when 2 days old; pupal development ceased at the black-eye stage without wing formation.

Fig. 2. Female moths that had emerged from 4-day-old pupae exposed to 10 krad; note the twisted and reduced wings.

Fig. 3. Male moths that had emerged from 4-day-old pupae exposed to 10 krad; note the deformed wings and extended abdominal segments with external genitalia protruding from the last segment.

PLATE II

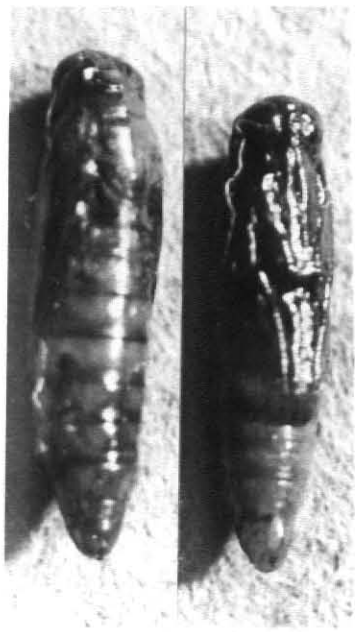

Fig. 1

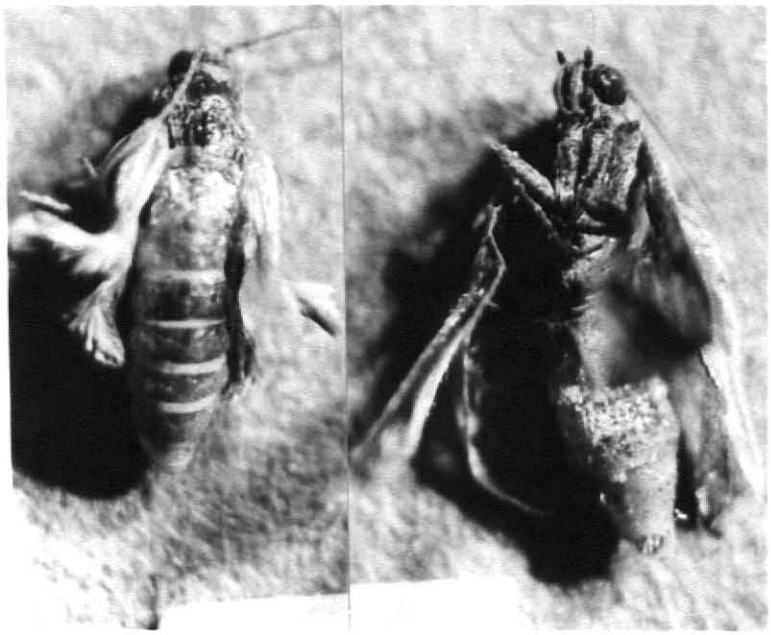

Fig. 2

Fig. 3

the adults were deformed (Plate II, Figs. 2 and 4) and all died within 48 hr after emergence. Although adult features were visible in the developing pupae, adult eclosion was prevented from 4-day-old pupae exposed to 20 or 30 krad.

As shown in Fig. 1, the mean % adult emergence from pupae 6 and 8 days old exposed to 10 or 20 krad was between 87 and 97% and was not significantly different from the % emerging from pupae irradiated with 0 krad. Moth emergence was significantly reduced when 6-day-old pupae were exposed to 30 krad. Those that emerged had deformed wings or extended abdominal segments (Plate II, Figs. 2 and 3).

For almond moths that emerged, radiation did not significantly affect length of the mean pupal period, which for males was 9.7 days, significantly longer than for females (9.2 days).

Adult deformities were expressed by abnormalities in the wings (Plate II, Fig. 3), abdominal segments, and the external genitalia (Plate II, Fig. 3). The % of deformed adults emerging from pupae irradiated when 6 and 8 days old indicated an age/dose interaction but sex was not a factor. When the dosage for 6-day-old pupae was increased from 20 to 30 krad, 6 times as many deformed adults emerged (Table 1). Increasing the dosage for 8-day-old pupae did not cause a significant increase in deformities in adults emerging, probably because pupae by that time had completely developed.

Effects of Radiation on Adult Mortality

As shown in Table 2, adults emerging from pupae exposed when 6 or 8 days old to 0, 10, or 20 krad did not show a large increase in

Fig. 1. Effects of irradiation on mean % adult emergence from 4-, 6-, and 8-day-old pupae exposed to 0, 10, 20, and 30 krad.

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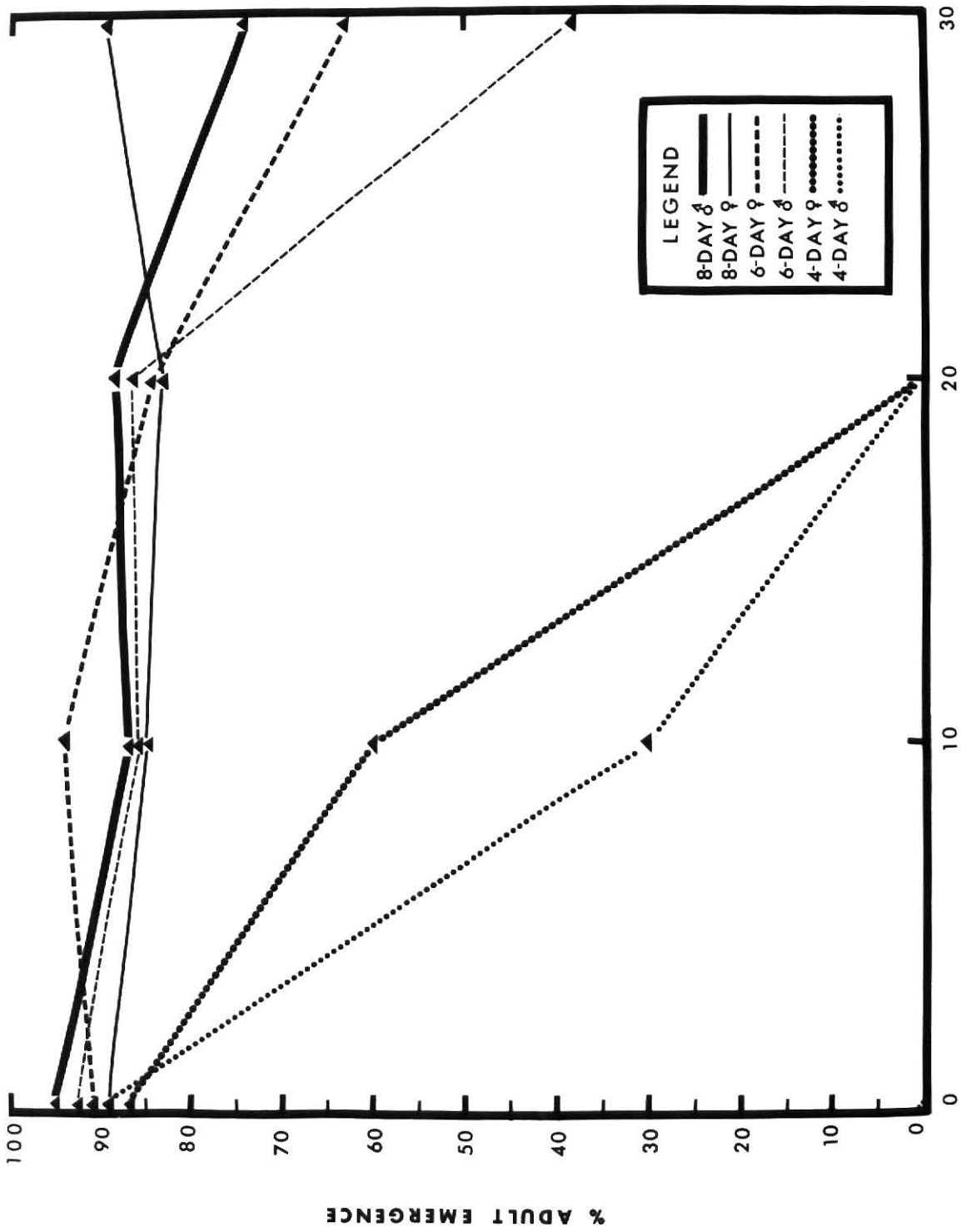
% ADULT EMERGENCE
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
DOSAGE (KRAD)
LEGEND
8-DAY ♂
8-DAY ♀
6-DAY ♀
6-DAY ♂
4-DAY ♀
4-DAY ♂

Fig. 1

Table 1. Mean % of adults deformed on emergence from pupae irradiated when 4, 6, and 8 days old.

Dose (krad)	% adults deformed ♂♂	% adults deformed ♀♀
	4-day-old pupae	
0	1	0
10	100	100
20	a	a
30	a	a
	6-day-old pupae	
0	2	1
10	2	0
20	13	16
30	90	83
	8-day-old pupae	
0	0	0
10	0	0
20	0	0
30	10	2

[a] Moths did not emerge from pupae irradiated at this level.

Table 2. Mean % mortality of male and female adult moths on the 2nd and 6th day after emergence from irradiated 6- and 8-day-old pupae.

Dose (krads)	% Adult mortality			
	2 Days after emergence		6 Days after emergence	
	6-day-old pupae	8-day-old pupae	6-day-old pupae	8-day-old pupae
		♂♂		
0	0	0	7	3
10	0	0	15	3
20	2	2	27	3
30	90	5	100	16
		♀♀		
0	2	1	10	2
10	2	0	15	9
20	3	0	26	14
30	72	1	100	21

mortality from the 2nd to 6th day after emergence. However, mortality was greater 6 days after emergence in adults emerging from 6-day-old pupae. Adults emerging from pupae irradiated with 30 krad when 6 days old had a mean % mortality of 72 and 90% for males and females, respectively, on the 2nd day, and 100% mortality on the 6th day after emergence. Deaths recorded for adults emerging from pupae exposed to 30 krad when 6 days old coincided with the high % of adult deformities (Table 1).

Effects of Radiation on Moth Fecundity

The effects of irradiation on the egg-laying potential of irradiated moths crossed with normal moths are summarized in Table 3. Using analysis of variance and testing for least significant difference, the results showed that the radiation effect on fecundity of ovipositing females reflected interaction of age, sex, and dose. Compared with oviposition of females emerging from non-irradiated pupae, only 1/3 as many eggs were oviposited by females emerging from pupae exposed to 10 krad when 6 days old and only 1/5 as many when exposed to 20 krad. Fecundity of adults that emerged from 6- and 8-day-old pupae irradiated with 30 krad was much reduced, as was the number of ovipositing adults.

Fecundity of the adults emerging from pupae irradiated when 6 days old was more significantly affected than was that of those emerging from pupae irradiated when 8 days old. Results in Table 3 also show that the oviposition potential of irradiated females was more significantly reduced than was that of normal females crossed with irradiated males, perhaps because radiation affected the oogenesis

Table 3. Effects of irradiation dosage on fecundity of irradiated females (I♀) crossed with normal males (N♂), and irradiated males (I♂) crossed with normal females (N♀).

Dosage (krad)	I♀ x N♂			I♂ x N♀		
	No. of females	Average no. of eggs/♀♀	Total no. of eggs laid	No. of females	Average no. of eggs/♀♀	Total no. of eggs laid
			6-day-old pupae			
0	42	199	8358	57	200	11,400
10	32	70	2240	38	142	5396
20	21	37	770	16	52	832
30	3	23	69	6	32	192
			8-day-old pupae			
0	50	181	9050	58	201	11,658
10	53	133	7049	34	168	5712
20	54	54	2946	35	115	4025
30	28	31	868	31	43	1333

of the developing females. Ovaries of irradiated 6-day-old female pupae were in most cases smaller than normal.

Effects of Radiation on Fertility

Using the mean % hatchability of eggs as a criterion for reduced sterility, it is obvious from data presented in Fig. 2 that fertility in both sexes decreased when radiation was increased. The increase in sterility was also greater in females than in males. Dosages of 20 and 30 krad induced greater sterility in adults emerging from pupae irradiated when 6 days old than from pupae irradiated when 8 days old.

Twenty krad was considered the dosage that induced sterility in 6-day-old pupae, and 30 krad the dosage required to induce the same level of sterility in 8-day-old pupae.

DISCUSSION AND CONCLUSION

Results obtained in this study on the almond moth indicate that the age of the pupae at the time of irradiation influenced adult emergence. The younger the pupae when irradiated, the more the radiosensitivity. Similar findings were obtained by Qureshi et al. (1968) on the Angoumois grain moth, Sitotroga cerealella (Olivier), Ouye et al. (1964) on pink bollworm, Pectinophora gossipiella (Saunders), and by Cogburn et al. (1966) on the Angoumois grain moth and the Indian meal moth, Plodia interpunctella (Hubner).

Between the 4th and 6th day, the almond moth pupae decreased markedly in susceptibility to radiation. Adults emerging from pupae exposed to

Fig. 2. Hatchability of eggs laid by adults emerging from irradiated 6- and 8-day-old pupae.

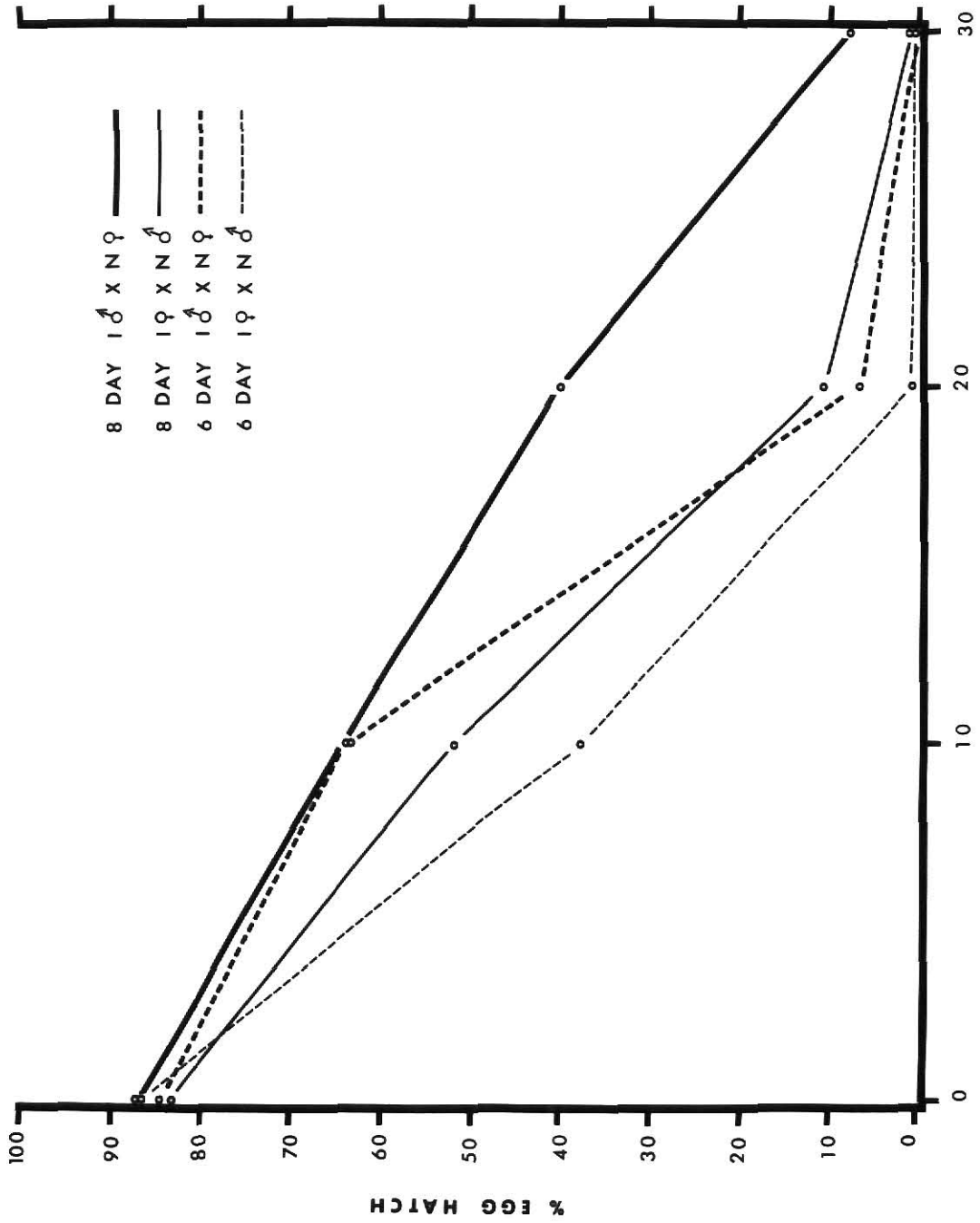
8 DAY 1♂ X N ♀
8 DAY 1♀ X N ♂
6 DAY 1♂ X N ♀
6 DAY 1♀ X N ♂
% EGG HATCH
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
DOSAGE (KRAD)

Fig. 2

10 or 20 krad when 6 days old were less affected than adults emerging from pupae exposed to 10 krad when 4 days old. Adults did not emerge from 4-day-old pupae exposed to 20 or 30 krad. Ouye et al. (1964) obtained similar results with the pink bollworm, in which pupae 5 days old marked the beginning of decrease in radiation susceptibility. However, Cogburn et al. (1973) obtained 67.8% almond moth emergence from 4-day-old pupae irradiated with 30 krad, a dosage level that prevented adult emergence in the present study. Hooper (1970), reviewing work on the Mediterranean fruit fly, Ceratitis capitata (Wied.), noted that, whereas "no emergence occurred when 5-10 krad were given to pupae 5 days before emergence, better than 50% emergence was obtained with the same dosage one day later."

The interaction of the radiation dose and age of the pupae significantly influenced the level of adult deformities. When 4-day-old pupae were irradiated with 10 krad, 100% of the emerged adults that had deformed wings and/or distended abdomens; many had the genitalia extended. A few of the adults emerging from 8-day-old pupae were deformed when irradiated with 30 krad, but more than 83% of the adults emerging from pupae irradiated when 6 days old were deformed at the same dosage level. Similar results were obtained with other lepidopterous insects (Cogburn et al. 1966, Qureshi et al. 1968, Ouye et al. 1964, Flint and Kressin 1967, and Pendlebury et al. 1966).

Longevity of moths emerging from 4-day-old pupae irradiated with 10 krad and 6-day-old pupae irradiated with 30 krad was 6 days. Less than 21% of adults emerging from 8-day-old pupae irradiated with 30 krad died during the first 6 days after emergence. These results coincided

with the high number of deformed adults emerging from the younger pupae and was probably due to the somatic injuries caused by the radiation.

Fecundity of irradiated moths mated with normal moths was reduced as the dosage level was increased. Age of the pupae at the time of irradiation also affected fecundity; when females from irradiated 6-day-old pupae were crossed with normal males (I♀ x N♂) the average number of eggs per mated female was significantly reduced from 199 to 70 eggs at 0 to 10 krad, respectively, a reduction of ca. half the average number of eggs laid by females from irradiated 8-day-old pupae at 10 krad. In the receiprocal crosses (I♂ x N♀), the average number of eggs laid by normal females mater to males from 6- and 8-day-old pupae treated with 10 krad were 142 and 168 eggs, respectively. Fecundity of irradiated females mated with normal males was more significantly reduced than that of normal females mated with irradiated males. Similar results have been obtained by North and Holt (1970) on the cabbage looper, Trichoplusia ni (Hubner), Qureshi et al. (1968) on the Angoumois grain moth, and Cogburn et al. (1966) on the Angoumois grain moth and the Indian meal moth. Calderon and Gonen (1971) also observed the fecundity of almond moth females irradiated at adult stage to be more significantly reduced than that of normal females mated to irradiated males at different dosage ranges of 0 to 45 krad. White and Hutt (1970) observed significant reduction in fecundity of irradiated female codling moths, Laspeyresia pomonella (L.), but fecundity reduction was not affected by the increase in radiation dose.

High doses (20 and 30 krad) of gamma radiation significantly reduced the mating ability of both sexes of the almond moth when irradiated as

6-day-old pupae. However, the mating ability in the adults emerging from irradiated 8-day-old pupae was not significantly reduced. North and Holt (1970) observed similar reduced mating in males of the cabbage looper emerging from pupae irradiated with various doses of gamma radiation 5 days before emergence. They also found that the fecundity reduction in the normal females was due to failure of irradiated males to transfer enough sperm to elicit an ovipositional response. Joubert (1964) found that in Angoumois grain moth pupae the germarium of the ovariole ceased activity in the mid-pupal stage or just before that stage was reached, thus limiting the activity to the number of oocytes in the pupal ovariole. Ashrafi et al. (1972) observed that when last instar larvae of the Indian meal moth were irradiated, high radiation doses caused significant somatic and gonial injuries; spermatocytes and spermatids were in that order next most sensitive.

Results obtained in this study showed that there was a progressive decrease in fecundity of the almond moth with increase in radiation dose in both types of mating combinations (irradiated males crossed with normal females and irradiated females crossed with normal males). The reduced fecundity in the almond moth was probably due to: (1) reduced transfer of active sperms by irradiated males in normal females, as shown by North and Holt (1970, 1971) in the cabbage looper, and (2) limited production of oocytes in the developing female. Dissections revealed that ovaries in irradiated 6-day-old female pupae were in most cases smaller than those in untreated pupae.

Twenty krad gave 99 and 93% sterility in females and male moths, respectively, emerging from pupae irradiated when 6 days old and 30 krad were required to give the same level of sterility in moths emerging from pupae irradiated when 8 days old. These sterility levels for 6- and 8-day-old pupae did not cause any significant injuries or deaths, and were comparatively lower than those required to sterilize almond moth adults (Calderon and Gonen 1971, Cogburn et al 1973, Tilton and Brower 1973). Female moths were sterilized at a comparatively lower radiation dose than were males, agreeing with the results reported by Calderon and Gonen (1971), Cogburn et al. (1973), and Pendlebury et al. (1966).

Irradiation of pupae either 6 or 8 days old, will probably fit into a radiation control program. Lower radiation dosages are required to induce sterility in 6- and 8-day-old almond moth pupae, than for adults. However, further research needs to be conducted before use in such a program can be established.

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INFLUENCE OF PUPAL AGE ON THE RESPONSE OF THE ALMOND MOTH, CADRA CAUTELLA (WALKER), TO GAMMA RADIATION

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Almond moth (Cadra cautella Walk.) pupae, 2, 4, 6, and 8 days old were irradiated in a Co-60 Gammacell 220, at dosage levels of 0, 10, 20, and 30 krad. Four replications of 30 pupae each were used for each age group and sex at each dosage level. After irradiation all insects were placed in a culture room maintained at 27 ± 1°C and 65 ± 5% R.H. Observations were made to determine its effects on the length of the pupal stage, percent adult emergence, level of adult deformity, and adult mortality after emergence.

Irradiated females were mated with normal males and irradiated males were mated with normal females. Fecundity of the ovipositing females and percent egg hatch were determined.

Adult emergence from irradiated pupae was affected by the interaction of age, dose, and sex. Irradiated 2-day-old pupae were the most susceptible with no adults emerging, followed by 4-day-old pupae. Mean % adult emergence from pupae exposed when 6 and 8 days old to 10 or 20 krad was not affected; exposure to 30 krad significantly reduced emergence from 6-day-old pupae. Increasing the dosage from 20 to 30 krad increased the number of deformed adults emerging from 6-day-old pupae, but had no apparent effect on adults emerging from pupae irradiated when 8 days old.

Moths emerging from pupae exposed when 6 or 8 days old to 0, 10, or 20 krad, did not show a significant increase in mortality from the 2nd to the 6th day after emergence. The high mortality of moths emerging from pupae exposed to 30 krad when 6 days old coincided with the high % of adult deformities.

Fecundity of the adults emerging from the irradiated 6-day-old pupae was more significantly affected than was that of the adults emerging from irradiated 8-day-old pupae. Oviposition potential of irradiated females mated with normal males was more significantly reduced than was that of normal females mated with irradiated males.

When females from irradiated 6-day-old pupae were crossed with normal males (I♀ x N♂) the mean egg hatch per mated female was 86.4, 37.7, 0.6, and 0% at 0, 10, 20, or 30 krads, respectively. In reciprocal crosses (I♂ x N♀) the mean egg hatch was 84.6, 63.4, 7.6, and 0.6%. Crossing females from irradiated 8-day-old pupae and normal males (I♀ x N♂) resulted in a mean egg hatch of 83.2, 51.9, 10.6, and 0.2% at 0, 10, 20, or 30 krad, respectively. In the reciprocal crosses (I♂ x N♀) the mean egg hatch was 86.5, 63.9, 40.0, and 7.8%. Analysis of variance and least significant difference indicated that the dose/sex interactions were significant at the 5% level. Increase in the dose resulted in increased sterility in both sexes, with the increase greater in females than in males.

Developing almond moth pupae were more susceptible to radiation during earlier pupal ages, with a pronounced reduction in susceptibility between 4 and 6 days of age. Results indicated that effects on adults emerging from pupae irradiated with 20 krad when 6 days old and on those emerging from pupae exposed to 30 krad when 8 days old were not significantly different.