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Fred Robert Slater Nelson
University of Massachusetts Amherst

COMPARATIVE STERILITY EFFECTS OF GAMMA RADIATION AND A CHEMOSTERILANT ON *DROSOPHILA MELANOGASTER* MEIGEN

A Dissertation Presented

By

FRED R.S. NELSON

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Approved as to style and content by:

Peter C. Steel
(Chairman of Committee)

William E. Tomlinson Jr.
(Member)

Frank R. Shaw
(Member)

T Michael Peters
(Head of Department)

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INTRODUCTION

The concept of utilizing sexually-sterilized insects to act as agents in the reduction or elimination of a species offers challenging opportunities for more effective and desirable means of insect management. Increasingly, there appears to be widespread acceptance of the sterility concept as a promising approach to insect population reduction. This method of insect population eradication is unique in that it utilizes the inherent mating instinct to the detriment of a species with minimal harm to other species in the environment, with perhaps the exception of obligatory parasites of the species under attack.

The sterility approach involves two entirely different techniques. One involves the continued release of sterile insects for several generations into the natural population. The declining fertile individuals in the natural population experience progressively greater odds in encountering fertile mates, until the chances for successful fertile matings reach zero. The other method involves the sterilization of a portion of the natural population by exposing them to chemosterilants, essentially in the manner by which insects are exposed to insecticides.

At the beginning of the 20th century, scientists began genetic studies with Drosophila and have since utilized it as a useful tool in various areas of biological research. However,

in the tomato canning industry Drosophila is especially undesirable since the presence of different stages, or fragments of these insects in tomato products is considered as contamination by the Food and Drug Administration.

The high reproductive potential of Drosophila, the ease with which they may be reared on artificial diets, and the lack of adequate conventional field control methods qualify them as suitable organisms for sterile-male technique investigations. This research was undertaken to determine the levels of gamma rays and a chemosterilant necessary to induce sterility in males of Drosophila melanogaster, without adversely affecting mating behaviour and longevity.

REVIEW OF LITERATURE

This literature review cites examples of progress made in inducing sterility in insects by means of irradiation and chemosterilants. Although emphasis is placed on Drosophila, the discussions also deal with other insects of economic and medical importance.

Biology of Drosophila

Demeric and Kaufmann (1965) gave a detailed and comprehensive account of the developmental stages of Drosophila as follows:

Egg. The egg is approximately 0.5 of a millimeter in length. The chorion is opaque and shows a pattern of hexagonal markings. A pair of filaments, extending from the anteriodorsal surface, keeps the egg from sinking into soft food on which it may be laid. Penetration of spermatozoa into the egg occurs through the micropyle, in the conical protrusion at the anterior end, as the egg passes through the uterus. The eggs may be laid by the female shortly after they are penetrated by the sperm, or they may be retained in the uterus during the early stages of embryonic development. Each female may lay over 300 eggs and the duration of the egg stage at 25°C is about three days.

Larva. The larval stage consists of three instars and at the end of the final instar may attain a length of 4.5 millimeters. The larvae are intensely active and voracious feeders and the culture medium usually becomes heavily channeled and

furrowed. This "working" of larvae is the simplest criterion for deciding, at a glance, after egg laying, whether or not the expected generation is developing successfully.

Gonads are located in the "fat bodies" that lie along the sides in the posterior portion of the larva. Because the testes of male larvae are much larger than ovaries of female larvae of corresponding or even greater size, there is no difficulty in determining the sex of individual larvae. The average length of the larval stage is five days.

Pupa. Larvae preparing to pupate, usually crawl from the culture medium and adhere to relatively dry surfaces. Drosophila pupates within the last pupal skin. The pupa at first is soft and white but slowly hardens and darkens. The length of the pupal period at 25°C is four days.

Adults. The adult forces its way through the anterior end of the pupal case. Upon emergence, the flies are relatively light in colour, but darken in a few hours. The tip of the female abdomen is more elongated and pointed, while that of the male is more rounded. As the female ages, the abdomen becomes distended with mature eggs. In many strains, the pattern of darker markings on the abdominal segments is sufficiently distinctive in the two sexes to permit separation on this basis. Males have the "sex comb," a fringe of about ten stout, black bristles on the distal surface of the basal tarsal joint of the foreleg. Such bristles are lacking in the female.

History of Insect Sterilants

Interest arose in the sterile-male techniques between the years 1937 and 1938, while Knipling was investigating the screw-worm, Cochliomyia hominivorax, in the southern parts of the United States. This worker was greatly impressed by the life history, biology and population dynamics of the screw-worm fly. At about the same time, progress was also made in producing sterility in D. melanogaster by the use of X-rays. Although the possibilities of sterile-male technique were discussed, conditions were not favourable for the pursuance of the investigation until 1946 through the renewed effort of Knipling. However, many scientists, especially geneticists, were skeptical regarding the feasibility of this approach and expressed doubts as to the ability of sterile flies to mix and compete in nature with normal individuals. Bushland, (1948-49) demonstrated within a relatively short period of time that it was possible to completely sterilize both male and female screw-worm flies with X-rays at 3000 and 4000r respectively.

Extensive investigations in the control of tsetse fly by insecticides and cultural methods were not fully successful. Research was conducted and it was ascertained that tsetse fly could be controlled with both 6,000 and 12,000r of gamma rays. However, one objection to the sterile-male release was that when males of some species attempted to mate with females of other species, they injured the abdomen of the females and thus prevented them from carrying on normal reproduction.

The dramatic success of the sterile-male release program in Curacao and southeastern United States stimulated research for a substitute method to replace radiation to obtain a more flexible and economic method of achieving sterility. By the year 1961 research workers detected that it was possible to sterilize insects by chemicals possessing sterilant activity, now known as chemosterilants. This approach precludes the necessity of rearing and sterilizing astronomical numbers of a species that after release might injure a crop or could be a disease vector.

To-day, the sterile-male technique is becoming an important part of insect suppression in many parts of the world.

Sterilization Methods

X-ray. Runner (1916) first demonstrated sterility in insect populations by showing that cigarette beetles produce infertile eggs when exposed to X-rays. Muller (1927) demonstrated similar effects in fruit flies, Drosophila melanogaster.

Bushland et al. (1951) demonstrated that screw-worm males could be made sexually sterile by exposing the pupae to X-rays or gamma rays without serious adverse effects to the mating behaviour of the insect. The investigation showed that the female screw-worm fly normally mates only once and when mated to a sexually sterile male her reproductive potential was completely destroyed.

Yanders et al. (1959) demonstrated that sperm of some Drosophila survived doses of X-rays in excess of 50,000r. Studies with Habrobracon showed conclusively that the dose

required for complete inactivation of sperm was considerably in excess of that required to achieve 100% dominant lethality (Whiting, 1949).

The effects of X-rays on European corn borer Ostrinia nubilalis (Hubner) exposed either in the pupal or adult stages, were investigated by Walker and Brindley, 1963. Male adults, less than twenty-four hours old, were sterilized by doses of 32,000r. When these males were mated with untreated females, less than 1% of the eggs hatched. Longevity was about the same for the irradiated and non-irradiated corn borers and irradiated males competed equally with normal males for females.

Gamma Ray. The use of sexually-sterilized males is a highly effective approach to the control or eradication of certain insect populations (Knipling 1960, Baumhover et al. 1955). The screw-worm fly Cochliomyia hominivorax (Coquerel) was successfully eradicated from Curacao and the southeastern United States following the release of males sterilized by ionizing radiation.

Eggs, larvae, pupae, and adults of codling moth, Carpocapsa pomonella (L.) were irradiated in a Cobalt-60 source at various dosages (Proverbs et al., 1962). Decreased larval vitality, high pupal mortality and deformed adults, occurred when eggs and larvae were irradiated at 25,000 and 50,000r. When irradiation was carried out during the pupal stage at a dose of 20,000r, female moths produced no viable eggs. Male moths were less sensitive to radiation since a 25% hatch was obtained in matings with normal females. Mature pupae within

24 hours of emergence or adult moths 0 to 24 hours old (anesthetized with CO_2 for convenience in handling) were irradiated at 40,000r and 98% sterility was obtained with no undesirable side effects.

Field cage tests using 20:1:1 and 20:20:1:1 ratios of treated moths (40,000r) versus normal moths indicated a 10% greater reduction of the F_1 generation when the irradiated females were not present (Proverbs et al. 1962).

Morlan et al. (1962) released sterilized Aedes aegypti males in several areas in Florida. The results were not conclusive since mosquito populations decreased in both the tests and check areas. The investigators concluded that before the sterile-male technique can be adopted for mosquito control, additional investigations of mosquito biology are required, especially with regard to male dispersal under field conditions.

Fay et al. (1963) developed techniques for rearing and irradiating A. aegypti. Mosquitoes were reared in large trays, each containing about 8,000 larvae in 6 to 12 liters of water. Pupae were separated from the larvae by an ingenious technique adopted from Bar-Zeev and Galum, whereby magnetic iron oxide was added to the larval medium. Larvae ingested the oxide and then were separated by means of an electromagnet from the pupae and prepupae which do not feed. Batches of 60,000 pupae were irradiated by placing them around a Cobalt-60 point source. The basic irradiation procedure described by McCray was modified for two reasons: a) because of the large variations in dosage (from 9,400 to 18,750) which were obtained, b) because

of the decrease in emergence and in longevity noted at dosages above 12,000r. By changing the geometry of the pupae holders in relation to the point source, the limits of the dosage range were narrowed down to between 8,800 and 9,500r and pupal survival was increased to 94%. These workers also tested the mating competitiveness of sterile and normal males two to three days of age at 21:1:1 and 5:1:1 ratios. The sterilized males were less competitive as they became older compared to normal males.

Henneberry (1963) found that untreated females of D. melanogaster mated with males, exposed to 4 Kr. of gamma radiation in the larval, 16 Kr. in the pupal or adult stages, deposited the normal number of eggs, but none hatched. Females, irradiated in the pupal or adult stage with 8 or 16 Kr. and mated with untreated males, produced few or no eggs. Females, irradiated in the larval stage, produced fewer eggs after exposure to high doses of gamma radiation than untreated females, but showed no reduction in the percentage of emerging adults.

Longevity of males or females exposed in the pupal or adult stage was not affected by irradiation. However, males and females irradiated in the larval stage were shorter lived than untreated insects.

Untreated females mated with irradiated males (16 Kr.) produced sterile eggs, but when mated a second time with untreated males produced viable eggs. Untreated females mated with normal males produced viable eggs, and when subsequently

mated with irradiated males continued to produce viable eggs. Sterile males confined with normal males and females reduced the number of progeny.

Henneberry et al. (1963) showed that three to four day old Drosophila males exposed to 16 Kr. of gamma radiation did not mate as readily or as many times with virgin females as untreated males or males exposed to 8 Kr. However, males exposed to 16 Kr. recovered within twenty-four hours and normal mating frequency and behaviour occurred. When males, exposed to 16 Kr., were held for twenty-four hours prior to mating, the number of matings per day was not reduced nor was the behaviour of these males affected as compared to untreated males.

Results of multiple mating tests, in which one male was confined with ten virgin females, indicated that on the average, both irradiated and non-irradiated males mated about seven times.

The effects of radiation on the fertility of Drosophila were also investigated by Henneberry and McGovern (1963). Normal females mated with adult males irradiated with 16,000r at one, five, or ten days after emergence produced approximately the same number of eggs as the controls but very few or no adult progeny emerged. However, females irradiated when ten days old, produced more eggs than females treated when one to five days old.

Also virgin females mated to irradiated males, that were held six days after being irradiated at 16,000r, produced the same number of eggs as those mated to males immediately after

irradiation. Thus, sperm viability was not restored in the males during these six days.

In further tests, males exposed to 8,000r or 16,000r were allowed to mate five successive times with virgin females either on the same day or five and ten days after treatment. Fewer eggs were deposited by the fourth and fifth female than the first and second in each series, an indication of sperm depletion.

In an investigation to compare the mating ability and reproductive potential of the yellow fever mosquito Aedes aegypti, Weidhaas and Schmidt (1963) reported that males treated as pupa, either with chemosterilant or with gamma radiation at 8,000 or 10,000r, and tested at a 4:1:1 ratio, were not fully competitive. The reason for the lack of competitiveness was not apparent, since male vigor, longevity and mating behaviour was similar to that of normal males.

LaChance et al. (1963) reported on the cytopathology of normal and irradiated screw-worm ovaries when irradiation (2,000 or 4,000r) was applied during various developmental stages. The cytology of the reproductive system, from five-day-old pupae to the sexually mature female, four to five days old, is described in detail. They found the most radiosensitive stage to be the period during which the egg chambers contained nurse cells undergoing endomitotic replication of chromosomal material.

The effects of gamma irradiation on the horn fly Haematobia irritans were investigated by Lewis and Eddy, 1964. Both sexes of the horn fly were sterilized at a dosage of

5,000r by irradiating pupa with a Cobalt-60 source. Longevity of adults was not affected at this dosage level. However, at higher dosages of 10,000 and 25,000r, adults were weakened and had a much shorter lifespan.

To determine the competitiveness of irradiated males, irradiated pupae and untreated pupae at a 10:1 ratio were caged together. Females laid 66% fewer eggs than the controls. Thus, the irradiated males did not fully compete with the untreated males as the reduction in female fertility was less than predicted theoretical values. However, such data might not represent an accurate analysis of the effects of radiation on female fertility, since they were derived from only one-tenth of the females and the irradiated females did not lay eggs.

Ouye et al. (1964) investigated the effects of gamma radiation on pupae and adults of pink bollworm, Pectinophora gossypiella. Fewer side effects, such as deformed wings, occurred when the treatments were made during the later stages of pupal development than when they were made to one and three-day old pupae. Seven-day old pupae required a radiation dose of 40,000r to sterilize females, whereas, a much higher dosage of 55,000r was required for males.

The longevity of treated males was approximately one-half that of males in the controls. However, the investigators pointed out that differences in longevity between treated and untreated males may not be a great disadvantage because of the mating habits of the moth. The important factor would be the

competitiveness of the irradiated males. Ouye indicated that males from seven-day old pupae treated with 34,000r were only partially competitive when released in ratios of 19:1:1 into large cages containing cotton plants. The mean reduction in population was only 52% instead of the expected 96%.

Aerial releases of sterile screw-worm flies, Cochliomyia hominivorax were made in field tests in northern Mexico, at a rate of 4,000 flies per square mile (Davis et al., 1967). The results were evaluated on the basis of the numbers of sterile egg masses recovered from wounded sheep penned in the area. Approximately 70% of egg masses collected from the test area were sterile when the population of wild flies was low. However, the sterile flies were ineffective in preventing a population increase of wild flies with favourable weather conditions. Furthermore, effective control was not obtained in persistent population centers nor in unisolated populations. Release of sterile flies so far apart may be of value in areas where wild fly population density is low and scattered for an extended period.

Abdel-Malek et al. (1967) conducted experiments to investigate the sperm activity in irradiated males using non-irradiated female Anopheles pharoensis and normal or irradiated males at 12,000r in cages. Normal or irradiated males, after complete matings with females, were replaced by irradiated or normal males and egg production and hatchability were recorded daily for the first ten days. When normal males were replaced by irradiated males there was a decrease in egg hatchability

Sterilization of males with 12,000r did not damage the sperm, since sperm of irradiated males competed successfully with that from normal males.

Chemosterilant. One of the first reports on the induction of sterility in insects by chemicals was that of Goldsmith and Frank, (1952). These workers found that an antimetabolite, amenopterin, fed to adult Drosophila for seven days reduced oviposition and, in many instances, prevented the treated females from laying eggs. Mitlin et al. (1957) extended these observations with aminopterin to the house fly Musca domestica (L.). These workers successfully induced sterility in female house flies by feeding mechlorethamine or colchicine. Sublethal doses of nitrogen mustards (Bird, 1950), or esters of methane sulfonic acid (Fahmy and Fahmy, 1961), administered by feeding or injection, completely sterilized males of Drosophila.

LaBrecque (1961) found that both male and female house flies were sterilized after the insects had fed on food treated with the aziridinyl derivatives, apholate, tepa or aphomide. Weidhaas et al. (1961) induced sterility in Aedes aegypti and Anopheles quadrimaculatus by feeding the adult mosquitoes on honey solution containing 0.1% of apholate.

Weidhaas (1962) demonstrated that the exposure of Aedes aegypti (L.) larvae from the third instar to pupation, in water containing 10 parts apholate per million produced approximately 90% sterility in the ensuing males, about 50% sterility in females. When both sexes were treated, sterility was 98%.

Plapp et al. (1962) investigating the metabolic fate of methapoxide found that degradation of the compound was complete within forty-eight hours of administration in adults and larvae of Culex tarsalis (Coquillett).

LaBrecque et al. (1963) applied baits containing 0.5% of metepa to droppings in a poultry house to evaluate the chemosterilant techniques for controlling house flies. Several applications were made at weekly intervals, for nine weeks, and then semi-weekly. Granular corn meal baits were most effective. The abundance of flies decreased sharply in the treated area and less than 10% of all eggs collected from females did not hatch.

Male and female house flies were successfully sterilized by tarsal contact with residues on glass surfaces treated with metepa and tepa but not by apholate or 5-fluororotic acid (Meifert et al., 1963).

Gains et al. (1964) studied the toxic effects of metepa on rats and found that the acute oral LD50 was 136 mg/Kg. in males and 213 in females. An oral dosage of 5 mg/Kg, per day produced severe reduction in fertility of males within 70 days and testicular atrophy within 77 days. A dosage of 2.5 mg/Kg. per day produced some reduction in fertility and partial testicular atrophy in 197 days. Dosages of 1.25 mg/Kg. per day for 197 days produced no detectable effect on fertility of males or histological changes in the testis.

Murvosh et al. (1964) studied the relationship between the concentration of metepa, apholate, and tepa in the diet and the degree of sterility induced in the adult house flies, Musca

domestica. They found that a wider variation resulted than would be expected from similar tests with insecticides, but construction of valid concentration/sterility regression lines was still possible. The calculated sterility concentrations (SC50 and SC90) of metepa and apholate were similar; while tepa sterilized at lower concentrations. Metepa and apholate at 1% substantially shortened the life span, although a slight delay in initial male mortality occurred. More than 90% of males survived the first ten days, a time span probably sufficient to allow mating with most of the females that emerge at the same time as the males.

In topical application experiments Chamberlain (1964) compared the level of metepa necessary to sterilize the stable fly and screw-worm. The male screw-worm required 5.5 times as much as the stable fly and the female screw-worm fly required 18 times as much as the female stable fly. The comparative values for feeding treatments of the screw-worm fly and stable fly were 3.9 and 6.2 times, respectively, for males and females.

Dame et al. (1964) found that P^{32}-labeled metepa was rapidly absorbed from glass surfaces by the mosquitoes Anopheles quadrimaculatus (Say) and Aedes aegypti and the house fly Musca domestica. The house flies and A. quadrimaculatus absorbed approximately 7 µg per insect during a four-hour exposure on surfaces treated at 10 mg/ft^2 whereas A. aegypti picked up 2.5 µg. This uptake resulted in a severe reduction of mating ability in mosquitoes, coupled with 99% sterility in house fly

and A. aegypti males. Metepa was found to be quite volatile on glass surfaces and highly sorptive on masonite. Under similar conditions A. quadrimaculatus absorbed 27 mμg from masonite treated at 100 mg/ft^2 and 7600 mμg from glass treated at 10 mg/ft^2.

At the end of three days feeding on treated food, the amount of chemosterilant, expressed in μg-equivalents of P^{32} metepa, were 3.0, 3.7, and 1.7 in A. quadrimaculatus, Musca domestica and A. aegypti, respectively. These doses caused sterility in all species without any reduction in male vigor. Exposure of mosquito larvae from the third instar through pupation in water treated at 10 PPM resulted in low metepa uptake and very little induced sterility. Distribution of metepa in the insects was rapid and apparently non-selective.

Excretion of metepa was rapid in insects exposed to residual deposits of 10 mg/ft^2. Insects exposed to treated larval medium and food retained a high percentage of their original radioactivity over prolonged periods. This activity undoubtedly represented detoxified metepa.

Gouck (1964) induced sterility in house flies by dipping different ages of the pupae in apholate, metepa, and tepa at concentrations of 2.5% and 5% for 30 to 300 seconds.

Hazard et al. (1964) found that certain insects could develop resistance to a chemosterilant. Increased resistance to the sterilizing effects of apholate was observed in two colonies of A. aegypti exposed in the larval stage of each generation to concentrations of apholate that induced about

90 to 49% sterility in the eggs laid by the ensuing adults.

Painter et al. (1964) tested fifteen compounds for chemosterilant activity against M. domestica. The sterilants were fed at levels up to 1% to newly emerged adult insects for forty-eight hours. Six of the compounds tested were effective. The compounds methotrexate 0.10% and 5-fluorodeoxyuridine 1.0% induced sterility with no oviposition. Methotrexate 0.01%, 5-fluorouracil and 6-methyluracil were temporary sterilants, and apholate and theotepa induced permanent sterility with oviposition.

Oviposition was prevented in house flies by concentration of 0.25% tepa, 0.5% metepa and 0.125% 1-methamesulforylaziridine (Parish et al. 1965). When flies were exposed to lower concentrations of sterilants non-fertile eggs were deposited.

Howland et al. (1965) found that cabbage looper was sterilized by feeding on a diet containing apholate, tepa or metepa in sugar solution or by exposure to metepa or tepa. Males were sterilized when fed 0.06% apholate or 0.02% tepa solutions whereas 0.25% apholate or 1% tepa was necessary to induce sterility in females. Complete control of reproduction was obtained when moths were fed 0.02% or higher concentration of metepa.

Ouye et al. (1965) treated one-day old male adults of pink bollworm, Pectinophora gossypiella (Saunders) with metepa on the mesosternum. These workers determined that males treated with 10 µg each, reduced egg hatch only 69.1%. At 15 µg, sterilized males were fully competitive with normal

males. Competitiveness decreased appreciably when males were treated with 35 and 50 µg. Reduced competitiveness was due to an overdose of metepa, which resulted in reduced mating by sterile males as indicated by examination of a small number of females. The number of spermatophores produced by this sample of meta-sterilized males (20 males, 35 µ each), a measure of mating frequency as demonstrated by Ouye et al. (1955b) showed that the sterile males mated 2.4 times as compared with 4.1 times for normal males.

Ratcliffe et al. (1965) conducted outdoor cage tests with apholate, metepa, and 4-bifunctional aziridine chemicals against the house fly, Musca domestica. The tests evaluated the effectiveness of five apholate formulations and granular sugar baits of the six chemicals under variable environmental conditions and moderately high house fly populations. The performance of apholate formulations ranked in this order of effectiveness: liquid and granular baits, impregnated ribbon, impregnated string, and residual treatments on plywood.

The effectiveness of the baits was associated with good attractiveness and more rapid availability of the sterilant. High sterility was obtained with each of the six chemicals as granular sugar baits. Metepa and a bis aziridene diphosphaspirodioxide compound, which were one-third as effective as apholate in the laboratory, gave promising results at field concentrations.

Collier and Downey (1965) tested metepa, tepa and apholate against eggs, pupae and adults of the gypsy moth,

Porthetria dispar. These investigators found no reduction in the number of eggs hatched, and with the exception of tepa, pupal dips were not effective. Concentrations at 1, 4, and 8 µg/moth were ineffective when applied topically to both sexes. Residual films of both metepa and tepa caused significant sterility of male moths. At high residual levels apholate caused sterility to both sexes.

Sterility was induced in both sexes of one or more species of tephritid flies without toxic effects by treating food and water with tepa, metepa, apholate or tretamine (Keiser et al., 1965). These compounds were applied topically to pupae or adults. Only females were sterilized when adults were exposed to deposits of the chemosterilants, methotrexate, aminopterin, colchicine, and 5-fluorouracil treatments.

Tepa, apholate, and tretamine sterilized as effectively and efficiently as ionizing radiation. Treatments were most effective against newly emerged flies, but deposition of hatched eggs by old gravid fertile females was inhibited within twenty-four to forty hours after treatment.

Chang (1965) found that 1 µ of tepa injected into male house flies reached 50% sterilization effectiveness in twenty-three minutes, and full effectiveness in three and one-half hours. Males remained sterile for one week. Partial restoration of male fertility occurred thereafter. Tepa was equally effective in sterilizing males of different ages.

Meifert et al. (1967) studied the effects of metepa, apholate and an insecticide trichlorofon against house flies

on three islands in the West Indies. They found that a 1% metepa liquid bait induced sterility in excess of 80% and reduced fly abundance more than 90% over a period of eighteen months. At similar concentration apholate gave 60-80% sterility and reduced fly abundance 50-80%.

Crystal (1965) and Fye et al. (1966) demonstrated the effectiveness of N,N[1] tetramethylenelus (1-aziridine carboxamide) as a chemosterilant of screw-worm and house fly. Crystal (1965) also found that the sterility of male screw-worm flies was greatly reduced by copulating with topically treated females.

Creighton et al. (1966) found that metepa-fed female adults of the banded cucumber beetle, Diabrotica balteate (LeConte), deposited significantly fewer eggs in laboratory cages than untreated beetles. Metepa, apholate and tepa fed to male adults gave significantly varying degrees of sterility. In laboratory studies with metepa, apholate and tepa, Ladd (1966) showed that topically-treated adults of Japanese beetle, Popilla japonica, deposited varying numbers of infertile eggs. Tepa was evaluated as the most effective.

Bhalla et al. (1966) fed nymphs of the pea aphids, Alyrthosiphon pisum, a chemical diet containing tepa, apholate or metepa. Reproduction was inhibited at dosage levels between 0.005 to 0.12, but there was no mortality to feeding nymphs at dosage levels between 0.001 and 0.12. Tepa also inhibited reproduction at dosage levels between 0.0025 and 0.1% but mortality was observed at dosages above 0.025%. Metepa was found toxic to nymphs.

Harris et al. (1966) found that apholate, tepa and an aziridiryl benzoquinone compound induced sterility in adult male and female horn flies, Haematobia irritans, when applied topically or mixed with the diet. A diet containing 0.05% tepa or 0.01% apholate produced complete sterility when it was given to adult males and females as a single overnight feeding. Horn flies fed continuously on a diet containing 5 PPM of tepa were completely sterilized. Mating tests indicated that horn flies sterilized with tepa were not as competitive as untreated males.

Toppozoda et al. (1966) determined the effects of apholate, metepa, and tepa on the larvae and the adults of the Egyptian cotton worm, Prodenia litura. The three sterilants were equally toxic to fourth instar larvae and gave partial sterility only to adults. Adults fed chemosterilants in sugar solution developed 100% sterility with concentrations of 1.1% metepa, 1.2% apholate, or 0.08% tepa. Tepa was found much more effective than metepa and apholate.

Three compounds, tepa, metepa or apholate were fed to moths of the cabbage looper, Trichoplusia ni (Henneberry et al. (1966). Tepa-fed male moths did not mate as frequently as untreated males. Apholate and metepa were less effective than tepa in sterilizing both sexes.

Klassen et al. (1966) found that, through selection, a population of Aedes aegypti may respond to metepa and develop resistance within a few generations when treated in the larval stages. Selection for resistance was made by exposing large

numbers of early fourth instar larvae to metepa solutions until pupation. Adults were placed in a cage and maintained for egg production. Batches of approximately 50 to 100 larvae were placed in 250 ml. water at 22°C to which 1 ml. ethanolic solution of metepa was added.

The first two generations were selected with 16 P.P.M.; the third, fourth and fifth with 32 P.P.M. and the sixth, seventh and eighth with 64 P.P.M. Selection with metepa for eight generations produced a strain which laid only viable eggs. The F_8 generation was observed to develop a low measure of resistance.

McCray, Jr. et al. (1967) investigated the comparative effectiveness of apholate, tepa and metepa on male southern house mosquitoes, Culex pipiens quinquefasciatus (Say). In a modified Hoskins-Caldwell spray chamber virgin males four days old were exposed to mists of the three chemosterilants in an 85:15 mixture of ethanol-glycerol. After twenty-four hours the males were introduced to virgin females and maintained as small colonies. Egg rafts were collected daily, and subsequent hatch was determined. A 2% concentration of apholate produced sterility of 95% or better. A 4% concentration of tepa and 8% concentration of metepa were required to produce similar levels of male sterility.

In feeding experiments Sato et al. (1967) found that the compounds, apholate and metepa induced partial to complete sterility in the adult bollworm Heliothis zea, and the tobacco budworm, H. virescens. At all levels metepa was more effective

than apholate. Both chemosterilants caused reduction in mating frequency and oviposition at high dosages.

When tobacco budworm moths were fed 1% apholate for one, two, four and eight days, results indicated little difference in effect among two, four and eight-day feeding period. The percent hatch was 6.8 for the insects exposed for one day, while exposure for two, four, and eight-day periods resulted in 0.4, 0.3 and 0% hatch, respectively. When both sexes were treated the effects were cumulative. Ovarioles of both species were significantly reduced in size when adults were fed the chemosterilants.

Morgan et al. (1967) observed a general loss in fertility in colonies of house flies exposed to low concentrations of chemosterilants. Fye et al. (1967) tested twenty-four different chemosterilants in sugar syrup against house flies. The flies were given simultaneous access to untreated food. The concentration of the chemosterilants ranged from 0.01 to 1% and were usually near the minimum at which each compound produced sterility in previous tests where no food was offered. Metepa and hempa at 1% concentration and fifteen other sterilants produced complete sterility in some or all tests with treated males mated to females, untreated females or both. The remaining sterilants induced 76 to 99% sterility.

Meifert et al. (1967) found that the treatment of female house flies with N,N'-tetramethylenelies (1-aziridine carboxamide) was an effective method of sterilizing males. Females which carried treated pads attached to the abdomen or were

treated directly on the dorsum induced sterility in male flies that were subsequently caged with them.

Crystal (1967) investigated the effects of N,N'-tetramethylenelies (1-aziridine carboxamide) against various stages of screw-worm, C. hominivorax. Sterility was not induced by incorporating the chemosterilant in the larval medium or by immersing prepupae in a solution. The pupae were effectively sterilized by immersing in solutions, and the adults by topical application, oral treatment, tarsal contact with residual film and injection. Oral administration to adults reduced survival of treated flies to at least half. Topically sterilized males were fully competitive sexually, but males sterilized by tarsal contact or intrathoracic inoculation were less competitive than normal males.

Crystal (1967) also treated screw-worm flies orally with 1-[bis (1-aziridinyl) phosphinyl]-3 (3,4-dichlorophenyl) urea and found that egg hatchability decreased progressively as the length of time the males fed on 5% chemosterilant increased from one to twenty-four hours. When the length of time males fed on 1% was increased from one to twenty-four hours, egg hatchability decreased more rapidly, if saturated sugar syrup was used as the vehicle than if 0.1% saturated sugar syrup was used.

Maitlen et al. (1967) chemosterilized the codling moth, Carpocapsa pomonella with an aerosol of tepa at levels of 4.5 and 22.8 µg per moth. Young et al. (1967) fed tepa to corn earworm, Heliothis zea (Boddie); the armyworm, Pseudaletia

unipuncta (Haworth) and the granulate cutworm, Feltia subterranea (F.). Males of each species were sterilized when fed 53 µg of tepa. Partial sterility of females was obtained with 53 or 106 µg of the compound.

Henneberry et al. (1967) found that untreated female D. melanogaster Meigen mated to irradiated or apholate-fed males produced about the same number of eggs as females of untreated pairs, but that most of the eggs laid were nonviable. Irradiated or apholate fed females laid fewer eggs than untreated females, and most of the eggs did not hatch. The age of males or females when treated appeared to have little effect on results obtained after radiation exposure. Apholate appeared to induce a higher degree of sterility when males or females were older at the time of treatment.

When females producing fertile eggs were mated to irradiated (16Kr) males, adult emergence was markedly reduced. Females mated first to irradiated (16Kr) males produced nonviable eggs. However, subsequent mating with untreated males resulted in the production of fertile eggs.

Suppression of Drosophila field populations in one-quarter acre tomato field plots was accomplished by using adults of both sexes sterilized with 1% aqueous solution of apholate, and released at a ratio of twenty sterile males to one native male (Mason et al. 1968). Releases made in two separate areas resulted in maximum reduction of 86 and 44%, respectively, in the number of adults developing from eggs laid by trapped native females collected from these areas. Subsequent releases resulted

in a 50% maximum reduction in the development of adult progeny from the eggs of similar females and an average suppression of native flies of about 80% for seven weeks in the field plots.

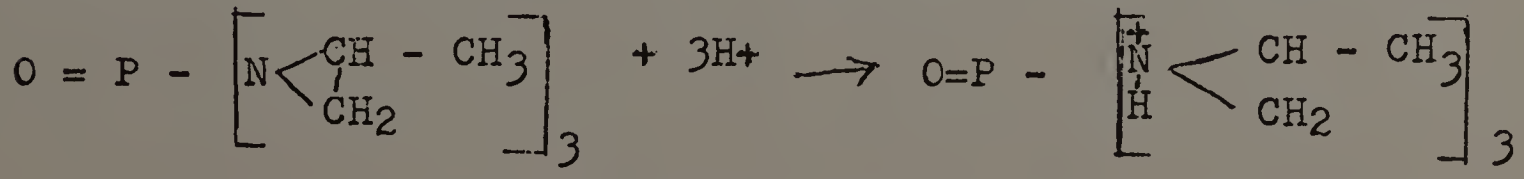
Properties of Metepa

The following properties of metepa are cited from Interchemical Co. Bulletin No. CD-107R.

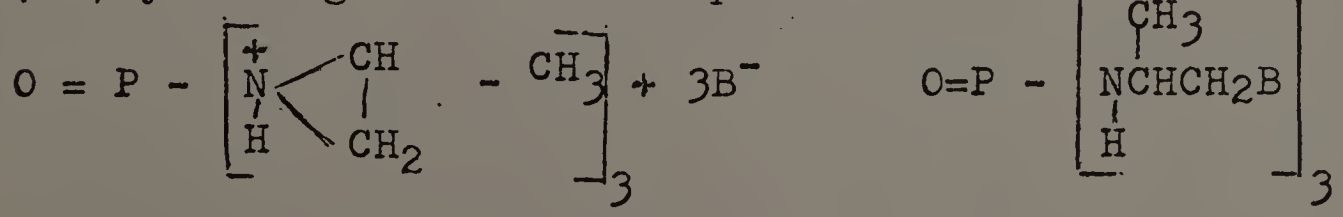
Physical properties. The typical physical properties are:

Molecular weight	215
Physical form	Liquid
Colour	Straw
Odour	High boiling amine
Reactive imine by analysis, wt. %	Min. 92
Volatiles, wt. %	Max. 0.5
Boiling point, 760 mm.	Polymerizes
1 mm.	118°-125°C.
Specific gravity, 25°/25°C.	1.079
Refractive index, $n^{25}D$	1.4798
Solubility	Completely soluble in water and all common organic solvents.

Chemical properties. Metepa is a highly reactive compound which undergoes addition and polymerization reactions via ring opening of the three membered imine ring. The ring opening is subject to catalysis by both H+ and OH- ions. With acid the reaction is believed to occur in two steps. Step one involves the formation of an immonium ion by protonation:



In step two the ring is opened by a suitable nucleophilic agent (B^-) yielding the addition product:



Physiological properties. While the physiological properties of metepa have not been fully investigated, it is known to be toxic by skin absorption and probably by ingestion.

Toxicity by absorption. In tests conducted with 30% solution applied to the skin of rabbits, a fatality did not occur until after an exposure for three days and the death of all animals did not result until after ten days. By exposure contrast similar tests with the ethylene imine analogue, tri(1-aziridinyl) phosphine oxide (APO), resulted in 100% fatalaties within twenty-four hours. Although the dosage required to kill small animals by skin absorption was above any amount likely to be encountered by a careful worker, it is evident that contact of this compound with the skin should be avoided.

Oral toxicity. No data on the toxicity of this compound by oral ingestion is available, but it is probably toxic.

Toxicity by inhalation. No data on the toxicity of compound by inhalation is available. Although metepa is a relatively non-volatile liquid, care should be taken to work with it only in well ventilated areas or in fume hoods.

Hazards and precautions. When using metepa the following precautions should be observed.

1. Do not swallow. If accidentally taken internally induce vomiting and obtain medical attention immediately.
2. Do not get in eyes, mouth, on skin or on clothing. Wear synthetic rubber gloves, eye goggles, and protective clothing. In case of contact with skin, immediately flush skin with water and wash thoroughly with plenty of soap and water.

3. Remove contaminated clothing and wash before re-use.

4. The empty containers should be thoroughly rinsed out with water before discarding and should never be re-used.

SUMMARY OF LITERATURE REVIEW

Drosophila

A. Untreated female Drosophila mated with irradiated males produced sterile eggs, but when mated a second time with untreated males, produced viable eggs (Henneberry et al. 1963).

B. Males exposed to gamma radiation did not mate as readily or as many times with virgin females as untreated males and this mating behaviour was adversely affected as gamma radiation levels increased (Henneberry et al. 1963).

C. Virgin females mated to irradiated males that were held six days after exposure to 16,000r produced the same number of eggs as those mated to males immediately after irradiation (Henneberry et al. 1963).

D. Suppression of Drosophila field population in a 1/4 acre tomato field plot was accomplished by using adults of both sexes sterilized with 1% aqueous solution of apholate and released at a ratio of 20 sterile males to 1 native male (Mason et al. 1968).

E. The age of males or females when exposed to radiation appeared to have little effect on the results obtained after radiation (Henneberry et al. 1967).

F. Chemosterilant (apholate) appeared to induce a higher degree of sterility when males or females were older at time of treatment (Henneberry et al. 1967).

G. Sterilization of Drosophila may be accomplished in the larval, pupal or adult stages (Henneberry et al. 1963).

General

H. Insects can develop resistance to chemosterilants (Klassen et al. 1966).

I. A 1% metepa liquid bait was more effective in inducing sterility in the house fly than apholate at the same concentration (Meifert et al. 1966).

J. When Pectinophora gossypiella was exposed to gamma radiation in the later stages of development, fewer side effects, such as deformed wings occurred (Ouye et al. 1964).

K. Males are sterilized at lower concentration with chemosterilants than females (Howland et al. 1965).

L. Great care should be exercised in the handling or application of chemosterilants, because of potential hazards to non-target species (Interchem. Bull. No. CD-107R).

MATERIALS AND METHODS

Rearing Procedures

Rearing medium. The several different formulations of Drosophila media cited in the literature suggest that the principal requirements of a good rearing medium are sufficient sugar to feed the larvae and promote the growth of yeast, and a proper consistency. The medium fed to adults and larvae of Drosophila was prepared by adding 9 gms. of agar (1.2%) and 100 cc. of Karo syrup to 500 cc. of distilled water. These ingredients were heated almost to boiling with constant stirring. Brown corn meal (40 gms.) and Brewer's yeast (15 gms.), mixed in 150 cc. of distilled water, was added to the hot mixture and allowed to reach the boiling point. Heat was then removed and after ten minutes of cooling, 2.5 cc. of propionic acid was added as mold inhibitor and thoroughly mixed.

This amount of medium was sufficient for 120 shell vials or 18 half-pint milk bottles. Forty-five minutes after the medium was added to individual culture or holding containers, a small amount of Fleischmann's active dry yeast was lightly sprinkled over the surface. When the yeast suspension on the sides of the containers dried, the containers were plugged firmly with cotton, packed in plastic bags and stored under refrigeration. The medium was aged for a day before offered to adults or larvae of Drosophila.

Culture containers. Wide mouth, one gallon glass jars were used as culture containers in the early part of the

experiments. These proved impractical and half-pint milk bottles, each containing 5 ml. of medium, were used for maintaining stock cultures (Fig. 1). The mouth of each bottle was tightly plugged with cotton. Cultures were established every three or four weeks depending on needs.

Flies were transferred from old to new culture bottles by tapping the bottom of old culture bottles several times on the table until most of the flies settled at the bottom. The new culture bottles were immediately inverted over the mouth of the old culture bottles under the illumination of a desk lamp. The light source attracted the flies into the new culture bottles. Usually several new cultures were established from a single culture. This technique of transferral avoided contamination of new cultures by dead adults and larvae.

Test vials. Shell vials (70x21 mm.), containing about 3 ml. of media, were used as test vials. Their wide straight tops facilitated easy cleaning and transfer of test adults. All vials were thoroughly washed and dried for several hours at 80°F. prior to use in experiments.

Holding racks. Groups of shell vials, containing mating pairs, ovipositing females and eggs were held in one-quart ice cream containers during the first third of these tests. Each container included a replicate of 11 vials tied together by rubber bands and held upright. Containers were held in an incubator as close as possible to 78°F.

Fig. 1.--Stock cultures of Drosophila in half-pint milk jars.

Plywood racks, 15 1/2 ft. x 9 1/2 ft. x 2 ins. high were substituted for ice cream containers for the remaining two-thirds of the tests. The design was such that all five replicates of each treatment were held in a single rack at the same time (Fig. 2). The racks had the advantage of easily maintaining the identity of each replicate. Each vial in these holding racks was individually numbered.

Culture populations. Fifteen individual cultures were maintained concurrently during the testing period. Old culture populations were affected by molds. Tegosept M (methyl-p-hydroxybenzoate) was first used in the medium as a mold inhibitor, but several cultures became contaminated with molds seven to ten days later. Subsequently, growth of molds was more effectively suppressed by the substitution of 2.5 cc. of propionic acid.

Overpopulation was averted by transferring limited numbers of Drosophila to new culture bottles (Fig. 3). Offspring selected from these uncrowded cultures showed less variation with respect to age.

Ecological Considerations

Temperature. Treated adult flies were held at 78°F. in an incubator during the winter months. At this temperature the reproductive potential of Drosophila is greatest. Treated adults were also held in the open laboratory in the spring and summer months when conditions allowed.

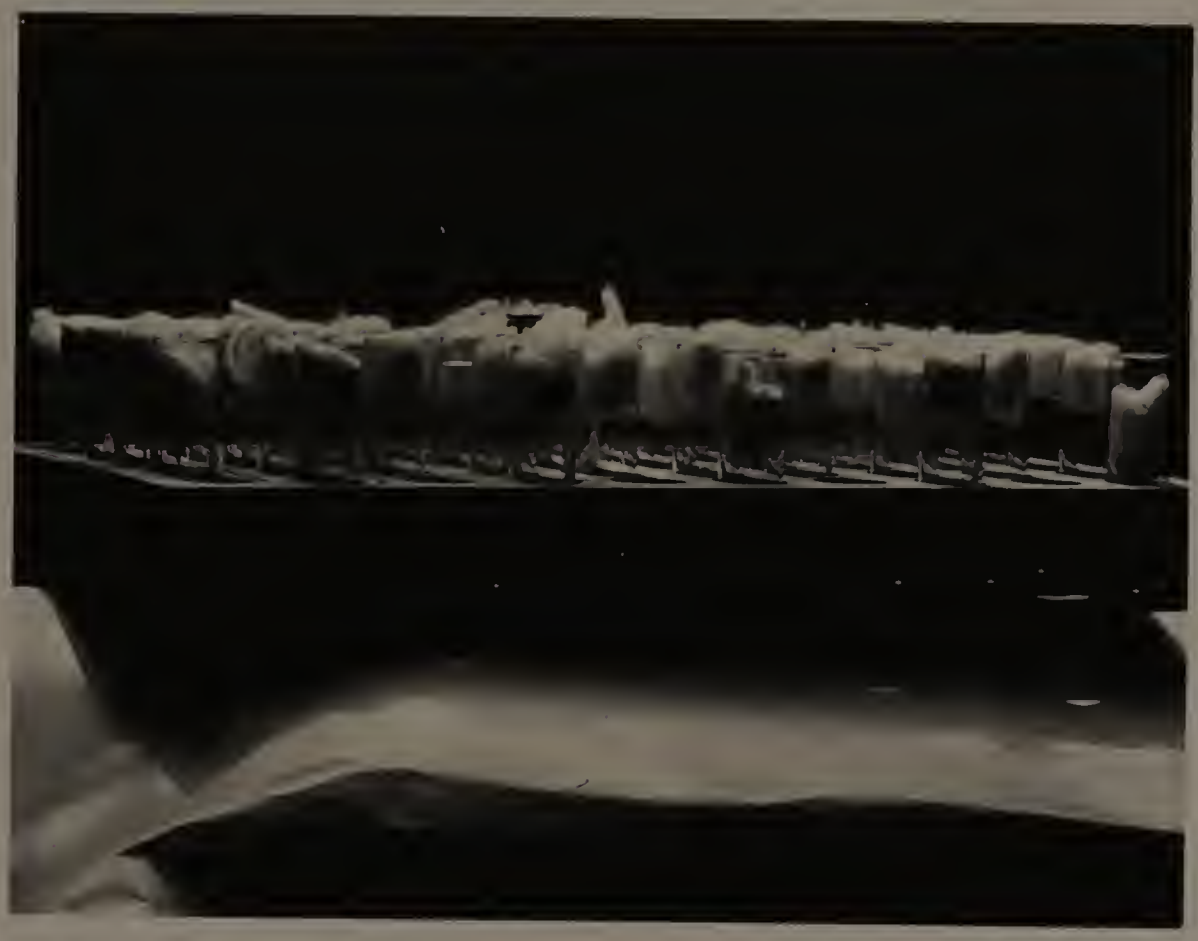


Fig. 2.--Plywood holding rack with test vials containing eggs laid by virgin females mated to treated males.

Fig. 3.--Technique of transferring flies from old (bottom) to new (top) culture jar.

Relative humidity. A hydro-thermograph in the laboratory recorded a range of relative humidity between 30 and 50 percent. Air moisture was increased by placing several open pans of water in the laboratory or incubator to raise the relative humidity when necessary.

Holding Procedures

Sex determination. The basic procedure used to determine the sex of D. melanogaster without recourse to the microscope was as follows: a) first generation larvae of various instars were selected from culture bottles and were transferred individually to separate shell vials containing 3 ml. of rearing medium; b) the two-day old adults which emerged in these vials were anesthetized and examined on a white background; c) those flies with dark abdominal markings were classified as males and others as virgin females. With this strain of Drosophila, this system of sex determination proved accurate, but should not be relied on entirely for adults less than 24 hours old.

Anesthesia. Flies were anesthetized with carbon dioxide prior to counting, transferral and sex determined. Adults were anesthetized by holding one end of a rubber tubing over the cotton plug of each container and allowing the carbon dioxide to seep in until flies became immobilized. Adults recovered quickly from the carbon dioxide treatment.

Ether was substituted for carbon dioxide after adults were exposed to metepa, to immobilize them for a longer period which was needed to count and separate males from females.

Etherized adults required a longer time recovering than those anesthetized with carbon dioxide.

Mating scheme. Each male was paired singly with a virgin female in a cotton plugged shell vial which contained approximately 3 ml. of medium. At the end of a two-day mating period, each female was transferred to a new shell vial containing fresh medium for oviposition. Using the same male, this treatment was replicated five times. Thus each experiment consisted of 50 treated and 5 untreated males mated with virgin females. There were four 2-day mating periods and at the end of each period, 55 new untreated virgin females were introduced to the males.

Oviposition period. Oviposition periods were of 2-day intervals. At the end of each period, females were anesthetized and removed from oviposition vials and each vial examined for the presence of eggs. Vials were held for 16 days to determine the number of F_1 generation adults which might emerge. The numbers of eggs in oviposition vials were not counted.

Counting. Test results were derived from counts of the number of adult offspring emerged in the oviposition vials. Normally all adults emerged in 14 days at 78°F. Occasionally the media showed signs of drying, which made it essential to count on days 10 and 12, as well as day 14. Adults in each vial were anesthetized with carbon dioxide and counted over a white background.

Longevity determination. At the end of the fourth mating period, males were held for longevity studies. Each treated male was introduced into a separate vial containing medium and observed daily. Males were transferred weekly to vials containing fresh media. A fifty-day holding period was considered adequate for this study. Treated males out-living this period were designated 50+ days. Death was considered as the point where prolonged involuntary movement was no longer observed. An occasional leg twitch did not disqualify a fly from being counted as dead (Fischang, 1963).

Sterilization Treatment

Gamma irradiation. One or two-day old males were irradiated with gamma radiation from a Ce^{137} (Cesium) source (Fig. 4) provided by Amherst College. Males were held in small gelatin capsules and exposed to dosage levels of 5,000, 12,000 and 15,000r for 22 minutes, 53 minutes and 66 minutes, respectively. All males were exposed to radiation when fully recovered from the effects of anesthesia. Each irradiated male was then immediately paired with a virgin female for mating and subsequent isolation of the female to oviposition vial.

Chemosterilant. All Drosophila tested under this treatment were exposed to baits containing various concentrations of chemosterilant. Half-pint milk bottles contained dental rolls soaked in a solution of 0.25, 0.5, or 1.% metepa in a 10% granulated sugar and 4% Fleischmann's active dry yeast.

Fig. 4.--Ce^{137} Radiation unit used for irradiation of Drosophila males.

In preliminary tests, dental rolls were placed at the bottom of the bait bottles. Several males became entangled between the rolls and died. Later adults were exposed to metepa by tying three dental rolls, one above the other, at one end of a cord, with the other end of the cord firmly fastened between the mouth of the bottle and the cotton plug. Baits were held approximately one-half inch from the bottom of the container (Fig. 5). As Drosophila are strongly attracted to light, the treated adults were retrieved under a strong light source by removing the cotton plug and inverting a clean, dry, half-pint milk bottle over the bait bottle. Adults were then sexed and males retained for experimentation.

Competition between treated and untreated males. Males exposed to 1.0% metepa were confined in vials with untreated males and females for a two-day mating period, at the following ratios:

*		**		***
0	:	1	:	1
1	:	1	:	1
5	:	1	:	1
10	:	1	:	1
25	:	1	:	1

At the end of the period each female was isolated in separate oviposition vial. Each ratio was replicated 5 times.

* Exposed males
** Unexposed males
*** Unexposed females

Fig. 5.--Males and females of Drosophila exposed to metepa baits impregnated on dental rolls.

RESULTS AND DISCUSSION

Radiation Treatments

Prior to detailed tests reported herein, preliminary experiments were conducted at 20,000 and 25,000r. No adult progeny emerged within the sixteen-day holding period from the hundreds of eggs oviposited by females. Copulation was not observed during the two-day mating periods at either treatment level. Also, the irradiated males appeared comparatively weaker than untreated males. Hundreds of adults emerged from eggs deposited by females used as controls.

The results of these preliminary experiments indicated that lower levels of gamma rays should be explored to obtain meaningful results. Exposure of Drosophila to radiation levels approximately 20,000 and 25,000r adversely affected the mating potential of males, thus preventing copulation.

Effects of 5Kr on Fecundity. Males irradiated at 5Kr were immediately paired with untreated virgin females, each pair in separate mating vials. Results of these tests are presented in Table 1. Untreated virgin females mated with irradiated males produced an average of 24 progeny. Those mated to untreated males in the controls produced an average of 94 progeny. In both the treated and untreated replicates, copulation was observed within a period of 15 to 20 minutes after males were introduced. Overall, the reduction in progeny averaged 68.1% (Fig. 6).

Table 1.--First generation adults from virgin females mated for a 2-day period with males exposed to 5Kr gamma radiation.

Males	No. of adult progeny* Replicate					Total***	Avg. progeny per female
	1	2	3	4	5		
1	102**	85	63	85	66	401	20.5
2	109	90	94	85	116	494	24.70
3	139	104	95	94	135	567	28.35
4	94	111	90	133	73	501	25.05
5	59	75	112	109	153	508	25.40
6	99	91	119	115	71	495	24.73
7	134	91	77	120	48	470	23.50
8	97	85	98	114	106	500	24.00
9	67	108	58	103	95	431	21.55
10	144	70	73	71	75	433	21.65
Check	384	400	406	331	362	1883	94.25

*Over a 16-day period.
**Total progeny from 4 females.
***Represents total progeny from 20 females.

All females deposited a considerable number of eggs. However, a lower number of F_1 generation developed from females mated to irradiated males, indicating that these females did lay some nonviable eggs.

Effects of 12Kr on Fecundity. Results of 12Kr on fecundity are shown in Table 2. A comparison of results obtained at 5 and 12Kr levels shows that substantially fewer numbers of adults emerged as irradiation increased. Copulation was observed 15 to 20 minutes after introduction.

Females mated to irradiated males deposited an abundance of eggs with no indication that egg production was reduced. Females mated to males exposed to 12Kr produced an average of less than 1.0 adult. The number of individuals in the F_1 generation decreased 99.3% as a result of this treatment (Fig. 6).

Effects of 15Kr on Fecundity. Results of 15Kr treated males on fecundity are shown in Table 3. A comparison of the data in Tables 2 and 3 shows only a slight difference between the numbers emerged at 12 and 15Kr levels. Untreated females mated to irradiated males were observed to deposit a considerable number of eggs at the 15 Kr level. However, fewer adults emerged at this treatment level, indicating that a great number of eggs deposited were nonviable. A similar trend relative to reduction of first generation offspring with increased levels of radiation was noted by Henneberry et al. (1963). An average of less than 1 adult emerged at this level indicating a reduction of 99.8% in terms of first generation offspring (Fig. 6).

Table 2.--First generation adults from virgin females mated for a 2-day period with males exposed to 12Kr gamma radiation.

Males	No. of adult progeny* Replicate					Total***	Avg. progeny per female
	1	2	3	4	5		
1	1**	2	5	4	3	15	0.75
2	2	0	5	0	3	10	0.50
3	0	5	2	1	4	12	0.60
4	0	0	0	5	0	5	0.25
5	5	3	6	1	1	16	0.80
6	0	2	0	0	2	5	0.25
7	0	0	8	5	5	18	0.90
8	3	1	2	0	0	6	0.30
9	0	0	0	7	0	7	0.35
10	3	2	4	0	5	13	0.65
Check	269	283	340	263	318	1473	73.65

*Over a 16-day period.
**Total progeny from 4 females.
***Represents total progeny from 20 females.

Table 3.--First generation adults from virgin females mated for a 2-day period with males exposed to 15Kr gamma radiation.

Males	No. of adult progeny* Replicate					Total***	Avg. progeny per female
	1	2	3	4	5		
1	0**	1	2	0	0	3	0.15
2	1	2	0	0	1	4	0.20
3	0	1	2	0	0	3	0.15
4	0	1	0	1	0	2	0.10
5	0	2	0	0	1	3	0.15
6	2	1	2	0	0	5	0.25
7	2	2	0	0	0	4	0.20
8	0	1	0	0	0	1	0.05
9	0	1	0	0	1	2	0.20
10	4	1	0	0	0	5	0.25
Check	392	322	353	320	299	1686	84.30

*Over a 16-day period.
**Total progeny from 4 females.
***Represents total progeny from 20 females.

Statistical Analysis. Using the analysis of variance procedure, statistical analysis of results in Tables 1, 2, and 3 indicated a high degree of significance between the treatment levels (Table I).

Effects of Radiation on Longevity

Males used in these experiments were held for 50 days to determine the effects of irradiation on longevity.

Effects of 5Kr on Longevity. Results of exposure to 5Kr are shown in Table 4. The mortality rate in replicates 1, 2 and 3 was comparatively higher than in replicates 4 and 5. The high mortality rates in these replicates was probably not the direct result of irradiation, since males in replicates 1, 2 and 3 were exposed to radiation three weeks in advance to those of replicates 4 and 5, and several died as a result of holding them in media for 21 days, which often became too dry. Also, some became entrapped in fresh media shortly after they were transferred.

Males of replicates 4 and 5, and subsequent males held for longevity tests, were introduced to fresh media weekly. With the exception of two individuals all males of replicates 4 and 5 outlived the 50-day holding period.

The percent reduction in days was 10.4 (Fig. 7), indicating that male longevity was not adversely affected by the radiation treatment.

Table 4.--Effects of 5Kr gamma radiation on longevity of males.

Replicate	Days to expiration, Individual male										Check	Avg. longevity of treated males
	1	2	3	4	5	6	7	8	9	10		
1	33	34	50+	35	47	35	35	49	50+	35	50+	40.3
2	35	50+	50+	46	44	46	41	44	50+	36	50+	44.2
3	50+	34	50+	44	50+	33	43	44	34	35	50+	41.7
4	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+	50.0
5	50+	50+	50+	35	46	50+	50+	50+	50+	50+	50+	48.1

Effects of 12Kr on Longevity. Results of longevity studies on males exposed at 12Kr are shown in Table 5. Fewer numbers of individuals died from extraneous factors in these tests. Average longevity of the irradiated males was 42.6 days. Only 16 percent of the males lived beyond the 50-day holding period. These results indicate that the 12Kr radiation level had a deleterious effect on the longevity of some males, reducing average male longevity by 14.8 days (Fig. 7).

Effects of 15Kr on Longevity. Results of these tests are presented in Table 6. Although there was considerable variation in mortality at each treatment level, males exposed to 15Kr were shorter lived than those at 5 and 12Kr. Two of the males used as controls died from extraneous factors at 37 and 18 days, respectively. The average longevity of treated males was 37 days and the percent reduction in days was 9.4 (Fig. 7). This reduction in lifespan indicates that irradiation at this level had a significantly adverse effect on the longevity of Drosophila males.

Statistical Analysis. The results in Tables 4, 5, and 6 show significant differences between the effects of radiation treatment levels on longevity (Table II).

Table 5.--Effects of 12Kr gamma radiation on longevity of males.

Replicate	Days to expiration Individual male										Check	Avg. longevity of treated males
	1	2	3	4	5	6	7	8	9	10		
1	50+	29	48	50+	50+	48	42	50+	45	8	50+	42.0
2	14	37	45	50+	48	50+	45	37	45	41	50+	41.2
3	16	23	31	50+	37	37	45	29	50+	50+	50+	36.8
4	45	45	48	50+	50+	50+	31	48	50+	50+	50+	46.7
5	16	50+	50+	49	50+	50+	50+	50+	50+	50+	50+	46.5

Table 6.--Effects of 15Kr gamma radiation on longevity of males.

Replicate	Days to expiration, Individual male										Check	Avg. longevity of treated males
	1	2	3	4	5	6	7	8	9	10		
1	50+	50+	31	27	16	32	45	12	33	21	50+	31.7
2	48	48	48	45	39	31	50+	47	31	39	37	42.6
3	50+	12	34	21	25	48	44	28	9	45	18	31.6
4	50+	50+	50+	50+	10	41	37	50+	40	50+	50+	42.8
5	44	24	45	32	49	17	37	28	47	49	50+	37.2

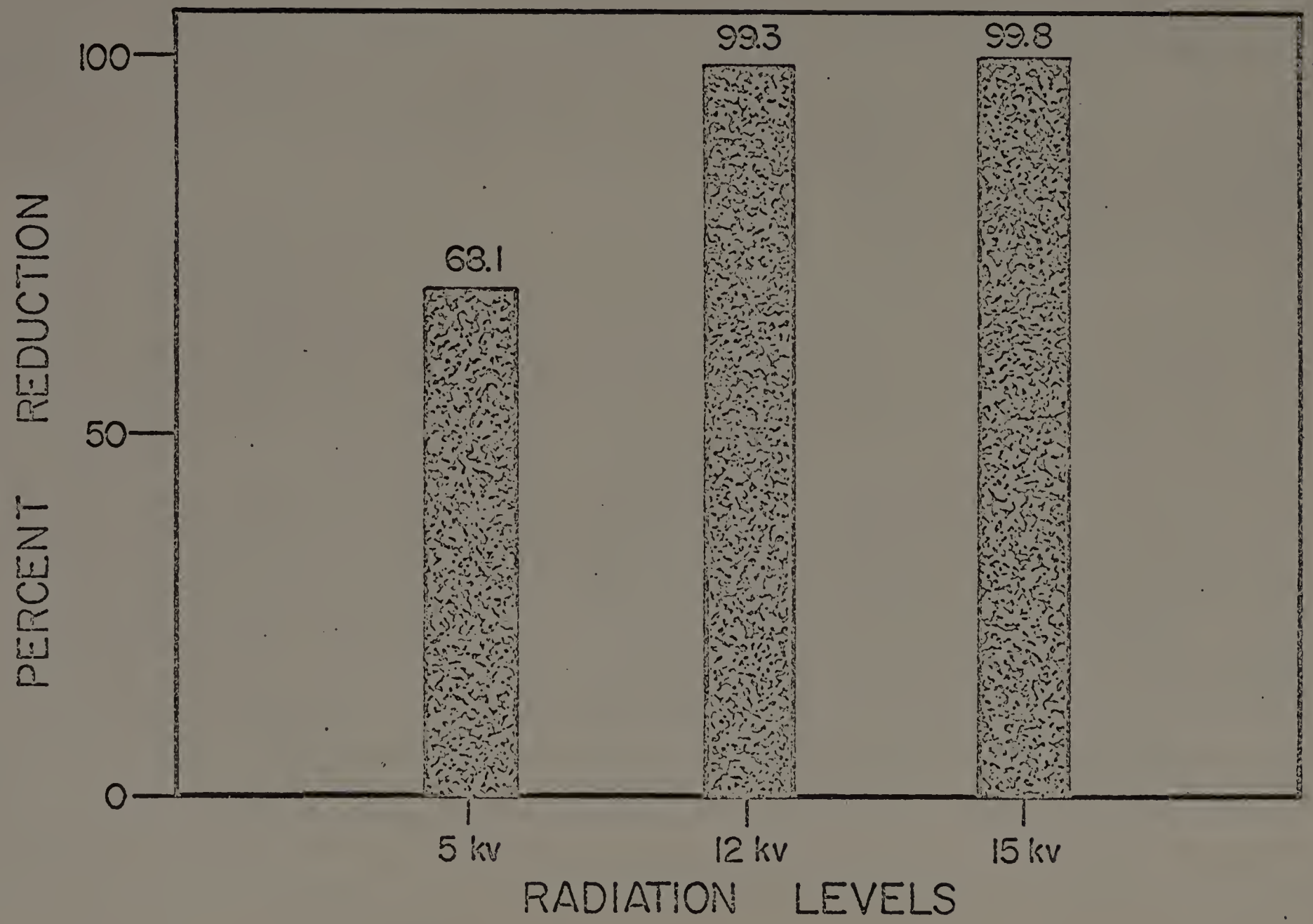


Fig. 6.--Reduction in progeny resulting from exposure of males to gamma radiation.

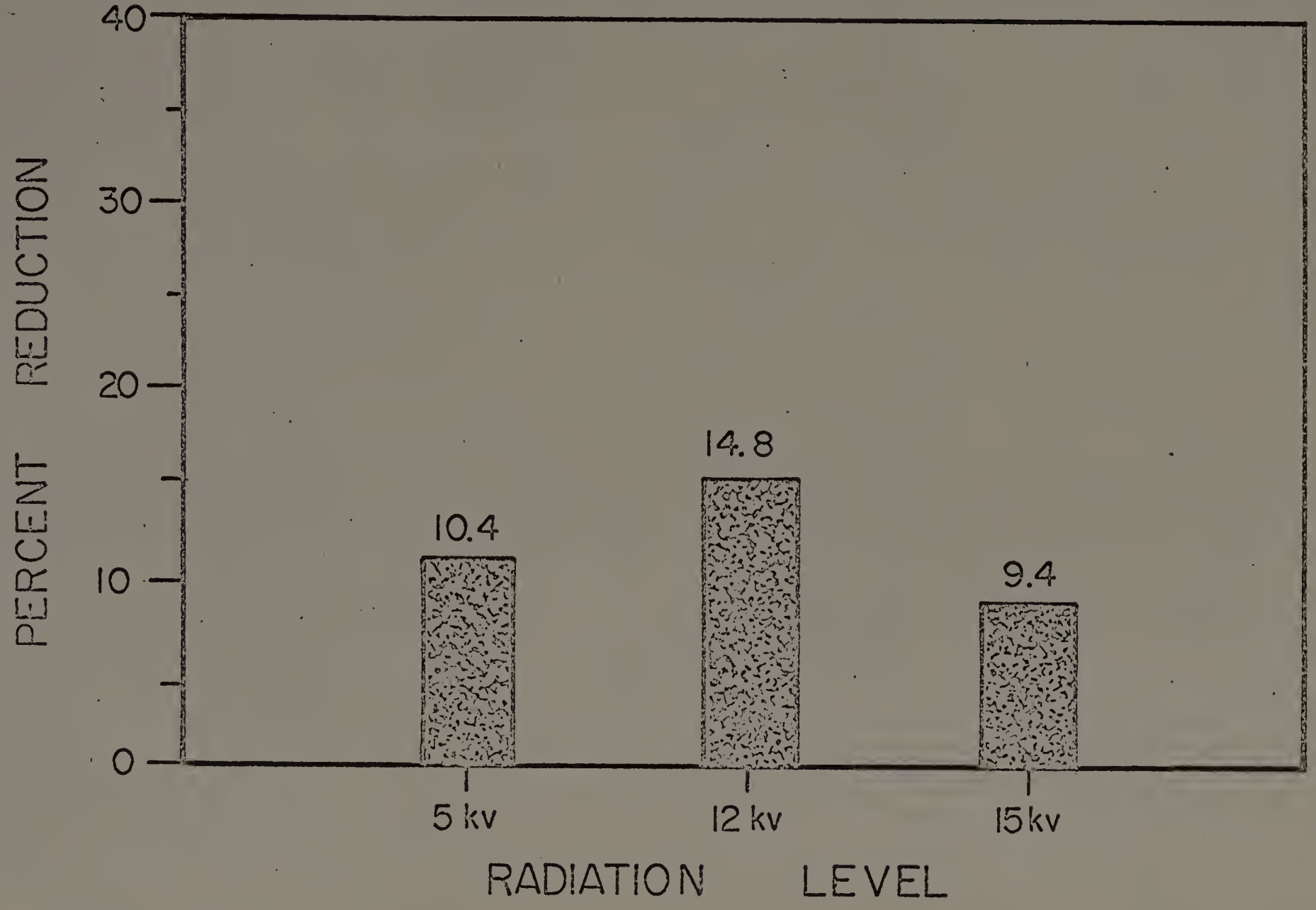


Fig. 7.--Reduction of male longevity resulting from exposure to gamma radiation.

Chemosterilization

Effects of 0.25% metepa bait on Fecundity. The effects of 0.25 percent metepa bait on fecundity is shown in Table 7. Females mated to treated males produced an average of 67.70 adult progeny from eggs laid by females mated to untreated males. There was a 45.3 percent reduction in the number of F_1 generation emerged as a result of mating untreated females to treated males (Fig. 8).

Copulation was observed shortly after flies were paired for testing. Females, mated to treated males, appeared to deposit a similar number of eggs as compared with untreated paired flies. The reduction in the number of adults emerged suggests that a number of eggs deposited by females mated to treated males were nonviable.

Effects of 0.50% metepa bait on Fecundity. Results of this treatment are presented in Table 8. An average of 10.5 adult progeny emerged from females mated to treated males. This number of offspring was substantially less than at the 0.25 percent level. Overall reduction in the number of first generation offspring was 85.6 percent (Fig. 8). Copulation was observed and the number of eggs laid by females mated to metepa-treated males appeared to be similar in number to those deposited by females mated to untreated males. The 0.50 percent concentration of metepa was sufficient to induce a fairly high degree of sterility in Drosophila males.

Table 7.--First generation adults from virgin females mated for a 2-day period with males exposed to 0.25% metepa bait for 24 hours.

Males	No. of adult progeny* Replicate					Total***	Avg. progeny per female
	1	2	3	4	5		
1	53**	77	0	134	102	366	18.30
2	49	86	66	162	63	426	21.20
3	47	115	59	90	193	504	25.20
4	99	71	237	117	110	634	31.70
5	78	68	1	88	51	286	14.30
6	135	124	60	99	109	527	26.35
7	85	150	151	98	59	543	27.15
8	99	147	255	77	64	642	32.10
9	120	67	81	205	69	542	27.10
10	131	71	90	121	220	633	31.68
Check	294	243	266	267	284	1354	67.70

*Over a 16-day period.
**Total progeny from 4 females.
***Represents total progeny from 20 females.

Table 8.--First generation adults from virgin females mated for a 2-day period with males exposed to 0.5% metepa bait for 24 hours.

Males	No. of adult progeny* Replicate					Total***	Avg. progeny per female
	1	2	3	4	5		
1	31**	29	28	41	45	174	8.85
2	29	35	54	43	51	212	10.60
3	49	51	35	46	47	228	11.40
4	41	39	49	41	36	206	10.30
5	33	45	38	48	52	216	10.80
6	39	23	50	36	37	185	9.25
7	50	46	54	45	49	244	12.20
8	27	42	43	53	51	216	10.80
9	44	31	39	47	30	191	9.55
10	47	49	59	49	37	241	12.05
Check	322	280	380	287	297	1566	78.30

*Over a 16-day period.
**Total progeny from 4 females.
***Represents total progeny from 20 females.

Effects of 1.0% metepa bait on Fecundity. Table 9 indicates that increasing the concentration of metepa to 1.0 percent resulted in a substantial decrease in the numbers of F_1 generation emerged. An average of 2.45 adults emerged from females mated to treated males. This was equivalent to a 97.2 percent reduction in the number of F_1 generation (Fig. 8). Even at this high concentration of metepa, the mating potential of males apparently was not impaired, since males copulated at various intervals.

Comparatively fewer adults emerged at this level than at 0.25 and 0.50 percent concentration, indicating that 1.0 percent metepa was most effective in reducing Drosophila population.

Statistical Analysis. Results presented in Tables 7, 8 and 9 show a high degree of significance between the treatment levels (Table III).

Effects of Metepa Bait on Longevity

Similar methods and procedures were used in longevity studies of chemosterilized males as for those irradiated.

Effects of 0.25% metepa bait on longevity. Detailed examination of data presented in Table 10 reveals that this treatment level had no observable effect on lifespan of males. Both treated and untreated males used as controls outlived the 50-day holding period. Males showed no sign of weakness during or at the end of experimentation.

Table 9.--First generation adults from virgin females mated for a 2-day period with males exposed to 1.0% metepa bait for 24 hours.

Males	No. of adult progeny* Replicate					Total***	Avg. progeny per female
	1	2	3	4	5		
1	1**	9	20	15	13	58	2.90
2	0	25	3	15	7	50	2.50
3	28	0	13	19	8	68	3.40
4	30	16	21	0	1	68	3.40
5	0	4	1	15	2	22	1.10
6	0	0	0	0	19	19	0.95
7	14	10	7	3	10	44	2.20
8	0	24	19	1	12	56	2.80
9	5	1	0	23	5	34	1.70
10	17	3	12	16	24	72	3.60
Check	259	295	283	260	343	1440	72.00

*Over a 16-day period.
**Total progeny from 4 females.
***Represents total progeny from 20 females.

Effects of 0.50% metepa bait on longevity. Inspection of Table 11 indicates that four treated individuals died within the 49-day period. However, these deaths occurred as a result of physical injuries in the holding vials. With the exception of these four individuals, males at this treatment were not shorter lived than those of the control, indicating that longevity was not affected. The percent reduction in longevity was 1.8 days (Fig. 9).

Effects of 1.0% metepa bait on longevity. The highest mortality occurred at this level but again a few of these deaths were caused from physical injuries in the holding vials. However, more than 80 percent of the treated males outlived the 50-day testing period (Table 12). At this level the average longevity was 48.7 days and the percent reduction in days was 3.6 (Fig. 9). It is evident from these tests that concentrations as high as 1.0 percent metepa are not deleterious to the lifespan of Drosophila males.

Statistical Analysis. The results in Tables 10, 11 and 12 show significant differences between the concentration levels of metepa on longevity (Table IV).

Table 10.--Effects of 0.25% metepa on the longevity of males.

Replicate	Days to expiration, Individual male										Check	Avg. longevity of treated males
	1	2	3	4	5	6	7	8	9	10		
1	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+
2	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+
3	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+
4	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+
5	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+

Table 11.--Effects of 0.5 metepa on the longevity of males.

Replicate	Days to expiration										Check	Avg. longevity of treated males
	Individual male											
	1	2	3	4	5	6	7	8	9	10		
1	50+	50+	50+	50+	50+	50+	50+	49	50+	50+	50+	49.9
2	50+	41	50+	50+	50+	50+	50+	34	50+	50+	50+	47.5
3	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+	50.0
4	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+	50+	50.0
5	50+	34+	50+	50+	50+	50+	50+	50+	50+	50+	50+	48.4

Table 12.--Effects of 1.0% metepa on the longevity of males.

Replicate	Days to expiration, Individual male										Check	Avg. longevity of treated males
	1	2	3	4	5	6	7	8	9	10		
1	50+	49	50+	50+	50+	50+	50+	30	50+	48	50+	47.7
2	50+	50+	50+	50+	50+	47	50+	48	50+	50+	50+	49.5
3	48	50+	50+	42	50+	50+	50+	50+	50+	49	50+	48.9
4	49	50+	50+	50+	50+	50+	39	50+	50+	50+	50+	49.8
5	50+	26	50+	50+	50+	50+	50+	50+	50+	50+	50+	47.6

Competition between Metepa Treated Males and Untreated Males

The data in Table 13 indicates an inverse relationship between the treatment ratio and number of offspring. It is likely that the treated males in all ratios were successful in mating first with the females. As the number of treated males increased in the ratios, the chances of the single, virile male mating with the female became less.

The average number of F_1 progeny from treatment ratios 1:1:1, 5:1:1, 10:1:1 and 25:1:1 were 42.6, 21.6, 15.8 and 10.1 percent, respectively. The percent reduction of adult progeny from these ratios were 38.1, 68.7, 77.1 and 85.5. Perhaps if the proportions of treated to untreated males were increased to ratios of 40:1 the percent reduction in reproduction would exceed 90 percent, which is thought necessary for economic reduction of field populations.

Statistical analysis. The results presented in Table 13 show significant differences between the treatment ratios (Table V).

Table 13.--Effect of introducing males exposed to 1% metepa bait to mixtures of unexposed males and females.

Treatment Rates EM* : UM :** UP***	Number of adult progeny Replicate 1	2	3	4	5	Total	female	% Reduction
0 : 1 : 1	56	81	77	61	69	344	68.8	
1 : 1 : 1	46	41	47	39	40	213	42.6	38.1
5 : 1 : 1	23	20	19	25	21	108	21.6	68.7
10 : 1 : 1	16	18	14	19	12	79	15.8	77.5
25 : 1 : 1	13	9	11	13	4	50	10.0	85.5

*
Exposed males
**Unexposed males
***Unexposed females

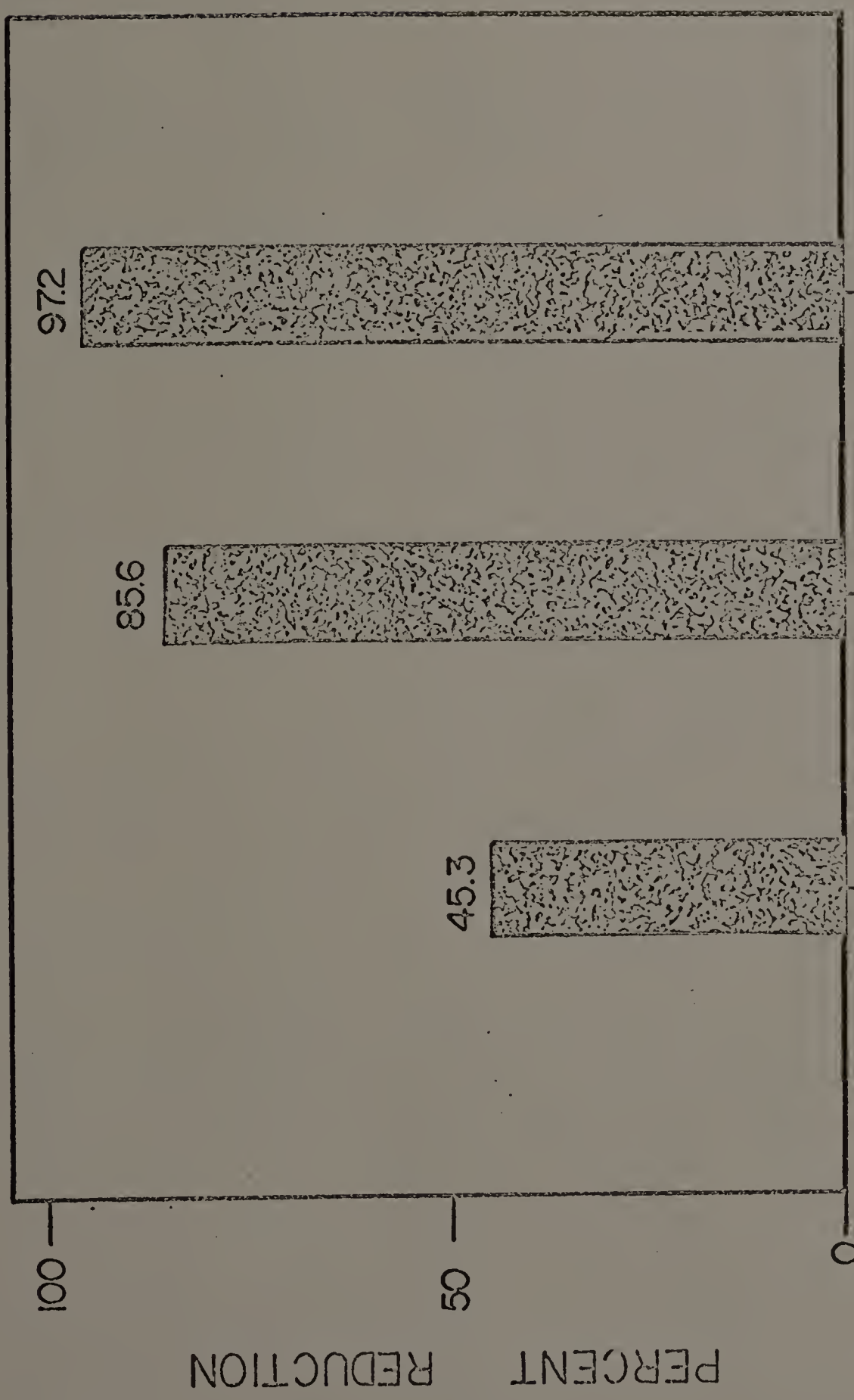


Fig. 8.--Reduction in progeny resulting from exposure of males to metepa baits.

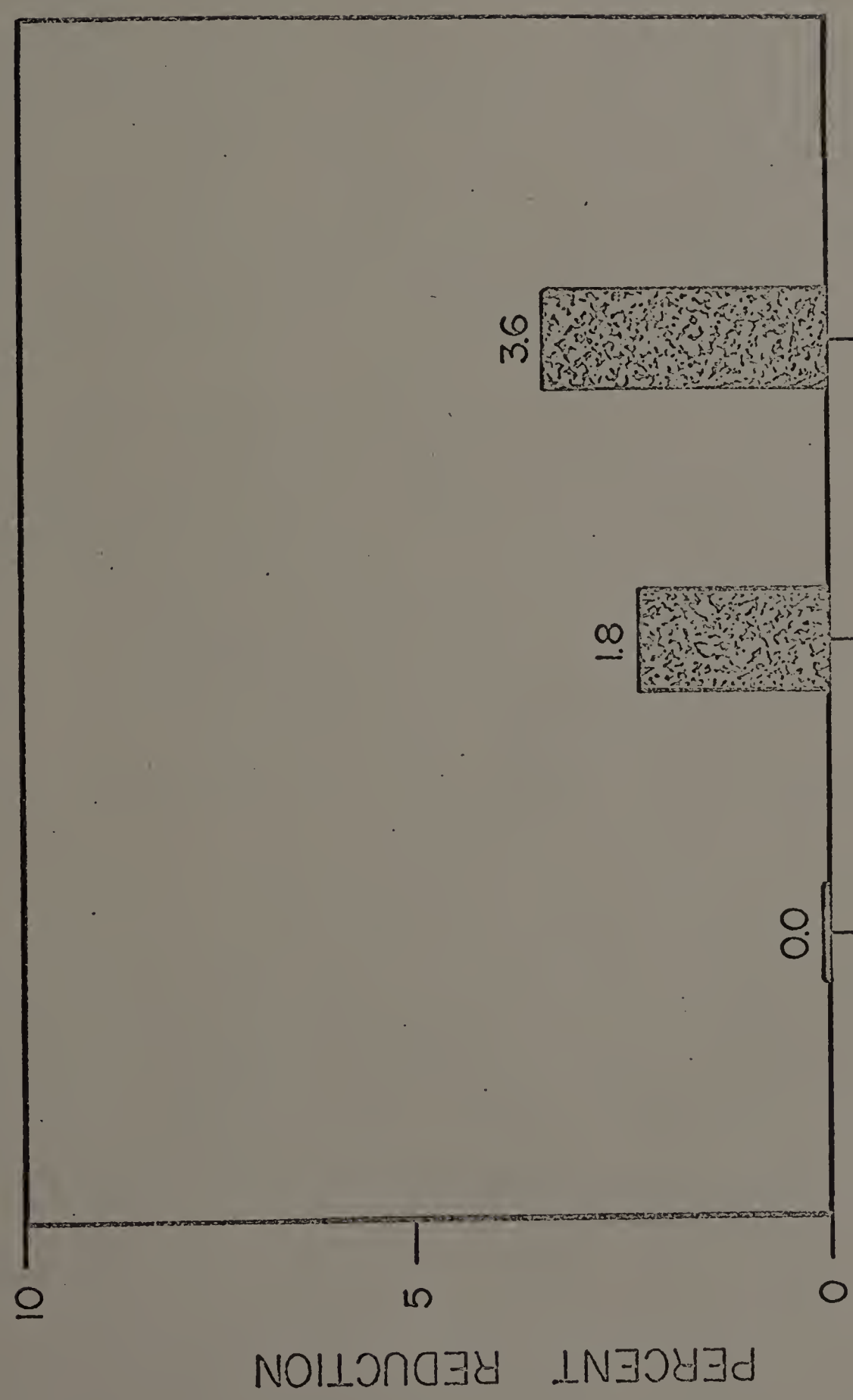


Fig. 9.--Reduction of male longevity resulting from exposure to metepa baits.

SUMMARY

Laboratory tests were conducted with gamma radiation and a chemosterilant, metepa, to determine dosage levels needed to induce sterility in Drosophila melanogaster, without adversely affecting the mating potential and longevity. The flies were reared on corn meal-agar medium and propionic acid was added to prevent molds infestation. Usually, only two-day old first generation males and females were selected for these tests.

Both irradiated and chemosterilized males were individually offered untreated virgin females on the same day and different females on the third, fifth and seventh day. Each mating period lasted two days and at the end of each period the mating vials were discarded and females were individually placed into oviposition vials with fresh medium. At the termination of each two-day laying period the females were discarded and the oviposition vials held for sixteen days to ascertain the number of offspring emerging from deposited eggs.

Males selected for radiation treatments were confined in small gelatin capsules and exposed at dosage levels of 5,000, 12,000 and 15,000r for 22 minutes, 53 minutes and 66 minutes, respectively. Radiation level at 15,000r was most effective in inducing sterility in Drosophila. When males were exposed at this level very few offspring emerged. A 12,000r level was nearly as effective with an average of less than one adult

emerged from the treatments. A 5,000r level was not very effective on the fecundity of Drosophila. The sterilizing dosages of 15,000 and 12,000r shortened the lifespan of some exposed males. At 5,000r the longevity of males were unaffected.

In the chemosterilization tests, both males and females were exposed together to baits containing 0.25, 0.50 and 1.0 percent concentration metepa for one-day period. At the end of the exposure period the adults were sexed, females were discarded, and the males retained for experimentation. Metepa concentration at 1.0 percent was most effective in inducing sterility. An average of 2.45 adults emerged from the treatments. A 0.50 percent concentration metepa was second best with an average emergence of 10.58 adults. A 0.25 percent metepa concentration did not appreciably affect the fecundity of Drosophila. An average of 23.99 adults emerged from these treatments.

None of the three concentration levels of metepa bait used had any deleterious effect on the lifespan of Drosophila males. When males exposed to 1.0 percent metepa bait, were confined at different proportions with untreated male and female the number of offspring decreased as the proportion of treated males increased.

CONCLUSIONS

Based on the experimental procedures and conditions described in this dissertation, the following conclusions were drawn.

1. Radiation levels approximating 20,000 and 25,000r adversely altered copulatory behaviour, while no such effect was observed with metepa baits used in concentrations up to 1 per cent.
2. Without affecting copulatory behavior radiation levels of 12,000 and 15,000r effectively reduced laboratory populations of Drosophila melanogaster below the level of economic importance. Similar results were obtained when populations were exposed to 1 percent metepa baits.
3. Radiation levels above 12,000r had deleterious effect on the longevity of males, but the lifespan of males was not adversely affected by metepa baits when used in concentrations of up to 1 percent.

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APPENDIX

Table I.--Statistical analysis of radiation-fecundity data presented in Tables 1, 2, and 3

Analysis of Variance				
Source of variation	Sums of squares	Degree of freedom	Mean squares	F
T	285943.3200	2	142971.6600	17.70*
R(T)	10050.2400	12	837.5200	

*Significant at 1% level.

Table II.--Statistical analysis of radiation-longevity data presented in Tables 4, 5, and 6

Analysis of Variance				
Source of variation	Sums of squares	Degree of freedom	Mean squares	F
T	1562.0400	2	781.0200	3.62*
R(T)	2582.3200	12	215.1933	

*Significant at 1% level.

Table III.--Statistical analysis of chemosterilant-fecundity data presented in Tables 7, 8, and 9.

Source of variation	Analysis of Variance: Sums of squares	Degree of freedom	Mean squares	F
T	215914.7200	2	107957.3600	
R(T)	15914.5200	12	1326.2100	81.40*

*Significant at 1% level.

Table IV.--Statistical analysis of chemosterilant-longevity data presented in Tables 10, 11, and 12.

Source of variation	Analysis of Variance: Sums of squares	Degree of freedom	Mean squares	F
T	56.5200	2	28.2600	
R(T)	79.9200	12	6.6600	4,24*

*Significant at 1% level.

Table V.--Statistical analysis of competition date presented in Table 13.

Source of variation	Analysis of Variance: Sums of squares	Degree of freedom	Mean squares	F
T	11604.5600	4	2901.1400	
R(T)	606.0000	20	30.3000	95.74*

*Significant at 1% level.

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