THE INFLUENCE OF PHYSIOLOGICAL
AND PHYSICAL FACTORS ON THE
RADIOSensitivity OF THE CODLING
MOTH: LASPEYRESIA POMONELLA (L.)

by

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ABSTRACT

Diapaus ing and nondiapausing 5th instar larvae of Laspeyresia pomonella were irradiated with various doses of gamma rays to determine whether the physiological state of the larvae at the time of irradiation would influence the expression of radiation damage. Pupal mortality, adult emergence, fecundity and sterility were the parameters studied. Although pupal mortality increased and adult emergence were reduced with increasing radiation dose, diapause did not have a significant effect on radiation sensitivity as measured by either parameter. In both cases females were more sensitive than males.

Three parameters were used as measures of fecundity, viz: mean egg lay, percentage of matings resulting in eggs laid and percentage of matings resulting in over fifty eggs laid. The mean egg lay of irradiated diapausing insects was significantly higher than that of irradiated nondiapausing insects. Females had a lower mean egg lay when irradiated and mated with normal males than when not irradiated and mated with irradiated males. The mean egg lay was inversely proportional to the dose. The percentage of successful matings in which more than fifty eggs were laid
was significantly higher for irradiated diapause females than for the irradiated nondiapause females. Normal females mated with irradiated males laid over fifty eggs more often than irradiated females mated with normal males. Successful matings which resulted in no eggs laid formed a larger percentage of the nondiapause irradiated matings than that of the diapause irradiated matings. Irradiated diapause males when mated with normal females had a lower percentage of matings resulting in no eggs than irradiated nondiapause females when mated with normal males. These three parameters indicate that the diapausing larvae are significantly less sensitive to radiation than the nondiapausing larvae. In other words, diapause appears to offer protection against radiation damage. However, the percent sterility of incubated eggs increased with increasing radiation dose and was not reduced in eggs laid by or fertilized by irradiated diapausing insects. Therefore, diapause does not offer protection against the induction of sterility.

To examine the effect of dose rate on radiosensitivity, nondiapause female larvae were irradiated with 2,500 and 10,000 rads. Each dose was delivered at four dose rates, viz; 233, 358, 742 and 1,375 rads/minute. Dose rates used had no influence on pupal mortality, adult emergence, fecundity or
sterility.

Radiation appears to have a stimulating effect on diapause breaking mechanism. Irradiation with 2.5 and 5 k rads significantly reduced the duration of larval diapause. At 10 k rads the stimulating effect was masked by radiation damage.
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INTRODUCTION

The study of radiation effects on biological systems began with Muller's (1927) experiments on *Drosophila melanogaster* but reached general interest only after the use of the atomic bomb in 1945. Since then, the radiation sensitivity of many organisms has been examined with the result that considerable information exists on which to build a study on the biological effects of radiation.

The most striking effect of radiation is the lethal effect, and it is used as a measure of radiation on different organisms. Higher plants are relatively sensitive. Exposure to 5 R per day over several years proved lethal to 90% of the trees examined, whereas lower plants were not so seriously affected (Woodwell 1967). The $\text{LD}_{50}^{(30 \text{ days})}$ of other organisms such as *Infusoria* and *Paramecium* may be as high as 350,000 rads. The $\text{LD}_{50}$ for a tortoise is 1,500 rads, whereas it ranges between 600 to 700 rads for a man. Mammals are the most sensitive among vertebrates to radiation. The arthropods, especially the insects, are characteristically more resistant to radiation damage than many other animals (Bacq and Alexander 1961, Menhenick and Crossley 1969).

The effect of radiation on insects has been reviewed
by Hilchey (1957), Grosch (1962), O'Brien and Wolfe (1964) and Proverbs (1969). The effects of radiation on the physiology of the insect are of special interest to this study. Radiation effects may be variably lethal or may be expressed in the induction of dominant lethal mutations in the insect germ cells.

The sensitivity of an insect to radiation injury decreases with age. Early developmental stages are more sensitive than later stages. In the wasp *Bracon hebetor* lethal doses are 100 R for cleavage stage embryos, 100,000 R for old pupae and 300,000 R for adults (O'Brien and Wolfe 1964). The $LD_{50}$ for *Drosophila* sp. are 200 to 900 R for eggs, 1,300 R for larvae, 3,000 R for pupae and 95,000 R for adults (King 1954). Similarly the susceptibility of the tobacco budworm *Heliothis virescens* decreased as development proceeded (El Sayed and Graves 1969), and eggs of the bagworm *Thyridopteryx ephemeraeformis* are the most sensitive stage (Reichle 1969). In addition sensitivity of a single developmental stage decreases with age. Nair (1962) showed that the sensitivity of the housefly pupae (*Musca domestica*) decreased with increase in age. Similar results were obtained by Donnelly (1965) in the pupae of the blowfly *Lucilia sericata*. 
Jobin et al. (1970) showed that Acheta domesticus embryos became less sensitive to radiation as they became older.

The sensitivity of insects has been found to vary with the sex of the insect that has been treated. In Periplaneta americana (Wharton and Wharton 1959) and Cochliomyia hominivorax (Bushland and Hopkins 1953) and Tribolium castaneum (Park et al. 1958) males are reported to be more sensitive to radiation injury than females. However, female Khapra beetles (Trogoderma granarium) were sterilized by a dose less than one third as high as that required to sterilize the males (Carney 1959). Similarly, females are the most sensitive sex in Laspeyresia pomonella (Proverbs and Newton 1962b) and Lucilia sericata (Donnelly 1965). The differential sensitivity is apparently related to the genome number in Bracon sp. in which the diploid females are only one third as sensitive as the haploid males (O'Brien and Wolfe 1964).

This explanation does not apply to all other insects, however, since relatively few orders have haploid sex determination and most insects display a sex differential in radiation sensitivity. Lower sensitivity in females (when it occurs) may be due to their larger store of nutrients (Grosch 1962).
Temperature is known to have an effect on radiation sensitivity of insects. Low temperature moderated the effect of radiation on the eggs of Bombyx mori (Paulov 1961), the sensitivity of Dahlbominus fuscipennis to radiation damage increased with increasing temperature (Baldwin 1956) and experiments on Sitophilus granarius larvae showed that they were more sensitive to radiation at higher temperatures (Cornwell 1966).

The concentration of oxygen in the irradiating atmosphere also has an effect on the radiation sensitivity of an insect. Rhodnius showed a reduced area of radiation burn when it was irradiated in a nitrogen atmosphere rather than oxygen (Baldwin and Salthouse 1959). Irradiation in a nitrogen atmosphere maintained the relative radiation sensitivity of several insect types but all types were less sensitive than in the air (Clark and Herr 1965).

The effects of radiation may also be influenced by the dose rate. Villee (1946) found that Drosophila could survive much higher doses of X-rays at very low dose rates than if they were irradiated at high dose rates. The same effect is found when the dose is subdivided as a series of low doses. A series of low doses is less effective than a single dose which is
the sum of the fractionated doses. Nair and Subramanyam (1963) showed that increasing the dose rate resulted in a decrease in the number of eggs laid by *Tribolium castaneum* and also a decrease in the viability of the eggs laid. The effects of radiation may be expressed as somatic damage. These effects may also be expressed in the disruption of the genetic makeup of the insect. These mutations are induced by radiation of all levels and it has been found that there is a direct proportionality between the amount of radiation and the frequency of induced mutation. It has also been demonstrated that there is no threshold below which radiation does not induce mutations. The effectiveness of a particular dose in inducing mutation is independent of the time required for the irradiation (Grosch 1962). Dominant lethal mutations give rise to at least one type of sterility found in insects (LaChance *et al.* 1967). The dominant lethal mutations produced by irradiation are a major cause of reproductive failure in insects and they may occur in the male or in the female gamete. These are then passed on to the offspring of the next generation. Lethality in these cases usually occurs during the embryogenesis or prior to hatching. Two types of mutations are thought to occur in insects, the first being
the "point mutation" which may result in the change of one or a few nucleotides. The second most common lethal mutation resulting from chromosome breakage and faulty recombination (Grosch 1962).

Another physiological factor that has been examined is diapause. Bodine and Evans (1934) claimed that diapausing mud dauber wasp larvae (*Sceliphron caementarium*) were not as radiosensitive as the nondiapausing larvae. On the other hand Lassota (1963) found that the diapausing eggs of *Bombyx mori* were more sensitive to radiation injury than the nondiapausing eggs. Other researchers have examined this problem with varying results. Nair and Rahalkar (1963) found that in the larvae of *Trogoderma granarium*, which has a weak facultative diapause, the radiation damage was postponed and was expressed only when the diapause was terminated by increasing the ambient temperature.

Diapause can be defined as a developmental arrest enforced by a physiological mechanism (Beck and Hanec 1960). It is this arrest in development that permits insects and some other arthropods to survive during periods of environmental extremes. Diapause can occur at any stage in the development of an insect but with few exceptions it occurs at a single
point in the development of a given species (Danilevskii 1965, deWilde 1962). Diapause can be of two types, facultative or obligatory. Insects with a facultative diapause have two or more generations per year and are called multivoltine, the initiation and termination of diapause being governed by photoperiod. Insects with an obligatory diapause have only one generation per year and are called univoltine (Beck et al. 1962).

In most cases diapause is induced by short day photoperiod (8 to 12 hours of light) whereas long photoperiods (12 to 16 hours of light) will maintain most insects in a state of continuous development (deWilde 1962, Lees 1956). In cases where photoperiod has been shown to control the induction of diapause, the dark phase or scotophase is often as important as the light or photophase. In an experiment with Ostrinia nubilalis diapause was induced by a 12 hour scotophase in combination with photophase ranging from 3.5 to 32 hours (Beck 1962). This scotophase is, however, not the controlling factor in all cases. Studies on the parasitic wasp Nasonia vitripennis in which the dark phase was interrupted by light show that diapause induction involves the whole light:dark cycle (Saunders 1969).
The critical photoperiod for induction of diapause varies from species to species. In the pink bollworm *Pectinophora gossypiella* at 27°C, diapause is induced by photoperiods of 13.5 hours or more (Adkisson 1966). The timing of the photoperiod is of extreme importance. In the European corn borer *Ostrinia nubilalis* it was found that exposure to a short photoperiod (9.5L:13.5D) would induce diapause only if the larva was in the final two days of its penultimate instar. The photoperiod would have to be maintained for the first five days of the final instar, after which the larvae would be committed to diapause (Mutchmor and Beckel 1959, Beck and Hanec 1960).

The reaction of arthropods to photoperiodism appears to be independent of light intensity above a certain threshold (Lees 1956). This threshold varies between species from a low of 0.1 ergs/cm²/sec in the larva of *Metriocnemus knabi* to a high of 40 to 80 ergs/cm²/sec in the red spider mite *Meta tetranychus ulmi* (deWilde 1962).

Blue and blue green regions of the visible spectrum are most effective for diapause induction. Most insects show no response at all to light at the red end of the spectrum (Lees 1956). Studies on the action spectrum of diapause
indicate a maximum sensitivity at 415 nm with limited
sensitivity ranging from 365 to 500 nm (deWilde 1962). Many
pigments have been proposed as the photoreceptor; porphyrins,
omochromes, flavins, carotenoids, melanins, pteridines and
xanthophylls all of which absorb light in the range required.
It has been proposed that riboflavin or a flavoprotein would
be the most likely receptor (Hayes et al. 1968). Norris
et al. (1969) suggests that more than one pigment may be
acting and the effect required to induce diapause may be an
additive one.

Experiments have been performed to demonstrate that the
compound eye is unnecessary as a photoreceptor for induction
of diapause (deWilde 1962). Compound eyes have been removed,
cauterized and painted with black lacquer without affecting
the induction of diapause (deWilde 1969). The brain has
been suggested as the receptor site for the photoperiodic
stimulus (Williams et al. 1965). In experiments with
Antheraea pernyi the brain was removed from the anterior of
the pupae and implanted in the posterior. Exposure of the
anterior to long day photoperiods would not terminate the
pupal diapause but exposure of the posterior containing the
implanted brain resulted in termination of diapause (Williams
and Adkisson 1964, Williams et al. 1965).

In many cases temperature plays an important part in diapause, suppressing induction when the temperature is high and enhancing induction at low temperatures (Lees 1956). Some researchers have found that photoperiod alone controls the diapause in insects. In the mosquito Aedes atropalpus, which diapauses as an embryo, it was shown that a short photoperiod induced diapause independent of temperature (Anderson 1968). In other insects, diapause induction is independent of temperature within certain ranges (McLeod 1963, McLeod and Beck 1963, Mutchmor and Beckel 1959).

Diapause can be terminated by a long photoperiod although this can be moderated by temperature. Termination of diapause is only possible after a period of diapause development. It has been proposed that some insects required a chilling period to initiate diapause development (Lees 1956). This hypothesis has been disputed by some researchers (McLeod and Beck 1963) who claim that photoperiod alone stimulated diapause development in Ostrinia nubilalis without the need for chilling. It has been demonstrated that several insects, Antheraea pernyi, Samia cynthia and Hyalophora cecropia require low temperature for several months of their diapause in order to
begin diapause development (Williams 1969). Peterson and Hamner (1968) found that they could break diapause in *Laspeyresia pomonella* by manipulating the photoperiod alone but also found that chilling facilitated diapause development.

Water has also been shown to play an important role in the termination of diapause in insects. It has been noted that dessication occurs in diapausing European corn borers. These insects were observed to lose up to 28% of their body water due to dessication. On termination of diapause, active imbibition of water was observed, returning the insect to its prediapause weight (Beck 1967). It is thought that development cannot occur until the water balance is restored due to an inhibition of the secretory activity of the insect endocrine system (Lees 1956, Beck 1967).

Diapause is thought to have hormonal controls. This was first proposed by Dickson (1949), in a two step process that synthesized a diapause hormone. The proposed hormone would be partially synthesized in the light and partly in the dark, as the days became longer or shorter the quantity of hormone would determine the physiological state of the insect. However, experiments using ligation, transplant, parabiosis and administration of tissue extracts have indicated that in
most species no "diapause hormone" exists. The notable exception to this occurs in Bombyx mori in which diapause does act upon the ovary to produce diapausing eggs (Beck 1968).

In larval and pupal diapause the hormonal mechanisms involved are those of normal growth and development. The secretions of the median neurosecretory cells of the insect brain are transmitted to the corpora cardiaca which then releases brain hormone to the haemolymph. Brain hormone acts on the prothoracic glands initiating secretion of ecdysone, which then promotes growth and molting (Wigglesworth 1965). Diapause in L. pomonella is probably a response of some pigment (Norris et al. 1969, Hayes et al. 1968) to a short day photoperiod which triggers the termination of neurosecretions and hence, the cessation of development. Development resumes when the diapausing larvae are subjected to a long photoperiod.

One of the striking characteristics of diapause is the reduced rate of oxygen consumption. This reduced metabolic rate permits the insect to utilize reserves in the fat body at a very slow rate and thus it can survive for a prolonged period. The ability of diapausing insects to withstand
prolonged exposure to metabolic inhibitors such as cyanide and carbon monoxide led Schneiderman and Williams (1954) to hypothesize that the electron transport system was interrupted and that an additional cytochrome provides a separate pathway to terminal oxygen. The resistance to cyanide of an organism appears to also bring with it a resistance to radiation injury (Bacq et al. 1952). It has been shown that diapausing insects show a resistance to cyanide (Schneiderman and Williams 1954).

The codling moth *L. pomonella* was chosen for this study since it is an insect on which much radiation research has already been done (Proverbs and Newton 1962 a, b). This enabled me to pursue my studies using this published ground work. *L. pomonella* may also be reared in and out of diapause in artificial light by manipulating the photoperiod (Hamner 1969, Peterson and Hamner 1968, Hansen and Harwood 1968, Norris et al. 1969). If the *L. pomonella* is resistant to cyanide while in diapause, and if this resistance to cyanide confirms resistance to radiation injury as suggested by Bacq et al. (1952) then the diapausing *L. pomonella* may suffer less somatic damage from the irradiation than the nondiapausing larvae.
The objective of this study was to determine if there was a difference in the radiation sensitivity of diapausing and nondiapausing insects and if there was sufficient evidence to support the hypothesis that diapause offered radioprotection or that it postponed the development of radiation injury. Various parameters have been examined to ascertain whether diapause influences the radiation sensitivity of the codling moth larvae, viz; pupal mortality, adult emergence, fecundity and sterility. The results are presented in this thesis.
Rearing procedure for *L. pomonella*

*L. pomonella* were reared on apple thinnings in constant temperature incubators under controlled light regimes. Diapausing insects were reared in a short day photoperiod which consisted of ten hours of light and fourteen hours of darkness (10L:14D). Nondiapausing insects were reared in a long day photoperiod (16L:8D). All incubators were maintained at 27±3°C and at a relative humidity (R. H.) of 80±10%. Washed eggs of *L. pomonella* were placed on the apple thinnings in enamel trays 2 inches deep when the eggs were in the "blackhead" stage of development. Within 24 hours the first instar larvae entered the apples. After 10 to 15 days the fifth instar larvae emerged from the apples and spun cocoons in strips of corrugated cardboard placed on top of the apple trays. These larvae were removed daily. They were sexed according to the method of Proverbs and Newton (1962a) and then placed in gelatin capsules until pupation. Pupation occurred in 2 to 10 days in nondiapausing insects. Upon pupation the pupae were removed from the gelatin capsules and placed in 3 dram snap cap vials with perforated caps. Adult emergence occurred 6 to 10 days after pupation. Moths
were maintained in the vials for 24 hours to ensure reproductive maturity, after which they were mated in single pairs in tetrahedral shaped perforated waxed paper cages of approximately 750 ml volume. Matings continued for one week and after oviposition was completed the adults were preserved in 10% formalin for later examination of spermatophores and external genetalia. The mating cages were opened into flat sheets and all eggs laid were counted. The egg papers (opened mating cages) were then incubated for a further 4 days to ensure sufficient time for maximum egg hatch. From this the percent egg hatch was determined. This procedure was modified for diapausing L. pomonella. The control and irradiated diapausing larvae were chilled at 4°C for 58 days after which they were removed from refrigeration and incubated at 27±3°C and at 80±10% R. H. They were then treated as previously described.

Irradiation Procedures

Nondiapausing larvae were irradiated in a Gammarcell 200 one day after spinning a cocoon. The larvae, enclosed in gelatin capsules, were placed in groups of five in 3 dram snap cap vials and irradiated. All irradiations were carried out in the central position of the irradiation chamber using the
6 cm center rod. After irradiation the larvae were placed in groups of ten in Petri dishes and returned to the long day incubator to await pupation. After pupation the treatment was the same as that described in the rearing procedure.

The diapausing larvae were maintained in the short day incubator for 12 days after emergence from the apples to ensure that each individual was in diapause. If pupation did not occur in this time the larvae were irradiated using the previously described procedure. After irradiation the larvae, in gelatin capsules, were placed in Petri dishes, 10 larvae to a dish, and refrigerated for 58 days at 4°C. After chilling the larvae were returned to the long day incubator to await pupation.

Spermatophores were dissected from the mated L. pomonella females, from one to four months after preservation in 10% formalin as preservation does not change the structure of the spermatophore (Pesho 1961). The spermatophores were excised by removing the abdomen from the thorax, making an incision in the lower abdomen and teasing the spermatophores from the genitalia with a dissecting needle. The spermatophores were counted and examined for differences in structure that might be related to the dose received or the physiological
condition of the insects.

The external genitalia of both sexes were examined to determine if any damage had resulted due to irradiation. The genitalia were removed from the preserved adults, cleaned overnight in a 10% solution of potassium hydroxide and mounted in cavity slides for examination.

**Radiation Dosimetry**

Measurement of radiation dose was carried out using a chemical dosimeter in aqueous solution. The Fricke–Miller ferrous ammonium sulphate dosimeter was used (Battaerd and Tregear 1966). The oxidation of ferrous ion to ferric ion was measured spectrophotometrically. The added ammonium ion suppressed some side reactions, as well as suppressing the effect of organic impurities. The oxidation of the dosimeter took place by ten reactions (Battaerd and Tregear 1966) (See Appendix I).

All glassware used in preparation of the dosimeter was cleaned overnight in chromic acid and rinsed for several hours in a water jet to remove all traces of chromic acid. Before use all glassware was rinsed thrice in distilled water and once in triple distilled water. Triple distilled water was prepared by double distillation of a solution of 0.01M
potassium hydroxide together with 0.01M potassium permanganate in distilled water. The 0.001M ferrous ammonium sulphate dosimeter was prepared from 0.8N sulphuric acid in triple distilled water. The sulphuric acid solution was aerated overnight by reducing pressure in the solution container. The air for aeration was filtered through a cotton filter and a water trap. The dosimeter was prepared immediately prior to irradiation and was maintained in a covered box before and after irradiation. Five ml of the dosimeter was placed in 3 dram snap cap vials which were irradiated at 1 cm intervals from the bottom of the sample chamber. A variable sample carrier was constructed. This carrier consisted of an aluminum disc the same diameter as the sample chamber, mounted centrally by means of a brass screw to rods 1 to 10 cm in height. These rods were seated in the drain of the sample chamber. The vial containing the dosimeter was positioned by a styrofoam spacer disc with holes provided for one or more vials. This sample chamber contained an additional two discs to fit two of three lead attenuators. The irradiated dosimeter was measured spectrophotometrically using a Unicam SP 500 Series 2 spectrophotometer at 304 nm. The absolute dose and the dose rate were calculated using the formula from Attix
and Roesch (1966) (See Appendix II). Because of the repetitive nature of this calculation it was programmed for an I. B. M. 360 computer with provision for handling replicates and yielding absolute dose, dose rate/min., dose rate/hour, mean and standard deviation for each position. Using this method the dose was reproducible within 3%. Due to the decay of Co-60 the dose rate changed from 1,550 rads/min to 1,300 rads/min over the experimental time (Fig. 1). The snap cap vials containing dosimeter were arranged across the sample chamber to show the variation in the dose from one side to another. This was repeated at 1 cm levels above the bottom of the sample chamber. This was repeated arranging the vials from back to front. Fig. 2 shows the approximate isodose curves as a cross section of the sample chamber. From the results in Fig. 2 and from data collected in the other plane it was evident that the maximum dose occurred at mid elevations close to the sides of the sample chamber. It would seem likely that more radioactive material was loaded in the forward right hand side of the source surrounding the sample chamber.

The three attenuators were also calibrated, although isodose curves were not drawn from these reduced sample chambers. The attenuators reduced the absolute dose as
Figure 1. Changes in dose rate of Gammarcell 200 as a function of time. Dosimetry was carried out by ferrous ammonium sulphate dosimetry. Time begins in May 1968.
Figure 2. Isodose curves in the sample chamber of the Gammacell 200 as determined by ferrous ammonium sulphate dosimetry. All doses expressed as percentages of the central dose.
TABLE I. Reduced dose in Gammasol 200 due to lead attenuators in the sample chamber.

<table>
<thead>
<tr>
<th>Attenuator number</th>
<th>Dose rate (rads/min)</th>
<th>Reduction in dose (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>1,375</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>742</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>358</td>
<td>74</td>
</tr>
<tr>
<td>3</td>
<td>233</td>
<td>83</td>
</tr>
</tbody>
</table>
Effects of Variable Dose Rate

The effects of the different dose rates were examined by irradiating larvae at the same absolute dose using the lead attenuators. Larvae were treated as previously described except for the lowest dose rate in which the sample chamber was reduced in size so that only three larvae could be irradiated at one time. All larvae used in determining the effect of dose rate were nondiapause females. Two different doses were used in this study, viz; 2.5 k rads and 10 k rads. Larvae were irradiated with four dose rates, viz; 233 rads/min, 358 rads/min, 742 rads/min and 1,375 rads/min (Table I). Larvae were treated as described previously for nondiapause L. pomonella. The effects of dose rate were assessed using the same parameters as previously described, viz; pupal mortality, adult emergence, fecundity and sterility induced.

Data Analysis

In order to determine whether diapause had any effect on the radiosensitivity of the codling moth L. pomonella, the previously described procedures were carried out. Five parameters were examined, viz; pupal mortality, adult emergence, femal
fecundity, sterility and development time. Each parameter was examined for three variables and the relationship between the variables. The three variables were: physiological condition, diapause or nondiapause; dose, 0, 2.5, 5 or 10 k rads and sex, male or female. It was the intention of this work to determine if an overall relationship between the parameters and the variables existed.

The data were subjected to three way analysis of variance, the variables being; dose, sex and physiological condition. Those interactions which caused rejection of the null hypothesis at $P=0.05\%$ were considered significant in this study. In those cases where data was all-or-none (i.e. either an adult emerges or it does not) as in the cases of pupal mortality and adult emergence, number of matings in which eggs were laid, number of matings in which over 50 eggs were laid, the analysis of variance was computed using a positive reaction as one and a negative reaction as zero. The results of analysis of variance are given as means of effects of variables. This indicates that the effect of a variable on the parameter is given as a mean or average of all of the data at that dose. For example, the effect of diapause on the manifestation of 2.5 k rads of irradiation includes the meaned data from the
males and the females. Some accumulated data was subjected to probit analysis to determine the time required for 50% pupation.
RESULTS

All *L. pomonella* were irradiated in the fifth larval instar but the effects of such irradiation were evaluated from later developmental stages. Diapause occurs only in the fifth larval instar but any protection it may have against radiation damage may be displayed in later developmental stages. Therefore, "an irradiated diapause pupa" in these results will refer to a pupa that was irradiated as a fifth instar larva while in diapause.

**Pupal Mortality**

The percent pupal mortality is shown in Fig. 3. It can be seen that as radiation dose increases the pupal mortality increases as well. This increase in pupal mortality with increasing dose is significant (Table II). There is no significant difference in mortality between diapause and nondiapause groups. The females are more sensitive than males (Fig. 3). Analysis of variance of the data indicates that females have a significantly higher pupal mortality than males. This is particularly evident at 10 k rads.

**Adult Emergence**

All irradiations were carried out while the *L. pomonella* was in the fifth larval instar. Therefore, in the following
Figure 3. Percentage of pupal mortality of those larvae pupating after treatment with 0, 2.5, 5 or 10 k rads in the fifth larval instar.
TABLE II. Means of effects of variables on the percentage of pupal mortality of *L. pomonella* after irradiation at 0, 2.5, 5 and 10 k rads in the fifth larval instar. (a) (b)

<table>
<thead>
<tr>
<th>Effects on pupal mortality of</th>
<th>Dose (k rads)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Dose independent of variables</td>
<td>19.2</td>
</tr>
<tr>
<td>Diapause</td>
<td>24.0</td>
</tr>
<tr>
<td>Nondiapause</td>
<td>14.4</td>
</tr>
<tr>
<td>Male</td>
<td>21.3</td>
</tr>
<tr>
<td>Female</td>
<td>17.1</td>
</tr>
</tbody>
</table>

(a) Means adjusted in computer to exclude the effects of other variables.

(b) The number of pupae involved in these experiments ranged from 27 to 57.

(c) Those variables having an effect significant at the 0.05% level of probability are indicated by an asterisk. Significance is indicated over the range of doses used, not at any particular dose.
Figure 4. Percentage of adult emergence after treatment with 0, 2.5, 5 or 10 k rads in the fifth larval instar. Adult emergence is a percentage of those larvae pupating that emerge as adults. Since there was no adult emergence at 10 k rads for nondiapause females it is not recorded in the figures.
TABLE III. Means of effects of variables on the percentage of adult emergence of *L. pomonella* after irradiation at 0, 2.5, 5 and 10 k rads in the fifth larval instar. (a) (b)

<table>
<thead>
<tr>
<th>Effects on adult emergence of</th>
<th>Dose (k rads)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Dose independent of variables</td>
<td>80.8</td>
</tr>
<tr>
<td>Diapause</td>
<td>76.0</td>
</tr>
<tr>
<td>Nondiapause</td>
<td>85.6</td>
</tr>
<tr>
<td>Male</td>
<td>78.7</td>
</tr>
<tr>
<td>Female</td>
<td>82.9</td>
</tr>
</tbody>
</table>

(a) Means adjusted in computer to exclude the effects of other variables.

(b) The number of pupae involved in these experiments ranged from 27 to 57.

(c) Those variables having an effect significant at the 0.05% level of probability are indicated by an asterisk. Significance is indicated over the range of doses used, not at any particular dose.
results a "2.5 k rads diapause male" refers to an adult emerged from a diapausing larva irradiated with 2.5 k rads while in the fifth larval instar. As the radiation dose increased the adult emergence was reduced. At 10 k rads no nondiapause females emerged at all (Fig. 4).

The results of the analysis of data on adult emergence are shown in Table III. This shows that as radiation dose increased adult emergence was significantly reduced. There was no significant interaction between diapause and radiation with respect to adult emergence in those larvae that were irradiated as nondiapause larvae. The male adult emergence was significantly higher after irradiation as larvae than was the female adult emergence.

**Fecundity**

Three measures of fecundity were used in this study, viz;

1. mean egg lay or the mean number of eggs laid per mated female;
2. the percentage of successful matings in which eggs were laid;
3. the percentage of matings in which over fifty eggs were laid. In the results which follow, the mean egg lay of a diapause female irradiated with 10 k rads refers to the mean number of eggs laid by an adult female emerged from a diapausing fifth instar larva irradiated
with 10 k rads and later mated to a normal male.

As radiation dose increases the mean egg lay is reduced except in the diapause female (Fig. 5). The reduction in mean egg lay is significant over the range of doses examined (Table IV). It indicates also that the irradiated diapause insects have a higher mean egg lay than the irradiated nondiapause insects. Although there are apparently large differences between diapause and nondiapause males irradiated with 0, 2.5 and 5 k rads, these differences are not significant to a "t" test. However, the differences in the mean egg lay between irradiated diapause and nondiapause females were found to be significant. It can be concluded from Table IV that L. pomonella irradiated in diapause laid a significantly greater number of eggs per female than those nondiapausing codling moths that were irradiated. Table IV indicates that the female is more sensitive to radiation injury than the male; a dose of 5 k rads results in infecundity in the nondiapause female (Fig. 5).

The second measure of fecundity in this study is the percentage of successfully mated pairs which laid eggs. A successfully mated pair is any pair in which the male has deposited a spermatophore in the female bursa copulatrix. Not all of the successfully mated females laid eggs.
Figure 5. Mean number and standard error of eggs laid either by females treated with 0, 2.5, 5 or 10 k rads in the fifth larval instar and mated to normal males or by normal females mated to males irradiated with the above doses. Nondiapause females laid no eggs after treatment with 5 k rads. Females treated at 10 k rads were not successfully mated and are not represented on this figure.
TABLE IV. Means of effects of variables on the mean egg lay of *L. pomonella* after irradiation at 0, 2.5, 5 and 10 k rads in the fifth larval instar. (a) (b)

<table>
<thead>
<tr>
<th>Effects on mean egg lay of</th>
<th>Dose (k rads)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Dose independent of variables</td>
<td>111.1</td>
</tr>
<tr>
<td>Diapause</td>
<td>89.8</td>
</tr>
<tr>
<td>Nondiapause</td>
<td>132.5</td>
</tr>
<tr>
<td>Male</td>
<td>107.6</td>
</tr>
<tr>
<td>Female</td>
<td>114.7</td>
</tr>
</tbody>
</table>

(a) Means adjusted in computer to exclude the effects of other variables.

(b) The number of pairs mated for each experiment ranged from 6 to 35. Where no successful matings were completed a dash (--) signifies no data was available. Irradiated males were mated to normal females and irradiated females were mated to normal males.

(c) Those variables having an effect significant at the 0.05% level of probability are indicated by an asterisk. Significance is indicated over the range of doses used, not at any particular dose.
Therefore, this parameter might be useful as an indicator of radiation injury in this study. Table V shows that the percentage of successful matings in which eggs are laid is reduced as the radiation dose increases. It can be seen that very little difference occurs between the males in the controls and those irradiated at 2.5 and 5 k rads (Fig. 6). But irradiation of nondiapausing males with 10 k rads caused a considerable reduction in the percentage of matings resulting in eggs. The most significant effect of radiation is on the nondiapausing female. At 5 k rads they mated but no eggs were deposited and at 10 k rads even mating was impaired. From Table V the following are evident: (1) the percent laying eggs after a successful mating is reduced by an increase in radiation dose; (2) the diapausing insects are less sensitive to radiation injury than the nondiapausing insects since they have a higher percent laying eggs after irradiation and; (3) females are more sensitive to radiation injury than the males since irradiated females lay a lower percentage of eggs when mated to normal males than normal females that have been mated to an irradiated male.

The third measure of fecundity is the number of successfully mated pairs laying over fifty eggs. This, like the
Figure 6. Percentage of matings of *L. pomonella* treated with 0, 2.5, 5 or 10 k rads in the fifth larval instar in which eggs were laid. Treated males were mated to normal females and treated females were mated to normal males. Nondiapause females treated with 5 k rads laid no eggs. Females treated with 10 k rads could not be mated.
TABLE V. Means of effects of variables on the percentage of successful matings in which eggs were laid.

<table>
<thead>
<tr>
<th>Effects on egg lay of</th>
<th>Dose (k rads)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Dose independent of variables</td>
<td>91.7</td>
</tr>
<tr>
<td>Diapause</td>
<td>90.9</td>
</tr>
<tr>
<td>Nondiapause</td>
<td>97.6</td>
</tr>
<tr>
<td>Male</td>
<td>94.3</td>
</tr>
<tr>
<td>Female</td>
<td>89.1</td>
</tr>
</tbody>
</table>

(a) Means adjusted in computer to exclude the effects of other variables.

(b) The number of pairs mated for each experiment ranged from 6 to 35. Where no successful matings were completed a dash (--) signifies no data was available. Irradiated males were mated with normal females and irradiated females were mated with normal males. All matings included one member irradiated at 0, 2.5, 5 or 10 k rads.

(c) Those variables having an effect significant at the 0.05% level of probability are indicated by an asterisk. Significance is indicated over the range of doses used, not at any particular dose.
other measures of fecundity, shows that the fecundity is significantly reduced with an increase in radiation dose (Fig. 5, Table VI). Those larvae, in diapause at the time of irradiation produced adults that, when mated to normal mates laid over fifty eggs in a significantly higher percentage of mating pairs than those larvae that were nondiapausing at the time of irradiation. The sensitivity of the female codling moth to gamma radiation is significantly greater than that of the male.

Sterility

The eggs laid by control and the irradiated *L. pomonella* were examined to determine the percentage of sterility induced by irradiation. As radiation dose is increased the percentage of sterility is increased as well (Fig. 8). Further, the data also show that diapause and sex have no significant effect on the percentage of sterility induced by radiation (Table VI).

Development Time

The time required for 50% of the larvae to pupate is shown in Fig. 9. The nondiapause larvae pupated shortly after treatment ranging from two days for the nondiapause male control to 9 days for the nondiapause female irradiated with 10 k rads. Although the $T_{50}$ in the diapause control ranged from 72 to
Figure 7. Percentage of matings of *L. pomonella* treated with 0, 2.5, 5 or 10 k rads in the fifth larval instar in which over fifty eggs were laid. Treated males were mated to normal females and treated females were mated to normal males. Nondiapause females treated with 5 k rads laid no eggs. Females treated with 10 k rads could not be mated. The normal female mates of nondiapause males treated with 10 k rads did not lay more than fifty eggs in any mating.
TABLE VI. Mean of effects of variables on the percentage of matings of *L. pomonella* in which more than fifty eggs were laid. (a) (b)

<table>
<thead>
<tr>
<th>Effects of egg lay of</th>
<th>Dose (k rads)</th>
<th>0</th>
<th>2.5</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose independent of variables</td>
<td>70.4</td>
<td>58.6</td>
<td>28.1</td>
<td>8.9 * (c)</td>
<td></td>
</tr>
<tr>
<td>Diapause</td>
<td>64.0</td>
<td>61.1</td>
<td>49.0</td>
<td>17.0 * (c)</td>
<td></td>
</tr>
<tr>
<td>Nondiapause</td>
<td>76.8</td>
<td>56.2</td>
<td>7.1</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>62.2</td>
<td>68.8</td>
<td>26.2</td>
<td>8.9 * (c)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>78.6</td>
<td>48.5</td>
<td>30.0</td>
<td>--</td>
<td></td>
</tr>
</tbody>
</table>

(a) Means adjusted in computer to exclude the effects of other variables.

(b) The number of pairs mated for each experiment ranged from 6 to 35. Where no successful matings were completed a dash (--) signifies no data was available. Irradiated males were mated with normal females and irradiated females were mated with normal males. All matings included one member irradiated at 0, 2.5, 5 or 10 k rads.

(c) Those variables having an effect significant at the 0.05% level of probability are indicated by an asterisk. Significance is indicated over the range of doses used, not at any particular dose.
Figure 8. Percentage of sterility of eggs laid by *L. pomonella* after treatment with 0, 2.5, 5 or 10 k rads in the fifth larval instar. Treated males were mated to normal females and treated females were mated to normal males. Nondiapause females treated with 5 k rads laid no eggs. No data was available for females treated with 10 k rads.
TABLE VII. Means of effects of variables on the percentage of sterile eggs laid by *L. pomonella* after irradiation at 0.2.5, 5 or 10 k rads in the fifth larval instar. (a) (b)

<table>
<thead>
<tr>
<th>Effects on sterility of</th>
<th>Dose (k rads)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Dose independent of variables</td>
<td>28.1</td>
</tr>
<tr>
<td>Diapause</td>
<td>30.3</td>
</tr>
<tr>
<td>Nondiapause</td>
<td>26.3</td>
</tr>
<tr>
<td>Male</td>
<td>25.8</td>
</tr>
<tr>
<td>Female</td>
<td>30.4</td>
</tr>
</tbody>
</table>

(a) Means adjusted in computer to exclude the effects of other variables.

(b) The number of pairs mated for each experiment ranged from 6 to 35. Where no eggs were laid for a given experiment a dash (--) signifies no data was available. Irradiated males were mated to normal females and irradiated females were mated to normal males.

(c) Those variables having an effect significant at the 0.05% level of probability are indicated by an asterisk. Significance is indicated over the range of doses used, not at any particular dose.
114 days this time was considerably reduced when irradiated with 2.5 and 5 k rads. Fifty percent of the diapause males irradiated with 2.5 k rads have pupated in less than one third of the time required for fifty percent of the diapause control to pupate. Irradiation with 5 k rads results in a reduction of the same order. Irradiation with 10 k rads returns the pupation time to very near that of the control. (There is no significant difference between the control diapause male and the 10 k rads diapause male). The diapause female presents an even more interesting example since the T50 pupation of the female groups irradiated with 2.5 and 5 k rads is almost 100 days shorter than the control. The female irradiated with 10 k rads, has a significantly longer T50 pupation than the other irradiated groups but it is also significantly less than that of the control.

**Spermatophore and Genitalia Examination**

There was no correlation between the number of spermatophores deposited and the number of eggs laid by the females. The structure of the spermatophores did not differ with any doses nor did it change for insects in different physiological conditions. External genitalia were undamaged at the highest doses in both sexes, the structure of the genitalia did not differ noticeably from the control through to 10 k rads.
Figure 9. Time in days and the fiducial limits for fifty percent of the larvae to pupate in *L. pomonella* treated with 0, 2.5, 5 or 10 k rads. Fiducial limits were not plotted on the nondiapause experiments as these limits were too small.
Effects of Variable Dose Rate

Nondiapause female larvae were irradiated with 2.5 and 10 k rads at four dose rates viz; 233 rads/min, 358 rads/min, 742 rads/min and 1,375 rads/min (Table II). After adult emergence the females irradiated as larvae were mated to normal males. The effect of irradiation at different dose rates was examined using four different parameters viz; (1) pupal mortality, (2) adult emergence, (3) mean egg lay and (4) sterility.

It is apparent from examination of the results (Table VIII) that there is no correlation between the dose rate and any of the parameters measured.
TABLE VIII. The effect of different dose rates on radiation sensitivity of L. pomonella.

<table>
<thead>
<tr>
<th>Percent</th>
<th>Total dose</th>
<th>233</th>
<th>358</th>
<th>742</th>
<th>1,375</th>
</tr>
</thead>
<tbody>
<tr>
<td>pupal</td>
<td>2.5 k rads</td>
<td>22.1%</td>
<td>16.7%</td>
<td>28.0%</td>
<td>19.6%</td>
</tr>
<tr>
<td>mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adult</td>
<td>2.5 k rads</td>
<td>77.9%</td>
<td>83.3%</td>
<td>72.0%</td>
<td>80.4%</td>
</tr>
<tr>
<td>emergence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean egg</td>
<td>2.5 k rads</td>
<td>47.8</td>
<td>35.3</td>
<td>44.5</td>
<td>39.2</td>
</tr>
<tr>
<td>lay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent</td>
<td>2.5 k rads</td>
<td>38.6%</td>
<td>43.0%</td>
<td>40.0%</td>
<td>35.9%</td>
</tr>
<tr>
<td>sterility</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent</td>
<td>10 k rads</td>
<td>97.8%</td>
<td>-- (a)</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>pupal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent</td>
<td>10 k rads</td>
<td>2.2%</td>
<td>-- (a)</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>adult</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>emergence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a) No larvae were irradiated with 10 k rads at a dose rate of 358 rads/min.
DISCUSSION

The first two parameters studied did not show any protective effects of diapause. Pupal mortality and adult emergence showed an increase with increasing radiation dose. It was also evident that female *L. pomonella* were more susceptible to radiation injury than males. The third parameter, fecundity, showed in all of its three measures that diapause has a protective effect against radiation injury. This parameter also showed that fecundity was reduced with an increase in radiation dose and that female *L. pomonella* were more susceptible to radiation injury than males. The fourth parameter examined showed that although sterility increased with increasing radiation dose diapause had no protective effect and males were as susceptible as females to genetic injury. The effect of radiation on the development time of the insect showed a stimulation of development in the diapausing *L. pomonella*.

Since there was no significant variation in pupal mortality between diapause and nondiapause irradiated insects (Fig. 3, Table II) diapause apparently did not offer radiation protection but only postponed the development of radiation injury. In other words, the damage remained latent and it
expressed itself at critical stages of development. One such critical stage in the development of insects is the pupal-adult apolysis followed by ecdysis. It was observed that in the unemerged irradiated samples, the pupae differentiated into fully formed adults, but were unable to break out of the pupal skin. Proverbs and Newton (1962b) found that adult moths unable to emerge survived for up to two weeks within the pupal skin. Nair (1962) observed that irradiation of young house fly pupa inhibited adult emergence although the pharate adult appeared very similar in external morphology to emerged adults. Subsequent studies (Nair et al. 1967, Sivasubramanian et al. 1970) showed that the absence of emergence in the irradiated pupae was due to radiation damage to the differentiating myoblasts. As emergence is a muscular phenomenon it is reasonable to assume that the failure of some of the irradiated codling moth larvae to emerge may be due to damage to the differentiating muscles leading to their dystrophy. It is evident also that the females are much more sensitive than the males to gamma radiation. This damage differential is evident in both the diapausing and nondiapausing forms. Proverbs and Newton (1962b) had also observed this damage differential between the two sexes in the nondiapausing codling moth larvae. Various factors appear to contribute to
this differential susceptibility between males and females. Among these the one that seems most plausible is the body volume. The females are approximately 25% larger in volume than the males. Hence, it is presumed that the total energy deposited by the gamma-rays is correspondingly more in the female than in the male. Nair and Rahalkar (1963) observed that the female Khapra beetle larva which is about three times as large as the male was three times more sensitive to gamma radiation than the males. Cole et al. (1959) determined the $LD_{50}$ of insects of various body size and found that the $LD_{50}$ decreased with the increase in body size. It is, therefore reasonable to expect a greater biological damage for the same dose of gamma-rays in the female codling moth larva than in the male larva.

That diapause does not offer radio protection is evident also from the data on adult emergence. In both diapause and nondiapause, adult emergence decreased with increase in dose, but the data on the interaction between the physiological condition (diapause and nondiapause) and radiation show that the differences are not significant. This confirms my earlier observation that radiation damage is latent during the larval and pupal stages but expresses itself at the time
of the metamorphic molt. These observations are at variance with those obtained by Raun et al. (1967) in their studies on irradiation of diapausing larvae of the European corn borer *Ostrinia nubilalis*. These authors claimed that radio-protection occurs when *O. nubilalis* larvae are irradiated in a diapausing state since adult emergence and mating were normal even when they were irradiated with doses as high as 5 k rads. It is pertinent to mention here that Raun et al. (1967) used field collected diapausing larvae and compared their radiosensitivity to laboratory reared nondiapause larvae. Beck and Chippendale (1968) reported that *O. nubilalis* of a natural population are hardier than laboratory reared individuals. This may account for the differential sensitivity between the diapausing and nondiapausing corn borer larvae. It should also be noted that these diapausing larvae were irradiated after 3½ to 5½ months of chilling, whereas in my study all irradiations were carried out prior to chilling. These and probably other factors might account for the differences in these findings. My data are in agreement with those of Nair and Rahalkar (1963), who observed that mortality in the irradiated diapausing Khapra beetle larvae was remarkably low, but when the diapause was broken the extent of
mortality was similar to that observed in the irradiated nondiapause Khapra beetle larvae. Further studies showed that even increases in the post irradiation diapause period had no significant effect on the post diapause survival time (Rahalkar and Nair 1968).

Hibernation in vertebrates is a specialized physiological state that can be compared to diapause in insects; both are forms of suspended animation seasonally controlled by hormones. When hibernating mammals such as marmots (Smith and Grenan 1951) and squirrels (Doull and Dubois 1953) were irradiated mortality was much less than for those nonhibernating mammals which were irradiated. But when the survivors were awakened the animals died after the same period of sickness as nonhibernating controls. My results conform with the observations made on irradiated hibernating animals since diapause, a state of suspended animation, did not seem to offer radioprotection as far as pupal mortality and adult emergence are concerned.

The third parameter that was examined was fecundity. This had three measures; (1) mean egg lay, (2) the percentage of successful mating pairs in which over fifty eggs were laid, and (3) the percentage of successful mating pairs that laid eggs. The data on all of these measures show that
irradiated nondiapause *L. pomonella* had reduced fecundity when compared to those of the irradiated diapause group.

It appears from the data on the mean egg lay that there is a threshold dose below which there is no effect on egg lay and above which there is a rapid decline in egg lay in both the nondiapause and diapause groups. Statistical analysis of the data by using the "t" test shows that the nondiapause male appears unaffected by 2.5 k rads but 5 k rads reduced the mean egg lay of its normal female mate to less than 19% of the control. Further increase in dose did not reduce the mean egg lay of its normal female mate indicating that the threshold dose lies between 2.5 and 5 k rads. Although the diapause male shows an increase in mean egg lay of its normal female mate when the male is irradiated with 2.5 k rads, analysis of the data shows that the difference is not statistically significant. Irradiation with a dose of 5 k rads reduced the mean egg lay of its normal female mate to about 60% of the control. There was further reduction when the diapause male was irradiated with 10 k rads.

The nondiapause female shows a significant reduction to 30% of the control mean egg lay when it is irradiated with 2.5 k rads and the mean egg lay is reduced to zero by 5 k rads.
rads. These data indicate that the threshold lies between 0 and 2.5 k rads for the nondiapause female. The diapause female, however, shows no appreciable reduction in mean egg lay even when irradiated with 5 k rads, indicating the reduction threshold in this case lies between 5 and 10 k rads.

Analysis of variance of the overall interaction between radiation and physiological condition (diapause and nondiapause) shows that the irradiated diapausing insects had a significantly greater mean egg lay than the irradiated nondiapause insects. That diapause offers radioprotection is further illustrated by the fact that the maximum reduction in mean egg lay occurs at 10 k rads in the diapause group whereas in the nondiapause group it is seen at 5 k rads. These observations suggest that there is a twofold increase in resistance in the diapause group over the nondiapause group as far as mean egg lay is concerned.

The second measure of fecundity was the percentage of successfully mated pairs that laid over fifty eggs. Using this measure the nondiapause male shows a decline in this value with increase in dose. None of the nondiapause mating pairs laid over fifty eggs after irradiation with 10 k rads. On the other hand the data from the diapausing males show that
even at 10 k rads about 20% of the female mates laid more than fifty eggs per female. Moreover, the reduction in these values is more gradual with increase in dose when compared to the drastic reduction in the nondiapause males.

In the case of the nondiapause females, there is a drastic reduction in the percentage of successful mating pairs laying over fifty eggs after irradiation with 2.5 k rads. After irradiation with 5 k rads no eggs were laid by the nondiapause females. The diapause females showed little reduction in this value even after irradiation with 5 k rads, but laid no eggs after irradiation with 10 k rads. The data on the interaction of the variables (Table IV) further illustrates the radioprotective nature of diapause.

Irradiation of the nondiapause males resulted in a lowering of the percentage of mating pairs that laid eggs with increase in dose, whereas irradiation of diapause males did not (Fig. 6, Table V). This difference in the radiosensitivity between nondiapause and diapause groups becomes even more evident when the females of the mating pairs were irradiated (Fig. 6, Table V). These data further support the earlier contention that as far as fecundity is concerned diapause offers radioprotection against a reduction in
The protective effect of diapause was evident only when the females were irradiated. The reason for this protection can be ascertained from histology of the ovaries of the diapausing and the nondiapausing groups. According to Beck (1968) the ovaries of diapausing larvae are relatively undifferentiated, whereas those of nondiapausing larvae show various stages of oogenesis. In a recent paper Hansen and Harwood (1968) reported that the ovaries of diapausing L. pomonella larvae were smaller in size and contained fewer oocytes than those of nondiapausing larvae. From these observations it is reasonable to assume that the gonial cells in the diapausing ovary are physiologically different from those of the nondiapausing ovary. Fecundity in insects is dependent upon the differentiation of oocytes from oogonia and involves also the proper function of the nurse cells. Prior to yolk deposition the nurse cells undergo polyploidy. It has been shown that at certain times during egg maturation these nurse cells are highly sensitive to radiation, whereas at other times they are highly resistant (LaChance et al. 1967). Since the diapausing ovary of L. pomonella is in an undifferentiated state at the time of irradiation one would
expect the damaging effect of irradiation to be less. On the other hand the ovaries of a nondiapausing *L. pomonella* are mitotically active and irradiation in this stage would cause severe damage resulting in reduced fecundity. This probably accounts for the damage differential observed between the nondiapause and diapause females.

Although the percentage of sterility increased with increase in radiation dose in both the diapause and the nondiapause groups there was no significant difference between the diapause and nondiapause groups. This implies that as far as the induction of dominant lethals are concerned diapause does not offer any protection from radiation injury. von Borstel (1963) has observed that irradiation of insects after treatment with radioprotective agents would minimize somatic damage but genetic damage is not changed. My results show that although diapause offers protection as far as fecundity is concerned the induction of dominant lethal mutations seems unaffected.

One very interesting observation that has emerged from this study is the stimulating effect of gamma radiation on diapause breaking mechanisms. The reduction in pupation time of larvae irradiated with 2.5 and 5 k rads is significant.
The data indicates that larvae irradiated with low doses break diapause sooner than the control and sooner than those larvae irradiated with 10 k rads. Diapause has long been recognized as a response to a photoperiodic stimulus, both in its induction and in its termination (Lees 1956). Later investigators examined the action spectrum of diapause to determine if the diapause response was restricted to particular wavelengths of light and to determine if this light acted on a particular organ in the insect (Williams and Adkisson 1964, Williams et al. 1965). They found that the photoreceptor in the oak silkworm *Antheraea pernyi* was located in the brain of the insect and also that it reacted only to light in the blue end of the spectrum. Other workers (Norris et al. 1969) examined this same problem and found that *A. pernyi* responded by breaking diapause to wavelengths between 400 and 500 nm. These same workers also found that *L. pomonella* responded to wavelengths between 430 and 470 nm. They found that *L. pomonella* contains a pigment which absorbs light in the 400 to 500 nm region. Is it possible that this photoreceptor pigment could have been activated by the energy deposited by gamma rays during the lower dose of irradiation? Stimulating effects of radiation on insects have been observed before.
Tahmisian (1949) found that irradiation of the diapausing eggs of Melanoplus differentialis increased the oxygen consumption of the eggs and hence increased the metabolic rate. Melville (1958) found an increase in egg production in the flour mite Tyroglyphus farinae. A similar effect was found in the mosquito Anopheles pharoensis which laid more eggs than the control when irradiated with low doses (Abdel-Malek et al. 1966). The female flour beetle Tribolium castaneum has a higher fecundity when mated to an irradiated male than by mating to a nonirradiated male (K. K. Nair personal communication). This higher fecundity is thought to be due to an increase in the frequency or duration of mating as was found by Baldwin and Shaver (1963) who showed that the male Rhodnius prolixus increased its coitus time after receiving a low dose of X-rays. In all of the reported cases except for that reported by Melville (1958), the stimulation occurred at a relatively low dose and was reversed by higher doses. In my study the stimulation occurs only after irradiation with 2.5 and 5 k rads whereas a dose of 10 k rads increases the T_{50} pupation to the control level for the diapause male and to about one half of the control for the diapause female. It would appear that the stimulation found at the lower doses is
counteracted here by the inhibition of development caused by irradiation with 10 k rads. It is difficult to ascertain from the present study whether the stimulating effect was due to radiation effects on the photoreceptor or on the neuroendocrine system or both. Further studies are required to resolve this problem.

The effects of varying the dose rate was studied in order to determine whether a reduction in the somatic damage could be gained while maintaining an adequate induction of dominant lethal mutations. The dose rate was varied over four dose rates varying from 233 rads/min to 1,375 rads/min. Two doses were used in this study; 2.5 and 10 k rads. There was no significant difference found for any of the dose rates using the four parameters used in the study viz; pupal mortality, adult emergence, mean egg lay and percent sterility. The highest dose rate used in this study was approximately six times higher than the lowest dose rate and no differences in sensitivity were found. Proverbs and Newton (1962b) found a higher adult emergence and a lower induced sterility in *L. pomonella* irradiated with a similar dose level and with a dose rate approximately one twenty fourth as high as the highest dose rate used in my study. Nair and Subramanyam
(1963) irradiated newly emerged *T. castaneum* with a number of different dose rates. They found that as the dose rate increased, the fecundity and the fertility of the insect was reduced. These researchers were able to use dose rates as low as 126 rads/hour to achieve their effect and the range between their highest and lowest dose was approximately a 1,000 fold increase. Other workers have examined the dose rate effect using the mutation rate in the spermatogonia of mice as a parameter (Russel *et al.* 1958). They required a 10,000 fold difference between the highest and the lowest dose rates in order to find an observable difference in radiation sensitivity. One might expect that an increase in dose rate would give a change in the radiation sensitivity of *L. pomonella*. However, the range of doses possible using the Gammacell 200 did not show any significant effect.
CONCLUSION

Diapause does not offer any protective effect against radiation injury in *L. pomonella* larvae. There is, however, a delay in the expression of this injury and a protective effect on the reproductive systems of the survivors is evident, and is displayed in the higher fecundity of the diapausing *L. pomonella*. Female *L. pomonella* are less susceptible to radiation injury than males, this may be due to the difference in body size, the females being larger. The induction of dominant lethal mutations in *L. pomonella* using gamma radiation is independent of the sex, with equal sterility induced in each group. Radiation stimulates the development of diapausing *L. pomonella* at low doses.

*L. pomonella* is a very serious pest to orchard areas of the world, this work is a contribution to the research being done on the radiation sterilization of this insect (Proverbs and Newton 1962a, b, and c, Proverbs et al. 1966, 1967, 1969, Hathaway 1966). If it had been found that after irradiation the adult emergence of diapausing *L. pomonella* was higher than that of the nondiapausing group this might have been a practical method of sterilizing the male. The major problem in sterilizing insects during development is...
their higher susceptibility in the earlier stages. If the high mortality found from irradiation in the larval stage could be overcome by using radioprotective agents this could be worked into a practical pest control program. The examination of the effects of different dose rates on *L. pomonella* was another effort to reduce the somatic damage while still maintaining an adequate level of sterility. The effect of different dose rates on radiation sensitivity of *L. pomonella* would be of value to the sterile male control program since there may be an optimum dose rate at which genetic damage is maximized and somatic damage is minimized. If this dose rate exists it occurs at dose rates lower than those available in the Gammasell 200 used in this study.

Several interesting and important problems arose out of this study which warrant further research. The stimulation of diapause development by radiation should be further investigated to determine if the radiation is acting on the photoreceptor or if the stimulation is general and simply overrides this system.

The effect of diapause on radiation sensitivity should also be studied further. It would be of interest to maintain irradiated diapausing insects in diapause for different periods
of time to determine if some long term mechanism repairs radiation damage.
LITERATURE CITED


APPENDIX 1

The chemical reactions of radiation on the ferrous ammonium sulphate dosimeter.

(1) \[ H_2O \rightarrow H^+ + OH^- \]

(2) \[ 2H_2O \rightarrow H_2 + H_2O_2 \]

(3) \[ H + O_2 \rightarrow HO^- \]

(4) \[ H^+ + H^+ \rightarrow H_2 \]

(5) \[ H_2^+ + Fe^{2+} \rightarrow Fe^{3+} + OH^- \]

(6) \[ OH^- + Fe^{2+} \rightarrow Fe^{3+} + OH^- \]

(7) \[ HO^- + Fe^{2+} \rightarrow Fe^{3+} + HO_2^- \]

(8) \[ HO_2^- + H \rightarrow H_2O_2 \]

(9) \[ H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH^- \]

(10) \[ H^+ + H^- \rightarrow H_2 \]

(Battaerd and Tregear 1966)
APPENDIX 2

Formula for computing the dose for gamma irradiation of the ferrous ammonium sulphate dosimeter.

\[ \text{Dose (rads)} = \frac{N[A(OD) + 100]}{(\Delta \epsilon) \times 10^3 G(\text{Fe}^{3+}) \, \text{fpl}} \]

where \( N = 6.02 \times 10^{23} \) molecules/mole

\( \Delta (OD) = \) Optical density difference

\( \Delta \epsilon = \) Extinction coefficient difference

between \( \text{Fe}^{2+} \) and \( \text{Fe}^{3+} \) @ 304 nm

@ 25°C = 2.197 M\(^{-1}\) cm\(^{-1}\)

\( G = 15.6 \) ions/100 eV

\( f = 6.24 \times 10^{13} \) eV/rad

\( p = \) density of irradiated solution =

1.024 for 0.8N H\(_2\)SO\(_4\)

\( l = \) optical path length = 1 cm

which for use in this study can be expressed as:

\[ D = \frac{0.944 \times 10^9 \Delta (OD)}{(2.197) (15.6)} \]

(Attix and Roesch 1966)
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