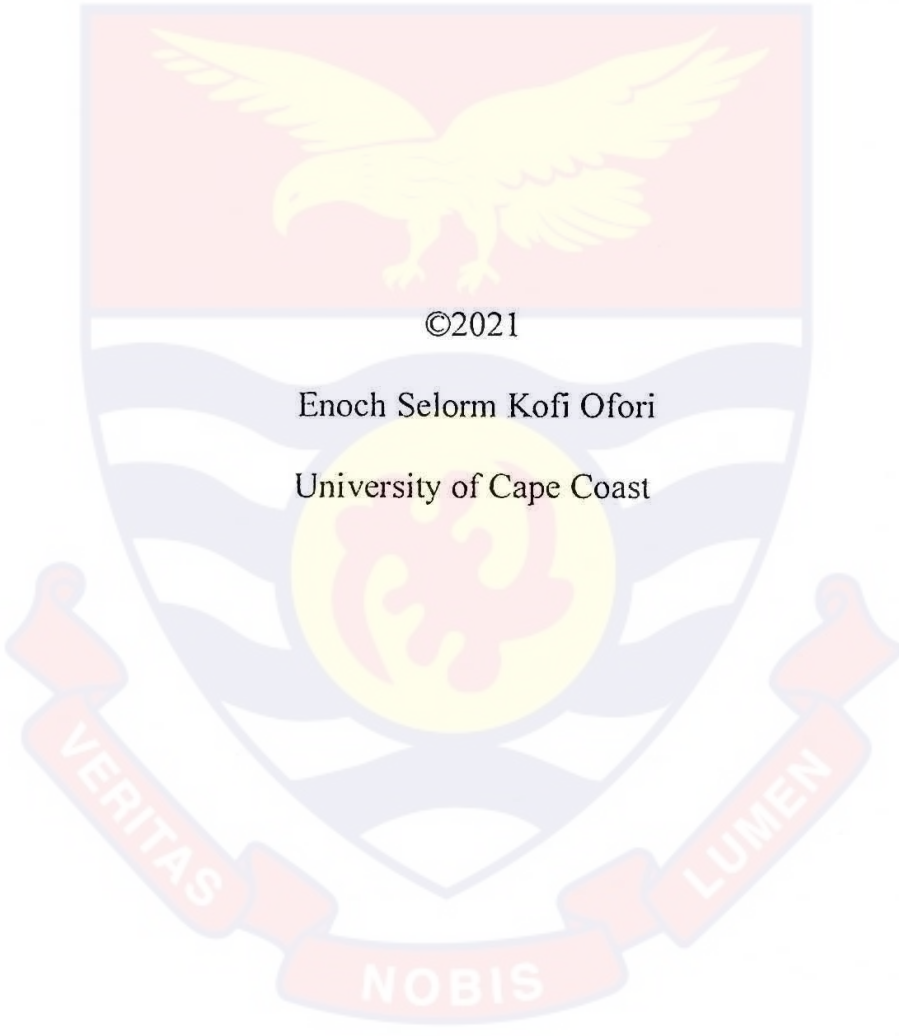

UNIVERSITY OF CAPE COAST

DEVELOPMENT OF TRAP-IRRADIATE-RELEASE/STERILE INSECT TECHNIQUE AS A TOOL FOR INTEGRATED PEST MANAGEMENT OF *Bactrocera dorsalis* HENDEL (DIPTERA: TEPHRITIDAE) IN MANGO ORCHARDS

BY

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Thesis submitted to the Department of Conservation Biology and Entomology, School of Biological Sciences of the College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfillment of the requirements for award of Doctor of Philosophy degree in Entomology

DECEMBER, 2021

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

I 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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ABSTRACT

One major important fruit fly causing widespread damage to fruits and vegetables in Ghana is the oriental fruit fly, *Bactrocera dorsalis* Hendel. Current management strategies such as male annihilation technique, bait application technique and insecticide applications are unable to eliminate the challenges posed by *B. dorsalis*. This study therefore sought to determine a new strategy in which *B. dorsalis* might be managed in a more integrated fashion. Over a period of one year, the fruit fly population in the south-eastern mango enclave of Ghana was monitored using baited traps to ascertain their diversity and population dynamics. This was followed by another study to determine the most efficient trap types and time of day to mass trap *B. dorsalis* for irradiation studies. In another study, the optimum dose of gamma irradiations to cause sterility in adult male *B. dorsalis* for trap-irradiate-release/sterile insect technique was investigated. Furthermore, the behavioural response of adult males of *B. dorsalis* that have been pre-exposed or un-exposed to methyl eugenol (ME) to ME-baited traps was investigated. Ten fruit fly species were identified in the study area with *B. dorsalis* being the most dominant. Through this study, *Dacus langi* and *Dacus longistylus* were detected and recorded for the first time in Ghana. Ecoman traps were most efficient for trapping large numbers of adult *B. dorsalis* and evening-captured flies survived better. Moreover, trapping of flies that were un-exposed to ME yielded a higher recovery rate compared with flies that were pre-exposed to ME. This study has demonstrated that, a large population of adult male *B. dorsalis* exist in the south-eastern mango enclave of Ghana that could be trapped in the evenings with Ecoman traps for irradiation and TIR technique of insect pest management. Furthermore, TIR has a great

potential to be successful since the males trapped with ME-baited traps and irradiated are less likely to be re-trapped in ME-baited traps.

KEY WORDS

Oriental Fruit fly

Tephritid

Trap type

Irradiation

Methyl eugenol

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DEDICATION

I dedicate this work first to God Almighty and my mother, Madam Margaret Agbinku for her prayer support and upbringing.

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CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Oriental fruit fly (*Bactrocera dorsalis* Hendel) is a pest that infests over 300 cultivated and wild fruits, including mangoes (*Mangifera indica* L.). *Bactrocera dorsalis* is a common mango fruit fly problem (Drew et al., 2005; Ekesi and Billah 2006). Depending on the cultivar, region, and season, damage might range from 30 to 80 percent (Ekesi et al., 2006; Rwomushana et al., 2008; Vayssières et al., 2009). *Bactrocera dorsalis* has been stated to be of economic significance in parts of South Asia and Sub-Saharan Africa.

Oriental fruit flies cause crop damage by laying their eggs in fruits and soft tissues of plants, feeding by larvae, and degrading tissues of plant via feeding by larvae (Sarwar, 2015). Young fruits that have been infested become deformed and calloused, and they usually drop; mature fruits that have been infested have a water-soaked look. When just a few larvae reach adult stage, the damage consists of an unattractive look and impaired market value as a result of egg laying punctures or tissue collapse as a result of decay (Steiner, 1957). In addition to the direct losses, the quarantine limitation on fruit fly-infested goods has resulted in massive indirect losses and limited export to big profitable markets in Japan, United States, Europe and the Middle East, where the insect pest is designated as restricted pests. *Bactrocera dorsalis* was found in many cultivated fruit species in African countries making it to be prohibited from being imported into the United States under a federal order issued by the United States, thereby significantly restricting the trade of horticulture goods between Africa and the United States (USDA-APHIS, 2008; Ekesi et al., 2016). Since

the advent of *B. dorsalis* (Guichard, 2009), rejections of African mangoes in Europe have increased significantly, from 21 rejections in 2008 to 38 in August 2009. Several documented reports of interceptions were recorded from Burkina Faso, Côte d'Ivoire, Gambia and Ghana as well as Mali, Senegal and Cameroon. More than a billion people throughout Africa's mango value chain are directly or indirectly affected by the harm caused by *B. dorsalis* and other tephritid pest species. The European Union's tight quarantine standards and maximum residue level (MRL) have exacerbated this problem by threatening the export of mangoes from Africa, which are worth an estimated $35,000–40,000 per year and more than $42 million over 8 years (Lux et al., 2003b). As a consequence of a number of nations banning imports owing to fruit flies, the market value of mango has diminished (Ekesi, 2010).

Further, it has been observed that this pest is expanding its geographic and/or host crop range. Low-altitude settings with a mild temperature and the presence of the cultivated mango host are chosen by *Bactrocera dorsalis* and where it reaches its maximum abundance (Rwomushana et al., 2008; De Meyer et al., 2010, Geurts et al., 2012; Vayssières et al., 2014).

Currently, there are not any technologies that can accurately anticipate when management measures may be implemented to reduce the *B. dorsalis* pest population on mango orchards, which has exacerbated the problem.

Bactrocera dorsalis populations peak in the Guinea Savanna zone of Ghana in May and June, which corresponds with the maturity, ripening and harvesting of major mango cultivars (Badii et al., 2015a; Kannan & Venugopala, 2006).

Temperature, relative humidity, rainfall, and their dispersion patterns of fruit flies have been shown to significantly impact the variety and population dynamics of tephritid fruit flies throughout the course of the season. Therefore, it is vital and necessary to understand the relationships between the fruit fly activities and the surrounding environment. Therefore, this study will establish the diversity and population dynamics of fruit flies in the SouthEastern mango enclave of Ghana, determine the most efficient trap types and time of the day to collect large numbers of *Bactrocera dorsalis* for irradiation studies, investigate the optimum dose of gamma irradiations to cause sterility in adult male *B. dorsalis* for trap-irradiate-release/sterile insect technique and investigate the behavioural response of adult males of *B. dorsalis* that have been pre-exposed or unexposed to methyl eugenol (ME) to ME-baited traps.

1.2 Statement of the Problem

True fruit fly is one of the devastating pests in the world. In regions where fruit flies are prevalent, they play a significant role in crop losses (Goergen et al., 2011). There are several fruit flies that have been introduced to Africa, but the dominant one causing widespread damage in Ghana is the oriental fruit fly, *Bactrocera dorsalis* Hendel (Tephritidae). It was originally found in Kenya in 2003 (Lux et al., 2003b; Drew et al., 2005) and in the shores of Ghana in 2005. (Billah et al., 2006). The insect has established itself in Ghana, inflicting considerable damage to mango, citrus, avocado, and other fruits. Other indigenous fruit fly species, such as *Ceratitis cosyra* Walker, *C. capitata* Wiedemann, and *C. ditissima*, compete with the aforementioned insect. The two major categories of mango fruit flies in Africa are based on their origin, namely invasive and indigenous species. The invasive species include *B. dorsalis*, *B.*

zonata, and *Zeugodacus cucurbitae* whiles the indigenous species include *C. anonae*, *C. capitata*, *C. catoirii*, *C. ditissima*, *Dacus ciliatus* that are native to the continent (Ekesi et al., 2009, Rwomushana & Tanga, 2016). *Bactrocera dorsalis* is a crucial pest in mango production, but there is evidence that it may also harm peaches and plums. In both native and invasive species, *B. dorsalis* has been shown to be the most damaging, according (Ekesi, 2010, Ekesi et al., 2009). The fruit and vegetable business in sub-Saharan Africa has been severely damaged by this insect pest, which has resulted in losses of up to eighty (80) percent (Ekesi, 2010, Ekesi et al., 2009). Consequently, farmers tend to apply broad spectrum insecticides to protect their crops. Such excessive use of toxic chemicals has negatively impacted on the environment. Furthermore, they constitute health hazards to both farmers and consumers. Pesticides kill natural enemies, thus resulting in the emergence of secondary pests such as spider mites, scales, mealy bugs and leaf miners amongst others (Hardin et al., 1995). Therefore, the need to develop an integrated pest management strategy for *B. dorsalis* in mango orchards. Trap-irradiate-release offers a good strategy for this.

1.3 Purpose of the Study

The purpose of the study was to:

- ascertain diversed types of fruit flies in some selected mango orchards in the south- eastern mango enclave.
- develop an effective system for mass trapping of adult male *B. dorsalis*
- establish an optimum dose for sterilizing *B. dorsalis* adult males.
- study the response and attraction of *B. dorsalis* to Methyl Eugenol baited traps.

1.4 Research Objectives

The aim of this research is to develop an integrated pest management strategy for *Bactrocera dorsalis* in mango orchards using trap-irradiate-release/sterile insect technique. Specific objectives of the study were to determine:

1. the diversity and population dynamics of fruit flies on mango orchards in the SouthEastern mango enclave of Ghana.
2. the efficacy of three trap types, period of the day and influence of weather conditions for mass trapping adult *Bactrocera dorsalis* for irradiation studies.
3. the optimum radiation dose for sterilizing adult male *Bactrocera dorsalis* and its effect on the fecundity of non-irradiated females.
4. the effects of pre-exposure to methyl eugenol (ME) on the attractiveness of *Bactrocera dorsalis* to ME-baited traps.

1.5 Significance of the Study

The results from this study seek to help farmers to know when to begin preparation for fruit fly control. Fruit flies pose a great danger to the fruit and vegetable market. Control of these fruit flies in an environmentally friendly manner will help prevent the introduction of harmful chemicals into the environment thus preventing air pollution and maintaining a clean atmosphere for horticultural production. Large numbers of fruit flies exist in the mango production enclaves and these fruit flies are major pests which reduce the quality of fruit and vegetables for export. Trapping of these flies vis a vis the trap type and period of the day is important in removing large numbers of the flies in the production area therefore reducing the danger they pose to these

horticultural markets. In effect controlling the pests in a friendly environment helps to improve nutrient in the form of vitamins and minerals for consumption from the fruits and vegetables.

1.6 Delimitations

This research is focused on developing an effective control strategy of TIR-SIT as a major tool in the integrated pest management strategy of fruit flies in the horticultural production areas. This extends to mainly where fruits and vegetables are produced and the problem, they face with fruit fly infestation. The development of TIR-SIT is important in reducing the incidence of fruit fly infestation in areas where technical and logistical constraints make the application of conventional SIT impossible. If probably integrated with the already existing fruit fly control measures such as Male Annihilation Technique (MAT), sanitation, Bait Application Technique (BAT), the menace of the fruit flies could be overcome. The scope of the research is geared towards using an efficient and effective means for mass trapping of adult male *B. dorsalis* populations, stabilizing them in the insectary and determining the most effective dose to cause sterility in the adult males. This is aimed at achieving the strategy of SIT where continuous releases of these mass trapped and sterilized adult males will eventually reduce the wild populations of *B. dorsalis.*

The irradiation studies were conducted at the Gamma Irradiation Facility (GIF) located at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), Accra, Ghana.

1.7 Limitations

This research was limited to the population studies to determine the diversity and abundance of fruit flies in three (3) farms situated in the SouthEastern mango enclave of Ghana. These farms include Enyonam, Power of Trinity (POT) and Modest Step farms. The three farms have different cultural practices such as picking and destroying dropped and rotten fruits, weed control, pruning, harrowing, fertilizer application for their mango production. The hygienic nature of the farm is left entirely to the farmer. For instance, Modest Step and Enyonam Farms practice good hygienic condition on their farms. Power of Trinity usually leaves dropped and rotten fruits on the ground which can serve as a source of breeding ground for fruit flies, leading to cross-infestation of nearby farms. POT has bushes in and around the farm which fruit flies may use as an alternate host. The afore mentioned factors are key determinants in fruit fly population dynamics. In brief, the cultural practices in each farm differs from the other and this can affect the overall fruit fly population in the study location. Key strategic information was passed to farmers to adopt the best farming practices to help reduce fruit fly population build up in the farms. This has to be done in synchrony.

1.8 Definition of Terms

BNARI	Biotechnology and Nuclear Agriculture Research Institute
EU	European Union
GAEC	Ghana Atomic Energy Commission
GIF	Gamma Irradiation Facility
ME	Methyl eugenol
MRL	Maximum Residue Level
POT	Power of Trinity
SIT	Sterile Insect Technique
TIR	Trap-Irradiate-Release

1.9 Organization of the Study

The dissertation has been arranged in seven chapters. Chapter one introduces the research, provides background information, the objectives and describes the scope of work. Chapter two largely reviews and discusses the biology of *B. dorsalis*, Fruit fly monitoring techniques, Fruit fly management, ionizing radiation and lastly the concept of SIT. Chapter three reports the diversity and abundance of fruit flies in order to ascertain their population dynamics in the SouthEastern mango enclave. Chapter four, looks at the effective means of mass trapping *B. dorsalis* in terms of trap type and time of day for optimum trapping. Chapter five looks at the determination of an optimum dose to cause sterility in the adult male *B. dorsalis* population without affecting other functional activities of the fly. These includes irradiating at doses from 0-100Gy in 3 replicates, mating with a laboratory reared female and subsequently determining hatchability. Chapter six looks at the response of *B. dorsalis* fed on ME and attractiveness to ME-baited trap. Finally, chapter seven, the concluding chapter, highlights the major findings and provides a summary, implications of the findings and recommendations derived from the work.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The aim of this research was to develop a fruit fly control strategy called the Trap-Irradiate-Release- SIT as one of the tools to use in a compatible manner with existing strategies to combat the menace caused by fruit flies, especially *B. dorsalis* in fruit and vegetable production business. This research will set the pace to reduce the nuisance caused by *B. dorsalis* by helping to boost the nutritional and market value of horticultural crops in Ghana. The optimum period and trap to use in collecting large numbers of *B. dorsalis* for irradiation studies and finally how ionizing radiation can safely be applied to cause sterility in adult male *B. dorsalis*. A large portion of the information in this chapter is derived from secondary sources, such as published journal articles and books, conference proceedings and reports on relevant studies, as well as information from the internet and official government papers. Again, this chapter reviews project documents from various sources such as theses and dissertations, Universities, Government and Departments to provide a comprehensive literature review. The purpose of the review is to provide insight into problems pertaining to the relative abundance and variety of fruit flies in Ghana's SsouthEastern mango enclave, as well as to determine their trends over a period. In addition, the review provides information on the biology and ecology of *B. dorsalis*, fruit fly monitoring techniques, management options for fruit fly, ionizing radiations, uses and challenges of SIT. It was also necessary to include personal opinions in order to provide a complete discussion of the numerous themes under consideration. In case of situations where there is limited previous

studies to extract data for this study, information from similar studies using different organism have been used since such organisms are also arthropods with similar biology and economic importance. Moreover, knowledge gaps were identified which merit further investigation.

2.2 Theoretical Framework

The concept of irradiating fruit flies to cause sterility and subsequently unleashing the irradiated flies back into the wild where a specified host plant is cultivated to eventually reduces the pest population over time leading to food security formed the main theoretical basis of this research. The Sterile Insect Technique (SIT), which is analogous to birth control, served as the idea for this method of insect infestation management. Using the SIT, vast numbers of a target insect pest species are produced, sterilized, and introduced into the environment. Sterilized insects can be released in large numbers to manage a broad variety of pests, including certain tephritid (Dyck et al., 2005). The Sterile Insect Technique is a pest management method that is both ecofriendly and species-specific. A modification to this technique is the Trap-Irradiate-Release (TIR) method. Trap-Irradiate-Release technique entails trapping of adult males of a target fruit fly intended to be controlled, irradiating them at an optimum dose and releasing these irradiated adult males back into the wild population. Conventional SIT requires rearing the target insect pest in an insectary in large numbers to obtain pupae. The pupae are then irradiated at an optimum dose and released into the wild to control the wild population. Due to competition for available females, the success rate of successful mating is lowered when this method is used. Because wild mating results in non-viable offspring, the overall population is lowered. By mating wild females with sterile males, the objective

is to reduce the natural pest population by the deposition of infertile eggs. A self-destructive activity, the SIT eliminates the use of insecticides, making it an ecologically friendly strategy that has led to its widespread use (Enkerlin, 2005).

In this current study TIR has an advantage over the conventional SIT in that adult male flies are trapped directly from the wild population and sterilized compared with the case in conventional SIT where the insect is reared in the insectary before use. Conventional SIT is laborious and expensive in terms of feeding of the insect colony. Trapping of flies in TIR reduces the adult male population in the wild which in itself helps to reduce the chances of an adult female in the wild from mating with a wild adult male.

The irradiation of the trapped flies and subsequent release into the wild on a large-scale basis helps to assure a reduction in the wild population of the targeted pest, overall assisting in the production of healthy fruits and vegetables for the local and international markets.

2.3 The Biology of oriental fruit fly, *Bactrocera dorsalis* Hendel

Bactrocera dorsalis eggs are thin, white, and small in size (0.8 mm x 0.2 mm, Figure 2.1). White maggots are the larvae. Adults have two dark patches beneath each antenna on their faces. There are noticeable black marks along the anterior margin of the wing. The sides of two elevated regions directly beneath the wing base are painted yellow. The thorax bears a pair of lateral broad yellow stripes. Tergites III-IV have wide, nearly rectangular-shaped lateral markings, and the midline of the abdomen is a clear, dark line from tergite III-V. Males are drawn to methyl eugenol, which is a distinct feature (Ekesi & Muchugu, 2007; Allotey et al., 2010). It was found that the Bangladeshi species had a wide range of scutum colour pattern variation, mostly black, according to Leblanc et

al. (2013). The scutum colour pattern variation recorded in Sri Lanka's *B. dorsalis* was comparable to this. *Bactrocera dorsalis* is a multivoltine insect, with a penchant for year-round hosts. Females deposit eggs in the fruit pulp (Vargas et al., 1996). Larvae hatch in 1-2 days from the eggs and feed on the host plant's fruit pulp. After around 11 days, the adult larvae depart from the fruit. The larvae eventually become passive and longitudinally shortened themselves towards the post-feeding periods. The absence of constrictions between segments. resulted in a smooth cuticular surface and increased diameter (Jing et al., 2019). Brown or black puparia measuring up to 12 mm in length are buried to a depth of 2-5 cm (Figure 2.1) under the soil of the host plants (Pena & Mohyuddin, 1997). The pupal stage lasts between 10 and 20 days, depending on the environmental circumstances (Allotey et al., 2010). Vargas et al. (1996) reported that pupation in the soil under the host plant can take 12 days at 24°C and 60% RH, but may be delayed for up to 26 days under cool temperatures. Under chilly circumstances, pupation may persist up to 90 days (CABI, 2007). *Bactrocera dorsalis* develops intrapuparially in stages that include larval-pupal apolysis, cryptocephalic pupa, phanerocephalic pupa, pharate adult, and emerging adult. The process from larval-pupal apolysis to adult emergence may take up to 246 hours at 27 °C (Jing et al., 2019). When the winged adults emerge, they infest the fruits, where the females need protein to mature their eggs (Pena & Mohyuddin, 1997).

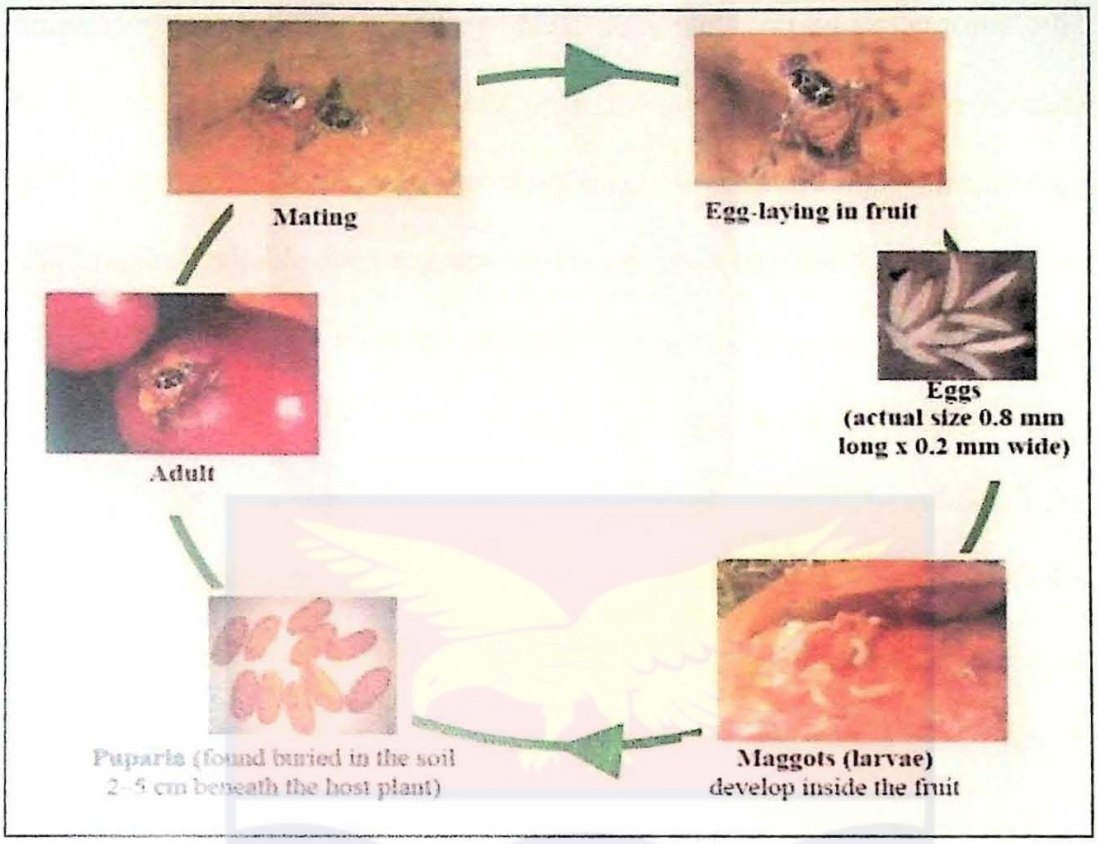

Figure 2.1: Generalised life cycle of Tephritid fruit flies (Source: Ekesi & Billah, 2009)

2.4 Fruit fly trapping technique

Trapping survey is a procedure that is carried out at a set period of time in order to analyse pest population or the distribution of different species (IAEA, 2003). Trapping is used for three purposes: detection, delimitation, and monitoring (IAEA, 2003). A trapping system includes pheromones, para-pheromones, and food attractants, killing agents (dry or wet), and trapping devices (IAEA, 2003). The attractants include male specific/para-pheromones and female biased/food baits. Male-specific para-pheromones include Trimedlure, Methyl eugenol, and cue lure (IAEA 2003; Manrakhan 2006). Para-pheromones are very volatile and typically available in controlled release formulations for field application (Cunningham 1989; Tan et al., 2014). Para-pheromones may also be attached to the panels using an adhesive substance. In

comparison to other food-based synthetic attractants, liquid protein and other food-based synthetic attractants last only a few days depending on climatic factors, catch only a small number of nontarget insects and male flies, making this attractant suitable for programmes that release irradiated and sterile flies (IAEA, 2003). The food-based attractants are not species- or sex-specific (Epsky et al., 2014). Dichlorvos, malathion, spinosad, and pyrethroids are some of the sticky or toxic substances used to kill pests (IAEA, 2013). Adding 1.5 to 2 g of borax to the liquid protein attractants used to trap fruit flies ensures that the flies will be preserved. There are borax-based protein attractants, thus no extra borax is needed. For flies, 10% propylene glycol is added when water is used. There are three major kinds of traps based on the killing agent:

i. Dry trap-The fly is either captured on a sticky board or chemically destroyed. Open bottom dry trap (OBDT) or Phase IV, Red sphere, Steiner, and yellow panel/Rebell are extensively utilized dry traps (Cunningham, 1989; IAEA, 2003),
ii. Wet trap-Water with surfactant or attractant solution drowns the insect. The McPhail trap is one of the most extensively utilised wet traps on the market (Cunningham, 1989; IAEA, 2003).
iii. Dry or wet traps-In either dry or wet conditions, these traps may be deployed. Most popular dry traps include Easy trap, Multilure trap, and Tephri trap (Cunningham, 1989; IAEA, 2003).

Trap design has a major importance in the success of trapping (Candia et al., 2019; Abu-Ragheef et al., 2020). Trap placement and deployment is important in fruit fly trapping (IAEA, 2003). The optimal height for capturing flies is determined on the size of the tree. For *B. dorsalis*, 4 m height of 8 m

mango trees (Ye et al., 2012). for *B. zonata*. 3 m height of 10 m tall guava trees (Siddiqui et al., 2003). Traps are often applied for a variety of purposes, as well as for monitoring (IAEA, 2003; IAEA, 2018). Monitoring The traps for monitoring fruit fly population are essential components of integrated pest management systems. When used in conjunction with a potent lure (parapheromone), male fruit flies will be powerfully drawn into the trap, which will aid in the detection of activities of fruit flies in the vicinity. Collected fruit flies might give you a rough idea of the degree of activity, but it should only be used as a guide. The same traps used for monitoring may be used for mass trapping, albeit at a considerably greater rate. It is the goal of this strategy to capture as many flying insects as possible. However, many traps utilise lures designed to attract male fruit flies alone, leaving egg-laying females untouched. It must be utilised with other established strategies such as protein bait sprays. Mass male fly trapping is akin to the Male Annihilation Technique (MAT).

2.5 Fruit Fly Monitoring

The concept of fruit fly monitoring is the deprivation of resources such as protein meal (protein bait control) necessary for egg laying by female flies, or lures that exclude fruit fly males from the region under study (Dhillon et al., 2005). The monitoring of fruit fly pest species helps to a) identify fruit fly pest species in an area, b) determine the distribution of insect pest species, c) identify local hot spots with high populations of the pest, d) track population changes, e) determine the efficiency of control measures, and f) aid in identification of new fruit fly pests in a specific region before they become established (Manrakhan, 2006). Fruit fly monitoring tools include attractant-based traps and host fruit surveys, which are both important components of the process (Manrakhan,

2006). Without reliable information, it is impossible to design an effective plan with respect to the peak period of fruit fly activity. For example, knowing the time and size of a pest outbreak may help control measures work more effectively (Ekesi & Billah, 2006). In order to make management choices, it is necessary to assess pest abundance or to track changes in pest populations over time. Automatic fruit fly monitoring technologies, which have recently been developed, have the potential to dramatically increase the efficacy and efficiency of fruit fly monitoring. Monitoring fly populations is the most appropriate means of managing the fruit fly population, independent of the quality of the fruit on the orchard (Goldshtein et al., 2017).

2.5.1 Attractants

The two most common forms of attractants employed in fruit fly monitoring are parapheromones or male specific lures, and food baits, which are both utilised in conjunction with one another (IAEA, 2013). The lures may be in either liquid or polymeric form, and they can be used to catch flies. According to Ekesi and Billah (2006), depending on the kind of bait used, the lure might last up to six weeks. Only male flies are attracted by para-pheromones (Cunningham, 1989). Because the flies are drawn to the traps from such a short distance, it is anticipated that the number of flies present in the surrounding region will be accurately estimated using these monitoring traps.

2.5.1.1 Male specific lures

Pheromones or parapheromones are the most widely used attractants and are male-specific (IAEA, 2013). Parapheromones are substances that resemble and elicit reactions comparable to real pheromones, but they are not naturally employed in intraspecific communication. Controlled-release versions of male specific lures are available in both liquid and polymeric forms (IAEA, 2013). The parapheromone methyl eugenol (ME) captures a large population of species of the genus *Bactrocera* (including *B. dorsalis*, *B. zonata*, *B. carambolae*, *B. philippinensis* and *B. musae*) and some species in the *Ceratitis* genera such as *C. dittisima*. The pheromone Spiroketal® captures *B. oleae*. The parapheromone trimedlure (TML) captures species of the genus *Ceratitis* (including *C. capitata* and *C. rosa*) (IAEA, 2013). The parapheromone cuelure (CUL) attracts large numbers of *Dacus* and *Zeugodacus* species, including *Z. cucurbitae* and *D. ciliatus*, *D. bivittatus*, *D. punctatifrons* D. *ciliatus* etc. Parapheromones are often extremely volatile, and they may be used in conjunction with a wide range of traps to attract flies. Controlled-release formulations of TML, CUE, and ME are available, allowing for a longer-lasting attractant to be used in the field (IAEA, 2013). It should be noted that the field activity of pheromone and parapheromone attractants may be affected by prevailing environmental conditions (Heuskin et al., 2011; Hafsi et al., 2020b). Additionally, parapheromones may be combined with an adhesive and applied to panel surfaces (Manrakhan, 2006; IAEA, 2013).

2.5.1.2 Food baits

Food or host scents are employed as female-biased attractants. Depending on the kind, they might be natural or synthetic. Many different fruit

fly species have been caught using liquid protein attractants in the past. Males and females alike are enticed by protein-rich liquids. The sensitivity of these liquid attractants is lower than that of parapheromones (White & Elson-Harris, 1992). With the application of liquid attractants, many nontarget insects are caught as well (Uchida et al., 2006; Leblanc et al., 2010). Ammonia and its compounds have been used to generate a variety of food-based synthetic attractants.

The baits are rich with critical nutrients for the growth and sexual maturation of flies (Perez-Staples et al., 2007), and the flies consume them in large quantities. This may help to limit the amount of nontarget insects that are caught in the traps. As an example, a synthetic food attractant consisting of three components—ammonium acetate, putrescine, and trimethylamine— captures *C. capitata* and other species (Lux et al., 2003a; IAEA, 2003; Ekesi & Billah, 2006.) Synthetic attractants may be used in sterile fruit fly release programmes because they are long-lasting (up to 10 weeks depending on climate conditions), catch a smaller number of non-target insects, and catch much fewer male fruit flies. Several novel synthetic food attractant technologies are now available and ready for use, including long-lasting three- and two-component combinations in the same patch, as well as three components contained in a single cone-shaped plug (Lux et al., 2003a). Female fruit flies may be identified earlier and at lower population levels thanks to synthetic food attractants, which are more successful than liquid protein attractants in attracting them while they are still sexually immature adults (IAEA, 2013).

2.6 Fruit Fly Management

2.6.1 Sanitation

In order to minimize the populations of fruit flies from spreading, any damaged and rotten fruit that has fallen to the ground should be gathered and disposed of (Hill, 1987; CABI, 2007). The dropped fruit may be buried or cooked or fed to farm animals. It is also a good idea to harrow the soil under the trees in order to make larvae and pupa visible to ants, chickens, among other creatures.

2.6.2 Picking fruits

Harvesting whole crop from an orchard has historically been utilized largely in eradication campaigns. This strategy has been deployed in orchards in California. It is necessary to harvest all of the fruit from the trees in order to eradicate any ovipositional sites that may be available for the fruit fly population to continue to flourish (Sharp et al., 1989; Jacobi et al., 2001). In Ghana, fruit picking at commercial mango farms is not well practiced thereby leading to fruit fly pest build ups in such farms leading to infestation.

2.6.3 Wild host destruction

In eradication programmes, it is desirable to eliminate hosts that are not economically valuable (Messing, 1999; Smith, 2001). In some cases, wild host promote the multiplication of fruit fly population density. When the cultivated hosts are absent or not fruiting, the fruits of the wild hosts provide a source of sustenance.

2.6.4 Fruit bagging

Many home gardeners and small farmers in Hawaii utilise fruit bagging to avoid fruit fly oviposition. Prior to harvest, the bag is removed to enable the fruit's natural colour to develop. The paper bag must be punctured with small holes in order to allow air to pass through. The usage of plastic bags is discouraged. When it comes to high-value fruits that are exported or backyard fruits that are used by the family, mechanical fruit protection is an excellent solution (Ekesi et al., 2007). Other studies have shown that fruit bagging is 100% effective in controlling fruit pests (Estradea, 2004; Graaf, 2010).

The Ugandan National Agricultural Research Organization (NARO) investigated the possibility of controlling fruit flies by bagging fruit before it is fully mature. Preliminary data show that bagged fruit has fewer fruit fly infections than unbagged fruit (Nankinga et al., 2014). Fruit bagging is not common in Ghana and other African nations because of its labor-intensive nature (Badii et al., 2015b).

2.6.5 Biological control

Fruit fly parasitoids, predators, and diseases are utilised in conjunction with other biological controls to mitigate the harm caused by a pest (Elzinga, 2004; Ekesi et al., 2007). With regard to *B. dorsalis*, the most remarkable successes in traditional biological management against fruit flies may be attributed to the use of the egg parasitoid, *Fopius arisanus* against the fruit fly larvae, which resulted in the eradication of the pest (Rousse et al., 2005; Mohamed et al., 2010). According to a preliminary survey conducted by Badii et al. (2016), *Psyttalia cosyrae* (Wilkinson), *Psyttalia concolor* (Szépligeti), and *Diachasmimorpha fullawayi* (Silvestri) were identified as parasitoids in some

areas of Northern Ghana. *Fopius caudatus* (Szépligeti) was found as the parasitoid with the highest prevalence, followed by *Psyttalia*. Combining these indigenous parasitoids with a comprehensive integrated pest management (IPM) programme in the region, *B. dorsalis*, the continent's most deadly tephritid pest, should see a dramatic reduction in its population levels (Badii et al., 2016).

Psyttalia cosyrae and *P. concolor*, as well as *Dirhinus giffaardi*, *Fopius caudatus*, *Spalangia* sp., and other parasitoids and predators are abundant in fruit and vegetable crops, which may help to reduce the fruit fly population. The African weaver ant, *Oecophylla longinoda*, Latreille, impedes the fruit fly's ability to lay eggs (Van Mele et al., 2007; Vayssières et al., 2013). *Oecophylla longinoda* is extensively utilised in several countries (Van Mele et al., 2007; 2009). however, its usage in Ghana is very restricted and rigorously regulated (Ativor et al., 2012; Abunyewah et al., 2015). Weaver ant usage has been hindered by the widespread belief among Ghanaian mango producers that the ants' stings are painfully excruciating (Ativor et al., 2012; Abunyewah et al., 2015). However, the potential still exists due to the fact that the existence of ants in the immediate environment has been demonstrated to prevent fruit flies from settling on fruits and laying their eggs, therefore minimising the incidence of fruit puncturing and the need for early harvesting, both of which are beneficial. When fruits are allowed to ripen on the tree for a longer duration before being harvested, the brix quality of the fruit increases (Akoto et al., 2011; Ativor et al., 2012). Using the predatory weaver ant to guard mango and citrus fruits against fruit fly damage is possible, given that the tree hosts offer the ant with food sources (Akoto et al., 2011; Ativor et al., 2012; Vayssieres et al., 2013). However, parasitoids and predators are not considered helpful because

of the poor fertility of parasitoids in contrast to fruit flies and the restricted ability of parasitoids to seek out larval and pupal populations of fruit flies in their natural environment (Nadeem et al., 2014). *Metarhizium anisopliae* and *Beauveria bassiana*, two potent fungal pathogen isolates, have been shown to be effective against the pupariating larvae and adult stages of the major fruit fly species, including *B. dorsalis*, *B. cucurbitae*, *C. cosyra*, *C. fasciventris*, *C. rosa*, *C. capitata*, and *C. anonae* (Ekesi et al., 2007). Marri et al. (2016) conducted an evaluation of a commercial formulation of the entomopathogenic fungus, *B. bassiana*, to control *B. dorsalis* in the southern part of Ghana. An optimal dosage of 26.5×10^6 spores/mL killed about 50 percent of adult flies in 4–5 days and about 99 percent of adult flies in 8–9 days when administered at the recommended rate (Marri et. al., 2016). Application of *B. bassiana* to tephritid traps in mango canopies, rather than soil surface spraying, is a more successful method of controlling fruit flies in the field (Marri et. al., 2016).

2.6.6 Sterile Insect Technique (SIT)

The sterile insect technique (SIT) is a strategy for containing, excluding, and eradicating fruit fly populations. The SIT's objective is to inundate well-defined geographic borders with sterile males, where they will mate with any wild female in the population, leading to infertilised eggs production. Since the 1960s, the potential for SIT to be used to manage pests has been recognized. SIT provides many benefits over insecticidal control approaches, including improved specificity and the ability to target afflicted locations (Knipling, 1959). Historically, SIT initiatives have failed because of persistent immigration into the regions targeted. Irradiation is the most often utilized technique for sterilizing fruit flies for SIT programmes. Irradiation is most effective around

70% pupal completion (Gilchrist & Crisafulli, 2006). In an SIT programme, efficient dosage of irradiation should render the male infertile without impairing its reproductive competitiveness. Irradiation dosage has been shown to have no effect on sterility induction, but a larger dose may produce stress, which can result in death. When irradiating male insects for SIT control operations, the "lowest practicable" dosage should be employed. In terms of females re-mating, males that have been irradiated do not have a reproductive advantage over normal males (Harmer et al., 2006). There is no difference in the proportions of successfully copulating males between irradiated and untreated flies despite the fact that irradiating males modify the timing of their calls and wooing calls (Mankin et al., 2008). For the treatment of *C. capitata* a sterile insect technique has been effective in countries such as Italy, Mexico, Nicaragua, Peru, Spain, Tunisia and California (CABI, 2007; IAEA, 2013). In 1963, and in Guam, SIT for *B. dorsalis* and *B. cucurbitae* were also successful (Hill, 1987).

2.6.7 Bait Application Technique (BAT)

Using this technique of fruit fly management, a dilute protein solution and an insecticide are combined and sprayed on the fruit fly larvae. Bait stations are also useful in attracting a sizable number of male and female flies. The bait with protein component acts as an attractant, and when the fruit fly consumes the protein combination, the insecticide component causes the fruit fly's death. This approach is effective against both male and female fruit flies. For example, in the Fruit Fly Exclusion Zone of Eastern Australia, a density of 100 spot sprays per hectare (about 6 to 8 spot sprays per residential home) is applied (Gilchrist & Crisafulli, 2006). The spray quantity is believed to be successful since a bait site is within the "daily roaming range of each fly inside the treatment area."

Avemectins, spinosads and Neonicotinoids are probably most commonly used as killing agent in baits since they have low mammalian toxicity compared with organophosphates such as Malathion. Spot sprays lose some of their potency over time as rain washes away the bait and the insecticide degrades. Several novel methods have been developed to overcome this challenge. For example, bait stations that protect the bait spray from direct rain and the use of sticking agents to reduce wash-off by rain. Bait spraying is the most effective method of population reduction, and it should be used in conjunction with other management approaches to have the most effectiveness. Chemical amounts used by bait sprays are often significantly lower than those used by cover sprays. Bait sprays are often administered to the foliage rather than the fruit itself (Dominiak, 2007). Considering that the equipment for spreading the bait is straightforward, this strategy is ideal for controlling fruit flies at both small and large scale (Allwood & Drew, 1997). In plots treated with GF-120, larval infestations of *B. dorsalis* and other local fruit fly species were much lower than in untreated control plots in Benin (Vayssières et al., 2009). The Plant Protection and Regulatory Services Division (PPRSD) and the Environmental Protection Agency (EPA) of Ghana, agreed to add this substance to the list of permitted products in Ghana, and Bait Application Technique was formally included in Ghana's IPM package against *B. dorsalis*. SUCCESS® Appat (GF-120) study was done in all of Ghana's key agro-ecological zones. Billah et al. (2009) reported that GF-120 was generally effective in obtaining acceptable and clean marketable fruits ranging from 38.5 to 84.5 percent in mangoes and 41.4–96.0 percent in citrus. Following the successful testing of GF-120 in Ghana, two more baits, the Ceratrap lure and the Great Fruit Fly Bait (GFFB), were

imported and evaluated. On farms that used GFFB, an increase of 93.6–96.8 percent in clean marketable fruits was reached, with an average increase of 95.2% in mango, whereas a range of 80.7-80.9 percent was obtained on farms that used SUCCESS® Appat (Billah et al. 2014). However, a significant barrier to the adoption of baits in Ghana is their high cost, making them unaffordable to a large number of fruit and vegetable producers in the SouthEastern mango enclave (Badii et al., 2015b).

2.6.8 Male Annihilation Technique (MAT)

A prevalent way of eliminating male insects is to use male lures like cuelure, methyl eugenol, terpinyl acetate, and trimedlure in combination with a legal killing agent. Insecticide-impregnated substrate and parapheromones constitute the foundation of the male annihilation process. Methyl eugenol traps are among the most efficient ways to get rid of fruit flies. In Ghana, MAT appears to be the most preferred technique for monitoring, management and control of fruit fly populations (Billah et al., 2006). Male specific lure, methyl eugenol has been demonstrated to have both olfactory and phagostimulatory effects on fruit flies, and it has been shown to attract fruit flies at a distance spanning more than 500 meters (Shelly and Edu 2010; N'Da 2018). It has been shown that the male annihilation strategy may be utilized effectively for the control and eradication of many *Bactrocera* species across India and Pakistan (Ravikumar & Viraktamath, 2007; Singh et al., 2014). For example, a nationwide campaign was launched to remove *B. dorsalis* from Taiwan in 1994. It was estimated that a substantial quantity of ME (40 metric tonnes) had been applied to subdue 75% of the tephritid population by the year 2002 (Vargas et al., 2010). When used with an area-wide suppression approach, the method is

helpful (Cunningham, 1989). The use of para-pheromones for monitoring led to the development of the notion of male annihilation. Several male lure traps are put in a certain region in order to capture most of the male in that area. As an example, a novel attract-kill formulation containing a male attractant and spinosad that has been developed as part of the specialised pheromone and lure application technology (SPLAT) has recently been shown to be promising for the effective suppression of fruit flies while posing no negative environmental impact (Vargas et al., 2009, Vargas et al., 2014). SPLAT-MAT-ME trap captures of marked male *B. dorsalis* released in Hilo, Hawaii, were compared under three experimental site density levels (110, 220 and 440 per km^2) to test the efficacy of different densities as well as how weathering of the SPLAT-MAT-ME formulation affected any density effects. It has been discovered that increasing trap density resulted in a decrease in efficacy (percent kill). Male fertility drops as a result of a scarcity of males, which causes the population to steadily diminish as a result of males being scarce. Reducing the males in a group reduces the likelihood of reproducing and regenerating successfully in that population. Male Annihilation Technique is ultimately applied to completely eradicate and eliminate the population of male fruit flies from the region.

2.6.9 Ground spraying

Ground spraying is used to control fruit flies infesting trees. The ground underneath affected trees is sprayed with a suitable pesticide, such as chlorpyrifos. Tree trunks and outer canopy edges are all treated with pesticides to protect the ground from other pests. All compost piles made from fallen and abandoned fruits in the surrounding area are also treated with pesticide. Most of

the time, no more than two ground sprays are required beneath a single tree. The larvae and emerging adults in the soil are the objectives of this procedure (Dominiak, 2007). In trials done at the ICIPE, it was discovered that the administration of a combination of Nulure/spinosad bait spray and soil inoculation of *M. anisopliae* decreased the number of *B. dorsalis* by about 79 percent when compared to a control treatment. Mango fruit infestations averaged 10% in bait-and-fungus treatment plots and 73% in untreated control plots. Field testing during the 2006-2007 mango season found that the combination of *M. anisopliae* and GF-120 spinosad bait spray reduced *B. dorsalis* by 92.1 percent compared to a control (Papadopoulos, 2010).

2.6.10 Postharvest (Regulatory Control)

A number of nations, including the mainland United States, prohibit the importation of vulnerable fruit unless rigorous post-harvest treatment has been performed by the exporter (CABI, 2007). To be able to move host fruits from regions having fruit flies into areas which are pest free, commodity treatments are required. Fumigants and even deadly temperatures are among the methods used.

To serve significant European retail chains, mango producers and exporters must adhere to GLOBALGAP, a private standard established by major European retail chains. It was observed by Akotsen-Mensah et al. (2017) in Ghana's southern mango enclave that, 80 percent of the mango farmers in the investigated region subscribed to GLOBALGAP standards. This validates claims that Ghanaian fruits have adhered to the GLOBALGAP criteria in previous years (Zakari, 2012; GlobalGAP, 2016). Farmers in Ghana's mango industry must be able to fulfil strict customer standards for pristine quality

mangoes with no chemical residues or quarantine bugs to maintain and profitably produce mangoes. Even local customers are becoming more knowledgeable of the environmental and health hazards linked with the use of pesticides in food crop cultivation, which is a positive development (Diedhiou et al., 2007. Braimah & Van Emden, 2010).

2.6.11 Fumigation

Toxic gases or vapours produced by fumigants may harm insects, microorganisms or rodents. There was a time when methyl bromide and ethylene dibromide were utilised, but they have since been discontinued.

2.6.12 Lethal temperatures

Insect and commodity thermal tolerance is taken into consideration while determining fatal temperatures. Temperature and timing are important factors in determining mortality. There are a variety of therapies available for those who are exposed to fatal temperatures. There are many types of vapour heat treatment, the most common of which is heating air that has been saturated with steam. The steam maintains a specified temperature for a predetermined amount of time on the item being heated (Self et al. 2012). Papaya fruit flies have been successfully treated using this method, as mango fruit flies have also been found in other regions of the globe. Using hot and cold therapies, such as baths, is an additional option (Thomas & Shellie, 2000). These papayas were soaked in hot water (49°C) for 20 minutes and then stored at 5- 6°C for 10 days. Mangoes, for example, may be treated with hot water for 67.5 minutes at 45.9-47.1°C to destroy fly eggs and larvae (Baldo & Raga, 2021). In order to destroy *C. capitata* eggs and larvae, it is required by US Department of Agriculture that

cold treatment for 10 days at 0°C or less, 11 days at 0.5°C or less, 12 days at 1.11°C or less, 14 days at 1.66°C or less, or 16 days at 2.22°C or less should be applied (Fletcher, 1987). Fruits that can be utilised after freezing may be disinfected by quick freezing.

2.7 Fruit fly SIT

Sterile Insect Technique (SIT) is defined as "a method of pest control using area wide inundative releases of sterile insects to reduce reproduction in a field population of the same species" (FAO, 2007). Large-scale production of pest species, irradiation sterilisation, and release of sterile insects into the environment are all components of SIT. Using a large number of sterilised insects released in a controlled environment (Dyck et al., 2005), this strategy may be used to manage a huge range of nuisance insect pests, including some tephritid. Since sterile males compete with wild males for wild females, rate of mating success is lowered while using this method of reproduction. The mating of a sterile to a wild animal creates non-viable eggs, and as a result, no progeny, which reduces the overall population. Since this autocidal action is carried out without the use of insecticides, the SIT is considered an ecologically friendly technique, which has resulted in its widespread adoption throughout the globe (Enkerlin, 2005). For the SIT to work effectively, sterile males outcompete wild males in order to accomplish mating with wild females, and this is where the most of the risk lies (Calkins, 1984). The SIT has been utilised against agriculturally significant species of *Bactrocera*, including the oriental fruit fly *Bactrocera dorsalis* (Hendel) and the melon fly *B. cucurbitae*, but to a lesser degree than other methods. The effectiveness of SIT, like with other area-wide pest management strategies, is improved when applied over a big region

(Klassen, 2005). SIT, in contrast to chemical control, has no unintended consequences for the environment or human health, making it appropriate for organic agriculture methods (Wimmer, 2005; Hendrichs et al., 2007). When it comes to fruit flies, gamma irradiation may be utilised to destroy all stages of the insect. It takes between 150 to 500 Gray to kill fruit flies at the recommended doses. However, there are still some uncertainties regarding whether or not irradiated food would be accepted by consumers (Sharp et al., 1989; Jacobi et al., 2001).

In North and Central America (Klassen & Curtis, 2005) including Libya (Lindquist et al., 1993), sterile male approach has been very successful in eradicating the New World Screwworm (NWS, *Cochliomyia hominivorax* Coquerel); the tsetse fly (*Glossina austeni* Newst.) from Unguja Island in Zanzibar, Tanzania (Vreysen et. al., 2000); the melon fly (*Bactrocera cucurbitae* Coquillett) from Japan (Kuba et al., 1996); the pink bollworm (*Pectinophora gossypiella* Saunders) from the San Joaquin Valley of California, USA (Staten et al., 1993; Staten & Walters, 2021); and the Queensland fruit fly (*Bactrocera tryoni* Froggatt) (Sproule et al., 1992). The Mediterranean fruit fly (*Ceratitis capitata* Wied.) was eradicated by SIT in California and Florida (USA) (Dowell et al., 2000; Barry et al., 2004), Mexico (Hendrichs et al., 1983), and Chile (Hendrichs et al., 1983; Esparza Duque, 1999; Gonzalez & Troncoso, 2007).

2.8 Challenges of SIT

The SIT is an effective method of eliminating a particular insect pest. However, there are obstacles that must be overcome before SIT may be widely used. Some pests provide a barrier in that there is no inexpensive, quick, or

efficient method to filter through a huge number of flies and identify whether they are male or female. For example, it is difficult to separate the pupae of *Bactrocera dorsalis* into male or female and therefore, irradiation of the fly results in the release of both male and females in the wild population after the emergence of the adult, resulting in increasing the pest situation in the wild. Additionally, the quantity of radiation utilised to sterilise the insects is too harmful to the insects, and they will just die as a result. As a result, insect sterilisation is a difficult operation that must be carried out by professionals who have received extensive training (Sullivan, 1964). Another issue is the mass rearing of target species which in most cases is laborious and capital intensive. This makes it difficult for resource poor countries to apply the technique in dealing with a specific pest problem. This problem can be circumvented by mass trapping live adult males using dry pheromones and with an appropriate trap since males are the target species in most SIT programmes. The live male flies can then be sterilized with an optimum dose and released back to the wild to compete with the wild males for females. This technique forms the basis of the trap-irradiate-release/ sterile insect technique being developed.

2.9 Fruit fly irradiation for SIT

Irradiation treatment has become the technique for sterilizing insects such that they are infertile. For SIT, additional issues like as penetration, cost, and product throughput influence the selection of an irradiation source (IDIDAS, 2018). Gamma radiation is most often employed to sterilise insects from isotopic sources like cobalt-60 or cesium-137 (UNSCEAR, 2010). X-rays and high-energy electrons may also be used to expose insects to radiation, which can be beneficial (Bakri et al., 2005). When it comes to sterilising insects using

radiation, the quantity of radiation that is absorbed by the insect is the most crucial component to consider. If the insects are unable to reproduce, but are still powerful enough to mate and compete with other wild insects, this element is incredibly crucial and must be handled very attentively, else they would die.

Although many of the unfertilized eggs do not survive any further stages, males tend to be less radiosensitive, and, in many species, eliminating a residual egg hatch of 1 percent (or less) from fertile females mated to irradiated males requires doses that significantly reduce the ability of males to compete with wild populations and thus induce sterility in wild populations (Fisher, 1997; Toledo et al., 2004).

The quantity of radiation absorbed by insects varies. It ranges from 5 Gy to 300 Gy, with some exceeding that. The problematic part is that elements like the flies' age or oxygen levels come into play when estimating how much radiation each insect species will absorb. It was reported by Collins & Taylor (2011) that full developed *B. tryoni* pupae may be sterilized by gamma radiation in a range of 20-70 Gy, while still providing a sufficient safe margin above radiation dosages at which remaining fertility can be predicted. Also, Guerfali et al. (2011) suggested 50 – 145 Gy of gamma irradiation of full-grown pupae. In Ghana, Ogaugwu et al. (2012) discovered that a 75 Gy gamma radiation dose made male *B. invadens* entirely sterile, while doses of 25 and 50 Gy produced partial sterility. It is critical to determine the optimal amount of radiation that achieves the needed degree of sterility without compromising the overall fitness of the released insect (Robinson, 2002).

2.10 Research Gaps

- There is lack of recent data on the tephritid fruit fly composition in the southeastern mango enclave of Ghana. This work will help to understand the composition of tephritid fruit flies in those areas.
- Conventional SIT is laborious and expensive especially in terms of larval diet preparation and logistics. A less expensive technique needed to be evaluated and integrated in other management strategies in Ghana.
- Lack of knowledge on the pre-exposure of *B. dorsalis* to ME and its effect on their behaviour. This is important for the use of trap-irradiate-release methods.
- Irradiation studies for SIT is mainly evaluated for pupae. We do not know what irradiation dose will cause sterility in adult male *B. dorsalis*.

CHAPTER THREE

ASCERTAIN DIVERSE TYPES OF FRUIT FLIES IN SOME SELECTED MANGO ORCHARDS IN THE SOUTHEASTERN MANGO ENCLAVE

3.1 Introduction

The complex of phytophagous insect pests, including numerous species in the Tephritidae family, is a major danger to the horticulture industry in various regions of Ghana (Badii et al., 2015a; Abbas et al., 2018). Tephritid fruit flies include more than 4,000 species and 500 genera (White & Elson-Harris, 1992). About 200 fruit fly species are regarded as serious pests because of direct losses they cause to a wide variety of fruit crops (Norrbom et al., 2012; Oliveira et al., 2013; Qin et al., 2015). Fruit flies are considered devastating pests in most fruit and vegetable growing areas. In addition to being very polyphagous, they are extremely fecund, and they have the potential to swiftly spread across a large region (Gillani et al., 2002; Nugnes et al., 2018; Mutamiswa et al., 2021). Many studies have confirmed that *Ceratitis* and *Bactrocera* genera are economically significant insects that infest tropical fruits in Africa, and this has been extensively documented (Billah et al., 2006; Mwatawala et al., 2009; Badii et al., 2015a). In Ghana, it is estimated that fruit loss due to fruit flies accounts for 65 percent of total fruit loss (Billah, 2007).

Soon after the detection of *Bactrocera dorsalis* between the borders of Kenya and Tanzania in 2003, the insect expanded to other African nations, including Nigeria (Lux et al., 2003a). *Bactrocera dorsalis* came to Ghana in 2005, less than two years after it was first discovered in Africa (Billah et al., 2006), and has since established itself as a serious pest of mango fruits in the

country. More than 250 host plants from forty families may be infested by this pest, including several varieties of commercial fruits (Allwood et al., 1999; Leblanc et al., 2013; Liquido et al., 2015; Stewart, 2017; Theron et al., 2017; Mutamiswa, 2021). The mango fruit fly, *Ceratitis cosyra*, was touted as the principal insect pest of mango before *B. dorsalis* invaded Sub-Saharan Africa, causing up to 30 percent of the region's mango fruit to be lost (Lux et al., 2003b). As a result of its introduction, *B. dorsalis* has spread rapidly across the area, generating significant direct and indirect consequences including agricultural losses, quarantine restrictions, and the displacement of native fruit flies such as *Ceratitis cosyra* (Walker) from their natural habitat (Ekesi et al., 2009). As a result of its vast range of host species, high fertility, and severe harm to agricultural goods, *Bactrocera dorsalis* ranks as a significant quarantine pest in most nations (Bateman, 1972; Fletcher, 1989; Alyokhin et al., 2001). As of 2018 (CABI, 2018; Mutamiswa et al., 2021), *B. dorsalis* was documented in 35 nations in Sub-Saharan Africa, including the Comoros and Mauritius islands (CABI, 2018; Mutamiswa et al., 2021), establishing itself as a serious insect pest of economically significant fruits (Ekesi et al., 2006; Mwatawala et al., 2006; Vayssieres et al., 2015; Hanna et al., 2020). Currently, *B. dorsalis* can be found in 65 nations throughout the globe (CABI, 2020), with Italy's Campania Region serving as the first confirmed location for the species (Nugnes et al., 2018).

Variations in abiotic conditions namely relative humidity, temperature, and rainfall as well as factors like the time of year when plants are planted, when fruits ripen, and how much vegetation is present all play an important role in

species diversity, population dynamics, and dispersal patterns (Ghanim, 2017; Khan & Naveed, 2017; Bota et al., 2018; Amin et al., 2019).

In Ghana, mango production is a major commercial activity in the coastal savannah agro-ecological zone. Farmers often have to adopt several strategies including insecticide application to manage tephritid insect pests in the orchards. It is necessary to have a thorough knowledge of the population dynamics of the species present at a given area, as well as influence of the prevalent biotic and abiotic variables, before efficient management of these flies in mango orchards can be implemented. Thus, control actions will be focused during times of maximal population surges and/or at the most vulnerable stage of the crop, ensuring that the most effective control measures are implemented (Ekesi & Billah, 2009; Mwatawala et al., 2006). The present study was conducted to determine the diversity and population dynamics of economically important fruit fly species associated with mango production in the Coastal-Savannah agro-ecological zone of Ghana.

3.2 Materials and Methods

3.2.1 Study Location

Sampling for fruit flies was carried out between July 2018 and June 2019 in three commercial mango orchards namely Enyonam Farm (5°56'59" N; 0°1'10" W) in the Shai Osudoku District of the Greater Accra Region, Modest Step Farm (6°2'19" N; 0°0'9" W) and Power of Trinity Farm (6°6'12" N; 0°0'7" W) in Yilo Krobo district of the Eastern Region (Figure 3.1). The study area lies within the Coastal Savannah agro-ecological zone of Ghana with a humid climate. The mean annual minimum and maximum temperatures in the area

was 25°C and 35°C respectively. The average relative humidity is between 60 - 75%. The major rainy season in the study area is between March and July with mean annual rainfall of 436 – 1,507 mm and 100 – 110 planting days. The study area has a minor rainy season from September – October with an annual rainfall of 59 – 603 mm and 50 planting days per year (Asare-Nuamah and Botchway 2019). The vegetation is predominantly short grasses with small clusters of shrubs and a few trees (FAO, 2005). Data pertaining to the weather for three variables, relative humidity (RH) at 15.00 h GMT, maximum temperature at 09.00 h, and total monthly rainfall were obtained daily from a small meteorological station in the vicinity of one of the farms in the study area. The farms were cultivated with a mixture of Keith and Kent varieties of mangoes and the main cultural activities were pruning after harvest, and mechanical weed control. No insecticides or fertilizers were applied to the orchards. Mango trees in the orchard were at the economic fruit-bearing age of eight years or more.

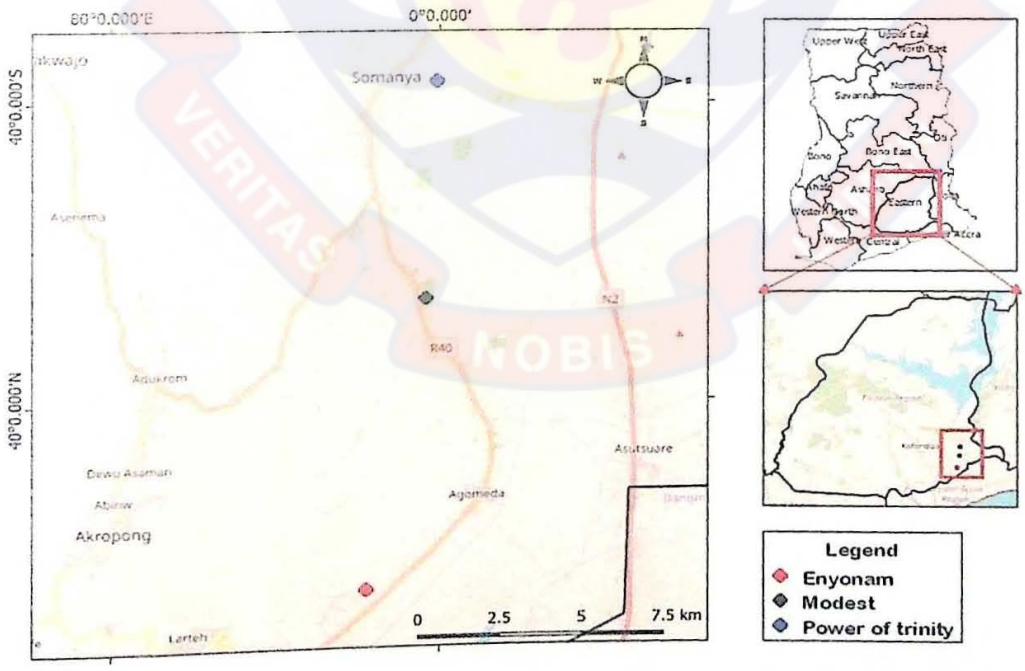

Figure 3. 1: Map of the SouthEastern mango enclave of Ghana showing the three farms under study.

3.2.2 Field preparation and demarcation

Fruit flies were sampled following the method described by Ekesi & Billah (2009) with some modifications. In each farm, an area of 990 m^2 within 4,047 m^2 was demarcated in a rectangular form for fruit fly sampling. The longer side of the rectangle was 55 m while the shorter side measured 18 m. The planting distance was 9 m × 9 m. The four trees at the corners of the rectangle and another in the middle were tagged and baited traps placed on them for fruit fly collection (Figure 3.2). Per the planting distance, there were 21 mango trees in the demarcated area for fruit fly collection. This setup was replicated in all three farms, which were separated from each other by a distance of not less than 30 km. This ensured that three independent replicates were obtained.

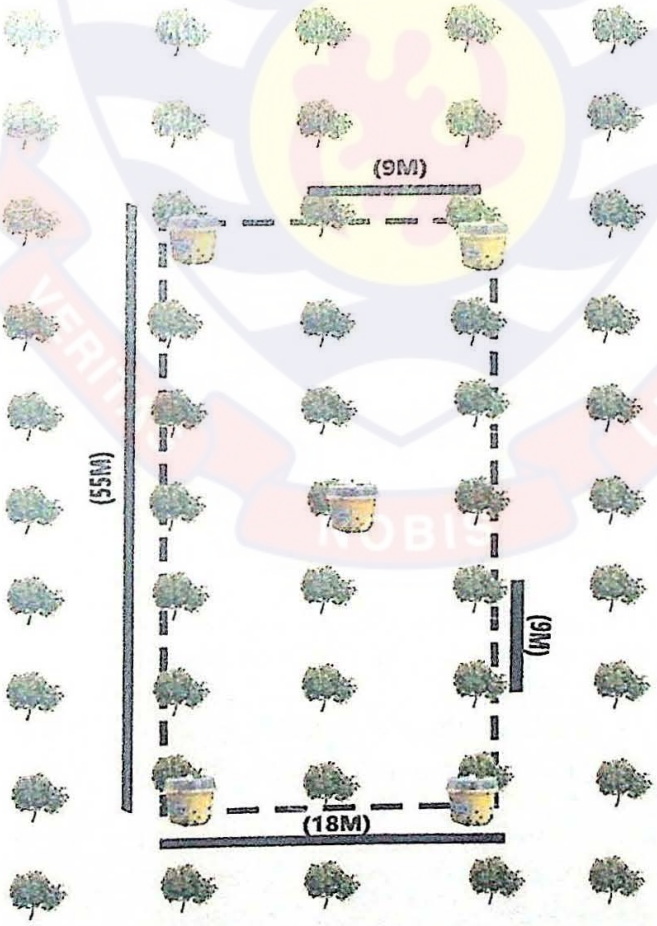

Figure 3. 2: Trap placement in the commercial mango orchard.

3.2.3 Attractants

Four (4) parapheromones and one (1) food bait were used as attractants in this study (Table 3.1). In general, parapheromones are very volatile and may be employed in a wide range of traps. The attractants, TML, TA, ME, and CUL (Figure 3.3*a*, *b*, *c* and *d*) respectively were in slow-release polymeric gel formulations while TY (Figure 3.3*f*) was in pellets. Strips of Dimethyl 2, 2-DichloroVinyl Phosphate (DDVP) (Plato Industries Ltd, Houston, TX) were used as killing agents in all the traps except the TY traps in which 200 ml of water was used as a drowning medium. The TY pellets were formulated with borax to serve as preservative for dead flies in the wet medium. Traps were recharged fortnightly by changing the attractants/baits and killing agents. In addition, Tephri traps were thoroughly cleaned to ensure that no trap is contaminated with the disposed attractants.

Table 3. 1: Attractants used in the study

Attractants	Chemical formular	Source	Target species
Cuelure (CUL)	4-(3 Oxobutyl) phenyl acetate	Scentry Biologicals, Inc, Billings, MT, USA	*B. tryoni, Zeugodacus cucurbitae*
Methyl eugenol (ME)	1,2-dimethoxy-4-(prop-2-en-1-yl) benzene	Scentry Biologicals, Inc, Billings, MT, USA	*Bactrocera* e.g. *B. dorsalis*, *B. zonata*, *B. carambolae*, *B. philippinensis* and *B. musae*
Terpinyl acetate (TA)	2-(4-methylcyclohex-3-en-1-yl) propan-2-yl acetate	Farma Tech International Corp, USA	*Ceratitis* e.g *C. capitata* and *C. rosa.*
Torula yeast (TY)	*Cyberlindnera jadinii* Minter	Scentry Biologicals, Inc, Billings, MT, USA	all types of fruit fly species
Trimedlure (TML)	tert-butyl 4 (and 5)-chloro-2-methylcyclohexane-ane-1-carboxylate	Scentry Biologicals, Inc, Billings, MT, USA	*Ceratitis* e.g *C. capitata* and *C. rosa.*

Source: (Ekesi & Billah, 2009)

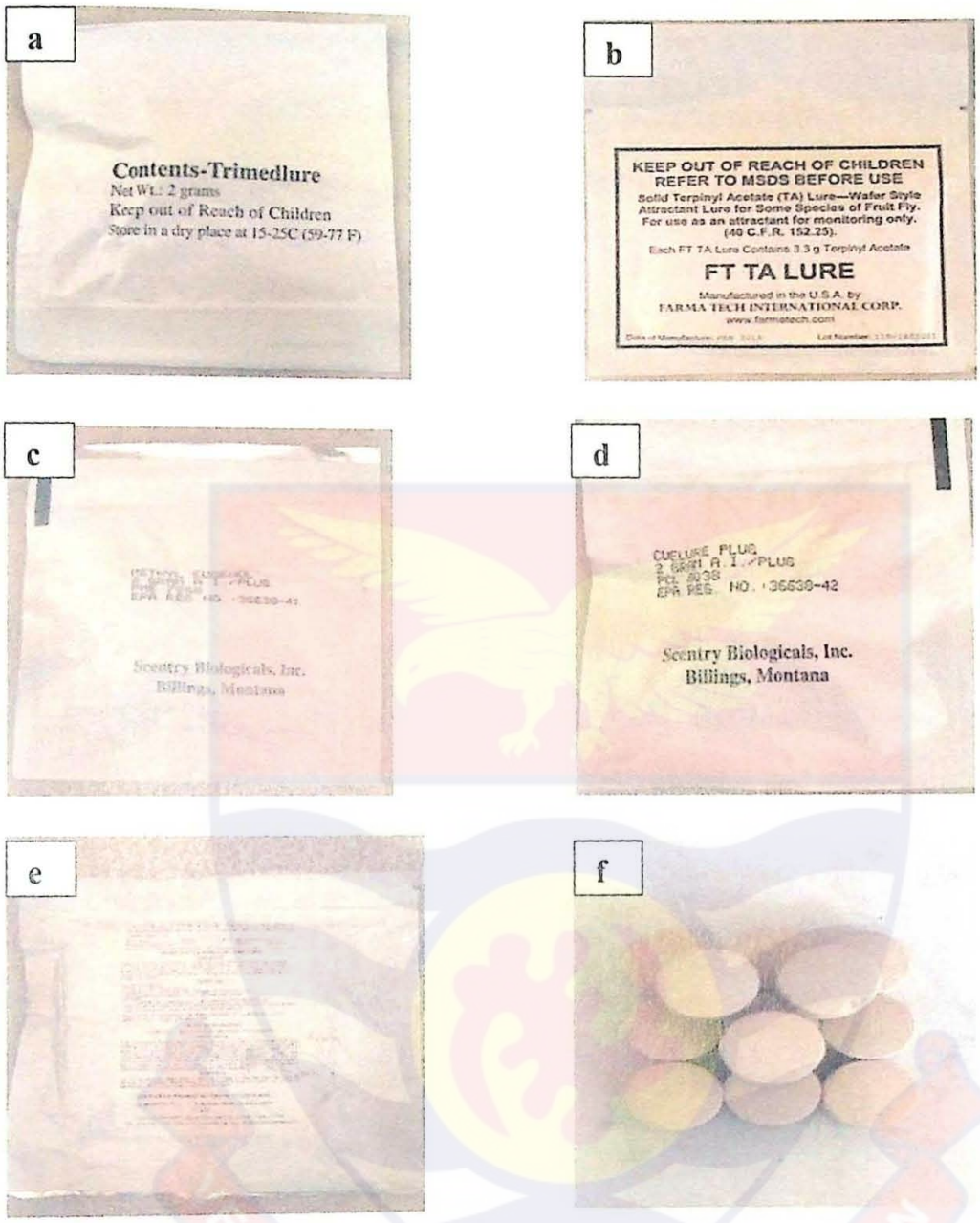

Figure 3. 3: Attractant and killing agent used to collect fruit flies, a) Trimedlure plug b) Terpinyl acetate c) Methyl eugenol plug d) Cuelure plug e) Dichlorvos strip f) Torula yeast pellet.

3.2.4 Fruit fly sampling and monitoring

A typical Tephri trap (SORYGAR, Madrid, Spain) consists of a 15 cm high vertical cylinder, of 12 cm diameter at the base with capacity to hold 450 ml of liquid. It has an invaginated aperture in the bottom which allows for easy service. The base is yellow with a transparent cover that is detachable allowing for easier servicing (Figure 3.4). Inside the top cover is a platform to hold

attractants. A rope hanger, placed on top of the trap body, is used to hang the trap from tree branches. To keep ants and other predators away from the insect captures, grease was added to the first one-third of the thread attached to the branch. Every month, traps were rotated so that they would not interfere with their performance. ME, CUL, TA, TML or TY were used as attractants in each trap. According to the tree's design, the traps were set 1.5 to 4 m above the ground and in semi-shaded and upwind parts of the canopy (Ekesi & Billah, 2009). The tephri traps were emptied weekly into transparent cylindrical plastic insect collection vials containing 70% ethanol or brown paper envelops and sealed. For identification, sorting, and counting, the insects were sent to a laboratory where they were examined. To get the total number of tephritid flies that were caught in a given month, the weekly catches were added together.

Figure 3. 4: Tephri trap used in the study.

3.2.5 Identification of trap catches

Identification of collected insects was done to species level based on morphological characteristics, using taxonomic keys developed by the African Fruit Fly Initiative (AFFI) (Ekesi & Billah, 2009). The specimen was viewed under a dissecting microscope (GX Microscopes, GT Vision Ltd, Suffolk, UK) at 20× magnification, non-tephritid flies were identified to Order or possibly Family levels. Samples of the identified insects except *Dacus langi and Dacus longistylus* have been deposited at the Radiation Entomology and Pest Management Center (REPMC) under the Biotechnology and Nuclear Agriculture Research Institute of Ghana Atomic Energy Commission. The identification of *C. penicillata*, *D. langi*, and *D. longistylus* was verified using morphological and molecular (DNA barcoding) methods at the Royal Museum for Central Africa (RMCA), Tervuren, Belgium. The RMCA received voucher specimens of the verified fruit fly specimens.

3.3 Data Analyses

The total number of flies were calculated and the percentages based on the different attractants in the three mango orchards were computed in Microsoft Excel for trend analysis throughout the experiment. For relative fly abundance, counts were expressed as number of flies per trap per day (FTD) (IAEA, 2003) to facilitate comparison across the different localities. Means obtained were correlated with weather parameters and presented in bar graphs (Microsoft Excel). Total number of fruit flies caught by the different baited traps from the field were subjected to one-way analysis of variance using GenStat statistical software, 12th edition (GenStat, 2009). The least significant difference (LSD) test at a probability level of 5% was used to separate treatment means.

Correlation and regression analysis were also performed between the fruit fly species and climatic factors measured during the experiment. Non-target captures were analysed and represented in percentages.

3.4 Results

3.4.1 Fruit fly abundance and diversity

A total of 172,617 fruit flies were collected in baited traps at the time of conducting this study. Out of this total number, ME-baited traps captured 156,728 (90.80%), TY-baited traps captured 11,156 (6.46%), the CUL-baited traps captured 4,417 (2.56 %), TA-baited traps captured 284 (0.16 %) and TML-baited traps captured 32 (0.02 %). Ten different species of fruit flies namely *B. dorsalis*. *C. cosyra*. C. *capitata*, *C. penicillata*, *D. bivittatus*, *D. langi*, *D. punctatifrons*, *D. ciliatus*, *D. longistylus* and *Z. cucurbitae* belonging to four genera (*Bactrocera*. *Ceratitis*, *Dacus*, and *Zeugodacus*) were identified from the three commercial orchards during the one-year trapping period (Figure 3.5). All the fruit flies captured in the ME-baited traps were *B. dorsalis*. Out of the total number of fruit flies captured in the CUE-baited traps, 3001 were *Z. cucurbitae*, 594 were *D. bivittatus*, 419 were *B. dorsalis*, 400 were *D. punctatifrons*, two were *D. langi* and one was *D. longystylus*. Of the total number of flies captured in the TML-baited traps, 24 were *B. dorsalis*, seven were *C. capitata* and one was *C. cosyra*. Out of 11,156 fruit flies captured in TY-baited traps, 10, 897 were *B. dorsalis*, 258 were *Z. cucurbitae*, and one was *D. bivittatus*. Of the total number of fruit flies captured in the TA-baited traps, 242 were *B. dorsalis*, 21 were *C. cosyra*, 12 were *C. capitata*, eight were *C. penicillata*, and one was *D. ciliatus* (Table 3.2).

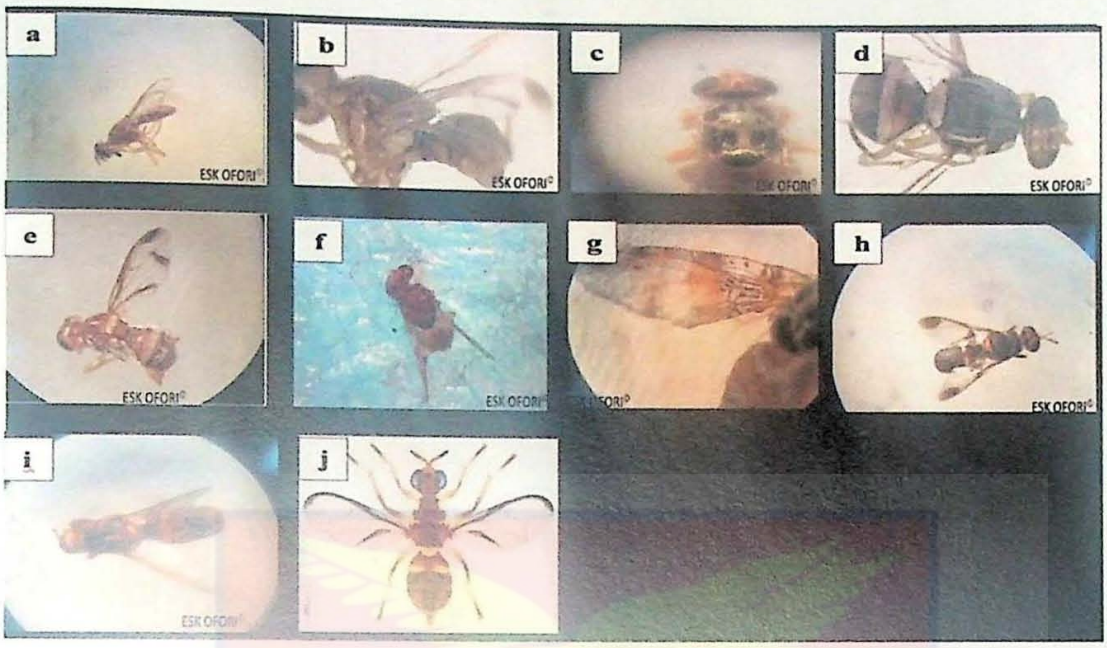

Figure 3. 5: Fruit fly species captured in baited traps in the study area. a) *Dacus punctatifrons*, b) *Dacus bivittatus*, c) *Ceratitis capitata*, d) *Bactrocera dorsalis*, e) *Zeugodacus cucurbitae*, f) *Ceratitis cosyra*, g) *Ceratitis penicillata*, h) *Dacus langi*, i) *Dacus longistylus*, j) *Dacus ciliatus* (Photo credit j: G. Goergen/IITA, Photo credit a-i: E.S.K. Ofori)

Table 3. 2: Number of Fruit flies captured by traps baited with 5 different attractants in 3 mango orchards at the SouthEastern mango enclave of Ghana.

	Fruit fly Species										
Attractants	*Bactrocera dorsalis*	*Ceratitis cosyra*	*Ceratitis capitata*	*Ceratitis penicillata*	*Dacus bivittatus*	*Dacus ciliatus*	*Dacus longistylus*	*Dacus langi*	*Dacus punctatifrons*	*Zeugodacus cucurbitae*	Total
ME	156728	0	0	0	0	0	0	0	0	0	156728
CUL	419	0	0	0	594	0	1	2	400	3001	4417
TY	10897	0	0	0	1	0	0	0	0	258	11156
TML	24	1	7	0	0	0	0	0	0	0	32
TA	242	21	12	8	0	1	0	0	0	0	284
Total	168,310	22	19	8	595	1	1	2	400	3259	172617

ME-Methyl eugenol, CUL-Cuelure, TY-Torula yeast, TML-Trimedlure, TA-Terpinyl acetate,

Methyl eugenol-baited traps had the highest mean catche of 1005±388. TY-, CUL-, TA- and TML-baited traps had mean catches of 72±31, 28±24, 2±1.5 and 0.2±0.1 respectively. There were significant differences in the mean catches between ME-baited traps and other traps (df= 4, 8, F = 6.66, p = .012; Table 3.3). However, there were no significant differences in the mean trap catches between CUL-baited traps and TY-baited traps, CUL-baited traps and TML-baited traps, CUL-baited traps and TA-baited traps (p > .05), TY-baited traps and TML-baited traps (p > .05), TY-baited traps and TA-baited traps (p > .05). and between TML-baited traps and TA-baited traps (p > .05). In terms of attractiveness of the baited traps, across the 3 different farms, Methyl eugenol had the highest FTD (143.10). This was followed by Torula yeast-baited traps (10.19). Culure-baited traps had FTD of 4.03, Terpinyl acetate-baited traps had 0.26 FTD and Trimedlure-baited traps had the least FTD of 0.03 (Table 3.3).

Table 3. 3: Fruit fly catches by 5 different attractants in the SouthEastern mango enclave of Ghana.

Attractants	No. of flies	No. of traps	Exposure period (days)	FTD	Mean catches ± SE
ME	156,728	3	365	143.10	1005 ± 388a
CUL	4,417	3	365	4.03	28 ± 24b
TY	11,156	3	365	10.19	72± 31b
TML	32	3	365	0.03	0.2 ± 0.1b
TA	284	3	365	0.26	2 ± 1.5b
P-value					0.012
LSD (P<0.05)					554.7
Total	172.617				

ME-Methyl eugenol, CUL-Cuelure, TY-Torula yeast, TML-Trimedlure, TA-Terpinyl acetate

Mean ± SD followed by the same letter in the last column are not significantly different (P < .05), Tukey's HSD test

3.4.2 Nontarget captures

A total of 1,985 nontarget insects in five (5) families (Muscidae, Nerridae, Lonchaeidae, Platystomatidae, Curculionidae) were captured in the baited traps with 65.1% (1292) coming from the TY-baited traps. This was followed by ME-, CUL-, TML- and TA-baited traps contributing 30.6% (607), 3.9% (78), 0.3% (5) and 0.3% (3) respectively (Table 3.4). Whiles the target insects formed 98.9% of the total number of insects collected (172,617 + 1985), the non-target insects formed only 1.1% of the total. Platystomatidae had the highest contribution (44.7%), followed by Muscidae (40.8), Lonchaeidae (9.5%), Nerridae (4.9%) and Curculionidae (0.1%) (Table 3.4).

Table 3. 4: Number of nontarget catches from traps baited with five different attractants in mango orchards at the south eastern mango enclave of Ghana (% in brackets).

	Catches per trap					
Family	ME-baited trap	CUL-baited trap	TY-baited trap	TA-baited trap	TML-baited trap	Total
Muscidae	284	16	509	0	0	809 (40.8)
Nerridae	50	7	41	0	0	98 (4.9)
Lonchaeidae	76	20	89	3	0	188 (9.5)
Platystomatidae	197	35	653	0	4	889 (44.7)
Curculionidae	0	0	0	0	1	1 (0.1)
Total	607 (30.6)	78 (3.9)	1292 (65.1)	3 (0.3)	5(0.3)	1985

ME-Methyl eugenol, CUL-Cuelure, TY-Torula yeast, TML-Trimedlure, TA-Terpinyl acetate

3.4.3 Flies per trap per day (FTD) variation of fruit flies with climatic factors

During the start of the minor mango season in July 2018, the FTD for *B. dorsalis* caught by ME-baited traps was 265.43. The FTD for *B. dorsalis*

dropped considerably to 0.476 in October 2018 and went up again from November 2018, reaching a peak of 45.86 FTD in February 2019 (Figure 3.6). In the major mango season, i.e., March 2019, FTD was 105.24 and it reached a peak of 332.17 in April 2019. In May and June 2019, the FTD of *B. dorsalis* were 269.34 and 358.88 respectively (Figure 3.6). Moreover, at the beginning of the minor mango season, the FTD for *D. bivittatus* trapped in cuelure-baited traps was 1.64. This dropped to 0.36 in August 2018. The highest FTD of *D. bivittatus* recorded was 2.23 in October 2018. The FTDs of *D. bivittatus* declined consistently through both the minor and major mango seasons (Figure 3.7). For *Z. cucurbitae*, during the minor mango seasons, FTD in July 2018 was 4.10. In October 2018, the FTD rose to 11.69. The FTD remained low until the end of February 2018, which marked the end of the minor mango season (Figure 3.8). During the start of the major mango season in March 2019, there was a slight increase in the *Z. cucurbitae* FTD (4.36) but the numbers dropped during the remaining period (Figure 3.8). The FTD for *D. punctatifrons* during the start of the minor mango season in July 2018 was 0.7. This increased to 1.4 in October 2018 before declining throughout the rest of the season (Figure 3.9). Similarly, FTDs were low for *D. punctatifrons* throughout the major mango season (Figure 3.9). In Torula yeast baited traps, FTD for *B. dorsalis* was 23.80 in the minor mango season in July 2018. The numbers of *B. dorsalis* in TY-baited traps remained low until the end of the minor mango season in February 2019. In the major mango season, *B. dorsalis* numbers remained low from March 2019 to June 2019 (Figure 3.10).

Correlation analyses conducted between the major fruit flies and weather parameter show that between *B. dorsalis* and temperature ($r = 0.0888$; $p = .7837$),

relative humidity (r = 0.4704; p = .1223) and rainfall (r = 0.4419; p = .1503) were positively correlated. However, there was no significance difference in the relationship between the weather parameters and abundance of *B. dorsalis* (Table 3.5). There were weak and non-significant correlation between the abundance of *D. punctatifrons* and temperature (r = 0.2764; p = .3844), relative humidity (r = 0.4492; p = .2060) and rainfall (r = 0.0382; p = .09060) (Table 3.6). Similar correlations were observed between *D. bivittatus* and temperature (r = 0.2259; p = .4801), relative humidity (r = 0.2416; p = .0583) and rainfall (r = 0.0542; p = .8672) (Table 3.7) and between *Z. cucurbitae* and temperature (r = 0.0867; p = .7888), relative humidity (r = 0.1039; p = .7479) and rainfall, (r = 0.0305; p = .9250) (Table 3.8).

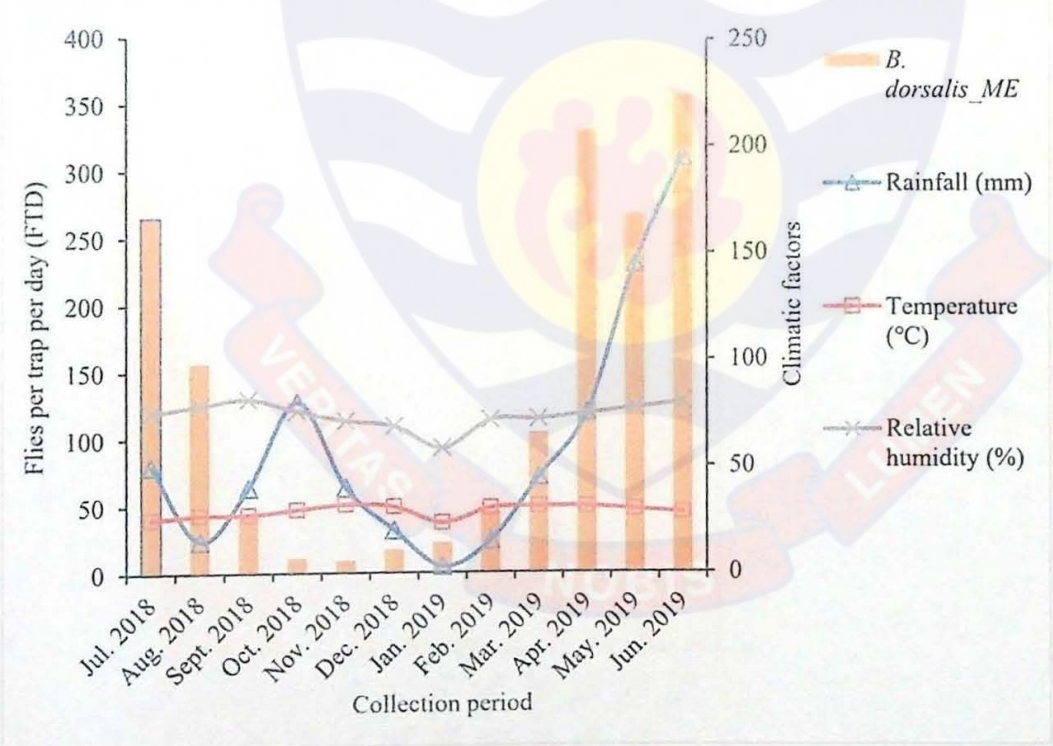

Figure 3. 6: Relationship between flies per trap per day (FTD) of *Bactrocera dorsalis* caught in the methyl eugenol-baited traps and some climatic factors (Rainfall, temperature, relative humidity) at the SouthEastern mango enclave of Ghana.

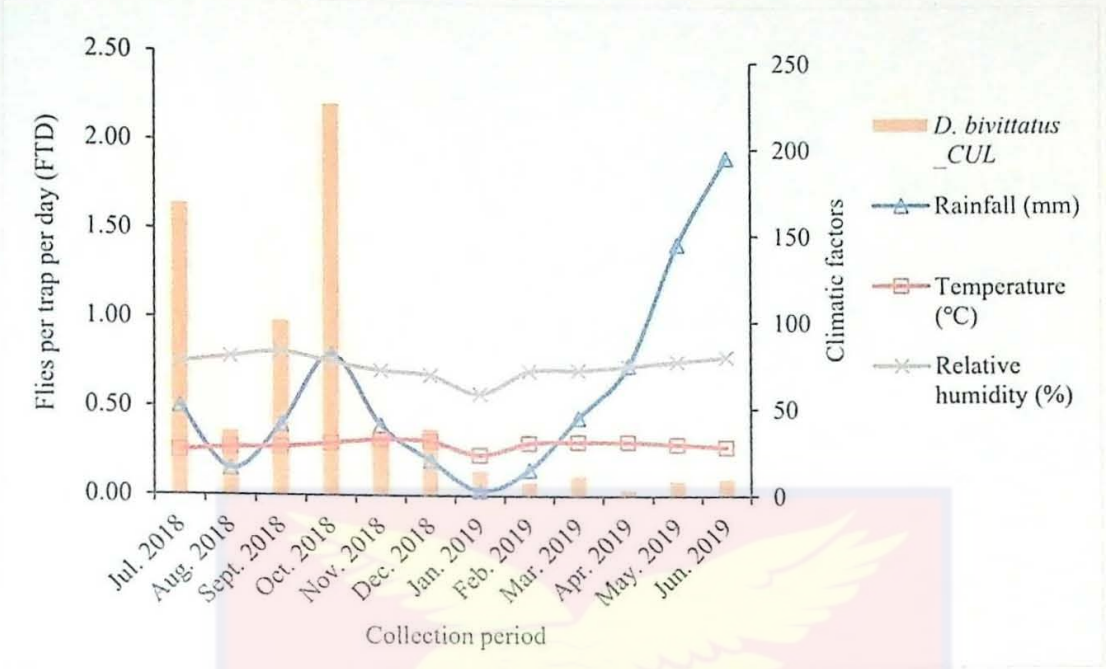

Figure 3. 7: Relationship between flies per trap per day (FTD) of *Dacus bivittatus* caught in the cuelure-baited traps and some climatic factors (Rainfall, temperature, relative humidity) at the SouthEastern mango enclave of Ghana.

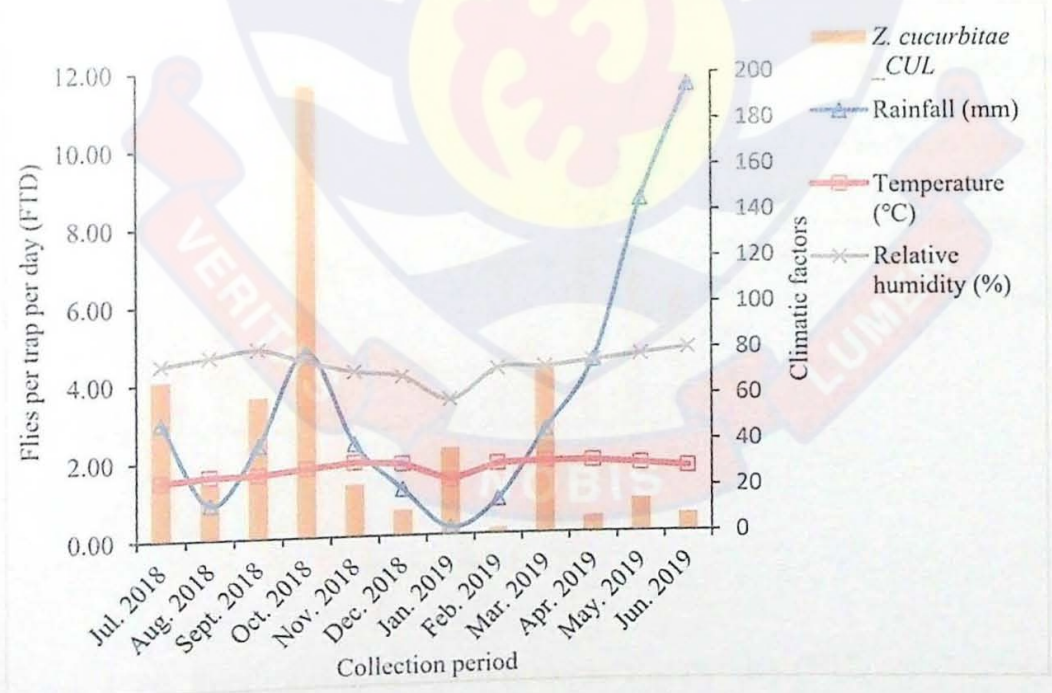

Figure 3. 8: Relationship between flies per trap per day (FTD) of *Zeugodacus cucurbitae* caught in the cuelure-baited traps and some climatic factors (Rainfall, temperature, relative humidity) at the SouthEastern mango enclave of Ghana.

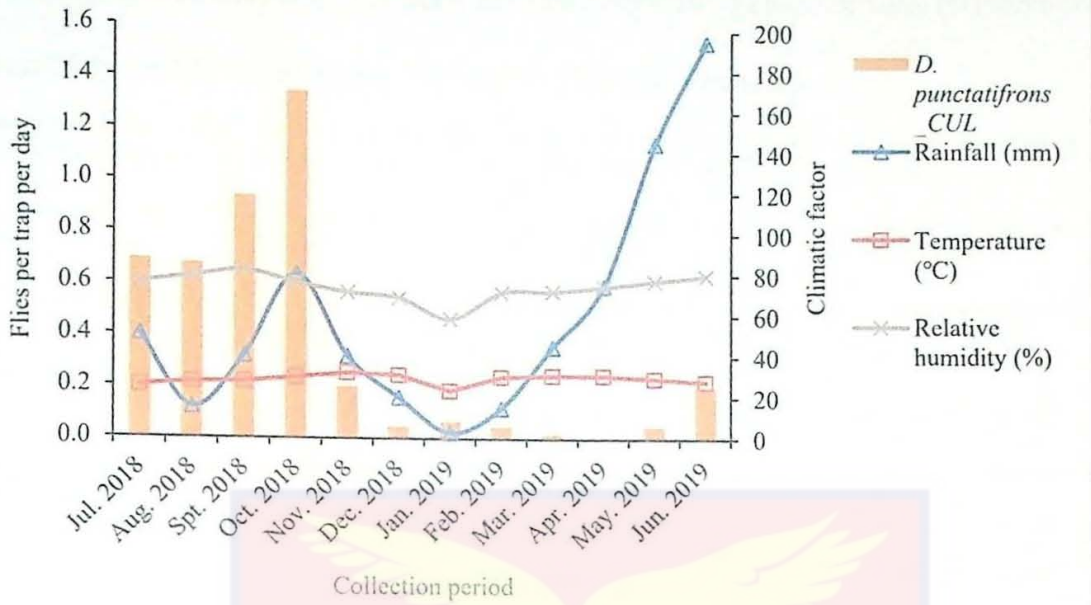

Figure 3. 9: Relationship between flies per trap per day (FTD) of *Dacus punctatifrons* caught in the cuelure-baited traps and some climatic factors (Rainfall, temperature, relative humidity) at the SouthEastern mango enclave of Ghana

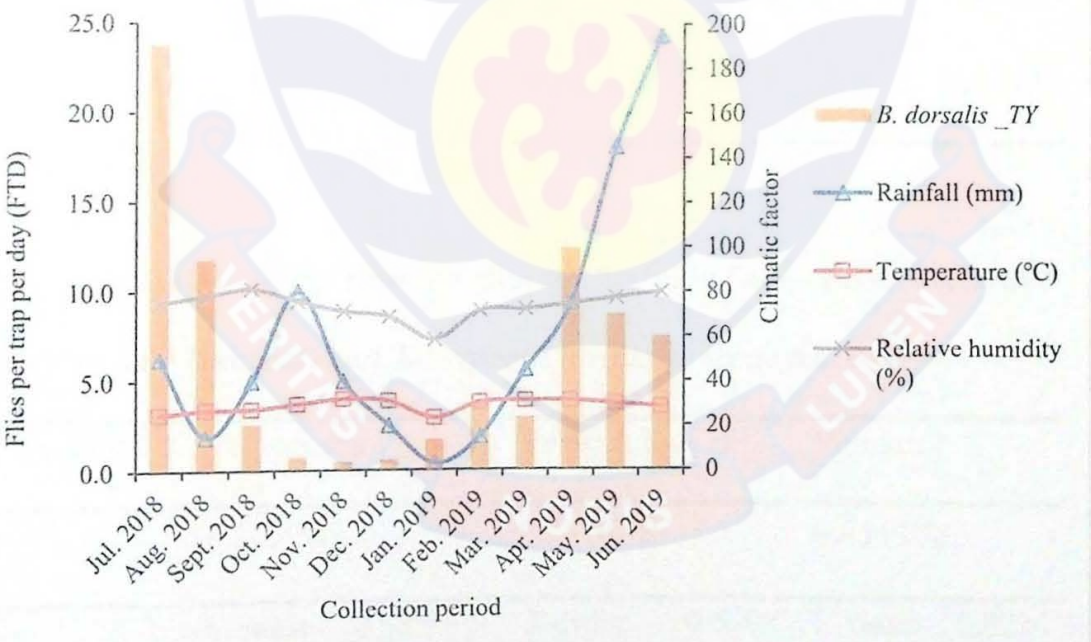

Figure 3. 10: Relationship between flies per trap per day (FTD) of *Bactrocera dorsalis* caught in the cue Torula yeast-baited traps and some climatic factors (Rainfall, temperature, relative humidity) at the SouthEastern mango enclave of Ghana.

Table 3. 5: Relationship between climatic factors (Temperature, Relative humidity and Rainfall) and *Bactrocera dorsalis* abundance.

Correlation	Temperature		Relative humidity		Rainfall	
r	0.0888NS		0.4704NS		0.4419NS	
	F-value	0.07945	F-value	2.842	F-value	2.427
	P-value	0.7837	P-value	0.1223	P-value	0.1503

NS-Non-significant. All P-value at .05

Table 3. 6: Relationship between climatic factors (Temperature, Relative humidity and Rainfall) and *Dacus punctatifrons* abundance.

Correlation	Temperature		Relative humidity		Rainfall	
r	0.2764NS		0.4492NS		0.0382NS	
	F-value	0.8273	F-value	2.5272	F-value	0.0146
	P-value	0.3844	P-value	0.2060	P-value	0.9060

NS-Non-significant. All P-value at .05

Table 3. 7: Relationship between climatic factors (Temperature, Relative humidity and Rainfall) and *Bactrocera bivittatus* abundance.

Correlation	Temperature		Relative humidity		Rainfall	
r	0.2259NS		0.2416NS		0.0542NS	
	F-value	0.5379	F-value	0.6198	F-value	0.0294
	P-value	0.4801	P-value	0.4493	P-value	0.8672

NS-Non-significant. All P-value at .05

Table 3. 8. Relationship between climatic factors (Temperature, Relative humidity and Rainfall) and *Zeugodacus cucurbitae* abundance.

Correlation	Temperature		Relative humidity		Rainfall	
r	0.0867NS		0.1039 NS		0.0305 NS	
	F-value	0.0757	F-value	0.1091	F-value	0.0093
	P-value	0.7888	P-value	0.7479	P-value	0.9250

NS-Non-significant. All P-value at .05

3.5 Discussion

3.5.1 Diversity of fruit flies

This study has shown that, at least ten fruit fly species namely *B. dorsalis*, *C. cosyra*, C. *capitata*, *C. penicillata*, *D. bivittatus*, *D. langi*, *D. punctatifrons*, *D. ciliatus*, *D. longistylus* and *Z. cucurbitae* inhabit the south-eastern mango enclave in Ghana. The ten fruit fly species captured in this study belong to four genera; *Bactrocera*, *Ceratitis*, *Dacus* and *Zeugodacus* (Thompson, 1998). These genera of fruit flies are known to be of major economic importance in several African countries (White & Elson-Harris, 1992; Thompson, 1998; Billah et al., 2006; Ekesi & Billah, 2006; Bota et al., 2018; 2020; N'Da, 2018; Zida et al., 2020; Amevoin, 2021). Vayssières et al. (2015) emphasized that growing mangoes in West Africa has been significantly hampered due to the presence of several fruit fly species, which have significantly limited the potential economic advantages of doing so.

Moreover, this is the first time *D. langi* and *D. longistylus* have been detected and reported in Ghana. *Dacus langi* was previously documented in Togo, Benin and Côte d'Ivoire, while *D. longistylus* was reported previously in Benin and

Nigeria (De Meyer et al., 2013). *Dacus longistylus* was also recently detected on Sodom apple in Côte d'Ivoire (N'Dépo, 2019).

Ceratitis, *Carpophthoromyia* and *Trirhiithrum* species in particular are relatively abundant in Ghana (De Meyer et al., 2013). In the current study, three (3) and five (5) fruit fly species in the *Ceratitis* and *Dacus* genera have been reported respectively. Mostly, tephritid fruit flies identified in this work are of native origin, except of *B. dorsalis* and *Z. cucurbitae* which were reported as exotic and invasive (Goergen et al., 2011; De Meyer et al., 2013).

3.5.2 Abundance of fruit flies

The SouthEastern mango enclave of Ghana forms a major part of the over 128,127,521m^2 of mango cultivation in Ghana (Zakari, 2012, Baidoo-William, 2017). This mainstay of the inhabitants of the area is beset by fruit fly infestation. *Bactrocera dorsalis* is a major tephritid fruit fly species whose presence and activities pose a challenge to the production of mango in the area. In fact, over 97.5% of the fruit flies captured in all traps in the study region were *B. dorsalis*. This makes *B. dorsalis* the dominant tephritid in SouthEastern enclave followed by *Z. cucurbitae*. The fact that TY-baited traps, which is not a species-specific attractant captured over 97.6% *B. dorsalis* underscore their dominance in the area.

Methyl eugenol turned out to be the most effective attractant to detect the presence of *B. dorsalis* in mango orchards as traps baited with it captured 90.80% of all the fruit flies captured in this study. Methyl eugenol-baited traps also had the highest fly densities across the study areas. This was not surprising as methyl eugenol is targeted at *B. dorsalis*. Indeed, methyl eugenol is a highly specific parapheromone (Manrakhan, 2006). It has olfactory and

phagostimulatory properties and can lure fruit flies from a distance of 500 m and lasts long (Shelly & Edu, 2010). Our finding together with others confirms that ME is very effective for monitoring and trapping *B. dorsalis* (White & Elson-Harris, 1992, Roomi et al., 1993; Billah et al., 2006). The high numbers of *B. dorsalis* confirms the reports of Lux et al. (2003a); Billah et al. (2006); Mwatawala et al. (2009) and Nboyine et al. (2012) that *B. dorsalis* is still dominant and very highly competitive with other species of fruit flies. The extremely high numbers and frequency of *Bactrocera dorsalis* in all of the mango farms in this research indicate that it has established itself in Ghana and poses danger to mango and other fruit produce.

The other attractants which are not target-specific also performed relatively well, at least, in detecting *B. dorsalis*. In most of the baited traps, *B. dorsalis* was found to dominate except in the CUL-baited traps where *Z. cucurbitae* was dominant. The CUL-baited trap was also effective to detect *D. langi* and *D. longistylus* for the first time in Ghana. It is not surprising that *B. dorsalis* dominates the fruit fly counts in the SouthEastern mango enclave in Ghana. In fact, previous works have reported that introduced exotic tephritid species are able to out-compete native species resulting in decrease in population and niches of native species (Duyck et al., 2004; Ekesi et al., 2009; Mwatawala et al., 2009). In Ghana, *C. cosyra* was the major insect pest of mango (Lux et al., 2003b) whiles *C. capitata* (Wiedemann) was a major insect pest of citrus (Afreh-Nuamah, 1999).

3.5.3 Population dynamics

High FTD values were recorded in April, May, June and July during the study period. This is likely due to the presence of matured and ripe mango fruits

on the orchards. Matured and ripe fruits tend to attract fruit flies for oviposition. Tan & Serit (1994), concluded that the availability of preferred hosts is the variable that most influences the size of the population of adults of *B. dorsalis* in Malaysia. Previous studies have reported high fruit fly population densities in an orchard where matured mangoes were found in April to May (Foba et al., 2012).

The current study detected that in April, May, June and July, the fruit fly populations fluctuate between *B. dorsalis* and *Z. cucurbitae*. This could be due to the capacity of *B. dorsalis* to exist together with overly competitive invasive species such as *Z. cucurbitae* (Goergen et al., 2011). The aggression of species like *Z. cucurbitae* and the ability of *B. dorsalis* to co-exist might have resulted in the displacement of indigenous species such as *C. cosyra*. In months (April, May, June) where *B. dorsalis* population was high, the population of *Z. cucurbitae* was low. The population of *B. dorsalis* peaks from April to July. This coincided with the major mango fruiting season in the SouthEastern mango enclave. The highest population (358.88 FTD) was observed in June during the major mango season, which coincides with the advancement of fruit maturity and harvesting. This observation is similar to findings from previous studies e.g., Jhala et al. (1989); Kumar et al. (1997) and Vayssières et al. (2014) in which high populations of fruit flies were found to coincide with ripening and harvesting of fruits.

In this study, a high proportion of *B. dorsalis* was recorded between March and August. A similar observation was made previously in early June by Vayssières et al. (2014). The relative fly abundance (358.88 FTD) recorded for *B. dorsalis* in the current study was higher than what was recorded by Nboyine

et al. (2012) where 0.02-22.25 and 0.08-121.39 FTD were recorded for the years 2009 and 2010 respectively. Vayssières et al. (2014) reported an FTD of 322 in Botim farms, Sunyani, Ghana but this was from the Guinea Savannah agro-ecological zone. Meanwhile, low FTDs (2.38) have also been recorded previously in the Coastal Savannah agro-ecological zone (Adzim et al., 2016). Moreover, in the current study, low numbers of *B. dorsalis* were recorded between September and February with the lowest (9 FTD) being recorded in November. This current relative fly density is about 3 folds the FTD recorded by Nboyine et al. (2012) in 2010 and could be due to poor sanitation of the farms i.e., dropped and rotten fruits on the orchard floor.

The prevailing environmental conditions in the mango orchards could have contributed to the high FTDs. For instance, the average temperature, relative humidity and rainfall recorded for the whole study period were 28.9°C, 74% and 60.3 mm respectively. Meanwhile, the optimum range of temperature for *B. dorsalis* development is 20°C – 28°C (Christenson & Foot, 1960; Bateman, 1972; Vargas et al., 1996; Wang, 1996; Wu et al., 2000). This makes it very conducive for *B. dorsalis* to multiply in the study area. During March to August where a high population of *B. dorsalis* was documented, the temperature was 25°C - 31°C, humidity was 72% - 80% RH and rainfall was 50 mm – 195 mm. Coledonio-Hurtado et al. (1995); Tan and Serit (1994) and Vayssières et al. (2005), concluded that the availability of hosts, combined with climatic factors such as temperature and rainfall, play a significant role in the fluctuation of population of fruit flies. Coupled with the closed canopies of the mango orchards, likely, optimum conditions were met to influence the harbouring of *B. dorsalis* during the major mango growing season. Meanwhile, the minimum

temperature during September to February where lower populations were recorded dropped slightly from 25°C to 23°C. These optimum temperatures, humidity and the high fecundity of adult female *B. dorsalis* result in the laying of about 3000 eggs per female, preferably in ripe fruits during their lifetime (Ekesi et al., 2006; Weems et al., 2016; Shahzad et al., 2017; Gui et al., 2018). The high trap catches of *B. dorsalis* during the period of high rainfall is corroborated by Vayssierres et al. (2005, 2009) in a study in which an increase in trap catches of *B. dorsalis* was observed shortly after the onset of the rainy season. It has also been suggested that *B. dorsalis* thrives well in moist weather and high temperatures, hence the high numbers of *B. dorsalis* during high rainfall seasons (Rwomushana et al., 2008). According to Cugala (2011), during hot and rainy season, the population of *B. dorsalis* increases until the end of the mango season. It is crucial to understand the link between population variations and biotic and abiotic variables, which may help producers estimate population expansion and take preventative actions.

Data on the various fruit fly species identified show positive correlation with climatic data. The present study supports the works of Bagle and Prasad (1983); Agrawal and Kumar (2005) and Sahoo et al. (2016) who observed positive correlation of fruit fly populations with temperature on mango plant. The present result is also in agreement with the finding of Rai et al. (2008), who reported that fruit fly population had positive but non-significant correlation with total rainfall in guava orchard. The fluctuation in these weather parameters have direct effect on the mango fruit fly population growth and development in the southeastern mango enclave.

3.5.4 Nontarget captures

Non-target insects caught included phorids, lonchaeids, neriids, muscids, and carrion-related species (families linked with rotting, decomposing, and/or fermenting organic materials). These were mostly large to microscopic insects that were drawn to the dead fruit flies and torula yeast in the traps in great numbers.

3.6 Conclusion

In conclusion, this study has provided important information about population dynamics of fruit flies inhabiting the south-eastern mango enclave of Ghana that could be utilized for the best management of the insect pests identified.

- The study has identified ten different fruit fly species belonging to four economically important genera.
- *Bactrocera dorsalis* has been identified as the most abundant fruit fly species attracted by both Methyl eugenol- and Torula yeast-baited traps. This is followed by *Z. cucurbitae* which was attracted by CUL-baited traps. The presence of these two tephritids results in extensive damage to mango fruits. The two flies are aggressive in attacking mango fruits during peak and off-peak fruiting seasons.
- It is worth noting that, *D. langi* and *D. longistylus* have been detected, identified and documented for the first time in Ghana through this study.
- The high efficacy of ME-baited traps makes it a candidate for attract-and-kill technique for controlling *B. dorsalis*. This could be complemented with good sanitation to reduce oviposition substrates for gravid females.

- Furthermore, the seasonal variation in the population of fruit flies can be targeted for effective management of the major fruit fly pests in the study area.
- Practical management of *B. dorsalis* populations should be intensified during April through July, which is the periods of highest *B. dorsalis* population and activity. Management efforts should start at the beginning of the major rainy season when the population happen to be most vulnerable.

CHAPTER FOUR

DEVELOPMENT OF AN EFFECTIVE SYSTEM FOR MASS TRAPPING OF ADULT MALE *Bactrocera dorsalis*

4.1 Introduction

A fruit fly trapping system is one of the most often used techniques in bio-systematics research and the most expensive. The efficiency of trapping systems varies according to the trap type used for trapping pest, the concentration and type of lure used, the population density of the pest, weather conditions, hanging height from the ground and direction of the trap (Rizk et al., 2014). It is imperative to note that the mass trapping approach is a preventative strategy that relies on attracting and killing adult fruit flies before they reach the fruit in order to lay eggs. The primary benefit of using the mass trapping approach is the exclusion of fruits and the prevention of total canopy contamination by pesticides during trapping. The mass trapping methods can be applied using traps of different constructions, which have to be set on the tree canopy. In addition, the traps may be filled with various kinds of attractants that have been treated with pesticide, or they may be filled with a water solution that contains both attractants and insecticides (Barcley & Haniotakis, 1991; Bjeliš, 2006; Kleiber et al., 2014; Gregg et al., 2018; Hafsi et al., 2020a). It is evident that mass trapping is more effective than bait sprays and less expensive to implement (Broumas et al., 1998; Bjeliš, 2006; Flores et al., 2017; Hafsi et al., 2020b; Stupp et al., 2021). To capture fruit flies with great efficiency, various coloured and shaped traps are required (Broughton & Rahman, 2017; Tadeo et al., 2017; Sikandar et al., 2017; Candia et al., 2019; Abu-Ragheef et al., 2020). Methyl eugenol and the cue lure (4-(p-acetoxyphenyl)-2-butanone) are known

to elicit responses in *Bactrocera*, *Dacus*, and *Zeugodacus* (Royer & Mayer, 2018; Royer et al., 2020).

Successful Sterile Insect Technique programmes, which entail the release of sterilised field-trapped individuals to manage wild populations of an insect pest, are dependent on the success of programmes that catch a substantial part of the wild population of an insect problem (Klassen et al., 2021). By this, it is important to optimize trapping for such a technique. The type of trap to be used for such a technique is important as the capacity for capturing insects may differ among trap types. Therefore, there is a need to evaluate different traps to determine the proportion of the wild population of an insect pest they can capture out of the total catch by all traps. It is necessary to use specific trapping systems in accordance with the objectives of specific pest control programmes, the economic and technical feasibility, the species of fruit fly present, and the phytosanitary condition of the delimited areas, which can be either infested or infested but low in pest prevalence, or a pest-free area (IAEA, 2018). In this study, three different traps for capturing *Bactrocera dorsalis* were evaluated.

The adult stages of fruit flies are often the focus of fruit fly monitoring systems. Traps and attractants may be used to screen the population of adult fruit flies (IAEA, 2018). The kind of attractant is the most important factor in deciding which traps to use (IAEA, 2018). On the other hand, the selection of attractants is dictated by the species of interest and the aims of the trapping operation, which may include timely identification of new pests, delimitation of new pest arrival, suppression, and eradication of existing pest populations (IAEA, 2018).

Although there are known attractants for several Afrotropical fruit fly pests, the responses of flies to trap types, the ideal period for trapping and effects of weather conditions at the time of trapping are not adequately understood. This study therefore sought to evaluate the efficacy of three trap types and period for mass trapping adult *Bactrocera dorsalis* for irradiation studies. The study also investigated the influence of weather conditions (temperature, humidity and rainfall) on trap efficiency.

4.2 Materials and Methods

4.2.1 Study Location

Sampling for fruit flies was carried out at Power of Trinity Farm (POT) (6°6'12" N; 0°0'7" W) in the Yilo Krobo district of the Eastern Region of Ghana (Figure 4.1) from March 2019 to June 2019. The farm lies within the Coastal Savannah Agro-ecological zone of Ghana. It has a humid climate with a mean minimum temperature of 25°C and a mean maximum temperature of 35°C. The major rainy season in the area is between March and July with mean rainfall between 436 – 1507 mm. The minor rainy season is between September and October with mean rainfall between 59 – 603 mm (Asare-Nuamah & Botchway, 2019).

The POT farm was cultivated with a mixture of Keith and Kent varieties of mangoes. All the trees in the mango farm were at the economic fruit-bearing age of seven years and above at the time of the survey.

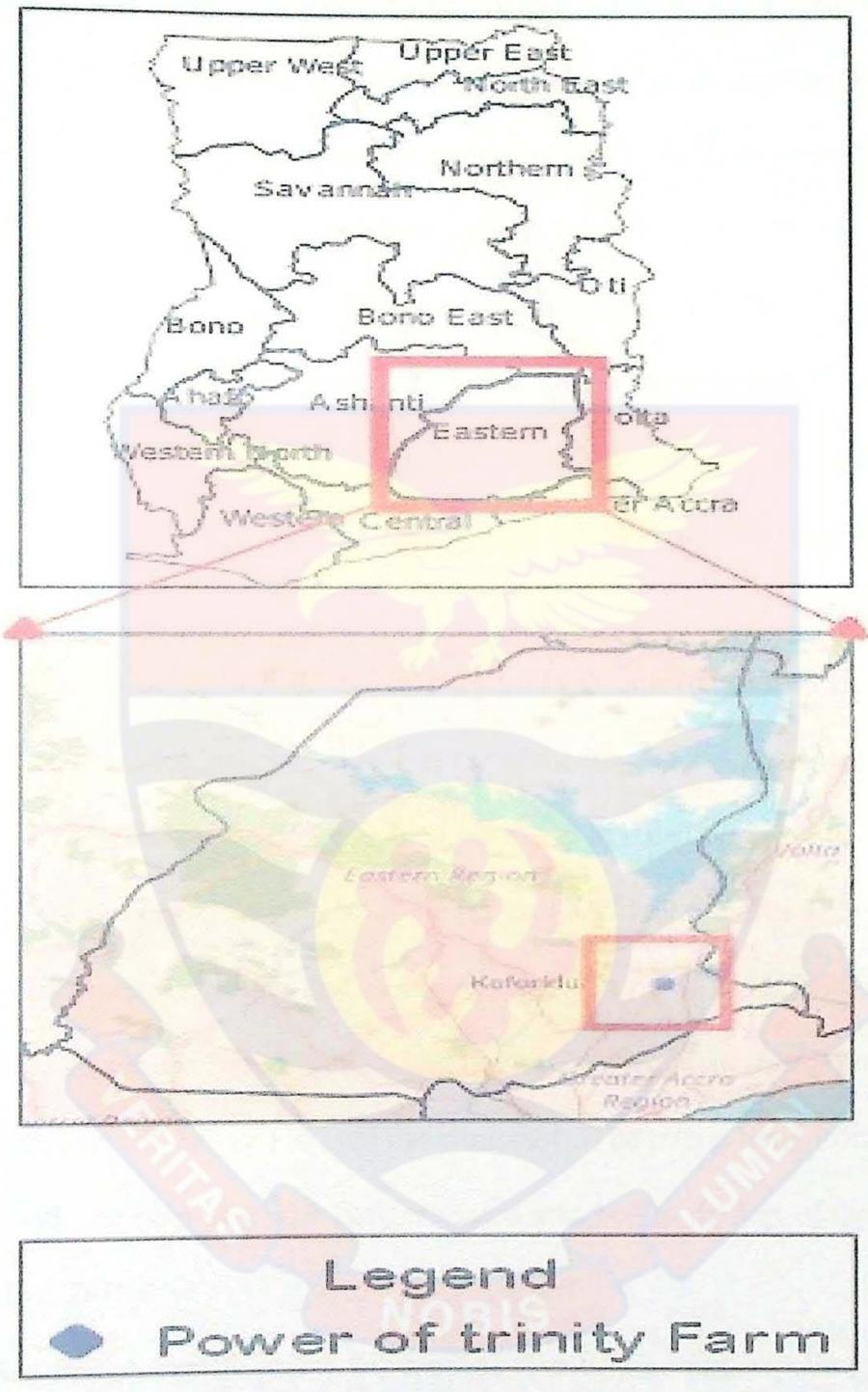

Figure 4. 1: Location of Power of Trinity orchard (marked in blue) in the Coastal savanna agro-ecological zone of Ghana.

4.2.2 Trap types under evaluation

Trapping was done according to IAEA guidelines (IAEA, 2018) with few modifications. Three different traps, Ecoman fruit fly trap (Ecoman

Biotech, Beijing, China), Tephri trap (SORYGAR, Madrid, Spain) and Bucket funnel trap (Insect Science, Tzaneen, South Africa) were evaluated to ascertain their efficiency and effectiveness in collecting large number of flies. Moreover, the trapped flies were monitored for their survival under laboratory conditions (i.e., 25±1°C; 75±5% RH; 12D:12L photoperiod).

The Ecoman fruit fly trap (Figure 4.2*a*) is a vertical cylinder 17 cm high with an average diameter of 0.83 cm at the top entrance. The total volume of the trap is about 404 ml. It has a white translucent bottle and a black cap (height, 0.65 cm and width, 0.70 cm) which can be unscrewed to facilitate servicing. The black cap is dome-shaped, with 4 spiral entry points (each 0.13 cm in diameter). The attractant is held in place by a plastic pin (0.6 cm in height), which is fastened to the inside of the top cover of the trap. The trap is suspended from a hook mounted on the dome-shaped black top of the structure.

The Tephri trap (Figure 4.2*b*) is similar to a McPhail trap. It's a 15-inch-tall, 12-inch-diameter cylinder that can store up to 450 ml of fluid. In order to make service easier, the base is yellow and the top is transparent. Inside the top is a platform that will be used to hold the attractants. The trap is hung from tree branches with the help of a wire hanger that is attached to the top of the trap body (IAEA, 2018).

The Bucket funnel trap (Figure 4.2*c*) consists of a tapered upper yellow pane (the funnel), white lower collection bucket, green lanyards/lid, white caps and green pheromone basket/cage. It is also a vertical cylinder 23 cm in height with a diameter of 17 cm. The green lid comes with two holes on top of the green lanyards with thread for hanging the trap. The traps were hung on the mango trees using nylon thread. To prevent ants from preying on the fruit fly captures,

a grease was put to the first one-third proximal area of the thread near the branch. Traps were swapped on a monthly basis to avoid a trap's position from interfering with its function.

Figure 4. 2: Traps used for sampling/collecting *Bactrocera dorsalis* males a) Ecoman trap b) Tephri trap c) Bucket funnel trap

4.2.3 Attractant

ME (Scentry Biologicals, Inc, Billings, MT) is a parapheromone that attracts and catches a large number of *Bactrocera* species as well as certain *Ceratitis* species. Parapheromones are often very volatile and may be used in

conjunction with a wide range of traps to attract fruit flies. The attractant, ME, was in a slow-releasing polymeric gel form. No killing agent was added to the attractant in the traps because the captured flies needed to be kept alive to be used for further experiments in this study. To avoid contamination from other odour sources, applicators hands were covered with disposable gloves before placing the attractants in traps. Only new traps were used. Methyl eugenol is known to attract fruit flies over long distances (Steiner et al., 1962; Roomi et al., 1993; Shelly & Edu, 2010; N'Da, 2018).

4.2.4 Fruit fly sampling and monitoring

An area of 12,141 m^2 within the 32,375 m^2 farm was demarcated as the sampling area. The mango trees were selected randomly to cover the area uniformly. Within the sampling area, three blocks containing 120 mango trees were demarcated with each block 10 meters apart. Within each block, a total of 15 trees, 60 m apart, were systematically selected and tagged. Traps were deployed on the selected trees at a height of 1.5 – 4.0 m above ground depending on the architecture of the trees (Ekesi & Billah, 2009). The deployment of the traps followed a 3 × 3 factorial arrangement. The 3 × 3 factorial multiplied by the three blocks or replication gave twenty-seven experimental units for the sampled area (12,141 m^2). The 15 traps per block consisted of 5 Ecoman traps, 5 Bucket funnel traps and 5 traps and they were charged with ME polymeric gel. The traps were placed in semi-shaded and upwind parts of the canopy by 7 am on sampling days. The traps were left in the field for 3 hours each in the morning, afternoon and evening. Flies caught between 7 am and 10 am were designated as morning catches. Those caught between 12 noon and 3 pm were designated as afternoon catches and those caught between 4 pm and 7 pm were designated

as evening catches. The catches of each trap for each designated period were carefully emptied into a cage. The flies were provided with enzymatic yeast hydrolysate, sugar (three parts yeast: one part sugar) and distilled water soaked in cotton wool in a small vial inside the cage (Ekesi et al., 2007). Another cotton wool soaked in water was placed on top of each cage to keep catches hydrated and the cage humid. While on the field, each cage was labeled with the respective trap type and time of catch and placed under shade. At the end of the day, the catches were transported under a temperature condition of 20°C to the laboratory for further studies. The flies were monitored for 30 days under laboratory conditions (25±1°C; 75±5% RH; 12D:12L photo period) for survival or mortality data. Artificial diet (three-part yeast: one part sugar) was introduced to the fruit flies when needed and water was replaced or topped up. Fruit fly sampling was replicated three times over the periods of March, May and June 2019.

4.2.5 Taxonomy and identification of trap catches

The captured fruit flies were identified to the species level based on morphological characteristics using taxonomic keys developed by the African Fruit Fly Initiative (Ekesi & Billah, 2009). The flies were viewed under a dissecting microscope (GX Microscopes, GT Vision Ltd, Suffolk, UK) at a magnification of 20×. Non-tephritid flies were identified to order or family levels. Samples of the identified insects were deposited at the Radiation Entomology and Pest Management Center under the Biotechnology and Nuclear Agriculture Research Institute of the Ghana Atomic Energy Commission.

4.3 Data Analyses

The responses measured in this experiment were subjected to analysis of variance (ANOVA) or Fisher's test. Effect of trap type and time of day on the number of fruit fly catches and percentage survival were tested with ANOVA. Data were input into Microsoft Excel to generate nine samples for the trap type and time of day, representing the sample size (n) used in ANOVA for the single factor effect using GenStat software (GenStat, 2009), by selecting general treatment structure to run the 3 × 3 factorial experiment. Data were log-transformed to normalize the initial distribution of raw data collected for fruit fly catches and percentage survival of flies in traps before performing the ANOVA test. Correlation and regression analysis were performed between the trap catches and climatic data measured during the experiment.

4.4 Results

4.4.1 Response of *Bactrocera dorsalis* to trap types

The trap types showed significant differences for the trap catches ($df = 2, 15, F = 26.44, p \leq .001$). After the 3×3 factorial analysis, the single factor effect, i.e., trap types, revealed Ecoman to be efficient in catching larger numbers of *B. dorsalis* compared with Tephri and Bucket Funnel traps (Figure 4.3). However, the Tephri trap caught more *B. dorsalis* compared with Bucket funnel trap.

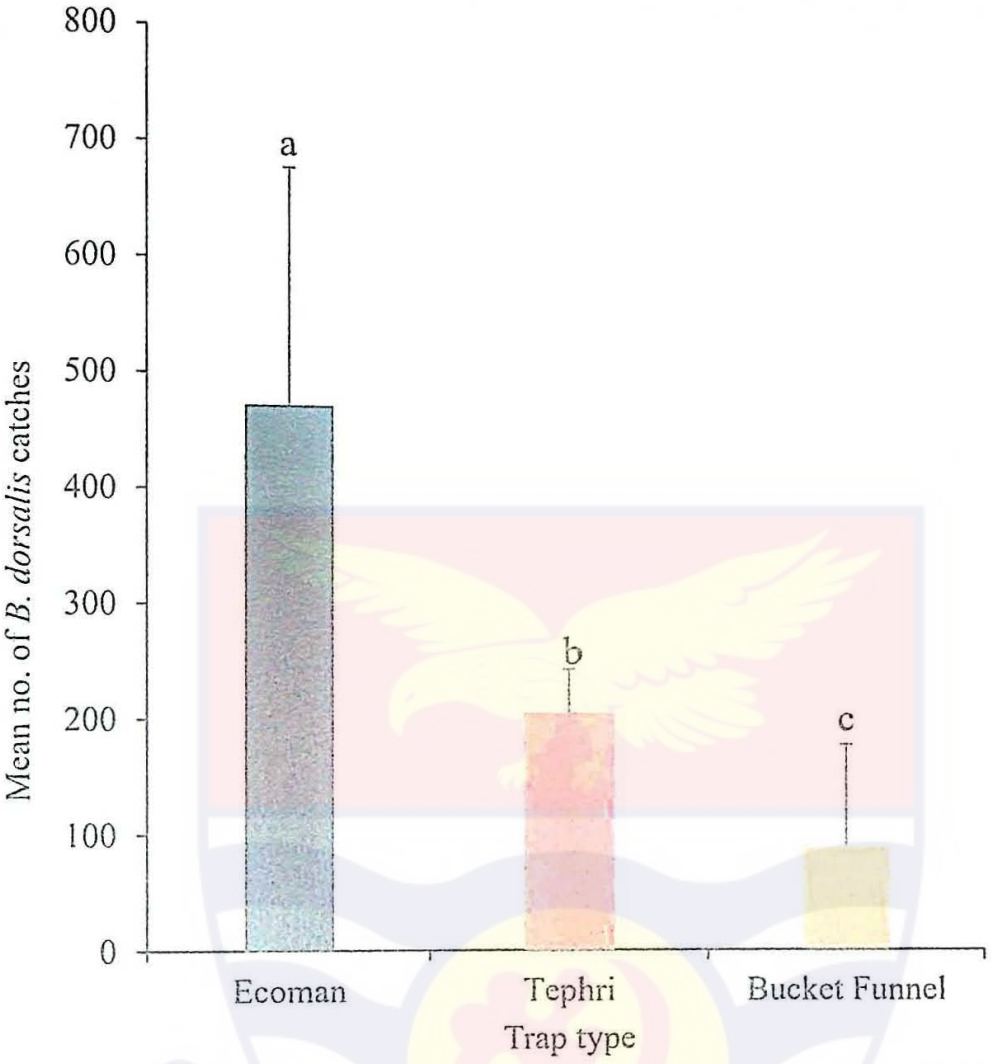

Figure 4. 3: Mean catches of *Bactrocera dorsalis* in three different trap types. Bars with different letters indicate significant differences at a Fisher's probability value of less than 0.001.

There were no significant differences in the survival of *B. dorsalis* in the three trap types under study (df = 2, 14, F = 0.08, p = .924) during a 24-hour study period. However, numerically more *B. dorsalis* survived in Ecoman followed by Bucket funnel trap and Tephri trap in that order (Figure 4.4).

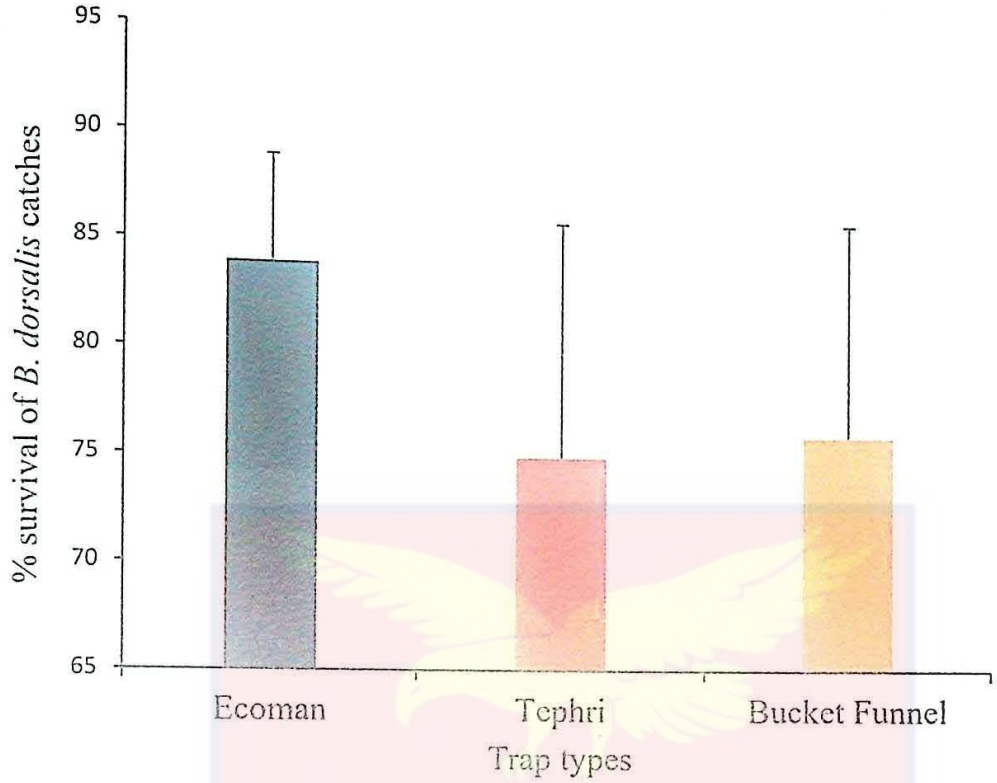

Figure 4. 4: Percentage survival of *Bactrocera dorsalis* in Ecoman, Tephri and Bucket funnel traps

In Ecoman traps, 69±10% of the *B. dorsalis* captured in the mornings survived. Of the number of *B. dorsalis* captured in the afternoon 88±8% survived whiles 95±2% of those captured in the evening survived. In Tephri trap, 73±17% of the *B. dorsalis* captured in the morning survived. In the afternoon captures, only 64±32% survived whiles the highest percentage survival in Tephri traps (87±10%) was observed in those captured in the evening. Ninety-two percent (92±5%) of the flies caught by the Bucket funnel trap in the evening survived, followed by afternoon (88±7%) and morning (49±25%) (Table 4.1). There were no significant differences in the percentage survival of *B. dorsalis* that were captured in the three trap types at the three different periods of the day after 24 hrs in the insectary (df =2 ,14, F = 0.10, p = .979). *Bactrocera dorsalis* catches in Ecoman at different periods of the day were not significantly different. In the Tephri traps as well, no significant differences were observed in the

percentage survival of *B. dorsalis* captured at different times of the day after 24 hrs in the insectary. Similarly, the captures by Bucket funnel trap showed no significant differences in percentage survival of *B. dorsalis* during the period of the day for the first 24 hrs. (Table 4.1).

Table 4. 1: Percentage Survival of *Bactrocera dorsalis* in three trap types after catches in 24 hrs on a mango plantation

Trap type	Time of Day	Mean survival (%) ± SE
Ecoman	Morning	69± 10[a]
	Afternoon	88± 8[a]
	Evening	95 ± 2[a]
Tephri	Morning	73 ± 17[a]
	Afternoon	64± 32[a]
	Evening	87 ± 10[a]
Bucket funnel	Morning	49± 25[a]
	Afternoon	88 ± 7[a]
	Evening	92± 5[a]

There were no significant differences among the trap types and period of the day (Fisher's probability value = 0.979). Means followed by the same letter within the column are not significantly different at 0.979

4.4.2 Interaction between trap catches and periods of the day

The 3×3 factorial analysis revealed that, there was significant differences in the interaction between the trap types and the periods of the day (df =4, 15, F= 6.69, $p < .003$). In the morning, Ecoman trap caught higher number of *B. dorsalis* compared with Tephri trap and Bucket funnel trap. Again, in the morning, the number of *B. dorsalis* caught by Tephri and Bucket funnel traps were not significantly different (Table 4.2). In the afternoon, there was no

significant differences in the mean catches by Ecoman, Tephri and Bucket funnel traps (Table 4.2). In the evening, Ecoman was efficient in catching larger numbers of *B. dorsalis* compared with Tephri and Bucket funnel traps. However, *B. dorsalis* caught by Tephri and Bucket funnel traps were not significantly different. (Table 4.2).

Table 4. 2: Trap catches of *Bactrocera dorsalis* at different periods of the day

	Period of Day		
Trap type	Morning (6am-9am) Mean ± SE	Afternoon (12pm-3pm) Mean ± SE	Evening (4pm-7pm) Mean ± SE
Ecoman	933 ± 546^{a}	126 ± 61^{bcd}	361 ± 187^{ab}
Tephri	231 ± 121^{bcd}	320 ± 239^{abc}	69 ± 45^{d}
Bucket Funnel	108 ± 59^{bcd}	125 ± 111^{cd}	38 ± 18^{d}

Means followed by the different letters within columns are significantly different at at $p < .005$ (Fisher's test).

4.4.3 Response of *Bactrocera dorsalis* to different period of the day

There were significant differences in the mean number of *B. dorsalis* caught at the different period ($df = 2, 15, F = 9.24, p < .002$). *Bactrocera dorsalis* caught in the morning was significantly higher compared to the catches in the afternoon and evening. However, the catches in the afternoon compared to those in the evening were not significantly different (Figure 4.5).

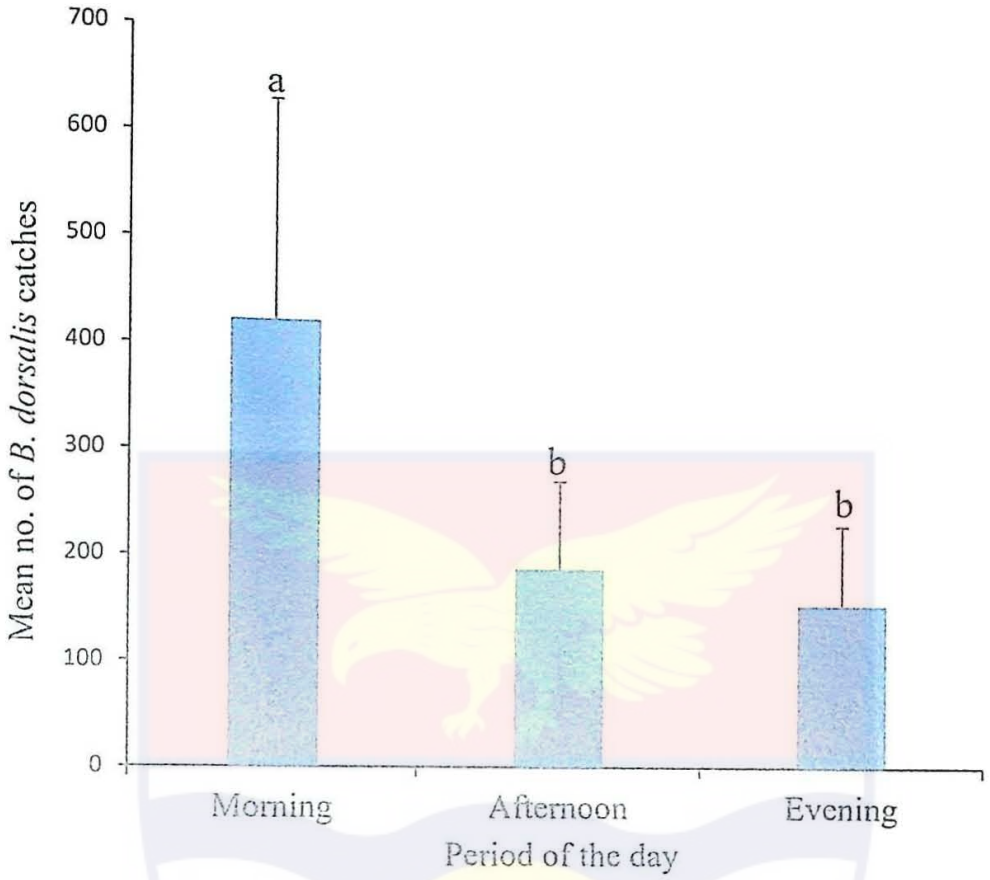

Figure 4. 5: Mean catches of *Bactrocera dorsalis* at different period of the day Means followed by the different letters within columns are significantly different at at $p < .005$ (Fisher's test).

There were significant differences in the percentage survival of *B. dorsalis* at different time of the day within a 24-hour period ($df = 2, 14$, $F = 8.83$, $p < .003$). Significantly higher number of *B. dorsalis* survived in the evening compared with morning. Similarly, significantly higher number of the flies survived in the traps set up in the afternoon compared with the morning. However, there was no significant difference between the flies caught in the afternoon and evening (Figure 4.6).

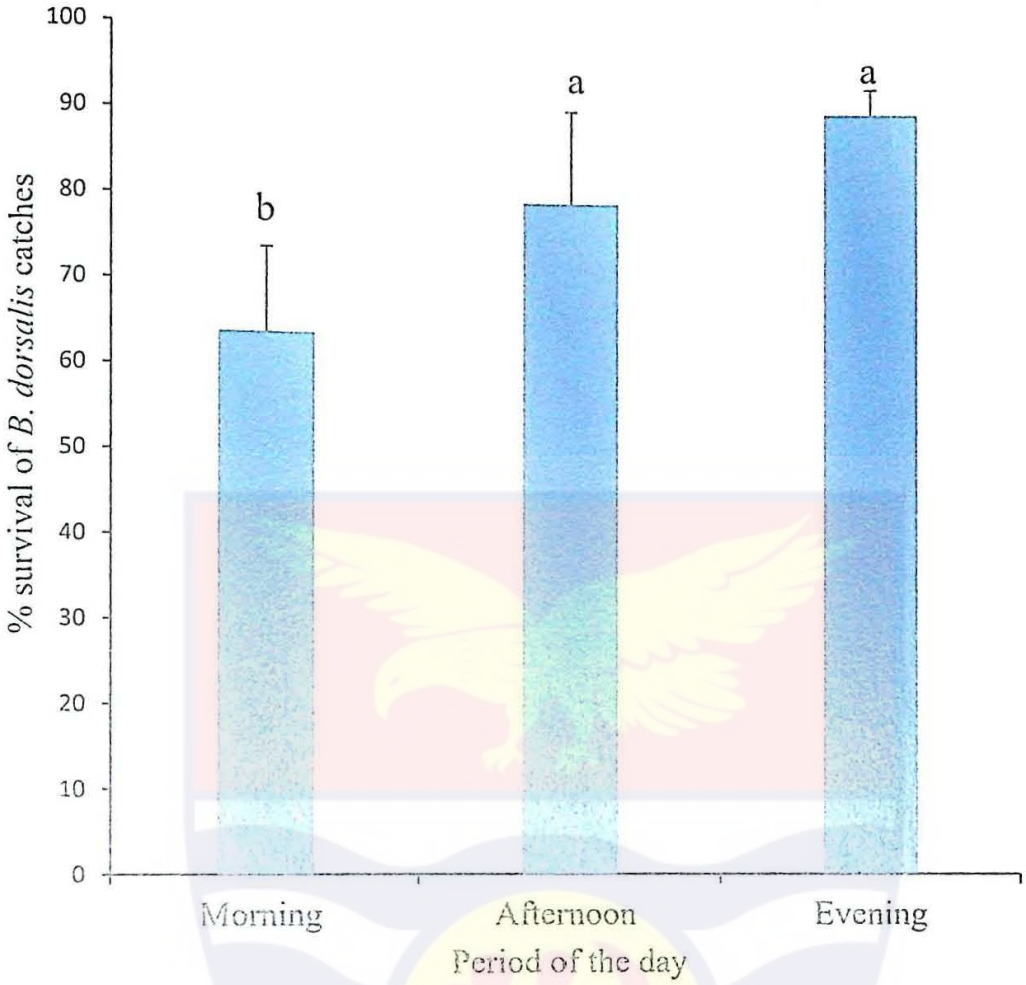

Figure 4. 6: Percentage survival of *Bactrocera dorsalis* in three different period of the day

Means followed by the different letters within columns are significantly different at at $p < .005$ (Fisher's test)

4.4.4 Interaction between trap catches and weather parameters

The average rainfall, temperature and relative humidity for the first trapping period in March 2019 were 45 mm, 31°C and 72% respectively. The average rainfall, temperature and relative humidity for the second trapping period in May 2019 were 145 mm, 30°C and 77% respectively. In June, the average rainfall, temperature and relative humidity for the third trapping study were 195 mm, 28°C and 80% respectively.

Correlation analyses between *B. dorsalis* trap catches and weather parameters showed that, temperature (r = 0.6638; p = .0668), relative humidity (r = 0.6192; p = .0754) and rainfall (r = 0.6182; p = .0760) were positively correlated with Ecoman traps. Generally, there were no significant differences in the relationship between the weather parameters and *B. dorsalis* catches in the Ecoman trap (Table 4.3). There was a strong positive correlation observed between the weather parameters and *B. dorsalis* catches for Tephri trap: Temperature (r = 0.7766; p < .0138), Relative humidity (r = 0.7220; p = .0281), Rainfall (r = 0.7196; p = .0138) (Table 4.3). A similar correlation was observed for the catches in the Bucket funnel traps. There was a strong and significant correlation between catches in the Bucket funnel traps and temperature (r=0.7286; p < .0404) as well as relative humidity (r=0.7001; p < .0354). On the contrary, the correlation between catches in the Bucket funnel traps and rainfall was not significant (r=0.6705; p = .0688) (Table 4.3).

Table 4. 3: Relationship between climatic factors (Temperature, Relative humidity and Rainfall) and *Bactrocera dorsalis* caught by Ecoman, Tephri and Bucket funnel traps

Trap type	Correlation	Temperature	Relative humidity	Rainfall
Ecoman	r	0.6338^{NS}	0.6192^{NS}	0.6182^{NS}
		F-value 4.7001 P-value 0.0668	F-value 4.3517 P-value 0.0754	F-value 4.330 P-value 0.0760
Tephri	r	0.7766^{S}	0.7220^{S}	0.7196^{S}
		F-value 10.6381 P-value 0.0138	F-value 7.6209 P-value 0.0281	F-value 7.5171 P-value 0.0289
Bucket funnel	r	0.7286^{S}	0.7001^{S}	0.6705^{NS}
		F-value 6.7886 P-value 0.0404	F-value 6.7623 P-value 0.0354	F-value 4.8993 P-value 0.0688

^{NS}-Non significant ^{S}-Significant

4.5 Discussion

4.5.1 Response of Bactrocera dorsalis to trap types

Traps and attractants may be used to monitor the population of adult fruit flies (JAEA, 2018). The selection of a trap for mass trapping is primarily determined by the type of attractant to be used, and the collection of flies in the field is determined by the combination of attractants and traps (IAEA, 2018). In the present study, adult *B. dorsalis* were collected at three different periods of the day (i.e., morning, afternoon and evening) in mango orchards using three different trap types with methyl eugenol as attractant. Methyl eugenol was

chosen because of the target pest. *Bactrocera dorsalis* collected in the evening with the baited traps had a higher survival rate for the first 24 hours after trapping compared to those collected in the morning and afternoon. This might be owing to the favourable weather conditions that prevailed throughout the evening. The evening has relatively low temperatures (28±1°C) and optimum humidity (77±5%) that might have aided the survival of the catches in the traps. The mean percentage survival of *B. dorsalis* catches in the Ecoman trap was 84%, followed by Bucket funnel trap (76%) and Tephri trap (74%). Motswagole et al. (2019) and Choi et al. (2020) reported 16.7°C to 34.9°C as possible climatic optimum temperature for the survival of flies during the time of capture. In this study, the temperature recorded in traps were at the optimum level which aid in the survival of the trap catches. Success in implementing a mass trapping strategy is dependent on the effectiveness of the traps and lures used (Cohen & Yuval, 2000). Because no deadly substance was introduced to the traps throughout this trial, the traps are not harmful to the environment (Manrakhan et al., 2017, 2021; Bali, 2021). Methyl eugenol is highly attractive but very specific in attracting fruit flies in the *Bactrocera* complex including *B. dorsalis*. In fact, earlier studies have shown that methyl eugenol is very efficient and effective in mass trapping *Bactrocera* species in mango orchards (Ishaq et al., 2004; Stonehouse et al., 2005; Jiji et al., 2009).

4.5.2 Response of *Bactrocera dorsalis* to period of the day

In all the three traps, more than 80% of *B. dorsalis* survived for the traps set in the evening. It is advisable to set the Ecoman trap in the evening for mass trapping of live *B. dorsalis* since it had the highest percentage survival of the flies. Our results are consistent with those of Siddiqui et al. (2003), who

discovered that fruit flies engaged in a diverse spectrum of diurnal activities. Control measures for this pest should be implemented in the early morning and late evening hours. These periods are the active periods of the fruit flies where they engage in all manner of activities like foraging, mating. This makes them available to be trapped. Kazi (1979) observed that fruit flies were most active between 10 and 11 a.m. and that adult flies spent most of the day sleeping on other plants in the proximity of cucurbit crops. The greatest number of adults were observed on these plants before 8 a.m. and after 5 p.m. The present observations are similar to those documented by Sarango et al. (2009) who suggested that *B. cucurbitae* (Coquillett) and *B. dorsalis* are active in the morning. A surge in activity was observed in *Bactrocera dorsalis* between 7:00 and 8:00 am. Rizk et al. (2014) also stated higher mean catches in Peach fruit flies (PFF) between 5 am and 7 am which is usually the mating activity period of Peach fruit flies.

4.5.3 Interaction between trap type and period of the day

This study has demonstrated that the population of *B. dorsalis* captured is affected by trap type and period of capture. The Ecoman trap captured and retained a large population of *B. dorsalis* in the mornings and evenings. The construction of the Ecoman fruit fly trap is such that the entry holes into the traps are spiral, preventing trapped flies from escaping. The Tephri trap employed in this research is a modified McPhail trap, in which entry holes are located around the topmost part of the yellow base's perimeter with an invaginated aperture located at the bottom of the traps. This design allows captured flies to escape if no killing agent is incorporated, making it unsuitable for trapping flies alive. The Bucket funnel trap has a wide space between the

upper yellow pane and the white bucket. This window allows trapped flies to easily escape, making it unsuitable for retaining the trapped flies. Earlier research has shown that varied trap designs, including different colours and forms, are necessary in order to achieve high effectiveness in fruit fly captures. The findings of this study confirm this hypothesis (Broughton & Rahman, 2017; Tadeo et al., 2017; Candia et al., 2019; Abu-Ragheef et al., 2020). Similarly, several authors have reported that Tephritid fruit fly traps vary in effectiveness depending on their size, colour, shape and the particular olfactory attractant used (Tadeo et al., 2017; Sikandar et al., 2017; Bajaj & Singh, 2017; Lasa et al., 2017; Manrakhan et al., 2017; Candia et al., 2019; Bali, 2021). The type of trap is important in mass trapping. Ecoman trap is good for collecting large numbers of *B. dorsalis* due to its trapping efficiency (Bawa et al., 2016). This trap, when used during the right time of the day can be used for mass trapping of live *B. dorsalis* for irradiation studies.

Stegeman et al. (1979) discovered that adult fruit flies were attracted to particular chemical lures earlier in the day in another investigation. Peak attractiveness and population occurred earlier in the day during the summer season than in the spring season. Although the Ecoman traps can retain the fruit flies very well, the retention of the flies in the trap should not be too long if one is interested in the live flies. Enough air might not reach the flies due to inadequate ventilation in the Ecoman traps. Traps should therefore be emptied into cages as soon as possible. Most of the catches in the afternoons by all traps were low. This could be because of the high temperature during that time of day and that *B. dorsalis* finds suitable refugia away from the heat of the sun.

4.5.4 Interaction between trap catches and weather parameters

All abiotic conditions substantially impact on the population of fruit flies captured using sex attractants, whether they are rising or decreasing. There was a positive relationship between the three trap types and environmental elements such as temperature, relative humidity, and rainfall, among others. According to this, an increase in temperature, relative humidity, and rainfall will also result in an increase in the fruit fly population. Ecoman trap catches have a non-significant positive correlation with climatic factors. Ecoman trap catches were not necessarily affected by an increase or decrease in temperature, relative humidity and rainfall. This could also account for the efficiency of the Ecoman trap in catching large populations of *B. dorsalis*. In another research, the amount of fruit flies collected using cuelure-baited traps was shown to be positively linked to all three abiotic parameters, namely temperature, humidity, and rainfall. These results were similar to those described above (Hasyim et al., 2008). Weather variations have a significant impact on the multiplication, growth, development, and dispersal of insects, as well as their population dynamics (Dhaliwal & Arora, 2001). Tephri traps on the other hand exhibited a strong positive correlation with all the climatic factors recorded. This validates the results of Khan et al. (2003), who discovered that weather conditions had a major influence on fruit fly population dynamics, with temperature and rainfall being the most critical factors affecting fruit fly population dynamics. For the Bucket funnel traps, both temperature and relative humidity had a significant positive correlation on trap catches except rainfall which had a positive but non-significant correlation. Patel et. al. (2013) and Bana et. al. (2017) found a positive link between temperature, relative humidity and rainfall and fruit fly

capture. Despite previous studies showing a nearly identical link between fruit fly infestation and meteorological variables, Adzim et al. (2016) found a negative association between *B. dorsalis* and rainfall and temperature in the coastal grassland region.

4.6 Conclusion

The three trap types evaluated had different shapes, colour and designs which made them unique in mass trapping of *B. dorsalis*.

- Survival was higher for *Bactrocera dorsalis* trapped in the evening with all the three trap types.
- On average, the survival rate of *B. dorsalis* in the traps is highest for Ecoman, followed by Bucket funnel and tephri trap.
- Ecoman trap has higher trapping efficiency compared with tephri trap and bucket funnel traps.
- Ecoman traps are therefore ideal for use in mass trapping of live *B. dorsalis* when conducting irradiation studies.
- Climatic factors influence the catches by the traps differently. Efforts should be made to set traps within areas of optimum temperature, relative humidity and rainfall to boost the efficiency of the traps in mass trapping.

CHAPTER FIVE

IRRADIATION STUDIES TO ESTABLISH AN OPTIMUM DOSE FOR STERILIZING *Bactrocera dorsalis* ADULT MALES

5.1 Introduction

The discharge of sterile insects is a species-specific, ecologically benign approach of insect management dubbed "birth control for insects." (Knipling, 1955). It entails the mass rearing of enormous numbers of target insects in an insectary, as well as the sterilisation of the males. These sterile males are then scattered throughout the contaminated regions, where they mate with wild females. The mating of a sterile male with a virgin wild female leads to the creation of non-fertile eggs, and the decrease of progeny results in the suppression or, in some cases, the local extermination of the wild population (Klassen, 2005). When using the Sterile Insect Technique (SIT), the capacity to mass-rear millions of sterile flies for release at the proper moment (when the pest population has not reached its peak) and across a large region is critical since the technique is designed to overwhelm the pest population (Tan, 2000). This results in a reduction in the reproductive capacity of the resident pest population, and eventually, the elimination of the pest population. Insect inundation technology is an ecologically friendly process that involves the successive discharge of sterile insects in a designated region. The released infertile males are in a competition with wild males for the right to mate with females in the wild. (Nation, 1974). For example, almost 1.5 billion flies were released in 1991 against *C. capitata* in the Kauai Coffee Plantation in Hawaii and that resulted in population suppression of 56%, compared with a control of no release (Vargas et al., 1994). Although, SIT is very effective, it could be

complex and expensive. Mass rearing is the highest contributor to the total cost of any SIT program. This high cost makes the application of SIT prohibitive to resource-poor countries, hence the need to explore alternative ways of applying the SIT under such conditions. One of the techniques that has the potential to eliminate the cost of mass rearing and hence reduce the overall cost of SIT is the Trap-Irradiate-Release (TIR)-Sterile Insect Technique (SIT). The Trap-Irradiate-Technique is a form of SIT that involves trapping wild males from a population, irradiating them with a specific dose of gamma radiations to cause sterility and subsequently releasing the sterile male flies into the wild to mate with wild females. Continuous trapping, irradiation, and release of sterile male flies will eventually bring the total population down to a minimum. In the early 1960s, Horber used the TIR-SIT strategy to eradicate the field cockchafer (*Melolontha vulgaris* F.) from 30 hectares of agricultural land in Switzerland (Horber, 1963).

One of the most challenging aspects of the SIT application is determining the radiation dosage, since the radiation doses used to induce reproductive sterility might differ across sexes and between species (Bakri et al., 2005; Williamson et al., 1985). In operations involving the release of sterile insects, the dosage employed to induce sterility is of critical relevance. Dosages that are too low result in insects that are not adequately sterile, whilst doses that are too high result in males that are poor competitors in mating with wild females as compared to wild males (Robinson et al., 2002). Optimizing the balance between somatic and reproductive fitness, as well as hereditary sterility, is consequently critical (Toledo et al., 2004). Though adequate information exists of the doses required for sterilizing males irradiated as pupae, information

of adult male irradiation is very scanty. This study was therefore set out to determine the optimum gamma radiation dose to cause sterility in adult male *Bactrocera dorsalis* for subsequent TIR research and the period of the year to collect large quantities of *B. dorsalis* for irradiation.

5.2 Materials and Methods

5.2.1 Study Location

The experiment was carried out at the laboratory of Radiation Entomology and Pest Management Center (REPMC) and Gamma Irradiation Facility (GIF) of Biotechnology and Nuclear Agriculture Research Institute (BNARI) under the Ghana Atomic Energy Commission (GAEC). GAEC is located about 20 km north of Accra (5°40′36.6″ N; 0°11′52.5″ W) and 76 m above sea level (Ewusie et al., 2010). Trapping studies were carried out in three commercial orchards namely Power of Trinity (POT) and Modestep Farm located in the Yilo Krobo district of the Eastern Region of Ghana and Enyonam farm in the Shai Osudoku district of the Greater Accra Region of Ghana (Figure 5.1) to determine the best period of the year to collect large numbers of *B. dorsalis* for irradiation studies. The study was carried out between February, 2018 and February, 2019 to cover the major and minor mango seasons in the Coastal Savanna agroecological zone. The climatic conditions of the orchards and types of vegetations cover have been previously described by Asare-Nuamah & Botchway (2019) and FAO (2005) respectively. Meteorological data for three variables, relative humidity (RH) at 15.00 h GMT, the maximum temperature at 09.00 h, and total monthly rainfall were obtained daily from a local meteorological station at one of the farms in the study area.

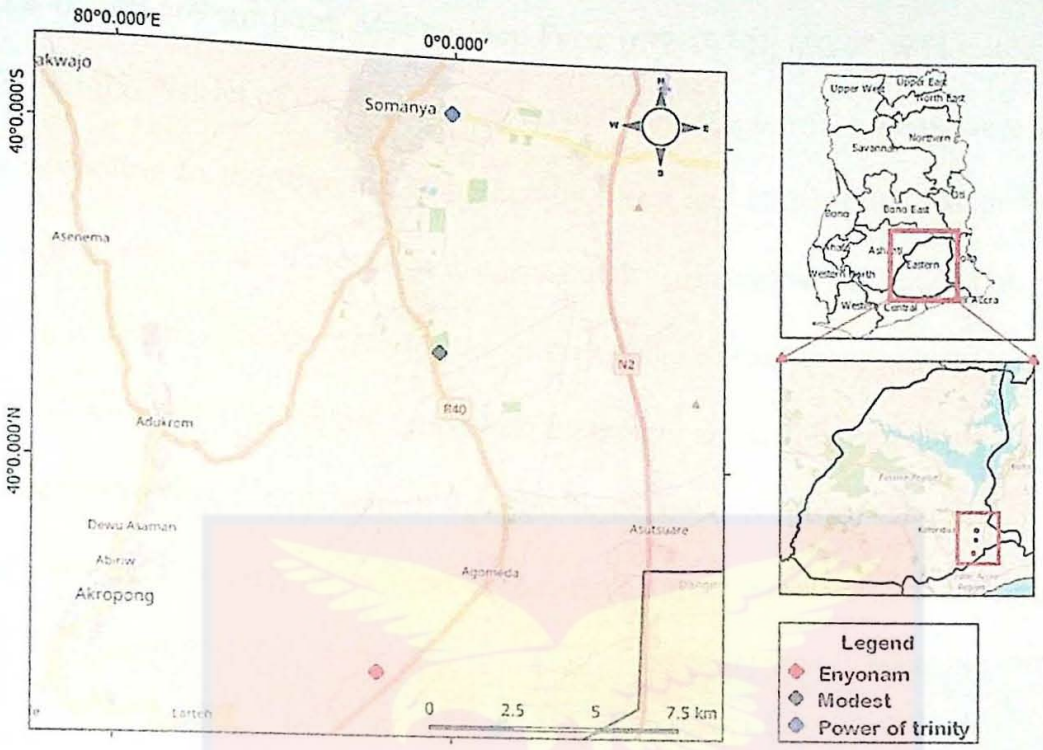

Figure 5. 1: Mango orchards for collecting year-round *Bactrocera dorsalis* flies in the SouthEastern mango enclave

5.2.2 Trap and attractant for collecting wild fruit flies

Ecoman trap (Ecoman Biotech Company, China) was used to collect live adult male *Bactrocera dorsalis*. Ecoman trap consists of a translucent white bottle of about 17 cm in height and width of 0.83 cm with a total volume of 404 cm^3 and a black cap of height, 0.65 cm and width, 0.70 cm. The Ecoman trap is designed in such a way that the black cap has 4 spiral entries with a hole each of 0.13 cm diameter, which opens into the translucent white base of the trap. The black cap has a plastic pin measuring 0.6 cm in length in the middle where attractants can be hanged. The outer top roof of the trap has a hook on which a thread can be attached to a tree. Once flies enter the trap through the spiral black cap, they are unable to escape. The parapheromone, methyl eugenol (ME), 2 g active ingredient per plug was used as an attractant in this study. The ME was sourced from Sentry Biologicals, Inc, USA. Fifteen Ecoman traps were set up

on a 12,140.569 m^2 farm, Modest Step Farm (6°2'19" N; 0°0'9" W) located at Yilo Krobo district of the Eastern Region of Ghana (Figure 5.1). Traps were set up according to the trapping technique by Ekesi and Billah (2009) with few modifications. Traps were set at distances of 50 cm apart and at heights of 1.5 m-2 m depending on the architecture of the mango trees. The traps were set up in such a way to avoid interference with each other and in the semi-shaded area of trees allowing flies to gain full access to the entry points of the traps. The traps were set up early in the morning around 6 am and emptied after one hour.

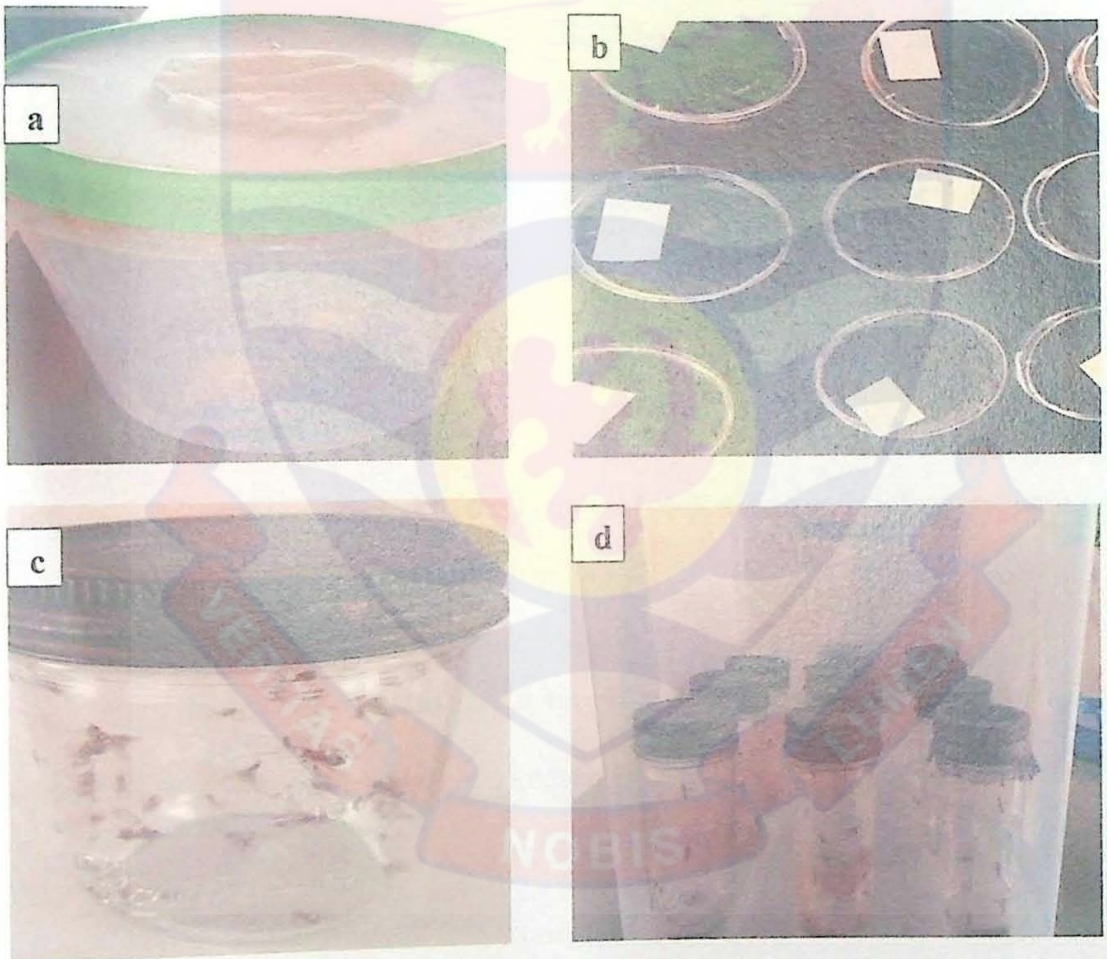

Figure 5. 2: Determination of the most efficient dose for sterilising *Bactrocera dorsalis*; a) Wild trapped flies being fed on yeast and sugar b) Eggs arranged in a petri dish for hatchability test c) Emergence glass jar with unirradiated male flies d) plastic basket with emergence bottle for irradiation.

5.2.3 Collection and preparation of flies for irradiation

Trapped flies were carefully emptied into four 6L-transparent plastic bucket cages and transported carefully under temperature conditions of 20°C to the REPMC laboratory for holding and stabilized before irradiation. In the laboratory, the adult wild *B. dorsalis* were maintained under 12-h photoperiod, 25±2°C temperature, 70±5% RH and fed with food made up of 3-part yeast: 1-part sugar and water soaked in cotton wool. The top net cover of the cage was also covered with cotton wool that had been soaked in water, in order to keep the flies hydrated. The flies were kept for 7 days to enable them acclimatize to the new environment (insectary) before being exposed to gamma irradiation.

Adult male *B. dorsalis* were stabilized in the laboratory for 7 days, they were transferred into glass jars of volume 250 ml (Figure 5.2*c*) with a metal cover having a wire mesh to allow for ventilation. Fifty (50) flies were transferred into each of 11 glass jars. The glass jars were placed in a bucket cage (Figure 5.2*d*) and transported to the Gamma Irradiation Facility for irradiation.

5.2.4 Dose-response calculation

Irradiation was carried out at the GIF, which uses a Cobalt 60 (^{60}Co) source with a strength of approximately 15.3 KCi. In a preliminary dose mapping irradiation, 50 male *B. dorsalis* held in a glass jar (Figure 5.2*c*) were placed on a 62 cm high stool at 90 cm from the cobalt 60 (^{60}Co) source rack. Ethanol chlorobenzene (ECB) Dosimeters were placed inside the glass jar for determination of actual dose delivered. To guarantee homogeneous distribution of the dosage provided under the same circumstances, the glass jar containing the flies and dosimeters was rotated 180° at half the processing time. After the irradiation time, the ECB dosimeters were withdrawn from the jar and the

absorbed dosage was calculated using a calibrated High-Frequency Dosimeter System (Model 2131, version 2.5, Sensolab Ltd, Göd, Hungary). The experimental samples were then individually subjected to the same conditions described above to deliver calculated doses of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 Gy at a dose rate of 160.4 Gy/hr. The adult males in the control treatment were exposed to the identical handling techniques as in the experimental treatment, but no irradiation was administered. The entire experiment was replicated three times.

5.2.5 Mating and fertility studies

Irradiated adult male flies were carefully transferred into adult holding cages labelled with the respective doses and fed with artificial diet and water (Figure 5.2a). The irradiated flies were allowed to stabilize (12D:12L photo period, 25±2°C temperature, 70±5% RH) for 3 days after which matured laboratory-reared virgin females were presented to them for mating at a ratio of 1:1. The set up was held in the insectary for 7 days to allow mating to occur.

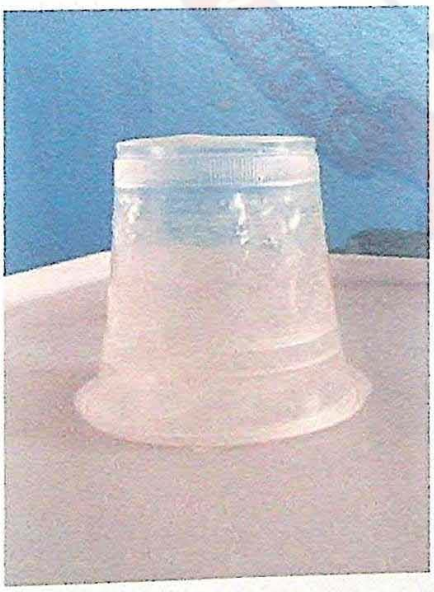

Figure 5. 3: Oviposition cups for harvesting eggs to determine percentage hatchability.

Sterilized oviposition cups (Figure 5.3) were placed in each of the eleven cages to harvest eggs from the females. The oviposition cup (470 ml) is a transparent plastic cup with the body and cover perforated by a 1mm needle to allow the ovipositor of the adult female to be inserted to lay eggs. The inner surface of each oviposition cup was sandpapered to make it rough to allow the oviposition attractant and eggs to stick to it. Mango juice was applied to the interior portion of the cup to serve as an oviposition attractant. The oviposition cups were left in the cages for 24 hours to enable enough eggs to be laid for the hatchability test.

To determine hatchability, a sample of 100 eggs was taken from each irradiated dose and with a small camel-hair brush, spread on moist denim cloth in a Petri dish. Four lines of eggs, each consisting of 25 eggs were spread on the moist denim cloth (Figure 5.4). After 2-3 days, the number of unhatched eggs was counted under a dissecting microscope (GX Microscopes, GT Vision Ltd, Suffolk, UK) at a magnification of 20× and the percentage of egg hatch was calculated (Equation 1). Hatched eggs look more transparent than the unhatched ones. The unhatched eggs look whitish in coloration (Figure 5.4a).

$$\%\text{Hatchability} = \frac{\text{Total number of hatched eggs}}{\text{Total number of egg set}} \text{x}100\%. \qquad (1)$$

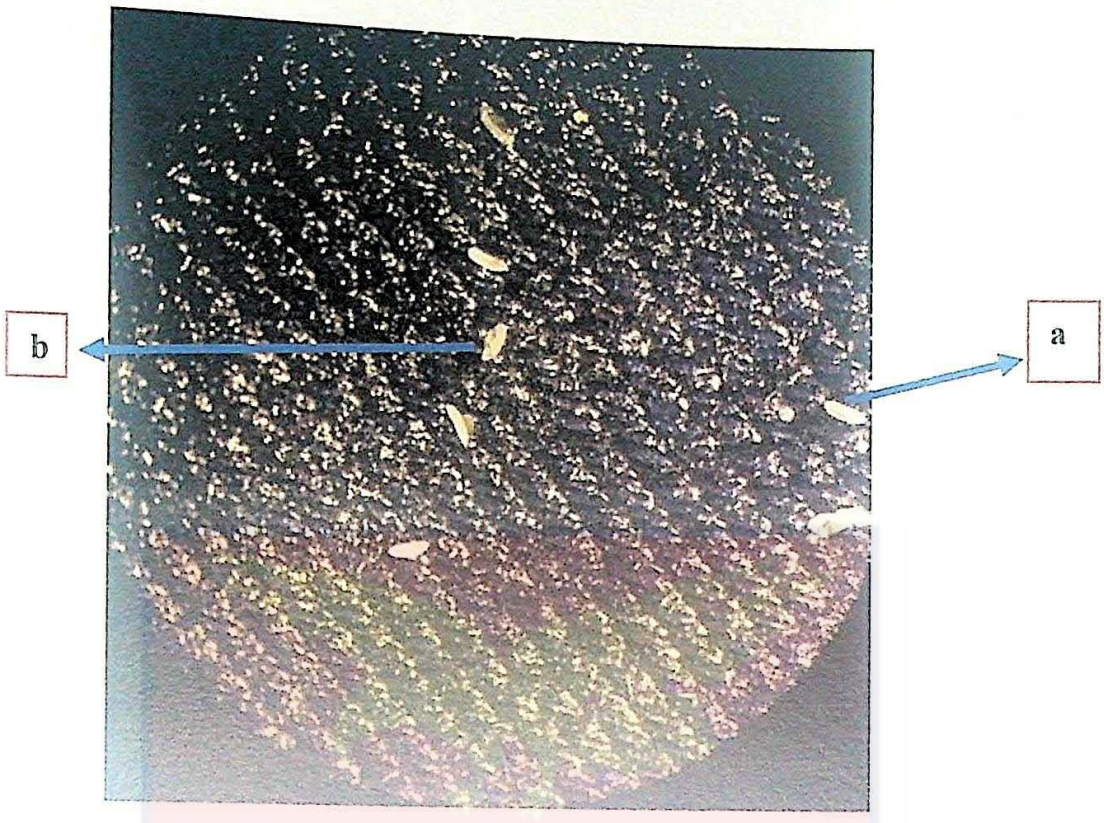

Figure 5. 4: Eggs of *Bactrocera dorsalis*; a) unhatched, b) hatched.

5.2.6 Trap layout and attractant

Trapping was carried out during the fruiting successive mango fruiting seasons in 2018. The trap used was Ecoman (Ecoman Biotech Company, China) and was baited with methyl eugenol (ME). The parapheromone methyl eugenol (1,2-dimethoxy-4-(prop-2-en-1-yl) benzene) (Scentry Biologicals) captures a large number of species of the genus *Bactrocera* (including *B. dorsalis*, *B. zonata*, *B. carambolae*, *B. philippinensis* and *B. musae*). Polymeric gels were used to release the attractant. The bottom of the trap was coated with a thin layer of Dimethyl 2, 2-DichloroVinyl Phosphate (DDVP), which served as a poison for any insects that stumbled into it. The trapping layout used was the technique described by Ekesi & Billah (2009). Each farm has 5 Ecoman traps deployed randomly. In all, 15 Ecoman traps were deployed, 15 ME attractants and 15 DDVP strips. Traps were examined for fruit flies and emptied on a weekly basis.

Specimens collected in traps were emptied in plastic vials which were labelled by farm and date and preserved in 70% alcohol. Attractants were replaced after 4 weeks. All Dichlorvos strips were replaced after 4 weeks.

5.2.7 Catches and identification of fly

Flies collected were transported to the laboratories of REPMC, where they were stored in a dark room to avoid discoloration of the catches. Identification of the catches were done under a dissecting microscope (GX Microscopes, GT Vision Ltd, Suffolk, UK) at a magnification 20×. The flies were identified to species at the REPMC laboratories using morphological characters as per published keys (Billah et al., 2009, De Meyer, 1996, 1998, 2000; De Meyer & Copeland, 2005; De Meyer & Freidberg, 2006; White, 2006). Voucher specimens of identified fruit flies were deposited at the laboratories of REPMC.

5.3 Data analyses

ANOVA was conducted to compare the different irradiation doses and percentage eggs hatched. Thus, F-test analysis was performed on all raw data summarized for parameters measured during the study. The least significant difference (LSD) test at a probability level of 5%, was used to separate means. All analysis were performed using one-way ANOVA described in the GenStats statistical software, 12th edition. Log-dose-probit analysis was used at a confidence level of 95% to calculate the Lethal Dose (LD-95) for eggs not to hatch using SPSS version 26. To make comparisons across locations easier, counts of flies per trap per day (F/T/D) (IAEA, 2003) were used to indicate relative fly abundance.

5.4 Results

5.4.1 Determination of optimum radiation dose for sterilizing adult male *Bactrocera dorsalis*

Generally, hatchability of the eggs laid by the female decreased with increasing irradiation doses except for the control (0Gy) which recorded an increased hatchability of 81±4.93%. This was followed by the 10Gy with percentage hatchability of 48±8.50. Next is 20Gy and 30Gy recording percentage hatchabilities of 32±8.33 and 28±2.03 respectively. Irradiation of adult males at 40Gy and 50Gy resulted in hatchabilities of 21±0.88% and 9±0.58% respectively. Irradiation doses at 60Gy, 70Gy, 80Gy, 90Gy and 100Gy resulted in decreased hatchabilities of 9±2.60%, 3±1.76%, 1±0.67%, 2±0.88% and 1±0.33% respectively (Table 5.1). Analysis of Variance (ANOVA) shows that there was significant difference (df = 10, 20, F = 40.21, $p < .001$) in the percentage hatchability between the 0Gy (control) and the rest of the irradiation doses (Table 5.1). There was a significant difference (df = 10, 20, F = 40.21, $p < .001$) in percentage hatchability between the 10Gy and 20Gy. There was no significant difference ($p > .05$) in percentage hatchability between the rest of the irradiation doses (Table 5.1)

Table 5. 1: Percentage hatchability of *Bactrocera dorsalis* at different irradiation doses

Dose (Gy)	Hatchability (%) ± SE
Control (0)	81 ± 4.93[a]
10	48 ± 8.50[b]
20	32 ± 8.33[c]
30	28 ± 2.03[c]
40	21 ±0.88[cd]
50	9 ± 0.58[de]
60	9 ± 2.60[e]
70	3 ± 1.76[e]
80	1 ± 0.67[e]
90	2 ± 0.88[e]
100	1 ± 0.33[e]

Means having the same letters under the same column are not significantly different at $p < .05$.

The mean number of percentage eggs hatched generally decreases with increasing doses. The equation for the regression line was 1 (r^2 =0.903; Figure 5.5). There was strong negative correlation between the irradiation dose and percentage hatchability ($r^2 = 0.903, p < .028$; Figure 5.5). From probit analysis, the effective dose that can cause 5% of eggs to hatch was 70Gy. An LD_{95} of 72.490Gy (95% CI: 56.4–106.4)Gy irradiation dose was calculated for unhatchability.

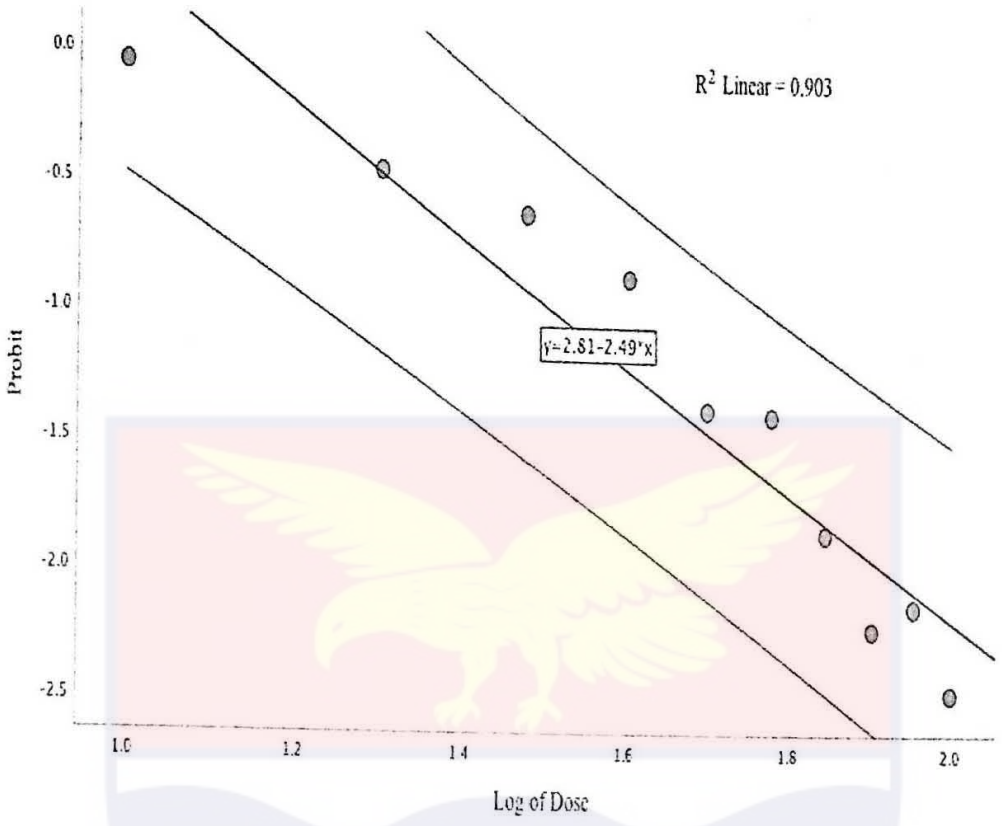

Figure 5. 5: Effect of irradiation dose on the egg hatchability of *Bactrocera dorsalis*

5.4.2 Fecundity and fertility of non-irradiated adult females mated with irradiated males

The number of eggs laid per female *Bactrocera dorsalis* with different irradiation doses was quite high. The highest mean number of eggs laid per female was 75.65±4.16 when males were irradiated at 70Gy. Irradiation at 0Gy produces a mean of 73.60 ±13.40 eggs per female. Irradiation at 80Gy produces 54.40±7.07 mean number of eggs laid per female (Table 2). There was however, no significant difference (df = 6, 13, F = 0.31, p = .913) in the number of eggs laid per female between the irradiation doses (0, 50, 60, 70, 80, 90, 100Gy). Irradiation at 0Gy produces the highest percentage hatchability (80.10±5.32). The lowest hatchability was 0.80±0.20% and 0.80±0.40% when males were

irradiated at 80Gy and 90Gy respectively. There were significant differences (df = 6, 13, F = 118.22, $p < .001$) in the percentage hatchability between 0Gy and the rest of the irradiated doses (50, 60, 70, 80, 90 and 100Gy) (Table 5.2)

Table 5. 2: Mean number of eggs laid per female and % hatchability of male *Bactrocera dorsalis* irradiated at different irradiation doses.

Dose (Gy)	No. of Eggs/Female	Hatchability (%)
Control	73.60 ± 13.40^{a}	80.10 ± 5.32^{a}
60	63.10 ± 13.90^{a}	4.60 ± 1.40^{b}
70	75.65 ± 4.16^{a}	1.40 ± 1.21^{b}
80	54.40 ± 7.07^{a}	0.80 ± 0.20^{b}
90	67.15 ± 5.37^{a}	0.80 ± 0.40^{b}
100	61.10 ± 0.50^{a}	4.60 ± 1.41^{b}

Mean values (±SE) within a column followed by the same letter are not significantly different ($p < .05$).

5.4.3 Time of trapping for irradiation studies

The flies per trap per day (FTD) at the end of the minor mango season (February) was low. The FTD recorded at this period was lower than 50. Relative fly density began to rise around April to June 2018 which marks the beginning of the major mango season. During these periods 138, 230 flies per trap per day were recorded for April and June 2018 respectively (Figure 5.6). The highest peak of captured flies was recorded in July 2018. In July 2018, 319 flies per trap per day were recorded. From the period in August 2018 to January 2019, which marks the minor mango season, the FTDs declined during these periods (Figure 5.6).

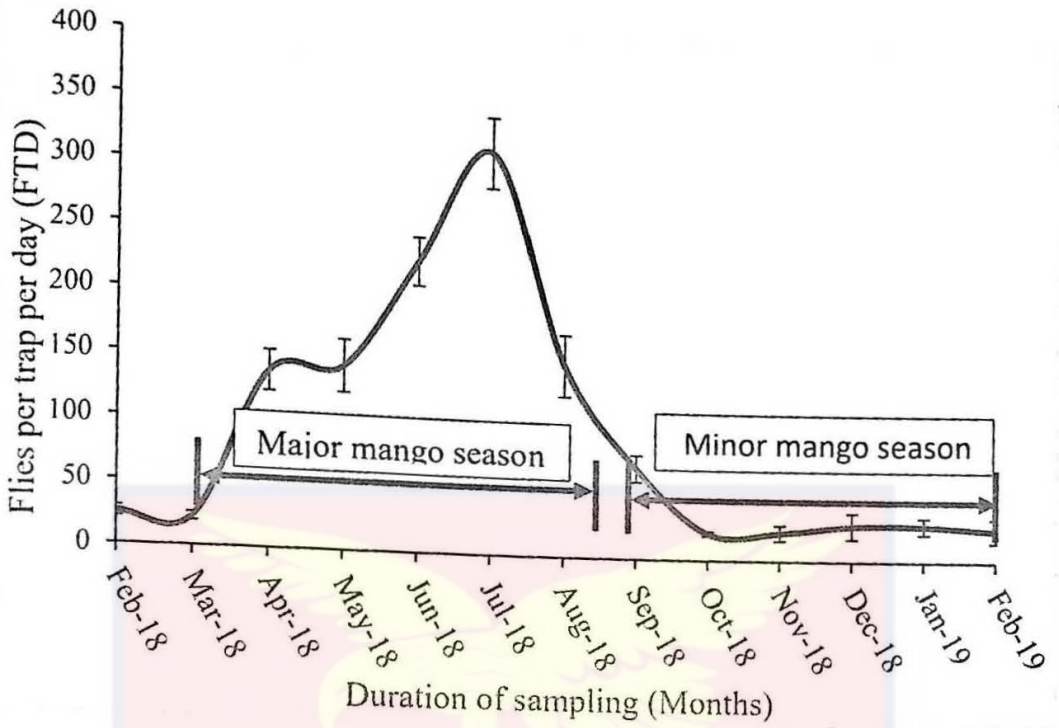

Figure 5. 6: Male *Bactrocera dorsalis* caught by Methyl eugenol traps between February 2018 to January 2019 within SouthEastern mango enclaves

5.5 Discussion

5.5.1 Determination of optimum irradiation dose for adult male *Bactrocera dorsalis* sterilization

The sterile insect technique is a major tool deployed to control tephritid fruit flies. The technique makes it easier to eradicate flies on an area-wide basis. In this study, the Trap-Irradiate-Release technique is being developed to see how effective and economical it can be in controlling *B. dorsalis*. In investigating the most suitable dose to sterilize adult *B. dorsalis*, adult males were exposed to irradiation doses from 10-100Gy. The percentage of eggs hatched after 72 hours decrease with increasing doses. To determine the most appropriate dose to administer to sterilize the adult males, probit analysis was carried out. The minimum dose that gave 5% hatchability was 70Gy which further decreases up to 100Gy. Dipteran groups get distinct sterility doses from

ionising irradiation, and these doses differ from one another (Bakri et al., 2005). According to Calkins and Parker (2005), sterility will rise as irradiation doses increase. Sensitivity of tephritid flies is moderate when compared with other dipteran groups, and the average dosage for sterilising this group is 65Gy. To establish sterility, two species, *B. philipinensis* and *C. capitata*, needed 100Gy (Bakri & Hendrich, 2002). Even though these doses (70-100Gy) achieved 95% sterility; it is not so different from other authors whose range is between 70-120Gy (Ohinata et. al., 1977; Nation et. al., 1995; Allinghi et. al., 2007; Younes et. al., 2009). Mating irradiated males with non-irradiated females of *B. zonata* did not decrease egg production, but did diminish hatchability, according to Mahmoud & Barta (2011). With a minimal dosage of 10Gy, 46-48 percent of eggs hatched, and the proportion of eggs hatched decreased even more as the dose increased.

Nasution et. al. (2018) and Klassen (2005) elaborated on the processes that results in gamma irradiation causing a male fruit fly to lose its fertility at a certain developmental stage of the fruit flies (Pupal stage). It is believed that 48 hours to the emergence of the adult from the pupal stage, germ cells are still actively dividing. The presence of irradiation disrupts the presently active process of cell division, causing cell damage and disrupting testicular development (Fletcher & Giannakakis, 1973). Sterility is caused by defective sperm cells being generated. Additionally, receiving high doses of gamma radiation may impair movement of sperm in an attempt to prevent fertilisation of an egg. If gamma rays strike the nucleus of a cell, they are likely to produce a deadly dominant mutation in the DNA base pair. Late in the pupal or early adult stages of many holometabolous species is a favourable period for

irradiation because germ tissues have developed (Anwar et al., 1971, Ohinata et al., 1971, 1977, 1978).

5.5.2 Fecundity and fertility of non-irradiated adult females mated with irradiated males

When non-irradiated females were mated with irradiated adult males, fecundity tests revealed that irradiation dosages had no effect on fertility. The fecundity of non-irradiated female fruit flies mated with non-irradiated male fruit flies were significantly different from those that were mated with irradiated adult males. Fruit fly egg production was unaffected by the degree of irradiation dosage during mating between an irradiated adult male fruit fly and a non-irradiated female fruit fly (Nasution et. al., 2018; Collins & Taylor, 2011). There was no statistically significant difference in the percentage mean fertility of non-irradiated males and those irradiated at 25 and 50 Gy, according to the findings of Ogaugwu et al (2012). However, egg laying rate showed a significant difference between females mated with irradiated males and females mated with non-irradiated males. For *B. zonata*, the same was claimed by Mahmoud & Barta (2011). According to Zahran et al. (2013), when non-irradiated female *B. zonata* eggs were mated with irradiated males at doses of 10, 30, 50, 70, and 90 Gy, the quantity of non-irradiated female *B. zonata* eggs was decreased. Fecundity in *B. dorsalis* is still fairly high, but it differs from fecundity in non-irradiated females (0Gy).

5.5.3 Time of trapping for irradiation studies

The major season lasted from March to August 2018. There were relatively high peaks within June and July (Vayssières et al., 2014) which coincides with the maturity and ripening of mango fruits. The high availability of resources (fruit) makes it easier for the high numbers of fruit flies captured during the period (Nboyine et al., 2012). This long window where there is the availability of fruit flies peak could be targeted to collect large numbers of *B. dorsalis* for irradiation. Narayanan & Batra (1960), Jhala et al. (1989) and Kumar et al. (1997) made similar observations to findings from previous studies e.g., where high populations of fruit flies were found to coincide with ripening and harvesting of fruits. The minor mango season which lasted from August to February 2018 has low populations of *B. dorsalis*. During the minor mango season, the production of mango was low. This was due to poor flowering and therefore mango production was greatly affected. This could be a major reason why low fly densities were recorded in the coastal savanna agro-ecological zone. The low numbers during the minor season can be targeted and irradiated flies can be released during this time to crash the population before the onset of the major mango season.

5.6 Conclusion

- The optimum irradiation dose to cause 5% egg hatchability is 70Gy.
- The higher the radiation doses, the lesser the percentage hatchability.
- Irradiation doses did not significantly affect fecundity.
- Low numbers of *B. dorsalis* were recorded at the minor mango season compared with the major mango season.

- The months of June and July have recorded higher numbers of *B. dorsalis* and this period can be targeted to collect large numbers of adult *B. dorsalis* for irradiation and releases during the minor mango seasons to manage the pest.

CHAPTER SIX

PRE-EXPOSURE OF ADULT MALE *Bactrocera dorsalis* Hendel TO METHYL EUGENOL BAITED TRAP

6.1 Introduction

Bactrocera dorsalis Hendel, sometimes known as the oriental fruit fly, is an Asian pest. It is very invasive, polyphagous, and harms a broad range of fruits and vegetables (Duyck et al., 2004; Clark et al., 2005). The fly was accidentally discovered in Kenya in 2003 while conducting standard fruit fly monitoring (Lux et al., 2003b) and 2005 in Ghana (Billah et al., 2006). Since its discovery, the insect has swiftly spread over the continent, wreaking havoc on huge commercial orchards as well as small-scale fruit-growing operations (Ekesi et al., 2006; Goergen et al., 2011; Vayssières et al., 2009). It is displacing native tephritids such as *Ceratitis cosyra* (Walker) and *C. capitata* (Wiedermann) (Lux et al., 2003b). Female *Bactrocera dorsalis* lay between 1200 and 1500 eggs throughout the course of their lives (Weems et al., 2012), making it an excellent dispersion candidate (Chen et al., 2015).

It is vital to use trapping technologies based on olfactory attractants in order to monitor and regulate *B. dorsalis* population growth. Methyl Eugenol (ME) (1, 2-dimethoxy-4-(2-propenyl) benzene), a particularly strong phytochemical lure for *B. dorsalis* males, has been used successfully for monitoring and eliminating adult males within *B. dorsalis* populations all over the globe, including in the United States (Liu et al., 2017; Shelly, 2017). It has been hypothesised in a previous study that male *B. dorsalis* are highly attracted to ME because it considerably boosts their mating performance and competitiveness (Shelly, 2010).

Chemicals consumed are either sequestered unaltered or converted into derivative chemicals, which are subsequently stored in male pheromone glands and released as pheromonal components that increase male mating competitiveness (Shelly & Dewire, 1994; Tan & Nishida, 1996; Khoo & Tan, 2000; Wee et al., 2007; Kumaran et al., 2013). Males which are provided ME attract much greater numbers of conspecific males than males who are not given ME (Diego et al., 2018). Methyl eugenol is a known powerful attractant of *B. dorsalis* males (Steiner, 1952; Steiner et al., 1965). In *B. dorsalis*, it serves as a precursor to the male sex pheromone and boosts the pheromone's attraction to females (Tan & Nishida, 1996; Shelly et al., 2000; Shelly, 2001). However, there is lack of knowledge concerning the effect of pre-exposure of *B. dorsalis* to ME and subsequent trapping using ME-baited traps in the wild. Therefore, this study sought to investigate the behavioural response of males pre-exposed to methyl eugenol and capture by ME-baited traps. This is important to determine whether males trapped with ME-baited traps for irradiation in the laboratory would be re-captured by ME-baited traps in the field and/or still respond to ME pheromones secreted by wild female. Findings from this study will have a significant impact on the effectiveness of TIR/SIT programme.

6.2 Materials and Methods

6.2.1 Study Location

The study was carried out at Enyonam Farm (5°56'59" N; 0°1'10" W) and Star Farm (5°59'4" N; 0°0'43" W) in the Shai Osudoku District of the Greater Accra Region; Modest Step Farm (6°2'19" N; 0°0'9" W) and Divine Field Farm (6°1'48" N; 0°0'9" E) in the Yilo Krobo district of the Eastern

Region of Ghana. The study area lies within the Coastal Savannah agro-ecological zone of Ghana with a humid climate as described earlier in Chapter Three.

6.2.2 Trap type for capturing fruit flies

Ecoman green trap (Ecoman Biotech Company, China) was used to collect live *Bactrocera dorsalis* males. The Ecoman trap and its functionality has been described in earlier chapters. The attractant used in this experiment was the parapheromone methyl eugenol (Scentry Biologicals, Inc, Billings, Montana, USA) as described in chapter 3.

Larvae from the initial stock were reared on banana and made to pupate in moist and sterilized fine sand. Emerged adults were separated immediately based on sex to avoid premature mating. Adults were fed on artificial diet (3:1 sugar/hydrolysed yeast) and water *ad libitum* for ca. 100 generations. Only adults at sexual maturity of 10–12 days old were used for experiments. They were reared under laboratory conditions of 25°C temperature, 65 ± 5% RH and 12L:12D photoperiod at the Radiation Entomology and Pest Management Center of the Ghana Atomic Energy Commission.

6.2.3 Methyl eugenol feeding

An amount of 4.64 g of ME (Scentry Biologicals, Inc, Billings, MT, USA) was placed onto a petri dish inside a 6L-cage containing unirradiated males to freely feed for 24 hrs following Wong et al. (1989).

6.2.4 Screen house Experiment

To investigate the behavioural response of pre-exposed and unexposed flies to ME-baited traps, the flies were marked with two different fluoresecent dyes, pink and green (Fujian Win-Mecode International Trading Co., Ltd, Fujian, China, 0.008g each; Figure 6.1*a* and *b*).

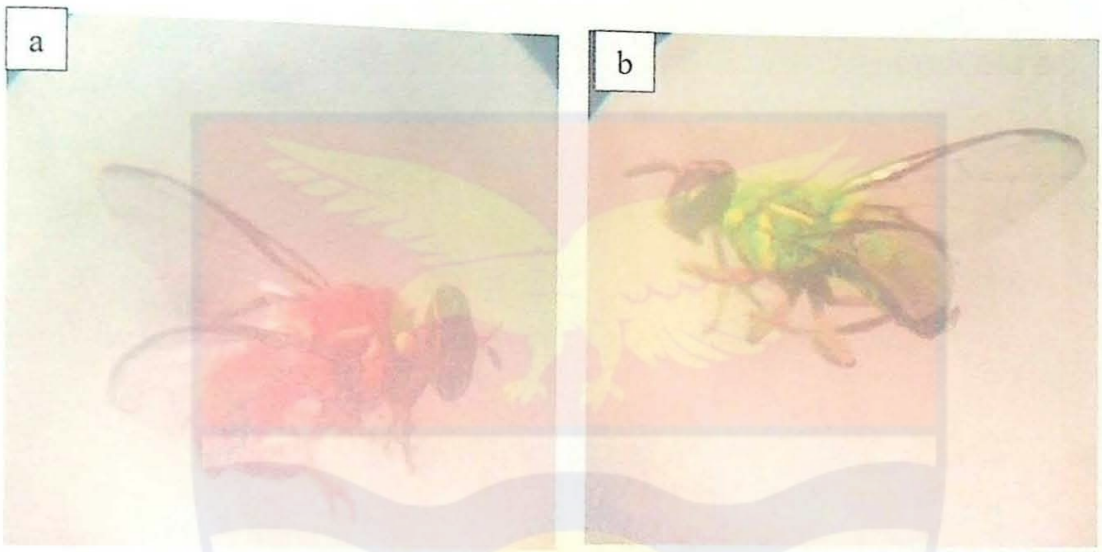

Figure 6.1: Adult male *Bactrocera dorsalis* stained by fluorescent dyes a) Pink dye b) Green dye

Fifty (50) marked and pre-exposed unirradiated laboratory-reared male *B. dorsalis* and fifty (50) marked and un-exposed unirradiated laboratory-reared *B. dorsalis* were placed in an emergence bottle separately and released from a corner of a screen house with asymmetrical plastic roof and a cemented floor area of 7.2 m^2 with a mesh netting surrounding the structure. The front chamber has an area of 1.35 m^2. The door measures 0.69m x 0.9m. The screen house conditions measured were 26 ± 2 °C, 70 ± 5 % RH and 12D: 12L and it is located at Radiation Entomology and Pest Management Center (REPMC (Figure 6.2). A Methyl Eugenol baited Ecoman trap was hung at the center of the roof of the screen house. The release points were alternated between the four corners of the screen house and replicated twice per corner. The dye used

to mark pre-exposed and unexposed flies was also alternated. The number of *B. dorsalis* males caught by the ME baited trap was recorded after 6, 12 and 24 hours. The total catches after 24 hours were expressed in percentages and used for analysis.

Figure 6. 2: Screen house for testing the attraction of adult male *Bactrocera dorsalis* to methyl eugenol.

6.2.5 On-Farm Experiment

Two hundred and fifty (250) marked and pre-exposed unirradiated laboratory-reared *B. dorsalis* males and two hundred and fifty (250) marked and un-exposed unirradiated laboratory-reared *B. dorsalis* males were released from the centre of a mango orchard. Ecoman traps set at distances of 10 m, 20 m, 30 m 40 m and 50 m along a radial transect (Figure 6.3). The number of *B. dorsalis* caught in the traps after forty-eight (48) hours was recorded. This was repeated in three (3) other orchards to get four independent replications. The total catches after 48 hours were expressed in percentages as recovery rate.

Figure 6. 3: Setup of field trapping experiment using Ecoman trap

6.3 Data analyses

The proportion of pre-exposed and un-exposed *B. dorsalis* that were recaptured by the ME-baited trap was converted into percentages and analysed. Student T-test was used to test for significant difference in recovery rates. All analyses were done using GenStat statistical software, 12th edition (GenStat, 2009). Where significant differences exist, Fisher's least significant difference (LSD) tests were used for multiple comparisons of means. Microsoft Excel was used to draw graphs where appropriate.

6.4 Results

6.4.1 Response of Bactrocera *dorsalis* to trapping distance

There were no significant differences in the percentage of trapped pre-exposed flies at different trapping distances (df = 4, 57, F = 1.04; $p = 0.395$). The highest percentage of captured pre-exposed flies (13%) was at 30 m from the centre of the orchard where flies were released. No flies were captured 40 m from the point of release (Figure 6.4).

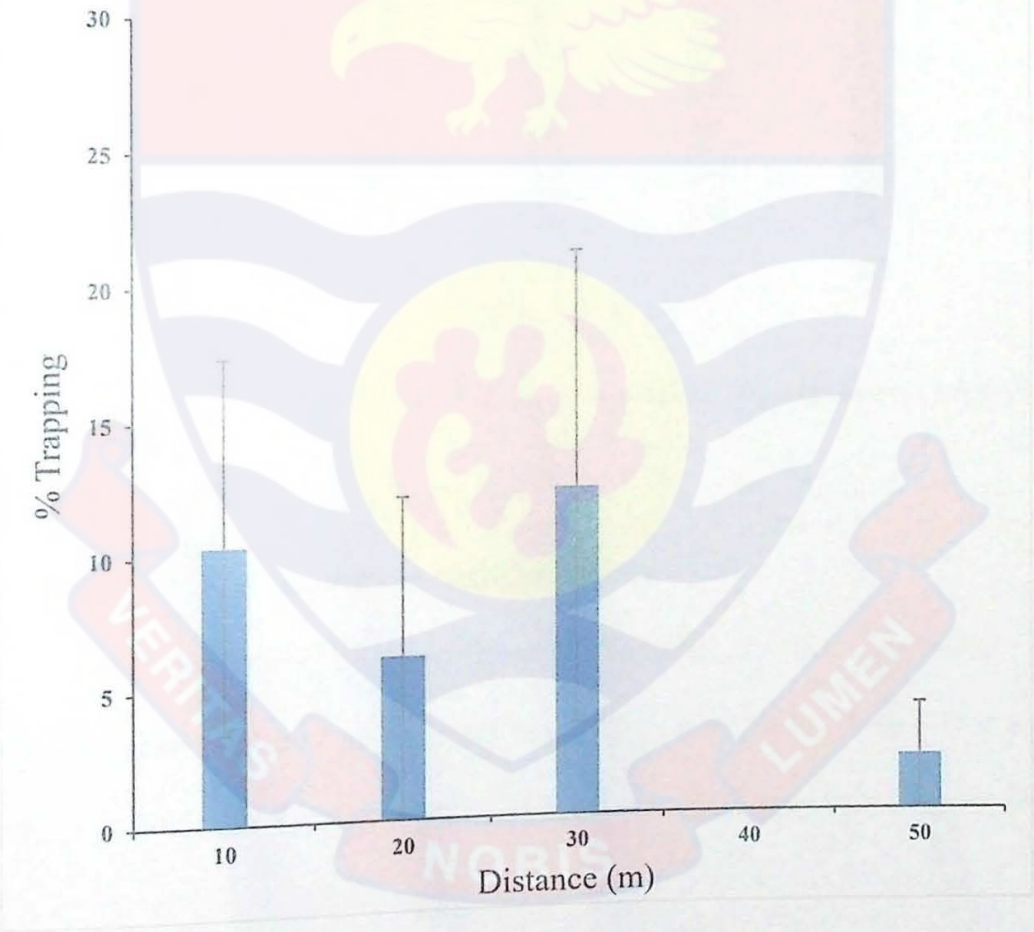

Figure 6. 4: Effect of distance on the dispersal of *Bactrocera dorsalis* pre-exposed to Methyl Eugenol.

There were significant differences between percentage of trapped unexposed flies at different trapping distances (F=6.17; df= 4, 57; $p < .001$).

More un-exposed flies were captured 10 m from the release point than distances further away (Figure 6.5).

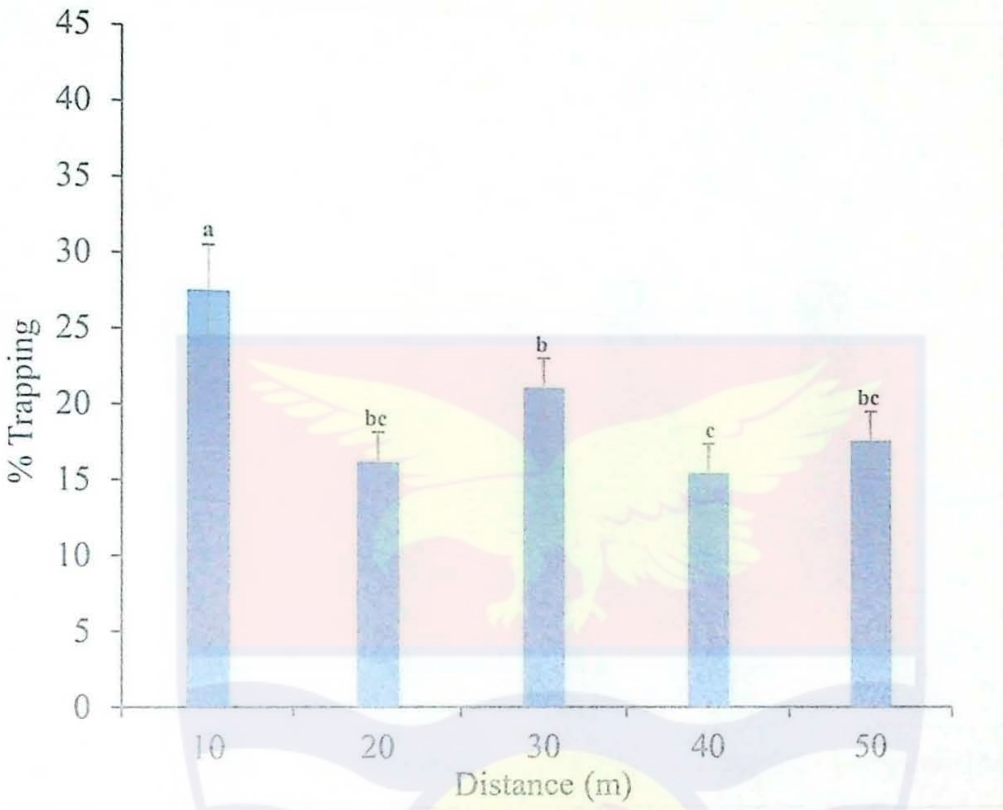

Figure 6. 5: Effect of distance on *Bactrocera dorsalis* un-exposed to Methyl Eugenol

6.4.2 Response of *B. dorsalis* to cardinal points

There were no significant differences in the percentage of unexposed flies captured at different cardinal points (North, East, West and South; ($F = 0.00$; df= 3, 57; $p < .001$). at the North, East and West (Figure 6.6).

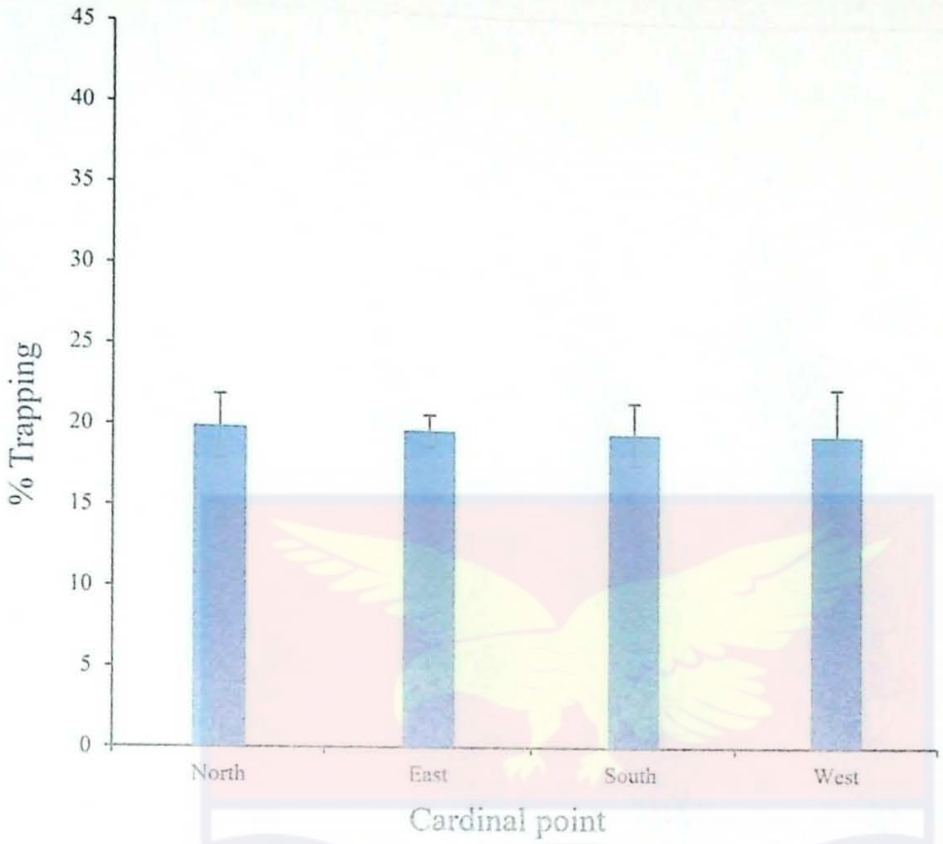

Figure 6. 6: Effect of cardinal points on *Bactrocera dorsalis* un-exposed to Methyl Eugenol

There were no significant differences in the percentage of trapped pre-exposed flies captured at different cardinal points ($df = 3, 57$; $F = 1.05$; $p = .376$). Flies in the study were not uniformly dispersed. Pre-exposed flies were more attracted to the ME baited traps set at east and west direction in the farms though not significantly different from flies captured in the other directions (Figure 6.7).

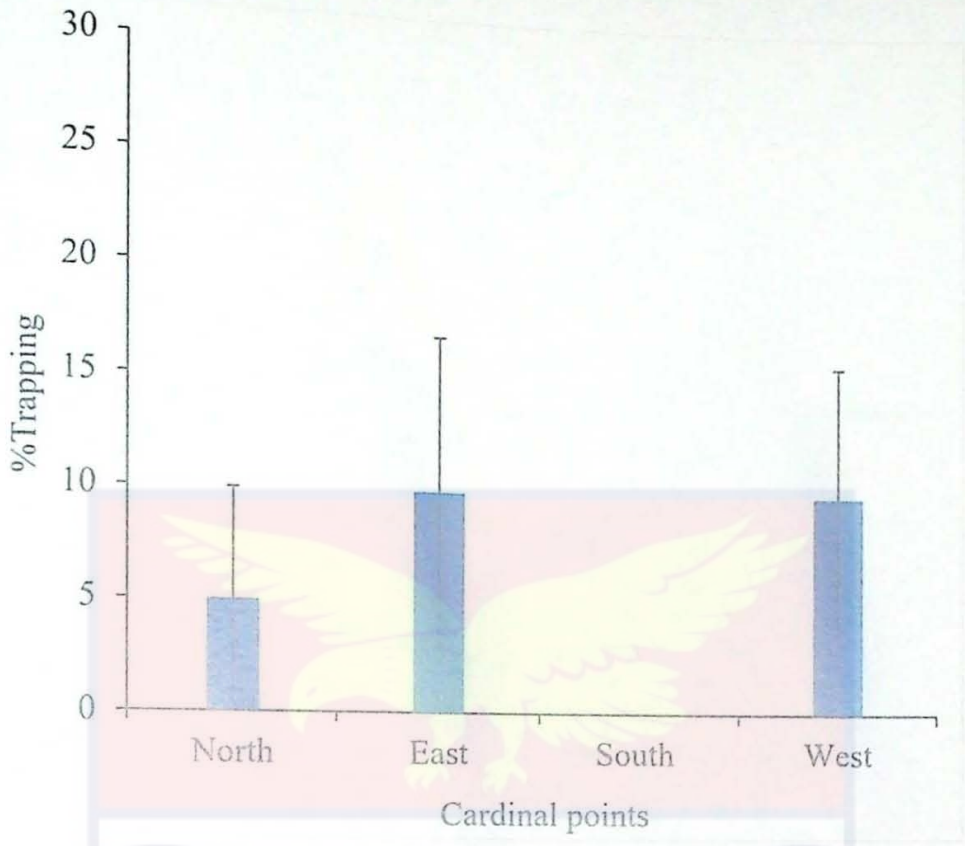

Figure 6. 7: Effect of cardinal points on *Bactrocera dorsalis* pre-exposed to Methyl Eugenol

6.4.3 Response of *B. dorsalis* to cardinal point and distance interaction

Higher proportions of the flies un-exposed to ME dispersed in the mango orchards. About 38% of the un-exposed flies were dispersed towards the 10 m radius and westward. This was followed by 30% being dispersed towards the 10 m radius and eastward. The least percentage of flies within the 10 m radius dispersed toward the north. Traps placed 50 m north, away from the center of the orchard captured highest percentage of flies (Figure 6.8).

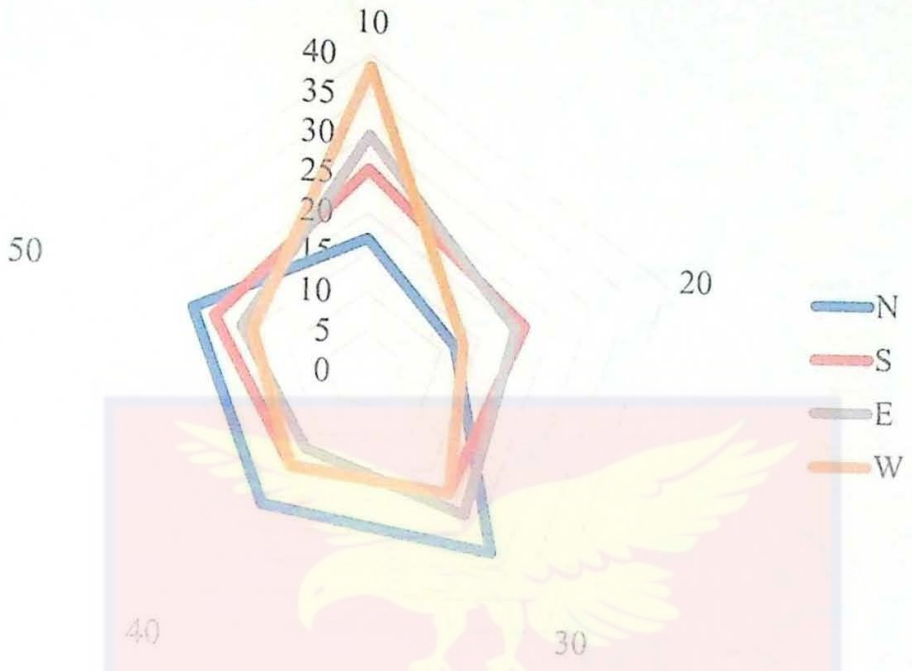

Figure 6. 8: Dispersal of marked adult male *Bactrocera dorsalis* un-exposed to Methyl Eugenol in orchard after 48 hours N=north, S=south, E=east, W=west

The dispersal of the pre-exposed flies seems to follow no particular direction. About 25% of the flies dispersed towards the 10 m radius and eastward. Similarly, 17% of the flies dispersed towards the 10 m radius and westward. At the 20 m radius, majority of the released flies were caught in the north direction (Figure 6.9).

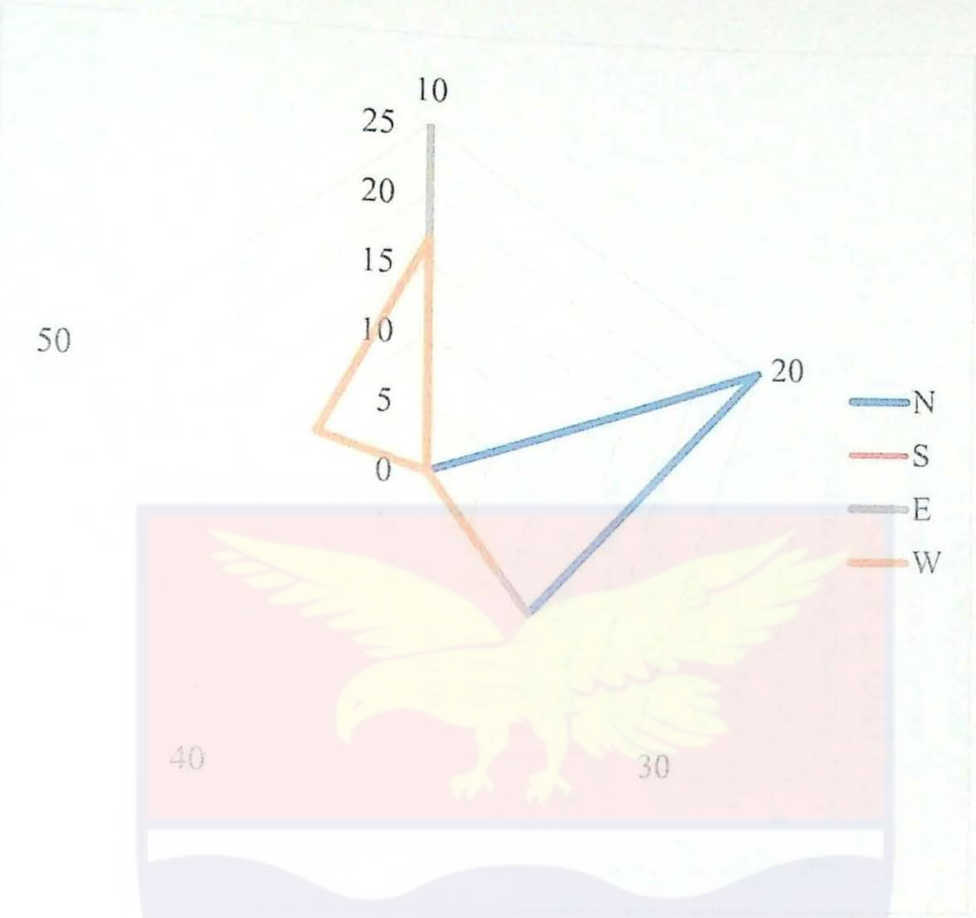

Figure 6. 9: Dispersal of marked adult male *Bactrocera dorsalis* pre-exposed to Methyl Eugenol in orchard after 48 hours N=north, S=south, E=east, W=west

There were significant differences in the percentage of un-exposed flies captured by the traps with regards to distance and cardinal point interaction ($df = 12, 57$; $F = 2.45$; $p < .012$). The highest percentage of flies were captured at West-10 (W10). This is followed by un-exposed flies caught at East-10 (E10). The lowest percentage of un-exposed flies were captured at North-20 (N20) and East-40 (E40) (Figure 6.10).

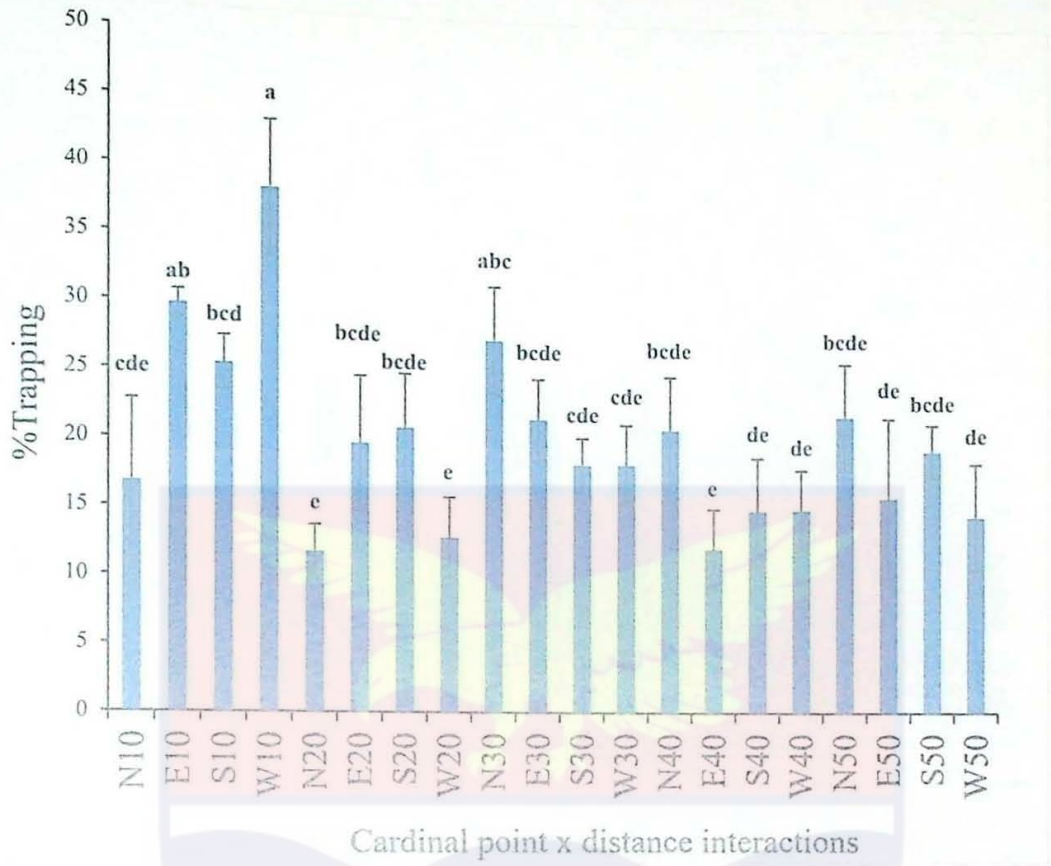

Figure 6. 10: Effect of cardinal points and distances on the capture of *Bactrocera dorsalis* un-exposed to Methyl Eugenol

N10= northwards at 10 meters, N20= northwards at 20 meters, N30=northwards at 30 meters, N40=northwards at 40 meters, N50=northwards at 50 meters, E10=eastwards at 10 meters, S10=southwards at 10 meters etc.

There were no significant differences in the percentage of trapped pre-exposed flies with regards to distance and cardinal point interaction (df= 12, 57; F = 0.97; p = .485). Similar high percentage of pre-exposed flies were captured at E10, W10, N20, E30 and W30 while the lowest percentage of pre-exposed flies were captured at W50 (Figure 6.11).

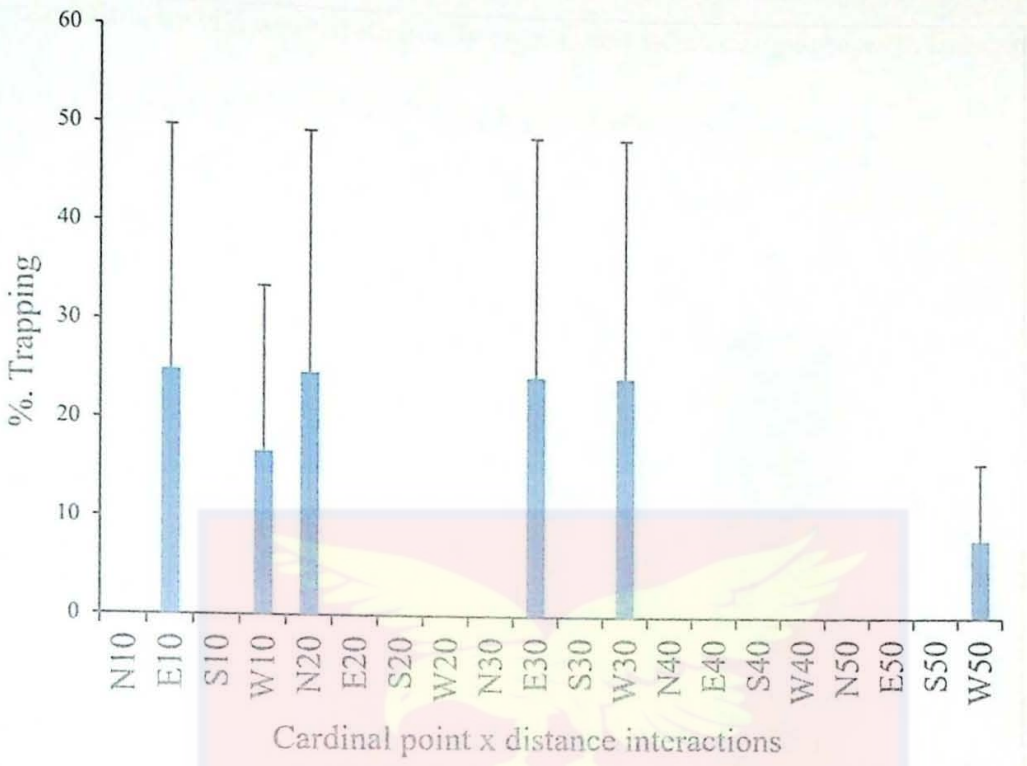

Figure 6. 11: Effect of cardinal point and distance on the capture of *Bactrocera dorsalis* pre-exposed to Methyl Eugenol.

N30=northwards at 30 meters, N40=northwards at 40 meters, N50=northwards at 50 meters, E10=eastwards at 10 meters, S10=southwards at 10 meters etc.

6.4.4 Effect of Pre- and Un-exposure of *B. dorsalis* to methyl eugenol

In the field, there were higher recovery rates for flies un-exposed to ME compared with flies pre-exposed to ME. Averagely, the recovery rate for the un-exposed fruit flies to ME lure was 2.41± 0.16% whiles 0.05±0.02% was estimated for pre-exposed. This recovery rate for the fruit flies un-exposed to ME was significantly different from fruit flies pre-exposed to ME (T-value = 5.65 df = 14, $p < .001$).

In the screen house, a higher percentage of the flies un-exposed to methyl eugenol were captured compared with flies pre-exposed to methyl eugenol. The proportion of *B. dorsalis* that fed on ME and captured were 22%, while the proportion of *B. dorsalis* un-exposed and captured were 57%. The un-

exposed flies to ME were statistically significant when compared with flies pre-exposed to ME after 24 hours of trapping (Figure 6.12).

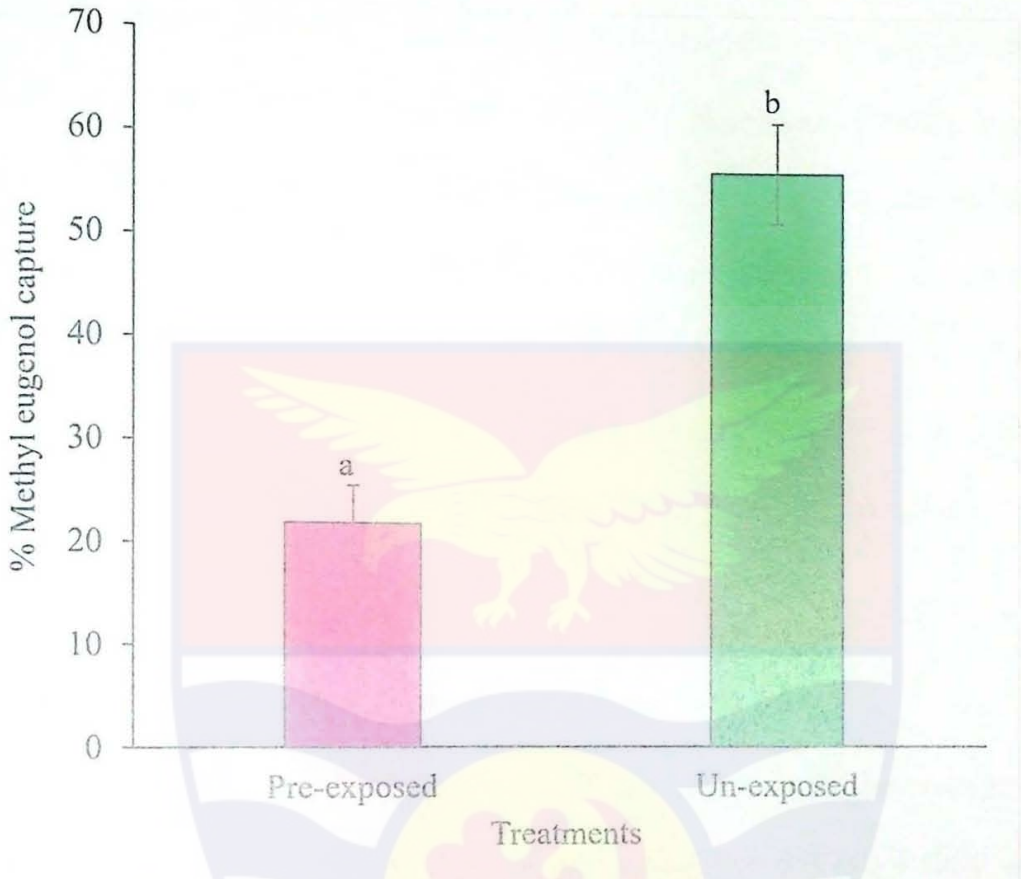

Figure 6. 12: *Bactrocera dorsalis* captured by Methyl Eugenol baited trap after 24 hours in a screen house

6.5 Discussion

6.5.1 Response of B. dorsalis to trapping distance

Bactrocera dorsalis males pre- and un-exposed to ME were trapped at shorter distances. The ME is a highly specific male pheromones and can attract at both short and long distances. The close proximity to which the flies were trapped in the ME traps is indicative of the stronger attraction of the pheromone. The percentage of pre- exposed *B. dorsalis* captured was lower than *B. dorsalis* un-exposed. There was likelihood of the ME not being fully sequestered by the flies pre-exposed to ME and therefore could affect their attraction to ME baited

traps due to the short exposure period in the wild (48 hours). This also goes to suggest that distance does not have a significant effect on flies that have been previously fed on ME. In a study in which *B. dorsalis* were marked with fluorescent powder, it was demonstrated that the flies could reach a flight distance of 97 km in 7 days (Chen & Ye, 2007). It is assumed that, during long flights, *B. dorsalis* replenish their nutrition and moisture as well as have intermittent rests. In this current study, the flies were fed over only 48 hours and this is likely to affect their ability to fly longer distances than 50 m. It is also known that, *B. dorsalis* cover long distances over a period of time and not at one go (Steiner, 1957; Chen & Ye, 2007).

6.5.2 Response of *B. dorsalis* to cardinal points

Cardinal points did not have a significant effect on the percentage of trapped (pre- and un-exposed) flies. Most of the flies did not follow any direction when moving out of the point of release. This could mean the flies might have gone in different directions when they were released. This could actually help in fair distribution of the flies especially during TIR/SIT studies.

6.5.3 Response of *B. dorsalis* to cardinal point and distance interaction

The distribution of the flies (pre-exposed and un-exposed) follows a particular direction. Most seem to be aggregated around the 10m westward and eastward direction from the point of release. This may be due to the behaviour of males calling in aggregation in order to attract females. Males calling singly is not effective (Shelly, 2001; Weldon, 2007). Methyl eugenol is a precursor for the male sex pheromone in *B. dorsalis* and increases the attractiveness of the pheromone to females (Tan & Nishida, 1996; Shelly et al., 2000; Shelly, 2001).

The collection of the flies at the short distance is indicative of the fact that the flies did not travel long during the first 48 hours after being released. *Bactrocera dorsalis* will likely require enough time to travel longer distance as usually is the case. The ME baited traps are highly attractive and will first of all capture flies within the release area.

6.5.4 Effect of Pre- and non-exposure of *B. dorsalis* to methyl eugenol

Both results from the field and screen house have shown that *Bactrocera dorsalis* fed on ME has a lower recovery rate compared with flies not fed on ME. The un-exposed flies on the other hand are highly responsive to ME-baited traps since they have not been previously fed on ME. Coupled with this, ME is highly specific to males in the *Bactrocera* genus and that makes it a candidate for trapping males in that genus. The success of TIR/SIT depends on males being competitive in searching for wild females to mate. The lower number of pre-exposed flies being captured helps to prevent the males from being trapped by ME-baited traps and therefore makes them to locate wild females early for copulation. Effort should be made to study the behaviour of pre-exposed flies to ME and determine the outcome of long exposure of ME-fed flies in the wild.

6.6 Conclusion

- *Bactrocera dorsalis* not exposed to ME is highly attracted to ME-baited traps than *B. dorsalis* fed on ME
- *Bactrocera dorsalis* tends to aggregate at short distance when released within a short period.
- *Bactrocera dorsalis* fed on ME has a lower recovery rate compared with flies not fed on ME.

CHAPTER SEVEN

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The oriental fruit fly, *B. dorsalis*, has remained a significant pest of fruits and vegetables across the globe, despite the introduction of new species. Different approaches have been exploited in dealing with the devastating nature of this fruit and vegetable-infesting pest. The development of a new strategy in the form of trap-irradiate-release, a form of Sterile Insect Technique could be one of the important means of solving pest problem, whiles at the same time protecting the environment from harmful release of chemicals into the environment. *Bactrocera dorsalis* thrives in tropical regions like Ghana, and is a major nuisance to exporters and local farmers. It is known to be highly competitive and has displaced major indigenous fruit flies like *C. cosyra*. This study was therefore initiated to seek an alternative solution to managing the pest by developing a new control tool to help manage the pest in an integrated pest management fashion.

7.1 Summary

To determine the population dynamics of fruit fly species in the south-eastern mango enclave in Ghana, trapping was done in three mango orchards for one year, spanning two fruiting seasons. Five traps baited with Methyl Eugenol (ME), Cuelure (CUL), Terpinyl Acetate (TA), Torula Yeast (TY), Trimedlure (TML) each were set in the three orchards. A total of 172, 617 fruit flies were collected from the traps with relative fly densities of 143.10, 10.19, 4.03, 0.26, and 0.03 flies per trap per day, for ME-, TY-, CUL-, TA-, and TML-baited trap respectively. Ten fruit fly species namely *Bactrocera dorsalis* Hendel, *Ceratitis cosyra* (Walker), *C. capitata* (Wiedemann), *C. penicillata*

(Bigot), *Dacus bivittatus* (Bigot), *D. punctatifrons* Karsch, *D. langi* Curran, *D. longistylus* Wiedemann *D. ciliatus* Loew, and *Zeugodacus cucurbitae* (Coquillett) were trapped on the orchards. *Dacus langi*, and *D. longistylus* were for the first time identified in Ghana by this study. Furthermore, from April to July, the population of the two most important species, *B. dorsalis* and *Z. cucurbitae*, changed significantly. Whiles *B. dorsalis* population peaked in April, May and June, that of *Z. cucurbitae* was low in those months. This peak population falls within the major mango fruiting season in the south-eastern mango enclave. Knowledge of this seasonal variation in the population of the major fruit fly pests could be harnessed for their effective management in the enclave. Particularly, interventions aimed at managing *B. dorsalis*, a major insect pest in the enclave could be executed from April to July, which is the period of peak activities of this insect pest.

In evaluating the efficacy of three trap types and period of the day in mass trapping *Bactrocera dorsalis* for irradiation studies, Ecoman caught the highest *Bactrocera dorsalis* in the mornings while Tephri caught the highest *Bactrocera dorsalis* in the afternoons although there was no significant difference in the mean catches ($p > .05$). There was no significant difference ($p > .05$) in the survival of *Bactrocera dorsalis* collected with the three trap types after holding them under controlled insectary conditions of 25 ± 2 °C, 75 ± 5 % RH and 12D: 12L light regime for 24 hours. The results of the correlation study revealed that climatic conditions had a substantial impact on trap catches. *Bactrocera dorsalis* caught in the evenings by all the three traps had a higher percentage survival compared to those caught during the morning or afternoon. Per the comparison made for the three trap types; Ecoman was the most efficient

for trapping *Bactrocera dorsalis* compared to Tephri and Bucket funnel. Therefore, Ecoman trap was recommended for mass trapping of *Bactrocera dorsalis* when conducting irradiation studies.

Sterile Insect Technique requires the mass rearing and release of large numbers of sterile males to compete with wild males to copulate with wild females. Trap-Irradiate-Release TIR/SIT is a technique that could cut the cost and time for mass rearing among other things. The purpose of this research was to determine the optimum radiation dose for sterilizing adult male *Bactrocera dorsalis* and its effect on fecundity of non-irradiated females. The research also determined the suitable period of the year to mass trap *B. dorsalis* for irradiation studies. Results from probit analysis showed that the optimum irradiation dose required to cause 95% sterility in male *B. dorsalis* was 70Gy and that percentage hatchability of eggs from laboratory reared virgin females mated by irradiated males was dose dependent. Thus, egg hatchability reduced with increasing radiation dose. Irradiation doses did not significantly affect fecundity.

Bactrocera dorsalis populations were low during the minor mango season in comparison to the major mango season. The months of June and July saw the greatest collection of *B. dorsalis*. Therefore, these months could be targeted for collection of large numbers of adult male *B. dorsalis* for irradiation and release during the minor mango season.

This research also assessed the effects and response of *Bactrocera dorsalis* to attractiveness to ME-baited traps. Results showed that both pre-exposed and un-exposed flies aggregate within a short distance of 10m after 48 hours. Fruit flies not fed (un-exposed) on ME has a higher recovery rate compared with fruit flies fed (pre-exposed) on ME.

7.2 Conclusions

Studies were conducted to develop an integrated pest management strategy for *Bactrocera dorsalis* Hendel (Diptera:Tephritidae) in mango orchards using Trap-Irradiate-Release/Sterile Insect Technique. The major findings were:

1. The study has identified ten different fruit fly species belonging to four economically important genera *Bactrocera, Ceratitis, Dacus* and *Zeugodacus*
2. *Bactrocera dorsalis* has been identified as the dominant fruit fly species attracted by both Methyl eugenol- and Torula yeast-baited traps. This is followed by *Z. cucurbitae* which was attracted by CUL-baited traps. These two species cause extensive damage to mango fruits. Both are aggressive in attacking mango fruits during peak and off-peak fruiting seasons.
3. It is worth noting that, *D. langi* and *D. longistylus* have been detected, identified and documented for the first time in Ghana through this study.
4. The high efficacy of ME-baited traps makes it a candidate for attract-and-kill technique for controlling *B. dorsalis*. This could be complemented with good orchard management practices to reduce oviposition substrates for gravid females.
5. During the months of April through July, when the *B. dorsalis* population and activity are at their peak, control operations should be ramped up to ensure effective management of these populations.
6. Survival was higher for *Bactrocera dorsalis* trapped in the evening with all the three trap types.

7. On average, the survival rate of *B. dorsalis* in the traps was highest for Ecoman, followed by Bucket funnel and Tephri trap.
8. Ecoman trap has higher trapping efficiency compared to Tephri trap and Bucket funnel traps.
9. Ecoman traps are therefore ideal for use in mass trapping of *B. dorsalis*.
10. The optimum irradiation dose to cause 5% egg hatchability in females mated by irradiated adult males is 70Gy.
11. The higher the radiation doses, the lesser the percentage hatchability.
12. Irradiation doses did not significantly affect fecundity.
13. When comparing the minor mango season to the major mango season, it was discovered that *B. dorsalis* populations were lower in the minor mango season.
14. The months of June and July recorded higher numbers of *B. dorsalis* and this period could be targeted to collect large numbers of adult *B. dorsalis* for irradiation and release during the minor mango seasons to manage the pest.
15. *Bactrocera dorsalis* not exposed to ME is more attracted to ME-baited traps than *B. dorsalis* fed on ME
16. *Bactrocera dorsalis* tend to aggregate at short distance when released within a short period.
17. *Bactrocera dorsalis* fed on ME has a lower recovery rate compared to flies not fed on ME

7.3 Recommendations

This research has provided additional information that TIR/SIT is technically feasible to be developed and deployed as an additional tool in managing the menace caused by *Bactrocera dorsalis* to commercial farmers in orchards in the SouthEastern enclave of Ghana.

1. Mass trapping should be carried out through the mango production season but can be intensified before fruits start maturing and ripening so as to prevent the multiplication of the flies leading to fruit attack. This will break their cycle and reduce the population.
2. Since Ecoman trap caught more *Bactrocera dorsalis* in the morning compared with catches in the evening, it is advisable to set Ecoman traps early in the morning to maximize catches and save time and energy.
3. In the current study, the optimum dose to cause 95% sterility in live *B. dorsalis* males was 70Gy but a further study should be carried out to evaluate the irradiation dose that can cause 100% sterility in the males without affecting the fitness and competitive nature of the live adult males for TIR/SIT.
4. Competitiveness and fitness studies should be carried out on irradiated live *B. dorsalis* so as to know their behaviour when released in the wild for the TIR/SIT. This was not studied in this research due to the breakdown of the Gamma Irradiation Facility (GIF) at GAEC.
5. Even though 70Gy was established to be the optimum dose to cause 95% sterility in males, a further study should be carried out using uninfected, sterilized mango fruit in a screen house to study the effect of the dose when irradiated males are paired with virgin females. The mango should be

dissected to ascertain the hatchability of the eggs. This will further prove the effectiveness of TIR/SIT.

6. *Bactrocera dorsalis* that was pre-exposed to ME and released in orchards should be left in the field a little longer than the 48-hours to ascertain if the effect of the methyl eugenol will wear off after a longer period of exposure. This might help the flies to respond more easily to ME baited traps.
7. Further research should be carried out to study with irradiated pre-exposed males to ME to ascertain their response and attraction to ME both in screen houses and on-farm fields.

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