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Sterilization of the Navel Orangeworm, *Paramyelois transitella* (Walker), by Gamma Radiation

(Lepidoptera: Phycitidae)

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This paper presents fundamental investigations on the navel orangeworm and on its control by the release of sterilized navel orangeworm moths.

The first essential for working with this insect was to develop effective techniques for its mass culture. The main problem, to induce mating under laboratory conditions, was solved by providing indirect air circulation, high relative humidity, temperatures between 10° and 16° C, and light intensity similar to that of the early-morning hours. The successful method is described in detail.

The effects of gamma radiation were tested on all stages of the navel orangeworm. Mature, eight-day-old pupae were the best able to tolerate dosages sufficient to make all individuals completely sterile. The effective dosage, established at 50,000 rads ± 3.5 per cent, did not affect mating, egg-laying, or longevity of the moths. Treatment with 40,000 rads reduced fertility drastically but did not give complete sterility. Treatment with 80,000 rads reduced the mating capacities of both sexes, and the treated females laid very few eggs. There was no evident difference between the sexes in the sterilizing dosage.

The introduction of sterilized female moths reduced the production of viable eggs in untreated laboratory populations at least as much as did introduction of sterilized males. Most significantly, when both sterilized females and sterilized males were added to untreated populations, the result was not to double but to square the amount of control effected by sterile moths of only one sex.

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INTRODUCTION

THE IMPORTANCE of the navel orangeworm, *Paramyelois transitella* (Walker) (figs. 1, 2), as a pest of almond and walnut in California is increasing steadily, and no effective method of control had been developed (Wade, 1961) before the present work. A number of considerations point to the use of sterile insects, called autocidal control, as a promising measure.

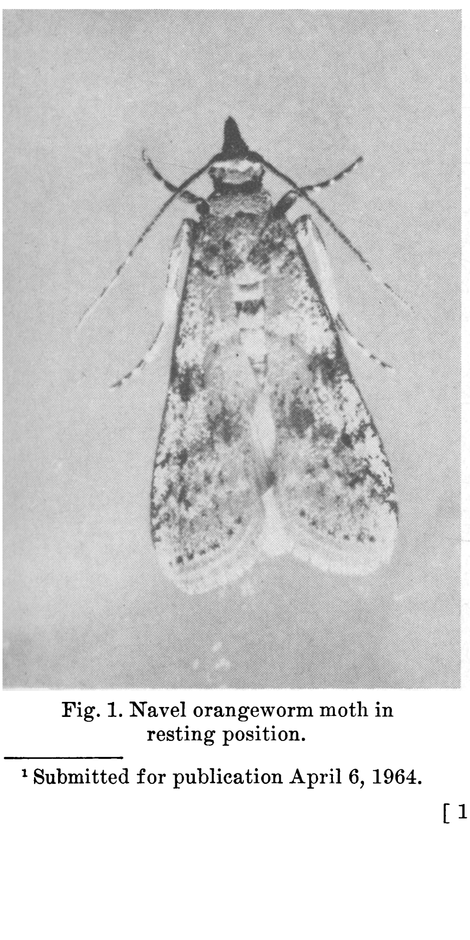

Fig. 1. Navel orangeworm moth in resting position.

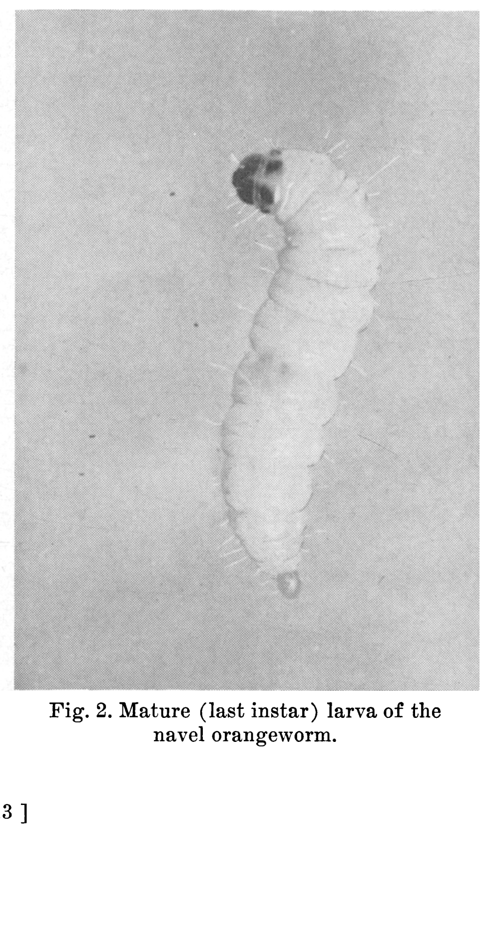

Fig. 2. Mature (last instar) larva of the navel orangeworm.

[1] Submitted for publication April 6, 1964.

Knipling (1955) suggested that large numbers of males of a given species, made sterile and released in the field, might effectively reduce the number of fertile eggs produced by the field population. The screw-worm, *Cochliomyia hominivorax* (Coq.), was actually eradicated on the island of Curaçao through the release of adults sterilized by gamma radiation (Baumhover *et al.*, 1955; Lindquist, 1955). Further discussions of the main factors that affect the success of insect control by this measure were published by Knipling (1959, 1960), Bushland (1960), Cornwell and Bull (1960), and von Borstel (1960).

The purpose of the present investigation was to explore the feasibility of autocidal control for the navel orangeworm. Experimental work covered the preliminaries necessary for actual field tests: some observations and experiments on the mating behavior of the moth, the development of techniques for mass culture, a study of radiation procedures and dosages, and tests of the effectiveness of sterilized moths in reducing fertile matings in a normal population.

MATING HABITS OF THE NAVEL ORANGEWORM

Knipling (1955) stated that the success of the sterile-male technique depended on the condition that normally females mate only once; otherwise the sperm of sterile males must compete with that of fertile males. Later he reported that the habit of one mating per female was not actually essential (Knipling, 1959). However, it is clear that mating habits are very important and should be considered fully in any investigation of control by sterile-insect release.

Determining the number of matings. In the mating of Lepidoptera the spermatophore, a membranous sac containing the sperm cells, is transferred by the male to the bursa copulatrix in the female reproductive duct. Williams (1941) grouped 61 American species into three classes. A female of Class *A*, which includes the family Phycitidae, may receive more than one spermatophore, and each spermatophore represents one copulation. Callahan and Chapin (1960) made a detailed study of the formation of spermatophores from the secretion of the male accessory glands in three species of Lepidoptera. Proverbs and Newton (1962) found that only one spermatophore was deposited during one copulation of the codling moth, *Carpocapsa pomonella* (L.)—also in Class *A*. Our observations on navel orangeworm moths confirm the above work, as it appears that only one spermatophore is deposited at each copulation.

The number of spermatophores found in the bursa copulatrix of a female was used to indicate the number of matings. Our practice at the end of each mating test was to preserve all the moths in 70 per cent alcohol and to dissect the females later for the spermatophore count (Gehring and Madsen, 1963). In navel orangeworm moths the spermatophore (fig. 3) is round, white, and rigid, 0.9 mm or less in diameter, with a tube about 0.7 mm long.

Field data. Adult moths were collected in a black-light trap at Walnut Creek, California, over the period from April to November, 1962. The sex ratio in these collections was of the order of seven males to one female. Of 100 females dissected, 11 contained no spermatophore, 81 had one each, and 8 had two each.

Laboratory data. To determine the effect of sex ratios on numbers of matings and on egg-laying of navel orangeworm moths and also for comparison with tests of irradiated insects, untreated virgin males and females in predetermined ratios were caged together for seven days (table 1). Subsequent dissection of the females showed some tendency for males to mate with unmated females and for each female to mate only once, especially when females outnumbered the males—although the one female with four spermatophores happened to be in the test with equal numbers of males and females. In general, the percentage of mated females and also the percentage of females with multiple matings increased regularly as the ratio of males to females increased. In the same populations the numbers of matings per male decreased regularly, and many males were unable to obtain

Fig. 3. Spermatophores dissected from female moths of navel orangeworm. *Above,* two spermatophores removed from bursa copulatrix; *below, left,* bursa copulatrix containing three spermatophores; *below, right,* bursa containing one spermatophore.

TABLE 1

EFFECT OF SEX RATIO ON MATING AND EGG-LAYING OF UNTREATED NAVEL ORANGEWORM MOTHS

Moths tested*		Eggs laid per female†		Number of spermatophores per female					Females mated		All matings	Matings per male
Male	Female	Viable	Non-viable	0	1	2	3	4	Single matings	Multiple matings		
no.	*no.*	*av. no.*	*av. no.*	*number of females*					*per cent*	*per cent*	*no.*	*av. no.*
5	30	18.4	24.2	19	11	0	0	0	36.7	0	11	2.20
5	15	39.9	43.6	8	5	2	0	0	33.3	13.3	9	1.80
10	10	18.5	39.6	4	3	1	1	1	30.0	30.0	12	1.20
15	5	51.6	27.0	0	2	2	1	0	40.0	60.0	9	0.60
30	5	38.6	20.8	0	1	2	2	0	20.0	80.0	11	0.37

* Virgin males and females, caged together within 24 hours of emergence from pupa case.
† During seven days in mating-oviposition cages.

mates when they outnumbered the females 6:1 or even 3:1. The data for six males to one female suggest a limitation on multiple matings of females even when many males must go unmated. The data for six females to one male suggest a limited mating capacity among the males, also, even with unlimited choice of mates. The highest average number of matings per male was 2.2, based on the spermatophore count in the female bursa. This average indicates a range from no matings to three per male; probably some males mated more times.

In this and other tests, 10 to 50 per cent or more of the untreated females did not mate at all during the seven days in the mating cages. One female mated five times. Our work gave no indication that multiple mating affected either the number of eggs laid by a female or the number of viable eggs.

MASS CULTURE OF THE NAVEL ORANGEWORM

A convenient and economical method of mass culture of an insect is essential, both for the preliminary investigations on autocidal control and for a practicable release program (Knipling, 1959). Attempts to culture the navel orangeworm in the laboratory have been made since 1947. Failure was generally attributed to difficulties in mating under laboratory conditions.[2] Wade (1961) studied the biology of this insect and tried again to rear it in the laboratory. He concluded that the moths were reluctant to mate indoors, although sporadic mating did occur.

In the summer of 1961 we developed techniques adapted to the needs of the navel orangeworm, so that large numbers of individuals were available throughout the year and could be obtained at a uniform stage in the life cycle.

The greenhouse colony was started with infested walnuts from an orchard at Walnut Creek, California. As soon as moths emerged they were transferred to mating-oviposition cages. During the preliminary study only 13 to 45 moths emerged over any four-day period so—to assure mating—the moths obtained during each four days were caged together. A week after the first emergence the eggs produced were transferred to a larva-rearing cylinder over fresh walnut meats, the nutrient medium used at that time.

The navel orangeworm has been reared in this laboratory continuously since September, 1961. The percentage of mating and the egg production per

[2] Personal communication from E. L. Atkins, Department of Entomology, Riverside.

female increased considerably in the last few generations of the experiment, either because of small improvements in rearing techniques or because of domestication of the colony. At the beginning 40 per cent mating was considered very good, but 70 to 90 per cent mating was obtained in later generations. The space needed for rearing is comparatively small and one man can handle large numbers of cultures easily.

Environmental conditions. An open dish or jar of water was supplied in every cage because the navel orangeworm at all stages requires high relative humidity—between 70 and 80 per cent. The egg stage is the most susceptible to desiccation. All cages except those for mating and oviposition were kept at a temperature close to 28° C, the optimum for development. There was provision for the necessary circulation of air, which may be difficult in the larva-rearing cylinders.

Larva-rearing cylinders (fig. 4) were of glass, 4 inches in diameter and 8 inches high. The upper ends were left open and the lower ends were closed with 24-mesh wire screen, held taut with plastic bands (larvae can eat their way through cloth or plastic screen). Cylinders half filled with rearing medium were set on heavy wire screening over a plastic vegetable tray filled with water. Six cylinders were placed on a tray and two trays in a cage (fig. 5).

Larva-rearing media. Originally we used offgrade walnut meats as the nutrient medium, but these were less satisfactory than clean walnut meats, probably because of contamination by a number of organisms. Sterilizing the walnut

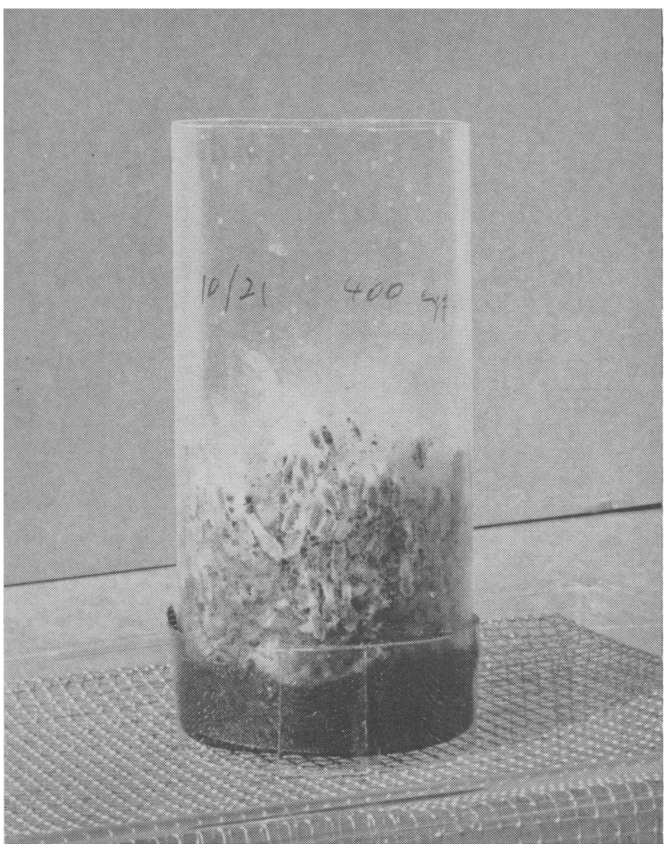

Fig. 4. Larva-rearing cylinder half filled with walnut meats, showing pupae in cocoons attached to the glass wall.

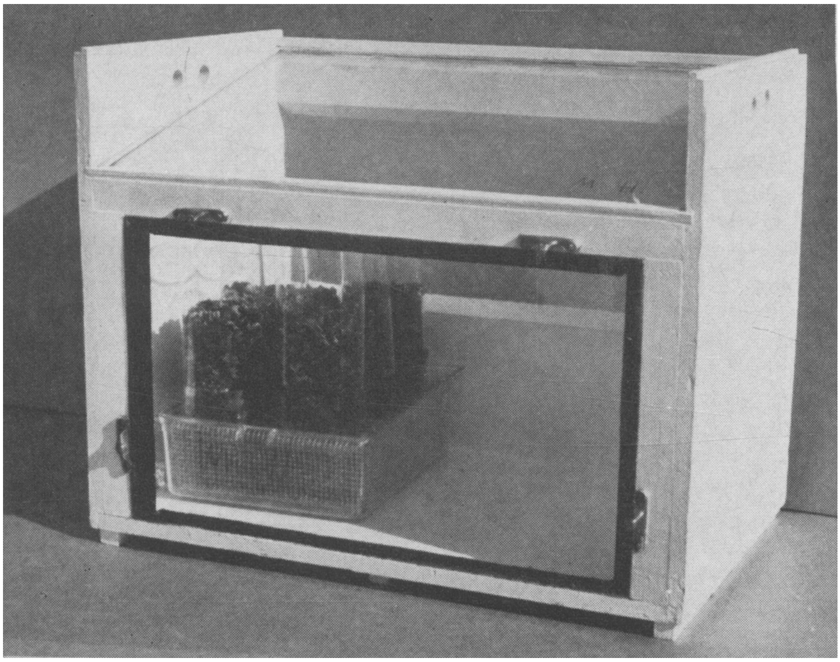

Fig. 5. Cage containing one plastic tray, with six larva-rearing cylinders half filled with walnut meats.

meats by autoclaving was too drastic and made the nuts unfit for food.

About 250 grams of walnut meats in each cylinder was more than enough to rear 400 larvae to pupation. Too large a population in a cylinder increases the temperature of the culture above the optimum. Almond meats were less satisfactory than walnuts, perhaps because the first-instar larvae could not penetrate the tough outer skins.

A mixture of 1,200 cc Pablum, 100 cc honey, 100 cc glycerine, and 50 cc water—enough for two cylinders—was a more satisfactory medium than nut meats. We used it for rearing all the insects used in our radiation experiments. The above formula—based on one given by Peterson (1959) for rearing the wax moth, *Galleria mellonella* (L.)—was adapted for navel orangeworm cultures in May, 1962, by Caltagirone, Shea, and Finney (1964).

Contamination and sanitation. *Bacillus entomocidus entomocidus* Heimpel and Angus[3], not previously reported on the navel orangeworm, nearly destroyed one of the early colonies. The source of contamination was not determined positively, but offgrade walnut meats used in the culture medium were suspected.

All equipment was cleaned and sterilized after each generation of larvae or of moths. Glass cylinders, wire screens, and implements used with the rearing medium were autoclaved for one hour at 5 pounds' pressure—about 109° C. This exposure killed all organisms, including the spores of *B. entomocidus entomocidus,* and the treatment reduced bacterial contamination and excessive growth of molds in the cultures. Cages, plastic materials, and other articles that could not be autoclaved were washed thoroughly and rinsed with a 5 per cent solution of formalin.

Sleeve cages (fig. 6) were of the glass-top type, similar to those described by Peterson (1959). Cultures were kept in the greenhouse. A 100-watt light bulb

[3] Identified by E. A. Steinhaus, formerly Professor of Insect Pathology, Berkeley, now Dean of Biological Sciences, Irvine.

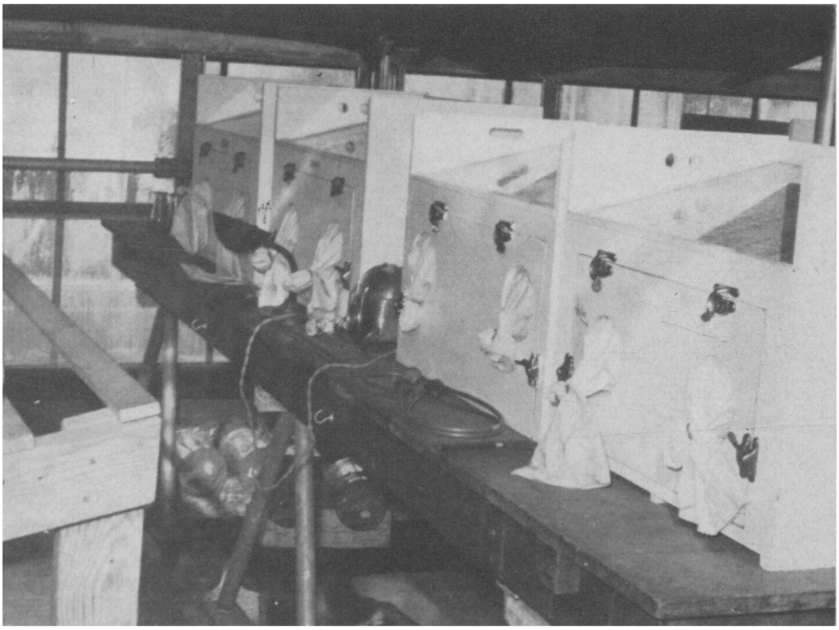

Fig. 6. Sleeve cages in the greenhouse. Newly emerged moths were transferred from these cages to the mating-oviposition cages shown under the table.

outside each cage gave additional heat when needed, especially at night.

To collect moths from the sleeve cages we used a simple suction device adapted from the one described by Dickson, Barnes, and Turzan (1952), with a 9-mm plastic tube leading through a rubber stopper to a plastic holding tube, approximately 1 inch × 4 inches. At the opposite end the holding tube was screened and connected by rubber tubing to a vacuum-cleaner tank, modified to produce just enough suction to capture the moths without injury (illustrated by Bailey and Madsen, 1964, fig. 2). Moths were collected easily during the day, when they were inactive.

The mating-oviposition cages (fig. 7) were cylinders of 1-mm plastic, 15 cm in diameter and 25 cm long, similar to cages used for rearing the codling moth (Sazama, 1932). Each cage was lined with a detachable cylinder of waxed paper. Plastic bands held the covers—perforated waxed paper at one end and gauze at the other end. A half-inch-diameter opening in the gauze, plugged with cotton, was used for placing moths in the cage and for adding water. A pad of wet cotton in a petri dish covered with plastic netting provided moisture.

Optimum conditions for mating require good ventilation and a temperature between 10° and 16° C. The mating-oviposition cages were sheltered from the sun under a large wooden table near a vent at the east side of the greenhouse, with a small fan directed onto wet burlap sacks under the cages (fig. 6). The perforated waxed paper, on which the eggs were laid, was oriented toward the morning light.

Oviposition. Most of the eggs were laid around the perforations in the waxed-paper cover of the mating cage (fig. 8). Female moths, whether mated or not, deposit eggs readily and even the mated females lay some unfertilized eggs. In these tests, egg production ranged from 10 to 150 per female during the first week after emergence; an average output of 80 eggs was considered good.

Development of eggs and larvae. A piece of the perforated waxed paper carrying about 600 eggs was placed in

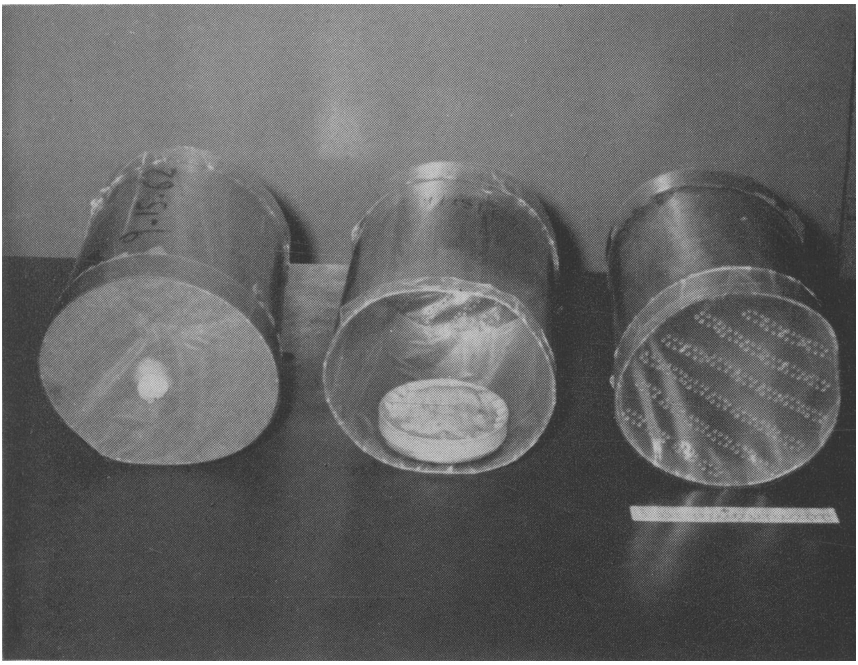

Fig. 7. Mating-oviposition cages, showing gauze cover at the left, dish for water in the center, and perforated waxed-paper cover at the right.

each rearing cylinder. At 28° C egg development takes at least five days. The newly hatched larvae crawl down into the culture medium and feed for at least 18 days if on the Pablum medium. On walnut meats the period of larval feeding is at least 26 days. Many of the eggs are nonviable, and some hatched larvae fail to complete their development in cultures. Hence the 600 eggs started in each cylinder have yielded an average of only 400 larvae and 350 moths. This yield compares favorably with yields in the mass rearing of other insects.

Pupation. Last-instar larvae spin cocoons against the glass wall of the cylinder above the culture medium. They use only a little silk on the smooth glass surface, so it is possible to observe changes during pupation. At 28° C pupation and pupal development take a minimum of eight days.

Table 2 gives the external color changes of pupae (fig. 9)—a dependable indication of their age. No significant color difference was observed between males and females. At 28° C pupae reached the black-eye stage in five days; they were considered mature when they became brownish black, usually the eighth day after pupation.

TABLE 2

UNTREATED PUPAE OF THE NAVEL ORANGEWORM, DEVELOPING AT 28°C AND 75 PER CENT RELATIVE HUMIDITY

(Emergence on ninth day)

Age	Color
days	
1	Yellowish white
2	Yellowish brown
3	Uniform light or reddish brown
4	Medium brown, with darker areas between segments and at tip of abdomen; eyes dark brown
5	Same, with black eyes
6	Dark brown with black eyes
7	Same, with a few dark spots on wings
8	Brownish black

Adult emergence. Moths usually emerged around twilight. The first moths appeared about 31 days after eggs were placed on the Pablum medium. Emergence reached a peak about six days later and continued

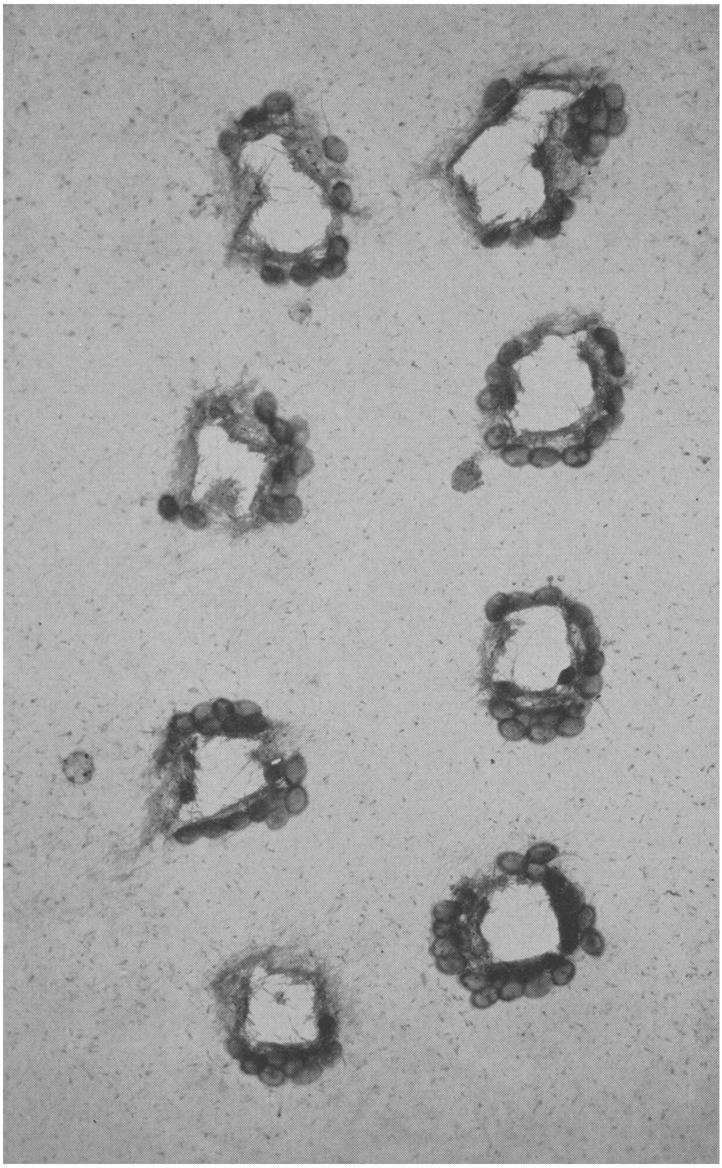

Fig. 8. Eggs of the navel orangeworm, laid around perforations in the waxed-paper cover of mating cage.

throughout the month, though most of the moths emerged in the first two weeks.

Sex ratio. Usually there were more males than females among the first moths that emerged from a culture, but when all moths had emerged the sex ratio was very close to 1:1. Goodwin and Madsen (1964) found the same ratio in large laboratory populations and also in moths reared from infested nuts collected in the field. Of 3,495 pupae from the laboratory colony that we sexed between August 8 and September 22, 1962, 1,770 were males and 1,725 were females. The percentage of males in the daily count varied from 45.0 to 64.1, with an average of 50.6.

Life cycle. From egg to egg the life cycle took from 35 to 65 days on the

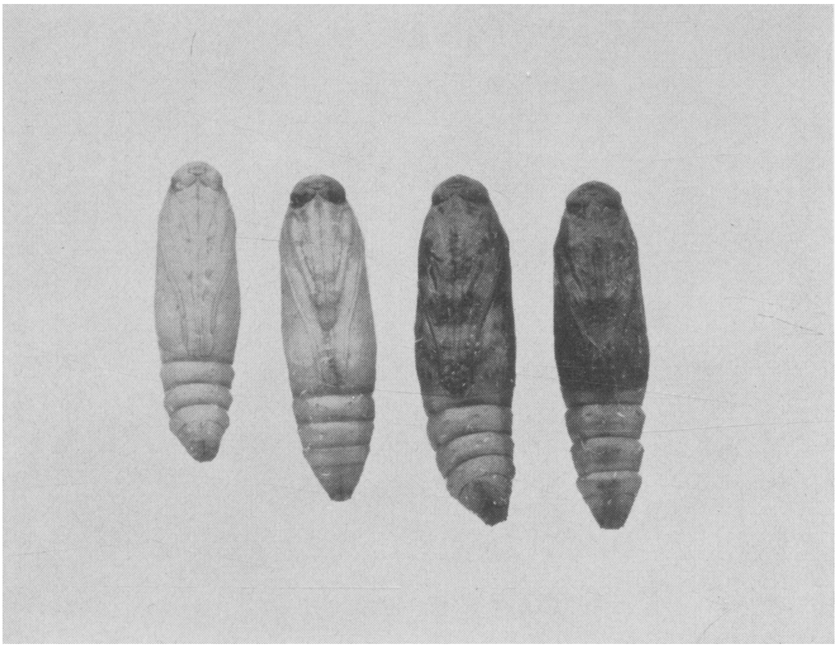

Fig. 9. Stages in the development of navel orangeworm pupae.

Pablum medium under the conditions described. For the majority it was about 40 days. During the present study the larvae never showed any tendency to undergo diapause, but they developed more slowly under unfavorable conditions. The culture medium had a definite influence on the rate of larval and later development. Insects reared on the Pablum mixture developed more rapidly and much more uniformly than those on walnut or almond meats. The average life span of adult males in the laboratory was 20.2 days, of females 24.9 days. A majority of the moths copulated within two days after emergence and most of the eggs were laid during the next three days.

GAMMA IRRADIATION

There are many types of apparatus for irradiation with cobalt-60. Darden, Maeyens, and Bushland (1954) and Proverbs and Newton (1962) have described some units constructed specifically for irradiation of insects.

The model 2 cobalt-60 irradiation unit in the Lawrence Radiation Laboratory at Berkeley (figs. 10, 11, 12) was used for all treatments reported here. The cobalt-60 source has a half-life of 5.25 years. We calibrated this source each month from November 1, 1961, to November 1, 1962, using the Fricke ferrous sulfate dosimeter and the procedure of Weiss, Allen, and Schwarz (1956). Even though the decay line is exponential (fig. 13) it is very close to a straight line because of the relatively slow decay rate of cobalt-60. The distance between the source and the rotating sample holder was adjusted for each treatment so as to give the specified dosage rate of 197,500 rads per hour ± 3.5 per cent (range from 190,605 to 204,405 rads per hour). Exposures at this dosage rate ranged from three minutes to one hour.

Procedures in Preparation and Care of Insects

All the insects were held under op-

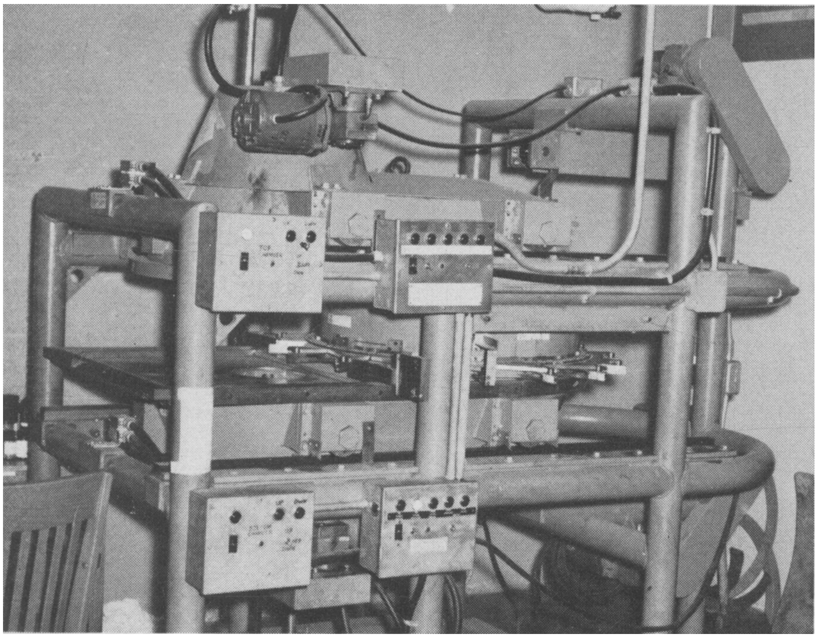

Fig. 10. Model 2 cobalt-60 irradiation unit in the Lawrence Radiation Laboratory, Berkeley.

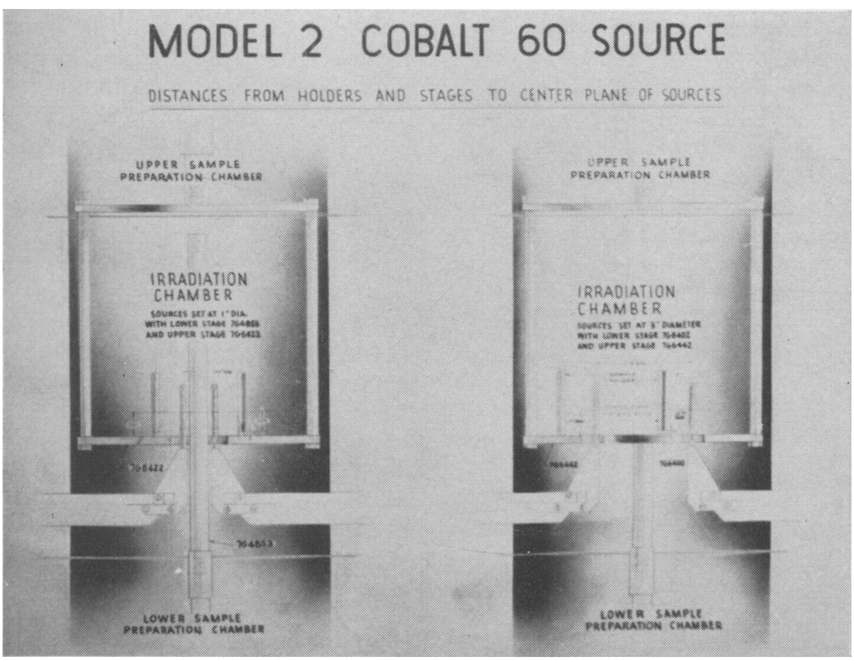

Fig. 11. Diagrammatic cross section of the irradiation chamber of model 2 cobalt-60 irradiation unit.

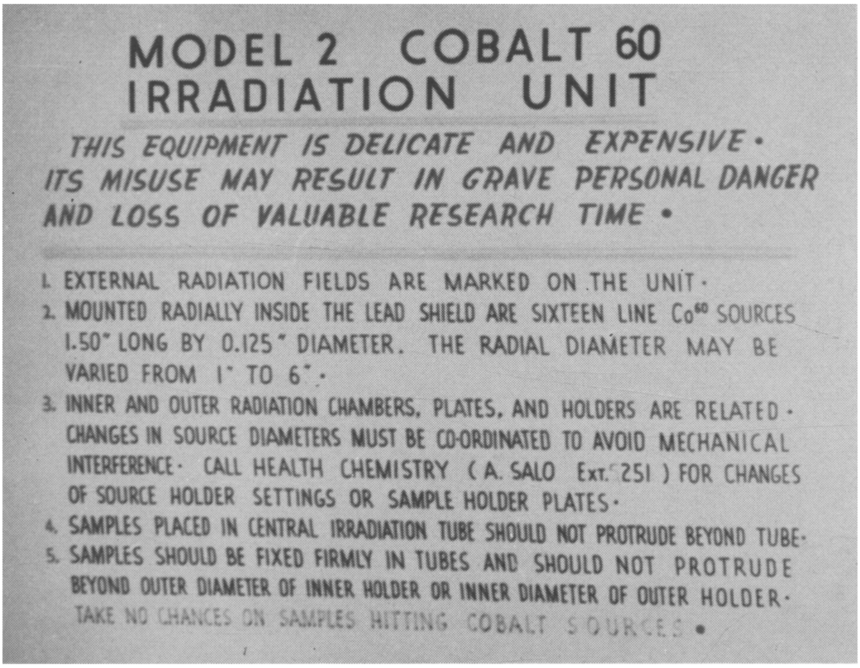

Fig. 12. Instructions for the use of model 2 cobalt-60 irradiation unit.

timum conditions before and after their irradiation.

Eggs. To obtain eggs of the same age, 40 males and 40 females were caged together for two days and then transferred to a clean cage. Eggs laid during the first 24 hours in the new cage were held under optimum conditions for four days before irradiation. Eggs were not

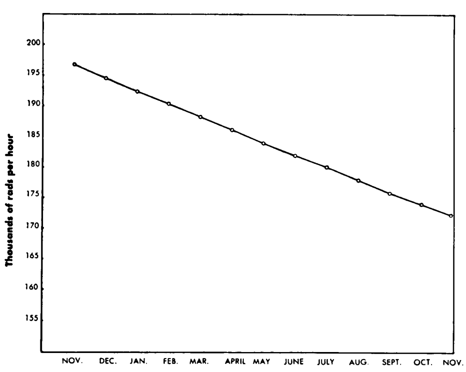

Fig. 13. Declining rate of radiation from the cobalt-60 irradiation unit. Each point is an average of four determinations made on the first of the month, using the Fricke ferrous sulfate dosimeter.

treated at earlier stages of development because the younger eggs are generally more susceptible to radiation injury (Tergian and Staller, 1958; Proverbs and Newton, 1962). Viable and non-viable eggs can be distinguished under a dissecting microscope after 48 hours' incubation at 28° C.

Larvae. Mature larvae were obtained from the rearing cylinders and were not sexed. As their exact age was not known, we added nutrient medium to the treatment vials after irradiation, so that all larvae could complete their feeding and development.

Pupae (figs. 14, 15). Cocoons from the larva-rearing cylinders, gathered into a 24-mesh wire basket, were immersed for one minute in water and then for 30 seconds in a solution containing one part of commercial bleach (5.25 per cent sodium hypochlorite) to one part of water. The pupae were rinsed thoroughly in tap water and placed on paper towels to dry. Larvae and pieces of the nutrient medium were picked off by hand. This treatment is a modification

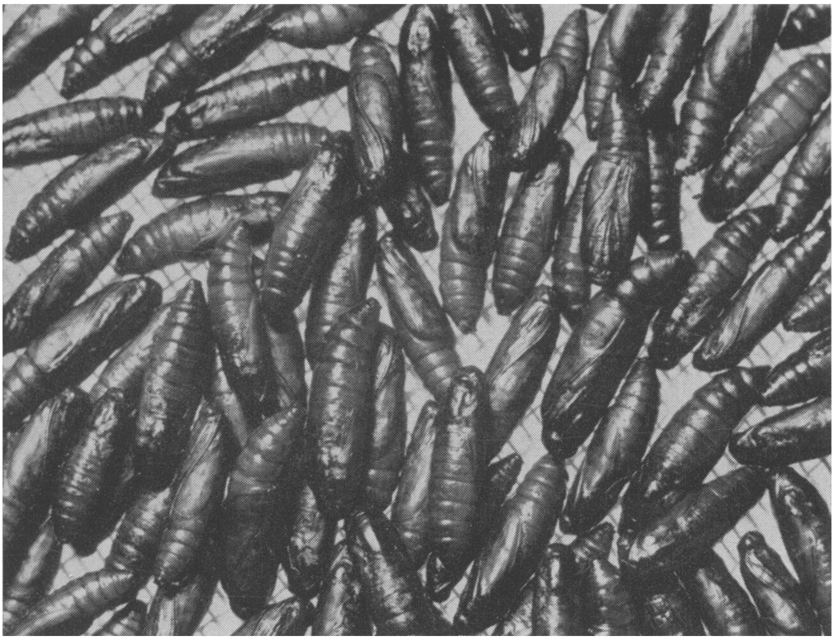

Fig. 14. Mature pupae of the navel orangeworm prepared for irradiation.

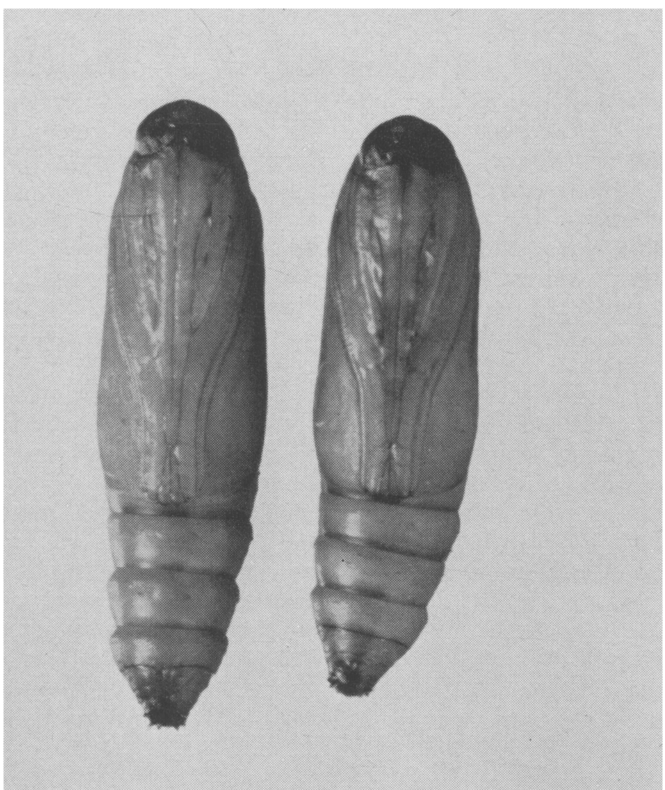

Fig. 15. Eight-day-old pupae of the navel orangeworm, ventral view. *Left,* female; *right,* male.

TABLE 3

EFFECT OF COLD STORAGE ON SUBSEQUENT DEVELOPMENT OF MATURE, UNTREATED PUPAE OF THE NAVEL ORANGEWORM

Exposure at 10°C*	Moths emerged†		Eggs laid‡		Females mated	Matings per male
	Male	Female	Viable	Nonviable		
days	*no.*	*no.*	*no.*	*no.*	*per cent*	*av. no.*
0	10	10	185	396	60	1.2
2	10	10	52	160	90	1.0
4	10	10	78	362	90	1.2
6	8	9	102	296	50	0.7
8	9	8	119	72	60	0.9

* Ten males and 10 females at each exposure.
† During the first 24 hours at 28°C.
‡ At 28°C, during the seven days in mating-oviposition cages.

of the technique used by Finney, Flanders, and Smith (1947). It dissolved the cocoons completely but caused no detectable damage to the pupae.

The sex of a pupa is recognized by the location of the gonopore—on the eighth segment in females and on the ninth segment in males. Male and female pupae were placed in separate vials for irradiation.

The purpose of this work was to achieve complete sterility with a minimum of injury. We used mature pupae of uniform age (eight days after pupation) because these were the best able to tolerate irradiation. In preliminary trials with 80,000 rads, emergence was 79 per cent from the mature pupae treated but only 30 per cent from the pupae treated at the black-eye stage (five days after pupation).

To obtain large numbers of mature pupae at the same time, it was necessary to hold some pupae in cold storage before treatment. When mature pupae were held from two to eight days at 10° C, with air and moisture provided, development was negligible and subsequent emergence, mating, and fertility were not affected (table 3). To avoid any possible complications, we used no pupae in irradiation tests that had been in cold storage longer than three days.

In all the work with irradiated pupae—unless otherwise specifically stated—the only moths we studied for mating behavior and fertility were those obtained during the first 24 hours of emergence from any lot of pupae. These moths were placed in mating-oviposition cages soon after emergence, with newly emerged mates—either treated or untreated. All mating and oviposition tests were discontinued after seven days.

In a few tests—where so indicated—we used moths obtained during the first four days of emergence from treated pupae. In these tests, only the first moths were held for the full seven days in the mating-oviposition cages. Moths added to these cages over the next three days were allowed progressively less time for mating and oviposition.

Adults (fig. 16). To obtain virgin adults for irradiation, male and female pupae were held in separate cages. The male and female moths were irradiated separately, within 24 hours of their emergence, and released promptly in the mating-oviposition cages. The adult male is recognized under a dissecting microscope by the characteristic claspers, the female by the pointed ovipositor. For counting, the moths in the collecting tube were immobilized by exposing them to CO_2 for a few seconds.

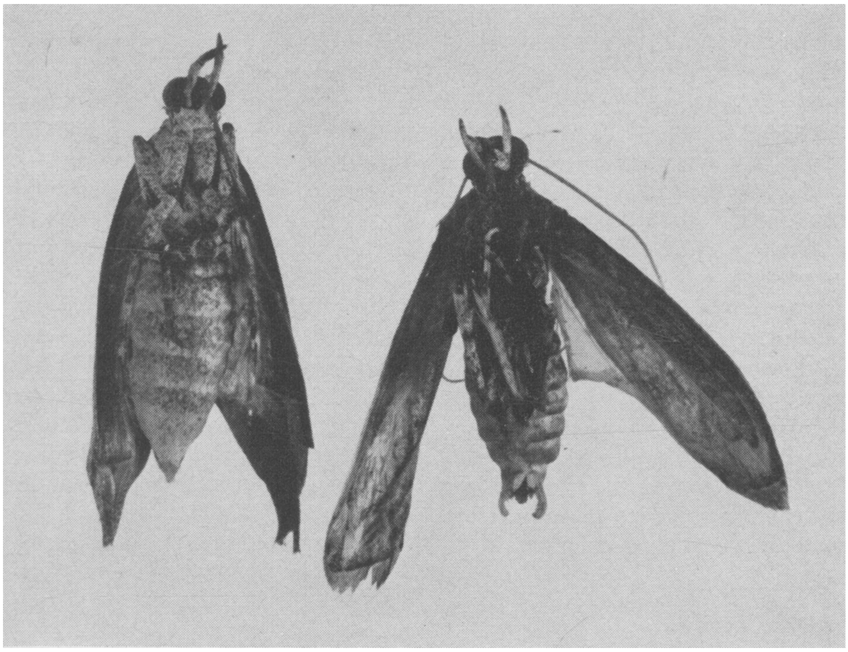

Fig. 16. Navel orangeworm moths, ventral view. *Left,* female; *right,* male.

EFFECTS OF GAMMA RADIATION ON NAVEL ORANGEWORM

Irradiated Eggs (table 4)

Exposure of four-day-old eggs to 3,000 rads reduced the hatch somewhat, 6,000 rads reduced it drastically, and no eggs hatched after exposure to 12,000 rads or more. Only eight eggs hatched out of the 191 treated at 6,000 rads, but seven of these eight larvae developed to maturity, giving a high percentage of adults from the larvae in this test—though with no particular significance. Even in the untreated lots of eggs, 15 per cent were either infertile or nonviable, and only 36 per cent of the larvae

TABLE 4
EFFECTS OF GAMMA RADIATION ON FOUR-DAY-OLD NAVEL ORANGEWORM EGGS

Dosage	Eggs treated	Eggs hatched		Adults developed				Moths caged*		F_1 eggs laid per female†	
				Male		Female		Male	Female	Viable	Nonviable
rads	*no.*	*no.*	*per cent*	*no.*	*per cent of hatch*	*no.*	*per cent of hatch*	*no.*	*no.*	*av. no.*	*av. no.*
0.......	255	216	84.7	42	19.4	36	16.7	25	24	8.9	34.9
1,500.......	250	190	76.0	34	17.9	27	14.2	20	10	4.7	49.2
3,000.......	169	102	60.4	16	15.7	10	9.8	7	4	0.3	60.5
6,000.......	191	8	4.2	5	62.5	2	25.0	0	0	...	....
12,000.......	101	0	....	..	....	..	....	..	..	...	....
24,000.......	180	0	....	..	....	..	....	..	..	...	....

* Adults obtained during the first four days of emergence, caged together.
† During a maximum of seven days in mating-oviposition cages.

that hatched developed to maturity and emerged as moths. This rate of mortality is usual in insect populations.

Second generation. All moths of either sex that appeared in each test lot during the first four days of emergence were caged together and held for a total of seven days. Under these conditions moths from 1,500-rad and 3,000-rad eggs produced fewer viable eggs per female but more nonviable eggs than did the controls. Four females from the 3,000-rad eggs produced only one viable egg among them. Six additional females emerged from the 3,000-rad eggs after the four-day limit and were not tested as potential parents. Because of the low survival rate, irradiation of eggs is not a feasible means of producing sterile moths.

Irradiated Larvae

Larvae were not sexed before irradiation, but those that were able to continue their development were sexed in the adult stage. We detected no consistent differences in radiosensitivity between the sexes.

Pupation and emergence (table 5). The injurious effects of gamma radiation on mature larvae were not evident immediately. Pupation took place within a week or not at all. Deformed pupae (fig. 17) were very common in this test, especially after exposure to 12,000 and 24,000 rads, but dosages up to 24,000 rads had little effect on the numbers of larvae that pupated. Injury from 12,000 rads was obvious when no moths emerged successfully. A few emerged partially and others completed their metamorphosis and remained alive in the pupal skin for seven days or more but died ultimately. At 30,000 rads fewer than half of the larvae were able to pupate and none completed pupal development.

Second generation (table 6). For each test of irradiated larvae, the male and female moths obtained during the first 24 hours of emergence were caged together for seven days. Exposure of larvae to 3,000 rads reduced subsequent mating and fertility somewhat. Following exposure of larvae to 6,000 rads there was only one mating among seven females, and no viable eggs were produced.

Irradiated Pupae

High doses (table 7). Injury to pupae from high dosages of gamma radiation was most conspicuous in the failure of adults to emerge. Some moths that did emerge were inactive, and most of these died in a few days. In the mortality counts—seven days after the first emergence in each test lot—moths were counted dead if they failed to fly or crawl when stimulated mechanically.

Doses of 160,000 rads and above killed all individuals before the end of the tests. Females seemed the more able to emerge, but at the end the females

TABLE 5

EFFECT OF GAMMA RADIATION ON MATURE LARVAE OF THE NAVEL ORANGEWORM

Dosage*	Larvae dead	Pupae dead	Adults deformed	Adults emerged	
				Male	Female
rads	*no.*	*no.*	*no.*	*no.*	*no.*
0	1	1	3	8	17
3,000	0	2	2	6	20
6,000	1	3	4	10	12
12,000	3	24	3	0	0
24,000	3	26	1	0	0
30,000	17†	13	0	0	0
40,000	25†	5	0	0	0

* Thirty larvae exposed at each dosage.
† Including two larvae that remained alive for four weeks without pupating.

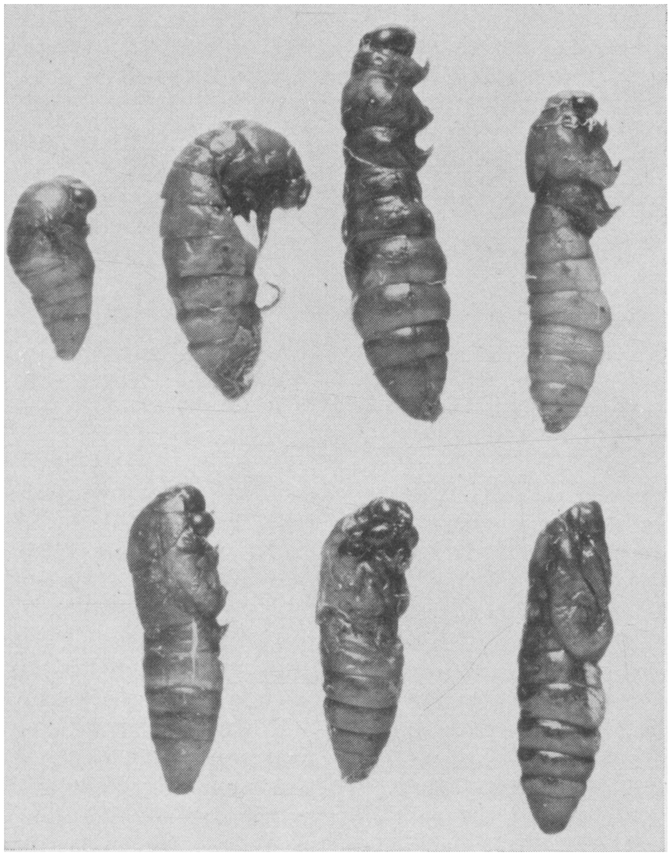

Fig. 17. Deformed pupae of the navel orangeworm, developed from mature larvae exposed to gamma radiation.

seemed slightly more susceptible to radiation injury than the males.

Moderate doses (tables 8, 9). No fertile eggs were produced in any test where either the male or the female parent had been exposed in the pupal stage to 50,000 rads or more. In these tests the treated moths of each sex were caged with untreated moths of the opposite sex. Forty thousand rads ± 3.5

TABLE 6

EFFECT OF GAMMA IRRADIATION OF MATURE LARVAE ON VIABILITY OF F_1 EGGS

Dosage*	Adults caged†		Eggs laid per female‡		Females mated		Matings per male
	Male	Female	Viable	Nonviable			
rads	*no.*	*no.*	*av. no.*	*av. no.*	*no.*	*per cent*	*av. no.*
0	6	14	17.6	49.5	6	42.9	1 00
750	4	15	22.1	52.7	7	46.6	1.75
1,500	10	19	22.0	31.9	11	57.9	1.10
3,000	6	12	13.1	30.2	4	33.3	0.66
6,000	10	7	0	5.0	1	14.3	0.10
12,000	0	0	....	....	..	....	....

* Thirty larvae exposed at each dosage.
† Moths obtained during the first 24 hours of emergence, caged together.
‡ During seven days in mating-oviposition cages.

TABLE 7

EFFECT OF HIGH DOSES OF GAMMA RADIATION ON MATURE PUPAE OF THE NAVEL ORANGEWORM

Dosage	Pupae treated		Pupae dead*		Total mortality†	
	Male	Female	Male	Female	Male	Female
rads	*no.*	*no.*	*per cent*	*per cent*	*per cent*	*per cent*
0	55	55	8	4	8	6
80,000	30	35	20	22	22	25
100,000	20	25	35	40	40	52
120,000	30	35	70	64	82	92
140,000	20	25	100	72	100	96
160,000	30	35	97	100	100	100
200,000	10	10	100	90	100	100

* No emergence during the seven days of the test.
† Seven days after the first emergence from each lot.

per cent was close to a sterilizing dosage for both sexes but not adequate for a control program; 30,000 rads reduced fertility drastically; 10,000 and 20,000 rads had little or no effect on fertility. There was no significant difference in fertility between the sexes at any dosage. Females treated with 80,000 rads produced only a few eggs. Egg output was reduced markedly, also, in preliminary tests where pupae at the black-eye stage were treated with 60,000 and 80,000 rads.

Mating behavior of moths from irradiated pupae (tables 10, 11). Mating was not reduced in either sex by treatment at 50,000 rads or less, but at 80,000 rads there was definitely less mating. All of the females irradiated with 50,000 rads were mated and four of them mated four times. Multiple matings were reduced when either sex was treated with 80,000 rads; moreover, 25 per cent of the females and 40 per cent of the males treated at this dosage failed to mate within the week.

Effect of substerilizing doses on the next generation (table 12). When mature pupae of either sex were exposed to substerilizing doses of gamma radiation, the effect on fertility was similar to that in the treatments shown in tables 8 and 9. As many as possible of the progeny of treated moths with untreated mates were reared to the adult stage. Except for one case, the number

TABLE 8

EFFECTS OF GAMMA RADIATION ON MATURE MALE PUPAE OF THE NAVEL ORANGEWORM

Dosage	Males tested*	Eggs laid†			Females mated		Viable eggs per mated female
		Total viable	Total nonviable	Per female			
rads	*no.*	*no.*	*no.*	*av. no.*	*no.*	*per cent*	*av. no.*
0	70	1,254	2,468	53.2	33	47.1	38.0
10,000	50	1,145	1,289	48.7	36	72.0	31.8
20,000	52	1,189	660	35.6	36	69.2	33.0
30,000	46	121	452	12.5	11	23.9	11.0
40,000	100	19	2,558	25.8	19	19.0	1.0
50,000	54	0	1,361	25.2	26	48.1	0
60,000	58	0	886	15.3	14	24.1	0
80,000	48	0	1,640	34.2	14	29.2	0

* Male moths from irradiated pupae plus equal numbers of untreated females, caged together within 24 hours of the first emergence from each lot of pupae. Totals of three to seven replicates, with 10 to 22 irradiated males per cage.
† During seven days in mating-oviposition cages.

TABLE 9

EFFECTS OF GAMMA RADIATION ON MATURE FEMALE PUPAE OF THE NAVEL ORANGEWORM

Dosage	Females tested*	Eggs laid†			Females mated		Viable eggs per mated female
		Total viable	Total nonviable	Per female			
rads	*no.*	*no.*	*no.*	*av. no.*	*no.*	*per cent*	*av. no.*
0	70	1,254	2,468	53.2	33	47.1	38.0
10,000	54	1,210	1,014	41.2	32	59.3	37.8
20,000	52	1,069	1,008	39.9	33	63.5	32.4
30,000	54	80	1,087	21.6	10	18.5	8.0
40,000	104	90	1,936	19.5	18	17.3	5.0
50,000	64	0	1,398	21.8	21	32.8	0
60,000	60	0	758	12.6	7	11.7	0
80,000	60	0	125	2.1	18	30.0	0

* Female moths from irradiated pupae plus equal numbers of untreated males, caged together within 24 hours of the first emergence from each lot of pupae. Totals of three to seven replicates, with 10 to 22 irradiated females per cage.
† During seven days in mating-oviposition cages.

TABLE 10

MATING BEHAVIOR OF MALE NAVEL ORANGEWORM MOTHS IRRADIATED IN THE MATURE PUPAL STAGE

Dosage	Males tested*	Number of spermatophores per female†						Females mated		All matings	Matings per male
		0	1	2	3	4	5	Single matings	Multiple matings		
rads	*no.*	*number of females*						*per cent*	*per cent*	*no.*	*av. no.*
0	20	3	8	5	3	1	0	40.0	45.0	31	1.55
10,000	40	5	16	11	6	2	0	40.0	47.5	64	1.60
20,000	40	6	16	10	6	1	1	40.0	45.0	63	1.58
50,000	20	2	8	5	4	1	0	40.0	50.0	34	1.70
80,000	20	9	10	1	0	0	0	50.0	5.0	12	0.60

* Male moths from irradiated pupae plus equal numbers of untreated females, caged together within 24 hours of the first emergence from each lot of pupae.
† After seven days in mating-oviposition cages.

TABLE 11

MATING BEHAVIOR OF FEMALE NAVEL ORANGEWORM MOTHS IRRADIATED IN THE MATURE PUPAL STAGE

Dosage	Females tested*	Number of spermatophores per female†					Females mated		All matings	Matings per male
		0	1	2	3	4	Single matings	Multiple matings		
rads	*no.*	*number of females*					*per cent*	*per cent*	*no.*	*av. no.*
0	20	3	8	5	3	1	40.0	45.0	31	1.55
10,000	40	8	16	10	3	3	40.0	40.0	57	1.43
20,000	40	8	19	10	3	0	47.5	32.5	48	1.20
50,000	20	0	13	2	1	4	65.0	35.0	36	1.80
80,000	20	5	12	2	1	0	60.0	15.0	19	0.95

* Female moths from irradiated pupae plus equal numbers of untreated males, caged together within 24 hours of the first emergence from each lot of pupae.
† After seven days in mating-oviposition cages.

TABLE 12

FERTILITY OF THE PROGENY OF NAVEL ORANGEWORM MOTHS TREATED IN THE MATURE PUPAL STAGE WITH LOW DOSAGES OF GAMMA RADIATION

Dosage	Irradiated insects*		Viable eggs laid†	F_1 adults developed		Eggs laid by F_1 moths‡	
				Male	Female	Viable	Nonviable
rads	*no.*	*sex*	*no.*	*no.*	*no.*	*no.*	*no.*
10,000	20	♂	157	24	21	108	318
10,000	20	♀	782	77	86	457	467
20,000	20	♂	235	27	16	29	94
20,000	20	♀	252	14	12	92	80
30,000	20	♂	41	4	1	0	7
30,000	20	♀	84	3	2	5	6
40,000	20	♂	41	5	3	0	0
40,000	20	♀	12	0	0	..	..

* Moths from irradiated pupae plus equal numbers of untreated moths of the opposite sex, caged together within 24 hours of emergence from each lot of pupae.
† During seven days in mating-oviposition cages.
‡ During a maximum of seven days in mating-oviposition cages.

of adult males in the F_1 slightly exceeded the number of females. The F_1 moths of both sexes obtained in the first four days of emergence from each test lot were caged together for a maximum of seven days but they produced relatively few eggs and these were of low viability. The adult progeny of males treated at 40,000 rads produced no viable eggs and the progeny of females treated at 40,000 rads failed to reach maturity.

Irradiated Moths (table 13)

When navel orangeworm adults were irradiated, the mortality at the end of seven days was no greater than in the controls. Survival beyond seven days was not considered, because these moths lay relatively few eggs after that period (Wade, 1961). None of the previous workers has suggested that sterilizing doses of gamma radiation affected adult longevity in the lepidopterous insects they studied (Rhode *et al.*, 1961; Vasilyan, 1961; Proverbs and Newton, 1962).

All of the males and females in this test were irradiated. The numbers of fertile eggs laid were reduced somewhat when both parents had received 18,000 rads and were reduced drastically after the 36,000-rad treatment. No fertile eggs were obtained after exposure of both parents to 54,000 rads, though this dosage reduced the production of infertile eggs only slightly. At each higher

TABLE 13

EFFECTS OF GAMMA RADIATION ON ONE-DAY-OLD NAVEL ORANGEWORM MOTHS

Dosage*	Moths dead after 7 days	Eggs laid†		Females mated	Matings per male
		Viable	Nonviable		
rads	*no.*	*no.*	*no.*	*per cent*	*av. no.*
0	9	275	301	65	0.85
18,000	6	130	423	70	1.15
36,000	4	7	930	65	0.70
54,000	10	0	429	55	0.75
72,000	4	0	228	40	0.50
90,000	9	0	180	30	0.35

* Twenty males and 20 females treated at each dosage. No untreated moths used except in the controls.
† During seven days in mating-oviposition cages.

dosage there was a pronounced reduction in numbers of eggs laid.

The incidence of matings after exposure to 54,000 rads was a little lower for both sexes than in the controls. At higher dosages it was markedly lower.

COMPETITION BY STERILE MOTHS IN NORMAL POPULATIONS

An important requirement for effective autocidal control is the ability of sterilized insects to compete for mates with normal individuals. To determine this competitive ability, male and female moths from pupae treated with 50,000 rads were caged in various ratios with untreated moths for the usual seven days.

The control tests, using untreated males and females in equal numbers, produced an average of 30.53 viable eggs per normal female. This average was used as the standard for measuring the effectiveness of the various ratios of normal moths to sterile moths. When the ratio is 1 normal male and 1 sterile male to 1 normal female, the female has 1 chance in 2 of mating with the normal male. With 1 normal male and 5 sterile males to 1 normal female, the female's chance of mating with the normal male is only 1 of the 6 possible matings plus a more remote chance among possible multiple matings. With 1 normal male to 1 normal female and 5 sterile females, the normal female still has 1 chance in 6 to mate with the normal male. With 1 normal male and 5 sterile males to 1 normal female and 5 sterile females, the chance of a mating between the two normal moths becomes 1 in 36 because the 1 chance in 6 of meeting a normal mate applies both to the normal male and to the normal female.

The calculation in table 14 of the numbers of fertile eggs to be expected in each test was based on the assumptions that sterile moths are as successful in competing for mates as are normal moths, that mating in the cages is entirely random, and that the capacity for mating is the same in both sexes. In all but one case the actual number of viable eggs per normal female was a little higher than the calculated number for the given ratio of sterile moths. However, the actual numbers were remarkably close to the calculated numbers, considering the normal variability in egg output. The result was especially close in the test with 200 sterile females,

TABLE 14

VIABILITY OF EGGS PRODUCED BY NAVEL ORANGEWORM MOTHS AT DIFFERENT RATIOS OF STERILE MALES AND STERILE FEMALES TO UNTREATED MALES AND FEMALES

Test population*				Viable eggs laid†		
Untreated moths		Sterilized moths		Total production	Production per normal female	
Male	Female	Male	Female		Actual‡	Calculated
no.	*no.*	*no.*	*no.*	*no.*	*av. no.*	*av. no.*
40	40	0	0	1,221	30.53	30.53
40	40	40	0	496	12.40	15.26
40	40	200	0	287	7.18	5.09
40	40	0	200	233	5.83	5.09
40	40	200	200	63	1.58	0.85

* Moths caged together within 24 hours of the first emergence from each lot of pupae. Totals of five replicates for each test.

† During seven days in mating-oviposition cages.

‡ L. S. D. at the 5 per cent level = 2.44.

whereas in the two tests with sterile males one result was higher than the calculated number and one was lower. In the test with sterile moths of both sexes, though the actual number of viable eggs was nearly twice the calculated number, still it was only a fraction of the number obtained with either sterile males or sterile females. It is clear that sterilized moths are as successful in mating as are untreated moths, that sterile females reduce the numbers of viable eggs quite as effectively as do sterile males, and that sterile moths of both sexes accomplish more reduction than do sterile moths of only one sex.

DISCUSSION

The most significant result of this investigation is the evidence that sterile females can be used in autocidal control as successfully as sterile males and that far better control may be obtained by using both together.

Most of the previous literature has ignored the possibility of using sterile females in a control program. However, Lindquist (1955) reported that sterile flies of both sexes were released in the eradication of the screw-worm on the island of Curaçao, to save the time and labor required for separating out the males. In our opinion, the striking success obtained in that work may have resulted from the use of both sexes.

Andrewartha and Birch (1960) made the general statement—but without explanation—that the extermination of an insect population might be more certain and might be achieved more economically if only sterile males were released.

Proverbs (1962) irradiated mature pupae of the codling moth with 30,000 rads and found that 50 irradiated male moths caged with 5 normal males and 5 normal females reduced the number of viable eggs by 98 per cent. In similar tests, 50 irradiated female moths gave only 60 per cent reduction and 100 irradiated moths (50 males and 50 females) gave only 66 per cent reduction. However, as the 30,000-rad dosage did not sterilize either sex completely, any fertile eggs deposited by the irradiated females would alter the results considerably. Proverbs (1962) used also a 40,000-rad dosage, which apparently sterilized all the treated females in his other tests. However, here again he obtained more fertile eggs when he introduced irradiated males and females with normal moths than when he introduced only irradiated males. In our tests, on the other hand, females sterilized with 50,000 rads were as effective as sterile males in reducing the numbers of viable eggs, and sterile moths of both sexes were still more effective.

Actually, the dosage that we found necessary for complete sterilization of mature navel orangeworm pupae—50,000 rads—is a little higher than that reported adequate for sterilization in the lepidopterous species investigated by Cornwell, Crook, and Bull (1957), and by Vasilyan (1961). Proverbs and Newton (1962) analyzed many factors affecting the results of irradiation and determined that exposure to 40,000 rads sterilized the female codling moth but that not even 75,000 rads made all the males completely sterile. They reported that female codling moths were not only more readily sterilized than males but also more sensitive to radiation injury. However, with other insects, Lindquist (1955), Jaynes and Godwin (1957), and Bletchly (1961) all reported that males were more easily sterilized by radiation than were females of the same species. We detected no consistent differences between the two sexes in dosage required for sterilization at the various stages.

Reexamination of the data of Bushland and Hopkins (1951), who caged sterilized screw-worm flies with untreated flies in various ratios, shows that when they added only sterile males to the normal population the number of viable eggs was lower than when they added only sterile females. This could be expected, because the female screw-worm fly mates only once, whereas the male mates several times. Moreover, adding sterile flies of both sexes reduced the numbers of viable eggs more than adding only sterile males.

The difference in efficiency of control with sterile males or sterile females may depend on the mating behavior of the insect in question. For a species in which a male normally mates more times than a female, the release of sterile males may be the more effective. Probably the reverse would be true for a species in which a female is able to mate more times than a male. The habit of multiple mating seems not to reduce the effectiveness of autocidal control for the navel orangeworm. Some moths of either sex may fail to mate but others may mate several times. We found a low percentage of multiple matings in female moths from black-light catches examined in the 1962 season. The more extensive studies of Goodwin and Madsen (1964) showed that multiple matings of navel orangeworm moths in the field increased as the season progressed.

SUMMARY

The above studies were oriented toward control of the navel orangeworm by the sterile-insect method. The first step—laboratory culture—required special conditions: high relative humidity and good indirect air circulation. Successful mating was obtained at temperatures between 10° and 16° C, with light intensity similar to that of the early-morning hours. Temperatures around 28° C favored growth and development.

Sterilizing treatments, using a cobalt-60 irradiation unit, were tested on all stages of the navel orangeworm. Irradiating either eggs or larvae did not give complete sterility without injurious effects and high mortality. Common injuries were failure to pass a critical stage of development, delayed metamorphosis, and deformed pupae or adults. We found that mature pupae, about eight days old, were best able to tolerate irradiation and also were convenient to handle.

Both sexes were sterilized completely by exposure of the mature pupae to 50,000 rads of gamma radiation ± 3.5 per cent. This treatment did not seem to affect mating habits, egg-laying, or longevity of the adults. A dosage of 40,000 rads very nearly sterilized most of the pupae treated, but not with sufficient certainty for use in a control project.

Multiple matings by navel orangeworm moths are fairly common but do not interfere with the success of a control program. At least one female mated five times and some males mated three times, probably more.

The most important result of this work is the demonstration that sterile females of the navel orangeworm can be used for autocidal control as successfully as sterile males, and that far better control—perhaps even eradication in some areas—may be obtained by using sterilized moths of both sexes at the same time. It might seem that the use of sterilized females would reduce the available number of sterile males competing with the normal males. However, statistical analysis shows that introducing sterile moths always reduces the probability of fertile matings and that sterile moths of either sex have independent competitive value. Thus, adding both sterile males and sterile females to a normal population gives two-way competition for the normal mates and does not simply add but actually multiplies the probabilities of sterile matings by the normal males and the normal females.

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