

GAMMA RAY INDUCTION OF TRANSLOCATIONS IN THE CORN EARWORM, *HELICOVERPA ARMIGERA* (HUBNER), (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Male pupae (pharate adults) of the corn earworm, *Helicoverpa armigera* (Hubner), were subjected to 100 and 150 Grey (Gy) of gamma irradiation. Karyological analyses of the normal homogametic male prepupae revealed diploid genomic complement $2n = 62$ (30 II + ZZ), small homomorphic chromosomes with mean relative length ranging from 0.022 to 0.044 and regular first meiotic behavior. The same were exhibited by the first generation progenies of the crosses between normal males and females. On the other hand, the progenies of matings involving normal females and 100 Gy or 150 Gy irradiated males possessed spermatocytes with heterozygous reciprocal translocations. These translocations appeared as chains and rings at diakinesis. At 100 Gy gamma radiation, the 900 spermatocytes of 30 first generation *H. armigera* exhibited 71.30% chain, 9.8% ring, 4.9% chain and ring and 14% normal; at 150 Gy gamma radiation, the spermatocytes showed 84.87% chain, 2.71% ring, 12.42% chain and ring and 1.67% normal. Thus, the inheritance of induced partial male sterility was inferred, translocations being markers of gametic infertility.

Key Words: *Helicoverpa armigera*, irradiation, inherited sterility, sterile insect technique, chromosomal aberrations, nuclear technique.

INTRODUCTION

The corn earworm, *Helicoverpa armigera* (Hubner), is one of the most destructive lepidopterous insect pests of agriculture. It is a polyphagous species which feeds and breeds on a wide variety of host plants. Deang (1971) cited 84 host plants among which, corn is most preferred.

To regulate populations of *H. armigera*, several control measures have been adopted and these include the use of insecticides, biological control or release of parasites, predators and pathogens, cultural control, and varietal resistance or use of resistant host plants.

Another alternative control strategy applicable to *H. armigera* is genetic control, a target-specific and non-polluting approach to insect population regulation. It requires mass rearing of insect species, sterilization of male insects by means of either radiation or chemicals and release of sterilized males to mate with wild females. Ultimately, the technique leads to reduction in fertility of the insect population.

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In this study, 100 Gy and 150 Gy of gamma radiation were utilized as partial sterilants to *H. armigera* males. The genetic effects of irradiations on spermatocytes were determined. Reciprocal heterozygote translocations which are markers of gonadal sterility were noted in irradiated spermatocytes. The study also determined the heritability of the induced partial male sterility.

Mass rearing and genetic crosses of normal and irradiated insects were done at the Toxicology Laboratory of the Department of Entomology, College of Agriculture. The cytogenetic analyses were conducted at the Genetics and Molecular Biology Laboratory, Institute of Biological Sciences, College of Arts and Sciences, UP Los Baños.

MATERIALS AND METHODS

Experiments were performed with the stock of wild-type moths held for mass rearing under laboratory conditions since August 1995. Cultures were reared in plastic cups containing meridic diet (Ceballo and Rejesus, 1983) at 27-29 °C and 60-70% r.h. Only mature pupae (pharate adults) were selected for irradiation. The insects were sexed in the pupal stage.

Irradiation of *H. armigera* males

Irradiation of male pupae was performed in the Philippine Nuclear Research Institute using a Gamma 220 Irradiator as ^{60}Co source. The doses of 100 and 150 Gy were delivered at a dose rate of about 4 Gy/min. For treatment, the insects were placed in glass tubes.

Genetic crosses of *H. armigera*

Two separate matings were made for *H. armigera*. One mating involved the normal adult females with normal males and the other mating was between the normal adult females with males irradiated with either 100 Gy or 150 Gy. Adults were mated inside ice cream pint containers with onion skin paper fitted inside and a small screw cap taped to the bottom center of the container. A cotton ball soaked in honey solution was placed on the cap for adult food. The emerging irradiated males were mated with untreated females at ratio of 3:3. The container was then covered with fine mesh where oviposition took place. Several mating cups were prepared and covered with wet cloth to increase humidity.

After two days, eggs were collected in bottles. Upon hatching, larvae were transferred to 50 ml plastic sauce cups with soybean-corn diet developed by Ceballo and Morallo-Rejesus (1983). Each larva was allowed to develop until the 6th instar. Larvae in the prepupal stage (insects had bored inside the diet) were collected for cytogenetic investigation.

Karyological Investigation on *H. armigera*

Fixation of testes of *H. armigera*. The prepupa was incised dorsally to extract the testes which appeared as yellow, elongated oval masses. The testes were placed in glass vials containing freshly prepared Carnoy's fluid (3 parts 95% ethanol: 1 part glacial acetic acid) for 24 hours. Then, they were transferred to vials containing 75% ethanol for storage. Vials were labelled as N (Normal) and IR (Irradiated).

Slide (Squash) preparation and staining. Each of the fixed testis was placed on a slide with a drop of lacto-aceto-orcein stain. Maceration was done using a bent needle. After two minutes, a cover slip was added and the preparation examined under a compound microscope. The cells were destained with 45% acetic acid placed on the sides of the cover slip. A small strip of filter paper was used to draw excess stain from the slide. Then, the preparation was passed over an alcohol flame 3 to 4 times to clear the cytoplasm and enhance stainability of chromosomes. Overheating of the specimen was prevented. A firm pressure was applied using a blunt pencil eraser to flatten the cells. The edges of the cover slip were sealed with paraffin mixed with Canada balsam. The slides were properly labelled and prepared for detailed viewing.

Microscopic Observations of Testicular Cells of *H. armigera*

Different stages of Meiosis I were observed from the testicular spermatocytes of the male prepupal progenies of both normal parents and of normal female and gamma-irradiated male *H. armigera* under oil immersion objective.

At least 30 cells for each stage of Meiosis I were observed and analyzed. Both normal and abnormal chromosome configurations were noted and their relative frequencies determined. Photomicrographs of the different meiotic stages were taken. Well-spread diakinetik chromosomes were observed and their numbers counted. Chromosomal lengths of diplotene-diakinetik chromosome pairs were measured in the photomicrographs using a metric ruler. Relative mean lengths of chromosomes at diakinesis were computed using the formula:

$$\text{Relative Length} = \frac{\text{Length of one chromosome}}{\text{Total length of all the chromosomes}}$$

The karyograms of the diakinetik chromosome pairs or bivalents of normal *H. armigera* and interpretative drawings of chromosome configurations at diakinesis of gamma-irradiated *H. armigera* were prepared. From 900 spermatocytes at diakinesis, occurrences of translocations in the form of chains and rings were noted.

RESULTS AND DISCUSSION

The prepupal stage of male *H. armigera* was ideal for karyological study. Lacto-aceto-orcein squash preparations of their testes showed active spermatogenesis particularly spermatocytes exhibiting the complete meiotic stages.

All stages of the reductional division phase, namely, Prophase I, Metaphase I, Anaphase I and Telophase I were observed in the spermatocytes of both normal and gamma-irradiated *H. armigera*.

Normal Male *H. armigera*. The normal parental male *H. armigera* and its first generation (F_1) male progeny exhibited regular cellular and chromosomal behavior.

Early Prophase I. The spherical spermatocytes measured 1.5 to 2.25 μ in diameter and they were observed to aggregate. The nucleus contained chromosomes which appeared as a network of darkly stained thin threads (Fig. 1a-c).

Diakinesis (Figs. 1c and 2). The chromosomes at diakinesis assumed maximal

condensation, being darkly stained and very distinct. The chromosome number was determined to be $n = 31$. All the chromosomes were bivalents, homomorphic or exhibiting almost similar shapes. With such homomorphic and symmetrical karyotype the sex-determining mechanism of male *H. armigera* was described to be homogametic.

The diploid genomic complement of male *H. armigera* was $2n = 62$ and its karyotype formula was $30\text{IIA} + \text{ZZ}$. In 1930, Beleajeff reported that a haploid number of 31 chromosomes is indeed the predominating number in several families of Lepidoptera and even hypothesized that this may be the ancestral number (Robinson, 1971).

The bivalent chromosomes were small, almost of similar sizes. The mean relative length ranged from 0.022 to 0.041 (Fig. 2)

No distinct centromere was observed in any one of the chromosomes. Early reports indicated that *H. armigera* like other lepidopterans possessed holocentric chromosomes or the centromeres were diffused (Baver, 1967; Soumalainen, 1969; Bigger, 1975).

Metaphase I (Fig. 1d). Bivalents aligned themselves parallel with each other at the equatorial region of the cell.

Anaphase I (Fig. 1e). Univalent chromosomes segregated towards opposite poles.

Telophase I (Fig. 1f). Chromosomes regrouped and coiled structures relaxed within the two nuclei of spermatocytes.

Irradiated Male *H. armigera*. The 100 and 150 Gy doses of gamma radiation induced partial heritable sterility in *H. armigera* males. These doses preserved the normal mating ability, behavior and competitiveness of male *H. armigera* to further produce subsequent generations in significantly reduced number (Ocampo *et al.*, 1996).

Gamma radiation specifically affected the chromosomes of the spermatocytes during the substages of Prophase I, the reduction division events of the meiotic cycle. These events were very active during spermatogenesis in the male prepupae of *H. armigera*. These genetic effects were observed at Diakinesis as chromosome associations in the form of chains and rings (Fig. 3).

Chromosome breaks were easily induced by radiation in *H. armigera* because of its holokinetic chromosomes. With this nature of chromosomes, the breaks will likely possess at least one centromere and can function independently. As these fragments reunite, they produce reciprocal translocations when exchange of segments occur between nonhomologues. The heterozygous translocations exhibit unique pairing configurations as manifested by chain or ring at Diakinesis. Chiasmata formation of the chromosome arms forms a ring and absence on one arm would form a chain. These chromosome translocations are the genetic markers for induced sterility in *H. armigera*.

From *H. armigera* males irradiated with 100 Gy, the 900 spermatocytes of the first generation progeny produced 71.3 % chain, 9.8 % ring and 4.9 % chain and ring. Only 14 % of the spermatocytes remained normal. On the other hand, spermatocytes of progenies of those irradiated with 150 Gy produced 83.2 % chain, 2.7 % ring and 12.4 % chain and ring. Only 1.7% of the spermatocytes remained normal or showing 31 bivalent chromosomes (Table 1). Thus, higher dose of gamma radiation resulted to higher occurrences of translocations.

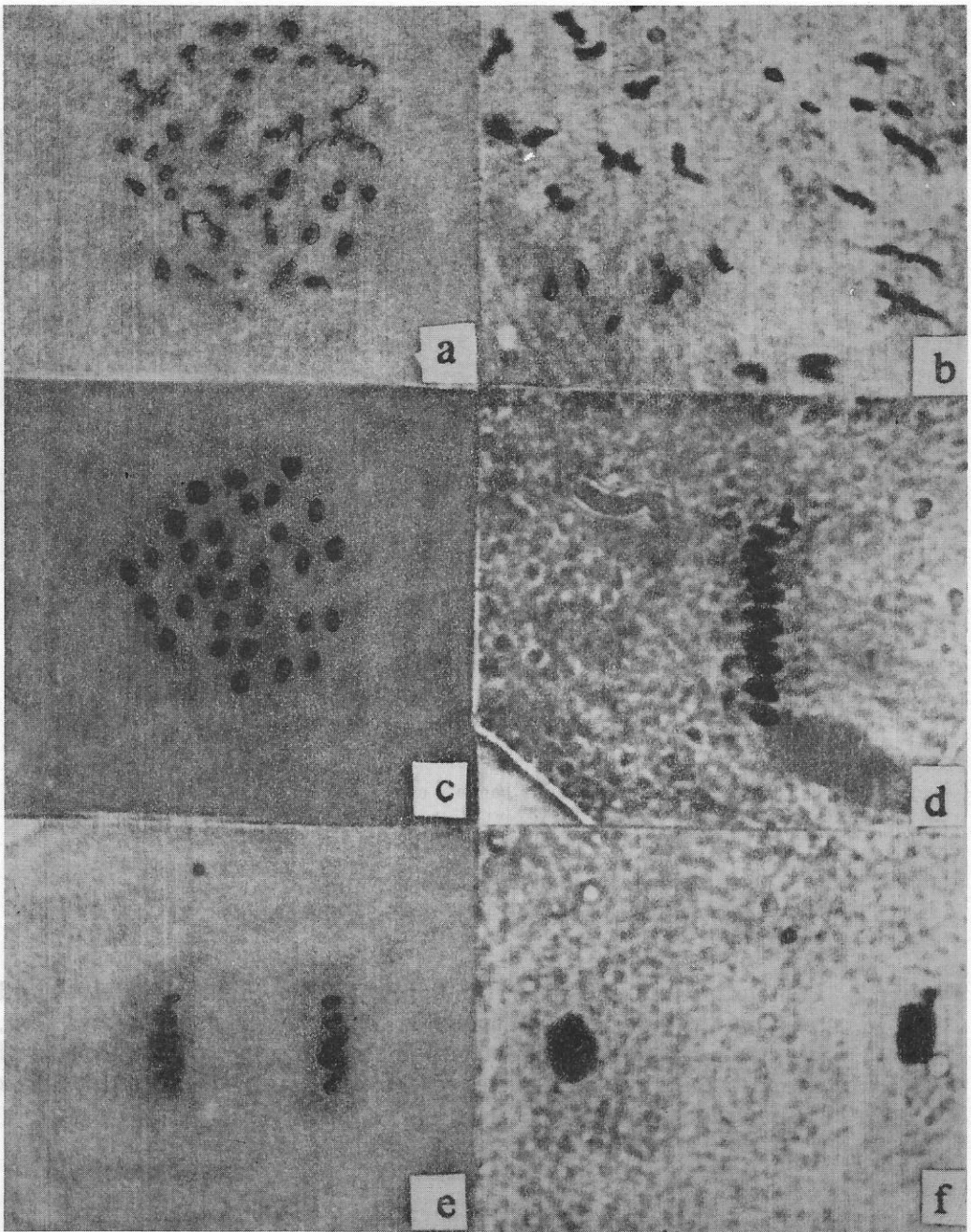


Figure 1. Photomicrographs showing stages of Meiosis I of the corn earworm, *Helicoverpa armigera* (Hubner).

- | | |
|---------------|----------------|
| a. Leptonema | d. Metaphase I |
| b. Zygonema | e. Anaphase I |
| c. Diakinesis | f. Telophase I |

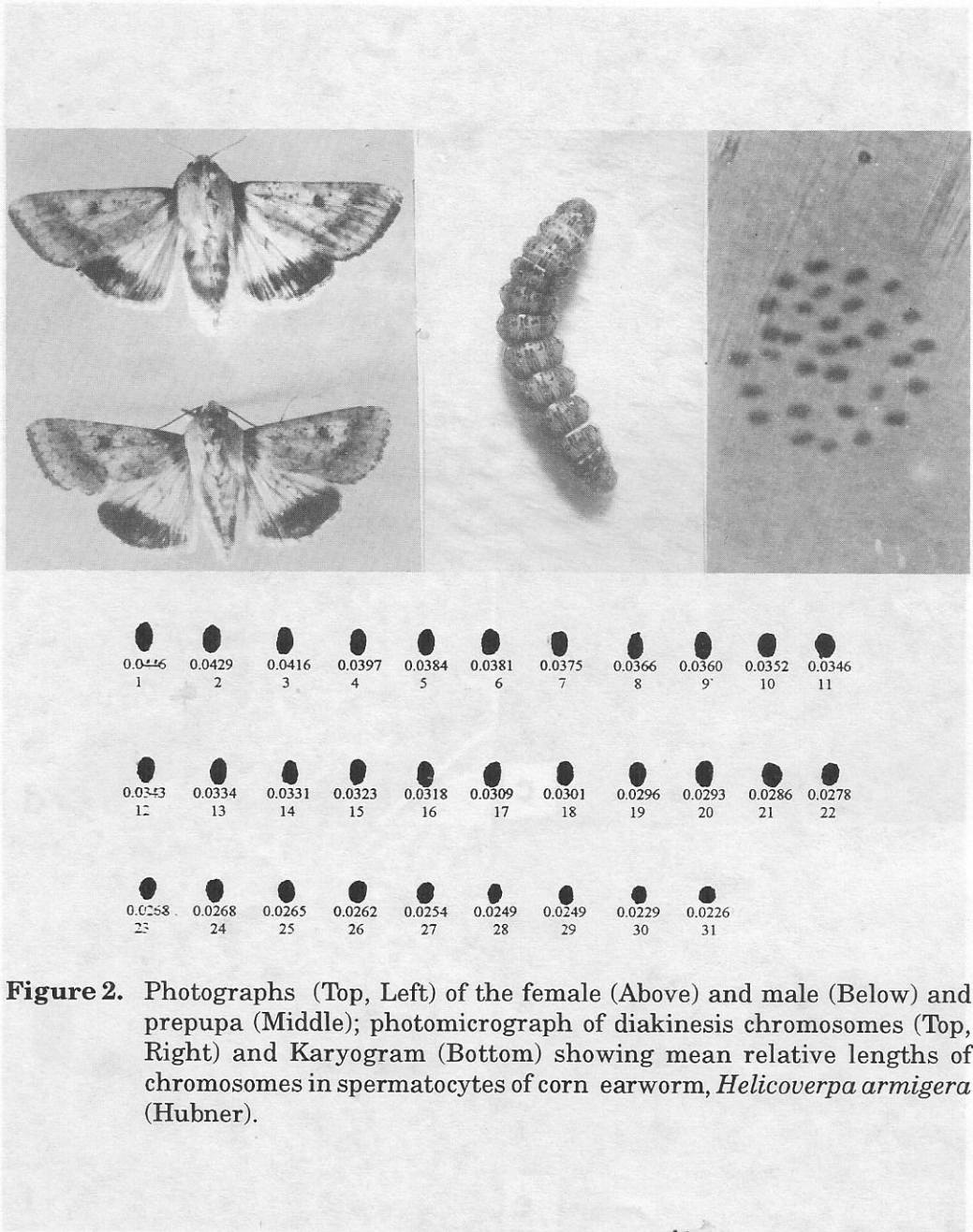


Figure 2. Photographs (Top, Left) of the female (Above) and male (Below) and prepupa (Middle); photomicrograph of diakinesis chromosomes (Top, Right) and Karyogram (Bottom) showing mean relative lengths of chromosomes in spermatocytes of corn earworm, *Helicoverpa armigera* (Hubner).

Figure 1. Photomicrographs showing stages of Meiosis I of the corn earworm, *Helicoverpa armigera* (Hubner).
 a. Leptonema
 b. Metaphase I
 c. Diakinesis
 d. Anaphase I
 e. Telophase I

Table I. Frequencies and proportions of the normal and abnormal (with translocations) spermatocytes of the first generation progenies from the crosses between the normal females and males of the corn earworm, *Helicoverpa armigera* (Hubner) irradiated with 100 and 150 Gy (gamma radiation).

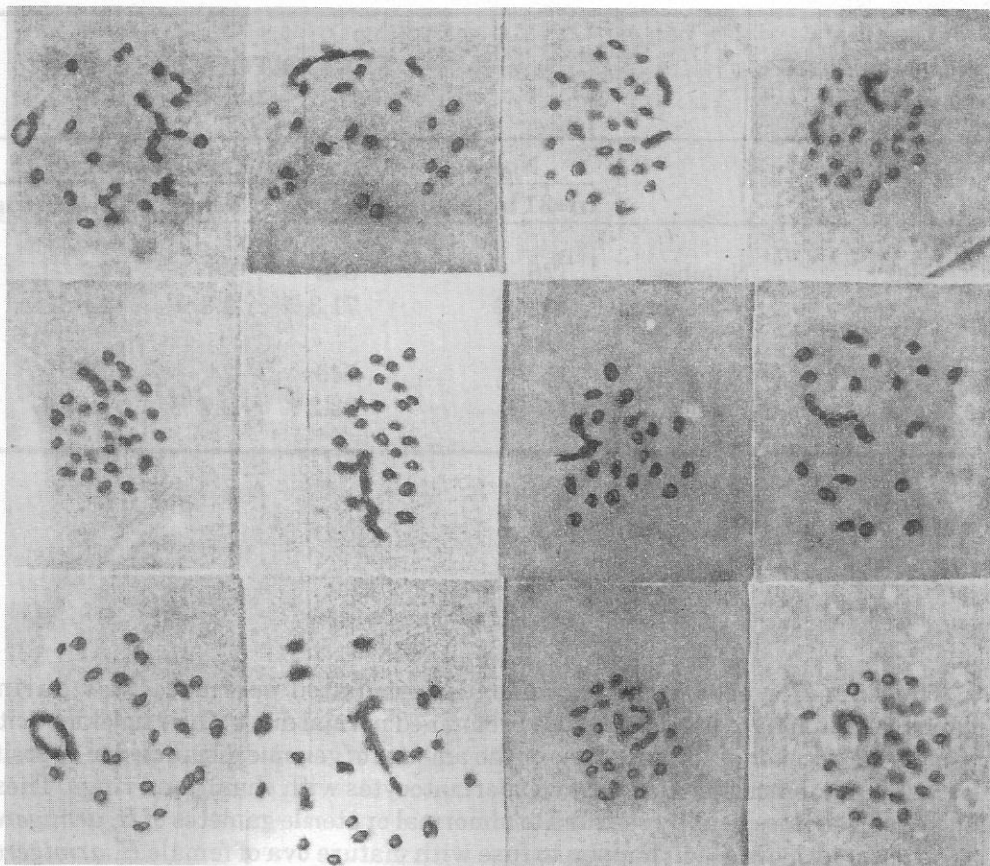


Figure 3. Photomicrographs and camera lucida drawings showing spermatocytes of the male prepupae of the corn earworm, *Helicoverpa armigera* (Hubner), irradiated with 100 Gy and 150 Gy containing translocation chains and rings.

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This paper is part of the results of the project entitled 'Evaluation of Population Suppression by Irradiated *Helicoverpa armigera* and its progeny' of which Dr. Virginia R. Ocampo is the Principal Scientific Investigator. This project is under the FAO/IAEA Coordinated Research Program entitled 'Evaluation of Population Suppression by Irradiated Lepidoptera and their Progeny'. The authors are grateful to the staff of the Philippine Nuclear Research Institute for the use of their ⁶⁰Co facility and to the International Atomic Energy Agency (IAEA) for the financial support to the project.

Table 1. Frequencies and proportions of the normal and abnormal (with translocations) spermatocytes of the first generation progenies from the crosses between the normal females and males of the corn earworm, *Helicoverpa armigera* (Hubner) irradiated with 100 and 150 Grey (Gy) gamma radiation.

Gamma radiation doses (Gy)		SPERMATOCYTES (900)			
		Normal (n=31 bivalents)	With Translocations		
			Chain	Ring	Chain+Ring
100	Number	126	642	88	44
	Percentage	14.0 %	71.3 %	9.8 %	4.9 %
150	Number	15	749	24	112
	Percentage	1.7 %	83.2 %	2.7 %	12.4 %

The behavior of the chromosomes in irradiated spermatocytes during Metaphase I, Anaphase I and Telophase I remained normal due to their holokineticity. However, the number of chromosomes or the amount of genome disjuncted to opposite poles were unbalanced particularly in spermatocytes with chains and rings. These unbalanced genomes usually resulted to abnormal or sterile gametes of *H. armigera*. If these aborted sperms will happen to fuse with mature ova of female *H. armigera*, no zygote will be formed. Thus, genetic control of *H. armigera* could be assured.

Results indicate that the first generation progeny obtained the chromosomal aberrations from their partially sterile male parents exposed to 100 and 150 Gy gamma radiation and thus, there is inheritance of induced partial sterility in *H. armigera*.

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LITERATURE CITED

- BAUER, H. 1967. Die Kinetische Organisation der Lepidopteren-chromosomen. *Chromosoma* 22: 102-125.
- BIGGER, T. 1975. Karyotypes of some Lepidoptera chromosomes and changes in their holokinetic organization as revealed by new cytological techniques. *Cytologia* 40: 713-726.
- CEBALLO, F. and B. MORALLO-REJESUS, 1983. Tryptophan and lysine supplemented diet for corn borer (*Ostrinia furnacalis* Guenee). *Philipp. Ent.* 6: 531-538.
- DEANG, R.T. 1971. Life History and Morphology of the *Helicoverpa armigera* Reared on Synthetic Diet and Topical Toxicity of Five Organic Insecticides to the Insects M.S. Thesis. UPLB, College, Laguna. 93 p.
- KNIPLING, E.F. 1970. Suppression of pest lepidoptera by releasing g partially sterile males: a theoretical appraisal. *BioScience* 20:465-470.
- NORTH, D.T. 1975. Inherited sterility in Lepidoptera. *Ann. Rev. Ent.* 20:169-182.
- OCAMPO, V.R., J.B. DE LEON and M. TABUR. 1996. Mass rearing and effects of substerilizing doses of radiation on the corn earworm, *Helicoverpa armigera* (Hubner) and its progeny. In: Working Material of the Second Research Co-ordination Meeting Within the FAO/IAEA Co-ordinated Research Programme. Vienna, Austria. Sept, 2-6. 57 p.
- ROBINSON, R. 1971. *Lepidopteran Genetics*. Oxford Pergamon Press. Ltd. 557-598.
- SOUMALAINEN, E. 1969. Chromosome evolution in the Lepidoptera. *Chromosomes Today* 2: 132-138.