UNIVERSITY OF CAPE COAST

THE DEVELOPMENT OF RADIATION-INDUCED INHERITED STERILITY FOR THE MANAGEMENT OF LEPIDOPTEROUS MAIZE STEM BORERS: *ELDANA SACCHARINA* WALKER (PYRALIDAE) AND *SESAMIA CALAMISTIS* HAMPSON (NOCTUIDAE)

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BY

CHARLES EMMANUEL ANNOH

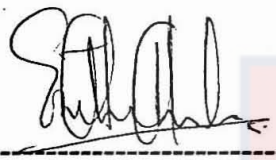
THESIS SUBMITTED TO THE DEPARTMENT OF ZOOLOGY OF THE FACULTY OF SCIENCE, UNIVERSITY OF CAPE COAST IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE IN ZOOLOGY.

MARCH 2003

DECLARATION

CANDIDATE'S DECLARATION:

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this University or elsewhere.



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SUPERVISORS' DECLARATION:

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

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PROF. KOBINA YANKSON
(SUPERVISOR)

DEDICATION

This thesis is dedicated to my dear up and coming sons and daughters,

Emmanuel Jnr.

Emma

Pearl and

Ebenezer

to aspire to higher heights in their academic pursuits and all endeavours.

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ACKNOWLEDGEMENTS

I wish to express my sincere thanks to the Principal Supervisor of this project, Dr. (Mrs.) M. A. Botchey of Zoology Department, University of Cape Coast, for her encouragement, relevant suggestions, corrections and guidance offered me throughout the period of research. Similar gratitude also goes to Prof. Kobina Yankson, 2nd Supervisor and presently Pro-Vice Chancellor of University of Cape Coast, for taking time off his tight schedule to offer suggestions and corrections in the review of the manuscript.

I am very grateful to Prof. Seth. K. Danso, Director of Ecological Laboratory (ECOLAB) of University of Ghana, through whose efforts some financial assistance was granted to start off and complete the research work. Similar gratitude is extended to the Director of Biotechnology and Nuclear Agriculture Research Institute (BNARI), Prof. George Klu for acquiring some assistance through the National Agriculture Research Project (NARP), for the use of vehicle and other logistics in support of the project.

My sincere appreciation also goes to Dr. (Mrs.) D. A. Adabie-Gomez, Head of Department of Animal Science (DAS) of BNARI for her pieces of advice, suggestions, corrections and comments on some aspects of the manuscript. The entire technical staff of DAS/BNARI, Messrs C. A. Asante, Albert Nkumsah, Fredrick Abroquah, Reinfred Aballey, Godfried Damnyang and E.A.Ewusie also deserve my sincere thanks for their invaluable technical services offered me, both on the field and in the laboratory.

I also thank the staff of Radiation Technology Centre (RTC) of Ghana Atomic Energy Commission (GAEC), Messrs, Emi Reynolds, Isaac Ennison, Marxwell Ofori Appiah, J. E.F. K. Ansah, S.N.Y. Annang and Arthur for their technical assistance in the radiation processes.

For typing the manuscripts, I thank Miss Sadia Moro of BNARI and Mr. E. K. Nyarko of Ghana Science Association (GSA) Secretariat. My sincere gratitude also goes to Mr. Samuel Timpo of BNARI for his assistance in statistical analysis and the photographers, Messrs Emmanuel Gbafa and Elvis Appiah of Crops Science, University of Ghana. I also thank Mr S.B. Dodou of Geography Department, University of Ghana for the drawings.

My appreciation also goes to Drs. B. A. Mensah and P. K. Kwapong all of Zoology Department, U.C.C. for their pieces of advice and encouragement. The technical staff of Zoology Department, U.C.C. with special mention of Messrs C. T. Agble and M. Eghan, deserve my thanks for their technical assistance. I sincerely thank the University of Cape Coast for offering me the opportunity to conduct the research for an award of a Doctorate degree.

My warmest appreciation goes to my God-given, suitable help-mate, Grace for her constant prayers and encouragement. Above all, my greatest indebtedness goes to the Almighty God for His supreme direction, protection and guidance throughout the period of the research and bringing it to a successful end.

ABSTRACT

Radio-sterilization study was conducted on the biology of two lepidopterous maize stem borers, *Eldana saccharina* Walker and *Sesamia calamistis* Hampson to induce inherited sterility for insect pest management programme in Ghana. Bioecology of the two borer species was also studied for a 3-year period, 1997-1999 at Medie, a predominantly maize growing community in the Ga District of Greater Accra Region, to determine the population dynamics and climatic factors influencing the population of the borer species. It was observed that *Sesamia* species usually attacked the young maize crop, with peak infestations occurring about 6-8 weeks after emergence of the crop. *Eldana* species preferred mature maize with peak infestations around 10-12 weeks after emergence of the maize crop. Larval numbers of *E. saccharina* showed inverse relations with rainfall, r= -0.5899; p= 0.043. Infestation levels of larvae of both species were relatively higher during the minor rainy season than the major season. Larvae and pupae of *E. saccharina* that developed separately on natural and artificial diets did not show significant difference in most of their biological parameters. In both borer species, pupal weights of natural dieters were slightly heavier than those of artificial dieters. Exposure of young pupae (less than 6 days old) of the two borer species to increasing doses of ionizing radiation, 80-180 Gy, resulted in high percentage of deformity and unemerged adults. Mature pupae of 6-8 days old were less susceptible to increased doses and exhibited fewer body deformities and unemerged adults. The mating capability of adults emerged from irradiated mature pupae of *E. saccharina* was not adversely affected. In the parent generation (P), fecundity and fertility decreased with increased doses of radiation for

crosses involving irradiated males and normal females as well as irradiated females and normal males. While the treatment in the former crosses resulted in partial fertility (40% at 180 Gy), the latter produced almost sterile individuals (96%, with doses above 100 Gy). In the F_1 generation, both fecundity and fertility were inversely related to increased doses of radiation. Crosses involving F_1 males showed lower fertility than their corresponding F_1 females. Fertility was reduced to 10% with radiation dose of 100 Gy in the former, whilst similar reduction in F_1 females required about 150 Gy or more. The overall sex ratio of emerged adults in F_1 and F_2 generations shifted in favour of males. Thirty pairs of chromosomes were observed in normal meoitic gametes (metaphase I) of *E. saccharina*. Chromosomal aberrations resulting from induced radiations were found in the form of fragments, rings and chains. In experimental field cages, moths treated with radiation dose of 150 Gy, resulted in decrease in fecundity and fertility of F_1 generation when the ratios of sterile to fertile moths was increased. Fecundity was reduced by 47% with ratio of 1 sterile to 1 fertile and 79% with mating ratio of 5 sterile to 1 fertile, as compared with the control of 1 fertile to 1 fertile. Since significant reduction in fecundity and fertility was observed with radiation doses between 120-180 Gy, it is suggested that this range could be used to induce inherited sterility in *E. saccharina*. The substantial reduction of population of the borer species due to induced sterility in the progeny implies that inherited sterility could be used as a control strategy to suppress the stem borer populations in insect pest management programme in Ghana.

CHAPTER ONE

GENERAL INTRODUCTION

LEPIDOPTEROUS MAIZE PEST AND SEARCH FOR SUSTAINABLE CONTROL MEASURES.

1.1 The stem borer – a threat to high yield in maize production.

Maize is one of the most important food crops cultivated throughout all the ecological zones in Ghana. It is grown from the coastal belt across the forest belt, forest –savannah transition, Guinea savannah to the Sudan savannah at the north –eastern corner of Ghana (NARP, 1993). It is reported in Ghana that current average yields of maize are in the order of 1.5 t/ha (PPMED, 1999). However, damage due to insect pest infestation has largely contributed to pre-and post-harvest losses, resulting in high demands of the commodity by consumers throughout the year.

Lepidopterous stem borers belonging to the families, Pyralidae and Noctuidae are the most important field pests of maize in Africa, including Ghana (Harris,1962; Atkinson, 1980, Van Rensburg *et al.*, 1988). The stem borers, *Eldana saccharina* Walker (Pyralidae), *Sesamia calamists* Hampson (Noctuidae), *Sesamia nonagrioides botanephega* Tam and Bowden (Noctuidae) and *Busseola fusca* Fuller (Noctuidae) cause substantial damage at practically all stages of the maize crop.

In Ghana, the most notorious field pest of maize are *E. saccharina* and *S. calamists* (Gounou *et. al.* 1994). Significant losses due to both pests have been reported from the forest zones (Bowden, 1976; Girling, 1980) and losses due to *E. saccharina* mainly from the coastal savannah (Endrody-Younga, 1968, Girling, 1980). Yield losses caused by stem borers have been estimated to range between 10% to 100% (Usua, 1968; Sampson and Kumar, 1985; Ampofo, 1986; Bosque-Perez and Mareck 1991). In Ghana Leyenaar and Hunter (1977) reported about 64% reduction in maize kernel size as a result of stem borer infestation. The borers are noted as a major pest problem to maize grown in the second cropping season (minor rains) and may result in devastation of entire field (Maafo, 1975). In some areas, farmers refuse to cultivate second season maize because of the problem of high infestation of stem borers.

1.2 Conventional control strategies

1.2.1 Chemical control

The control of maize stem borers is conducted almost entirely through the use of synthetic insecticides. Such control method is usually costly, non-selective and only effective at the target sites for a short period of time. In most cases the insecticides are ineffective because the borer larvae living inside the host plants are not reached by the chemicals. The cryptic feeding behaviour of stem borers makes insecticidal control difficult to accomplish (Overholt, 1998).

Chemical control of stem borers in cereals is unusually laborious because of the necessity for precise placement of the chemicals on the plant, except when systemic insecticides are applied (Minja, 1990). Duerden (1953) reported that to control maize stem borers, it was desirable to

deposit the insecticide inside the funnel shape of the developing leaves of the plant, because most larvae move through this region before entering the stem. Once the larva bores into the stem it is protected from the non-systemic insecticides. This means that the insecticides must be applied before downward migration of larvae. The period between crop-emergence and this migration is short and timing is critical (Minja, 1990).

Sithole (1990) observed that even application of carbofuran at planting time is not always effective against the borers. Mathez (1972) tried a number of insecticides including DDT, Carbaryl and BHC and found that Carbaryl was relatively effective. However, this could only be recommended if the potential yield reached about 2700 kg/ha of maize, combined with good husbandry, improved maize variety and fertilizer. All these are extra cost to the subsistence farmer and they cannot afford regular spraying. The frequent application of the chemicals has also resulted in relatively high levels of insecticide resistance developed by the stem borers and caused a great concern of pesticide pollution of the environment.

Some botanical pesticide extracts (herb extracts) have been tried on maize stem borers by local farmers in Tanzania (Minja, 1990). Leaf extracts (40%) of *Tephrosia vogelii*; *Neurautanenia mitis* and *Cassia didymobotrya* plants in water were quite competitive with commercial insecticides. It is known that the leaves of these plants are used locally in Tanzania to control stalk borers in maize by the farmers. The extracts from *Neurautanenia* and *Tephrosia* plants showed promising results (Mallya, 1985, 1986; Marandu *et al* 1987). In Ghana, the use of herbal extracts for the control of maize stem borers is not yet publicised. However, interacting with groups of local farmers in some parts of the country, revealed that the use of wood-ash through foliar-applied method could reduce early stages of larval infestation.

1.2.2 *Cultural control*

Cultural practices are usually an inherent component of normal crop management practices in maize field. These measures include crop rotation, crop sanitation, the adjustment of planting times, crop associations and many others, which adversely affect pest population in the field (Sithole, 1990). Cultural practices are widely used by subsistence farmers all over the country, however, little attention or no research has been given to this aspect of control strategy and there is the need for researchers to direct their serious attention to it.

1.2.3 *Host-plant resistance*

The use of resistant or tolerant crop cultivars forms one of the promising pest control measures. In eastern Africa, cereal improvement programmes initially paid more attention to yield improvement and few were directed towards resistance to stem-borers (Minja, 1990). Research at the International Centre of Insect Physiology and Ecology (ICIPE) has been conducted on the screening of maize and sorghum lines, (Omolo 1983; Seshu Reddy 1983; Ampofo *et. al.* 1986). Omolo (1983), and Omolo and Seshu Reddy (1985) identified new sources of stem borer resistance from local and exotic maize lines, which offered a wide scope in multiple resistance or multiline approach towards stem borer management. Ampofo *et al* (1986) further evaluated these lines against *Chilo partellus* oviposition and establishment and concluded that some of them were a source of good materials for selection of resistance to *C. partellus*.

Studies on the genetic basis of resistance in maize and sorghum to C. *partellus* infestation and damage have also been done. Studies on maize showed that both additive and non-additive and epistasis gene effects are important in the inheritance of dead-heart, while the gene effects for leaf feeding and stem-tunnel length was predominantly additive (Pathak, 1990).

In Ghana, the major objective for maize varietal development was to develop high yielding varieties that were resistant to stalk lodging, diseases and insect pests and that were adapted to the major ecological zones of Ghana (NARP, 1993). The initial approach of the programme was to develop full seasons (120 days) and intermediate (105 days) composite varieties with wide adaptation for growing throughout the country. Development of composite varieties remained the major thrust until in 1986, when a review of maize programme recommended that efforts should be devoted to developing hybrid maize.

Currently, International Institute of Tropical Agriculture (IITA) is conducting research into maize stem-borer resistance, from which Ghana also benefits through exchange of germplasm and collaborative research. Research into host-plant resistance to maize stem borer is on-going in Ghana and it is hoped that with more financial support the project would be successful.

1.2.4 Biological control

The use of other insects or organisms as natural enemies to control insect pest population is also another promising control strategy in Insect Pest Management. Research work on graminaceous stem borers has revealed that large numbers of species of natural enemies are available for trial against *Chilo* species. About 50 species of parasitoids have been listed for *Chilo* species, attacking maize, millet and sorghum, but only about 20 species are considered important (Mohyuddin, 1990).

In Ghana, Maafo (1975) performed laboratory mass rearing studies on three exotic parasitoids of *Tetrastichus* species, *T. inferens* Mani, *T. israeli* Kurian and *T. ayyari* Rohwer. These are all protelean pupal endoparasites, imported from India, which are highly effective biogents against graminaceous stem borers of maize and sugar cane. *T. israeli* has been released in Senegal against maize and sugar cane stem borers and found to be effective (Maafo, 1975).

Girling (1980) observed that two parasites of the tachinid group, *Sturmiopsis parasitica* (Curr.), common on *E. saccharina* and *Descampsina sesamiae* (Mesnil), mostly found on *S. calamistis*, were commonly collected at the Asutsuare sugar estates on the Volta delta. These parasitoids were not found at the Komenda sugar estates in the Central region. Unlike maize, sugar cane crop is present throughout the year, and therefore provides a more stable environment for *E. saccharina* and its natural enemies. Girling (1980) explained that biological control using introduced parasites was difficult in the maize crop because the stem borers were immigrants from other host plants into ephemeral environment, thus preventing a build up of parasite numbers and development of a stable interaction. Further studies on parasitoids are needed to discover if any are able to parasite on *E. saccharina* and other stem borers effectively.

1.3. Sterile insect technique (SIT) – to the rescue?

1.3.1 Historical background

The use of sterile insects to control insect pest populations is one of the revolutionary innovations in modern entomological research. SIT is one of the genetic control methods that has been developed and field tested against several insect pests. The origin of the idea and development of the techniques are closely linked to research on the screw-worm fly,

Cochliomyia hominivorax (Coquerel). It was initiated in an isolated laboratory in Texas when in 1937, Knipling observed that the female screw-worm flies appeared to mate once (LaChance *et al.*, 1967). Knipling suggested to his associates that if the males could be sterilised without impairing their mating activity, the sterilised males could be used to eradicate the isolated population of the pest in the south-eastern USA.

Later, A. W. Linquist of Corvallis, Oregon laboratory drew the attention of Knipling to a report by Muller (1950) that ionizing radiation could cause male sterility by inducing dominant lethal mutations in the sperm. Tests were performed on the screw-worm with X-rays (Bushland and Hopkins, 1951) and gamma radiation (Bushland and Hopkins, 1953). X-ray and gamma ray radiations proved equally effective and either adults or pupae could be sterilised. The most efficient method was to irradiate pupae about 2 days before adult emergence.

Following successful laboratory tests, field studies were undertaken in Florida, USA. Screw-worms were brought to the vanishing points by the release of 100 sterile males per square mile per week on Sanibel Island near Fort Myers, Florida (LaChance *et al.*, 1967). Baumhover *et al.*, (1955) also performed field experiment on the Island of Curacao and achieved eradication on that isolated 170 square mile islands.

As a result of the study on screw-worms, SIT has been extensively used to control many insects of economic importance including Mediterranean fruit fly, *Ceratitis capitata* (Wied.), melon fly, *Dacus cucurbitae*, tsetse fly *Glossina* species and other insects of the Diptera order (Nitzan *et al.*, 1993; Ofori, 1993; Rossler, 1997).

1.3.2. Theory of SIT

Sterile insect technique proposed by Knipling involves the rearing, sterilization and release of large numbers of the sterile insects to mix with and compete for mates with those of the natural population (Knipling, 1955). The theory in its simplest form asserts that the introduction of fully competitive sterile organisms into a natural population will reduce the reproductive potential of the natural population in proportion to the ratio of sterile to fertile insects present in the population after insects are released. If the ratio is 1:1 and the released sterile insects are fully competitive, the reproductive capacity of the natural population will be reduced by 50%. If the ratios is 9:1 the reproductive capacity of the natural population will be reduced by 90% (Knipling, 1955).

To contrast the difference between the SIT method and the conventional method of insecticidal killing of insect pest, it is noted that the continued use of the same treatment of insecticide for killing insects in subsequent generations will have the same percentage effect regardless of population density. On the other hand, the release of a constant number of sterile insects will cause a higher and higher percentage of control as the natural population density declines and the ratio of sterile to fertile insects increase. A hypothetical illustration by Knipling (1955) is shown in Table 1.0. below:

e 1.0: **Theoretical population decline in each generation by release of constant number of sterile males in natural population of 1 million each of females and males.**

Generation	No. of virgin females in each area	No. of sterile males released in each generation	Ratio of sterile: fertile males to virgin females	% Females mated to sterile males	Theoretical pop of fertile females
F_1	1,000,000	2,000,000	2 : 1	66.70	333,333
F_2	333,333	2,000,000	6 : 1	85.70	47,619
F_3	47,619	2,000,000	42 : 1	97.70	1,107
F_4	1,107	2,000,000	1,807 : 1	99.95	Less than 1

1.3.3. Radiation- induced inherited sterility (RIIS)

Radiation-induced inherited sterility, usually expressed as inherited sterility (IS) and also known as F_1 sterility, is one of the genetic control methods of insect pest populations. The principle is the same as SIT but with slight difference in radiation dose application. In IS, the radiation dose is considerably lowered so that the released insects (usually both males and females) are only partially sterile rather than completely sterile (LaChance, 1985). The radiation dose can also be adjusted so that when the released partially sterile males and females interbreed, few or no progeny are produced but when they out-cross with insects in nature, egg hatchability is decreased and the progeny that are produced are sterile.

One important advantage of SIT over most types of insecticide control is that the sterilised insects will seek out fertile individuals in environmental sites where they would be normally protected from insecticides. Another advantage of releasing large numbers of sterile insects is that natural resources such as food and shelter may be so over-strained that many of the native insects may be displaced into less suitable ecological habitats where they are unable to survive

(Andrewartha *et al.*, 1967). Sterilisation control methods would be particularly useful against insects with overlapping generation (Proverbs, 1969). Release of sterile insects should be used when native population is at low level, adults released should be preferably harmless and the insect easy to rear economically.

1.4 Justification

Each of the conventional control methods described above has drawbacks and application of these methods singularly has limited success. An integrated approach however, which involves the use of two or more strategies, with different modes of action, would be more diversified, effective and less vulnerable to failures. Many researchers have demonstrated the potential of inherited sterility to reduce the reproductive ability and suppress natural populations of the corn earworm, *Helicoverpa zea* (Boddie), the spotted stem borer, *Chilo pertallus* (Swinhoe) and many other lepidopterous pest species (LaChance, 1983; Okoth, 1990; Carpenter, 1993). The fact that inherited sterility has the capacity to perform compatibly and synergistically with other control strategies suggests that it could be developed and used as a major component of integrated approaches to managing maize stem borer pest population in Ghana.

1.5 General aim

This work is therefore generally aimed at developing radiation-induced inherited sterility in *Eldana saccharina* and *Sesamia calamistis* to reduce their reproductive capability and subsequently to suppress the pest population of the borer species for insect pest management programme in Ghana.

1. 6 Specific Objectives

i) To study and describe the population dynamics of the stem borer species in maize farming ecosystem in the Ga District of Greater Accra.

ii) To demonstrate the effect of substerilizing doses of ionizing radiation on the biology of *E. saccharina* and *S. calamistis* and determine the level of induced inherited sterility of the progeny.

iii) To develop and establish laboratory rearing facility for mass rearing of the two species of stem borers.

iv) To determine level of pest suppression on maize crops through field cage studies of different ratios of irradiated and non-irradiated stem-borer species.

1.7 Anticipated benefits

Results from this research project would enlighten researchers on the mechanism of induced inherited sterility of maize stem borers and other insect pests of economic importance. Its application in the control of maize stem borers would reduce the high incidence and infestation of stem borers in maize ecosystem in Ghana. This would lead to ensuring sustainable increased production of maize and other cereals, sufficient to feed the growing population of Ghana. Farmers in the study area, at Medie in the Ga District and the surrounding towns would be the immediate beneficiaries, if the subsequent phase of field trials of the project is successfully implemented.

CHAPTER TWO

LITERATURE REVIEW

INHERITED STERILITY IN LEPIDOPTEROUS MOTHS

2.1 Principles of inherited sterility in lepidopterous moths

Lepidoptera are one of the most destructive insect pests of agriculture known world-wide. Due to the increasing resistance to insecticides, coupled with public out-cry of environmental hazards caused by the misapplication of many chemical pesticides, there has been the tendency over the past years to look for alternative and innovative strategies for insect pest control (North, 1975).

Lepidoptera have often been referred to as being radio-resistant and require large doses of radiation to effect sterility compared with most other insects (LaChance *et al.*, 1967; North, 1967). When Lepidoptera are given sterilizing doses of radiation, induced physiological disturbances, such as insufficient sperm transfer and lack of mating are manifested (North and Holt, 1968; Holt and North, 1970; North and Holt, 1970; North and Holt, 1971). It was realised that these debilitating effects would limit the ability of sterile males to compete with natural males in suppressing a pest population. It was therefore found that insects would be more competitive by effecting partially sterilizing doses of radiation (North, 1967; Walker and Quintana-Munez, 1968a).

Inherited sterility (IS) or F_1 sterility was discovered by Proverbs in the course of his studies on the codling moth, *Carpocapsa pomonella* (Proverbs, 1962). This has since been established and documented in many lepidopteran species of economic importance (Makee and Saour, 1997). Proverbs (1962) was the first to observe that the progeny of irradiated males were semi- to completely sterile. The surviving progeny of irradiated parents inherit sufficient genetic material to make them partially or completely sterile. Inherited sterility in moths differs from sterile insect technique, in that the dose of ionizing radiation of the former is lowered so that the moths are only partially sterile. In other occasions, the dose is adjusted so that females are completely sterile and males are partially sterile (LaChance, 1985). Because the dose of radiation is lowered, partially sterile insects are generally more competitive than fully sterile insects.

The release of partially sterile insects offers far greater suppressive potential than the release of fully sterile insects. Knipling (1970) was the first to recognize the potential of inherited sterility over SIT. In his initial models comparing the two control strategies, (IS vrs. SIT), he estimated that to achieve the same degree of suppression in the native population over three generations, four times as many sterile insects as partially sterile insects would have to be released. In addition, infusion of inherited sterility into the parental generation produces F_1 progeny with varying degrees of sterility, thus allowing for production of sterile insects in the field (Carpenter and Layton, 1993). All theoretical models comparing IS with SIT have shown that partially sterilized insects suppress the native population more effectively than do an equal number of fully sterile insects (LaChance, 1985).

2.2 Methods of inducing inherited sterility

Various types of ionizing radiations have been used to sterilize insects. These include alpha, beta, and gamma radiations of radioactive substances, X-rays and neutrons (Proverbs, 1969). Beta rays are not efficient for external application because of their poor penetrating power. Alpha particles have a short range and creates difficulties (Rogers, 1955). Neutron sources are not very common and not used extensively. However they have been shown to be quite efficient in inducing recessive lethals in silkworm and other insects (Murakami *et al.*, 1965; Lightly, 1971). Gamma and X- rays have great penetrating power and are most useful for inducing insect sterility. X- ray tubes are known to be expensive and usually over heat and burn out during prolonged operation (Proverb, 1969). Radiation sterilization in insect control programmes is therefore commonly performed by gamma rays, usually from Cobalt-60 source and sometimes Cesium- 137 (North, 1975).

The other practical method of inducing insect sterility is by chemical treatment. However due to the environmental hazards that are associated with the general use of chemicals, there has been limited use of chemosterilants in inducing inherited sterility (Stimmann, 1971).

2.3 Life stages irradiated for inherited sterility

It has often been a common practice of many researchers studying radiation- induced sterility in insects to irradiate all life stages. Irradiation of early stages (prior to pupa) to induce sterility has not been efficient because the induced damage is not included in the newly formed gametes but rather selected against (North, 1975). However some attempts have been made to induce sterility in the early stages of insects.

2.3.1 *Embryos/Eggs*

Attempts to induce sufficient genetic damage to effect inherited sterility by irradiating embryos have generally not yielded satisfactory results (Lassota, 1963; El Sayed and Graves, 1969; Murakami, 1969; Ercelik and Holt, 1972; Bartlett *et al.* 1973). Varied results were observed because heterogeneous embryonic stages were irradiated. Generally as the dose of radiation increases, embryonic mortality also increases and in most cases there was no control over such developmental influences such as temperature and photoperiod. Adults resulting from these are often malformed and fail to mate, but some of the moths that do mate are partially sterile. The female appears to be more radiosensitive when irradiated as an embryo. Some researchers (Hough, 1963; Nielsen, 1971) have observed the persistence of sterility for several generations beyond the parent (P) of irradiated eggs. These observations give room for further exploration of embryonic irradiation as a means of inducing inherited sterility. However, future studies should take into consideration irradiation of precise stages of embryonic development and exercise control over environmental factors such as temperature and photoperiod (Deseo, 1973).

2.3.2 *Larva*

Larvae irradiations have proved unsatisfactory in several cases because the adults resulting from irradiated larvae usually are not capable of reproducing (El Sayed and Graves, 1969; Qureshi *et al.*, 1970; Walker *et al.*, 1971; Ercelik and Holt, 1972). Relatively low doses (35 Gy) given to fifth instar larvae of the Indian meal moths, *Plodia interpunctella* Hubner induced sufficient genetic damage to spermatogonia to induce sterility (Ashrafi *et al.* 1972). The aberrations induced by the low doses are incorporated into the mature spermatozoa, and

semisterility is transmitted by the female. First instar of the greater wax moth, *Galleria mellonella* larvae given 40 Gy of gamma radiation developed into partially sterile adult males. This sterility persisted through, at least two generations of the male line and nearly all of the later populations were male (Nielsen, 1971). Chromosome injury by radiation is almost the same in somatic cells as in germ cells and may lead to cell death (Rai, 1964; George and Brown, 1967). Chromosome aberrations are induced most easily in interphase cells that are preparing for division (LaChance *et al.*, 1968). Where there is much proliferating tissue present as usually found in larval development, it is expected to get a large amount of somatic injury. It is well known that ionizing radiation reduces rate of development and may completely or partially inhibit metamorphosis.

Studies have shown, (Kuzin *et al,* 1968), with *Ephestia* that inhibition of pupation in irradiated larvae results not from a disturbance of the DNA of the hypodermis as was formerly believed, but from the absence of the pupation hormone, ecdysome. Its absence is believed to be the result of irradiation damage to neuro-secretory cells.

Riemann (1967) observed in the screwworm that the cells most killed were the primary spermatocytes, followed by secondary spermatogonia, primary spermatogonia and secondary spermatocytes; spermatids and spermatozoa were uninjured. A high proportion of spermatogonia die at interphase or at the following mitotic division (Clayton, 1962).

2.3.3. Germ cells

Germ cells are particularly sensitive at early stages of meiosis (Savhagen, 1963). Germ cell sensitivity in many female insects is complicated by the presence of nurse cells; if these cells are severely damaged, the germ cells are unable to complete development. Nurse cells are most subject to injury when their chromosomes are undergoing endomitotic activity; small

doses of radiation at this time disrupt the proper degree of ploidy and the size required for normal vitello-genesis (LaChance and Leverich, 1962).

The severity of radiation injury to germ cells may also be influenced by factors such as temperature, oxygen tension, genetic make up of the insect, its age and stage of development. The effects of these factors on radiation injury are well documented by many researchers including Lea, 1962; Mandl, 1964; Cornwell, 1966. Oogenesis occurs later than spermatogenesis in Lepidoptera; irradiation of early stages (before fifth instar) therefore, would completely destroy the developing germ cells of the ovaries (Miya *et al.*,1970) and thus produce infecund females.

Irradiation of early cell stages of spermatogenesis in Lepidoptera would not be expected to yield genetic damage that would be inherited in any sufficient amount required to be useful in control (Sado, 1963; Virkki, 1963; Rule *et al.*, 1965; Sugai and Iijima, 1967; Ashrafi *et al.*, 1972). Testes containing only gonia cells irradiated with 50 Gy had the definitive gonia killed but the predefinitive gonia were more resistant (Holt, 1968; Sugai and Suzuki, 1971). Consequently, the testes were repopulated with slightly damaged or undamaged cells. Researchers exploring the possibility of inducing inherited sterility by irradiation of larval and embryonic stages of Lepidoptera must be able to recognize the target germ cells damaged by the radiation and determine their ultimate fate. Induced genetic damage not incorporated into the sperms or ova because of cell death does not lead to inherited sterility.

2.3.4 Pupa

The reproductive capability of cabbage looper, *Trichoplusia ni* irradiated as pupae was similar to that when adults were irradiated when the dose were fractionated over several days (Toba and Kishaba, 1973). Generally female sterility was also higher when fractionated dose were given to pupae. Pupal irradiation is preferred because the pupal stage is the easiest to handle (North, 1975). However further studies of dose fractionation of pupal stages would have to be conducted.

2.3.5 Adult

The adult stage does not undergo metamorphosis and therefore a good radiation target, since it does not suffer as many of the debilitating effects of radiation (i.e. malformed wing, improper eclosion, inability to mate etc.) that are usually found in irradiated juvenils (Cogburn *et al.*, 1966; Godwin *et al.*, 1965; Qureshi *et al.*, 1967). Adult irradiation, however does not totally avoid physiological damage (Holt, 1968; North and Holt, 1970; North and Holt, 1971).

Reduced longevity is one of the most commonly observed responses caused by somatic damage. In some adult insects, the mid-gut epithelium is renewed periodically, but since radiation inhibits mitosis the degenerated cells cannot be replaced. This may lead to early death in the adult (Riemann and Flint, 1967). Irradiation may shorten the life span of an insect by increasing its susceptibility to attack by micro-organisms (Jafri, 1964). There is also the evidence that, following irradiation, certain protozoan parasites in the fat body destroy the fat which in turn leads to reduced longevity.

The progeny of moths irradiated as either adults or late pupae exhibits the same amount of inherited sterility (Nielsen, 1971; Debolt, 1973). The dose of radiation required to induce inherited sterility is between 125 Gy and 225 Gy for some lepidopteran species. The male progeny from irradiated parents in all species are more sterile than the female progeny, regardless of the stage irradiated.

2.4 Sex distortion and determination

Many researchers have generally observed that irradiated lepidopteran males produce more male than female progeny (Proverb, 1962; Husseiny and Madsen, 1964; North and Holt, 1969; Proshold and Bartell, 1970; Gonen and Calderon, 1971). Contrary to this, irradiated females produce progeny in a normal sex ratio (Proshold and Bartell, 1972). Studies conducted on tobacco budworms have shown that when all the progeny from irradiated females were grouped together, the sex distortion was in favour of males but if individual pairs were analysed, some lines showed distortion towards an excess of females (Proshold and Bartells, 1970). Sallam and Ibrahim (1993) performed gamma irradiation studies on male cotton leaf worm, *Spodoptera littorilis* (Biosd.) and observed that generally as dose increased, the sex ratio in the F_1 shifted in favour of the males and at the highest dose of 200 Gy, the male to female ratio was nearly 2:1.

In Lepidoptera the male is homogametic and the female is heterogametic. The sex chromosome of the male is designated as XX or ZZ and the female is XY or ZW depending on the nomenclature used for the sex chromosomes (Taxima, 1964; Mittwoch, 1967; Robinson, 1971). It is also known that there are many autosomal factors involved in sex determination of Lepidoptera (Taxima, 1964; Robinson, 1971; White, 1973). The reason that irradiated

males tend to produce more male progeny than female was probably due to the induction of recessive lethals on the X chromosome of the male (North and Holt, 1970; Proshold and Bartell, 1970).

2.5 Male-female sterility response

It is generally observed that the progeny from irradiated male moths are more sterile than their male parents. This observation, however, may differ from species to species. Walker *et al.* (1971) on irradiation of female sugarcane borers, *Diatraea saccharalis* (F.) with as little as 20 Gy, observed that the female progeny were sterile and the male progeny semi-sterile. F_1 males from irradiated female cabbage loopers, *Trichoplusia ni*, at a dose of 150 Gy were more sterile than the F_1 females (North and Holt, 1970). However, no difference was found in sterility between the F_1 males and females of irradiated tobacco budworm, *Heliothis virescens* females at any dose up to 225 Gy (Proshold and Bartell, 1973). This is inconsistent with the data reported for sugarcane borer, though it is difficult to visualise what difference in the species could be responsible for the large variance in the dose and why the F_1 females are more sterile than F_1 males. In tobacco budworm, the F_1 males from irradiated females did not transfer sperm as well as untreated males (Proshold and Bartell, 1973). Other researchers have also observed that this trait is characteristic of F_1 males from irradiated male parents (North and Holt, 1970; Proshold and Bartell, 1970; Ashrafi and Roppel, 1973; Riemann, 1973).

2.6 Reproductive system and sperm production and transfer

The reproductive systems of Lepidoptera are similar, but variation occurs in size, shape, and placement of female accessory organs. Almost every species has differently shaped bursa copulatrix and there is a distinctive variation in the size and shape of the spermatophore.

Callahan and Chapin (1960) did comparative studies on the morphology of the reproductive organs of three noctuids and noted that certain species are more likely to successfully complete the mechanics of copulation than others. The number of unsuccessful matings appeared to be directly correlated with the complexity of spermatophore inversion. Their study reported 2.8% aberrations in matings of untreated corn earworms, *Heliothis zea* (Boddie), 15.5% in armyworm, *Pseudaletia unipuncta* (Haworth) and 0.0% in the variegated cutworm, *Peridroma saucia* (Hubner). The relatively high percentage of aberrations in matings observed in the armyworm were attributed to the complexity of the spermatophore insertion as opposed to 0.0% in the variegated cutworm. North and Holt (1968) reported 22% aberrations for singly mated untreated male and female cabbage loopers; and Flint and Kressin (1968) reported 15% aberrations of sperm transmission in the untreated tobacco budworms. Studies of the reproductive systems of the above-mentioned species reveal considerable variations and complexities in sperm productions and transfers. These offer some explanation of the variation in sperm transfer under normal conditions and after irradiation.

Holt and North (1970) observed that irradiated male cabbage looper does not really fail to transfer sperm but, because of a disruption in the timing of the mating process, the sperms of the irradiated male are ejaculated directly into the bursa copulatrix rather than incorporated into the bulb of the spermatophore. They pointed out that though only a small percentage of the males actually have a normal complement of sperm incorporated in the spermatophore bulb, when the total sperm count of the ejaculate is considered, the number of sperm ejaculated for both control and irradiated males is the same.

2.6.1 Sperm-type

The type of sperm transferred in adult moths is important in ensuring egg fertilization or unfertilised egg (North and Holt, 1971). In Lepidoptera males two types of sperm are identified: (a) *eupyrene* and (b) *apyrene* sperms. Apyrene sperm are anucleate and incapable of fertilization. They are smaller but more mobile than eupyrene sperm (Iriki, 1941). Eupyrene sperm are nucleate and undergo gross morphological changes during spermiogenesis from the time they leave the testes until they reach the spermatheca (Riemann, 1970). The normal eupyrene to apyrene ratio is considered to be 2 eupyrene : 1 apyrene. Any ratio below 1:1 was considered abnormal in sperm found in the spermathecae of the females.

In the cabbage looper, Holt and North (1970) observed that the eupyrene sperm, which are nucleate and capable of fertilization in contrast with the anucleate apyrene sperm do not become mobile until they have reached the spermathecae of the female. Any eupyrene sperm, therefore, placed directly into the bursa copulatrix cannot find their way up the seminal duct. On the other hand, since apyrene sperm possess motility when incorporated into the ejaculate, they can find their way to the spermatheca by random chance, but could produce only unfertilized eggs (Chen, 1969). This explains why irradiated males often appear to transfer only apyrene sperm (North and Holt, 1971).

The male progeny from males given a partially sterilizing dose of radiation often fail to transfer sperm to the spermathecae of the female successfully (Proshold and Bartells, 1970; Gonen and Calderon, 1971; Chen and North, 1972; LaChance *et al.*, 1973). In the pink bollworm, *Pectinophora gossypiella*, studies were conducted on the relative amount and type of sperm transferred to the spermathecae by the F_1 males (Chen and North, 1972; LaChance *et. al.*, 1973). It was observed that F_1 males have a problem in sperm production and transfer.

The routine experimental procedure to determine the mating ability of irradiated moths has involved dissecting the female bursa copulatrix and counting the spermatophores (North and Holt, 1971). The procedure also involved the dissection of the spermatheca to determine the presence of sperm. This is usually performed after the completion of oviposition by the female.

2.7 Cytogenetics of inherited sterility

The lepidopteran karyotype is characteristically small and has symmetrical chromosomes without much morphological variation (Suomalainen, 1965; Barry *et al.*, 1967; Suomalainen, 1969a). The chromosomes are short and from the polar view the bivalents look round dots in reduction metaphase of meiosis. The chromosomes have diffuse centromeres (polycentric or holokinetic) a condition that allows chromosomal fragments from natural or artificial sources to survive as intact chromosomes (Guthrie *et al.*, 1965). These observations also serve as the basis to explain radioresistance and inherited sterility in Lepidoptera (LaChance *et al.*, 1967; North, 1967). When a moth is irradiated, most of the pieces from broken chromosomes are not lost during cell division, for each piece has its own site of attachment (Proverbs, 1969).

2.8 Practical applications of inherited sterility for population suppression

Many researchers have studied the possibilities of population suppression through the release of radiation-sterilized moths, trying to follow the success of the screw-worm fly, *Cochliomyia hominivorax* (Godwin *et al.*, 1965; LaChance *et al.*, 1967; Knipling, 1970; Bushland, 1971). Even though initially there were some failures in suppression of the pest population through the release of sterile moths, there have also been other successes like the control of the codling moth, *Laspeyresia pomonella* (Proverbs, 1970; Proverbs, 1971) in an isolated orchard test,

and the tobacco horn-worm, *Manduca sexta* (Snow *et al.*, 1974), which was controlled on the island of St. Croix, U.S. Virgin Islands. Partially sterile cabbage loopers were released on St. George Island, Florida, but there was no evidence that the suppression obtained for one month resulted directly from inherited sterility (Lingren *et al.*, 1972). North and Snow (1978) performed cytological analysis of corn earworms collected on St. Croix, U.S., Virgin Islands, during and after release of male and female moths given 250 Gy gamma dose. Larvae sampled from the surviving progeny showed that as high as 30% had chromosomal aberrations, indicating that they were progeny of an irradiated parent. These results show the extent of infusing F_1 into a natural population and clearly indicate that F_1 progeny were capable of establishing themselves in a natural population.

Field release of partially sterile insects has been investigated for many species of Lepidoptera by researchers. These moths include the corn earworm, *Helicoverpa zea* (Boddie) (Carpenter *et al.*, 1987; Carpenter and Gross, 1993), the gypsy moth, *Lymantria dispar* (L) (Mastro, 1993) and codling moth, *Cydia pomonella* (L.) (Proverbs *et al.*, 1978). For codling moth, both laboratory (Fossati *et. al.*, 1971) and field (Proverbs *et. al.*, 1978) results have shown that moths irradiated at a substerilizing dose of 250 Gy, are more competitive and bring about better control than fully sterile moths (400 Gy).

Bloem *et al.* (1999) conducting Sterile Insect Release (SIR) programme in the British Columbia, Canada, and using inherited sterility (IS) for control of codling moth *Cydia pomonella*, released moths treated with 350 Gy (Dyck *et al.*, 1993; Bloem and Bloem, 1996). The dose of gamma radiation has been reduced each year to improve codling moth competitiveness, with moths in 1997 receiving 250 Gy in spring and 300 Gy during summer releases. By the end of 1997, 91% of 623 orchard sampled throughout the treatment area has

no (0%) detectable codling moth damage at harvest (Bloem, 1997; Bloem and Bloem, 1996). Bloem *et al.*, (1998) observed that the SIR programme in British Columbia, Canada, is on-going and continually seeks ways to improve the performance of sterile codling moths, to decrease costs and to ensure that the goal of eradication is met successfully.

2.8.1 Models for integrated approach

Flexibility in application of the inherited sterility method has been described by LaChance (1985). This flexibility should be enhanced by the integration of other pest control methods, for management strategies to be modified as seasonal changes occur in the pest status and the host plant availability. The full potential of inherited sterility as an area wide control strategy for some Lepidopteran pests may be realised only when inherited sterility is integrated with other suppression methods (Carpenter, 1993).

Models developed by Knipling (1979; 1992) depicting different integration scenarios suggest that combining inaudative releases of parasitoids (natural enemies) with sterile insects will yield both additive and synergistic effects. Knipling (1992) calculated that combining sterile insects with parasites theoretically could be 10,000 times more effective than if each technique were used alone. Even greater suppression could be expected if parasite releases were combined with the inherited sterility technique. A theoretical model was designed by Carpenter (1993) using inherited sterility in combination with natural enemies of the corn earworm for pest suppression. From his model, inherited sterility technique produces sterile F_1 larvae that would provide an increased number of hosts for the parasites, thereby increasing the parasite population for the next corn earworm generation (Carpenter, 1993). The surviving sterile larvae would produce sterile *Heliothis zea* adults for the next generation to perform the

suppression action.

In the maize ecosystem in Ghana, some active parasitoids which are very effective on larvae and pupae of *Eldana saccharina* and *Sesamia calamistis* have been found (Girling, 1980). Researchers are currently conducting studies to identify and use these parasitoids as bioagents and subsequently integrate with F_1 sterility for stem borers control.

2.9 Highlights of write-up

The present research is therefore aimed at developing radiation-induced inherited sterility (RIIS) in *E. saccharina* and *S. calamistis* and demonstrate the technique as a potential biological control method for stem borer pest management programme in Ghana. Sub-sterilizing doses of ionizing radiation are performed on the two species of maize stem borers to determine the level of induced sterility of the F_1 progeny. Other studies conducted and included in the thesis are:

- Bio-ecological studies of the two borer species in maize farming system at Medie in the Ga District.
- Biology of the two species laboratory reared on artificial and natural diets.
- Comparison of the reproductive system and mating mechanisms of the moth species.
- Inherited sterility of *E. saccharina* with respect to mating, fecundity and fertility.
- Chromosomal aberrations of inherited sterility of *E. saccharina.*
- Field cage studies on sterile to fertile ratios of released moths for pest suppression.

Each of these studies constitutes the subsequent major chapters of the thesis and composes of an introduction, materials and methods, results, discussion and conclusion.

CHAPTER THREE

BIOECOLOGY OF *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE) AND *SESAMIA CALAMISTIS* HAMPSON (LEPIDOPTERA: NOCTUIDAE)

3.1 Introduction

Studies on the biology and ecology of crop pest species provide essential information for better understanding of the host plant-pest relationship and for planning effective control strategy. Better knowledge of the population dynamics of pest species would facilitate the development of appropriate control programme (Harris, 1990).

The potential of using inherited sterility as a component of pest management of Lepidoptera has been demonstrated by researchers on many moth species. These include the cabbage looper, *Trichoplusia ni* (Hubner) (North and Holt, 1969), spotted stem borer, *Chilo partellus* (Bughio,1988; Okoth, 1990), corn earworm, *Helicoverpa zea* Boddie (Carpenter *et al.*, 1987; Carpenter and Gross, 1993), codling moth, *Cydia pomonella* (L.) (Anisimov *et al.*, 1989; Bloem *et al.*, 1999) and the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Marec *et al.*, 1999). Most of these moth species are of temperate type and not of tropical origin. There is, therefore, the need to examine the tropical species, including *E. saccharina* and *Sesamia* species to assess the possibility of using inherited sterility to reduce the pest population for increased maize yields.

Bioecology of maize stem borers has been conducted in Ghana in the major ecological zones (Gounou *et al.*, 1994). There is however, little or no information of the two borer species in the forest-savanna transitional zone in the Ga District of Greater Accra Region. Cultivation of maize crop in this ecological zone is relatively in abundance and occurred almost throughout the year. The farming community however, reports of serious attacks of the maize crop by the borer species and this calls for pragmatic control measures. The present study therefore aims at determining the population levels of larvae/pupae of *E. saccharina* and *S. calamistis* and the influence of climatic factors, particularly rainfall pattern on the incidence of the pest species. Information from this study would enable forecasting the timing of releasing sterile moths into the natural pest population and ensure synchrony in mating behaviour between the released and the natural moths. The farming system of the community is also discussed.

3.2 Study Area

3.2.1 Location

Field sampling for population study of larvae/pupae of the two species of stem borers was conducted on maize farms at Medie, a small town in the Ga District. Medie is located at the northwestern section of Greater Accra Region, a distance of about 25 km. from the center of the city of Accra on the main Accra-Kumasi road. The town is situated between latitudes 5° and 6° North and longitudes 0° and 1° West of Accra (Fig. 3.1) and covers an area of about 1.5 square kilometres. Estimated population of the community is about 3,000.

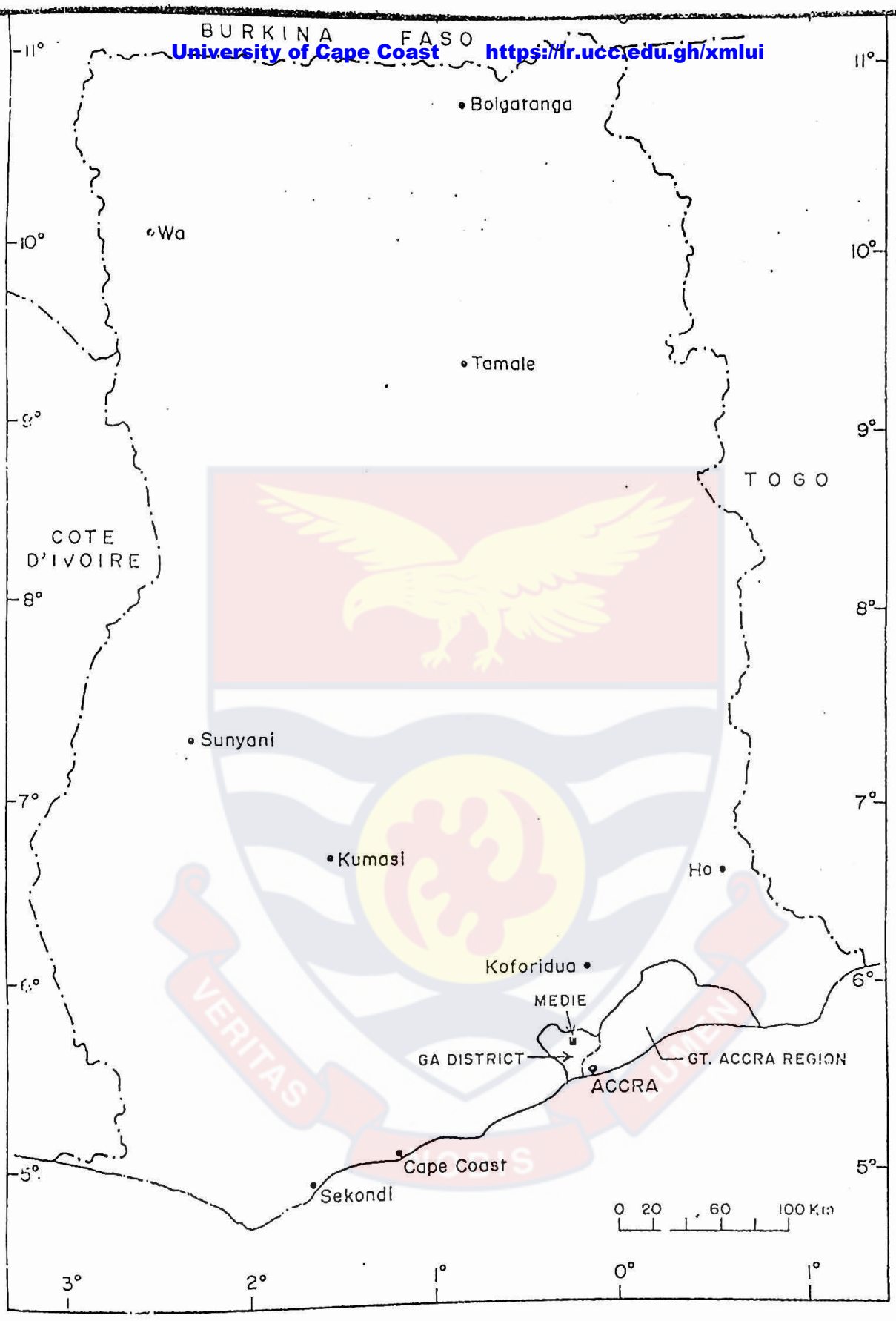


Fig. 3.1 Map of Ghana showing the location study area Medie in the Ga District of Greater Accra Region.

3.2.2 *Vegetation and land-use*

The study area falls within the forest-savanna transitional zone, where both forest and savanna plant species are found. Some forest trees (emergents) that are found include the silk cotton tree, *Ceiba pentandra* and bark cloth tree, *Antiaris africana* (Adu and Asiamah, 1992). The grasses are largely made up of guinea grass, *Panicum maximum*, the spear grass, *Imperata cylindrica*, *Andropogon* and *Heteropogon* species. Most part of the area is heavily farmed for maize and cassava. The farmers produce large quantities of fresh maize almost throughout the year. Vegetables like garden eggs, okro and pepper are also cultivated.

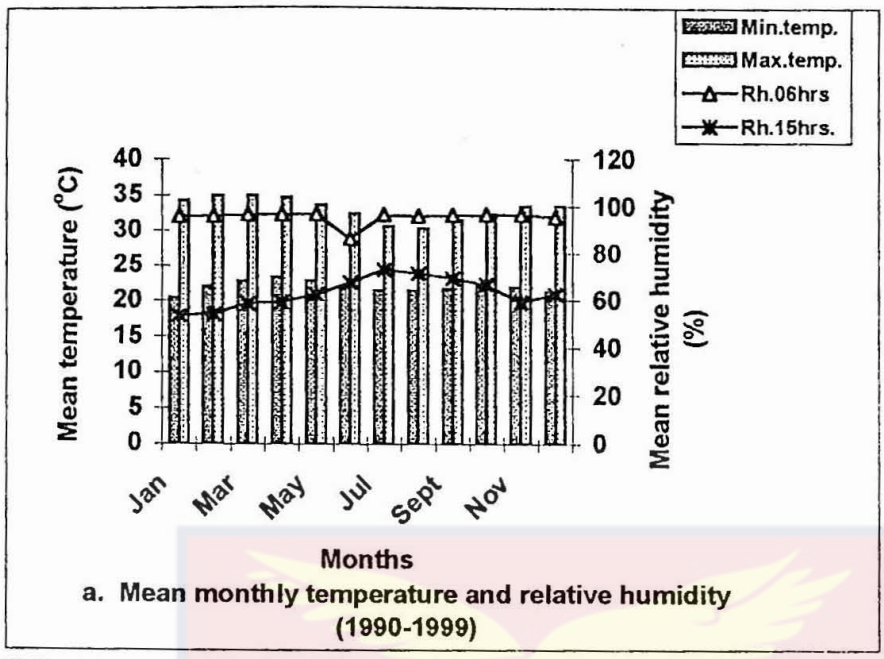
3.2.3 *Soil-type*

Adu and Asiamah (1992) identified and classified the soil-type as Densu series, developed in deep poorly drained, seasonally flooded, grey mottled brown alluvial clays found in valley bottoms. Densu soils have very slow internal drainage, slow run-off, very slow permeability and medium to high water-holding capacity. During severe dry seasons, the soils tend to dry out, becoming hard and compact, showing cracks. This soil-type is relatively well supplied with plant nutrients and suitable for rice, sugarcane and vegetables.

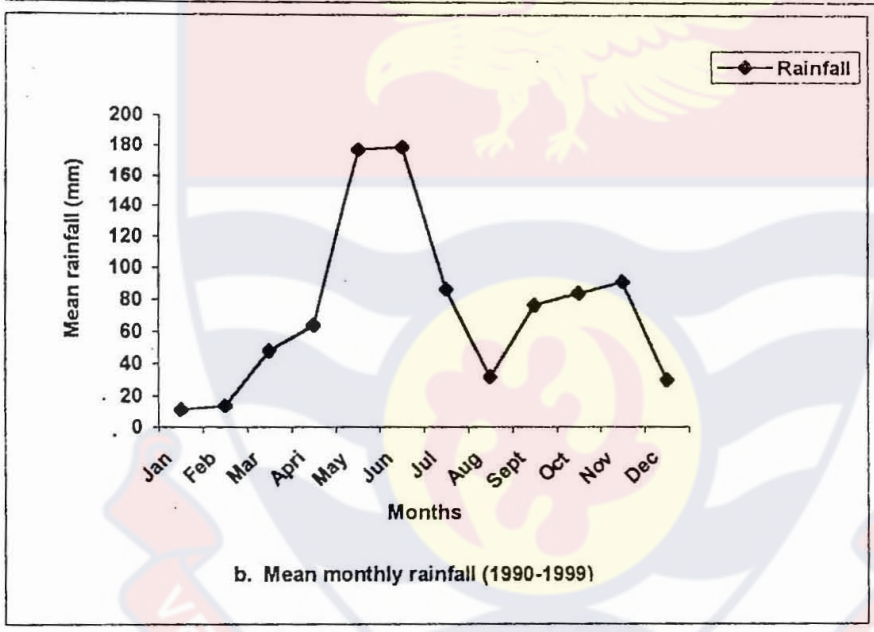
3.2.4. *Climatic conditions*

Data on the monthly mean temperature, relative humidity and rainfall for a ten-year period (1990-1999), as graphically represented in Fig. 3.2 (a,b), were recorded by the Meteorological Services Department at Pokuase, about 3 kilometres from Medie.

Annual rainfall pattern at Medie and its environs is bimodal with the peaks occurring in May/June (major rainy season) and in October/November (minor rainy season). Generally on the average, there is no month without rainfall in the year. Temperature in the field is fairly stable. Monthly mean temperature ranges between 26°C and 28°C. Hottest period in the year occurs in February/March with mean monthly maximum temperature of 35°C. Mean monthly minimum temperature ranged between 21°C and 23°C, occurring in January, July and August. The mean monthly relative humidity at 0600 hrs is 96% and between 54% and 72% at 1500 hrs.



a. Mean monthly temperature and relative humidity (1990-1999)



b. Mean monthly rainfall (1990-1999)

Fig. 3.2 Average temperature, relative humidity and rainfall at study area for a 10 year period (1990-1999).

3.3 Materials and Methods

3.3.1 *Maize agro-ecosystem practices*

Local farmers usually engage in extensive maize farming almost throughout the year at Medie. The major farm sites of the study area were broadly divided into 2 sections shown in Fig. 3.3. During the major rainy season, most farmers cultivated large acreage of maize crops on well-drained loamy soil (sampling sites A, B & C). In the minor season however, maize was cultivated on the low-lying water-logged soil at sites D, E & F. Farmlands were ploughed using hired tractors and sometimes farmhand labourers were hired to plant viable seeds in rows, 2-3 seeds per hill. Spacing between rows were about 60 cm. apart and 25 cm. between plants. Weeding through the farms was performed at least 2 times before formation of tassels of maize crops (Plate 3.1). Most farmers applied chemical fertilizers 4 weeks after maize emergence, to enhance crop yields (Plate 3.2). About 90-110 days after maize germination, most farmers began harvesting fresh corn for sale, leaving few grains in the field to dry up for storage and home consumption.

Experimental maize farms, about 0.2 ha each, were also ploughed at the research farm of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) at Kwabenya, Accra. This was to provide fresh maize stems for feeding the laboratory reared larvae and also to replenish the larval numbers of the laboratory stock.

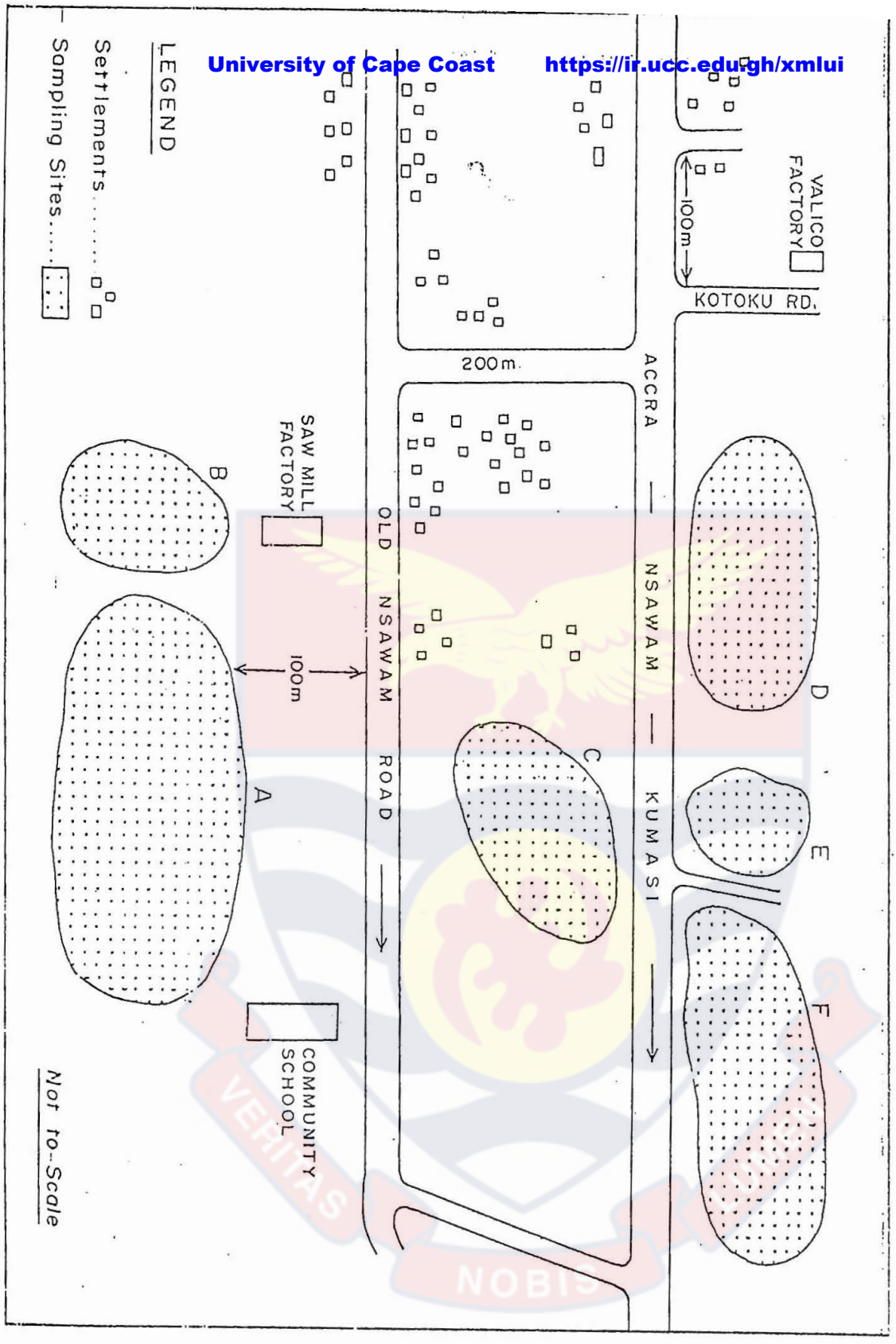


Fig. 3.3 Sampling sites of maize stem borers at Medie in the Ga District

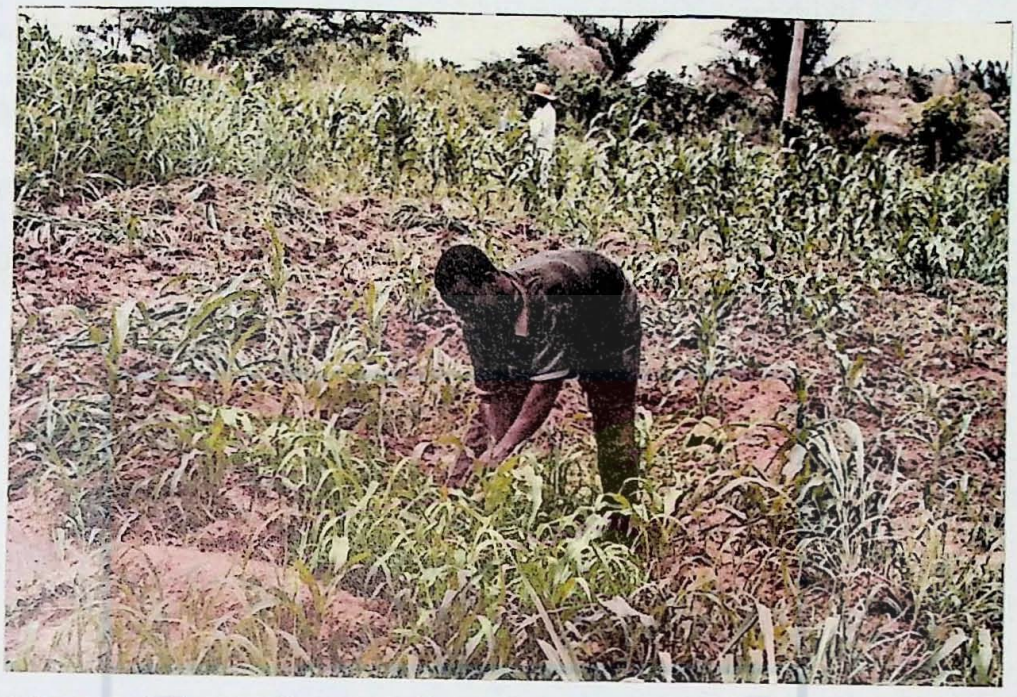


Plate 3.1 A farmer (foreground) weeding through maize farm

Plate 3.2 Blooming maize crops in a community farm (> 6 weeks old)

2 Sampling procedure

weekly field sampling of larvae/pupae to determine the population levels of the two species stem borers, was conducted in the maize farms of the study area during the period of 1997, 98 and 1999. During sampling, each farm was divided into three sections of almost equal es. The chief investigator was assigned to sample one section and two other field assistants ained in sampling procedures) worked in other two sections.

wo sampling procedures, a non-destructive presence-absence method and a destructive ethod were used (Gounou *et al.*, 1994). In the former, 50 plants per section were randomly elected to determine the proportion of damaged plants in the field, based on observation of xternal signs and symptoms of borer attacks, such as dead-hearts, bored holes, frass, leaf amage and stem lodging (Plates 3.3 and 3.4). The destructive sample method was used in the ssessment of the number of larvae or the extent of actual borer infestation. With this method, 25 plants were randomly re-sampled from each section and each stem was split open to identify and count larvae/pupae, dead or live. Live specimens (larvae/pupae) were taken to the laboratory for rearing and further studies. Plates 3.5 and 3.6 represent scenarios in field sampling procedures. Biological parameters estimated include percentage pest infestation and the relationship between rainfall, temperature and relative humidity on the incidence of larval/pupa numbers. Statistical software programme, *Statistica*, was used to determine correlation between the climatic factors and larval numbers.

Plate 3.3 Maize plant (foreground) showing symptoms of dead-heart caused by borer infestation

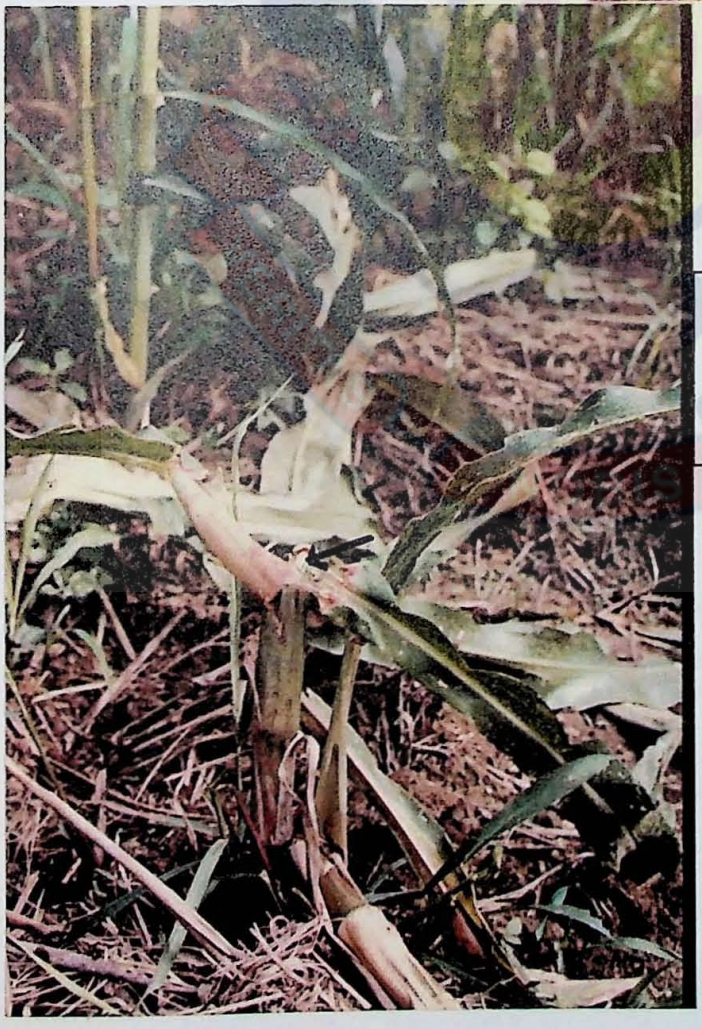


Plate 3.4 Maize stem destroyed by lodging activity of borers

Plate 3.5 A Field Assistant sampling in a maize farm

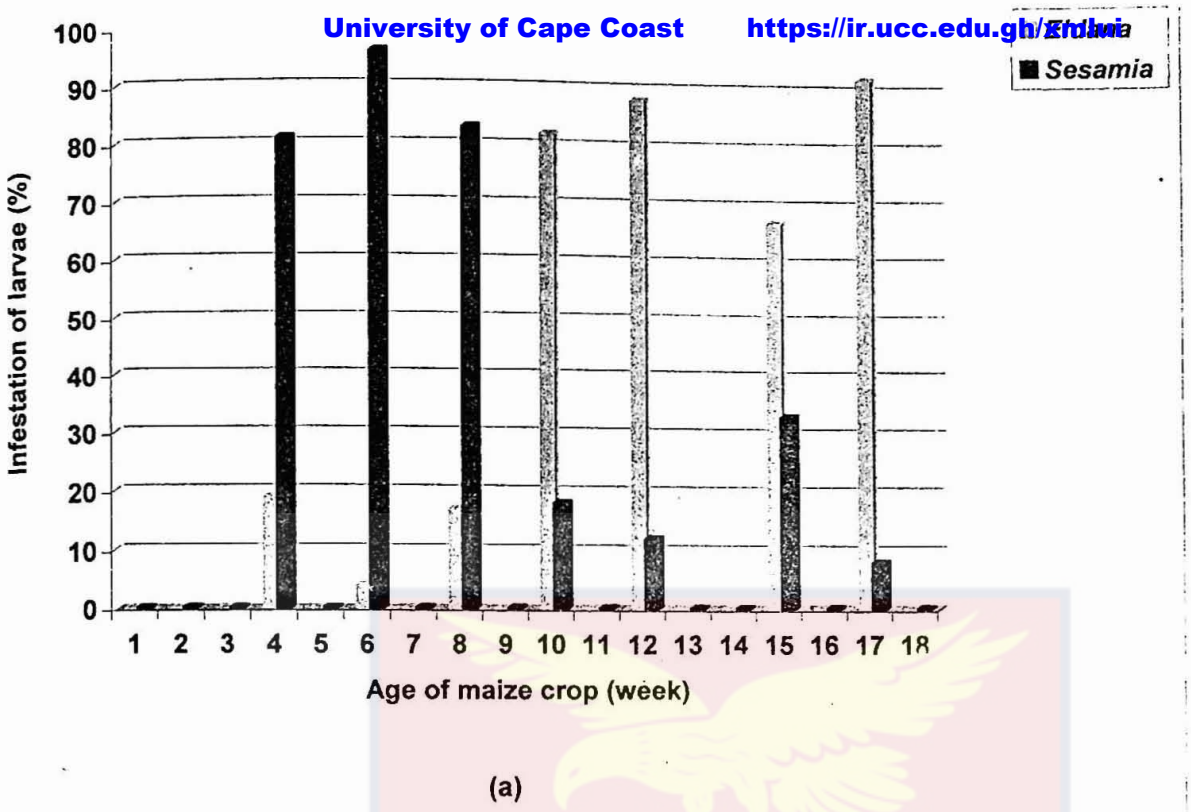
Plate 3.6 Dissected maize stem showing a stem borer species.

3.4. Results

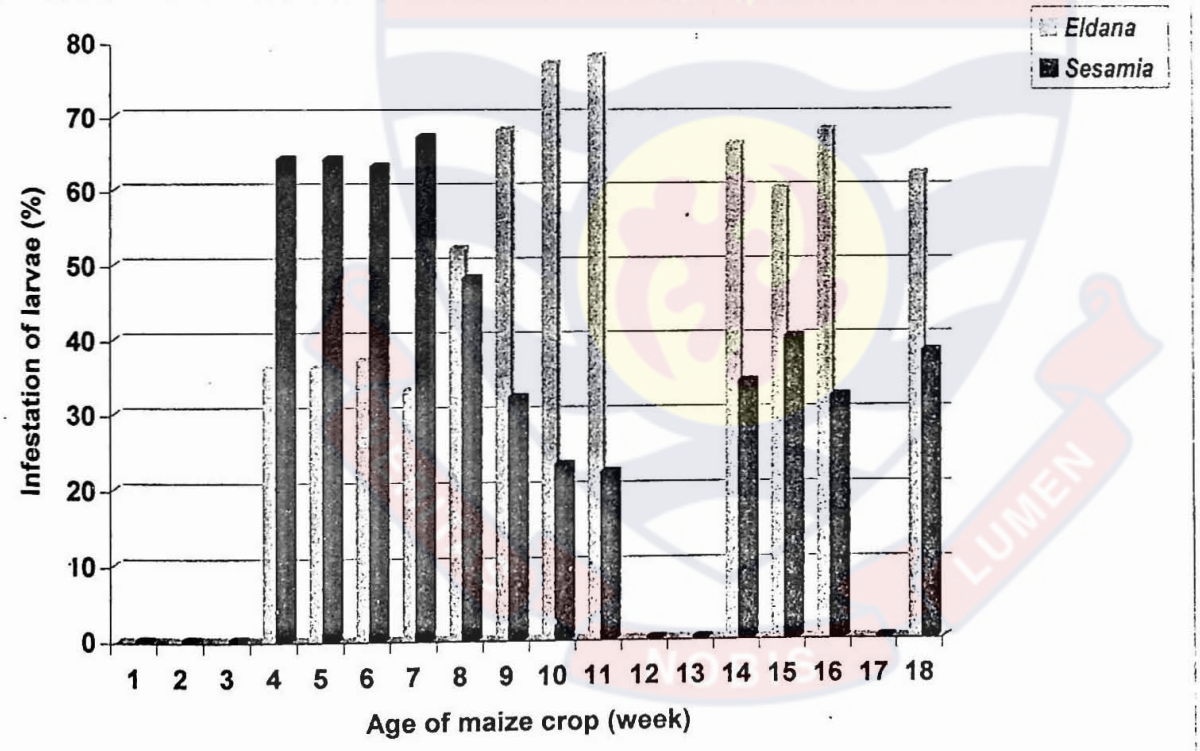
3.4.1. Age of maize plants and borer infestation

The age of the maize crop in relation to borer infestation is shown in Fig. 3.4, (a) and (b) for minor and major rainy seasons respectively. Larvae of *S. calamistis* usually attacked maize crops at the early age with peak infestation occurring between 6-8 weeks after germination. During the minor rainy season percentage infestation was 96% by the 6th week compared with 57% in the major rainy season in the 7th week.

E. saccharina larvae however, attacked the maize crop at a late growth stage, with percentage infestation peaks around 10-12 weeks after germination. In the minor rainy period the peak of infestation was 92% observed in week 17 and 78% by the 11th week in the major rainy season. This study has shown that larval infestation levels were relatively higher during the minor season than the major season for both *S. calamistis* and *E. saccharina*.



(a)



(b)

g. 3.4 Age of maize crop in relation to incidence of larvae in (a) minor season and (b) major season

3.4.2 *Incidence of larvae in relation to rainfall pattern.*

Farming activity of the people at Medie is largely dependent on the annual rainfall pattern of the area. Mean larval numbers in relation to the rainfall pattern during the period of investigation is shown in Fig. 3.5 (for period 1997 to 1999). It was observed that during the major rainy season, peak rainfall occurred in May/June, recording 299.0 mm. in June 1997, 223.0 mm. in May 1998 and 463.0 mm. in June 1999. The month of November recorded the highest rainfall amount during the minor rainy season, with 130 mm., 129 mm., and 90 mm. for 1997, 1998 and 1999 respectively.

Mean number of larvae for both pest species were however relatively low during the period of heavy rains. Conversely, when rainfall amounts were relatively low, mean larval numbers were comparatively high. In 1997, the mean number of larvae per 75 sampled stems was 0.70 in June for *E. saccharina* and 3.0 for *S. calamistis* in May. In 1998, *S. calamistis* recorded 63.0 and 58.0 larvae in August and September respectively (short dry period) and 52.0 larvae in December for *E. saccharina*. Mean number of larvae of *E. saccharina* was 55.0 in August and 26.0 in October for *S. calamistis* in 1999, when rainfall amounts were relatively low.

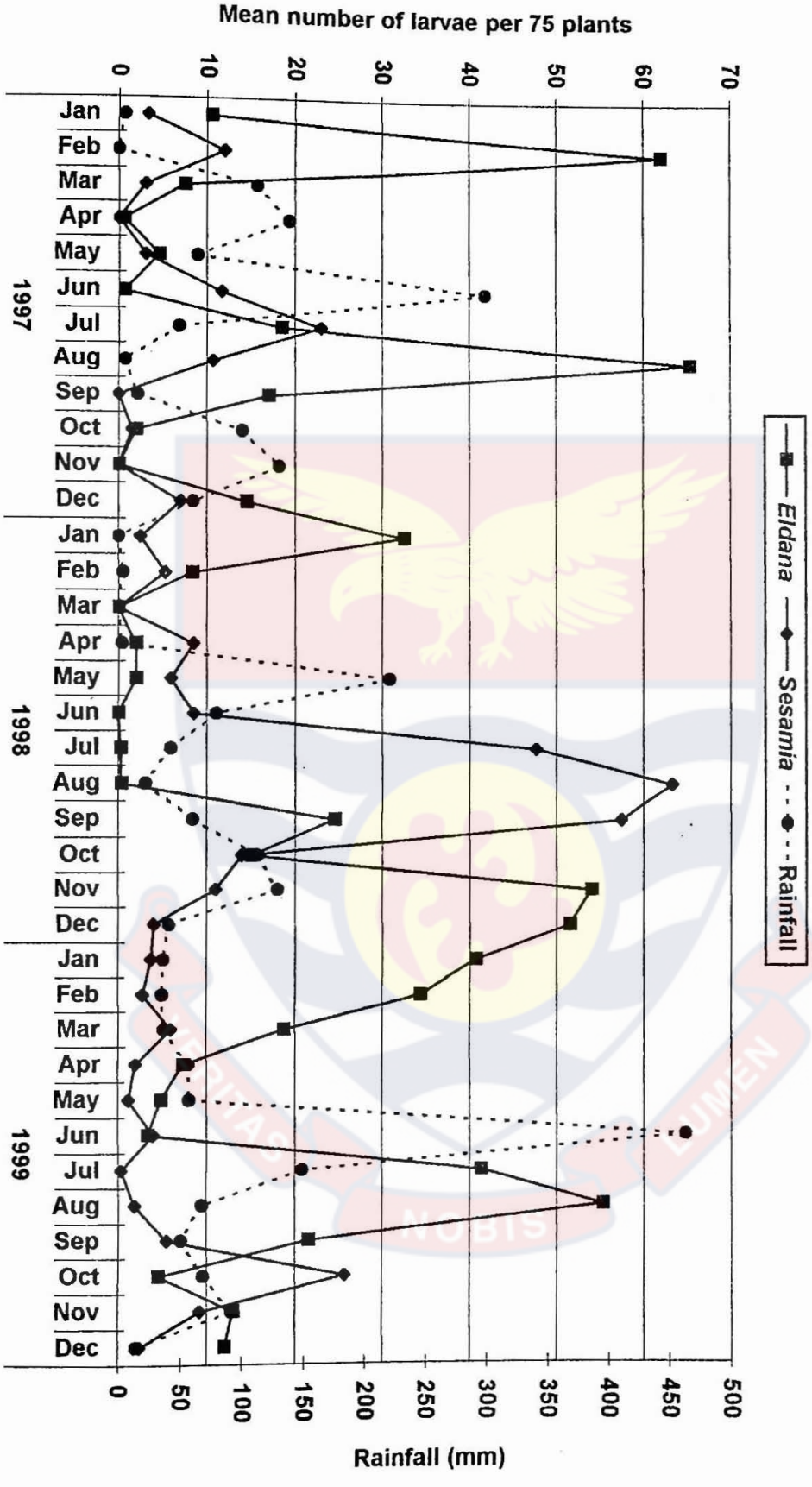


Fig 3.5 Incidence of larvae in relation to monthly rainfall in study area from 1997-1999

3.4.3 *Variations in temperature and relative humidity with larval incidence*

Mean monthly temperature and relatively humidity in relation to incidence of larvae of the two borer species are shown in Figs 3.6 and 3.7 respectively. Average temperature range was between minimum of 26.0°C in July, 1999 and maximum 31.0°C in April, 1998. Mean monthly relative humidity was 70% (minimum) in January, 1998 and 90% (maximum) in July, 1999.

There was a general inverse correlation between larval numbers of the two borer species with rainfall in the respective years. Differences in correlation between the larval numbers and the climatic factors were statistically not significant ($p > 0.05$). However, a few exceptions of significant values were observed. For instance, in 1997, increased larval numbers showed inverse correlation with rainfall amount ($r = -0.5899$; $p = 0.043$) for *E. saccharina* and the corresponding temperature also showed similar trend of correlation with larval numbers of *Sesamia calamistis* ($r = -0.7474$; $p = 0.005$).

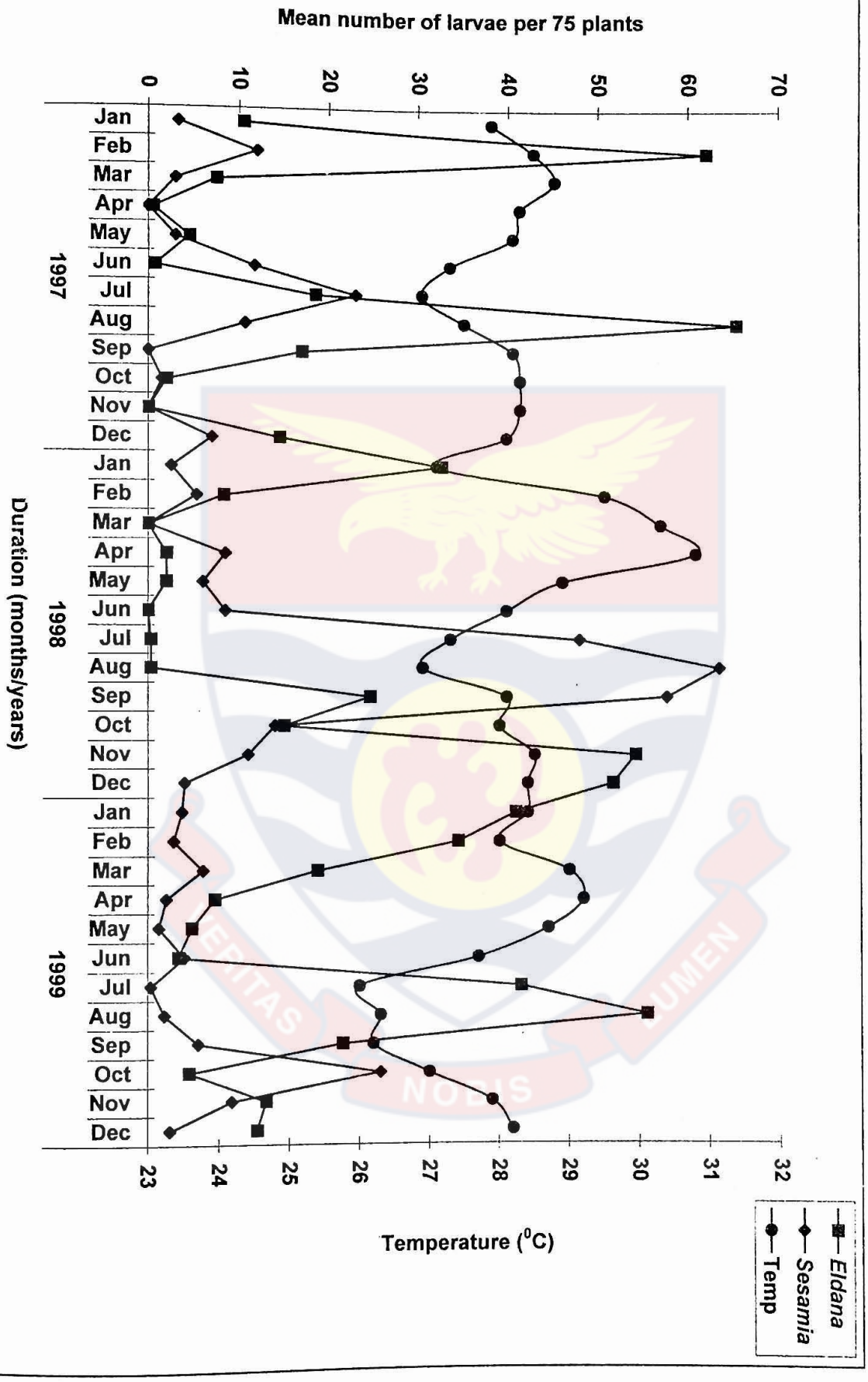


Fig. 3.6 Incidence of larvae in relation to temperature in study area from 1997-1999.

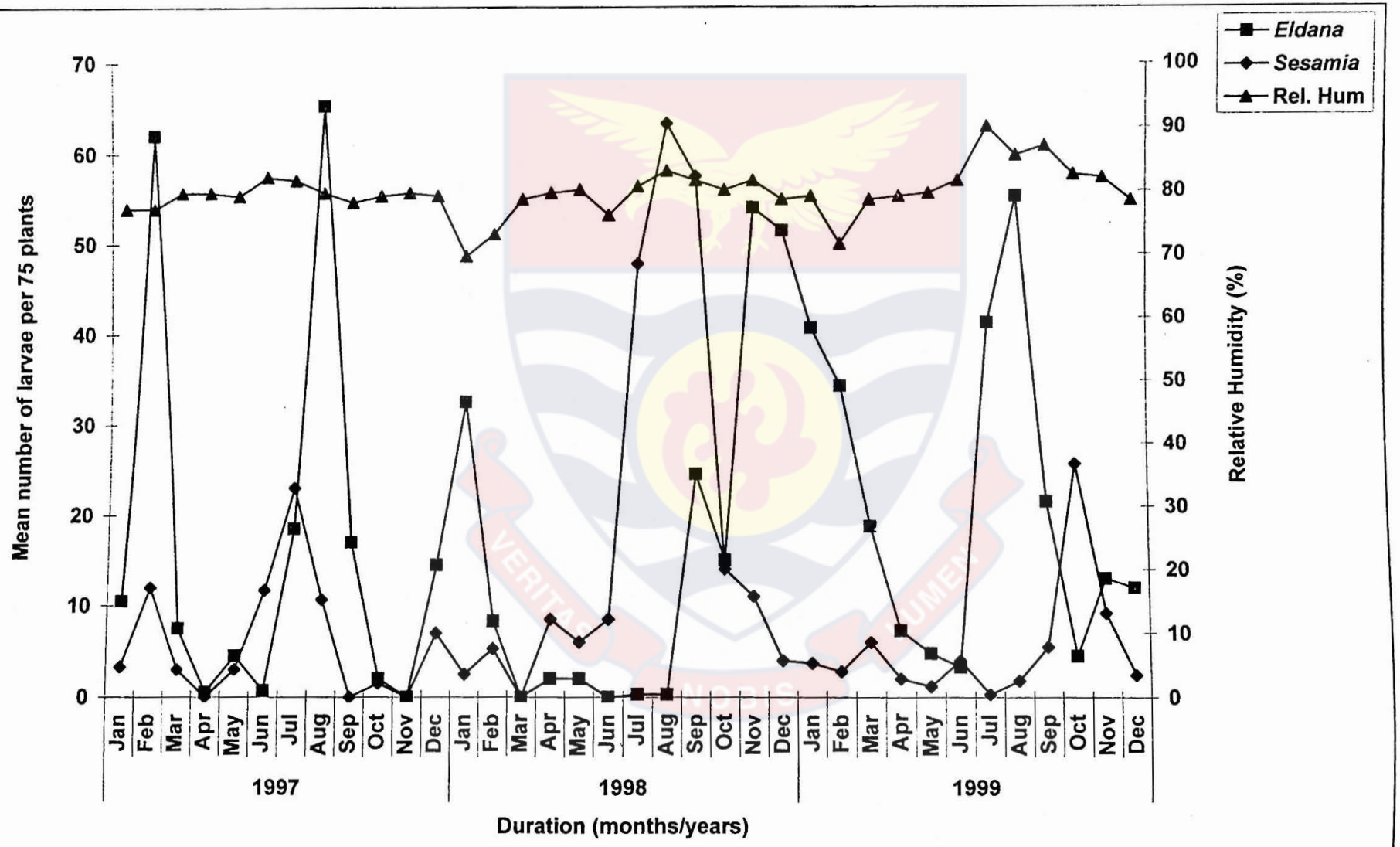


Fig. 3.7 Incidence of larvae in relation to relative humidity in study area from 1997-1999

The attack of *E. saccharina* larvae in preference for mature maize crops, with peaks occurring later than that of *S. calamistis* as observed in this study, is similar to the results of other researchers (Girling, 1978, 1980; Bosque-Perez and Mareck, 1991; Bosque-Perez, 1995). Egg laying of *S. calamistis* usually occurs from the time the maize crops are about 2-3 weeks old until plant flowering, with serious damage occurring at early plant stages (Bosque-Perez, 1995). *S. calamistis* normally appears soft and docile and may therefore find the early stages of the maize crops quite soft and easy to masticate. Possibly, during this stage, the borer could easily extract sufficient mineral salts from the fresh stem for physiological development than when dried. Adults which emerge at the beginning of the main cropping season (major rainy season) tend to be fewer in numbers and less fecund than those emerging later in the year (minor rainy season). The combined effects of fewer numbers and less fecund adults could explain the relatively lower incidence of larval numbers of *S. calamistis* during the first maize cropping season.

Adult females of *E. saccharina* begin laying eggs around flowering time of the maize plants (about 6-8 weeks after germination). Larval development in the field usually takes between 28-38 days and may account for the high larval infestation at the late stage of the maize crop.

Bosque-Perez and Mareck (1991) performed infestation studies of *E. saccharina* larvae on yield of maize at International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. They observed that percentage of plant damaged in plots with natural infestation for the major rainy season was 51%, whilst in the minor rainy season it was 100%. Previous studies have also

own *E. saccharina* to be very abundant in the second rainy season at IITA, Ibadan (Bosque-ez and Mareck, 1990). This might be attributed to rainfall-induced larval mortality, since first rainy season was heavier than the second season. Heavy and frequent rains in the first son could wash off some number of immature stages of stem borers (ie. eggs, neonate-rae), leaving fewer numbers to infest the maize crops. This may account for the general erse relation between rainfall and mean larval numbers of the two borer species.

nperature and relative humidity may not have direct influence on larval numbers of the er species, since the latter live inside maize stems for greater part of their life span. The istical significant difference observed in 1997 with temperature on *Sesamia* could be due to nce and possibly attributed to other factors such as inadequate sample size of borer species.

amou *et al.* (1993) also observed that with increasing acreage of maize cultivation, stem er problems would increase. The yearly fluctuations of stem borer infestation levels in an could also be attributed to maize cultivating practices of the farmers. This assertion seems orroborate with the pattern of maize farming system engaged by the farmers at Medie, re maize is grown almost throughout the year. Farmers cultivate maize in the low-lying erlogged soil during the minor season and move to well-drained loamy soil in the major y season. This creates overlapping maize cropping, which increases the chances of urrence of different age groups of maize, favourable for oviposition and infestation of the borer species. *S. calamistis* for example, would oviposit before tassel formation of e, while *E. saccharina* preferred ovipositing during tassel/post-tassel stage. With this ation created, emerging adults of the borer species would always encounter suitable host ts for oviposition and thereby maintain the survival of progenies of the borers in the area.

This could explain the incidence of the stem borer species in the study area almost throughout the year. This therefore calls for strategic control approach to suppress the pest population, in order to enhance maize yields to sustain the livelihood of the farmers and the people of the community.

The application of inherited sterility as a control strategy involving releases of sterile moths, could be adopted after acquiring knowledge of population dynamics of the pest species, influence of environmental factors like climate and farming practices of the people of the area. From the above observations and discussion, it is suggested that subsequent releases of sterile moths could be performed at periods when generally, the natural populations are relatively low and other environmental factors such as rainfall may not be too frequent to wash them off. These conditions would ensure that the released sterile moths overwhelmingly overcome the natural population and infect the sterility factor into the population. Further investigations in field cage experiments and pilot field trials need be conducted in order to develop a more conclusive protocol on the control strategy.

3.6 Conclusion

Larvae of *S. calamistis* usually attacked maize crops at the early stages of the plant growth and therefore became serious pest at early plant stages. *E. saccharina* however, preferred attacking mature maize plants and therefore larval numbers peaked at late stages of plant. Incidence of larvae of both *E. saccharina* and *S. calamistis* was greatly influenced by the rainfall pattern and farming practices in the study area. Suggested periods of releases of sterile moths may be conducted when larval numbers of the natural population are relatively low and rainfall has subsided. Further study is needed before drawing up a conclusive protocol on control strategy for inherited sterility.

CHAPTER FOUR

EMALE REPRODUCTIVE SYSTEM AND MATING MECHANISMS OF *ELDANA* ICCHARINA AND *SESAMIA CALAMISTIS*

1 Introduction

formation on complete description of the reproductive systems and mating behaviour in pidopteran moths are lacking in many contemporary scientific literature. However, some vestigations on reproductive morphology and mating characters in some species of moths ay be found in a few literature. These include studies on the Mediterranean flour moth, *phestia kuehniella* (William, 1938), corn earworm, *Heliothis zea* (Boddie) (Callahan, 1958), armyworm, *Pseudaletia unipuncta* (Haw.) and variegated cutworm, *Peridroma argaritosa* (Haw.) by Callahan and Chapin (1960) and on a codling moth, *Laspeyresia omonella* (L.) (Ferro and Akre, 1975).

lthough the general reproductive systems of lepidopterous moths are fairly uniform, the ariations in shape, placement and size of the various female accessory organs are extremely ariable (Callahan, 1958). Almost every species has a differently shaped bursa copulatrix and ther structural modifications, which could influence the reproductive behaviour of the female. Male reproductive systems, on the other hand, are quite similar with a few or no norphological and functional differences.

n this chapter, studies were conducted to examine the internal morphology of the female eproductive systems of *E. saccharina* and *S. calamistis*, in order to understand more

thoroughly the reproductive biology and mating behaviour of the two species of stem borers. Attempts were also made to observe and describe mating mechanisms and spermatophore formation, which are essential processes in ensuring mating success and sperm transfer of moths in application of inherited sterility for population control.

4.2 Materials and Methods

4.2.1 Rearing procedure

Live specimen larvae of *E. saccharina* and *S. calamistis* collected from the field were brought to the laboratory. These were reared on fresh maize stem cuttings in transparent plastic containers (dimensions 6 x 6 x 9 cm) until pupal formation. Pupae were removed from the breeding containers and incubated in separate plastic containers for adult emergence.

Moths that emerged were sexed and placed separately in oviposition cages, each consisted of a 3.5-liter transparent plastic bottle with a nylon netting at the sides/bottom for ventilation. Each oviposition cage was provided with a folded cone-shaped white paper for moths to rest or oviposit on. Drinking water/food was provided in the form of 10% sugar solution soaked in cotton wool. A piece of dry white paper was placed in the cage as oviposition substrate.

The initial separation of females from males was to obtain a number of "virgins" or unmated females for dissection, after 2-3 days of emergence, to study the internal reproductive systems. For studies on mating mechanism and spermatophore formation, moths were paired in ratios of 1 female to 1 male in each cage. On certain occasions, special surveillance was kept between the hours of 20 hrs and 04 hrs (the peak of mating) to observe copulating pairs. Such copulating pairs were observed for 10-15 minutes intervals within a period of 90 minutes.

Serial pairs from such surveillance were removed from the cages, labelled and kept in deep freezers for dissection later. The remaining pairings, after the oviposition period of 5-7 days, were removed from the cages for dissection. Eggs oviposited were collected from the cages and incubated to develop into larvae to sustain the laboratory colony.

4.2.2. Dissections and drawings

All specimens were dissected using Belar's saline solution composed of 6 g Sodium Chloride, 0.2 g Potassium Chloride, 0.29 g Calcium Chloride, 0.29 g Sodium Carbonate and water to make 1-liter (Ferro and Akre, 1975). Specimens were dissected in waxed petri-dish placed under dissecting microscope. Some specimens had their abdomens excised and dropped into test-tubes containing Potassium Hydroxide solution (30%), boiled in water bath to remove the soft tissue. This technique allowed a clearer examination of the genitalia and spermatophore in bursa copulatrix after mating.

For each species, 12-15 dissected specimens were used to illustrate the general internal reproductive systems, mating mechanism and spermatophore formation. With the aid of a camera- lucida, the morphology of the internal reproductive structures of the species specimens were drawn and labelled. Additionally, photographs of the reproductive system were taken and the pictures are shown in Plates 4.1- 4.4.

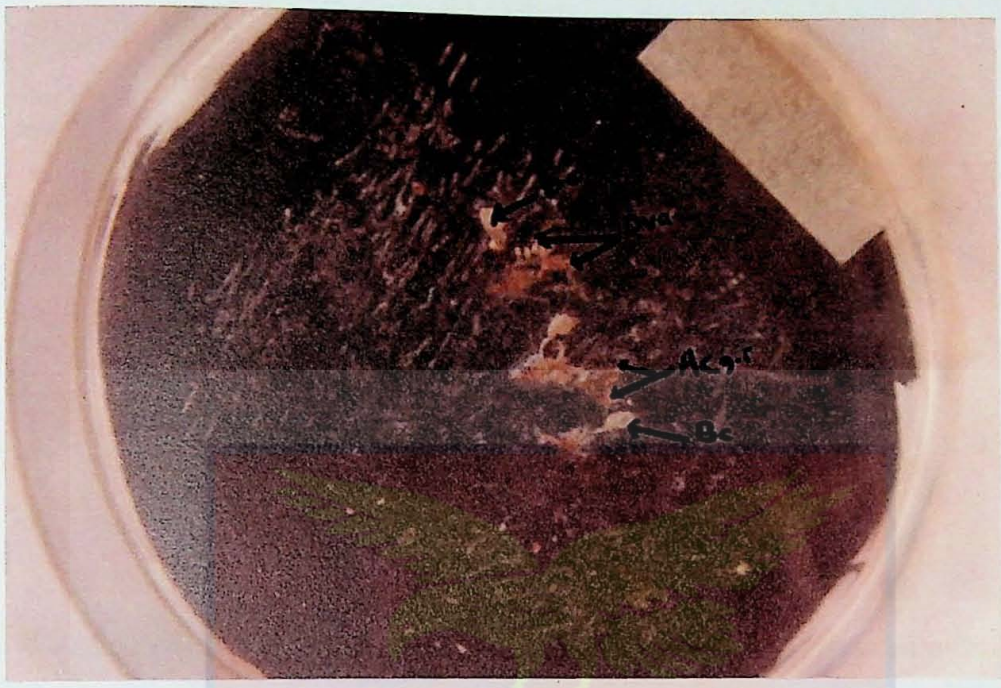


Plate 4.1 ***E. saccharina*** **female reproductive system showing some essential parts.**

Ac. g. r.- ***Paired Accessory gland reservoirs (filled);*** **Bc-** ***Bursa corpulatrix;***

Ova-*Ovary.*

Plate 4.2 ***E. saccharina*** **bursa copulatrix with inseminated spermatophore**

(whitish view). Bc- ***Bursa copulatrix.***

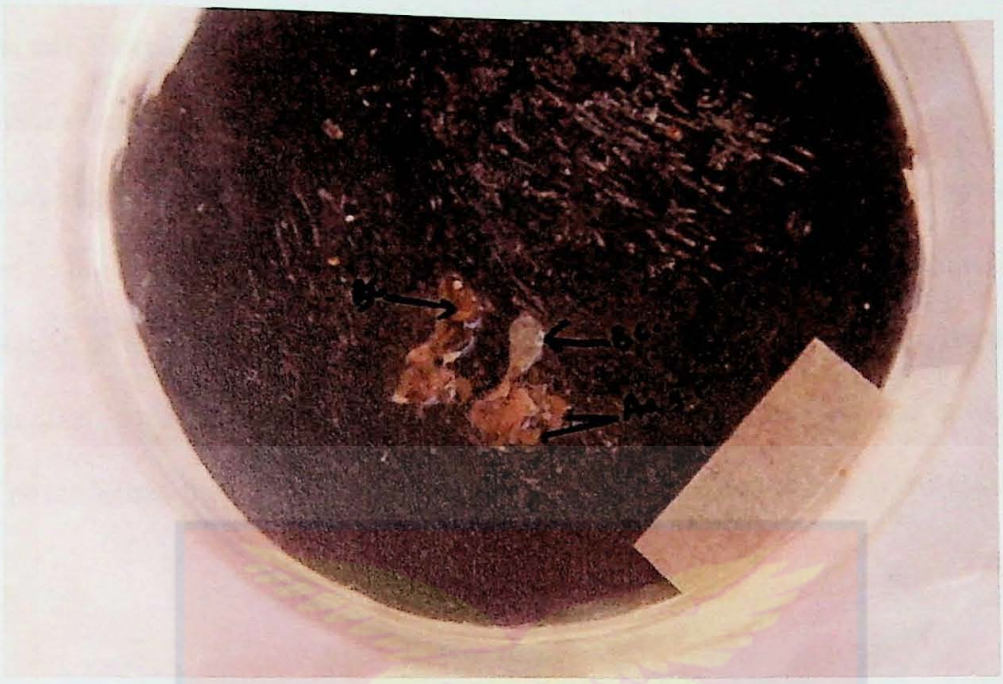


Plate 4.3 *S. calamistis* **female reproductive system showing some essential parts**

Ac. g. r.- ***Paired Accessory gland reserviors (filled)*****; Bc.-** ***Bursa corpulatrix.***

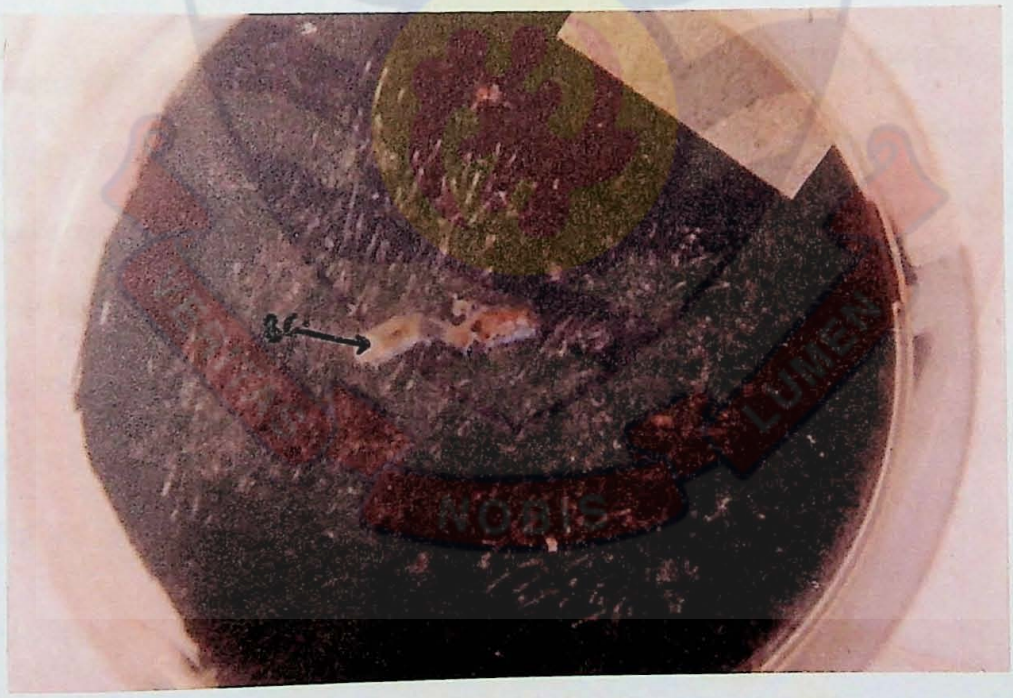


Plate 4.4 *S. calamistis* **bursa copulatrix with inseminated spermatophore**

(whitish view). Bc- ***Bursa copulatrix.***

4.3 Results and Discussion

4.3.1 Structural reproductive system and functions.

The gross morphology of the female reproductive systems of *E. saccharina* and *S. calamistis* are illustrated in Fig. 4.1 and Fig. 4.2 respectively. The diagrams may not necessarily show the actual orientation of the system *in situ*, but are oriented to best display the important organs. Even though there could be a few omissions due to limitations in dissecting and drawing techniques, nevertheless, the illustrations are fairly good representations of specimens used in this study.

4.3.1.1 Bursa corpulatrix

The bursa corpulatrix is considered as the largest and most conspicuous organ in the female reproductive system. It was found lying within the 4^{th}-6^{th} abdominal segments. It could be divided into 3 sections, comprising of the balloon-like expanded portion called the ***corpus bursae***; a narrow but slightly expanded portion towards the anterior end, the ***cervix bursae*** and finally the ***ductus bursae***, leading to the vulva. The corpus bursae receives the spermatophore and may have sclerotized spines or signa on the interior wall as was found in *E. saccharina* but not in *S. calamistis*. The entire bursa corpulatrix of *E. saccharina* appeared sheathed by tough muscles whilst that of *S. calamistis* was membranous. Bursa corpulatrix inseminated with spermatophores for *E. saccharina* and *S. calamistis* are illustrated in Fig. 4.3. and Fig. 4.4 respectively.

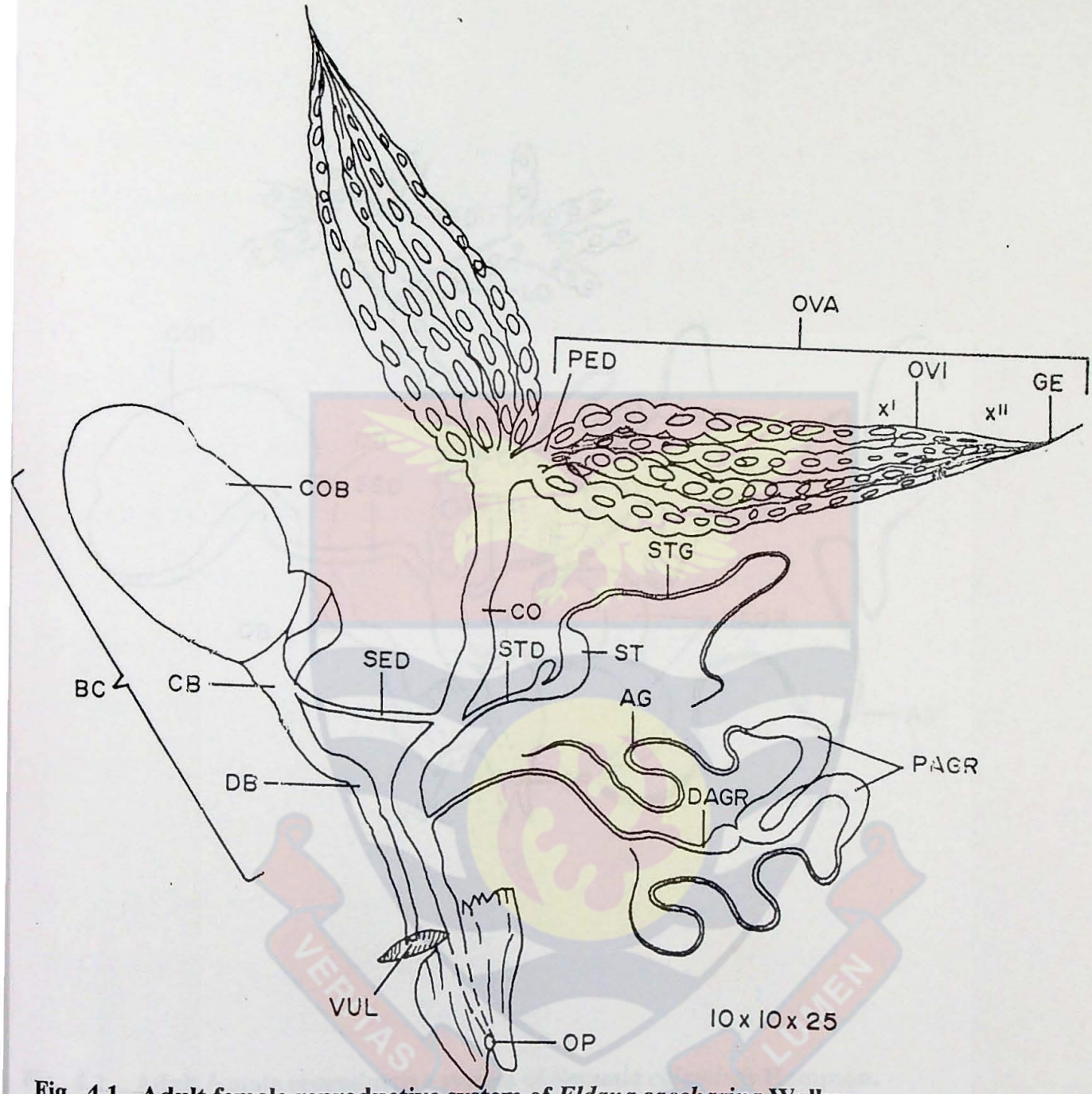


Fig. 4.1 Adult female reproductive system of *Eldana saccharina* Walker

AG- ***Accessory gland*****; BC-** ***Bursa copulatrix*****; CB-** ***Cervix bursae*****;**

CO- ***Common oviduct*** **; COB-** ***Corpus bursae*** **; DAGR-** ***Duct of accessory gland reservoirs*****; DB-** ***Ductus bursae*****; GE-** ***Germarium*****; PAGR-** ***Paired accessory gland reservoirs*****; OP-** ***Ovipore*****; OVA-** ***Ovary*****; OVI-** ***Ovriole*****; PED-** ***Pedicel*****; SED-***Seminal duct*****;**

ST- ***Spermathecae*****; STD-** ***Spermathecal duct;*** **STG-** ***Spermathecal gland*****; VUL-** ***Vulva.***

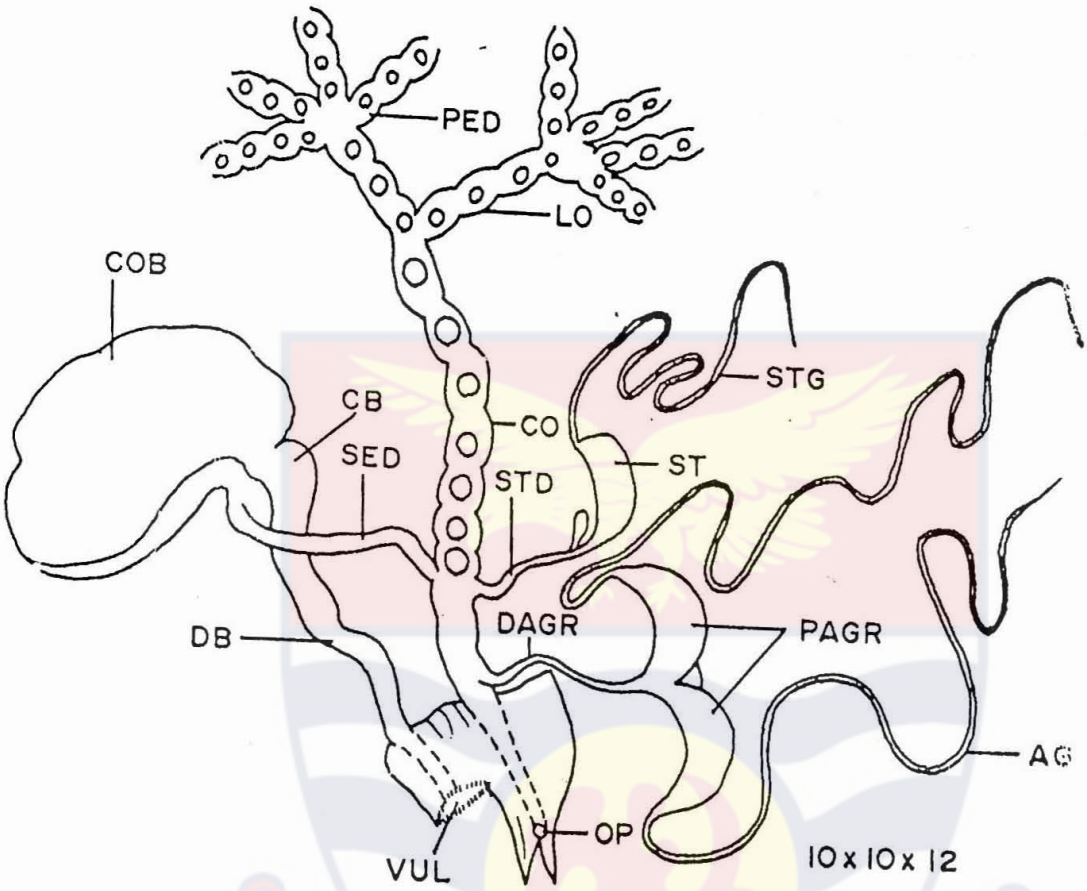


Fig. 4.2 **Adult female reproductive system of *Sesamia calamistis* Hampson.**

AG- ***Accessory gland;*** **CB-** ***Cervix bursae;*** **CO-** ***Common oviduct;***

COB- ***Corpus bursae;*** **DAGR-** ***Duct of accessory gland reservoirs;*** **DB-** ***Ductus bursae;***

LO- ***Lateral oviduct;*** **OP-** ***Ovipore;*** **PAGR-** ***Paired accessory gland reservoirs;***

PED- ***Pedicel;*** **SED-** ***Seminal duct;*** **ST-*Spermathecae;*** **STG-** ***Spermathecal gland;***

STD- ***Spermathecal duct;*** **VUL-** ***Vulva.***

4.3.1.2 Seminal duct

This duct connects the anterior end of the cervix bursae and the ductus bursae and links up with the common oviduct. Sperm discharged from the spermtophore in the corpus bursae are passed through the seminal duct and stored in the spermathecae

4.3.1.3 Spermathecae

In both species, the spermathecae consists of long tubular spermathecal gland, connected to relatively large lobe (***utriculus***) and to a smaller lobe (***lagena***), which is finally joined to the common oviduct by the spermathecal duct. In the cabbage loopers, *Trichoplusia ni*, the apyrene sperm are primarily stored in the lagena region of the spermathecae and the eupyrene sperm located in the utriculus (Holt and North, 1970). This assertion was not investigated in this study due to time constraint and lack of relevant techniques.

4.3.1.4 Accessory glands

In *S. calamistis* (Fig.4.2) the bi-lobed accessory gland reservoirs are joined at their base into a duct to link to the common oviduct, a few millimeters below the points where the seminal and spermathecal ducts also enter the common oviduct. From the apex of the reservoirs emerge two long accessory glands which are known to secrete the adhesive substance used for attaching eggs to surfaces during oviposition (Ferro and Akre, 1975). In *E. saccharina* (Fig.4.1), the accessory gland reservoirs appear some-what "S" shaped. In both species, when "virgin" or unmated females were dissected, the accessory gland reservoirs were very conspicuous and found to be filled with greyish fluid.

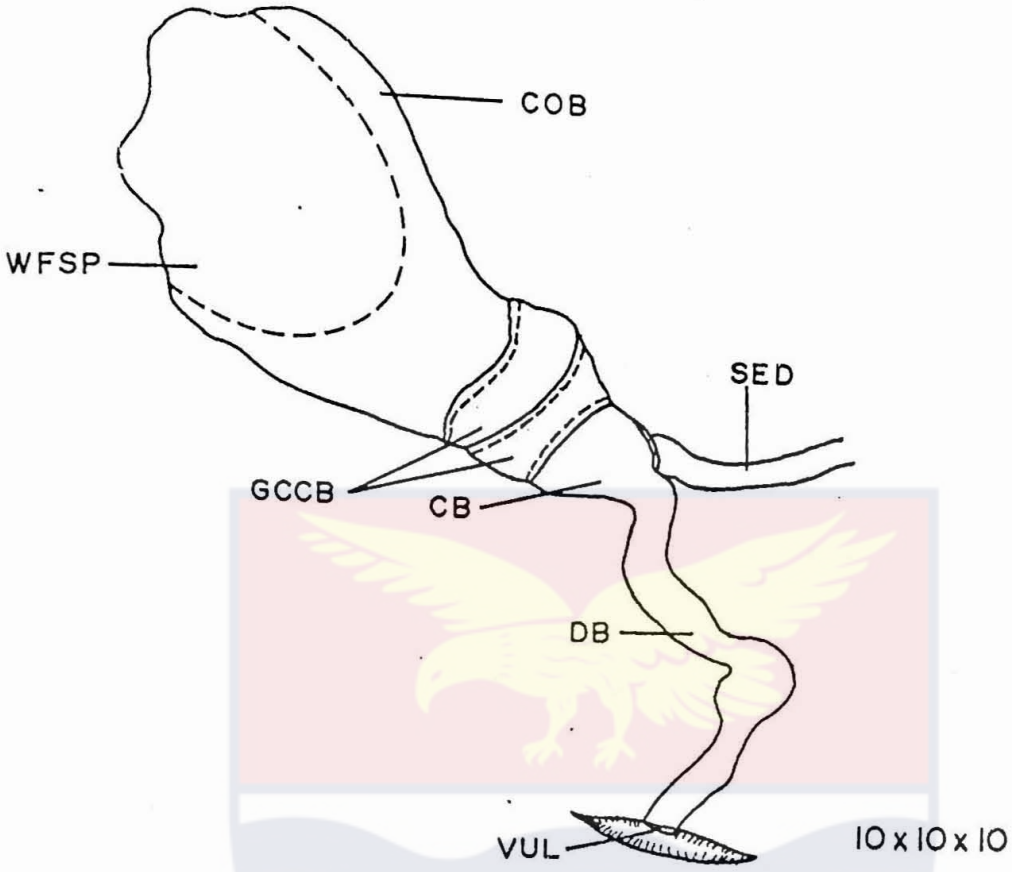


Fig. 4.3 Bursa copulatrix of *Eldana saccharina* expanded by inseminated spermatophore

CB- *Cervix bursae*; COB- *Corpus bursae*; DB- *Dactus bursae*;

GCCB- *Gelatinuous coiling on cervix bursae*; SED- *Seminal duct*;

WFSP- *Whitish transluscent fluid of inseminated spermatophore*;

VUL- *Vulva*.

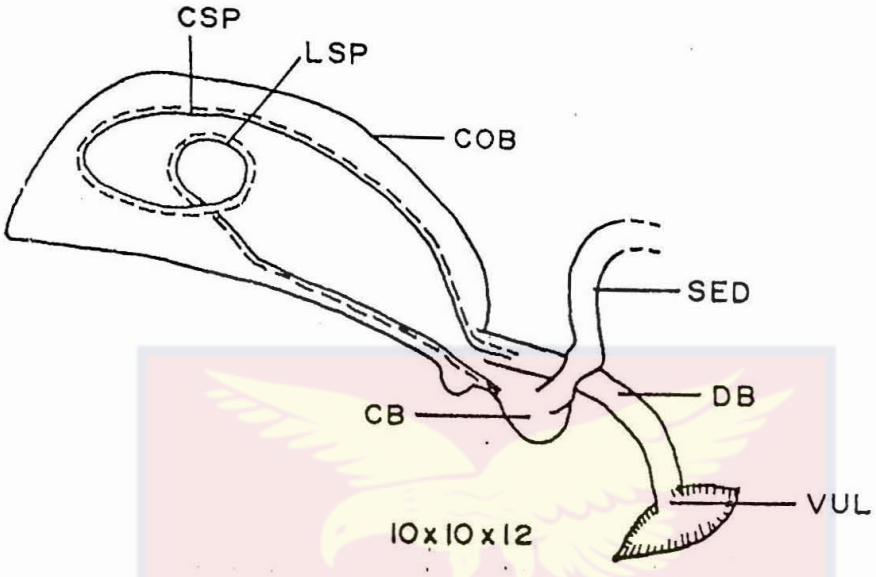


Fig. 4.4 Expanded bursa copulatrix of *Sesamia calamistis* inseminated with spermatophore.

CB- ***Cervix bursae*****; COB-** ***Corpus bursae*****; CSP-** ***Collumn of spermatophore*** **;**

DB- ***Ductus bursae*****; LSP-** ***Loop of spermatophore*****; SED-** ***Seminal duct*****;**

VUL- ***Vulva.***

4.3.1.5 *Ovaries*

Each ovary comprises of four polytrophic ovarioles typical of the Lepidoptera. The ovaries are found lying dorso-laterally along the abdominal wall and extend anterior to the 2nd-3rd abdominal segments. The apical half of each ovary is tightly coiled upon itself and the entire ovaries are held in place by tracheal mesh and fat bodies. Using the illustration of *E. saccharina* (Fig.4.1) in this description, each ovariole can be divided into 3 main sections. The first section stretches from the pedicel to X^{I}, where in mature moths, fully matured eggs are usually stored. This region, also known as the egg chamber contains mature eggs with chorion (averagely 6-10). The second region extends from X^{I} to X^{II}, the vitellarium, where oocytes alternated with nurse cells (trophocytes). The remaining portion, the germarium is where oogonia are formed from follicle cells (cystocytes) in the germ cells. The four germaria of each ovary were tied closely together by connective tissue.

4.3.1.6 *Ovipositor/Ovipore*

The common oviduct makes an exit at the most posterior end (9th-10th segments), forming the ovipositor lobes, eversible structure of the female. Eggs are deposited through the ovipore.

4.3.2 *Mating and spermatophore formation*

In general, the male reproductive systems of the two borer species are quite similar without any significant morphological and functional differences. Thus, like most lepidopteran males, they all exhibited similar reproductive behaviour. During mating, males of *E. saccharina* and *S. calamistis*, as observed in many other moths, approaches females from the rear and grasps them with their claspers during copulation, leaving the pairs facing the opposite direction (Plate 4.5).

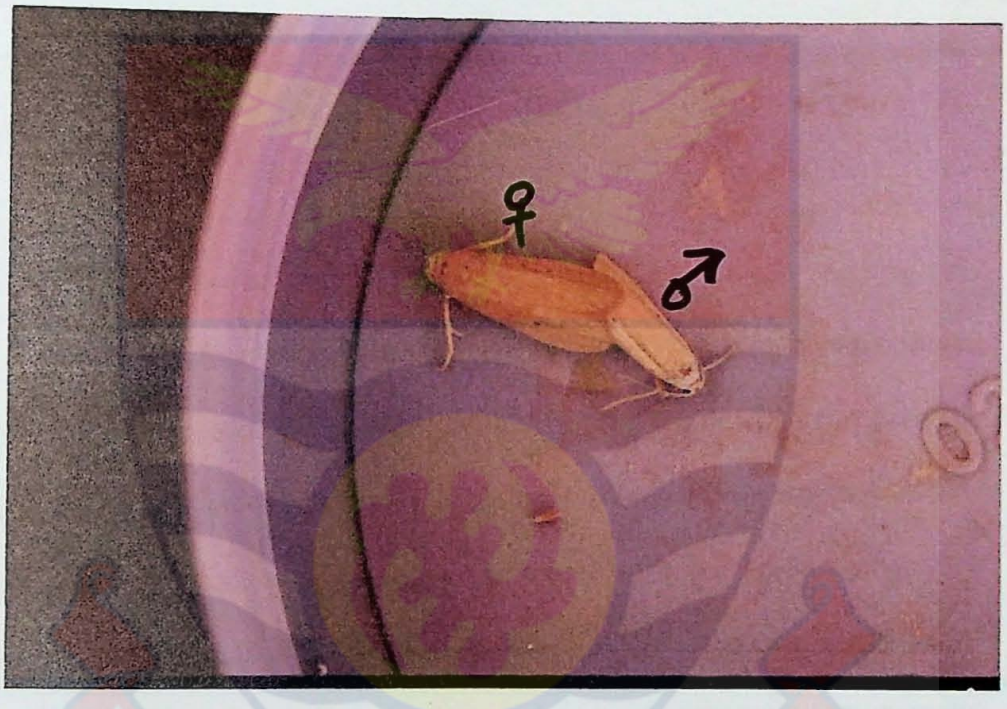


Plate 4.5 **Paired *E. saccharina* adults *in copula:* male (♂) and female (♀).**

Within a few minutes of initial stages of copulation, the aedeagus of the male was inserted through the vulva of the female and into the ductus bursae, with the everted endophallus in the cervix bursae. The cornuti (spines) on the endophallus attached themselves to the sclerotized plate in the cervix bursae, giving the male a firm grip on the female. At the same time, the primary simplex, a section of the male reproductive structure would be secreting the precusor of the spermatophore in a form of resilient tube into the cuticular simplex at the terminal end of the male reproductive system. This eventually formed the spermatophore, which would be forced into the bursae corpulatrix via the endophallus. As the spermatophore was being secreted, the signa in the corpus bursae would be pushed backwards with the spermatophore forming around the signa.

The spermatophore ultimately formed around the signa in a harden state, usually resulting in a coiled sclerous tube as was found in *E. saccharina* and similar to the codling moth, *Laspeyresia pomonella* (Ferro and Akre, 1975). In the codling moth, the signa aided in retaining the spermatophore in the corpus bursae as the male aedeagus was being withdrawn. Sometimes, mating pairs of *E. saccharina* were found stuck together and could not separate after copulation. This could be due to some aberrations in the structural formation of spermatophore. Possibly the inter-locking forces between the spines of the male cornuti on the endophallus and the signa in the corpus bursae could not easily unlock during withdrawal of the male aedeagus after mating.

4.3.3. Sperm transfer

In the codling moth, *L. pomonella*, it was reported that soon after the formation of the spermatophore, the male ejaculated the milky seminal fluid containing mostly apyrene sperm

into the spermatophore and immediately followed by the eupyrene sperm bundles, a more compact substance (Ferro and Akre, 1975). Whilst in the lumen of the spermatophore, the apyrene was highly motile, with eupyrene sperm bundles remaining inactive. After a few hours in the spermatophore, the eupyrene sperm bundles began to break down and was carried out of the corpus bursae into the cervix bursae through the medium of apyrene sperm. The sperm mass, composed of eupyrene surrounded largely by highly motile apyrene sperm, then entered the seminal duct and finally ended in the spermathecae, where they were stored for fertilization of matured eggs.

This trend of sperm transmission is similar to most lepidopteran moths (Callahan, 1958; Callahan and Chapin, 1960; Taylor, 1967; North and Holt, 1968; Holt and North, 1970) and *E. saccharina* and *S. calamistis* could also exhibit the same trend. In preliminary investigations made of the process of sperm transfer in the cabbage lopper, Holt and North (1970) showed that the apyrene bundles break down into individual sperm immediately they exit from the testis of the male. However, the eupyrene bundles did not break down until they have been transferred to the bursa copulatrix in the spermatophore. The eupyrene and apyrene sperms then migrated from the bursa copulatrix through the seminal duct to the spermathecae. Photographs of some features of apyrene and eupyrene sperms of *E. saccharina* are represented in Plates 4.6-4.7.

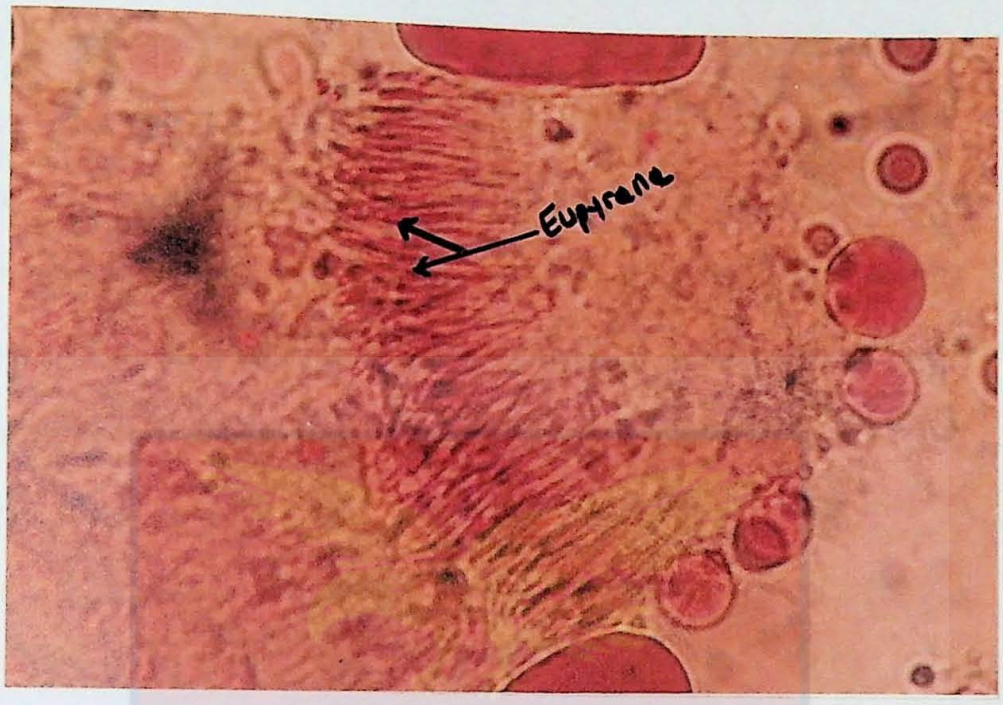


Plate 4.6 Sperm bundle of eupyrene of *E. saccharina* about to disperse

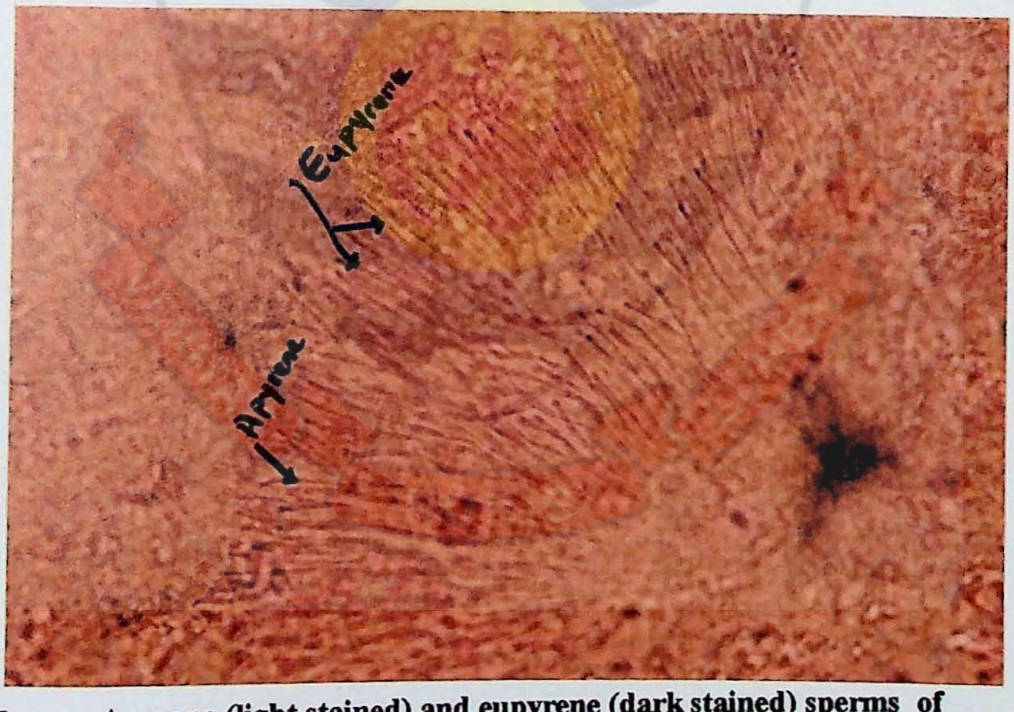


Plate 4.7 Apyrene (light stained) and eupyrene (dark stained) sperms of *E. saccharina* dispersed.

4.4 Conclusion

The female reproductive system in both *E. saccharina* and *S. calamistis* has the basic structural features as found in many moths, but show differences in shape, size and other modifications. This could account for some differences in reproductive biology of the two borer species. The male reproductive biology is however, not too different from that of other moth species, in relation to their mating mechanisms and sperm transfer. These characteristic features observed in this study for *Eldana* and *Sesamia* species, are comparable with other moths species that have stood the test of inherited sterility for population suppression. It could therefore be anticipated that *E. saccharina* and *S. calamistis* would equally offer themselves as viable specimens in the application of inherited sterility to suppress the insect populations in Ghana.

CHAPTER FIVE

BIOLOGICAL CHARACTERISTICS OF LABORATORY REARED NORMAL PUPAE AND ADULT EMERGENCE OF IRRADIATED PUPAE OF *ELDANA SACCHARINA* AND *SESAMIA CALAMISTIS*

5.1 Introduction

The pest problems caused by the two stem borer species are quite complex in nature and require multi-faceted approach to bring the situation to minimal levels. In general, damage caused by the borers reduces yields by destroying the growing points (dead-heart), causing early leaf senescence and lodging due to weakened stems (Bosque-Perez and Mareck, 1990). Some researchers have applied the principle of induced inherited sterility for suppression of lepidopterous pest populations (Carpenter *et al.*, 1989; Dyck *et al.*, 1993; Seth and Reynolds, 1993; Bloem and Bloem, 1996; 2000). The successful application of inherited sterility to suppress a wild population of the corn-ear worm, *Helicoverpa zea* (Boddie) during a pilot test in the USA, has since encouraged further development of this control strategy (Carpenter *et al.*, 1989).

One of the essential conditions for successfully implementing the inherited sterility principle in a control programme is to establish laboratory rearing facility for colonies of the pest species. There is also the need to determine the suitable developmental stage of the insect where optimal radiation doses could be applied to induce sterility. Many researchers studying radiation-induced sterility in insects have irradiated all life stages, including eggs, larvae, germ cells, pupae and adults (Savhagen, 1963; LaChance and Leverich, 1962; Ercelik and Holt, 1972; Bartlett *et al.*, 1973). In general, irradiation of early stages of insects to induce

sterility has great damaging consequences. The pupal stage of the lepidopterous insect is however, found to be suitable for irradiation (North, 1975). The pupa is easy to handle with least interference of bodily structures and also the most stable stage of the insect with little or no movement. Toba and Kishaba (1973) observed that the reproductive capability of cabbage looper, *Trichoplusia ni* irradiated as mature pupae was similar to that when adults were irradiated.

This chapter reports on the laboratory rearing of *E. saccharina* and *S. calamistis* larvae on both natural and artificial diets to compare their developmental differences. The study was also aimed at observing and collating information on some biological characteristics such as differences in weight, sizes, survival and adult emergence of pupae of the two borer species. Different pupal age-groups of the stem borer species were also irradiated at sub-lethal doses, (80-180 Gy), to determine the suitable age of effecting adult emergence with minimum or no bodily deformities for induced inherited sterility. Information from these studies would be essential for establishing laboratory mass rearing of the borer species and thereby enable the assessment of the competitiveness of laboratory reared adults emerged from irradiated pupae, to induce sterility in F_1 progeny for pest management programme.

5.1. Materials and Methods

5.2.1. *Natural Dieters*

Larvae of *E. saccharina* and *S. calamistis* collected from the field were brought to the laboratory and reared in transparent plastic containers (12 cm x 8 cm diameter), whose lids or bottoms were cut and covered with nylon netting to allow sufficient ventilation. About 3-5 larvae of the same age group were kept in each container. They were supplied with fresh

maize stem cuttings, two times a week and observed daily until they developed into pupae. Larval mortality, longevity, and instar numbers were noted and recorded.

On the first day of pupal formation, the length (size) of each pupa was measured with a graduated ruler and the weight recorded, using analytical electronic balance (max. wt. 310 g; decimal pt. approx. 0.1 mg). Subsequently, each pupa was weighed daily, placed singly in a glass test tube, and observed until adult emergence. The sexes of the emerged adults were observed and recorded. The emerged moths were paired in a ratio of 1 female to 1 male and placed in oviposition cages, each consisted of a 3.5-liter transparent plastic bottle. The sides or bottom of the cages were covered with nylon netting for ventilation. A piece of white paper was placed at the base of each cage as oviposition substrate, in addition to cone-shaped white paper in a slanting position for moths to rest as well as oviposit on. Food or drinking water was provided in a form of 10% sugar solution, soaked in a cotton wool and placed in each of the cages. Daily observation of oviposition, egg hatchability, and moth longevity was noted. For each borer species, cohorts of 25 larvae, pupae and adults, in 4 replications each, were set up and observed for studies.

5.2.2 *Artificial Dieters*

Some neonate larvae emerged from hatched eggs produced by mated adults as described above, were randomly selected and fed on artificially formulated diet in petri-dishes (10 cm. diameter) covered with transparent plastic cups (230 ml volume). Diet preparation was based on a modification of International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (after Bosque-Perez and Dabrowski, 1989). The actual composition of the diet used in this study is shown in Table 5.1.

Table 5.1 Composition of artificial diet for laboratory reared *Eldana saccharina* and *Sesamia calamistis* (100 ml ≈ 100 g. diet)

Ingredients	Function/Purpose	Quantity	Percentage
Water (distilled)	Solvent	43.00 ml	42.44
Soya bean flour	Proteins	4.00 g	3.95
Maize stem pith flour	Carbohydrates	6.00 g	5.92
Salt	Mineral salts	0.50 g	0.49
Sugar	Phagostimulant	1.50 g	1.48
Water for agar	Solvent	43.00 ml	42.44
Agar-agar	Binding/gelling agent	1.40 g	1.38
Ascorbic acid	Organic acid/vitamins	0.63 g	0.62
Tetracycline (1 cap)	Antibiotics	0.28 g	0.28
Streptomycin	Antibiotics	0.01 g	0.01
Acetic acid	Stabilizer/antioxidant	0.40 ml	0.39
Formaldehyde (37%)	Fungicide	0.10 ml	0.10
Potassium Hydroxide(10%)	Stabilizer/antioxidant	0.10 ml	0.10
Multivitaplex	Vitamins	0.40 ml	0.39
Folic acid tabs. (1 tab.)	Organic acid/vitamins	0.01 g	0.01

The larval feed was replenished regularly, at least, once in 5-7 days, depending on the rate of consumption and age group of larvae. As many as 10-20 (1^{st}-3^{rd} instars) larvae could be placed together in each petri-dish. The number of larvae was however reduced to 2-5 per dish at the late larval stages (4^{th}-6^{th} instars) to avoid larval mortality due to cannibalism exhibited especially in *E. saccharina*. Oven-heat sterilized petri-dishes and plastic cups were used as feeding containers throughout the period of study.

Larval development was monitored until pupal formation. The length of each pupa was measured and the weight recorded at daily intervals. On the day of adult eclosion, the sexes of the moths were noted. All biological parameters and replications described above (5.2.1) were similarly applied in artificial dieters. Some essential apparatus and instruments used for laboratory studies are shown in Plate 5.1

5.2.3. Irradiation of pupae

Different ages of pupae, 2, 4, 6 and 8 days old of each of the borer species from natural dieters were randomly selected and irradiated separately at sub-lethal doses of 80, 100, 120, 150 and 180 Gy. Pupae of natural dieters were chosen because they were readily available during the study. For irradiation treatment of a particular pupal age, cohorts of 20 pupae of each species were placed singly in separate plastic vials, (6 cm x 2.5 cm diameter), corked with dry cotton wool. The specimens were placed in the gamma source chamber for irradiation. Each treatment was replicated twice. Similar group of pupae was held as a control (0Gy).

Plate 5.1 Some essential apparatus used for laboratory rearing of stem borers.

Radiation treatments were conducted at the Radiation Technology Center (RTC) of Ghana Atomic Energy Commission at Kwabenya, Accra. All specimens were irradiated in air in a Cobalt-60 gamma source (Gamma-cell 220, Canada), delivering average dose rate of about 3.0 $Gy^{-min.}$. Dose calibration using Fricke dosimetry indicated a dose error of ± 5%. All irradiated pupae were taken to the laboratory for daily observation of adult emergence.

5.2.4. Laboratory conditions

All studies and observations were conducted in the laboratory with a temperature range between 26°C and 32°C, relative humidity of 65%-75% and photo-period of 12hrs light and 12hrs darkness. Statistical analysis used included the analysis of variance (ANOVA) and correlation analysis. No data transformation was performed during statistical analysis.

5.3 Results

5.3.1. General comparison of biological parameters

Biological parameters of larvae/ pupae and adults of *E. saccharina* reared on natural and artificial diets are shown in Tables 5.2 and 5.3 respectively. Biological parameters of larvae and pupae on both diets showed slight differences but not statistically significant ($p > 0.05$). Mean larval mortality was 32.5% and 28.3% on natural and artificial diets respectively (Table 5.2). Mean pupal mortality of artificial dieters was slightly higher than that of natural dieters, 22.2% and 17.5% respectively.

Table 5.2 Larvae of *E. saccharina* reared on natural and artificial diets and their resulting pupae (N=100)

Biological parameters	*Natural diet*	*Artificial diet*
Range of larval life period (days)	28-46	30-48
Mean larval life period (days)	37.2 ± 7.8	39.0 ± 5.5
Larval instars	5-6	5-6
Mean larval mortality (%)	32.5 ± 16.7	28.3 ± 15.4
Range of pupal life period (days)	7.0-10.0	7.0-9.0
Mean pupal life period (days)	10.0 ± 1.0	8.2 ± 1.7
Mean pupal mortality (%)	17.5 ± 7.5	22.2 ± 5.2

Table 5.3 The performance of adult *E. saccharina* from larvae reared on natural and artificial diets in the laboratory (N=50 pairs)

Biological parameters	*Natural diet*	*Artificial diet*
Range of adult life period (days)	4-10	3-10
Range of eggs laid per female	115-780	110-540
Mean fecundity (eggs per female)	425± 223	268± 110
Eggs incubation period (days)	4-6	4-6
Mean egg hatchability (%)	82.0 ± 13.9	78.0 ± 15.7
Mean adult emergence (%)	87.0 ± 13.8	77.0 ± 1.7
Sex ratio	1.2 female : 1.0 male	1.2 female : 1.0 male

Mean fecundity (eggs laid per female) on natural diet was higher than that on artificial diet, 425 and 268 respectively with their corresponding percentage of egg hatchability of 82% and 78% respectively (Table 5.3). Sex ratios of emerged adults of both diets was 1.2 female to 1.0 male, with slightly more females than males.

Mean larval mortality and pupal mortality of *S. calamistis* were comparatively higher in artificial dieters, 37.2% and 58% respectively than their corresponding values in natural dieters, 30% and 27% respectively (Table 5.4). Differences between pupal mortality of natural and artificial diets were significant ($p<0.05$).

In this study, eggs laid per female in *S. calamistis* was very low, 192.0 and 80.0 on natural and artificial diets respectively (Table 5.5), compared with that of *E. saccharina*. Adult emergence in natural dieters was 84% but drastically reduced to 42% in artificial dieters, probably due to deformities in the pupae. For sex ratio, more males than females were found in artificial dieters, 1.0 females to 1.6 males, than in the natural dieters, 1.0 females to 1.0 males.

Table 5.4 **Larvae of *S. calamistis* reared on natural and artificial diets and their resulting pupae (N=100)**

Biological parameters	*Natural diet*	*Artificial diet*
Range of larval life period (days)	38-43	41-51
Mean larval life period (days)	41.3 ± 2.3	44.5 ± 3.6
Larval instars	5-6	5-6
Mean larval mortality (%)	30.4 ± 12.2	37.2 ± 5.6
Range of pupal life period (days)	10-13	10-12
Mean pupal life period (days)	11.0 ± 1.4	10.3 ± 1.3
*Mean pupal mortality (%)	27.0 ± 16.0	58.0 ± 22.2

* Significant difference ($p < 0.05$)

Table 5.5 **The performance of adult *S. calamistis* from larvae reared on natural and artificial diets (N= 50 pairs)**

Biological parameters	*Natural diet*	*Artificial diet*
Range of adult life period (days)	3-13	4-12
Range of eggs laid per female	68-450	16-180
*Mean fecundity	192 ± 103	80.0 ± 54.3
Eggs incubation period (days)	4-6	5-7
Mean egg hatchability (%)	74.0 ± 17.9	65.4 ± 9.7
*Mean adult emergence (%)	84.0 ± 17.8	42.0 ± 9.0
Sex ratio	1.0 female : 1.0 male	1.0 female : 1.6 male

* Significant difference ($p < 0.05$)

5.3.2. *Some biological characteristics of pupae*

Variation in mean pupal weight in relation to pupal length of natural and artificial dieters of *E. saccharina* is shown in Fig. 5.1. Increase in pupal length was directly proportional with mean weight of both dieters. Pupal length for natural dieters of *E. saccharina* ranged from a minimum of 11 mm to maximum of 16 mm with their corresponding mean weights of $5.64 \pm 0.65 \times 10^{-2}$ g and $16.70 \pm 0.36 \times 10^{-2}$ g respectively. Artificial dieters recorded minimum length of 9 mm and maximum of 14 mm with their respective mean weights of $2.70 \pm 0.36 \times 10^{-2}$ g and $10.20 \pm 1.22 \times 10^{-2}$g.

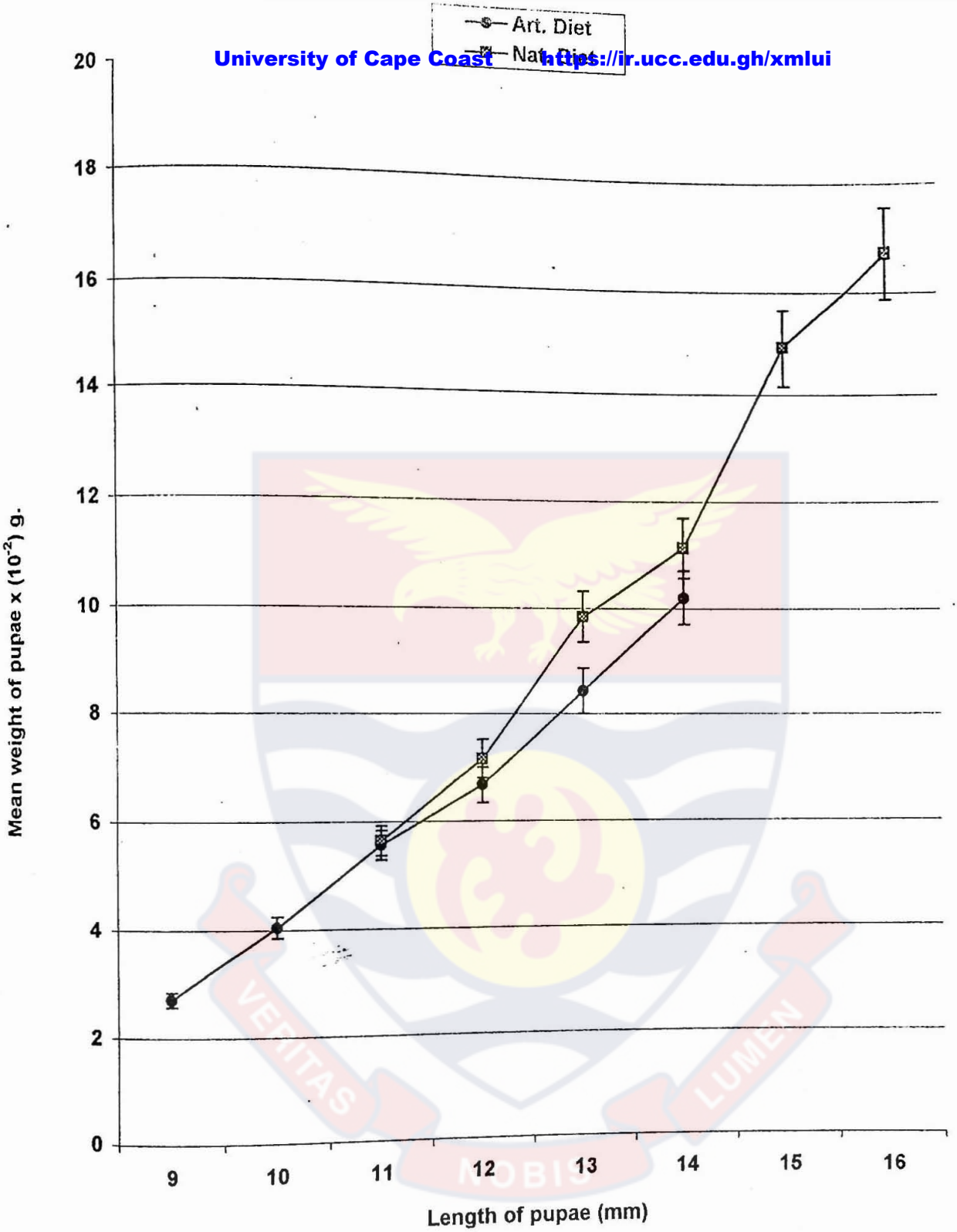


Fig. 5.1 Relationship between pupal weight and pupal length of *E. saccharina* reared on artificial and natural diets (Vertical bars =+/- 1.0 SD)

Mean pupal weight of natural and artificial dieters of *S. calamistis* generally increased with increased pupal length (Fig. 5.2). Pupal length of natural dieters ranged from minimum 12 mm to maximum 22 mm, with their respective mean weights of $8.96 \pm 0.41 \times 10^{-2}$ g and $32.46 \pm 1.54 \times 10^{-2}$ g. Artificial dieters have pupal lengths ranging between 11 mm minimum and 15 mm maximum with their corresponding mean weights of $6.96 \pm 0.23 \times 10^{-2}$ g and $13.31 \pm 0.30 \times 10^{-2}$g respectively

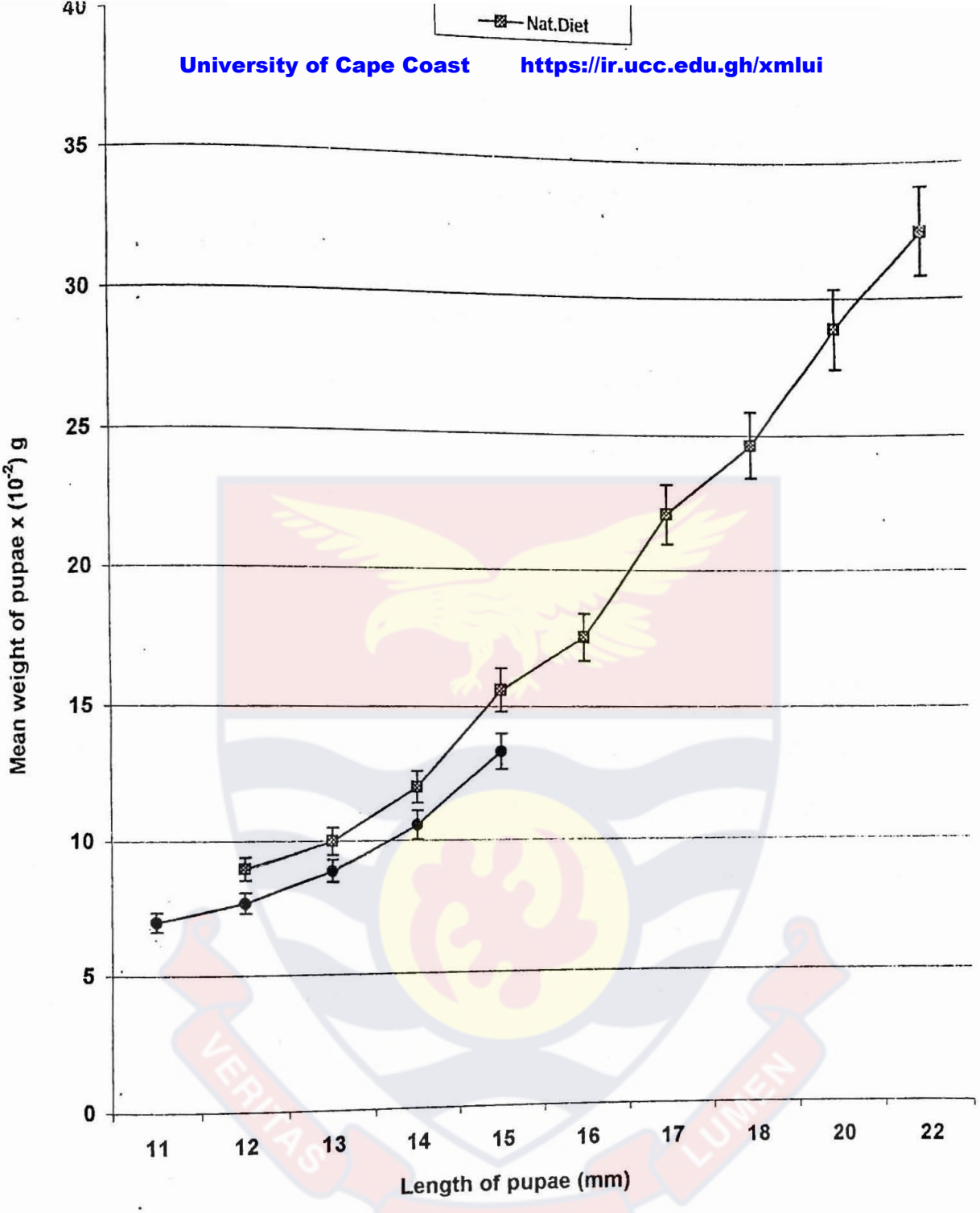


Fig. 5.2 Relationship between pupal weight and pupal length of *S. calamistis* reared on artificial and natural diets (Vertical bars = +/- 1.0 SD)

Correlation analysis revealed positive correlation between weight and length of pupae developed from larvae reared on both natural and artificial diets for the two borer species. For *Eldana*, the correlation coefficient values were r= 0.9943 and 0.9975 on natural and artificial diets respectively. The correlation coefficient values were r= 0.9932 and r= 0.9720 for *Sesamia* on natural and artificial diets respectively. Differences of mean among the weights were also significant at 5% level of probability, with p= 0.0275 and p= 0.0150 for *E. saccharina* and *S. calamistis* respectively.

Change in pupal weight with respect to age of pupae for lengths of 12 mm and 14 mm developed from larvae reared on natural and artificial diets for *E. saccharina* are represented in Fig. 5.3 and Fig. 5.4 respectively. The two pupal lengths/sizes (12 mm and 14 mm) were chosen to represent the average of the minimum and maximum sizes, since all the various pupal sizes of the species showed similar trends of increased in age with decrease in pupal weight. Correlation analysis performed for natural and artificial dieters (12 mm-sized pupae), showed negative correlation but difference of mean not significant (r= -0.9726 and p= 0.1483). Similar trend in correlation was observed for 14 mm-sized pupae with r= -0.9731 and p= 0.0454.

The regression equations for *E. saccharina* (12 mm-sized pupae) are given by: y= -0.404x + 8.68 (artificial dieters) and y= -0.304x + 8.69 (natural dieters), where y= mean pupal weight (g) and x= age of pupae (in days). Similarly, for 14 mm-sized pupae of artificial dieters y=-0.43x + 12.35 and y= -0.42x + 13.31 for natural dieters.

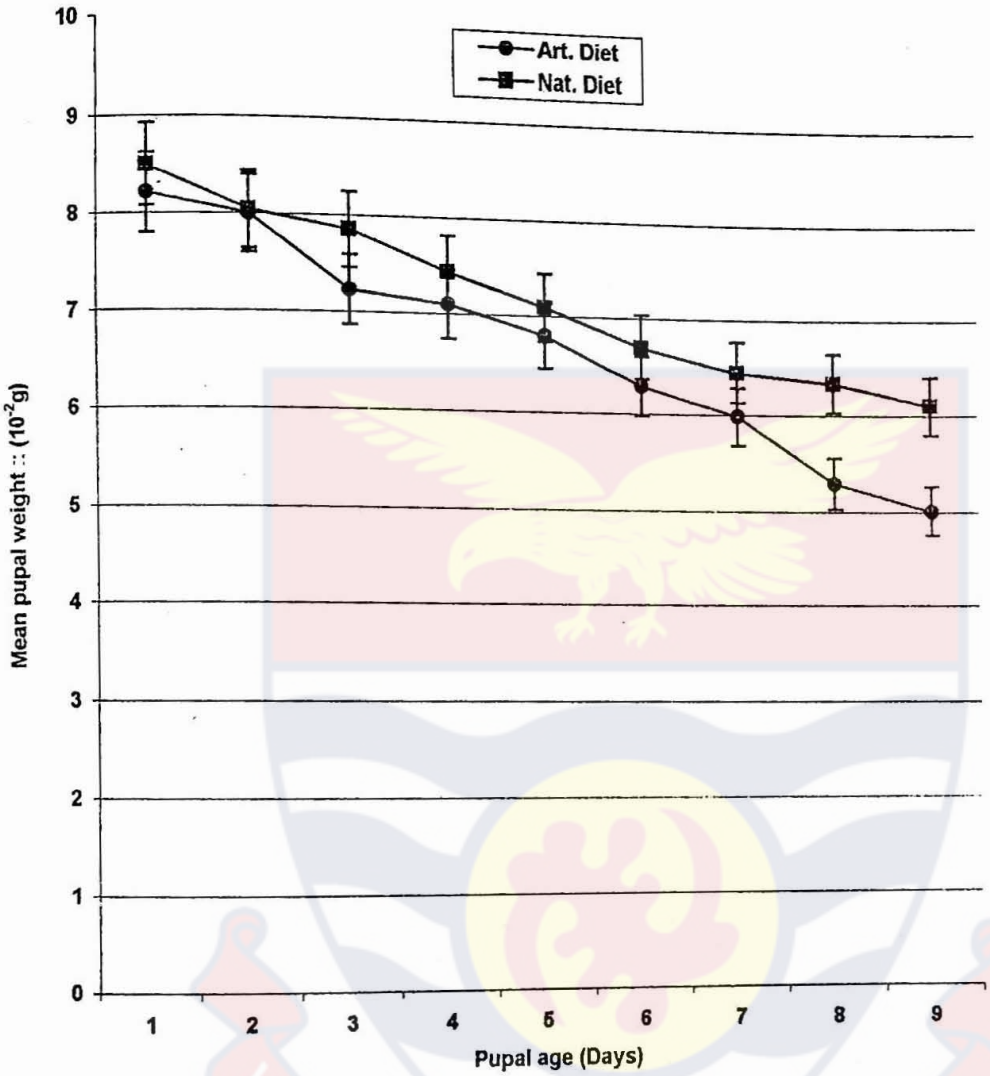


Fig. 5.3 Change of pupal weight with age of *E. saccharina* reared on artificial and natural diets (12 mm- sized pupae; Vertical bars = +/- 1.0 SD)

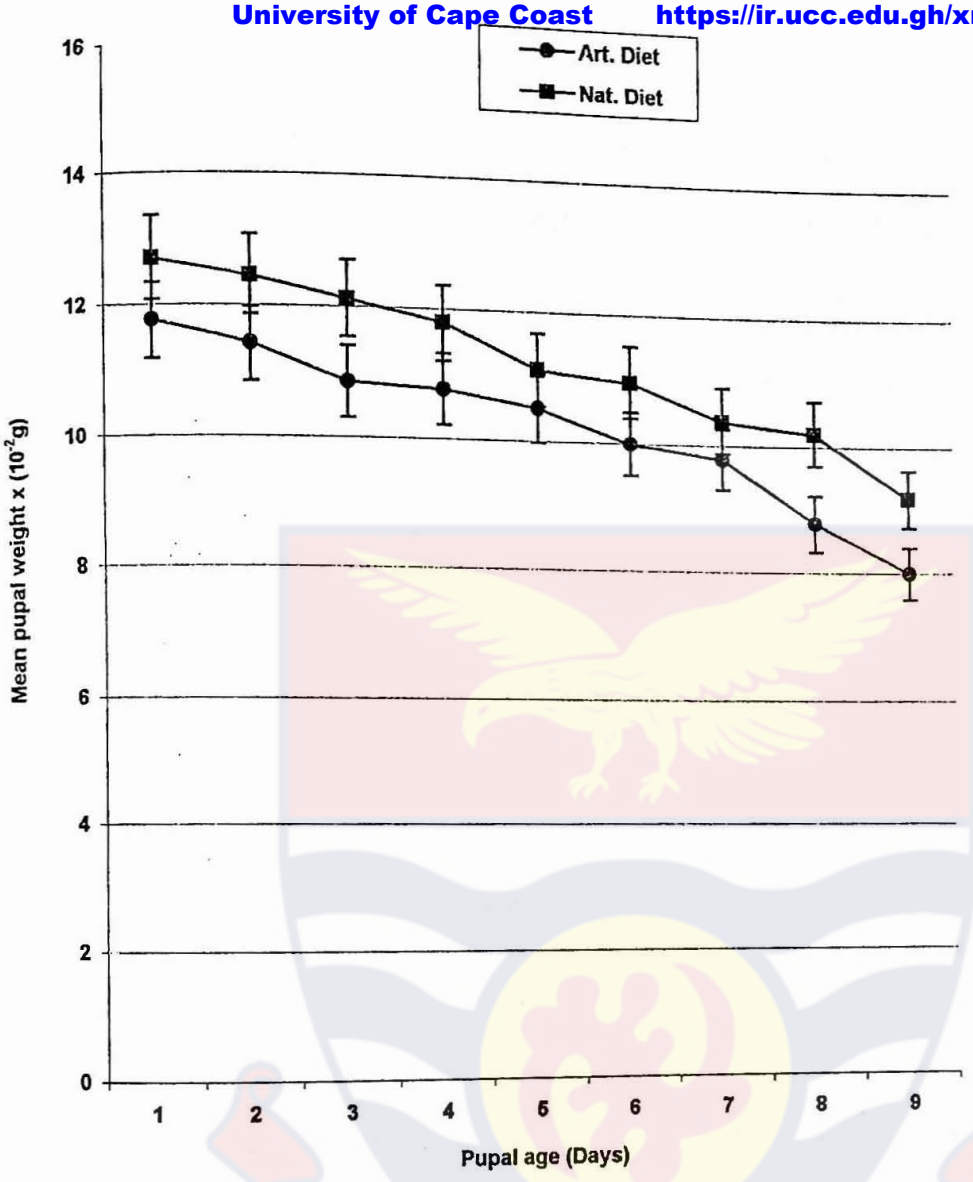


Fig. 5.4 Change of pupal weight with age of *E. saccharina* reared on artificial and natural diets (14 mm-sized pupae; Vertical bars = +/- 1.0 SD)

With *S. calamistis*, there was also decrease in pupal weight with increasing age of pupae of sizes 12 mm and 15 mm, (selected minimum and maximum sizes for *Sesamia*) as shown in Figs. 5.5 and 5.6 respectively. Results of correlation analysis for weights on natural and artificial diets were r= -0.9305 and p= 0.0002 for 12 mm-sized pupae; r= -0.9800 and p=0.000, for pupae of 15 mm-sized. Test for differences between means revealed that the natural dieters were significantly heavier than the artificial dieters ($p < 0.05$).

The regression equations for 12 mm-sized pupae of *S. calamistis* are expressed by: $y = -0.22x + 8.91$ (on artificial diet) and $y = -0.15x + 9.70$ (on natural diet), where y= mean pupal weight (g) and x= age of pupae (in days). For 15 mm-sized pupae, $y = -0.10x + 13.84$ for artificial dieters and $y = -0.23x + 16.76$ for natural dieters.

General observation from this study was that pupal weights of natural dieters were relatively heavier than those of artificial dieters for the two stem borer species, *E. saccharina* and *S. calamistis*.

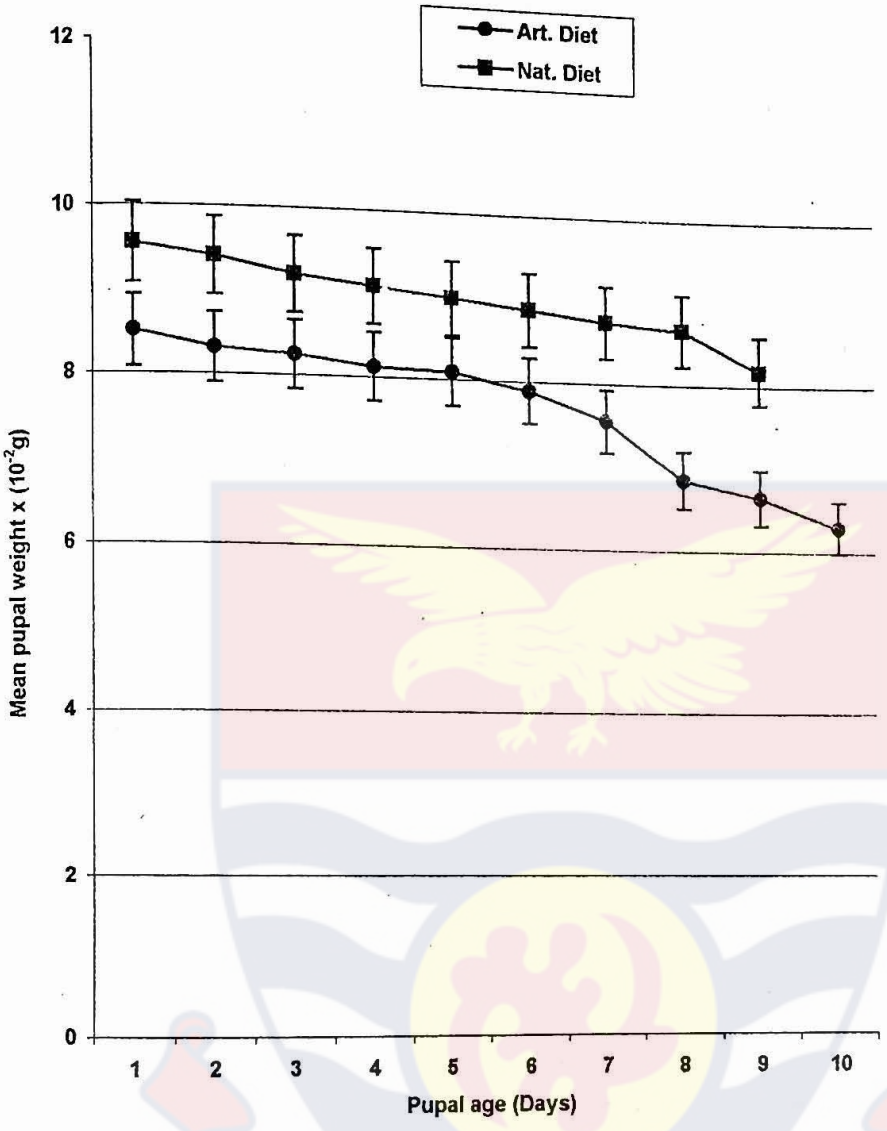


Fig. 5.5 Change of pupal weight with age of *S. calamistis* reared on artificial and natural diets (12 mm- sized pupae; Vertical bars = +/- 1.0 SD)

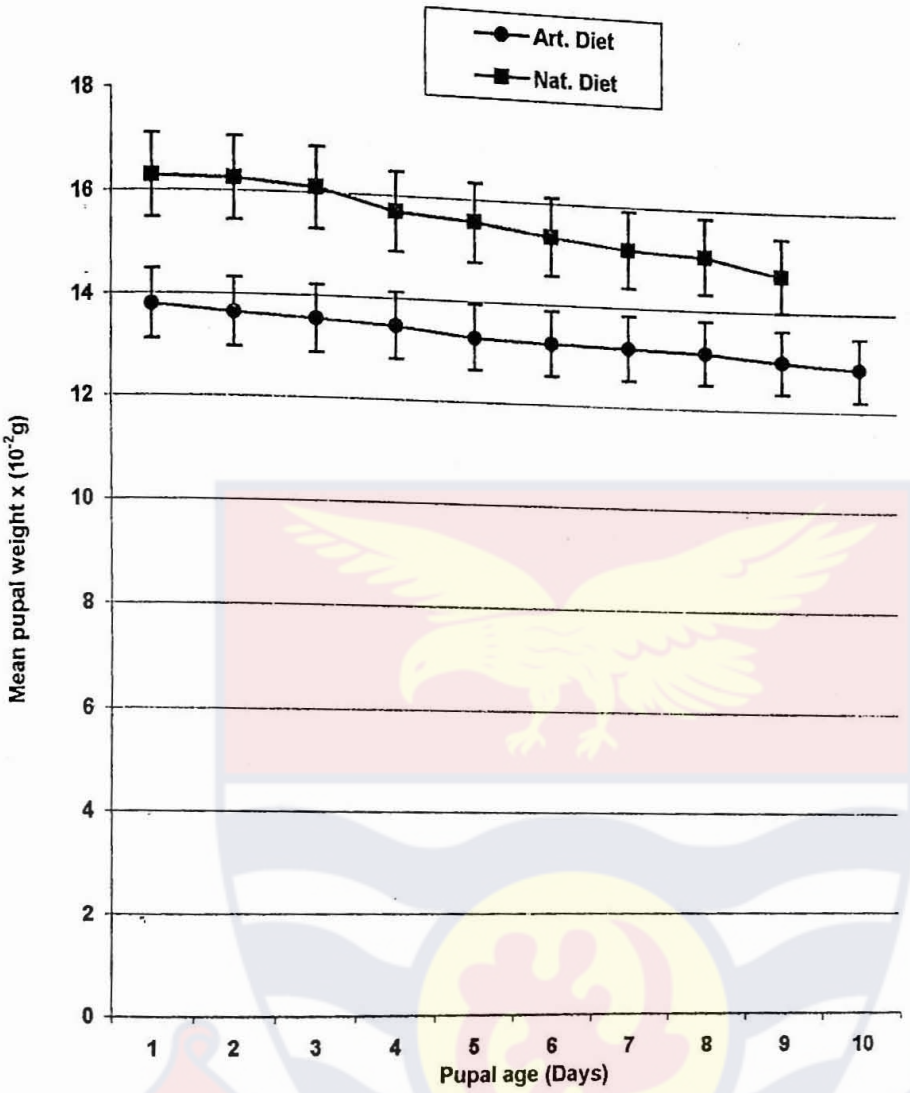


Fig. 5.6 Change of pupal weight with age of *S. calamistis* reared on artificial and natural diets (15 mm-sized pupae; Vertical bars = +/- 1.0 SD)

Variation of pupal sizes in relation to adult emergence, sex ratio, pupal mortality (unemerged) and pupal longevity of natural and artificial dieters of *E. saccharina* is shown in Table 5.6. High percentage of adult emergence (50%-100%) was found in both natural and artificial dieters of *E. saccharina*. Relatively smaller-sized pupae (9 mm- 10 mm) and (11 mm- 12 mm) of artificial and natural dieters respectively showed relatively high percentage of male emergence (50%-67%), while larger pupal sizes favoured emergence of more female (53%-100%), than males (25%-47%). Relatively more unemerged adults were found in artificial dieters in the form of lysed/hollow (dead) pupae, with percentage range of 8%-50%. Mean longevity for both natural and artificial dieters was between 8.0 ± 0.0 and 11.0 ± 0.0 days.

Table 5.6 Variation of pupal size of *Eldana saccharina* in relation to adult emergence, sex-ratio, pupal mortality and longevity

Pupal size (mm)	Total adult emergence (%)	Sex emergence (%)		Unemerged adults (%)			Eclosion period (Mean ± SE) (Day)
		Female	Male	Lysed/ Hollow	Partially developed		
					Female	Male	
Artificial Dieters							
9	60.0	33.3	66.7	50.0	0.0	50.0	8.5±0.5
10	50.0	40.0	60.0	40.0	0.0	60.0	8.0±1.0
11	81.3	69.2	30.8	18.7	40.3	41.0	8.0±0.5
12	91.7	54.5	45.5	8.3	50.0	41.7	9.0±0.6
13	100.0	75.0	25.0	0.0	0.0	0.0	8.9±1.4
14	100.0	100.0	0.0	0.0	0.0	0.0	10.0±0.0
Natural Dieters							
11	100.0	50.0	50.0	0.0	0.0	0.0	9.3±1.2
12	100.0	40.0	60.0	0.0	0.0	0.0	10.7±0.5
13	100.0	53.3	46.7	0.0	0.0	0.0	9.3±0.7
14	100.0	60.0	40.0	0.0	0.0	0.0	10.0±1.2
15	100.0	70.0	30.0	0.0	0.0	0.0	9.8±0.8
16	100.0	100.0	0.0	0.0	0.0	0.0	11.0±0.4

Adult emergence of natural and artificial dieters of *S. calamistis* was from 70% to 100% (Table 5.7). There was more of female emergence (50%-100%) of relatively larger sized pupae (14 mm-22 mm) in natural dieters than male emergence (0-50%). Artificial dieters of size/length 12-13 mm showed a total of 47% non- emerged adults (lysed/hollow pupae), whilst natural dieters of 13-14 mm in size scored a total of 35% non-emerged adults. Pupal longevity of *S. calamistis* ranged from 10.0 ± 0.8 to 13.3 ± 1.0 days.

Table 5.7 Variation of pupal size of *Sesamia calamistis* in relation to adult emergence, sex ratio, pupal mortality and longevity

Pupal size (mm)	Total adult emergence (%)	Sex emergence (%)		Unemerged adults (%)			Eclosion period (Mean ± SE) (Day)
		Female	Male	Lysed/ Hollow	Partially developed		
					Female	Male	
Artificial Dieters							
11	100.0	30.0	70.0	0.0	0.0	0.0	10.7±0.5
12	70.0	50.0	50.0	30.0	30.0	40.0	10.6±0.9
13	83.3	50.0	50.0	16.7	43.3	40.0	11.0±0.0
14	100.0	30.0	70.0	0.0	0.0	0.0	10.0±0.8
15	100.0	66.7	33.3	0.0	0.0	0.0	10.3±0.6
Natural Dieters							
12	100.0	33.3	66.7	0.0	0.0	0.0	10.3±0.5
13	75.0	33.3	66.7	25.0	35.0	40.0	11.3±0.6
14	90.0	77.7	22.3	10.0	50.0	40.0	11.0±0.8
15	100.0	50.0	50.0	0.0	0.0	0.0	10.2±0.4
16	100.0	80.0	20.0	0.0	0.0	0.0	11.8±1.6
17	100.0	83.3	16.7	0.0	0.0	0.0	11.3±0.8
18	100.0	83.3	16.7	0.0	0.0	0.0	10.3±0.6
22	100.0	100.0	0.0	0.0	0.0	0.0	13.3±1.0

5.3.3 *Adult emergence of irradiated pupae*

Moth emergence following irradiation of different age groups of pupae was classified into three categories: completely emerged, deformed/partially emerged and unemerged moths (Plates 5.2-5.5).

Immature pupae of ages 2-4 days old exposed to radiation doses of 150 and 180 Gy for both borer species were largely unemerged, crumpled and dead pupae. A few that emerged or partially emerged, within the same age group and radiation doses, showed deformed bodies with crumpled wings, feeble legs and distorted mouthparts.

Plate 5.2 **4 day- old irradiated pupae of *E. saccharina* with dose 180 Gy; *unemerged/crumpled (dead)* .**

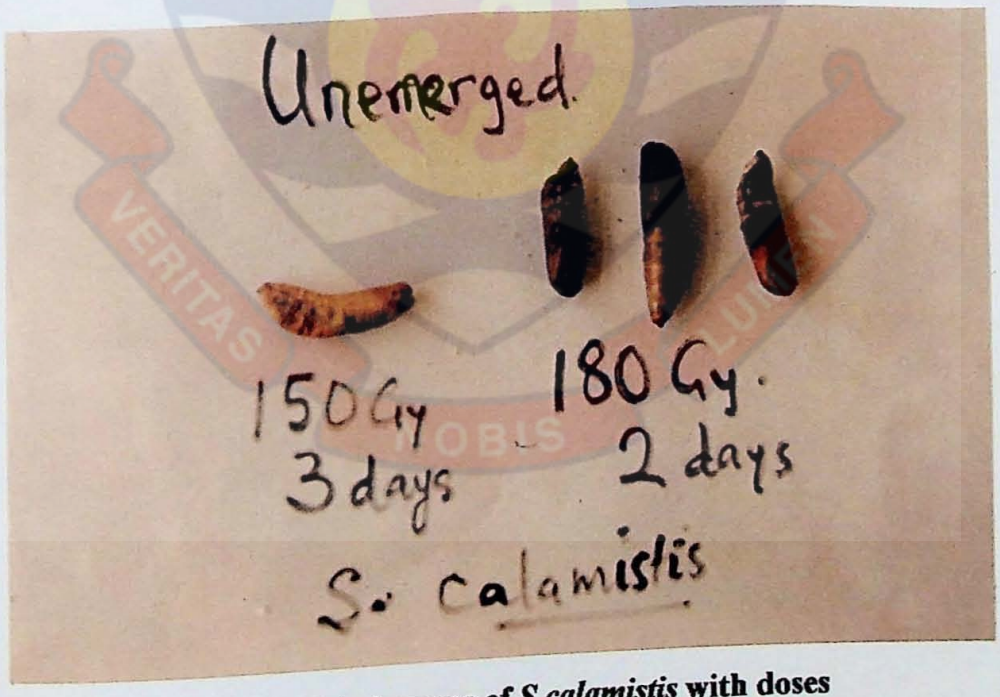


Plate 5.3 **2 and 3 day- old irradiated pupae of *S calamistis* with doses of 180 Gy and 150 Gy respectively; *unemerged/crumpled.***

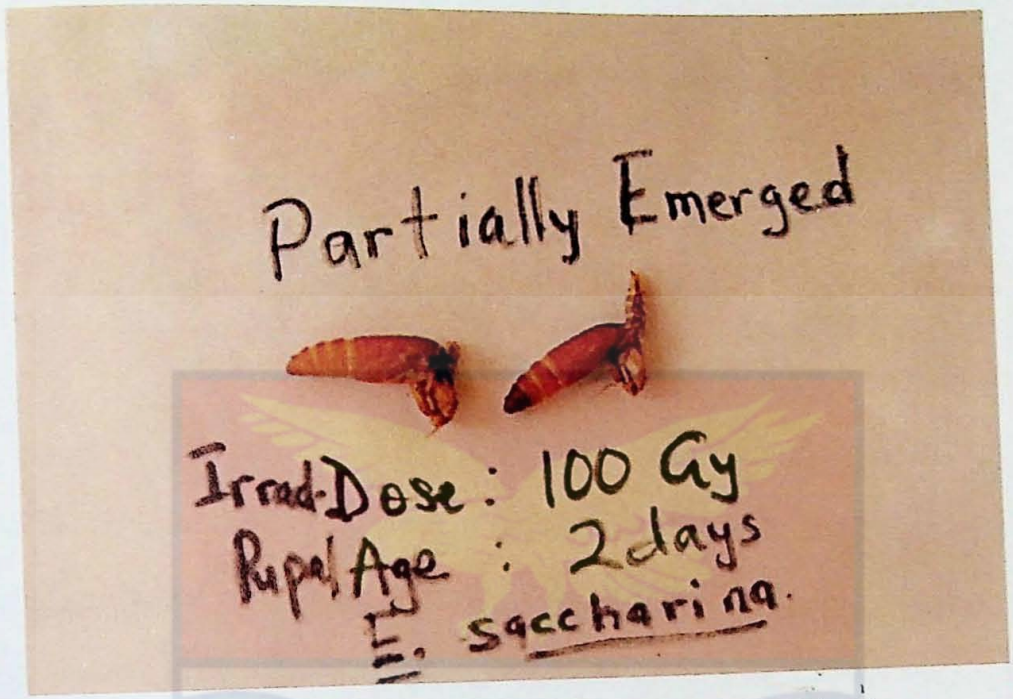
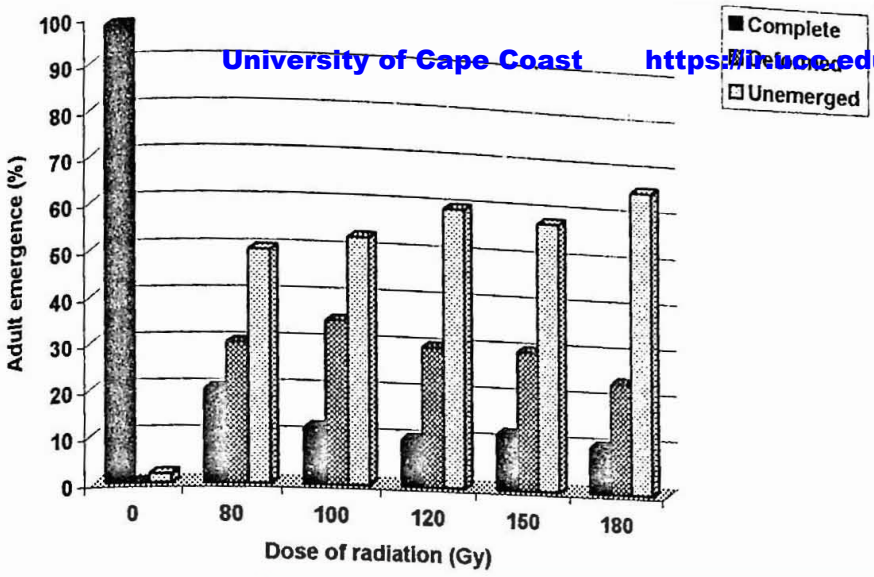


Plate 5.4 **2 day-old irradiated pupae of *E. saccharina* with dose of 100 Gy; *partially emerged.***

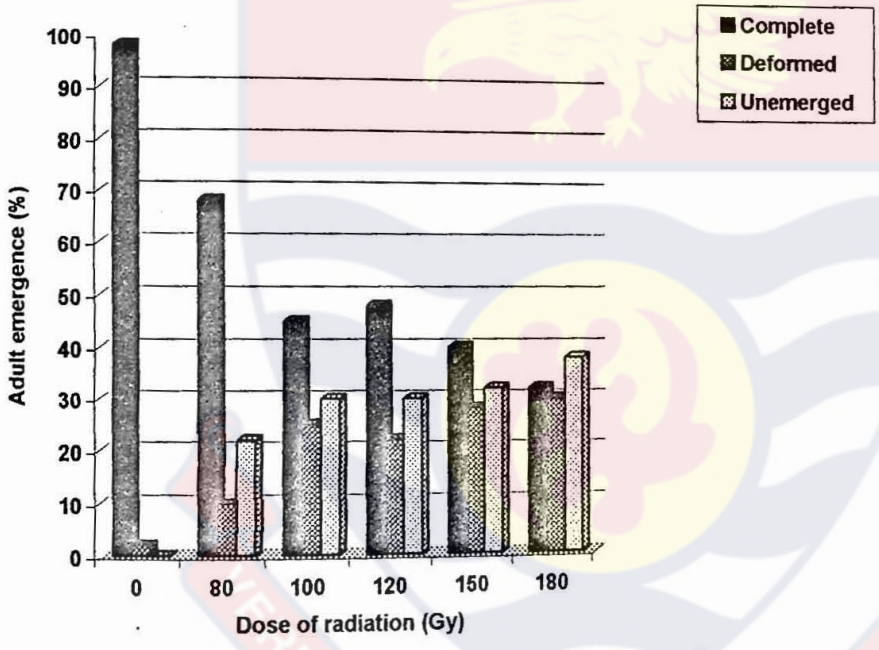
Plate 5.5 **Completely emerged moth of *S. calamistis* from 4 day-old pupa irradiated with dose of 180 Gy; *deformed body.***

Emergence of *E. saccharina* pupae of different age groups (2, 4, 6,and 8 days old) exposed to increasing doses of ionizing radiation is shown in Fig. 5.7 a, b, c and d respectively. The untreated pupae/control (0 Gy) showed high percentage adult emergence, 95%-100%, for all the age groups.

For two days old pupae, increasing doses of ionizing radiation resulted in gradual increases in unemerged moths from 50% at 80 Gy to 66% at 180 Gy respectively. Percentage of deformed moths ranged between 25% and 35%, whilst completely emerged fell below 20%. Four days old pupae exhibited slight reduction of unemerged moths, between 22% and 38%, but appreciable rise in completely emerged moths from 32% to 68% in inverse relationship with radiation doses. Higher percentage of completely emerged moths, 80% to 98%, was observed for 6 and 8 days old pupae with very few or no deformed and unemerged moths, averagely less than 10%.

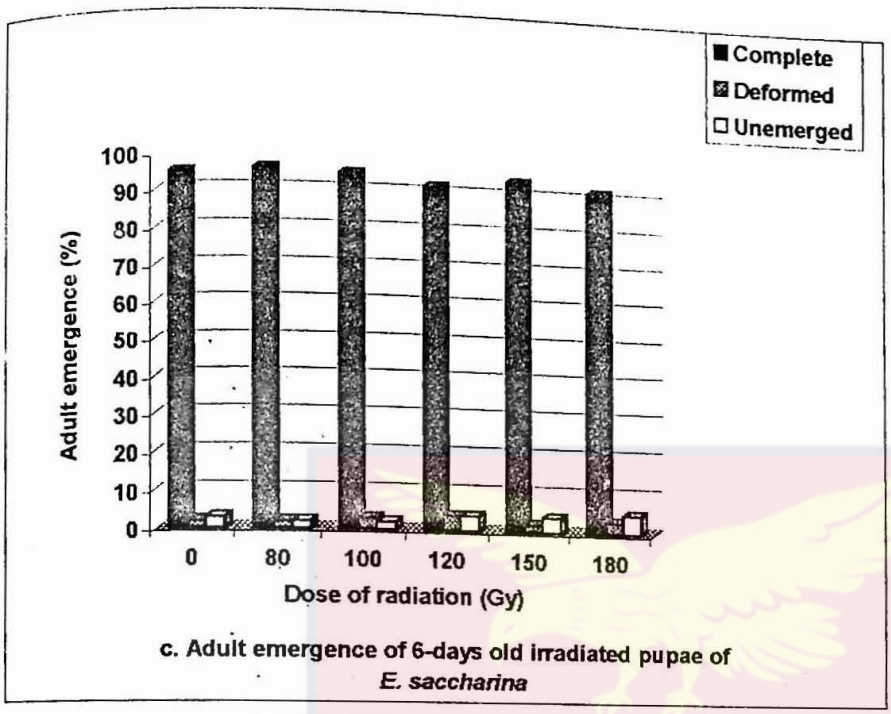


a. Adult emergence of 2-days old irradiated pupae of *E. saccharina*

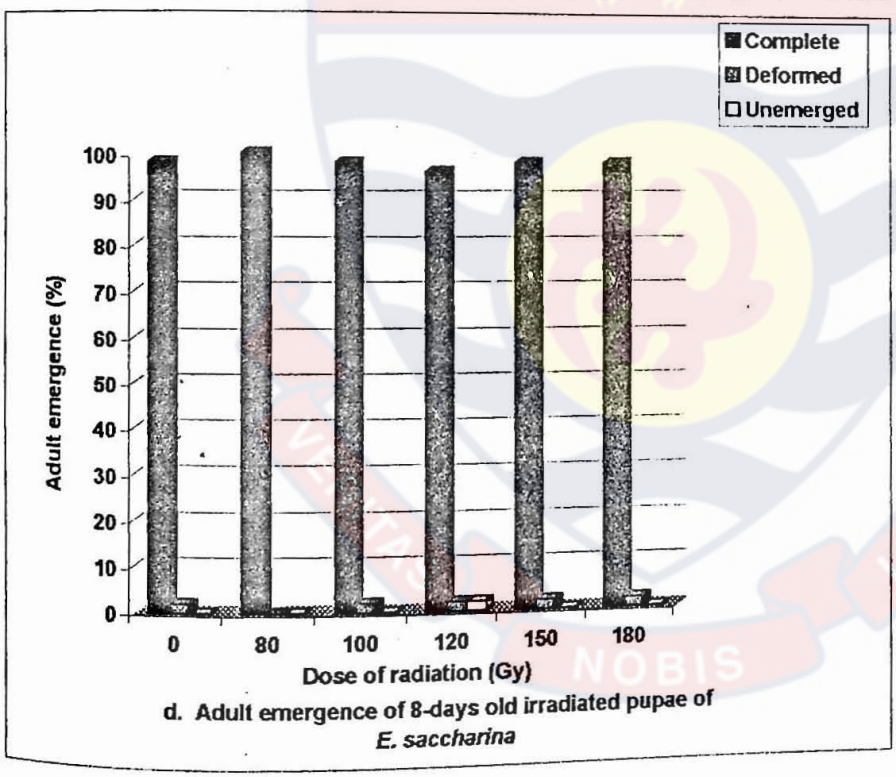


b. Adult emergence of 4-days old irradiated pupae of *E. saccharina*

Fig. 5.7 a,b, Adult emergence of *E. saccharina* following irradiation of irradiation of 2 and 4 day-old pupae.



c. Adult emergence of 6-days old irradiated pupae of *E. saccharina*

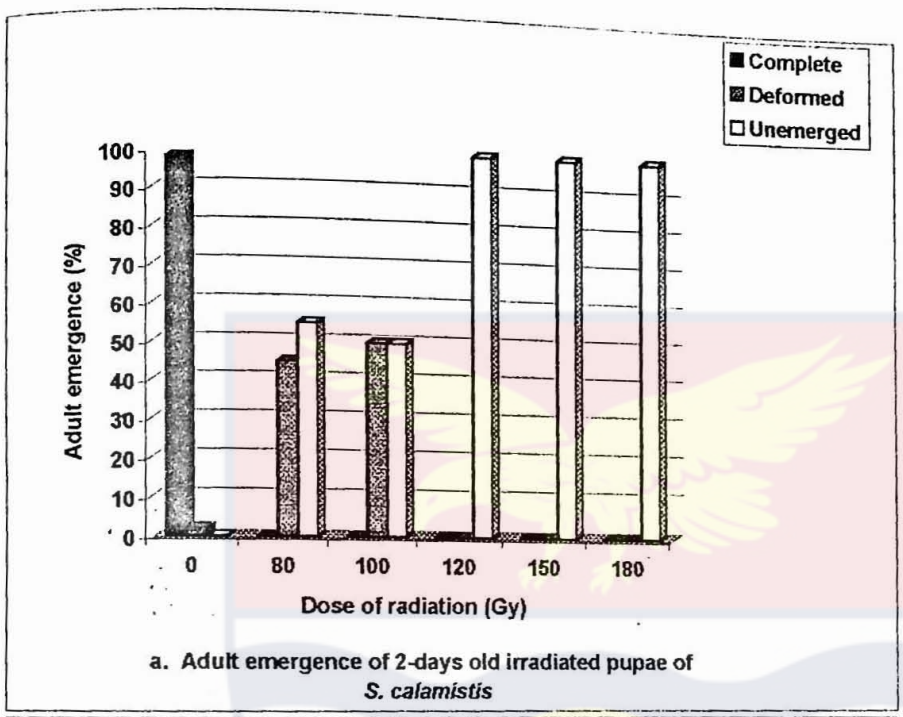


d. Adult emergence of 8-days old irradiated pupae of *E. saccharina*

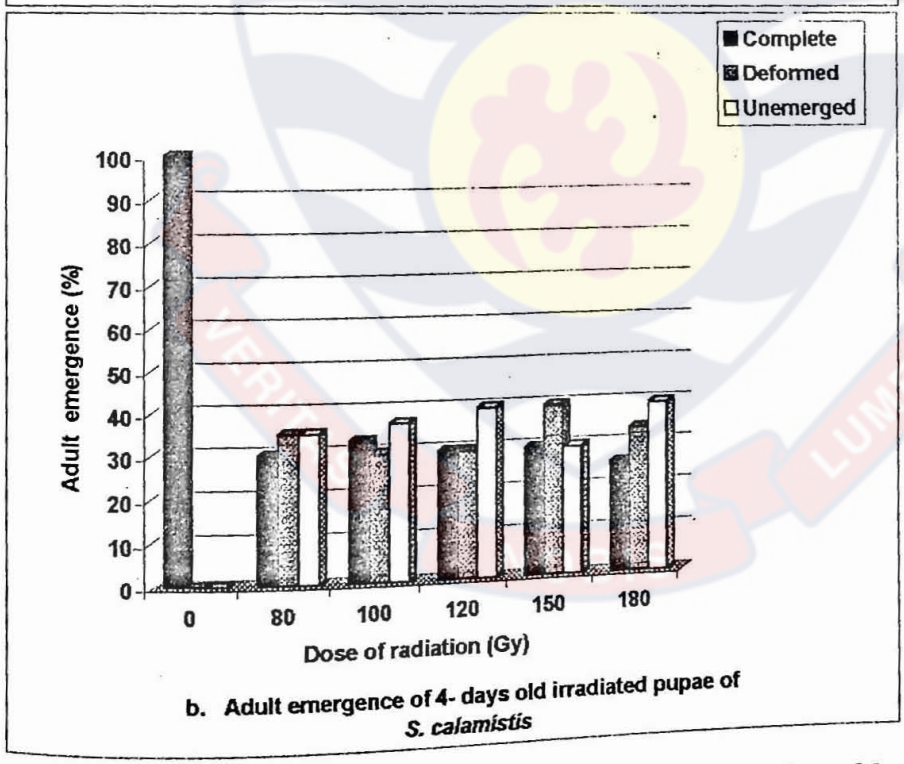
Fig. 5.7 c, d Adult emergence of *E. saccharina* following irradiation of 6 and 8 day- old pupae.

Adult emergence of *S. calamistis* following irradiation of different age groups (2, 4, 6, and 8 days old) of pupae is represented in Fig. 5.8 a, b, c and d respectively. Increasing doses of ionizing radiation for 2 days old pupae resulted in high percentage of unemerged adults, 50%-100% and about 45-50% for deformed moths. There was no completely emerged moths for doses between 80 Gy and 180 Gy. Four days old pupae registered between 30% and 40% unemerged and deformed moths and 26%-33% of completely emerged moths. Mature pupae of 6 days old and over had relatively low percentage of unemerged and deformed moths (below 20%), but higher percentage of completely emerged moths of 70%-92%. The control in all the groups was almost completely emerged between 98%-100%.

Generally, in both borer species, immature pupae (2-4 days old) exposed to increasing doses of irradiation, resulted in high percentages of deformed and unemerged moths and relatively lower percentage of completely emerged moths. With mature pupae (6-8 days old), increasing doses of irradiation resulted in fewer deformed and unemerged moths but higher percentage of adult emergence which appeared independent of dose variation.



a. Adult emergence of 2-days old irradiated pupae of *S. calamistis*



b. Adult emergence of 4- days old irradiated pupae of *S. calamistis*

Fig. 5.8 a, b **Adult emergence of *S. calamistis* following irradiation of 2 and 4 day-old pupae.**

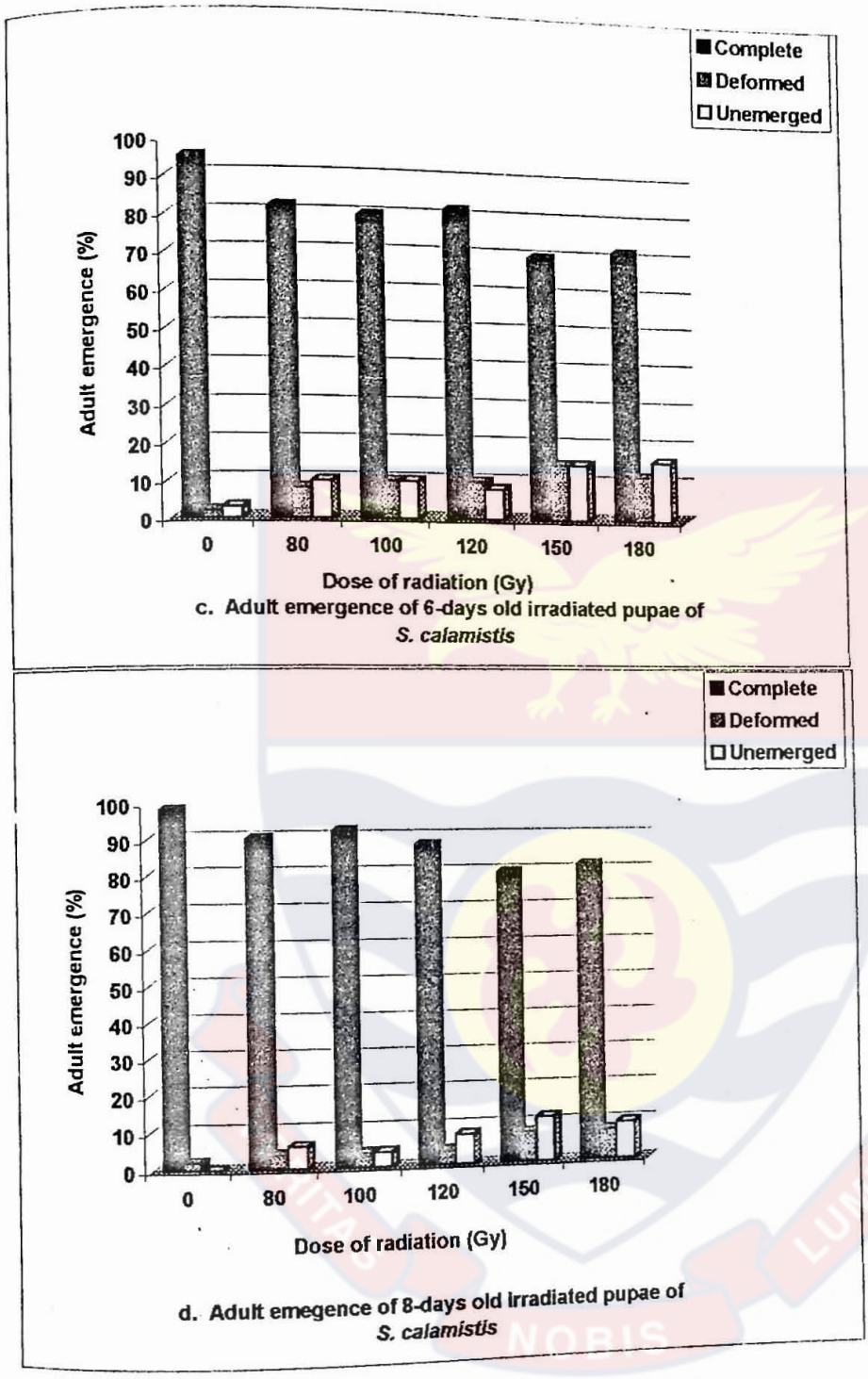


Fig. 5.8 c, d **Adult emergence of *S. calamistis* following irradiation of 6 and 8 day- old pupae.**

5.4 Discussion

In this study, the relatively high mortality of *E. saccharina* larvae on natural diet could be attributed to microbial infection and parasitoid attacks that must have been carried from the field to the laboratory. Mortality of artificial dieters could be due to fungal infection in the diet medium in the laboratory. In *S. calamistis* it was observed that on a few occasions, large numbers of pupae reared on artificial diet were deformed. This could be due to mineral imbalance in the ingredients of the diet prepared and might account for the mortality of larvae and pupae.

The pupa, in general, is a distinctive stage in holometabolous insects which occurs between the larva and adult stages. It is during the pupal stage that major internal reconstruction may occur, particularly involving the eversion and growth of the wings and development of the flight muscles (Chapman, 1973). The pupa is usually immobile and during this stage of the insect, feeding activity ceases almost entirely. However, the larval stage of the lepidopterous insect feeds voraciously and large amounts of food nutrients are accumulated by the larva to supply the pupal metabolic processes (Chapman, 1973). Larvae that feed more may increase in size and store more food reserves, thereby creating increase in mass (weight). This may account for increases in mean weight in proportion to increase in pupal sizes of both *E. saccharina* and *S. calamistis* fed on natural and artificial diets.

The fate of fat body during pupal metabolism depends on the degree of reconstruction of other body tissues. In the lepidopterans where reconstruction is extensive, the fat body may be almost or completely used up. Loughton and West (1965) observed in the Lepidoptera that there was significant drop in the concentration of haemolymph proteins at pupation, due to the passage of proteins into the tissue. It was suggested that these proteins could be involved in the transport of lipids and carbohydrates. It was also observed in the blow-fly that larval proteins were broken down to relatively complex peptides which were then rebuilt into the adult proteins (Chen and Levenbook, 1966). In the present study, the general trend of decrease in pupal weight with respect to pupal ageing for both borer species, may be explained by the fact that food reserves in the pupae were being metabolized to release energy for reconstruction of the tissues for the adults.

In host-plant feeding relationship, phytophagous insects normally obtain all their nutritional requirements for normal growth and development from the natural diet. In artificial formulated diet some essential nutrients for normal growth are present but may not be in their correct amounts. For example, in *Ephestia kuehniella* not less than 0.13 g of whole-meal flour is needed for normal development; any smaller amounts (as little as 0.04 g) will produce normal moth but smaller in size (Norris, 1933). *Schistocerca* also needs, at least, 20% sugar in the diet for normal growth (Dadd, 1960). In this study, the essential nutrients for growth in the artificial diet might not be in the right amounts and could account for relatively smaller size and lighter weight in artificial dieters compared with the natural dieters.

Percentage adult emergence was equally high in both natural and artificial dieters in relation to pupal size, indicating that dietal effect did not largely have adverse influence on emergence. Female moths were slightly larger in size than the males of the two species. This sexual dimorphism is common in nature. One of the reasons could be due to the presence of the reproductive structures of the female moths and their accessory organs for egg production and sperm storage.

Ouye *et al.* (1964) conducted studies on the effect of ionizing radiation on the pupae of the pink bollworm, *Pectinophora gossypiella* (Saunders), at ages of 1, 2, 3, 5 and 7 days with a dose of 100 Gy. They reported that the susceptibility to radiation decreased with the increase of age. Observations of the present study on irradiated pupae of *E. saccharina* and *S. calamistis* agree with those of the previous researchers. Increasing doses of irradiation on immature pupae might have caused severe injury to most of the developing cells and therefore unable to complete development, thereby resulting in their deformities and mortality (unemerged). Furthermore, Henneberry and Clayton (1988) irradiated pupae of the pink bollworm at ages of 2 to 6 days with doses of 50, 100 and 150 Gy. They also found that younger pupae were more susceptible to the effects of radiation than older ones and that radiation induced sterility in the F_1 generations. The present study also confirms what was observed in Henneberry and Clayton (1988).

The progeny of moths irradiated as either adults or mature pupae exhibited the same amount of inherited sterility (Nielsen, 1971, Debolt, 1973). The adult stage of moths does not undergo metamorphosis and could therefore be a good radiation target. However, Bartlett

(1978) stated that adult irradiation may have undesirable features including the lack of feeding in the first hours of adult life and the loss of scales during handling, which could handicap the sustained flight and survival capabilities under environmental stresses. Irradiated pupae are easier to handle and transport for releases, because there are no problems about mobility, anaesthetization or cooling for treatment.

5.5 Conclusion

Development of larvae and pupae of *E. saccharina* on both natural and artificial diets even though did show some differences in biological parameters, only few were statistically significant. Sex ratio of emerged adults from both diets was 1.2 females to 1.0 males. Mean larval mortality and pupal mortality of *S. calamistis* were significantly higher in artificial diet than in natural diet. Mean fecundity in *S. calamistis* was relatively low in both natural and artificial diets compared with *E saccharina*.

There was a general decrease in weight with increased in age of pupae of both borer species on natural and artificial diets. Pupal weights of natural dieters were slightly heavier than those of artificial dieters. Adult emergence of relatively smaller-sized pupae produced more males, whilst larger-sized pupae emerged into more female adults in both borer species.

Immature pupae of both borer species exposed to increased doses of radiation showed high percentage of deformed and unemerged adults. Mature pupae however, were less susceptible to increased doses of radiation, with fewer deformed and unemerged adult moths.

This study has shown that mature pupae (6-8 days old) of *E. saccharina* and *S. calamistis* exposed to ionizing gamma radiation had minimum or no adult deformities but high adult emergence. Due to limited resources and time constraint, *E. saccharina* was selected for further sterility studies. The next chapter reports on further application of gamma irradiation on mature pupae of *E. saccharina* to induce inherited sterility.

CHAPTER SIX

INHERITED STERILITY OF *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE): EFFECT OF IONIZING RADIATION ON MATING, FECUNDITY AND FERTILITY

6.1 Introduction

Radiation-induced inherited sterility, also known as F_1 sterility or inherited sterility for short, is considered to be one of the most promising genetic methods that has been developed and field tested for population suppression of lepidopterous pests (LaChance, 1985; Mastro and Schwalbe, 1988). In inherited sterility of lepidopterans, doses of ionizing radiation are lowered so that the insects are only partially sterile and generally more competitive than fully sterile ones (North, 1975).

Proverbs (1962) was the first to report the presence of inherited sterility in the F_1 progeny of the codling moth, *Carpocapsa pomonella* (L). Similar investigations were subsequently performed by researchers on other lepidopterans, including the cabbage lopper, *Trichoplusia ni* (Hubner) (North and Holt, 1970); the corn earworm, *Helicoverpa zea* (Boddie) (Carpenter, 1993; Carpenter and Layton, 1993) and the gypsy moth, *Lymantria dispar* (L.) (Mastro, 1993). Some general attributes of inherited sterility are that F_1 males and females are more sterile than the parents (P) males and females irradiated and that more F_1 male progeny than females are produced (LaChance, 1985). These attributes immensely contribute to the effectiveness of using inherited sterility to suppress lepidopterous pest populations.

In Ghana, *Eldana saccharina* Walker (Pyralidae) and other related stem borers ie. *Sesamia* species are the most destructive field pests of maize. Apart from maize, *E. saccharina* also attacks other cereals like millet, sorghum, and sugar cane (Sampson and Kumar, 1985; Bughio, 1988).

In view of the economic importance of *E. saccharina* and the difficulties encountered in controlling maize stem borers through conventional methods, it would be of great interest to use other alternative methods of control. One of such methods is to ascertain the effect of gamma radiation doses on the parent pest and the feasibility of applying F_1 sterility for suppression of the pest population. This chapter reports on the findings of the effect of sub-sterilizing doses of ionizing gamma radiation on mating, fecundity and fertility of *E. saccharina* Walker (Pyralidae). It is also to determine the effective doses or the optimal dose to induce inherited sterility in the F_1 progeny. The study also records observations on mortality of the larvae/pupae, longevity and sex ratios of the F_1 and F_2 generation.

6.2 Materials and Methods

6.2.1 *Insect stock*

Larvae of *E. saccharina* used in this study were originally collected from maize farms in the field and reared on fresh maize stem cuttings in plastic containers in the laboratory till pupation. Pupal development was observed for a period of 6-8 days (ie. black-eyed pupae/ pharate pupae), when they were matured and removed from cocoons ready for irradiation. Experiments were conducted in laboratory conditions of temperature 28.0 ± 2.5° C, relative humidity, 72.0 ± 10% and a 12 : 12 (light : dark) hours photoperiod.

6.2.2 *Irradiation*

All radiation process was conducted at the Radiation Technology Center (RTC) of Ghana Atomic Energy Commission (GAEC) at Kwabenya, near Accra, using a Cobalt-60 gamma source (Gamma-cell 220, Canada). The average dose rate of the gamma source was 3.0 $Gy.min.^{-1}$ and the pupae were exposed to sub-sterilizing doses of 80, 100, 120, 150 and 180 Gy respectively. Dose calibration using Fricke dosimetry showed a dose error of about ± 5%.

For each treatment of radiation dose, 20 pupae were singly placed in separate plastic vials, (6 cm. x 2.5 cm. diameter), corked with dry cotton wool and arranged in the source chamber for irradiation. Two replications for each dose treatment were conducted. A group of matured pupae was also held as the control, 0 Gy.

6.2.3 *Parents (P) and F_1 crosses.*

Irradiated pupae in plastic containers were conveyed to the laboratory for daily observation till adult emergence. Emerged moths, P, were transferred to separate oviposition cages and paired with non-irradiated moths of the opposite sex in 1:1 ratio. Non-irradiated paired moths were held as the control. Details of conditions in oviposition cages were as described in Chapter 5.

Mating behaviour and oviposition period were observed. Dead moths were removed from cages and females dissected to examine mating status ie. spermatophores present in the bursa copulatrix. After oviposition period (5-7 days after mating), all females that survived were dissected to determine number of spermatophore in bursa copulatrix and the presence/absence of sperms in the spermathecae. Males were also dissected to observe spermatozoa mobility in the testes/accessory organs. Mean adult longevity of both sexes were recorded.

Eggs laid were incubated in petri-dishes covered with transparent plastic cups and hatched into larvae after 3-5 days post-oviposition. Number of eggs laid per female (fecundity) and percentage egg-hatchability (fertility) for each dose treatment were recorded. Two types of unhatched eggs were distinguished during the study:

(i) Sterile eggs, which were unfertilized and without any detectable embryonic development. They also included eggs with early embryonic mortality.

(ii) Non-viable eggs were those in which the embryo died during different stages of embryogenesis or exhibited late embryonic mortality.

First filial generation (F_1) larvae developed through pupae to adult stages, and the resulting adults were used in F_1 crosses to give rise to the F_2 generation. Due to very low fecundity of irradiated parent females, very few larvae could develop to adults and therefore not sufficient for F_1 crosses. Only progeny of irradiated male parents were used for F_1 crosses.

6.2.4 Type of adult crosses.

Parents (P):

a) **NM x NF (Control)**

b) **TM x NF**

c) **NM x TF**

Where **N** = Non-irradiated/Normal; **T** = Treated/Irradiated; **M** = Male and **F** = Female.

F_1 progeny:

a) **NM x NF (Control)**

b) **F_1M x NF**

c) **NM x F_1F**

Where **F_1M** = males obtained from P males irradiated as mature pupae

and **F_1F** = females obtained from P males irradiated as mature pupae.

6.2.5 *Statistics*

Statistical analysis of data were performed with *Statistix* software programme. No transformation of data was done. One-way analysis of variance (ANOVA) was used to determine differences among the mean values of treated groups and the control. Linear regression analysis was also performed to examine the effect of radiation doses on fecundity and hatchability.

6.3 Results

6.3.1 *Irradiation effect on parents and larvae/pupae development.*

Percentage of successful mating and mean number of spermatophores per female were not significantly affected by increasing radiation doses on mature pupae of *E. saccharina* (Table 6.1). For the control crosses, 92% mating adults were recorded. Irradiated males crossed with normal females at 180 Gy registered 85% and their reciprocal cross with the same dose was 78%. In general, a single spermatophore was found in bursa copulatrix per mated female. However, since calculations included specimens with and without spermatophores, the average number of spermatophore per female for both control and irradiated pairings was less than 1.0.

Longevity of male moths emerged from irradiated pupae was between 7 and 9 days, whilst that of non-irradiated males ranged between 8-10 days. However, treated females showed relatively longer range of life-span (8-10 days) than untreated ones (6-9 days). Differences in adult longevity between the crosses, on the whole, were statistically insignificant ($p < 0.05$).

Table 6.1 Mating success and longevity of *Eldana saccharina* parent moths (P) emerged from irradiated mature pupae

Crosses/Dose (Gy)	Mating (%)	*Mean no. spermatophore per female	Longevity (days) female	male
*NF x NM 0 (Control)	92[a]	0.93± 0.03[a]	7.0±3.0[bc]	8.7±2.0[bc]
NF x TM 80	90[ab]	0.90±0.00[ab]	6.0±1.7[c]	7.3±2.0[ab]
100	92[a]	0.93±0.08[a]	7.7±3.0[bc]	7.8±2.0[ab]
120	90[ab]	0.90±0.05[ab]	8.3±1.8[ab]	8.8±1.8[bc]
150	88[ab]	0.88±0.08[ab]	8.1±2.8[ab]	8.1±2.5[bc]
180	85[ab]	0.85±0.05[ab]	9.1±2.8[a]	9.3±2.5[a]
TF x NM 80	88[ab]	0.88±0.03[ab]	8.0±0.0[ab]	10.5±2.2[a]
100	85[ab]	0.85±0.00[ab]	8.1±0.0[ab]	8.6±2.5[bc]
120	80[ab]	0.80±0.00[ab]	8.9±2.7[ab]	7.8±1.1[ab]
150	80[ab]	0.80±0.50[ab]	8.3±2.8[ab]	7.8±1.9[ab]
180	78[c]	0.78±0.03[c]	10.4±2.5[a]	10.0±2.0[a]

*N=normal; T=treated; F=female; M=male
* Each figure is mean/average of 20 pairs of mating moths per treatment.
* Means followed by the same letter(s) in the same column are not significantly different; Tukey (HSD) comparison of means $p<0.05$.

Irradiation effect reduced fecundity significantly compared with the control and there was a remarkable difference between the two reciprocal crosses, TM x NF and TF x NM (Table 6.2). In crosses of treated males, fecundity reduced by 40-44% compared with the control, but without clear dose-dependence. In contrast, fecundity was considerably reduced in crosses of treated females and this effect decreased drastically with increasing doses of radiation (Fig. 6.1). Females irradiated with doses of 150 and 180 Gy respectively laid only 3%-4% of eggs, in comparison with the control.

In both treated groups of TM and TF crosses, fertility decreased with increasing doses of radiation (Fig. 6.2). Treated males remained partially fertile (>40%) even at the highest dose of 180 Gy. However fertility in the reciprocal crosses (TF x NM) was drastically reduced so that females treated with doses higher than 100 Gy were almost sterile (>95%). In the treated male crosses, non-viable eggs were largely responsible for the reduction of fertility, whereas sterile eggs prevailed among unhatched eggs laid by treated females (Table 6.2).

Table 6.2 The effect of irradiation on fecundity and fertility of *Eldana saccharina* moths in the parent (P) generation

Dose/Crosses[#] (Gy)	Mean no. of eggs per female (±SD)*	(%) Mean egg hatchability (mean±SD) sterile	non-viable	hatched
NF x NM				
0 (Control)	355.4±120.6^{a}	8.3±1.0^{a}	0.0^{a}	91.7±5.8^{a}
NF x TM				
80	215.4±102.0^{b}	7.8±0.0^{a}	15.6±1.0^{b}	76.6±8.8^{b}
100	217.2±81.2^{b}	9.5±1.2^{a}	21.5±0.0^{b}	69.0±10.1^{bc}
120	208.0±101.2^{b}	10.0±0.0^{a}	22.6±1.6^{b}	67.4±10.5^{bc}
150	197.6±89.9^{b}	15.9±1.8^{ab}	28.7±1.5^{bc}	55.4±20.4^{cd}
180	199.7±95.5^{b}	19.1±2.1^{ab}	37.9±2.0^{cd}	43.0±25.4^{d}
TF x NM				
80	66.1±73.4^{c}	45.8±2.0^{c}	34.8±1.0^{c}	19.4±21.5^{e}
100	61.3±67.4^{c}	48.2±0.0^{c}	39.8±1.5^{cd}	12.0±18.0^{f}
120	27.2±67.0^{d}	52.6±2.0^{cd}	41.4±0.0^{cd}	6.0±15.5^{f}
150	10.5±25.9^{e}	55.8±1.0^{d}	40.3±1.0^{cd}	3.9±10.4^{f}
180	11.5±28.8^{e}	55.6±0.0^{d}	41.4±0.0^{cd}	3.0±9.4^{f}

[#] N, normal; T, treated; F, female; M, male.
*Each figure is a mean of 20 pairs of 2 replicates per treatment.
*Means followed by the same letter(s) in the same column are not significantly different at the 0.05 significance level, Tukey (HSD) comparison of means.

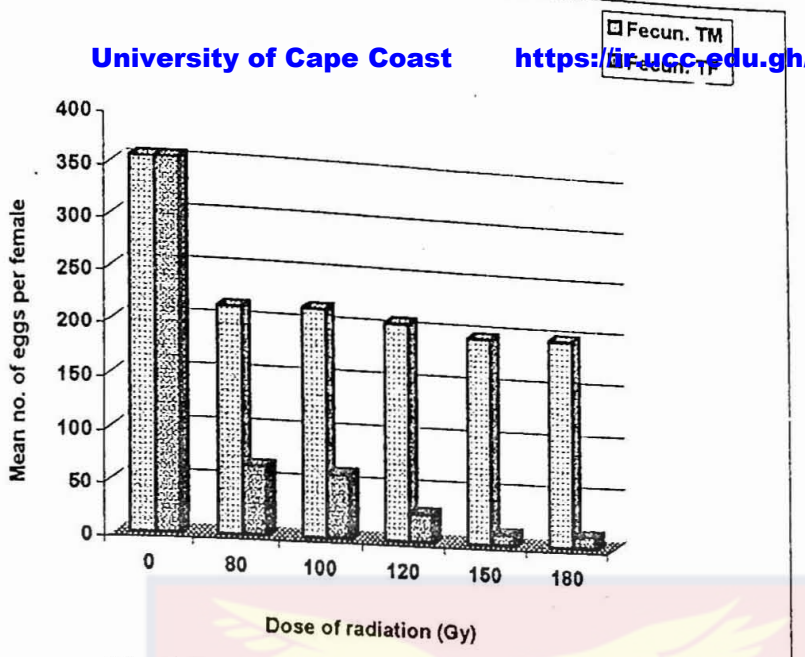


Fig. 6.1. Fecundity of irradiated mature pupae of *Eldana saccharina* parent (P)

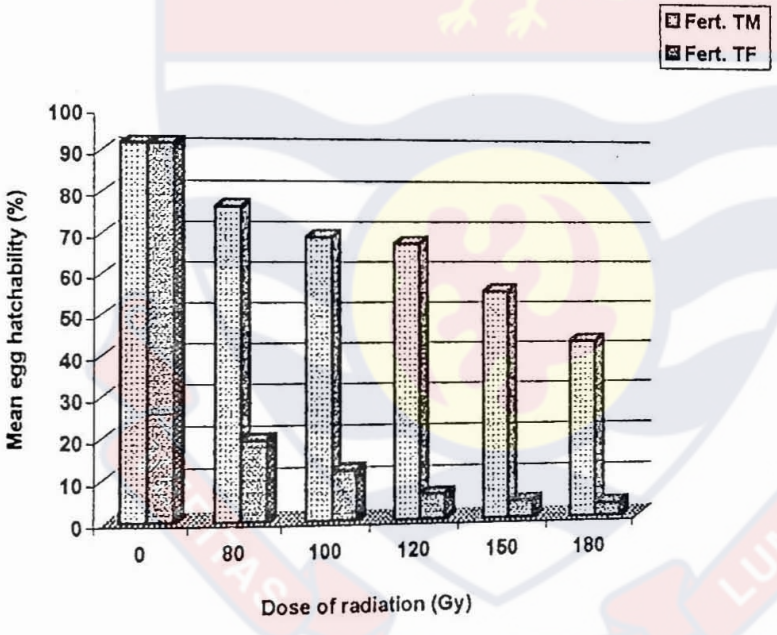


Fig. 6.2. Fertility of irradiated mature pupae of *Eldana saccharina* parent (P)

Table 6.3 shows a summary of biological parameters of irradiation effect on the development of larvae and pupae of *E. saccharina*. In the F_1 generation, mean larval life period slightly increased with increasing radiation doses, from 41 days with 0 Gy to 50.3 days with 180 Gy. Average larval mortality also increased with rise in doses of radiation, with 8% mortality among non-irradiated crosses (0 Gy) and 32% with 180 Gy. In this study, pupal mortality compared with that of larvae, was relatively low (4-12%), leading to relatively high adult emergence, ranging between 84-96% for both the control and the treated crosses.

Sex ratio of emerged adults was slightly skewed in favour of males with increasing doses of radiation. However, there was no significant differences among the crosses. The sex ratio was slightly dose-dependent and the trend was more distinct in F_1 than F_2 generations.

Table 6.3 DEVELOPMENT OF LARVAE AND PUPAE OF F_1 AND F_2 GENERATIONS OF *E. saccharina* FOLLOWING IRRADIATION OF MATURE MALE PARENT PUPAE

	Larval Development				Pupal Development				
Radiation dose (Gy)	Mean life period (±SE) days	Longevity range (days)	Percentage transformed to pupae (%)	Ave mortality (%)	Mean life period (±SE) (days)	Longevity range (days)	Percentage of adult emergence (%)	Average mortality (%)	Sex ratio female:male
			F_1 generation (P male x normal female)						
0 (control)	41.0 ± 4.8^a	30-48	92	8	8.7 ± 1.2^a	7-10	96	4	1.2 : 1.0
80	44.9 ± 6.4^{ab}	32-52	80	20	9.2 ± 1.2^a	7-11	95	5	1.0 : 1.3
100	46.4 ± 3.7^{ab}	41-52	76	24	9.5 ± 1.2^a	8-11	95	5	1.0 : 1.3
120	47.0 ± 5.3^c	38-56	80	20	8.8 ± 1.2^a	7-11	85	15	1.0 : 1.6
150	48.5 ± 7.3^c	39-70	76	24	9.5 ± 1.2^a	7-11	84	16	1.0 : 1.6
180	50.3 ± 9.0^c	36-68	68	32	9.5 ± 1.3^a	7-11	88	12	1.0 : 1.5
			F_2 generation (F_1 male x normal female)						
O (control)	43.3 ± 6.0^a	32-55	88	12	8.9 ± 1.3^a	7-11	91	9	1.0 : 1.0
80	44.2 ± 5.6^a	36-52	76	24	8.8 ± 1.4^a	7-11	89	11	1.1 : 1.0
100	48.8 ± 6.5^{ab}	36-61	68	32	9.1 ± 1.3^a	7-11	88	12	1.0 : 1.1
120	51.0 ± 7.4^c	38-62	56	44	9.7 ± 1.1^a	8-11	71	29	1.0 : 1.0
150	52.7 ± 7.0^c	42-64	60	40	9.1 ± 1.2^a	7-11	73	27	1.0 : 1.2
180	52.3 ± 6.0^c	45-64	64	36	9.8 ± 1.0^a	8-11	75	25	1.0 : 1.0

6.3.2 *Fecundity and fertility of F_1 progeny*

In both F_1M x NF and F_1F x NM crosses, the mean number of eggs per female and percentage of egg hatched decreased with increasing doses of irradiation (Figs. 6.3 and 6.4). Regression analysis revealed that for both treated crosses, fecundity decreased with increasing doses, with slopes of -9.6×10^{-1} for F_1M and -9.1×10^{-1} for F_1F crosses. The regression equation for F_1M is expressed as $y = -0.96x + 283.76$, where y=mean fecundity and x= irradiation dose. Similarly, that for F_1F is $y = -0.91x + 284.45$. Differences in fecundity were statistically significant, with $p = 1.43 \times 10^{-5}$ for F_1M and $p = 0.25 \times 10^{-5}$ for F_1F.

Fertility for both F_1M and F_1F crosses also decreased with increasing irradiation doses (Fig. 6.4). The slope of fitted regression lines for both F_1 males and F_1 females were almost identical, -4.8×10^{-1} and -4.7×10^{-1} respectively. Corresponding p- values were 7.0×10^{-3} for F_1M and 2.0×10^{-3} for F_1F, showing significant differences in percentage egg hatched ($p < 0.05$). The regression equation of fertility for F_1M is expressed as $y = -0.48x + 73.25$ and for F_1F, $y = -0.47x + 81.56$, where y= fertility/hatchability and x= irradiation dose.

Among unhatched eggs, both the sterile and non-viable eggs were observed in almost similar proportions (Table 6.4). At each irradiation dose, F_1 males showed lower fertility than the corresponding F_1 females. While in F_1 males a reduction of fertility to about 10% was observed at 100 Gy, dose of 150 Gy was required to obtain similar reduction of fertility in the F_1 females.

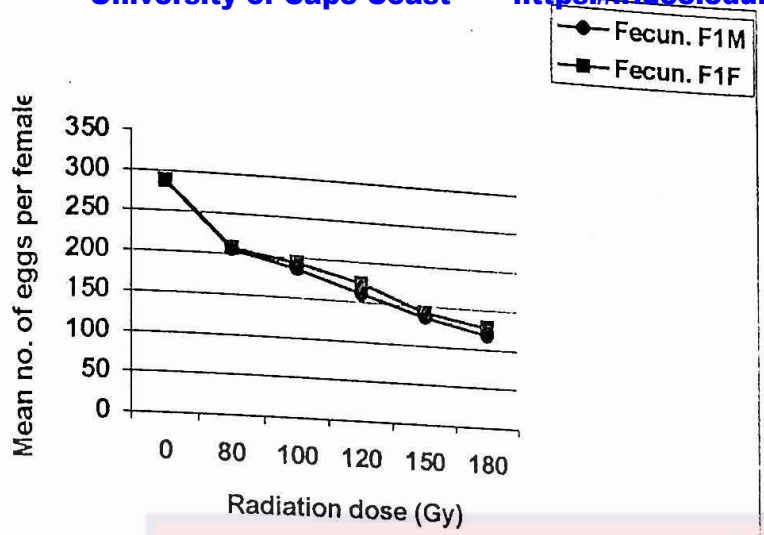


Fig. 6.3 Fecundity of F1 *E. saccharina* adult in response to radiation doses.

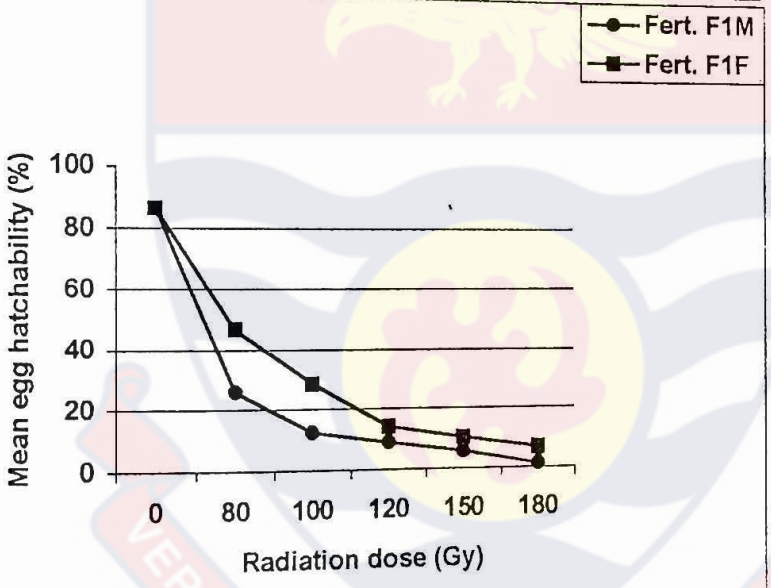


Fig. 6.4 Fertility of F1 *E.saccharina* adult in response to radiation doses.

Table 6.4 **Fecundity and fertility of F_1 *Eldana saccharina* moths from male parents (P) irradiated as matured pupae.**

Crosses[#] /Dose (Gy)	No. of eggs laid per female (Mean±SD)*	(%) Egg hatchability (Mean±SD)* sterile	non-viable	hatched
NF x NM				
0 (Control)	287.7±102.2^{a}	3.5±0.0^{a}	10.0±1.0^{a}	86.5±7.9^{a}
NF x F_1M				
80	205.6±82.5^{b}	41.7±1.0^{b}	32.6±0.0^{b}	25.7±20.1^{b}
100	184.8±76.0^{b}	39.3±1.2^{b}	48.7±1.5^{c}	12.0±14.8^{c}
120	159.8±92.4^{bc}	43.5±1.5^{b}	47.9±1.0^{c}	8.6±4.1^{c}
150	136.6±70.7^{bc}	40.5±2.0^{b}	54.0±0.1^{c}	5.5±7.8^{c}
180	117.7±71.0^{c}	45.8±1.0^{b}	52.9±0.0^{c}	1.3±3.9^{c}
F_1F x NM				
80	207.0±104.6^{b}	22.5±2.0^{c}	30.8±0.0^{b}	46.7±28.4^{d}
100	193.1±118.2^{b}	32.3±3.1^{cb}	39.5±1.5^{b}	28.2±29.5^{b}
120	173.0±106.1^{b}	40.3±2.0^{b}	46.0±1.0^{c}	13.7±19.4^{c}
150	141.8±90.2^{bc}	41.1±1.5^{b}	48.9±0.0^{c}	10.0±12.2^{c}
180	127.9±84.8^{bc}	40.5±1.0^{b}	53.0±0.5^{c}	6.5±9.6^{c}

[#] N, normal; F_1, progeny of treated parents; F, female; M, male.
*Each figure is a mean of 20 pairs of 2 replicates per treatment.
*Means followed by the same letter(s) in the same column are not significantly different at the 0.05 significance level, Tukey (HSD) comparison of means.

6.4 Discussion

This study reveals that sub-sterilizing doses of ionizing gamma radiation on mature pupae of *E saccharina* has not adversely affected the mating capability of the emerged adult moths. This is evident from the results that both irradiated (treated) and non-irradiated moths exhibited relatively high percentages of mating success and had almost equal number of spermatophores (1). Successful mating of treated moths is very essential in inherited sterility, by ensuring that large proportions of released moths can inseminate and transfer sterile sperms to the normal moths in the natural populations. If mating success is achieved, reproduction of the natural population would be disrupted, resulting in decline of the pest population.

The present study has also shown that *E. saccharina* females are more sensitive to sub-sterilizing doses of gamma radiation than their male counterparts. Females, irradiated as mature pupae with doses ranging from 80-180 Gy, produced very few eggs (11-66 eggs per female) when crossed with untreated males. However, fecundity of crosses between irradiated males and untreated females remained relatively high (about 200 eggs per female) for the same range of radiation doses. Similar observations of radio-sensitivity of females have been reported in other lepidopterous species such as the codling moth, *Cydia pomonella* (L.) (Anisimov *et al.*, 1989 ; Bloem *et al.*, 1999), pink bollworm, *Pectinophora gossypiella* (Saunders) (Qureshi and Hussain, 1993) and the Mediterranean flour moth, *Ephestia kuchniella* Zellar (Marec *et al.*, 1999).

Oogenesis proceeds later than spermatogenesis in Lepidoptera (North, 1975). Comparing female and male mature pupae of about the same age, it is possible to find germ cells of female ovaries in less advanced stage than germ cells of male, resulting in different radio-sensitivities of oocytes and spermatocytes respectively. Traut (1977) reports that in mature eupyrene sperm, the nuclei are in interphase, but in nuclei of mature oocytes, meiosis is arrested at metaphase I and does not proceed until after the eggs have been deposited. It is likely that the higher radio-sensitivity of females than males reflects the higher sensitivity of dividing oocytes nuclei in comparison with non-dividing interphase nuclei of sperm (Marec *et al.*, 1999).

In addition, the production of eggs in many insects is largely dependent on the differentiation of oocytes from oogonia and proper function of nutritive cells, also known as the nurse cells or trophocytes. Damage to the oogonia by radiation can result to reduction in egg production and damage to the nutritive cells as well (LaChance *et al.*, 1967). At certain times, however, after the egg cells have become fully differentiated and attained a high degree of polyploidy, even large doses of radiation will not affect the growth and production of eggs that appear normal, though the eggs may contain dominant lethal mutations (Grosch and Sullivan, 1954).

Reduction of egg hatchability in irradiated insects is usually caused by induced dominant lethal mutations, most probably through chromosomal aberrations (LaChance, 1967). Dominant lethal mutation may be described as a change occurring in the nucleus of the cell that can effect the death of the zygote, even though it is introduced by only one of the germ cells that unite at fertilization (LaChance *et al.*, 1967). Dominant lethal mutations are not

lethal to the treated cell, but lethal to its descendent and that is why they usually prevent the zygote from developing to maturity.

Some researchers have shown that majority of dominant lethal mutations in Lepidoptera are manifested late in embryonic development (Berg and LaChance, 1976; Bughio, 1988). From their observations, the induced late embryonic mortality, classified as non-viable eggs in the present study, resulted from dominant lethal mutations transferred from the parents to their progeny. The high percentages of non-viable eggs in the F_1 progeny of both F_1M and F_1F crosses contributed to very low fertility. It is however, possible that a fraction of the sterile eggs failed to develop because of induced dominant mutation that caused early embryonic mortality.

Other species of Lepidoptera, for example, the sugar cane borer, *Diatraea saccharalis*, exhibited early embryonic mortality on irradiation (Walker and Quintana-Munez, 1968b). This is an indication that not all species of lepidopterous moths may respond to irradiation in the same way in all respect. In this study, significant reduction in fecundity and fertility is observed between 120 Gy and 180 Gy of ionizing gamma radiation, suggesting that induced inherited sterility of *E. saccharina* could be effected within the dose range of 120-180 Gy.

6.5 Conclusion

The present study has demonstrated that sub-sterilizing doses of gamma radiation on mature pupae of male and female *E. saccharina* have not adversely affected the mating capability. Deleterious effects induced by gamma radiation in parents (P) are transmitted and expressed in the F_1 progeny, resulting in significant reduction of fecundity and fertility compared with the control. The optimal doses of gamma radiation to induce inherited sterility in *E. saccharina* could be between 120 Gy and 180 Gy. The suggestion that sterility could be caused through chromosomal aberration is further discussed in the next chapter. Inherited sterility as demonstrated in this study could be a potential tool in insect pest management and could be used to suppress the population of *E. saccharina* and other related stem borer species in Ghana.

CHAPTER SEVEN

CHROMOSOMAL ABERRATIONS CAUSING INHERITED STERILITY IN *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE)

7.1 Introduction

Ionizing-radiation injures not only the somatic cells of irradiated insects but also the chromosomes in the germ cells. The chromosomes contain genetic information that determines the general characteristics and features of the organism. Thus, the severity and magnitude of damage to germ cells can directly affect not only the fertility of the irradiated insects (Zhang *et al.*, 1993), but also viability and fertility of their progeny.

The genetic principle explaining the possible mechanism of inherited sterility in lepidopterans has been reviewed and discussed by a number of researchers (North, 1975; LaChance, 1985; Anisimov *et al.*, 1989; Marec *et al.*, 1999; Tothova and Marec, 2001).

Cytological studies conducted by researchers have indicated that F_1 progeny of moths have multiple chromosome translocations, resulting from radiation-induced chromosomal aberrations in the parent moths (North and Holt, 1968; North and Snow, 1978). LaChance (1985) explained further that usually the released parent moths (P), may have dominant lethal mutations in some of the sperm and ova and chromosome translocations in the remaining sperms and ova. When outcrossed to native moths, gametes bearing dominant lethal mutations would lead to the production of inviable zygotes. Gametes bearing chromosome translocations would result in the production of a reduced number of F_1 progeny that could be totally or partially sterile, depending on the radiation dose administered to the parent (P) insects.

Significantly, incidence of visible chromosomal aberrations in moths has been used to determine paternity in a natural population (Carpenter, 1992; Carpenter *et al.*, 1997). Chromosomal aberrations has also been used to determine the effectiveness of releasing partially sterile moths into a population and to detect interspecific hybrids in the population (North and Snow, 1978).

Chromosomes of Lepidoptera are very small in size, numerous in number and uniform in shape during cell division at metaphase. The chromosomes are usually spherical, lack distinct centromeres and sister chromatids separate by parallel disjunction at mitotic metaphase (Suomalainen, 1969b). Such characteristic features of Lepidoptera chromosomes are described as being holokinetic. It is believed that the high radio-resistance of lepidopterans could be attributed to this peculiar chromosome structure (Murakami and Imai, 1974).

The small sizes and numerous chromosomes of lepidoptera, coupled with the usual use of light compound microscopes (to observe chromosomes), which do not adequately reveal detailed chromosomal rearrangements, may have contributed to making cytological studies of chromosomes in these species not very extensive and probably less attractive to researchers. This could have accounted for little information on cytogenetics in moth species, with only a few examples like the fig moth, *Ephestia cautella* (Al-Taweel *et al.*, 1990), corn earworm, *Helicoverpa zea* (Carpenter, 1991), and the fall armyworm, *Spodoptera frugiperda* (Carpenter *et al.*, 1997).

Recently, a modified microspreading technique has been applied in studying lepidopteran chromosomes. This method allows visualization of the long synaptonemal complexes of spread pachytene nuclei at a high resolution in the electron microscope (Marec and Traut, 1993; Marec and Traut, 1994; Tothova and Marec, 2001). Nevertheless, the procedures involved in this technique are complex and the essential apparatus more expensive, unlike the use of squash and staining technique, viewed under light microscopes.

In Ghana, little or no information on cytogenetics of *Eldana saccharina* and other related lepidopterous maize stem borers is known. The present study was therefore aimed at employing the squash-staining technique and the use of light microscope to examine and identify the various chromosomal aberrations caused by a sub-lethal dose of gamma radiation in inherited sterility of *E. saccharina*. It was also to ascertain the chromosome number in the normal *E. saccharina*.

7.2 Materials and Methods

7.2.1 *Insect stock*

Larvae of *E. saccharina* used in this study were originally collected from maize farms in the study area at Medie. The larvae were reared on fresh maize stem cuttings in plastic containers, until they developed to mature pupae (black-eyed and dark brown pupae), ready for irradiation. Studies were conducted under laboratory conditions of temperature range 26^{0}C-30^{0}C, relative humidity between 60% and 85% and photoperiod of 12:12 light and dark hours.

7.2.2 *Irradiation of pupae and adult crosses*

Twenty five male mature pupae were randomly selected from the colony stock, singly placed in plastic tubes and conveyed to the gamma source for irradiation. Detailed process of irradiation was described in Chapter 6. The pupae were exposed to a sterilizing dose of 150 Gy, chosen as the mean of optimum dose between 120 Gy and 180 Gy. A group of similar number of mature pupae was also held as the control (0 Gy).

Adult moths that emerged from the treated pupae were crossed with normal virgin females, forming the parental (P) generation. The mated pairs were kept in oviposition cages where eggs laid subsequently hatched into larvae, constituting the F_1 generation. These larvae were reared on artificially formulated diet described in Chapter 5. The larvae were observed daily until they developed to $4^{th}/5^{th}$ instars, (28-35 days post-hatched larvae) when they were ready for dissection. Larvae that were not selected for dissection were allowed to develop through pupae to adult moths to sustain the laboratory colony. The F_1 males randomly selected were crossed with another set of normal virgin females in the colony to produce larvae of F_2 generation, whose $4^{th}/5^{th}$ instars were also selected for F_2 dissection. Control groups of untreated larvae were also set up for both F_1 and F_2 generations.

7.2.3 *Dissection and squash-staining technique*

The sexes of *E. saccharina* larvae cannot be distinguished morphologically. Larvae in $4^{th}/5^{th}$ instars (both treated and control) were randomly selected from the experimental colony and dissected in a physiological saline solution (Belar's solution) to identify the testes. The pair of testes are normally located in the 5^{th} abdominal segment, dorso-laterally placed and attached to

the tracheal tubes, usually surrounded by fatty tissue. In *E. saccharina* the testes are a pair of small whitish oval-shaped organs. The procedures of squash-staining technique (Marec *pers. comm.*) used in slide preparation of spermatocytes of *E. saccharina* male larvae were as follows:

- Testes identified in a dissected larva were carefully removed and dropped in a hypotonic solution of 0.075 molar of Potassium Chloride (0.075M KCl) for about 10 minutes. The solution caused the testes to swell up for easy staining and maceration.
- The testes were then stained by transferring into a drop of 2.5% lactic-acetic-orcein (LAC) for about 20 minutes.
- For maceration, a testis was transferred onto a slide, a drop of lactic-acetate added and mounted needles used to release clusters of spermatocytes, disposing off the sheaths of testis as well.
- The specimen on the slide was then covered with a cover-slip and squashed by using the thumb on a piece of filter paper, wrapped around the cover-slip to blot out excess stain.
- The slide preparation was temporarily mounted by sealing the margins of the cover-slip with nail varnish and this could be stored in a refrigerator for about 2 weeks or more.
- The slides were observed under light compound microscope, Reichert-Jung Micro Star 110.

In this study, photomicrographs of the prepared slides were taken using Olympus BX 50 fluorescence microscope at magnification 100 x 1.30 x 50 and fitted with camera Olympus C-3.5 AD-4. A total of 12 pairs of testes, each of F_1 and F_2 generations were squashed and

stained, with a similar number for the control. For each prepared slide observed under the microscope, the best view of actively dividing spermatocyte cells were examined and counted.

The cells with chromosomal aberrations were also identified and counted. Cells showing clear meiotic/mitotic divisions (metaphases) of normal chromosomes were noted and counted. Percentages of aberrant cells and normal cells were calculated.

7.3. Results

In the present study, the normal chromosome structure of *E. saccharina* appeared dot-like, as found in other species of Lepidoptera. The chromosome number counted was 30 pairs as observed in metaphase I of meiotic division of the spermatocytes (Plate 7.1). It was also observed that radiation-induced chromosomes in the spermatocytes of *E. saccharina* exhibited various forms of chromosomal aberrations in chains, rings and fragments (Plate 7.2).

Table 7.1. shows the percentage visible chromosomal aberrations of F_1 and F_2 generations, originated from irradiated parents (P) of *E. saccharina* with a dose of 150 Gy in comparison with the control. The F_1 generation showed more chromosomal aberrations, 97%, in irradiated spermatocyte cells than in the control, 3.0%. In F_2 generation however, chromosomal aberrations relatively reduced to 89% compared with the F_1 in irradiated cells but slightly increased to 11% in the control. The percentage of cells that appeared normal was 12% compared with 3.0% in F_1 generation.

Plate 7.1 **Normal spermatocytes of *E. saccharina* in meiotic division showing 30 chromosome pairs (metaphase I).**

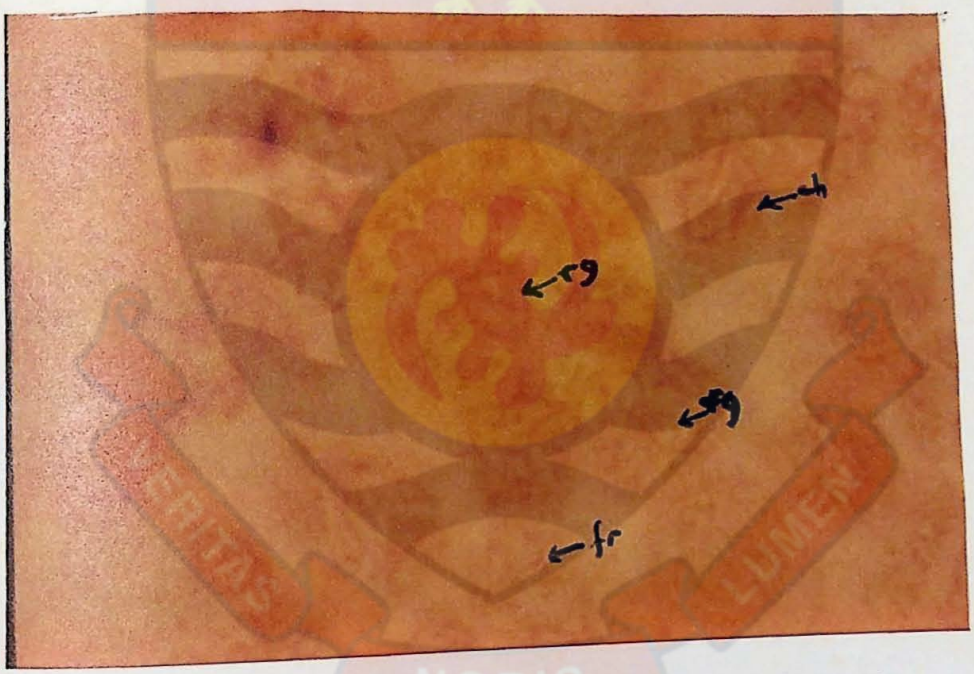


Plate 7.2 **Spermatocytes of *E. saccharina* F_1 male whose paternal parent received 150 Gy dose of radiation, showing chromosomal aberrations of *chains- ch*; *rings- rg*; *fragments- fr.* (metaphase I).**

Table 7.1. Chromosomal aberrations in spermatocyte cells of male larvae of *Eldana saccharina* in F_1 and F_2 generations

Spermatocyte cells in active cell division (metaphases)	Radiation dosage (Gy)	
	0 (Control)	150
	F_1 generation	
Total cells examined	131	229
No. of normal cells	127	7
No. of aberrant cells	4	222
Percentage of aberrant cells	3.1	96.9
Percentage of normal cells	96.9	3.1
	F_2 generation	
Total cells examined	179	209
No. of normal cells	160	24
No. of aberrant cells	19	185
Percentage of aberrant cells	10.6	88.5
Percentage of normal cells	89.4	11.5

7.4. Discussion

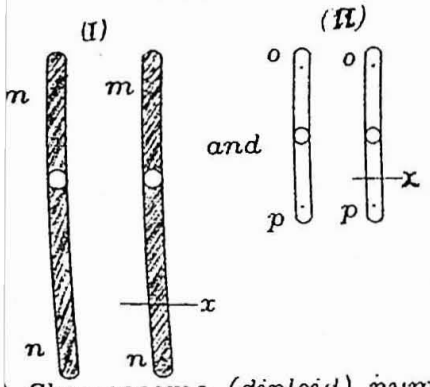
The haploid chromosome number of 30 recorded for *E. saccharina* in this study, confirms the usually large chromosome numbers characteristic of Lepidoptera (Virkki, 1963; Guthrie *et al.*, 1965). White (1954) listed diploid numbers of chromosomes of 58, 60, and 62 as common among lepidopteran species. The small sizes of chromosomes in the Lepidoptera may have resulted in large numbers of chromosomes in their nucleus. This is in contrast with the situation in Diptera, where the chromosomes are normally large in size and fewer in numbers, as it occurs in the fruit flies with only 4 pairs of chromosomes in the nucleus. The physiological implications of the chromosome numbers and sizes on the behavioural pattern of the insects are not known.

The present study has shown that F_1 and F_2 generations of *E. saccharina*, whose paternal parents were irradiated with sterilizing dose of 150 Gy, resulted in the formation of a number of chromosomal aberrations in the spermatocyte cells. Similar observations were also recorded in cytological studies on other moths like the fig moth, *Ephestia cautella* (Walker) (Al-Taweel *et al.*, 1990), the Asian corn borer, *Ostrinia furnacalis* (Guenee) (Zhang *et al.*, 1993) and the fall armyworm, *Spodoptera frugiperda* (Smith) (Carpenter *et al.*, 1997). In the F_2 generation, the percentage of normal cell was relatively higher than that of F_1 generation. This possibly could be an indication of gradual recovery of induced cells to normalcy in subsequent generations, a situation which could occur, if the frequent releases of the sterility factor into the natural population is discontinued.

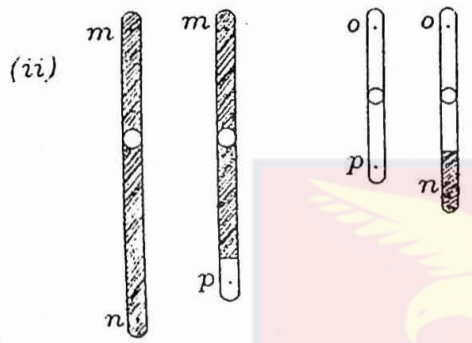
The formation of rings, chains and fragments of aberrant chromosomes in this study, could have resulted from multiple translocations in the germ cells due to irradiation in the

spermatocytes. Broken chromosomes may reunite in many ways. A typical chromosomal rearrangement is the reciprocal translocation in which parts of two different broken chromosomes have rejoined. In general, most reciprocal translocations can be transmitted to the offspring since each of them can have a centromere on them (Zhang *et al.*, 1993). Such chromosome rearrangement, however, may be fatal to a progeny in meiosis, because the gametes produced could be deficient or duplicated with genetic materials that are contained in the germ-cells.

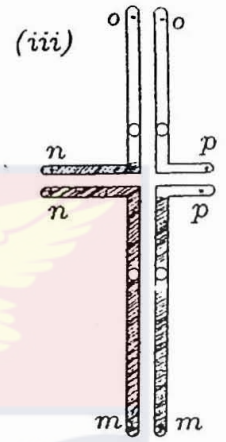
An illustration of a possible formation of translocations in chromosomal aberration is shown in Fig. 7.1. This illustration (modification of Pedder and Wynne, 1972) is further used to explain the possible mechanism of genetic sterility in the irradiated moths. During meiosis in prophase I, chromosomes in active dividing cells usually become prominent as they proceed from pachytene through diplotene to diakinesis. Homologous chromosomes after pairing, coiling and exchange of genetic materials, tend to repel from each other. Gene to gene pairing could cause the formation of a characteristic "Pachytene cross". Consequently, translocation complex, such as the "Metaphase ring complex" can be formed as well. At anaphase where chromosomes are pulled to opposite poles of the cell by spindle fibers, it is possible that adjacent chromosomes in the germ cell may move to the same pole. This results in the formation of gametes containing chromosomes with duplications and deletions (deficiency) of genes. Such gametes formed from these combinations would be inviable or (partially) sterile.



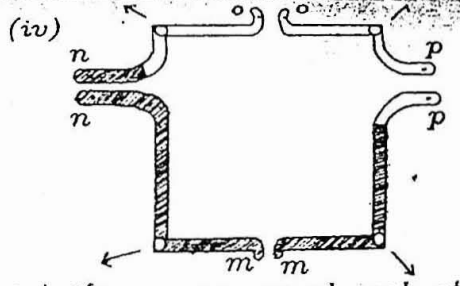
) Chromosome (diploid) number of
ur, made up of 2 shaded (I) and 2
nshaded (II) homologous chromosomes
espectively. Loci of allelic genes, m, n, o, p.
re shown on their respective chromosomes;
ie point of exchange of gene segments X,
tween non-homologous chromosomes.



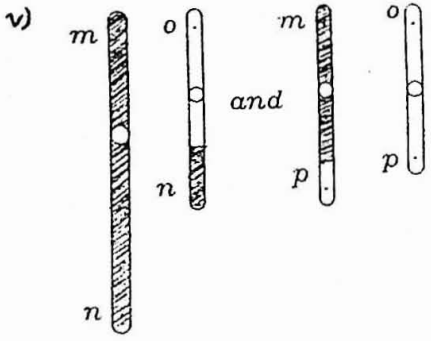
ii) Exchange of segments between non-homologous chromosomes, leading to chromosomes carrying translocated segments in meiotic division, at crossing over.

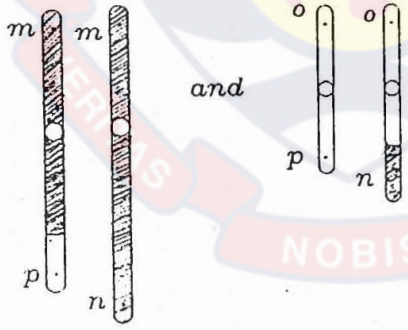


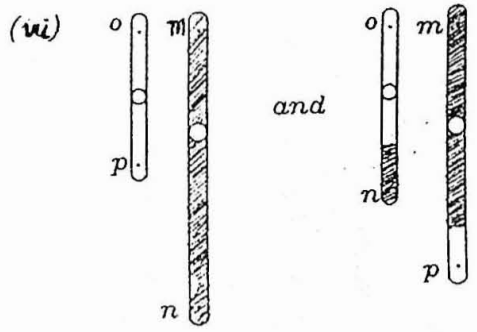
iii) Further pairing and coiling of chromosomes in Prophase I, may lead to the formation of characteristic "Pachytene cross"



iv) Chromosomes repel each other to form a translocation complex, "Metaphase Ring Complex"







If adjacent chromosomes in (iv) moved to the same pole, four types of gametes are formed, containing chromosomes with duplicated genes and some deletions. Gametes formed from these combinations are inviable, causing sterility in the offspring

If alternate chromosomes moved to the same pole, two types of gametes are formed, each with a full complement of genes m, n, o, p, respectively. Such combinations will be viable.

This explanation agrees with the views and observations of other researchers, who performed similar genetic studies on some lepidopteran species (LaChance, 1985) and on the Mediterranean flour moth, *Ephestia kuehniella* (Marec *et al.*, 1999; Tothova and Marec, 2001). It was suggested from their observations that the probable mechanism of inherited sterility in the Lepidoptera results from the production of chromosomally unbalanced gametes, caused by radiation-induced multiple translocations in the F_1, which are passed on to cause sterility of their carriers.

7.5 Conclusion

The study has shown that there was high percentage of visible chromosomal aberrations in the spermatocyte cells of F_1 and F_2 generations of *E. saccharina* male larvae, whose male parents were exposed to sterilizing dose of 150 Gy. Types of chromosomal aberrations in the form of rings, chains and fragments were found in the progeny. The probable mechanism of inherited sterility in the moth could be due to the production of gametes containing chromosomes with duplications and deletions of genes, caused by radiation-induced multiple translocations in the F_1 and transferred to the offspring. Evidence of visible chromosomal aberrations can therefore be essential tool for monitoring the success of releases of sterile moths for population suppression in the wild. Cytological analysis can be performed on sampled larvae from the suppressing population to ascertain the extent of infusing F_1 (sterility factor) into the natural population and the capability of establishing inherited sterility in a natural population.

CHAPTER EIGHT

RELEASES OF STERILE TO FERTILE MATING RATIOS OF *ELDANA SACCHARINA* AND DETERMINATION OF F_1 STERILITY IN FIELD CAGES

8.1 Introduction

Radio-sterilization studies have been performed and documented on a few tropical moth species, including the cocoa moth, *Cadra cautella* (Amu, 1971; Ahmed *et al.*, 1972), spotted stem borer, *Chilo partellus* (Bughio, 1988; Okoth, 1990) and the fig moth, *Ephestia cautella* (Al-Taweel *et al.*, 1990). All these studies however, were wholly performed under laboratory conditions and not in the field.

In order to embark on any meaningful field trials, prior to an area-wide control programme, it would be necessary to undertake field cage studies of the pest species. Field cage experiments are appropriate exercise to assess the quality of laboratory-reared insects compared to that of the wild insects, because they allow for natural environmental factors to affect the insects being tested rather than occur under laboratory conditions alone (Huettel, 1977).

The present study reports on releases of different mating ratios of sterile/fertile *E. saccharina* on potted maize plants in field cages and the determination of infestation levels of larvae on maize plants in the cages. The study also determined the F_1 sterility of *E. saccharina* released at the different mating ratios in the assigned field cages. The study was aimed at ascertaining the efficacy of inherited sterility for population suppression.

8.2 Materials and Methods

8.2.1 Treatments and releases of moths in field cages

Three field cages, each of dimension 1.0 m x 1.0 m x 2.0 m were constructed and placed in an enclosed farm-yard near the laboratory. Maize was grown in black polythene bags with 3 seed-plants per bag. About 35-40 bags were placed in each field cage at a time. Moth releases into the cages were usually effected 50 days (7 weeks) after maize emergence (germination) because *E. saccharina* was normally attracted to oviposit on mature maize crop.

Mature, 6-7 days old pupae of both sexes were randomly selected from the laboratory colony for the studies. Irradiation of pupae was performed with a dose of 150 Gy in air in gammacell-220 irradiator of Cobalt-60 source. The three mating ratios of emerged adults from the untreated/treated pupae in the assigned field cages were as follows:

1 fertile : 1 fertile = 6 fertile males + 6 fertile females.

1 sterile : 1 fertile = 6 males and 6 females both irradiated with 150 Gy + 6 fertile males and 6 fertile females

5 sterile : 1 fertile = 30 males and 30 females both irradiated with 150 Gy + 6 fertile males and 6 fertile females.

Only three mating ratios could be performed in this study due to time constraint and inadequate resources. The first treatment was the control, demonstrating the natural situation of almost equal proportions of fertile males mating with fertile females. The second treatment was to demonstrate a situation in field cages of equal ratios of fertile to sterile moths, whilst the third treatment was the situation where the number of sterile male/female moths released were 5 times more than the natural fertile population.

Emerged adults were released in the field cages between the hours of 1700 and 1800 GMT local time, due to their nocturnal activity. Seven days after releases, female moths were collected from the cages and dissected to examine their mating status.

8.2.2 *Determination of larval infestation and stem damage*

To determine larval infestation and extent of stem damage, random sampling of 100 maize plants was conducted in each field cage, 35 days after moth releases (for late instar larvae). The stems were then dissected and total number of larvae in the stems of each cage per treatment was counted. The larvae were put into separate plastic containers, labelled and conveyed to the laboratory for further studies. Each treatment was replicated three times with new sets of moth and potted maize plants. Percentages of stem damaged and larval infestations were calculated per treatment. Data were subjected to analysis of variance (ANOVA) for comparison of means of each treatment.

8.2.3 *Determination of F_1 sterility*

Larvae of F_1 generation collected from the field cages and carried to the laboratory, were fed on fresh maize stem cuttings. They were daily observed in the laboratory through pupal to adult stages. Laboratory conditions recorded during the period of study were photoperiod of 12 hrs darkness : 12 hrs light, temperature of $28^0C \pm 2.5$ and relative humidity of $70\% \pm 10.5$.

In this study, only males of emerged F_1 adults were used to cross fertile virgin females of the laboratory stock. Reciprocal crosses were not performed due to time constraint. Adults were

paired with the opposite sexes in 1:1 ratios and mating pairs placed in plastic oviposition cages, labelled according to the specified treatments as pertained in the field cages. All females after oviposition (5-7 days) were dissected to examine their mating status. For mating adult, five mating pairs were set up in separate oviposition cages and repeated four times.

Total number of eggs laid per female (fecundity) was calculated. Sterility for each mating ratio was determined by calculating the percentage of eggs that failed to hatch. Other biological parameters recorded include larval/pupal mortality and sex ratios of emerged F_1 adults.

8.3 Results

8.3.1 Damage/infestation caused by released moths in field cages

The percentage plant damaged and larval infestation caused by releases of *E. saccharina* in field cages are shown in Table 8.1. The mean number of larvae per plant was approximately 2 in the control with fertile moths. This was drastically reduced by 50% and 75%, as the ratios of sterile to fertile moths increased from 1 to 5 respectively. In similar trend, percentage stem damaged of 86% and larval infestation of 71% in the control significantly decreased to 24% and 10% respectively in 5 sterile : 1 fertile moths ratio. This corresponds to reduction by 72% and 86% in stem damaged and larval infestation respectively, in the latter compared with the control. Differences between the means were significant ($p < 0.05$).

Table 8.1 **Percentage damaged maize plants and larval infestation by *E. saccharina* moths released in field cages at different sterile to fertile ratios.**

Treatments/Ratios of released moths.	Mean number of larvae per plant	% stem damaged *(Mean ± SE)	%larval infestation (Mean ± SE)
1 fertile : 1 fertile	1.89 ± 0.5^a	86.0 ± 0.8^a	71.0 ± 0.6^a
1 sterile : 1 fertile	0.80 ± 0.1^b	46.0 ± 0.9^b	25.0 ± 1.0^b
5 sterile : 1 fertile	0.49 ± 0.1^c	24.0 ± 1.0^c	10.0 ± 0.8^c

*Means followed by different letter(s) in a column are significantly different at 0.05 level, LSD.

8.3.2 F_1 sterility

Data on F_1 sterility in field cages are represented in Table 8.2. Percentage mortality for both larvae and pupae increased with increased sterile to fertile ratios. The differences of means of 1 : 1 and 5 : 1 sterile to fertile ratios are however, not significant ($p>0.05$). The means of fecundity and hatchability decreased with increased sterile to fertile ratios. Moth ratio of 5 sterile : 1 fertile showed a reduction by 79% in fecundity compared with the control, whilst that of 1 sterile to 1 fertile was reduced by 47%. Mean of sterile eggs was 7.2% for the control, 65.6% and 83% for moth ratios of 1 sterile : 1 fertile and 5 sterile : 1 fertile respectively.

Table 8.2 F_1 sterility in progeny of *E. saccharina* released moths collected on maize plants in field cages

Treatments/Ratios of released moths	% Mortality *Larv; Pup	%Fecundity (Mean ±SE)	% Eggs hatchability (Mean ±SE)	% Eggs sterile (Mean ±SE)	Emerged F_1 adults sex ratio M : F
1 fertile : 1 fertile	25 ; 3.5	98.0±16.1	93.0 ± 10.3	7.2 ± 5.2	1.1 : 1.0
1sterile ; 1 fertile	36 ; 8.5	52.6±12.1	33.2 ± 4.0	65.6 ±3.6	1.7 :1.0
5 sterile : 1 fertile	39 ; 9.5	20.7± 6.2	18.0 ± 2.0	83.0±2.8	1.5 : 1.0

*Legend : Larv. = Larvae ; Pup. = Pupae

Sex ratios of emerged F_1 adults was approximately 2:1 in favour of more males than females in sterile to fertile moth ratios. For the control, the sex ratio is 1 male : 1 female.

8.4 Discussion

Normal mature moths when ready for reproduction, usually carried fertile sperms in the males and fertile eggs in the females. After mating, the females oviposited large proportions of fertile eggs that hatched into large number of larvae, infesting the host plants and consequently causing great damage to the stems. This could explain the situation in the field cages of the control which recorded relatively high fecundity, hatchability, percentage stem damaged and larval infestation levels.

This study has shown that with 150 Gy irradiation and moth released ratio of 5 sterile to 1 fertile into the population of *E. saccharina* in field cages, F_1 sterility could be used to reduce the reproductive ability by 79%. The field cage experiments have proved viable the use of F_1 sterility to suppress the population of the stem borer species. However, further studies on higher ratios of sterile to fertile released moths need be performed for a more conclusive prove of efficacy of F_1 sterility in pest management of stem borer species before embarking on field trials and area-wide control programme.

CHAPTER NINE

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

9.1 General Discussion

In applying inherited sterility as a control strategy in insect pest management, certain fundamental biological studies have to be undertaken in order to achieve the desired results. These include the biology/ecology of the target species, laboratory rearing of the insects for mass production, radiation biology of the insect species and other essential parameters of economic importance.

Most peasant farmers in the rural areas depend largely on rainfall for their farming activities. Maize crops cultivated in the study area during the major rainy and planting season experienced relatively lower larval infestations of *E. saccharina* and *S. calamistis*, compared with the higher levels of infestations in the minor season. The frequent rains could wash off some considerable number of eggs and neonate larvae, resulting in fewer of them establishing in the maize ecosystem.

This favourable condition could be of great advantage to farmers and the entire farming community to obtain relatively better yields in maize production. The people could take

advantage of the frequent rains to cultivate large acreage of maize, harvest and store enough for use during the lean season. During the minor rainy season, less frequent rains would lead to more of the larvae establishing on the maize crops, resulting in higher infestations and consequently poor maize yields. Due to limited time and inadequate logistics, trapping equipment could not be procured to undertake adult population study.

It is essential to study the ecobiology of the pest species in order to know the population fluctuations and behavioural pattern of the insect species. One of the ultimate benefits from the results of this study could be to predict or offer appropriate timing for field releases of sterile pupae/moths and ensure synchrony in the emergence of wild moths and the released ones for successful mating in a field control strategy.

The ultimate aim of laboratory rearing of the borer species is to produce large numbers of the species for conducting experimental studies and for performing field releases of sterile moths. Laboratory rearing of *E. saccharina* and *S. calamistis* in the present study was satisfactorily conducted at a minimum cost, since most of the rearing apparatus were plastics containers of moderate prices and already some infrastructures were in place. Both borer species were successfully colonized in the laboratory, feeding them on natural and artificial diets. However the colony of *S. calamistis* was not quite sustainable, probably due to its relatively high vulnerability to changes in laboratory conditions. The colony of *E. saccharina* was however self-sustaining and seemed to exhibit higher reproductive capacity, compared with the former. *Eldana* also appeared resilient and could better withstand changes in the laboratory conditions.

One observation during laboratory rearing of the two borer species was the relatively longer life span of about 50-60 days, starting from the egg stage through to the adult stage. The period of rearing the voraciously feeding larvae alone was averagely between 30-40 days, which could be a disadvantage in increasing the cost of feeding. The egg, pupa and adult stages were however short-lived and easy to handle.

Radio-sterilization studies of the two borer species was successfully performed through one of the essential resources, the ready availability of Cobalt-60 gamma facility (except for unforeseen technical problems). However, one short-coming of the gamma cell-220 facility was the low dose rate, which therefore prolonged the time of irradiation.

Irradiation effect on pupae of both borer species showed that *S. calamistis* sustained more bodily deformities and higher percentage of unemerged adults than that of *E. saccharina* with the same radiation doses. Probably the docile and soft nature of the former in contrast with the robust and hardier nature of the latter has made *Eldana* appeared more radio-resistant and therefore readily available for radiation experiments.

Increased doses of radiation (80-180 Gy) did not affect the mating ability and sperm insemination of *E. saccharina*. Fecundity and fertility reduced significantly with increased radiation doses for both F_1 male and F_1 female crosses. In order to throw more light on the possible mechanism of inherited sterility in the borer species, a study on cytogenetics of *E. saccharina* was performed, to observe the chromosome structure, the

estimated chromosome number of the dividing cells, and the chromosomal aberrations of partially sterile F_1 progeny. Cytogenetics of lepidopterous species is generally quite tedious due to the small sizes of the chromosomes, which therefore requires powerful microscope to reveal detailed structures. The present study was however conducted using the ordinary compound microscope and the chromosome number obtained was 30 pairs; chromosomal aberrations observed were in the form of rings, fragments and chains.

Field caged experiments performed on mating ratios of released moths of *E. saccharina* with 150 Gy, showed drastic reduction in both fecundity and fertility with increased sterile to fertile ratios in F_1 progeny. The field caged experiment in the present study was conducted up to the ratio of 5:1 due to time and financial constraints. If more resources were available, more cages could be constructed for performing experimental studies for ratios of 9 sterile to 1 fertile and 10 sterile to 1 fertile.

9.2 Conclusions

On the whole, studies conducted on the biology and irradiation of *Eldana saccharina* and *Sesamia calamistis* have shown that the two species could be suitable candidates in the application of induced inherited sterility for population suppression. Laboratory rearing of both species can be conducted using simple apparatus and the colony of *E. saccharina* is manageable and sustainable.

The study has proved the potential of using F_1 sterility to reduce the reproductive capability of *E. saccharina* to over 90% with optimum radiation dose range between 120 Gy and 180 Gy. Field cage experiment of *E. saccharina* with radiation dose of 150 Gy suppressed the pest population by over 80% with released moth ratio of 5 sterile : 1 fertile.

9.3 Recommendations

- Adequate funding should be granted for further studies on inherited sterility of the two borer species, especially on *Sesamia calamistis* for insect pest management.
- The present facility of the gamma cell-220 Cobalt-60 source should be up-graded or replaced with a bigger source for fast and more efficient results.
- The laboratory should be furnished with modern equipment, including microscopes of higher magnification power for performing detailed analysis in research work.
- It is acknowledged that more efficient results in insect pest management could be achieved through integrated approach. It is therefore anticipated that collaborative research is conducted by integrating F_1 sterility with other biological control strategy such as parasitoids, for more effective population suppression of the maize stem borer species.

1 A study was conducted on bioecology of *E. saccharina* and *S. calamistis* in maize farms at Medie in the Ga District of Greater Accra Region. The study area is located within a forest-savanna transition ecological zone of Ghana. Bi-weekly field sampling of larvae/pupae of the two borer species was undertaken for a 3-year period. The effect of rainfall, temperature and relative humidity on larval numbers was observed.

2 High rainfall amounts tended to reduce larval numbers on the maize crops, while larvae increased with low amounts of rainfall. Since larvae live inside the stem for greater part of their life span, temperature and relative humidity did not have direct influence on larval numbers of the borer species.

3 Laboratory rearing of *E. saccharina* and *S. calamistis* larvae/pupae on both natural and artificial diets was performed to compare their developmental differences.

4 Developing larvae/pupae of *E. saccharina* on both natural and artificial diets did not show significant differences in their biological parameters. Mean larval/pupal mortality of *S. calamistis* was significantly higher on artificial diet than on natural diet. Mean fecundity in *S. calamistis* was relatively low on both diets compared with that of *Eldana*.

5 In both borer species, pupal weights of natural dieters were slightly heavier than those of artificial dieters. Adult emergence of relatively smaller-sized pupae produced more males, while larger-sized pupae emerged into more female adults for both borer species.

6 Different ages of pupae (2, 4, 6 and 8 days old) of *E. saccharina* and *S. calamistis* were irradiated with sub-lethal gamma doses of 80, 100, 120, 150 and 180 Gy. This was to determine the suitable age of radiation-induced pupae, whose emerged adults showed minimum or no bodily deformities.

7 Younger pupae of both borer species exposed to increasing doses of ionizing radiation showed high percentage of deformities and unemerged adults. Older pupae however, were less susceptible to increased radiation doses and therefore showed fewer deformed pupae and unemerged adults.

8 The effects of sub-lethal doses of ionizing gamma radiation, (80-180 Gy) on mating, fecundity and fertility of *E. saccharina* was observed. The optimal doses of induced inherited sterility in the F_1 progeny was determined.

9 Increased doses of radiation did not adversely affect mating capacity of *E. saccharina*, resulting in 78-92% mating success for both irradiated and non-irradiated mating pairs. In crosses involving treated males and normal females of the parent generation, fecundity decreased (partially) by 40% compared with the control. With the

reciprocal crosses, fecundity drastically reduced by about 96% (almost completely sterile) with doses of 150 Gy or more. Similarly, fertility decreased with increased doses, such that with treated females crossed with normal males, fertility was almost completely sterile with doses higher than 100 Gy.

10 In F_1 generation, both fecundity and fertility followed similar trend of inverse relationship with increasing doses of radiation. F_1 males however, showed lower fertility than the corresponding F_1 females. While a reduction of fertility to about 10% was observed in F_1 males with 100 Gy, similar reduction in fertility was obtained in F_1 females with dose of 150 Gy.

11 Significant reduction in fecundity and fertility was observed with ionizing radiation dose above 100 Gy, suggesting that induced sterility in *E. saccharina* could be effected within the doses of 120 and 180 Gy.

12 Radiation on developing larvae/pupae of *E. saccharina* slightly prolonged larval life span, 41-50 days, with increasing radiation doses, 0-180 Gy. Average larval mortality also increased from 8% to 32% with 0 Gy and 180 Gy respectively. Pupal mortality was relatively low, 4-12%, compared with that of larvae.

13 Sex ratio of emerged adults was slightly skewed in favour of males than females with increased doses of radiation in F_1 progeny.

14 The chromosome number observed from normal meoitic spermatocytes of *E. saccharina* was estimated at 30 pairs of chromosomes. Chromosomal aberrations found in radiation-induced cells were in the form of fragments, rings and chains.

15 The probable mechanism of inherited sterility deduced from the chromosomal aberrations could be explained by formation of unbalanced gametes, containing chromosomes in duplications and deletions of genes, caused by radiation-induced multiple translocations and transferred to the progeny.

16 Field cage studies of *E. saccharina* on mating ratios of sterile to fertile released moths, irradiated with 150 Gy, revealed that fecundity, fertility and percentage larval infestation reduced with increasing ratios of the sterile to fertile released moths. Fecundity was reduced by 47% with ratio of 1 sterile to 1 fertile and 79% with ratio of 5 sterile to 1 fertile released moths. Corresponding fertility reduction was by 64% and 80% respectively.

17 From general observations, it is noted that applying sub-lethal doses of gamma radiation on mature pupae of *E. saccharina* invariably resulted in substantial reduction in fecundity and fertility of both parents and F_1 generations. This is indication of population reduction of the insect species, which could be used in a control strategy to suppress insect pest populations.

18 The general implication from the present study is that inherited sterility could be one of the significant innovations of peaceful uses of nuclear energy in biotechnology, that could be applied to mitigate the perennial problems of insect pest infestations in our developing world. This could be used as a control strategy in insect pest management to enhance productivity in agriculture and ensure sustainable food security.

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Appendix- 1

Data on Rainfall, Temperature and Relative Humidity during the study period

Year	Months	Monthly Rainfall (mm)	Mean Temperature (°C)	Mean Relative Humidity (%)
1997	January	4.80	27.90	77.00
	February	0.00	28.50	77.00
	March	112.10	28.80	79.50
	April	138.20	28.30	79.50
	May	63.70	28.20	79.00
	June	298.50	27.30	82.00
	July	48.80	26.90	81.50
	August	5.20	27.50	79.50
	September	14.80	28.20	78.00
	October	99.80	28.30	79.00
	November	128.80	28.30	79.50
	December	60.00	28.10	79.00
1998	January	0.00	27.10	69.50
	February	3.40	29.50	73.00
	March	0.00	30.30	78.50
	April	2.80	30.80	79.50
	May	222.50	28.90	80.00
	June	78.60	28.10	76.00
	July	41.70	27.30	80.50
	August	21.50	26.90	83.00
	September	59.90	28.10	81.50
	October	111.90	28.00	80.00
	November	128.50	28.50	81.50
	December	40.50	28.40	78.50
1999	January	35.70	28.40	79.00
	February	34.70	28.00	71.50
	March	36.60	29.00	78.50
	April	56.10	29.20	79.00
	May	57.20	28.70	79.50
	June	462.50	27.70	81.50
	July	148.70	26.00	90.00
	August	67.30	26.30	85.50
	September	50.70	26.20	87.00
	October	67.80	27.00	82.50
	November	90.80	27.90	82.00
	December	14.00	28.20	78.50

Appendix- 2

Correlation Coefficients of Larval Numbers in Relation to Rainfall, Temperature and Relative Humidity

Year/Species Type	Climatic Factors with Correlation Coefficients (r) and Probability Values (p)					
	Rainfall		Temperature		Relative Humidity	
	r	p	r	p	r	p
97 *Eldana saccharina*	-0.5899*	0.043*	-01191	0.712	-0.3176	0.314
Sesamia calamistis	-0.0522	0.872	-0.7474*	0.005*	0.4621	0.130
98 *Eldana saccharina*	0.0921	0.776	-0.1978	0.538	-0.0756	0.815
Sesamia calamistis	-0.0913	0.778	-0.5436	0.068	0.5731	0.051
99 *Eldana saccharina*	-0.2284	0.475	-0.3831	0.219	0.3947	0.204
Sesamia calamistis	-0.1078	0.739	-0.0743	0.818	-0.1291	0.689

Correlations are significant at p<0.0500