KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF AGRICULTURE AND NATURAL RESOURCES

FACULTY OF RENEWABLE NATURAL RESOURCES

DEPARTMENT OF WILDLIFE AND RANGE MANAGEMENT

POLLINATION ECOLOGY OF UPPER AMAZON COCOA AND BREEDING SUBSTRATES OF COCOA POLLINATORS IN THE EJISU-JUABENG DISTRICT OF THE ASHANTI REGION, GHANA

BY

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A thesis submitted to the Department of Wildlife and Range Management

Kwame Nkrumah University of Science and Technology

In partial fulfillment of the requirement for the degree of

DOCTOR OF PHILOSOPHY

Faculty of Renewable Natural Resources

College of Agriculture and Renewable Natural Resources

August, 2012

DECLARATION

I hereby declare that this submission is my own work towards the Ph.D, and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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Name of Head of Dept.   Signature   Date

THESIS ABSTRACT

Cocoa plays important role in the economy of Ghana as it is the second foreign exchange earner after gold. Lives of 800,000 farmers and their families depend on it. Although it has enjoyed a consistent growth Ghana's cocoa yields per hectare are still low by international standards. This is probably because cocoa pollination which has for a very long time been relegated to the background in the scheme of cocoa production. The study was undertaken at Kubease in the Ejisu-Juabeng district using ten farmer managed farms around the Bobiri Forest. It involved a study of the ecological importance of floral phenology of the Amazon cocoa which forms about 58% of cocoa varieties in Ghana; a survey of insects in the cocoa ecosystem to establish their pollinator status; the relative importance of the breeding substrates in the cocoa ecosystem was also investigated; and the contribution of natural pollination to cocoa production. Results indicated that cocoa trees exhibited seasonally-related phenological patterns of flowering and fruit-set involving overlapping cycles under both intrinsic and extrinsic control. Significant $(P<0.001)$ differences in the monthly floral production existed between the study farms. Optimum floral production occurred at temperature 22.5 $^{\circ}$C, light intensity 91.8 Fc, and rainfall of 141.1 mm per month; however, rainfall could be the most critical factor in the floral phenology. Flower stability was affected by seaons and pollination. 2721 insects belonging to 36 species and 7 orders found in the ten farm plots. About 52.81% did not visit the cocoa trees; 10.4% insects were found on the cocoa trees. The ceratopogonids visited ($F_{1,\ 30} = 28.79$, $P <0.05$) more than other insects. Only midges could carry 60.1 ± 13 pollen. Population of midges was significantly ($F = 0.65$, $P<0.05$) greater under rotten banana pseudostem than the other substrates. There were significant diferences between the number of seeds of naturally pollinated and that of artificially pollinated fruits of small size pods ($P< 0.04$), medium size pods ($P< 0.01$)

and the large sized pods (*P*<0.01). The study therefore demonstrated that cocoa has a specialised pollination mechanism, in which pollinators belong to just one pollinator class. Rotten banana pseudo stem preferred substrate implying that it might have provided a more condusive breeding microhabitat for the ceratopogonids. Natural pollination could contribute to cocoa productivity.
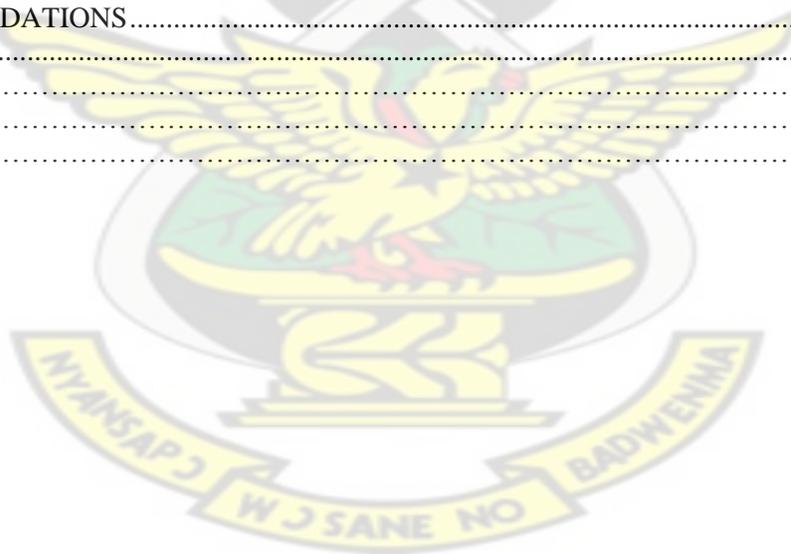
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ACKNOWLEDGEMENT

Undertaking a Doctoral study under the African Sun goes with a lot of daunting challenges, and it takes the support of people who are always there for you. I am very certain that I would never have been able to finish this study without the help of some individuals. It is therefore natural that I show appreciation as follows:

My premier thanks go to my God whom I love and serve. The Holy Spirit sustained me emotionally, physically (in terms of health) throughout all the trying moments. God was simply gracious to me.

I am greatly indebted to my supervisor Prof. William Oduro of the Department of Wildlife and Range Management, Faculty of Renewable Natural Resources, who single handedly helped me a great deal with the whole study. He went through my work with superb clarifications and precipitous guidance. I am really impressed with the patience and yet his meticulous approach to the review of my presentations at every level. Above all he prayed for me.

Dr Ben Banful of the Department of Horticulture, KNUST, was of great importance as he challenged me during the write up and publications in top scientific journals. His insight into research and skill in writing to me was invaluable.

Professor Theophilus C. Fleischer of Department of Herbal Medicine proved to be was friend for all seasons. Unperturbed he supported me both financially and emotionally. May he and his descendants never see lack in this life.

Dr Moses Mochiaa and Dr Mike Owusu-Achiaw (rtd), all of Entomology section, Crops Research Institute of Council for Scientific Industrial Research, Kwadaso helped with a thorough evaluation of my initial thesis proposal as well as read through my work with great perspicacity.

I would like to express my gratitude to the staff of Cocoa Research Institute of Ghana (CRIG) who took me through field work to enable me understand the uniqueness of the cocoa plant and

perculiarity of natural pollination of cocoa. Dr Emmanuel Dwomoh of the pathology section greatly encouraged me to carry on with the study at a time when I was confronted with uncooperative attitude of some individuals in the institute. He personaly intervened and linked me up with the field workers.

My heartfelt gratitude goes to Mr Ato Harry-Brew, a retired cocoa entomologist who assisted me with literature and shared his wealth of experience with the cocoa pollinators. His expositions greatly enlightened me as it helped me become conversant with the midges.

Reverend (Dr) Peter Ohene Kyei and his wife, Abigail Kyei were exceptionally supportive. May the Lord bless them from on high.

Above all, I would like to thank my dear wife Dinah Abena Adjaloo, and children Abigail Boadiwa, Anna Nyarko, Michael (Jnr) and Grace Nyiamh for their unequivocal support for an unavailable father during the days of struggle. I am grateful for all their sacrifices. Dinah always cheered me up and was my tower of strength. I know that mere expression of thanks does not suffice.
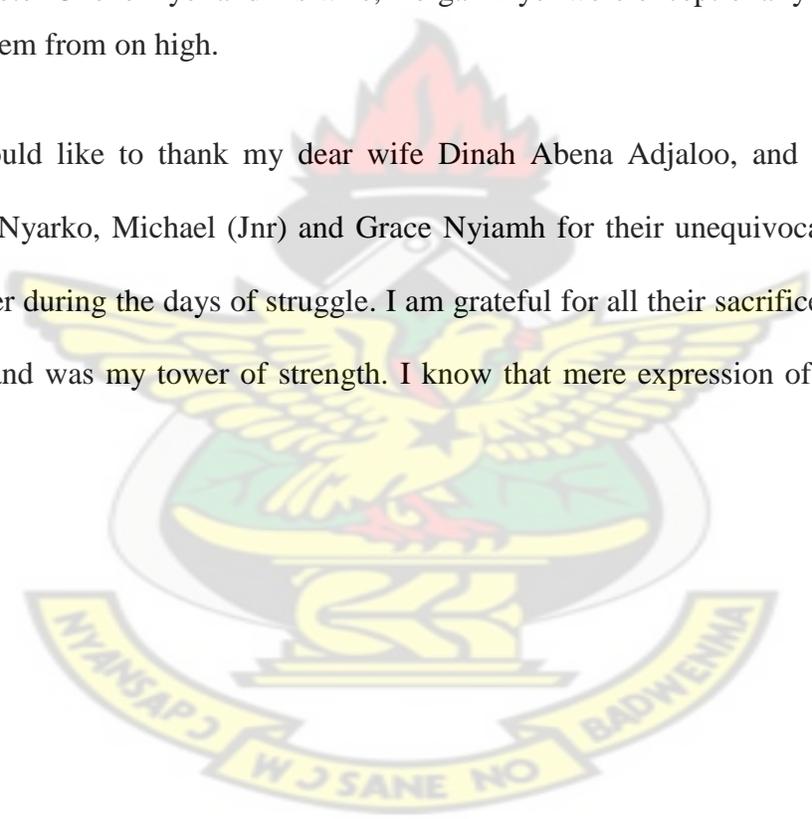
DEDICATION

I DEDICATE THIS WORK TO

MY LATE MOTHER: GRACE AMA BOADIWA ASARE,

WHO NURTURED ME, AND THOUGH AN ILLITERATE SHE SAW THE VALUE OF

EDUCATION AND SACRIFICED HER ALL FOR ME.

MAA!! WE WILL FOREVER REMEMBER YOU

KNUST

CHAPTER ONE

INTRODUCTION

1.1   Importance of Cocoa to World Economy

Cocoa, *Theobroma cacao* L (Sterculiaceae), also known as food of the gods, is an important economic tropical tree whose center of origin is thought to be in several native areas of the tropical rainforest of equatorial America or Upper Amazon (Allen and Lass, 1983; Motamayor et al., 2002; Bailey et al. 2005), and grows profitably within latitudes 20ºN and 20ºS of the equator. However, the bulk of the crop is produced within latitudes 10ºN and 10ºS (Anim-Kwapong et al., 2002). Cocoa is the world's third most important agricultural export commodity, after coffee and sugar, and a major earner of foreign income for the number of countries that dominate production. It is also a major cash crop in many tropical countries ranking among other key bean and nut commodities in terms of global scale of crop production (World Wildlife Fund, 2006; World Cocoa Foundation, 2010). Farmed on over 7.5 million hectares, cocoa provides a means of livelihood for an estimated 40-50 million people worldwide, including five million cocoa farmers (World Cocoa Foundation, 2010). Smallholder farmers with farms less than ten hectares (ha), grow over 90% of the world's cocoa, and make little or no use of fertilizers and agro-chemicals (International Cocoa Organization, 2005; 2010). Cocoa is a major economic resource to several tropical countries (Browna et al., 2007; Lanaud et al., 2009). The cocoa sector alone employs over 80,000 small holder farm families (Asamoah and Baah, 2003; Frimpong et al., 2007), representing 19 percent of rural households, and contributing between 70% - 100% of annual household incomes of smallholder farmers (Breisinger et al., 2008). Ghana's agricultural GDP attributed to cocoa increased from 13.7 percent in 2000 - 2004 to 18.9 percent in 2005/2006

(Breisinger et al., 2008). To sustain such remarkable growth in production, there is the need to take a more critical look at the ecosystem to improve on the features of the system which have positive impact on cocoa yields (Gockowski, 2007).

West Africa has been the center of cocoa cultivation for many decades as seventy percent of the world's cocoa is grown in this sub-region (World Cocoa Foundation, 2010), and its production has been documented in agricultural reports since 1556 (Johns, 1999). Compared to other agricultural activities, cocoa has been a leading subsector in the economic growth and development of several West African countries (Duguma et al., 2001). Ghana is the second largest cocoa producer after Ivory Coast (ICCO, 2007; Filou and Kenny, 2009). Together the two countries represent approximately 72% of world cocoa production (Vigneri, 2007).

1.2   Cocoa Production in Ghana and its importance to Ghana's Economy

Cocoa thrives in regions around the equator with high humidity, ample rainfall and year round high temperature (Mann, et al., 2010). This makes countries like Ghana ideal for its production. Ghana has six cocoa growing regions namely Ashanti, Brong Ahafo, Central, Eastern, Western, and Volta where rainfall is 1,000-1,500 millimeters per year (MoFA, 2003). An estimated 1,600,000 hectares of Ghana's land is cultivated with cocoa (Frimpong et al., 2007). Since 2001 the volume of cocoa produced in Ghana has grown at a yearly average of 11% between 1994 and 1999, and 16% in the following years 2000 and 2003 (MoFA, 2003; Vigneri, 2007). At the end of the 2008/2009 cocoa season (in October) cocoa production had increased by 4.2% which translated into 710,000 metric tonnes (Mhango, 2010). It was estimated at 903,646 metric tonnes in 2011 and 1 million metric tones in 2012 (COCOBOD Report, 2012). Cocoa exports account

for about 40% of total Ghanaian exports, and was the major export earner for the country in the year 2004 (Frimpong et al., 2007). In 2007 cocoa beans and refined sugar were the largest exports for Ghana (FAO, 2010).

Notwithstanding the above, yields of cocoa per hectare are generally lower in Ghana compared to other major producing countries (Dormon et. al., 2004; Vigneri, 2007). Whilst the average cocoa yields are 1,800 kg ha^{-1} in Malaysia, and 800 kg ha^{-1} in the Ivory Coast, it is only 360 kg ha^{-1} in Ghana (Anon., 1999). Surveys carried out by Government of Ghana Task Force and COCOBOD estimated productivity per hectare annually to be less than 250kg and ranged between 200 and 290kg (COCOBOD, 1998). It is estimated that on the average the potential yield of cocoa in Ghana ranges between 1000 and 1500kg/ha (MOFA, 2003). Reasons for the low productivity has been variously attributed to poor farm maintenance due to low prices paid to cocoa farmers, planting of low yielding varieties, and the incidence of pests and diseases (Anon.,1999; Abekoe et al., 2002). Brew (1984), however, attributed the low productivity to the low pollination due to some cultural practices which affect pollinators. Mabbett (1989) had also noted that pollination by insects is one of the many critical stages in the cocoa production cycle.

Due to the importance of cocoa to the economy, the Government of Ghana has prioritized cocoa as a commodity crop and aims to increase its production (Frimpong et al., 2007). To this end the government has over the years been implementing policies aimed at reforming the cocoa sector in an attempt to boost production. However, the policies merely introduce economic measures and do not include such important areas as pollination ecology. There is the need to involve all

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factors of productivity, including the pollinating agents. Pollination services will result in increased production and higher quality of the crop (Roubik, 1995).

1.3   Pollination Ecology of Cocoa

Pollination ecology is the scientific study of plant-pollinator relationships and it involves the life histories, floral phenology, energetics of foraging, distribution, and behavior of individual species as well as the structure and function of natural systems at the level of populations, communities, and ecosystems. The ecological importance of pollinators is crucial (Kearns *et al.,* 1998), but management of pollination systems is relatively new and untried (Kearns and Inouye, 1997). This applies to cocoa with its unique qualities such as the cauliflory and pollination mechanism which are shared by very few tropical plants (Bos et al., 2007). A significant issue of cocoa pollination ecology is the synchronization between pollinator population cycle, the floral phenology of cocoa tree, and the adequacy of pollination (Young, 1983).

Over the years, several workers have established that cocoa is entomophilous, dependent on cross-pollination, and the responsible insects are midges (*Forcipomyia* spp.) of the family Ceratopogonidae (Posnette, 1950; Entwistle, 1972; Kaufman, 1975; Cilas, 1987; Klein et al., 2008; Groeneveld et al., 2010). Moreover, Winder and Silva (1972) suspected that pollination of cocoa by insects was one extrinsic limiting factor regulating fruit set in cocoa. Cocoa pollination has since 1925 been a subject of interest [5] and yet very little is known about the mechanisms of pollination that contribute to the production of the fruits and subsequently the yield of the tree [6]. Stephenson (1981) and Bos et al., 2007 reported that over 90% of flowers produced by cocoa trees drop after opening. Consequently only 10% of the total flowers produced pollinate

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successfully. This low success percentage is even dependent on the activities of midges in the cocoa plantation. Winder (1977) observed that inadequate pollination of cocoa by midges in cocoa plantations occurred when rotten vegetation had been too fastidiously removed, and concluded that a knowledge of breeding sites could leverage to increase pollinator populations. Ismail and Ibrahim (1986) confirmed this assertion in Malaysia, where an increase in pollinator populations was observed when additional substrate of rotten palm trunks was provided on the cocoa farms. The results provided the first demonstration that pollinator abundance was limited by a lack of breeding sites which ultimately affected fruit set and yield. They further confirmed the need to increase insect pollinator population especially at the time of the year when pollination was most needed through some cultural techniques that could enhance their population in cocoa farms and subsequently increase the level of pollination. The reproductive system of cocoa (*Theobroma cacao* L) is said to be characterized by high numbers of flowers, of which generally no more than 5% develop into mature fruits (Entwistle, 1972; Stephenson, 1981; Free, 1993; Young, 1994; Bos et al., 2007). This implies that only a small proportion of the total number of flowers produced are successfully pollinated. There is obviously a delicate balance between crop success and failure. Perhaps pollinating insects may be a factor limiting yield in a number of cocoa growing environments (Brew, 1988). Achieving maximum pollination is necessary for optimum yield (Mabett, 1989). It is therefore essential to acquire a sound knowledge of the mechanism of pollination (Brew and Boorman, 1993). Winder and Silva (1972) have suggested that in order to enhance knowledge on natural pollination and hence the crop yield, the characteristics of the cocoa tree: the number of flowers produced, and the period of the year in which they are produced must be considered. Furthermore an understanding of factors underlying phenological patterns is said to assist conservation scientists in predicting

consequences of perturbations such as a typical climatic events (Foster, 1982) or global warming (Tutin and Fernandez, 1993). There is therefore the need to update knowledge of flower phenology of the cocoa trees, flower persistence and stability on the cocoa trees.

1.4   Problem Statement

Even though the economic importance of cocoa is globally acknowledged, very little is known about the factors that determine its yield (Bos et al., 2007). Most studies on cocoa pollination were done between 1950's and 1970's. Due to the nature of the known cocoa pollinators cocoa entomologists easily shift to other insects, thus less attention is given to cocoa pollination studies (Winder, 1972). The cumulative result is that cocoa pollinators are totally ignored; their identity (i.e.taxonomy) is less known; paucity of relevant literature on cocoa pollination and what is available is outdated. Due to lack of systematic dissemination of information on pollination farmers and extension workers are less informed of the value of cocoa pollinators. This in turn has led to lack of proper management of pollinators in cocoa production and methods for monitoring cocoa pollinators are both limited and inefficient.

In most West and Central African countries, growth in the cocoa subsector have been achieved by increasing the area cultivated rather than by improving yield (Kuwornu et al., 2011; Ofori-Bah and Asafu-Adjaye, 2011). Consequently, cocoa production has been among the most significant factors associated with the near disappearance of the West African Guinea rainforest as cocoa farms are increasingly encroaching on the rainforest ecosystem (Binam et al., 2008). The pollinator assemblage in the cocoa ecosystems and the relative importance of breeding substrates of cocoa pollinators are less researched. There is therefore the need for an update on

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the pollination ecology of cocoa, the activities of cocoa pollinators and their practical relevance to the cocoa farmer. The cocoa tree is visited by a myriad of insects which were viewed as potential pollinators. Some researchers (Billes, 1941; Posnette, 1944; Entwistle, 1972; Kaufman, 1973, 1974; Brew, 1984) had presented some claims about the possible involvement of other pollinator guild. However, they were quite unanimous on the pollinator status of the midges. This calls for further study to establish the pollinator status of other insect visitors to the cocoa flowers, especially the bees and thus, update the studies of natural pollination of cocoa. It is believed that the breeding substrates of recognised pollinators can illicit and increase the rate of pollination (Sarfo et al., 2003) yet no comprehensive study has been carried out on them.

To boost cocoa production Ghanaian farmers are clearing more forests while, the government of Ghana has offered free mass spraying since the year 2001, as a pest control measure, and fertilizers are also supplied to farmers. The result is the high level of forest depletion currently being experienced in Ghana. The critical ecological component of crop production i.e. pollination, is largely ignored (Winder, J. A. *n.d*).

1.5   Justification of Study

Globally there is great concern over the apparent reduction in populations of pollinators of all kinds due to its potential impact on agricultural productivity (Kevan and Philips, 2001; Biesmeijer et al., 2006; Chagnon, 2008). According to Gallai et al (2009) crops that appear to be most affected by dwindling pollinator numbers include stimulants (coffee, cocoa) with 39 % vulnerability, nuts (31 %), fruit (23.1 %), oilseed crops (16.3 %) and vegetables (12.2 %).

Worldwide recognition of the key role pollinators in health of ecosystem both in farmers' fields and in wild landscapes has led to the establishment of International Pollinator Initiative (IPI) of the United Nations' Food and Agriculture Organization (FAO). In collaboration with some member countries the IPI is developing strategies for pollinator conservation and management. Consequently, the African Pollinator Initiative was established in 2009 to promote pollination as an essential ecosystem service for sustainable livelihoods and the conservation of biological diversity in Africa. In this regard some detailed ecological study of cocoa pollinators is necessary to augument the growing body of knowledge. An improved understanding of natural pollination of cocoa can make a substantial contribution to two key sectors of Ghanaian economy i.e. agriculture and conservation (Rodger et al., 2004).

The Cocoa Research Institute of Ghana at Tafo and Bunso in the Eastern Region of Ghana employs supplementary hand (artificial) pollination to increase yield and also breed new varieties of cocoa. However, a reconnaissance survey conducted in cocoa gowing areas of Western, Eastern and Ashanti regions of Ghana during this study has revealed that the technique is not known to farmers who are the actual producers of the country's cocoa. The study therefore is expected to rekindle interest among cocoa entomologists and ultimately result in the integration of cocoa pollination into agricultural extension protocols.

1.6   Aim of Study

The aim of this study is to investigate some aspects of pollination ecology of Amazon variety of cocoa, and evaluate the relative importance of the breeding substrates of cocoa pollinators.

1.6.1 Specific Objectives:

1. To determine the floral phenology of the cocoa trees and its ecological significance.

2. To determine the pollinator status of insect assemblage in the cocoa ecosystems.

3. To determine the most suitable breeding substrate(s) of the cocoa pollinator (s)

4. To determine the relative contribution of natural pollination as against artificial pollination with regards to yield of cocoa.

1.6.2 Research Questions

The following research questions were addressed in the thesis:

1. What factors affect the floral phenology of the Upper Amazon cocoa? What is the ecological significance of the floral phenology?

2. What are the insect species associated with the cocoa trees in the study area? Which of them provide pollination as an ecological service to the cocoa trees?

3. Do the cocoa pollinators have substrate preference? Will increase in breeding substrates increase pollination?

4. What contribution does natural pollination make towards fruit production in cocoa? How does it compare with artificial pollination?

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Given the importance of cocoa to world economy it is not surprising that there is a plethora of documentation on its cultivation (Cabala- Rosand and Santana, 1982; Wood and Lass, 1985; Hepper, 1986; Gockowski et al (*unpublished*); Amoah,1997; Johns, 1999; Duguma, et al., 2001; Osei-Bonsu, 2002); production (FAO,1996; International Cocoa Organisation, 2000; Donald, 2004; Dormon et.al., 2004; International Cocoa Organisation, 2005), and its contribution to the global economy. Over the years most of the worldwide research on the productivity of cocoa has been skewed toward the socio-economic factors (Koczberski et al., 2001; Dormon et al., 2004), agronomical (e.g. Osei-Bonsu et. al., 2002), soils and fertilizer studies (e.g. Appiah et al., 2000), and integrated pest management (Konam et al., 2008).

For many years the efforts of the cocoa entomologists have been geared mainly towards studies on the ecology of insect-vectors of swollen shoot diseases viz: *Planococcoides njalensis* Laing, and *Planococcoides citri* Risso, neglecting entirely the studies on the ecology of insect pollinators, the *Forcipomyia* spp and related species. In contrast, however, fewer researchers have really worked on the pollination phenomenon, the agents of pollination and the faunal biodiversity whose activities, though imperceptible are crucial for the healthy growth and fitness of the cocoa crop. In South America where most of the cocoa trees are self–incompatible, some basic work on the cocoa pollinators has been carried out (Hernandez, 1965; Soria, 1970; Winder

and Silva, 1972; Young, 1985). Similarly, Kaufman (1973; 1975) has also made observations on the ecology of a number of insects associated with pollination of cocoa in West Africa. Though these studies have become subjects of scientific debate, they are major sources of reference.

Important gaps still remain in the knowledge of the biology and ecology of the cocoa pollinators (Winder, J.A. *unpublished*). For instance, the pollinator status of some insect species and impact of the breeding substrates on pollination of cocoa is not available. In order to augment cocoa yield through pollination, more information based on scientific experiments is required. Therefore the need for intensive studies on cocoa pollination and the pollinators cannot be over-emphasized. This literature review looked broadly at the ecological, biological and physiological factors that are important to the productivity of cocoa; the pollinator availability and activity in the midst of interactive factors. Specifically, the search focused on six thematic areas: Pollination as an Ecosystem Service; Cocoa and its distribution; productivity and production of cocoa; flowering and fruit setting; fruit loss in cocoa; and breeding substrates of the insect pollinators. These have provided the relevant basis for the thesis.

2.2 Pollination as an Ecosystem Service

Pollination refers to the transfer of pollen from the anther to the stigma of a flower, and this is the first step in the sexual reproduction of 70% to 90% of angiosperm species (Foutaine et al., 2006). It is an important ecosystem service for human welfare (Kremen et al., 2002; Klein et al., 2007), and is considered a critical factor affecting fruit set, yield and fruit quality (Al-Obeed and Soliman, 2011). Pollination strongly links productivity and sustainability in almost all terrestrial ecology (Kearns et al., 1998; Kevan, 1999) and is essential for the maintenance of diversity in

wild flowers (Westerkamp and Gottsberger, 2000; Goulson, 2003; Potts et al., 2005). Agricultural crops often depend, at least in part, on unmanaged or 'wild' pollinator populations for their productivity (Kevan, 1977; 2001; Klein et al., 2003; Kremen et al., 2002; 2004; Ricketts, 2004; Ricketts et al., 2004). Estimate has showed that the value of food crops directly consumed by humans attributed to insect pollination services in 2005 was US$ 153 billion, representing about 9.5% of total world production of human food (FAO, 2008). Beyond caloric intake Steffan-Dewenter et al., (2005) opined that our diet would be greatly impoverished both nutritionally and culturally if pollination services further declines.

Plant-pollinator interaction is viewed by some workers as one of the most essential and perhaps the bedrock of ecosystem services (Allen-Wardell et al., 1998; Kearns et al., 1998; Al-Obeed and Soliman, 2011). Free (1993) in an extensive literature survey noted that majority of the most important crops providing fruits or seeds for human consumption benefit from animal pollination. In fact, animal pollinators are thought to contribute between 15% and 30% of global food production (McGregor, 1976; Roubik, 1995). Crops with either specialized or generalized pollination systems have been shown to profit from high pollinator diversity (Klein et al., 2003). Furthermore, both self-compatible and self-incompatible plants can profit from pollination (Klein et al., 2003; Free, 1993; Moradin and Winston, 2005).

Kevan (1999) argued that pollinators need more recognition as bio-indicators because of their diversity, the importance of their population, activities of individuals, and their central place in agriculture and natural productivity. Cash crops grown in tropical agroforestry systems depend strongly on ecosystem services provided by naturally occurring species (Schroth et al., 2000;

Tylianakis et al., 2005). However, the economic, biodiversity and aesthetic value of pollinators is known for relatively few systems (Nabhan and Buchmann, 1997; Delaplane and Mayer, 2000). Various studies have highlighted the positive influences of some particular pollinators (Pimentel et al., 1997; Allen-Wardell et al., 1998; Kearns et al., 1998; Cunningham et al., 2002; Kremen et al., 2002), and that pollinator diversity and abundance can increase pollination rates and fruit or seed set (Rathcke and Jules, 1993; Aizen and Feinsinger, 1994; Steffan-Dewenter and Tscharntke, 1999; Kremen et al., 2002; Klein et al., 2003; Ricketts et al., 2004). Though pollinators do not directly affect ecosystem processes they have the potential to change the structure and diversity of plant communities thereby affecting ecosystem properties (Lundberg and Moberg, 2003).

Natural pollination is often overlooked but at a large cost factor in crop production. There are recorded instances of pollination deficit reducing agricultural productivity (Kevan and Phillips, 2001). In case of poor pollination, costly techniques, such as hand pollination, is applied to enforce fruit set and often with poor results (Westerkamp and Gottsberger, 2000). Fortunately, there is a global concern for pollinator decline and this has resulted in more global studies, in recent times. (Rathcke and Jules, 1993; Kearns et al ., 1998; Wilcock and Neiland, 2002; Ahmad et al. 2006; FAO, 2008).

In Africa pollination appears to be drastically understudied compared to Europe where information exists on a larger proportion of known species (Procter et al., 1996). An extensive review by Rodger et al (2004) revealed that there are still gaping inadequacies in knowledge and understanding of pollination in Africa. It is suggested that similar comparative studies be made

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to obtain information on pollinator conservation and also address the gaps identified (Rodger et al., 2004).

2.3   *Theobroma cacao* L.: Varieties and Mode of Cultivation

Cocoa (*Theobroma cacao* L.) is an allogamous tropical woody species formerly in the Sterculiaceae family and reclassified in the Malvaceae family (Alverson et al., 1999). Inspite of its rich history and continuing significant role in human consumption and potentially in human health, the *T.cacao* tree has received relatively little attention as a crop species (Bennett, 2003) especially in the realms of natural pollination.

Cultivated cocoas show great variability. Based on seed and fruit characteristics and the geographical distribution of cocoa, two racial groups known are: Criollo (or Fine cocoa) and Forastero (*Theobroma leiocarpa*). A third group called Trinitário (mixed group or Hybrid cocoa), probably originated from the hybridization between Forastero and Criollo (Cheesman, 1944; Gordon, 1976). Forasteros can be further subdivided into Upper and Lower Amazon, representing genetic materials responsible for 80% of the world cocoa production (Lerceteau et al., 1992; Marita, 1998). The Upper Amazon accessions, are important resistance sources to the main diseases of cocoa (Adomako and Adu-Ampomah, 2000; Iwaro et al., 2001 and Umaharan et al., 2001). The Amelonado type of Forastero formed the bulk of cocoa in Gold Coast. In the last two decades, Ghanaian cocoa farms have 17.83% Hybrid, 58.52% Amazon, 8.48% Amelonado, and 15.17% mixed group according the Survey data collected by Cocoa Swollen Shoot Virus Disease (CSSVD) Control Unit of the COCOBOD (*n.d*). Eskes (2001) estimated that only, 30% of *T.cacao* cultivated today is of selected varieties. The other 70% of trees are

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traditional populations, and these are grown increasingly from seeds collected by farmers from preferred trees and likely to be inbred (Adomako and Adu-Ampomah, 2000).

Most of the world's cocoa is from small farms and not in large plantations. According to International Cocoa Organisation (2005) 2.5 million farmers produce almost 90% of the world's cocoa on smallholdings between 5-10 acre holders and occur in the biodiversity-rich humid tropics. Even when the average size of holdings is large, such as in Brazil, the small farms greatly outnumber larger plantations. Cameroon and Ghana have typical distributions for farm size, with a predominance of holdings ranging in size from less than 1 ha to 5 or 10 ha. In Nigeria, the average farm size is 1.7 ha, and in Côte d'Ivoire, farms average 2.8 ha. Yet, for nearly all countries, the small-size farms dominate the cocoa landscape (Rice and Greenberg, 2000).

Preparation of cocoa farms follows a similar fashion throughout West Africa. Primary and secondary forests are selectively cleared to establish the cocoa farms (Duguma et al., 2001). This farming system has made cocoa a destroyer of tropical forests (WWF, 2006) as increased cocoa production is done through expansion into areas hitherto undisturbed (FAO, 1996). The trend should be reversed or at least moderated to reduce the rate of forest destruction. Extensive cultivation methods practiced by migrating cocoa farmers as a means of increasing production has, however, been identified as a major cause of deforestation and fragmentation in Ghana and West Africa (World Bank, 1987; MES, 2002; Asare, 2006). Currently, no un-worked forestland is available outside designated forest reserves in Ghana (Hepper, 1986). A viable alternative to

the expansion of uncultivated land as a means of boosting cocoa production is the use of efficient insect pollinators.

2.4   Cocoa Productivity and Production

Productivity, is a term used to describe the rate of production in an ecosystem, and is an important functional property for both the natural and agricultural ecosystems (Viglizzo and Roberto, 1997). The productivity of crops is determined by a set of yield defining factors (e.g. climate, atmospheric CO_2 concentration and crop characteristics), limiting factors (e.g. water and nitrogen supply) and restricting factors e.g. pest and diseases (Goudriaan and Zadoks, 1995; van Ittersum et al., 2003).

Studies on the cocoa productivity have focused mainly on economic factors of production such as land, labour, marketing systems, subsidies on inputs, and labour productivity (Teal, and Vigneri, 2004; Zeitlin, 2004). Dormon et al. (2004) observed that causes of low productivity could be classified into biological and socio-economic factors. The biological factors included the incidence of pests and diseases, most of which, according to the authors have received extensive research attention in Ghana. Scientifically, productivity of cocoa has largely been studied in terms of genetic improvement (e.g. Edwin and Masters, 2005); shade controls (Gockowski et al., 2004); soil improvement and fertilizer (extra nutrients) application and land use (Buresh and Tian, 1997). All these works, however, ignore the role of pollinators whose activities on the cocoa flowers result in the fertilization and subsequent fruit production.

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2.5   Cocoa Flowering and Fruit Setting

Cocoa has small, cauliflorous flowers which are hermaphroditic, with each of five anthers separated from the central stigma by a folded sepal, indicating the importance of vectors in its pollination (Motamayor et al., 2002). Flowers are present year-round, although peaks may occur in early wet seasons (Bos et al., 2007). However, cocoa's reproductive biology is characterized by very high flower: fruit ratios and fruit-set after natural pollination can be increased up to 10 fold by manual pollen supplementation (Valle et al., 1990; Falque et al., 1995). Parthenocarpy in cocoa is rare (Entwistle, 1974; Young et al., 1987).

Stephen (1981) has indicated that floral demographic studies can greatly augment the knowledge in pollination biology and ecology of various plant species, but that of cocoa has been largely understudied. Cocoa flowering is said to be usually abundant, particularly in full sun, with up to 125,000 flowers per tree each year (Lachenaud and Mossu, 1985). However, flowering intensity can vary among genotypes (Paulin et al., 1983). Empirically, flowering of cocoa in Ghana is scanty and spasmodic in the January to March dry seasons, then very heavy at the beginning of the rains usually in April. It then declines to become almost nil during the peak of the main-crop period, but increases somewhat as this crop is removed from the trees. Valle et al. (1990) explain this observation that generally, flowering intensity decreases with increasing number of developing pods per tree due to competition for assimilates. The study of flower phenology of cocoa in Ghana, however, has been very scantily documented in literature.

The influence of seasons on flowering pattern of cocoa (*T. cacao*) in various cocoa growing countries showed that flower production and subsequent pod setting is controlled, either directly

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or indirectly by environmental factors such as rainfall and temperature (Alvim, 1966, 1984; Mohr and Schopfer, 1994; Daymond and Hadley, 2008). It has, however, been observed that excessively dry or relatively cold periods may completely inhibit flowering in regions where seasonal variability in rainfall and temperature persist (Alvim, 1966; Asomaning et al., 1971).

It is generally observed that in plants the number of flowers exceed final numbers of mature fruits (Stephenson, 1981; Bos et al., 2007). The reproductive system of cocoa is characterized by high numbers of flowers, sometimes more than 50,000, of which generally no more than 5% produce fruit set and develop into mature fruits (Entwistle, 1972; Alvim, 1984; Free, 1993; Young, 1994; Bos, 2006). The flowers open about dawn and the anthers usually burst almost immediately (Brew, 1984), though Saunders (1959) observed a period in Trinidad when they did not burst until 09.00hrs. Unpollinated flowers fall within two days following anthesis, whereas hand-pollination can result in only 10% flower drop three days after pollinaton (Paulin, 1981). Falque et al. (1995) examined the distribution of the number of seeds per fruit and concluded that flower abortion and low number of seeds per pod were consequences of the small number of fertilized ovules resulting from insufficient pollination. Kaufman (1975) and Massaux et al. (1976) considered that pollination was adequate whenever the stigma received at least 35 pollen grains, but this figure was based only upon the number of ovules in the ovary (Young, and Sverson, 1994).

Cocoa has been classified as cauliflorous as the flowers and fruit of the *T.cacao* are produced not only on the branches, but also on the trunk/stem. Only a few tropical plants share this peculiar characteristic with cocoa (Alverson et al., 1999; Frimpong et al., 207). Also its pollination

mechanism and pollinating agents are not only unique but are also very controversial and still elusive (Brew, A.H. *unpublished*). The flower has also been reported to possess neither nectar nor fragrance to attract any insect (Cheesman, 1932; Urquhart, 1961). However, studies show that cocoa flowers possess a number of microscopic nectarines on the pedicels, sepals, ovaries, "guidelines" of the petals, and the staminodes. The nectarines secrete nectar which has fragrance that attracts various insects (McGregor, 1976). Cocoa flowers need pollen vectors because the stigmas are surrounded by a fence of staminodes and the anthers are hidden inside cave-shaped petal hoods; thus, anthers and stigmas are widely separated within the flower (Westerkamp and Gottsberger, 2000).

Self-incompatibility (SI) and cross-incompatibility in cocoa were recognized from the 1920's when the wilting and dropping of cherelles were attributed to incompatibility (Pound, 1932; Free, 1993; Dias and Resende, 2001). Latter investigations into the mechanisms by Knight and Rogers (1953 and 1955) and Cope (1958; 1959 and 1962) indicated that an incompatible pollination did not result in inhibition of pollen germination or pollen tube growth. Bouharmont (1960) noted that the pollen tube growth rates in cacao under both compatible and incompatible pollination conditions are similar. Other studies noted that despite the initially higher pod set by self-compatible clones the number of pods at maturity is similar to that in self-incompatible clones (Warren, 1994; and Warren et al., 1995; Jonathan et al, 2006).

The *Theobroma* type of SI is found to be unique in two ways a) the expression of s-gene (recognition) apparently occurs only after the haploid pollen tube and the ovules have come into contact, b) the rejection reaction results, in the abscission of the entire flower, and not the

deposition of callos in the pollen tube (de Nettancourt, 1977). This suggests that s-gene expression occurs at the pollen tube-ovule interface. Recent studies suggest recognition of self-pollen may occur earlier than previously reported. Thus, Theobroma may represent an example of an ovarian SI system, unlike the majority of SI plants. Later studies of Theobroma (Aneja et al., 1992; Baker, 1997) suggested that flower abscission occurs because incompatible pollination is initiated prior to the pollen tubes reaching the ovules. So, the breeding system of cocoa has a sporophytic with a gametophytic control (Ramos et al., 2005). The SI in cocoa therefore justifies the need for cross-pollination, and the nature of the flower makes the pollination process possible only by specialized insect pollinators. This may account for healthy cocoa trees which don't bear enough cocoa pods.

Pollination intensity (PI), the number of conspecific pollen grains received per stigma, was monitored in Ivory Coast (Parvais et. al., 1977; De Reffye et al., 1978) and Columbia (Tovar and Ortiz, 1991). These studies indicated that 53-76% of the stigmas were void of pollen. The authors also examined the distribution of the number of seeds per fruit and concluded that flower abortion and low number of seeds per pod were consequences of the small number of fertilized ovules resulting from insufficient pollination. Falque et al. (1995) studying cocoa aged between 20 and 24 years in Ivory Coast discovered that seed content of pods increased with increasing PI, and reached an average maximum of 58 seeds, an indication of a strong positive relationship between PI and the number of seeds per pod. In all pods, the number of seeds per pod was below the number of pollen grains deposited on the stigma. All pods contained at least nine seeds. The 64 flowers that received less than nine pollen grains all aborted. They also found that flower drop was mainly determined by PI.

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2.6   Fruit Loss in Cocoa

Fruit production in cocoa is beset by fruit losses. Cherelle wilt, the main cause of fruit loss is partially related to pollination intensity, probably because cherelles with few fertilized ovules constitute a weaker physiological sink than those with many fertilized ovules (Falque et al, 1995). Wood and Lass, (1985) and Valle, et al (1990) also concluded that cherelle wilt is a result of competition for nutritional resources. Thus cherelle wilt is important in regulating the number of fruit per tree (Wood and Lass, 1985; Valle et al., 1990; Lachenaud, 1991). Earlier studies have shown that conditions, which favour pod production, also coincide with conditions favourable to disease incidence (Meideros, 1976; Holderness, 1992). Many studies have been done to correlate the incidence of black pod disease in West Africa with environmental factors such as rainfall, temperature, and humidity (Thorold, 1967; Wood, 1974). Efombagn et al (2004) identified fruit aged 2-3 months as the highly susceptible stage of development in the most susceptible clone. Spatial distribution of pods on the trees showed that pods on the trunk were more likely to become diseased than those on the branches due to two main factors: closeness to the upper part of the soil layer (Opoku, 1994) and in shade tree roots (Opoku et al., 2002).

The role of rainfall in the host-pathogen interaction is critical. High rainfall is followed by high incidence of black pod, even in clones considered to have some level of field resistance (Efombagn et al., 2004). Cultural practices can affect the disease cycle for many pathogens. Weekly removal of diseased pods (as recommended by Cocoa Research Institute of Ghana, New Tafo) interrupts the fungal cycle, and eventually lowers disease pressure, by reducing the secondary inoculums source inside the plantation (Ndoumbe, 2002). Yet studies in Ghana shows that cultural control has little or no effect on the incidence of black pod due *P. megakarya*, and even chemical control was ineffective (Opoku et al., 2000).

2.7   Pollination and Pollinators of Cocoa

The pollination of cocoa remained imperfectly understood until Harland (1925) advanced the evidence of flying insects acting as pollinators, but since no one had actually observed a pollinator action, the evidence was widely regarded as an aberration. Harland (1925) and Posnette (1942) postulated that both crawling and flying insects were involved in the cocoa pollination. Billes (1941) made an important contribution by demonstrating that there was no pollination without insects and that some species of ceratopogonid flies the genus, *Forcipomyia* (Ceratopogonidae or biting midges) was the potential candidate in cocoa pollination. He also found that only the females were actively pollinating cocoa flowers, noting the characteristic manner in which midges deposited sticky lumps of pollen grains on the style. However, Billes (1941) never found *Forcipomyia* spp during the dry season and there was little or no fruit set. This confirmed Pound's (1932) suggestion that absence of a pollinator was responsible for low number of flower setting at a period of most abundant flowering. Hernandez (1965) also reported of pollination percentages ranging from 1 to 52% when he studied midges, bees, thrips, and ants. However, he did not indicate how pollination was accomplished. Studies by Winder (1978a) and Brew (1984) revealed that the *Forcipomyia* spp have the characteristics for effective pollination of cocoa. They observed pollinating cocoa in the field, carrying with them more than 35 or so pollen grains necessary for an effective pollination and can easily move between individual cocoa trees and rows.

Recent studies have confirmed that cocoa is entomophilous and dependent on cross-pollination by insect pollinators (Posnette 1950; Cilas 1988; Ibrahim 1988; Klein et al., 2007; Klein et al., 2008). This was corroborated by Groeneveld et al. (2010) who rated pollination in cocoa to be a

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higher order limiting factor in cocoa yield than agronomic resources. Though cocoa pollination is generally credited to midges (Entwistle 1972; Young 1994), insects such as, bees, ants, aphids and thrips are considered potential pollinators or collaborators of pollination (Entwistle, 1972; Young, 1994; See and Khoo, 1996). Their distinctive roles, however, have not been clearly established. Winder, J.A. (*unpublished*) noted that information on cocoa pollinators are important in different regions, but is still lacking even today. It is obvious that much more data on cocoa pollination and pollinators need to be gathered if enough knowledge could be acquired to solve future problems.

The pollinator status of bees, especially, in the cocoa ecosystems has been a subject both of debate and speculation. Young (1986) and Kaufman (1975) have insisted that bees were pollinators. Young (1986) hypothesized those large effective pollinators such as euglossine bees may be the original pollinators of cacao flowers, while midges would only be facultative alternate pollinators with low pollination efficiency. Kaufman (1975) discovered that three different families of Hymenoptera viz: Apidae, Megachilidae, and Halictidae gather pollen from *T. cacao* in Ghana. The author noted that among them the halictine bee, *Lasioglossum* sp was the most efficient pollinator at cocoa canopy level, 3-4m above the ground, pollinating 42% of 107 flowers. Studies in other systems, however, have also concluded that the commonly held conception that honeybees are the primary pollinators may not always be correct (Kevan et al.*,* 1990; Westerkamp, 1991) meaning that the honeybee may not pollinate many plants. It is therefore necessary to confirm the pollinator status of bees found in the cocoa plantations.

Several of the major volatile compounds found in the floral oils of *T. cacao* and other species of *Theobroma* occur in the mandibular and other exocrine glands in various bees, including stingless bees and halictids, known visitors of *Theobroma* flowers. These compounds are particularly present in noncultivated species of *Theobroma* and have much more noticeable fragrances than the seemingly scentless flowers of the cultivated *T. cacao* selected for agriculture. It is hypothesized that the floral attraction system of ancestral or wild (noncultivated) *T. cacao* and other species of *Theobroma* may have evolved to attract certain bees as their principal pollinators in natural habitats in the Neotropics, with a floral reward hypothesized as being sociochemicals needed by bees for mating, foraging, territorial defense etc. Because of the many generations of extensive selection by cloning for desired cultivars, *T. cacao* might have lost much of its original attraction system for bees, instead being pollinated opportunistically by dipterans in plantation habitats. This may explain why natural pollination in cultivated *T. cacao* is generally very poor relative to observed fruit-set in wild or non-cultivated species of *Theobroma.*

Ghana is said to have the most citations on individual species of positively identified pollinators of cocoa (65%), followed by Costa Rica (24%) and lastly Brazil (12%). Kaufman (1975) had discovered some 70 species of Ceratopogonidae; however, only eight (including four species of *Forcipomyia,* two species of *Stilobezzia,* and a species of *Clicoides* and *Atrichopogon*) were commonly found pollinating cocoa flowers. The sub-genus *F. falciniella* (Kieffer) and *F. nana* (Macfie) are definitely known to be pollinators in Ghana, Costa Rica and Trinidad, respectively (Winder and Silva, 1975). Also of the five sub-genera, in Microhelea only *F.inornitipennis* (Austen), two species of Lepidohelea: *F. clastrieri* Dessart and *F. squamipennis* are known to be

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a pollinators in Ghana (Kaufmann, 1974, 1975). Kaufmann (1975) noted that two of the commonest ceratopogonids, i.e. *Forcipomyia inornatipennis* and *F. squamipennis* were active pollinators in Ghana. Her conclusion was that other common ceratopognids were less efficient. Further observation indicated that most cocoa flowers receive very few pollen grains in cocoa farms where midges are the sole pollinators (Kaufmann, 1975). This was corroborated by Massaux et al. (1976) who determined that flies and midges visiting cacao flowers carried an average of only 16-23 pollen grains of cacao. This implies that the midges could not be the only pollinators. Brew (1984) working in the same Ghanaian environment made a counter claim. Examining the structure of the cocoa flower, ability of the insect to collect and deposit the minimum pollen required for successful pollination, and the capability of transferring pollen between trees based on adaptive structure and habits of the insect fauna associated with open cocoa flowers, he inferred that the *Forcipomyia* is the main and only pollinator of cocoa specifying that *F. Europrojoannisia, F.squamipennis, F.ashantii, F.castanea, Stilobezzia* and *Atrichopogon* spp are the most efficient species in cocoa pollination. Although this was contrary to previous claims made by other studies (Entwistle, 1972; and Kaufmann 1973 a, b), it confirmed technical review by Winder J.A (*unpublished*). Though pollinating ability has been assumed by caging adults on branches with open flowers, field observation followed by collection is the best way to demonstrate pollinating ability which is predominantly female activity (Winder, 1978b). Considering the overwhelming available evidence that *Forcipomyia* is the main pollinators of cocoa throughout cocoa growing regions of the world, claims about other potential pollinators need to be corroborated. To date, to the best of knowledge, this has not been done.

Insect collection by Brew (1984) in cocoa experimental farms shows that of the total number of insects present in the cocoa flowers of Tafo and Bunso in the Eastern Region of Ghana, over 80% were crawling insects. Aphids being the dominant species constituted about 30% of the overall total of the arthropods pooled together. The diptera comprised cecidomyiid, the gall midges, drosophila and ceratopogonids. Cecidomyiid, ceratopogonid and drosophila constituted 8%, 7% and 5% of the total insects collected respectively. Ceratopogonidae with all its three genera and eight species were uncommon; even though relatively high number of insects were found in the open cocoa flower.

The involvement of *Forcipomyia* midges in cocoa pollination has not been studied in detail, probably because of the inherent difficulty of observing them in the act of pollination and due to their apparent low numbers (Winder, J.A *unpublished*). In their attempt to collect midges in Costa Rica Saunders and Bowman (1956) failed to observe any *Forcipomyia* midge entering or leaving the flower. Three weeks of sweepnetting yielded only 73 adult biting midges, however, the specimens collected failed to show any pollen on their bodies. Saunders (1957), however, observed that although midges were scarce, and could be observed only by exceptional chance, they might still affect satisfactory fruit set over a period of time.

2.8   Breeding Substrates of Cocoa Pollinators

The breeding substrates or sites of potential and positively identified pollinators of cocoa has been a subject of great interest to scientists of cocoa pollination over the years, as knowledge of them is inseparable in the study of cocoa pollination and, could contribute to sustainable production of cocoa. Saunders (1959) described numerous favourite breeding substrates for

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Forcipomyia. Some extensive observations on the breeding sites have been made in Costa Rica (e.g. Hernandez, 1965; Soria, 1970), Brazil (Winder and Silva, 1972; 1975; Winder, 1977); and in Ghana (Kaufmann, 1973a, b, c). *F.inorenitipennis,* for example, is known to breed in rotten cocoa pods, banana stems, tree stumps, cow, sheep and goat dung in Ghana (Kaufmann, 1974, 1975). In an extensive technical review Winder, J.A. (*unpublished*), observed that ceratopogonid species identified on cocoa flowers breed in wide range of organic substrates, the most important of which are rotten cocoa pods, cocoa leaf litter, rotten banana stems or trunks, water-holding plants, such as bromeliads, and tree rot-holes. The *Forcipomyia* is known to be more cosmopolitan as it has been bred on 11 substrates, among which are rotten cocoa pods, banana stem, leaf litter and water-holding plants; the sub-genus *Euprojoannisia* has also been bred on 6 substrates with cocoa leaf litter being especially important. He however, conceded that breeding sites for pollinating *Forcipomyia* are still poorly studied and therefore largely unknown. Kaufman (1975) threw some light on this issue indicating that the ecological requirements of the midges were close spacing of the cocoa trees to create the right environment, a variety of breeding sites within the plantation including large shade trees with large buttresses, decomposing logs, old hollow tree stumps and cocoa husks. She added that the shade trees and ground substrates should be evenly distributed throughout the plantation, for observations show that cocoa trees within a radius of 5-6m surrounding a midge habitat often bear conspicuously large numbers of pods than trees outside this range.

Kaufmann (1975) proposed two ways to improve the contribution of midges in the pollination process: one is to make the cocoa plantation more attractive to the ceratopogonids, and the other is breeding them. According to the author once these midges take up residence in a cocoa

plantation they tend to remain in it throughout the year, provided that their ecological requirements are met.

Young (1986) suggested that cocoa grown close to forest, particularly large tracts of the natural forest, supports a greater diversity of the cocoa pollinators which may result in more pollination. Preliminary results demonstrated greater numbers of ceratopogonid species could be found in the heavier shaded, abandoned cocoa, and commercial farms with more heterogeneous shade (Young, 1982). Janzen (1973) observed a strong movement of insects into moist refugia in Costa Rica during the dry season. Janzen and Schoener (1968) observed that moist areas possessed more species. Winder (1978a) has suggested that it would seem good practice to intersperse commercial cocoa plantations with area of natural forest, forming a mosaic of refugia serving as a pool for ceratopogonid pollinators during the dry periods.

Ceratopogonids vary in their choice of habitats (Kaufman, 1974); some species are positively phototropic and tend to choose sunny environments, others are negatively phototropic so they live in cool, dark habitats, while still others are intermediate in their tendency toward the sun (Kaufman, 1974). The first group seldom or never visited cocoa flowers. The third group on the other hand inhabited a cocoa plantation at least part of the year and thus is occasional cocoa pollinators. Hence the most important are the negatively phototropic group since they are "permanent" residents of the plantation (Kaufman, 1974). Breeding of midges under laboratory conditions have been discussed by some workers (Winder, 1978b), and others have attempted to produce them with limited success (e.g. Kaufman, 1975; Brew, 1988). Kaufman (1975) has

suggested that a large scale culture breeding may be useful for release in the plantation, especially during the main flowering season.

There is the need to determine whether large scale breeding of the midges could result in more effective pollination, and fruit set and whether the midges have substrate preference. Brew (1988) has noted that piles of moist rotten pod husks dumped well over 120 m, away from cocoa farms have yielded diversity and abundance of midges, indicating that the adult midge species were attracted by the musty scent of the moist rotten pod husks and were able to fly there to breed. Whiles the breeding idea may be good in the controlled experimental environment no attempt has been made to produce them under the farmer's farm conditions. This is because the cocoa pathologists believe that it may lead to increase in the spread of black pod disease. Micro-organisms *Botrydioplodia theobroma* and *Rhizopus oryzae* have been isolated from cultures of midge larval stomach-contents and scrapings of moist rotten cocoa leaf-litter (Brew and Brew, *unpublished*). It is understandable therefore that the build-up of immature pollinator midges coincides with the peak of rains in April-May when populations of micro-organisms are at the peak (Ahenkorah et al., 1987).

From the review it could be inferred that to date important gaps still remain in the knowledge of the biology and ecology of the cocoa pollinators. These include studies of the breeding sites of recognized pollinators, pollinator status of insects which visit the cocoa trees, their efficiency and hence the pollinator importance. Therefore the need for intense studies on these cocoa pollinators cannot be overemphasized.

CHAPTER THREE

MATERIALS AND METHODS

3.1   Survey for Farms Selection

The entire study was preceded by a three months (February –April, 2005) survey in the Ashanti region of Ghana to select farms suitable for the studies. Cocoa farms were visited in Offinso, Kubease, Tano-Odumase, Jachie, Konongo, Odumase, Hiawu-Besease, and Nkawie, Toase, and Duampompo (Fig. 1) to assess their suitability. Factors considered were accessibility to the farm, age of farm, and cooperation of the farmers.

The survey was preceded with an introductory meeting held first with the chief cocoa farmer in the community, and later with the farmers. Briefing was done to explain the objective of the study. During the meetings farmers were allowed to express their concerns and to seek clarification with respect to the study to be undertaken. Farms were visited with the assistance of key informants who were mostly young men.
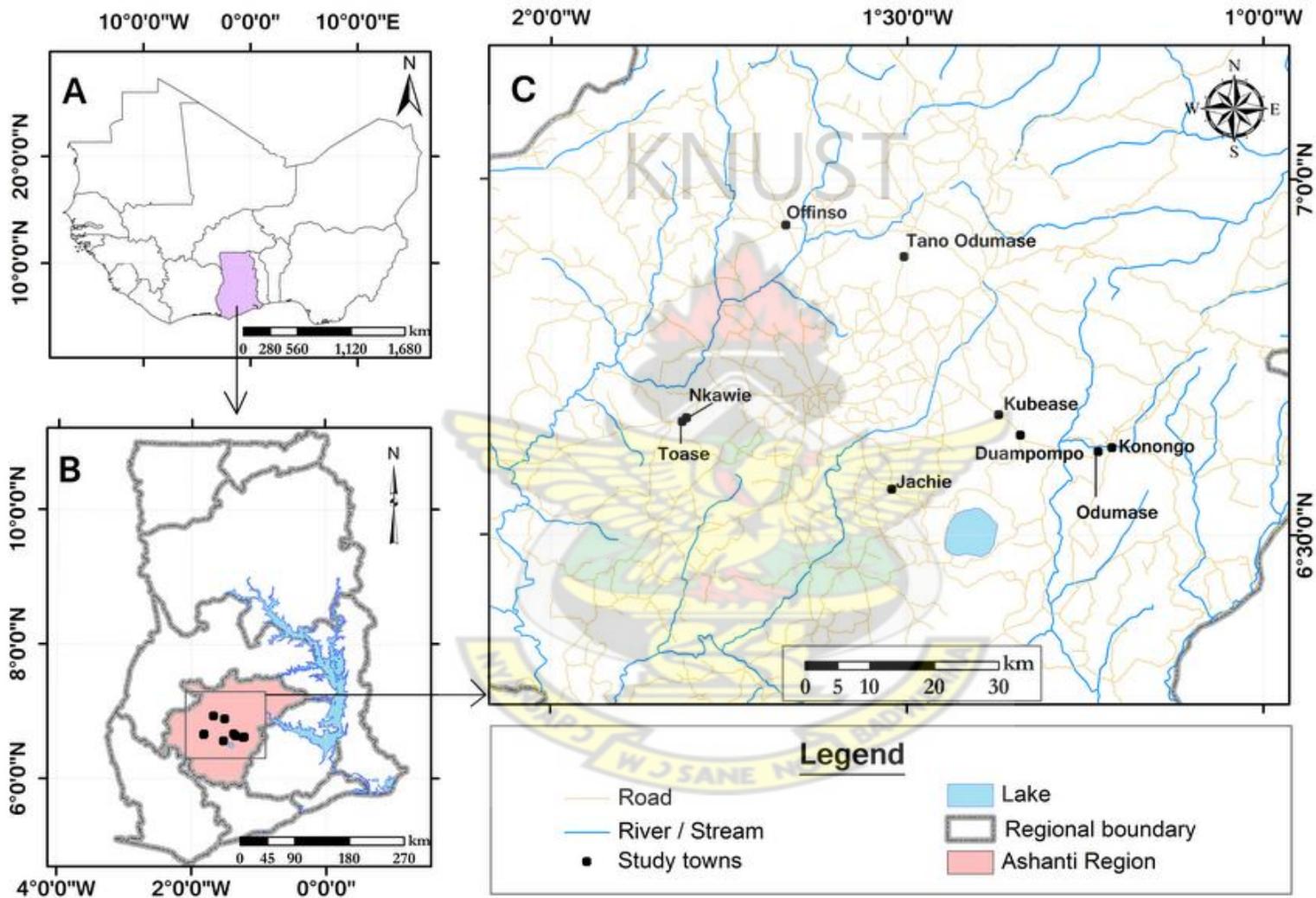
Figure 1: **Map showing the nine locations in Ashanti Region surveyed during the baseline studies.**

3.2 Selection of Study Area and Study Farms

3.2.1 Farm Accessibilty

Farms in eight (8) of the nine selected farming areas were not easily accessible, and were generally scattered. In Offinso for example, the available farms were 20-30mins walk away from the road, and inter-farm distances were very long. Farms in Konongo, Tano-Odumase, and Jachie were similarly distant away from the access roads and this could affect the data collection.

Kubease, in the Ejisu-Juabeng district, Ashanti region of Ghana, however, presented a special case. Of all the nine areas surveyed Kubease was the nearest to Kwame Nkrumah University of Science and Technology (KNUST). It is about 3 km North East of the main Kumasi-Accra Motor Road and 34 km from Kumasi. The farms were generally close to access roads, and were very accessible. Where one needed to walk was the case of Farms nos. 3, 4 and 5, and it took less than 10 mins to reach even the farthest of the farms.

3.2.2 Age of Farms

It was discovered that cocoa farms in the eight (8) districts- Offinso, Tano-Odumase, Jachie, Konongo, Odumase, Hiawu-Besease, and Nkawie, Toase, and Duampompo were very old (between 30 and 35 years). However, ten (10) cocoa farms in Kubease were between 20-25 years and were still productive. They were therefore selected for the study.

3.2.3   Cooperation of Farmers

Farmers in all the nine (9) farming districts in the region were generally friendly, some however, expressed their unhappiness that researchers always come to them for information and yet their research never reflected on them (farmers) in terms of higher production.

At Kubease great assistance was received from the chief cocoa farmer who happened to be a retired Agriculture Officer with the Ministry of Agriculture. The response of the farmers was good with some of them voluntarily offering their farms for the study. Key respondents assisted in the selection of farms as they provided vital information on the farm location, farm owners and the farm caretakers. Ten farms located around the Bobiri Forest Reserve were selected for all the studies. Kubease was therefore selected because of the location of the farms, the age of the farms, and general attitudes of the farmers.

3.4   The Biophysical Characteristics of the Study Area

The Bobiri Forest Reserve is situated in the Juaso Forest District within the administration of Juaso sub-district and Mampong in the Ashanti Region of Ghana. It lies between latitudes 6^0 44' and $6^0$40' North and longitudes $1^0$15' and $1^0$22' West, and about 180 to 240m above sea level (source: Gold Coast Survey Field Sheet No.129). The natural forest belongs to the *Triplochiton-Celtis* Association of the Tropical Moist Semi-Deciduous Formation, the South East Subtype (Hall and Swaine, 1981), and is the number 39 of the Forest Management Unit (FMU) according to the Forestry Commission of Ghana. The

Bobri Reserve Forest has an area of 54.6 km^2 and hosts a Butterfly Sanctuary which is a major tourist's attraction in the Ashanti Region.

The area has an annual average temperature of 26.5 ($\pm$ 1.44) ^{O}C, relative humidity of 86.1 ($\pm$ 3.55) %, and a mean monthly rainfall ranging between 19.1-235.1mm, with the peak of rainfall being in June. The area experiences two rainy seasons: the major season (April to July) and the minor season (August to October). Thus, there are clear seasonal fluctuations of wet and dry seasons, but there may be short, dry periods when rain does not fall. The climate is marked by high incidence of solar radiation and relatively little variation in day length.
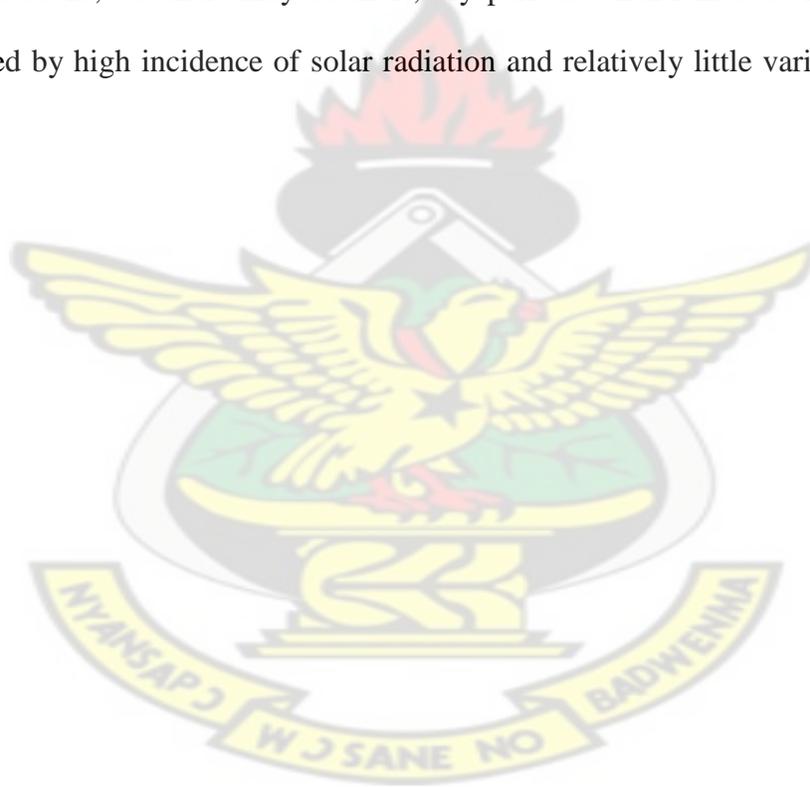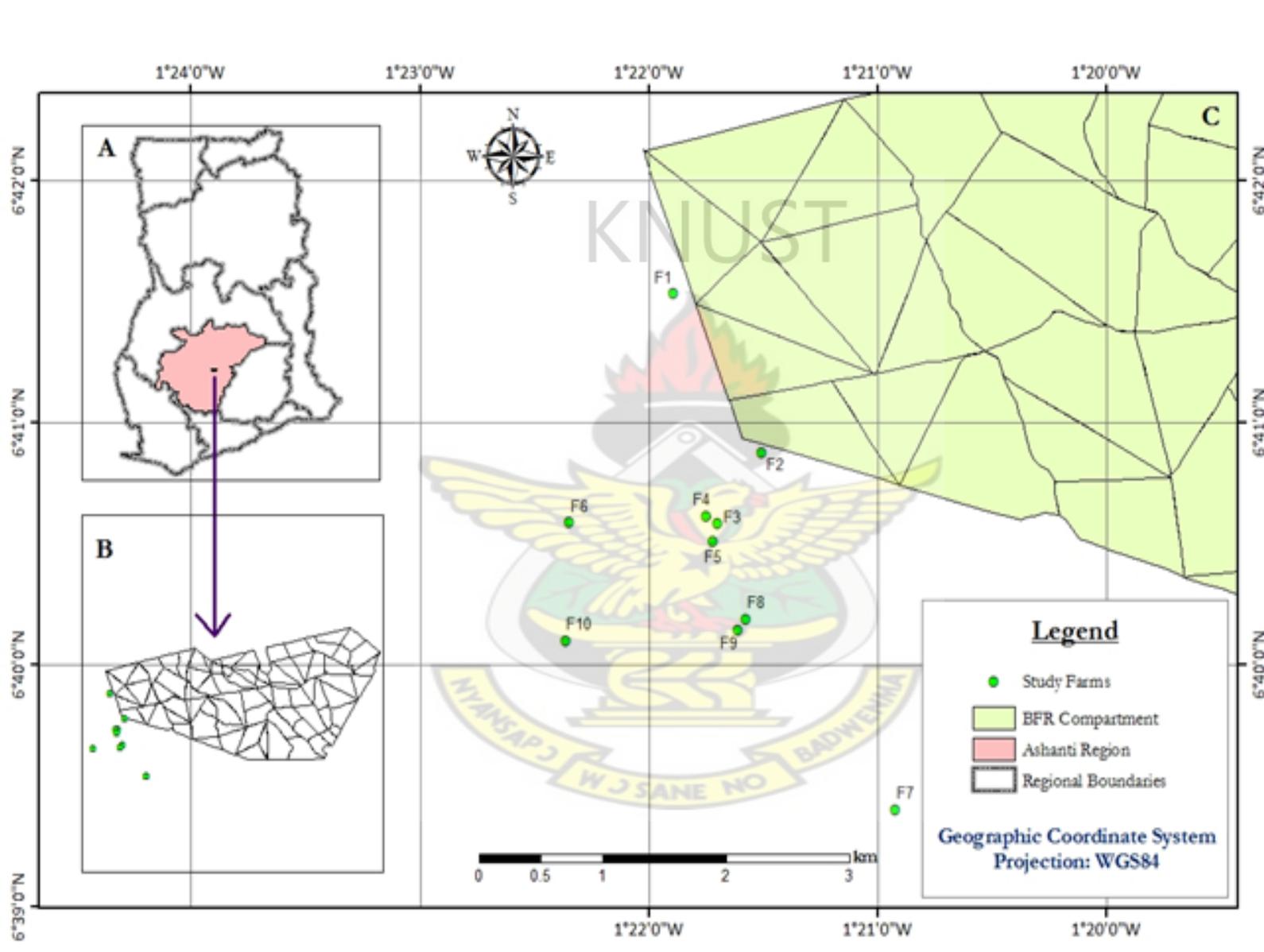
Figure 2: Map of Ghana (A) showing Study Area (B) and location of of farms in relation to the Bobiri Forest Reserve (C).

3. 5 Characteristics of Study Farms and Farm Plots

The selected farms were generally small, family-owned, characteristic of most cocoa farms in Ghana (Tiffen *et al.,* 2004), and typical of cocoa-producing landscapes world-wide, comprising of many small farms and not large plantations (International Cocoa Organisation, 2005). The sizes of the selected farms ranged from 0.2 to 2.5 ha (Table 1).

Table 1: Profile of the ten Selected Farms at Kubease

Farm no.	Age of farm (yrs)	Distance from forest Reserve (m)	Size of Farm(ha)	No. of Trees per Farmplot (400m^2)	Average inter-tree spacing (m)	Mean Height. of Cocoa Trees (m)
1	25	96	2.5	46	2.2	10.52 ± 0.63
2	20	171	0.6	49	2.5	7.30 ± 1.47
3	25	280	0.8	31	2.3	10.19 ± 0.97
4	25	290	0.2	47	2.8	8.56 ± 0.13
5	21	300	0.5	38	2.8	9.60 ± 0.78
6	25	1340	0.8	25	3.0	6.11 ± 0.38
7	25	1730	0.9	49	2.3	9.42 ± 0.34
8	23	670	0.6	54	2.4	7.91 ± 0.40
9	23	770	0.7	43	2.2	8.12 ± 0.66
10	25	1710	0.3	53	2.1	8.71 ± 0.46
Total number of cocoa trees per farm plot				**435**		

Source: Field work

Three of the ten Farms (nos.1, 7 and 6) were characterized by diverse shade trees of great economic value. Among them Farm no.1 was the most heavily shaded with thirty-one different tree species. This was followed by Farm no. 7 with twenty-five tree species and then Farm no. 6

with thirteen species. The other farms had scattered trees at the farm fringes with some banana and/or plantain trees interspersed with cocoa trees. To ensure standardization farm plots without shade trees were demarcated within the selected farms. This conforms to the preference of Ghanaian farmers for cocoa trees under unshaded conditions because of the higher short-term profitability. Unfortunately, however, cocoa trees under such unshaded conditions have much shorter growing cycle (Obiri *et al.*, 2007). It is therefore expected that findings of this study would be applicable to the present agricultural practices in the cocoa sector.

The number of cocoa trees per plot of the ten selected farms ranged from 25 to 54 trees (Table 1). Differences in the number of trees could be attributed to the fact that the cocoa trees were planted at random without regard for CRIG's recommended spatial arrangement. The average inter-tree spacing of farms ranged from 2.10m to 3.02m (Table 1), while the recommended spacing by the Cocoa Research Institute of Ghana is 3.00m x 3.00m (Osei-Bonsu *et. al.*, 2002). The apparent disregard for recommended planting distance between cocoa trees by the farmers is called the 'Atedwe' system. This is a planting method acceptable among cocoa farmers in Ghana, probably as a way of maximizing land-use.

The heights of the trees were generally high. Farm no. 6 had the lowest mean height of 6.11($\pm$ 0.38) m, whiles Farm no.1 with the tallest set of trees had an average height of 10.52 ($\pm$ 0.63) m (Table 1). Among the ten farm plots the tree height range (N=100) is 5.42 m to 11.52 m. Consistent with descriptions of flowering on older cocoa trees (Alvim, 1984; Young, 1986) the cocoa trees found in the area had considerable flowering on both the trunks and branches.

The predominant cocoa varieties found in the area were the Amazon cocoa genotype (Table 2) which is self-incompatible, with late-acting incompatibility system (Seavey and Bawa, 1986;

Lockwood, 1977). It is also known to be high-yielding and early maturing, and was of the same size, and overall healthiness. Self-compatibility and autogamy were associated with reduced pollen limitation, presumably because the capacity for self-fertilization decreases reliance on cross-pollination by pollinators (Larson and Barrett, 2000). Canopies of the trees were closed and overlapped each other, a common feature in cocoa plantations in Ghana. All the ten farms were well maintained by farm assistants or caretakers.

Table 2: Some characteristics of the 10 selected Farms[1]

Farm plots In The Study Area			GPS Data	Elevation (m)	Percentage of Cocoa Variety (%)	
Farm Group	Farm Plot	Distance from Forest edge (m)			Amazon	Hybrid
1	1	96	6^0 41'32.2s N 1^0 21'53.6s W	234	70	30
	2	171	6^0 40'52.5s N 1^0 21'30.5s W	232	60	40
	3	280	6^0 40'36.4s N 1^0 21'44.5s W	244	55	45
2	4	290	6^0 40'36.8s N 1^0 21'45.0s W	261	60	40
	5	300	6^0 40'30.5s N 1^0 21'43.3s W	214	76	24
	6	1,340	6^0 40'35.2s N 1^0 22'20.9s W	225	65	35
3	7	770	6^0 39'24.0s N 1^0 20'55.4s W	262	75	25
	8	670	6^0 40'11.3s N 1^0 21'34.6s W	248	75	25
	9	1,730	6^0 40'08.7s N 1^0 21'08.9s W	231	70	30
4	10	1,710	6^0 41'05.9s N 1^0 22'22.0s W	175	76	24
Mean Percentages of Cocoa Variety					**67.6 ± 3.1**	**32.4 ± 1.2**

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[1] Farms were grouped using distance from the Bobiri Forest Reserve.

Plate 1: A study farm with trunks of the trees marked for the studies.

3.6   General Sampling of Farms, Farm plots and Study Trees on the plots

Stratified sampling of the farms was carried out based on the distances of farms in relation to the

boundary of the Bobiri Forest Reserve. Distances from the reserve forest to the individual farms

and between farms were calculated using the Global Position System (12 Satellite Garmin e Trex

Venture) and data were transformed. Two of the farms (Farm 1 and 2) were located closest to the

forest boundary (Table 1) being 96m and 171m, respectively, away from the forest boundary.

They were followed by Farms 3, 4 and 5 (280m, 290m and 300m respectively). Farms 7 and 10

which were farthest were 1.73km and 1.71km respectively from the reserve (Table 1). The rest of the farms were situated at different distance ranges to the Reserve boundary. Inter-farm distances also varied greatly with Farms 3 and 4 having the shortest inter-farm distance of 20 m while distance between 5 and 6 was 1,160m. For the purpose of the study farms were grouped into 'distance blocks' (Table 2) thus creating a distance gradient. Due to the non-uniform shapes and different sizes of the farms, a standard plot of $400m^2$ (20m x 20m) was demarcated within the farms avoiding edge effects. Thus, ten farmplots were used in the entire study.

The cocoa trees for the study were also selected by simple random sampling. Trees on each plot were first censused and numbered. The numbers were recorded on paper slips and placed in a bowl. The slips were drawn randomly without replacement, avoiding any bias, and the number on it indicated the tree to be tagged for the study. Thus, a total of 100 cocoa trees were used in the study. The trees were tagged using number coded red ribbon. The red colour of the ribbon was consistent with the belief by the farmers that red materials or fabrics are anti-dote to squirrel attacks and therefore most acceptable to them. Small insects, including midges visiting cocoa flowers were easy to census in these habitats. Land-use and all the cultural practices by the farmers did not change within these core areas during the study period.

3.7   Study of Floral Phenology of the Cocoa[2] Trees

3.7.1   Floral Census

For three consecutive flowering years (2006, 2007, and 2008) monthly examination was made on each tree for counts of flowers, buds and fruit-set to determine their seasonality. The studies covered three rainy and three dry seasons. Incidence of flower bud, flower opening, and fruit

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[2] Refers to Upper Amazon cocoa which constituted 67.6 percent of cocoa varieties in the study area.

abortion were recorded as determinants of final yield. Based on preliminary floral census of fifty

cocoa trees on five farm plots was carried out each day. Data collection from each tree lasted

between 20 and 25mins. Thus, one complete cycle of flower census was done in two days.

Whole-plant flower census from trees with height ranging from 5.42-11.52 m was not possible.

To ensure standardisation the protocol developed by the Cocoa Research Institute of Ghana

(CRIG) whereby 1-m with upper and lower paint ring 30 cm from the base marked out on the

trunk for such tall trees (Sarfo et al., 2003) was adopted.

Data collected included numbers of mature flower buds, open flowers, new pods or cherelles

(which gave an estimate of pollination under field conditions), small pods (5-10cm), medium

pods (11-60cm), and large pods (>60cm); number of diseased pods, aborted pods (or cherelle

wilt) and vertebrate (squirrel) attacked pods. The different pod sizes were counted to track

changes in pod size over time, and to determine which pod size had the most vertebrate damage.

The data collection started from the onset of rains in April to the end of December each year for

three seasons.

3.7.2   The Flower Stability

The study was carried out during two dry and wet seasons prevailing in Ghana to determine the

flower stability (how long the flowers stayed when un-pollinated) of cocoa flowers. Stratified

sampling of the cocoa trees in the farm plots was used for the study based on distance gradient

determined earlier, and availability of flowers on the trees. The sample size for the farm plots in

group one within 96-171m was estimated at 22 cocoa trees (N=95). Similarly, sample size for

farmplots in group two (280-300m) was 27 trees (N=116), group three (670-1,340m) was 29

(N=128) and that for farmplots in group four (1,710-1,730m) was 33 trees (N=96). Thus the sample size was proportional to the size of the stratum.

Five freshly opened flowers per tree were selected and covered to prevent contact with any insect. Thus 110, 135, 145, and 165 flowers were used in the study for each set of observation. The number of both pollinated and unpollinated flowers that stayed after the second day was recorded each day till the fifth day. A second set of flowers (i.e. 110,135,145 and 165) were selected and manually pollinated following the standardised manual cross-pollination (Falque et al., 1995; Bos et al., 2006). It was carried out by rubbing three anthers from flowers of three different trees against the subject flower's stigma. This method ensured the likelihood of optimal pollination. The number of the pollinated or unpollinated flowers that remained after two days were recorded. The two treatments were then compared to determine the floral stability. The whole process was repeated for each season while observing all exogenous factors affecting floral stability.

3.7.3 Climatic factors

Data on temperatures and relative humidity were measured by means of Data logger Hobo Pro temp/relative humidity three times per habitat unit. The light intensity per study site was measured with Digital light meter (Extech model 401025) 0-2000 Foot Candle (Fc) range, under standardized conditions (on the ground and on sunny days, local time 09.00 a.m.). Rainfall values, however, were taken from the meteorological station of the Forest Research Institute of Ghana of the Council for Scientific and Industrial Research.

3.7.4 Data Analysis

The design of the experiment was Randomised Complete Block Design (RCBD) with ten replications. The count of flowers and other parameters taken during the three flowering seasons

were pooled together for all the ten farm plots. The flower count data were subjected to square-root ($\sqrt{X + 0.1}$) transformed before analysis. All the data were backtransformed to the original values before plotting of graphs and interpreting them. For the effect of climatic conditions (temperature, relative humidity, rainfall and light intensity) on floral production, regression analysis was performed between climatic factors and the flower count, and complemented by Pearson's correlation analysis. Estimated rate of fruit set per month is the new pods divided by the sum total of floral productions and expressed as a percentage. Stepwise Multiple Regression General Linear Models of climatic variables on floral production was carried out to determine the factors affecting the rate of floral production. All the data collected were subjected to GLM and the means separated using the Student Neuman Keul (SNK) at 5% significant level. Percent pod (fruit) loss was calculated by dividing the value of fruit loss by the sum total of the healthy and diseased pods. The flower-stability ratio was calculated as percentage of the initial number of flowers. All analysis was carried out using SAS, 2005, version 9.0 Monospace.

3.8.0 Insect Assemblage in the Cocoa Plantations and their Pollination Status in relation to the Cocoa Trees.

3.8.1 *Insect survey*

For three consecutive years (2006, 2007 and 2008) insect surveys were carried out in the ten study plots. The aim was to determine the insect species diversity and abundance on each plot, their relative proportions, and also determine whether they are pollinators or contribute to the pollination of cocoa. The sampling period which fell in the wet seasons (April-October) was based on earlier study of the floral phenology of the cocoa trees in the plantations when flowers were available on the tree trunks and therefore were more accessible to the actual and potential pollinators. Trees used were selected based on availability of flowers. For each day, two of the six

techniques listed below were employed in sampling the insects between 06.00 and 18.00 h GMT in each study farm. The pan trap sampling, however, was used on each day of sampling. The sampling tools were thus tested for their suitability for the various insect species.

a) *Hand height flower/insect collection*

Thirty randomly selected cocoa trees from farms 1 and 2 were subjected to the direct flower-related sampling. The hand-height flower insect collection protocol of the Cocoa Research Institute of Ghana (Brew, 1984) was followed. For every 15 min per tree (n=30) 100 cocoa flowers were randomly hand picked in a top-down fashion 24 h after opening of the new flowers. The insects were caught by trapping the flowers swiftly between a 2.5 cm tube and its stopper. They were kept and brought to the laboratory for microscopic examination for the presence of insects.

b) *Sweep netting sampling*

Arboreal insects close to the cocoa trees were sampled using sweep-net ($d =30$ cm). Insects were captured by three sweeps between the ten cocoa trees within each farm plot following the method by Klein et al. (2002). The catch was then aspirated into storage vials. Insect samples were rendered immobile by placing them in a cold chest for some few minutes before sorting out.

c) *Pan trap sampling*

Following the Standardised Toolkit for Monitoring Pollinators procedure (Potts, 2005), pan traps consisting of three different coloured plastic bowls (blue, white and yellow) of 20cm in diameter, containing water (about 80% full) mixed with several drops of detergent solution were placed in the open ground with no tree canopy directly overhead, and at distances of 5 m apart on sunny

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days. The traps were left on the farms for 2 days per treatment, and care was taken to avoid insect rot. This was the only sampling method that was used in both dry and wet seasons of the year.

d) *Aspirator sampling*

Adult flying insects fluttering around bark of cocoa trunks, including cocoa pods, and those resting festooned between buttresses of large shade trees were caught by sucking the air around them. Samples were treated like those captured by sweep net.

e) *Pirbright Light Trap sampling*

The Pirbright light trap which has been reported to be most efficient way of sampling the midges (Brew, 1984) was employed in further sampling of the insects overnight (19.00h GMT-6.00h GMT). This trap was operated with 12 V bulb, built-in fan and vehicle battery (13 plates).

Plate 2: Purbright light trap (arrowed) used for insect sampling[3].

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[3] The Purbright light trap was operated from 19.00h to 06.00h GMT

f) *Focal patch observation*

To determine the pollination mechanism of the Amazonia cocoa a complementary study of the regular floral insect visitors and assessment of their pollinator activity was done through a focal patch observation (Frankie et al., 2002; Potts, 2005). This method has been extensively used in agro-ecosystems (Pontin et al., 2006; Haynes and Crist, 2009).

Experiment 1

Within the 1-m trunk section of thirty (30) selected cocoa trees from the ten farm plots, insect visitors to the cocoa trees and flowers were carefully recorded together with the spot/place of capture. This was done for 10 min within four periods of the day (06.00-08.00 h GMT; 10.00-12.00 GMT h; 13.00-15.00 GMT h; 16.00-18.00 GMT h). Visiting insects were captured using the putter and the flowers they visited gently removed and further observed under laboratory conditions. To obtain unbiased data, each day was dedicated to a farm, but the order was varied. Thus, all the farms were treated every ten days during the flowering seasons.

Experiment 2

A further investigation was undertaken in the field to determine whether crawling insects were involved in the pollination of the cocoa flowers. Ten trees in Farm plots 1 and 2 which were infested with psyllids and ants were selected when flowers were in abundance. Fifty freshly opened flowers per tree were tagged, and grease-banded at their base. The grease was to prevent any crawling insect from reaching the flowers. An equal number of flowers were tagged but not grease-banded to serve as control. The flower set was observed every ten days for three months (May-July, 2008). In all cases the activities of insects that visited the flowers were carefully

observed. Those that carried pollen to the stigmas were noted. A "true visit" was defined as an insect landing on a flower and collecting nectar and/or pollen from it (Ricketts, 2004). In all a total of 200 observations were done in five months (May-September, 2008). Estimate of total pollen deposition per flower was used as proxy for pollination services (Kearns and Inouye, 1993), and hence the pollinator importance of insect visitors.

Experiment 3

Following the outcome of the focal patch observations above a detailed observational study was carried out during the peak of the flowering seasons (May and June) of the years 2008 and 2009 to confirm the pollinator status of the honey bees in the cocoa farms. Six cocoa farm plots were selected by stratified sampling based on the distant gradient defined as above. A total of 12 healthy, flower-laden cocoa trees (i.e. two trees per farm plot) were randomly selected from the farm plots. Following the protocol of Cocoa Research Institute of Ghana, Tafo (CRIG) flower patch within 1-m section of the tree trunk was earmarked for each cocoa tree. Fifty (50) fully opened flowers were selected and pin-marked on each tree for observation for a period of 15-minutes concurrently, with the help of an assistant. Thus, 100 flowers on two trees were concurrently observed per session. Based on previous results of insects' visit to the cocoa trees the observations were carried out between 06.00h GMT and 12.00h GMT and between 15.00h GMT and 17.00h GMT. The activities of the bees on these flowers were observed at a safe distance (ca. 0.5 m) ensuring minimal movement and avoiding shadows being cast on the patch.

3.8.2   *Insect Identification*

All the insects captured were identified to the morphospecies level and were stored temporarily in vials containing 70% ethanol. Insects that eluded identification were coded for later identification.

Further identification was done using reference collection at the Cocoa Research Institute of Ghana insectary. When identification was not possible because of missing parts or if there was uncertainty, insects were classified as "not identifiable" and left out of the analysis of species richness. Due to taxonomic difficulties the identification of the midges was limited to the genus. Total number of individual insect and species for each of the 10 farm plots were then recorded.

3.8.3 *Data Analysis*

The catch from all the various replicate sites for the entire sampling period were pooled together for analysis of the abundance and diversity of the insects in the ten cocoa farms. Data was tested for Poisson distribution as described by Clarke and Cooke (1992), and square root ($\sqrt{X+0.5}$) transformed to make their distributions approximately normal before analysis. They were then subjected to ANOVA and the means separated by Tukey's (*w*) test for pairwise comparison at α = 0.05 level. Multivariate analysis of variance (MANOVA) was used in the analysis of insect count data to determine the spatial distribution using farm plot location (within the three distance blocks) and population i.e. insect numbers sampled (nested within location).

The species diversity of samples on the farms was calculated as Shannon-Wiener diversity index *H'* (Magurran, 2004) using the Estimator SWin800 software package (Colwell, 2006). Widely used in ecological studies, *H'* is largely independent of sample size and is sensitive to the presence of rare species (Anderson, 1978).

Flower set was used to indicate pollination under the field conditions; while estimate of total pollen deposition per flower was used as proxy for pollination services (Kremen et al., 2004). Pollinator importance was calculated as the product of pollination efficiency and visitation rate of

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a given pollinator (Bloch et al., 2006). The pollination efficiency of different insect visitors was measured by the number of pollen grains deposited by a single pollinator species on stigma lobes, while the visitation rates/frequencies was estimated by counting visits/ abundance of foraging insects. Two variables were also estimated: i) species richness i.e. number of morphospecies per census, and ii) total visit frequency rate (by all potential pollinators) expressed as number of visit x $10mins^{-1}$ x $flower^{-1}$. Only insect visitors contacting stigma (i.e. those actually or potentially performing pollination) were included in the analysis.

All analysis was performed with the Statistical Analysis System (SAS version 9.0, SAS Institute, 2005) and SPSS version 17 and were considered at an overall significance level of $\alpha=0.05$.

3.9.0 Comparative Evaluation of Breeding Substrates for Cocoa Pollinator in Cocoa Farms

3.9.1 *Field Procedures*

Following Young (1982) a field experiment was conducted during two flowering seasons (June – July in both 2008 and 2009) using six farm plots represented in the distance blocks i.e. Farm plots 1, 3, 4, 5, 7, and 9 (Fig 2). No chemical inputs such as fertilizers or herbicides were used in these selected farms, a practice which is typical for the area. The experiment was a randomised complete block design (RCBD) with four treatments and replicated three times. Rotten cocoa leaf-litter, rotten fallen trunks of banana trees, and rotten cocoa pod husks all of which were readily available on all the farms in the study area were collected from different places within each farm and placed in a wooden quadrat measuring 1 x 1 m. For eight consecutive weeks a total of 160kg (20kg/tree/week) of rotten cocoa leaf-litter were distributed around each of three randomly selected cocoa trees in each farm. The gathering of substrates was started in the month

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of May barely two weeks into the beginning of the rainy season. Similarly, 160kg of rotten banana stem, and 160kg of rotten cocoa pod husks per farm, were collected in three replicates. The period chosen for this study was the wet season of the year, and the peak of flowering (Table 8; Fig 3). It is also the peak of breeding of pollinators and pollinating activities (Brew, 1984). Three cocoa trees left untreated served as the control in each farm. Thus, a total of 72 cocoa trees and 3600 freshly opened flowers (i.e. 50 flowers per tree) were used in the experiment each week. The targeted flowers were labelled with a number tag that was attached by needle to the tree. As the flowers had a high production turnover and followed a sequential flowering pattern, new flowers were tagged at the beginning of each week and mid-week in order to maintain the required number. The substrates were known breeding places of the midges therefore it was assumed that the population of the midges increased with the quantity of the substrates (Young, 1986).

3.9.2 *Data collection*

The relative importance of the substrates was evaluated using the following prematurity parameters of yield:

1) Flower set (an indication of successful pollination)
2) Flower abortion (a prematurity loss of flowers)
3) Fruit set (indicated by cherelle formation)
4) Fruit abortion (indicated by cherelle wilt).

The flower set was characterised by slight increase in purple coloration in the petals and sepals after 24 hours coupled with drying of the sepals and this is completed within 36 hours.

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Cherelle formation was counted every 48h aborted fruits (pod loss) were counted weekly for the eight weeks. This was based on their rate of production. The impact of the midges on cocoa pollination therefore, was determined by the number of targeted flowers that were pollinated as the substrates increased in quantities. Results were also used to determine which of the substrates was much utilized by the midges for breeding and pollination purposes.

Although experimental and control plots were generally mixed together in the farms it was assumed that cocoa trees with greater densities of midges breeding beneath them have far greater fruit set because midges generally visit the flowers of nearby trees first, before moving to others (Young, 1986). Presumably, trees in control plots had less population of midges compared to those with substrates. A greater amount of pollinating activity resulting from an increased abundance of ceratopogonid midges was expected to be manifested in higher level of fruit set (Young, 1986).
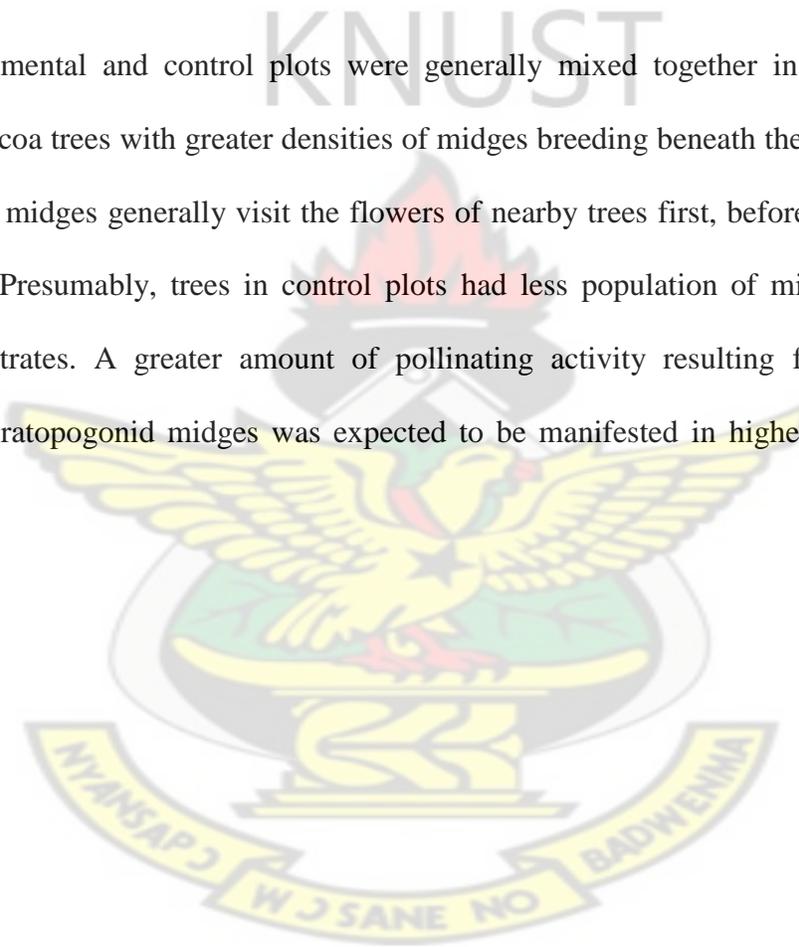
Plate 3 Decomposing cocoa leaf litter as breeding substrate of midges under a cocoa tree

Cocoa pod husks

Plate 4: Decomposing cocoa pod husks as breeding substrate

Banana pseudo stem

Plate 5: Decomposing banana stem as a breeding substrate of midges

Plate 6: Mature floral buds (arrowed) due for abscission[4]

3.9.3 *Laboratory procedures*

A laboratory experiment was designed to determine which substrate supported the highest population of the pollinating agents of cocoa. The design was completely randomized design with four replications.

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[4] Opened flowers were pollinated within 24 hours of opening.

Plant material: Rotten cocoa husks, rotten cocoa leaves, and rotten banana stock were collected from the study farms (Kubease) and kept in polythene bags in the laboratory.

Substrate preparation: Rotten cocoa leaves (50g) and husks, (50g) and rotten banana stocks (50g) were homogenized separately with 50 ml of water, and the liquid extracts obtained by filtration through gauze. The extracts obtained were kept in a refrigerator until needed for the experiments.

Bioassay: Six emergence boxes measuring 20cm x 20cm x 20cm each with 4 circular holes (d = 2cm) on the lid, were filled with 3kg of a common rotten substrate i.e. cocoa pod husk.

The boxes were subjected to the following treatments: 2g of cotton wool was wetted with 1ml of the rotten cocoa leaves extract and packed tightly at the bottom of a test tube (l =150cm; d = 24cm). A second test tube was similarly treated and positioned directly opposite the first test tube. The third and fourth test tubes were also packed but with cotton wool wetted with water to serve as controls. The four test tubes were placed upside down over the circular holes of the emergence box containing the substrate. An incandescent light was set about half a meter over and away from the box. The laboratory had a relative humidity ranging from 25 to 78% and temperature of 23 to 31°C during the time of study. The relative humidity was measured using hygrometer, whiles the temperature was measured by means of glass (mercury) thermometer after calibration. The set up was observed for 14 hours each day for 7 days per month for three months and the number of midges that emerged from the box into the test tubes were counted. The whole set up was thus repeated using extracts of rotten cocoa pod husks and subsequently the rotten banana stock, but with the same cocoa pod husk as substrate. The number of midges counted was used as an indication of which extract attracted most midges.

A follow up experiment was conducted to determine which substrate produced most adult midges. The six emergence boxes were divided among the three substrates i.e. two boxes for cocoa leaves, two boxes for cocoa pod husks, and two for banana pseudostem. All the four test tubes per box were packed with cotton wool wetted with distilled water, and placed on the boxes as in the first study, and observation made on midges' emergence for 10 days per month for two months. The substrates were periodically moistened with the distilled water to maintain suitable environment for the midges. The number of midges observed in the test tube indicated which substrate contained the highest population of midges.

Plate 7: Emergence boxes with substrates. Test tubes tubes with common attractant.

Plate 8: Emergence boxes containing substrates. Test with extracts (treatments) and water (control).

3.9.4 *Data analysis*:

The field data were analysed by analysis of variance (ANOVA), using Statistix 8 software. Least significant differences (LSD) were calculated and the probability of treatment means being significantly different was set at $P = 0.05$. The number of flower sets, cherelles and aborted fruits were count data and therefore the data was square root ($\sqrt{X + 0.1}$) transformed before analysis was done. Regression analyses were performed to determine relationships between substrates and flower sets, cherelles and aborted fruits.

The Laboratory study design was Completely Random Design with 4 replicates. Two sets of analysis were carried out after the data was square-root ($\sqrt{X + 0.1}$) transformed, to determine which of the substrates was most preferred for breeding. The first set of analysis was a series of t-tests to compare the attraction level of water and the substrate extracts. Since the base substrate cocoa pod husks, and the control, water were constant, the results would indicate which extract was preferable to the midges. Orthogonal contrasts of the water (control) against any two of the substrates were analysed. The second set of analysis involved a one-way ANOVA of midges' emergence from the six boxes. Determining the population of the midges in each substrate was not easy because of the size of the insects, and their dispersion within the substrate, therefore the population was indirectly determined by the rate of midge flight per hour (i.e. average number of midges that emerged from the substrate per 10 mins x 6) from each substrate. All laboratory analysis was done using the SAS 9.0 (2005) version.

3.10 Natural and Artificial Pollination

3.10.1 *Pollinator-exclusion experiment*:

A pollinator-exclusion experiment spanning six (6) consecutive weeks was carried out in the rainy season (May- June, 2009) to determine whether pollination is a limiting factor and thereby establish its importance for successful fruit-set in cocoa. To these ends five (5) cocoa trees bearing mature floral buds about to undergo anthesis in 24 hours were purposively sampled in Farm plot 1. For each week fifteen mature floral buds per tree were selected, allowed to flower and subjected to treatment. Observation was done for five (5) days. Each treatment was repeated the following week. Thus, thirty (30) flowers per tree were selected for a treatment every two (2) weeks. In all a total of 450 flowers i.e. 90 per tree were involved in the study. Three treatment regimes were carried out: 'open pollination' (i.e. pollinators had unhindered access to flowers), which served as control; hand-pollination of flowers following [18]; and total exclusion of pollinators using pollinator-exclusion tubes. These tubes had fine (1mm) nylon mesh covering the anterior, while the posterior was stuck in a gummy material applied around the flowers, on the stem of the cocoa. "Percent fruit set was used as the measure of fertility because pollen limitation has been reported most often in terms of fruit set" (Larson and Barrett 2000; Ashman et al., 2004).

3.10.2 *Natural Pollination Versus Artificial Pollination*

For two consecutive years a complimentary field experiment was carried out to compare fruits produced under natural pollination with that of artificial (hand) pollination conditions. The purpose was to answer the research question whether there was any significant difference in the number of seeds and weight of the fruits. In May, 2009 a total of sixty (60) flower-bearing cocoa trees were selected through systematic sampling from Farm plots 7 and 8. These trees were re-

marked for identification. Thirty (30) of them were artificially pollinated following Falque *et al* (1995), whiles the other thrity was left for natural pollination. Ninety mature fruits made up of small fruits (5-10cm), medium (11-60cm) fruits, and large (>60cm) fruits were randomly selected, harvested, and weighed with the help of manual balance in grams /kilograms, and the seeds counted between October and November, 2009. The whole experiment was repeated in 2010 to validate the results of the previous year.

3.10.3 *Proximate Analysis*

Proximate analysis using the Association of Official Analytical Chemists (2005) method was carried out at the Department of Biochemistry, of Kwame Nkrumah University of Science and Technology to determine whether there was any significant difference in the food constituents of the cocoa pod and seeds produced under the two pollination regimes. The analysis covered determination of moisture content, crude fat, crude fibre, ash, protein, and total carbohydrate:

Determination of moisture content: Five (5) grams of sample was transferred to previously dried and weighed dish and placed in an oven thermostatically controlled at $105^{o}C$ for 5hr. It was then removed and placed in a desiccator to cool to room temperature and weighed. This was repeated till a constant weight was achieved.

Determination of crude fat and crude fibre: 250ml round bottom flask was weighed after drying in an air oven at $100^{o}C$. Five (5) grams of sample was transferred to 22x 80mm paper thimble and a ball of cotton wool was placed into the thimble to prevent loss of the sample. About 150ml of petroleum spirit B.P. 60-80°C was added to the round bottom flask. Extraction was then carried out in a Soxhlet after which the thimble was removed to recover the solvent by distillation. The

flask and the fat (oil) were heated for 30mins in an oven at 103°C. These were cooled to room temperature in a desiccator. The weight of the fat collected was determined after weighing the flask. Crude fibre was reported as loss in weight on ignition of dry residue remaining after digesting the sample with 1.25% H_2SO_4 and 1.25% NAOH.

Determination of ash: Two grams of sample was placed into a crucible which had been previously ignited and cooled, and then weighed. The crucible (and its content) was ignited in a muffle furnace (pre-heated to 600°C) for 2hr. It was then removed, cooled in a desiccator and weighed.

Determination of protein: The protein content was determined by the Kjeldahl method. Five gram sample was heated in sulphuric acid in the presence of selenium-based catalyst tablets and a few anti-bumping agents. This was digested till carbon and hydrogen are oxidised, as the protein nitrogen was reduced and transformed into ammonium sulphate. Sodium hydroxide was added and the digest heated to drive off the liberated ammonia into a volume of standard acid solution. The unreacted acid was determined and by calculation the percentage nitrogen in the organic sample was determined. A conversion factor of 6.25 (equivalent of 16% Nitrogen per gram of protein) was used.

Determination of total carbohydrate: The total carbohydrate was determined as the difference between 100 and the sum values of the moisture, crude protein, and crude fat, crude fibre and ash contents in sample.

3.10.4 *Data Analysis*

Pollinator exclusion experiment

The pollen limitation was calculated using Larson and Barrett (2000) index as follows:

$[L=1-(P_o/P_s)]$, where P_o is the percentage of fruit set taken from open-pollinated controls while P_s is the percent fruit set by plants that were artificially pollinated; $L=0$ indicates no pollen limitation in the population under study.

Natural Pollination Verses Artificial Pollination

The two year data were subjected to t-test analysis at 95% confidence interval (each year) using the SPSS version 17.0 to determine any significant differences between the weights and number of seeds (beans) of the small, medium, and large pods produced by the two modes of pollination. The weight and number of seeds per fruit are measures of reproductive success (Steffan-Dewenter and Tscharntke, 1999; Holzschuh et al., 2011).

Proximate analysis using the Association of Official Analytical Chemists (AOAC, 2005) method was carried out at the Department of Biochemistry, of Kwame Nkrumah University of Science and Technology to determine whether there is any difference in the constituents of the cocoa pod and seeds.

CHAPTER FOUR

RESULTS

4.1 Floral Phenology of the Cocoa Trees

4.1.1 *Flowering patterns and Fruit production in the cocoa trees*

The overall phenological pattern of flowering of cocoa consisted of segmental flowering in repeated phases. Floral productions generally increased in the major rainy season which began in the month of April and peaked in July (Fig 3). During this period flowering was high and was borne on the branches and on trunks of the cocoa trees. Flowering generally reduced drastically in the minor rainy season from September to November, and was restricted to the canopy and branches leaving the trunks with little or no flower. Thus, there were fluctuations in the floral abundances with peaks and declines in flower production. The production of new pods increased during the major rainy season (June, July and August), but was evenly distributed from the minor season, through to the dry season. From December which marked the beginning of the dry season flowering was sparse even in the canopy whiles the trunks were completely flowerless.This trend continued throughout the dry season which ended in late March to mid- April when rainfall began. The production of new pods increased during the major rainy season (June, July and August), but was evenly distributed from the minor season, through to the dry season.
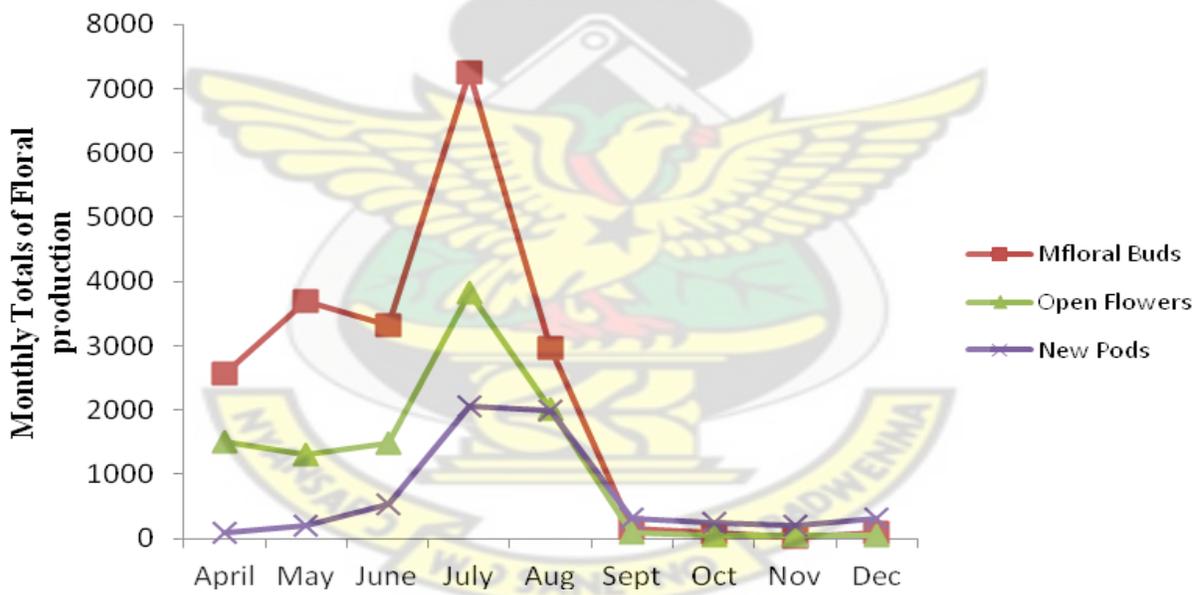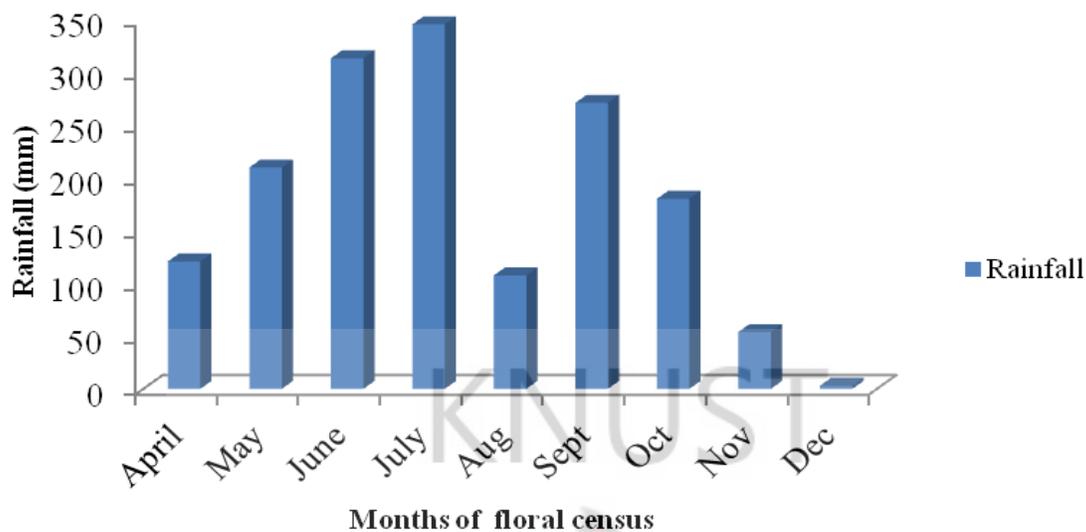
Figure 3: Phenological patterns of flower and fruit production in relation to rainfall pattern at Kubease in the Ejisu- Juabeng District.

Flowering appeared to have been affected by the level of fruiting in the preceding year. About 40% of selected trees (N=100) produced more fruits in the year 2006, leading to a good harvest by some farmers. In such situations there was late flowering of the trees in the ensuing year 2007

in spite of the onset of rains. Trees which had little fruiting in the previous year experienced early flowering, and sustained high flower production throughout the flowering periods. On the whole, the month with the most rain was also the most productive month (Figure 3; Table 3).

There were considerable tree-to-tree differences in the production of mature floral buds, and open flowers (Table 3). The monthly totals of mature buds ranged from 34 to 7246 (summed for all the 100 trees). Similarly, the monthly totals of open flowers and new pods or cherelles for the 100 trees ranged from 40 to 3818 and 102-2061 respectively.

Table 3: Phenological patterns of mature flower buds, open flowers, new pods, and percentage fruit-set of cocoa trees (N=100) in the ten farm plots.

Census Month	Mature Floral Buds Total[a]	Mature Floral Buds Mean ± SE	Open Flowers Total[a]	Open Flowers Mean ± SE	New Pods Total[a]	New Pods Mean ± SE	[b]Estimated fruit-set%
April	2573	131.15 ± 15.61	1511	75.61 ± 8.73	102	5.12 ± 0.71	2.44
May	3289	186.4 ± 7.55	1307	65.35 ± 7.11	194	9.73 ± 1.12	4.05
June	3313	165.65 ± 5.45	1480	74.21 ± 9.32	532	26.61 ± 3.14	9.99
July	7246	362.30 ± 8.16	3818	190.70 ± 10.58	2061	103.05 ± 13.8	15.7
August	2955	147.75 ± 6.08	2018	100.90 ± 8.60	1982	104.11 ± 11.61	28.48
September	34	1.77 ± 0.16	40	2.01 ± 0.99	206	10.31 ± 3.06	73.57
October	89	4.45 ± 0.51	51	2.55 ± 0.38	252	13.40 ± 2.37	64.28
November	157	7.85 ± 3.26	94	4.70 ± 1.08	317	17.05 ± 2.37	55.8
December	82	4.25 ± 0.11	44	2.24 ± 0.19	322	16.15 ± 3.80	71.87

[a] Total refers to the sum total of floral counts of floral production-floral buds, open flowers, and new pods (cherelles) during the three years (2006-2008) pooled together.
[b] Estimated fruit set per month calculated as the new pods divided by the sum total of floral productions and expressed as a percentage.

Fruit-set was low in April (2.4%) but increased till it peaked in September (173.6%) (Figure 4; Table 3).
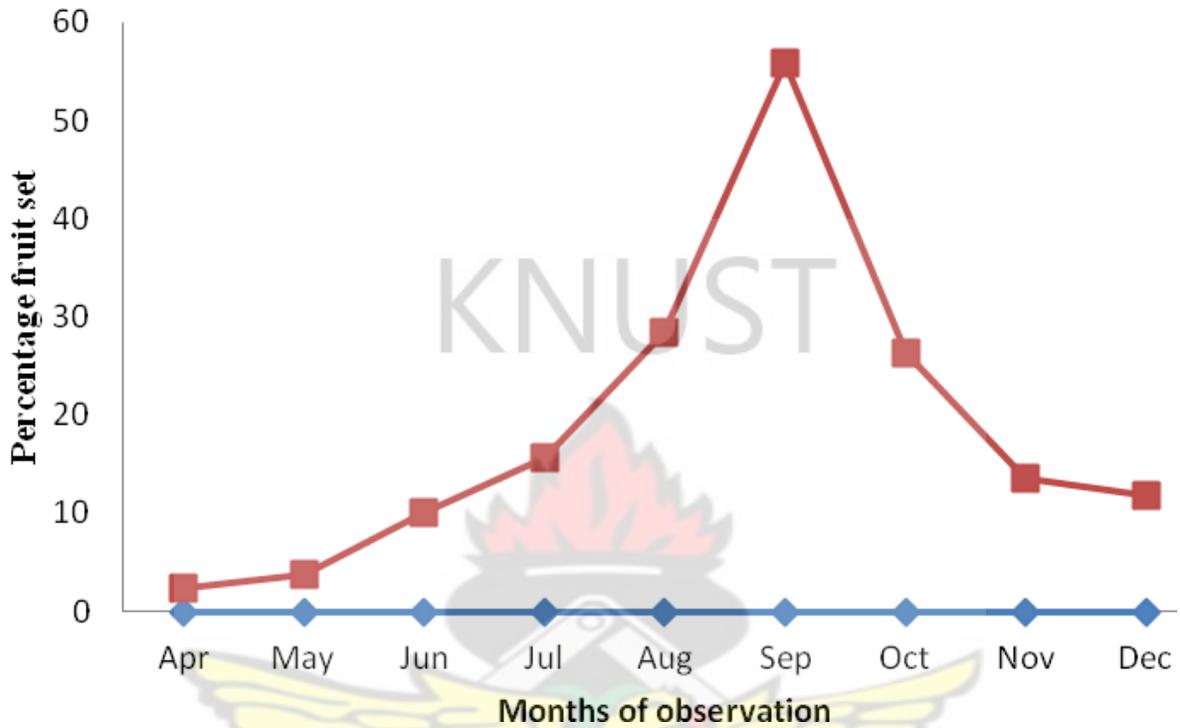
Figure 4:Variation in fruit -set production

Floral buds were produced well in excess above open flowers and new pods during the rainy seasons, but this declined as the rains subsided (Fig 5).
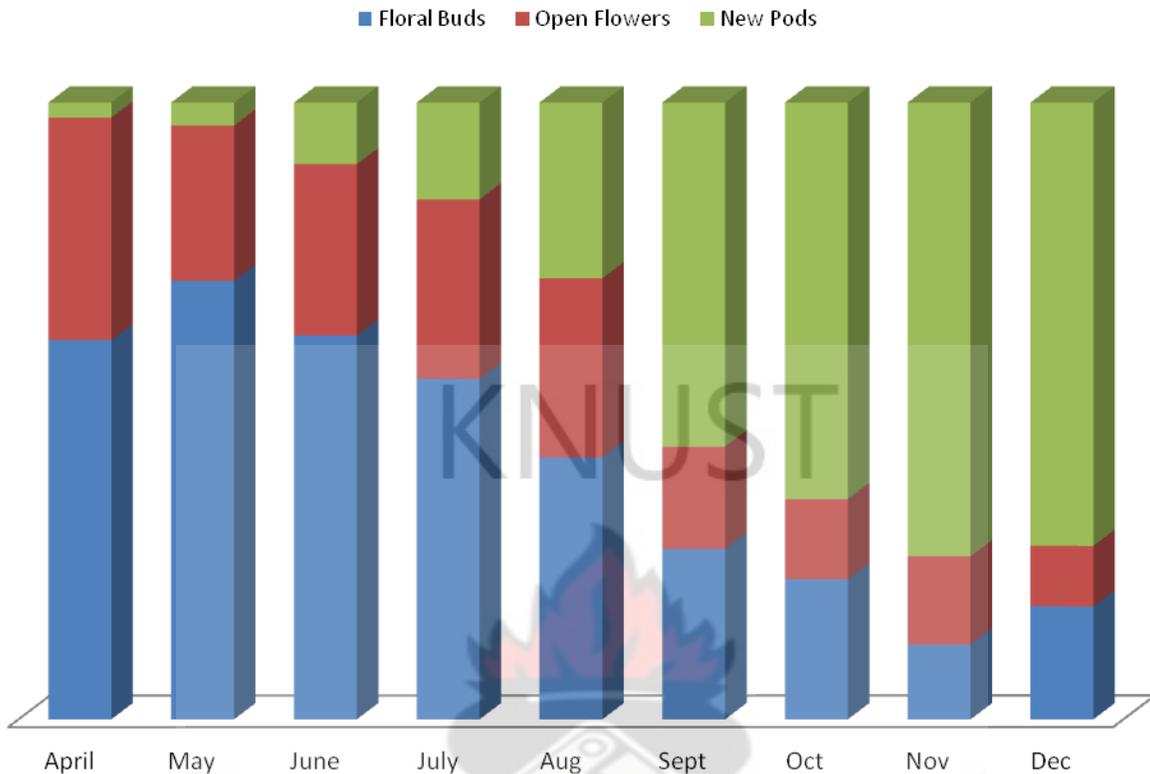
Figure 5: Monthly variations in production of floral buds, open flowers and new pods.

There was significant *(P<0.001)* differences in the monthly floral production between the study farm plots although there were some overlaps in the level of production (Table 4). In addition the monthly production of floral buds and open flowers also showed highly significant *(P<0.001)* differences (Table 4).

Monthly production of both mature flower buds and open flowers significantly (P<0.001) showed less variation from September to December among the farm plots (Table 4). Cherelles production levels were also significantly different *(P<0.05)* between the months (Table 4).

Table 4: Comparison of monthly production of Flower and Fruit in the ten (10) cocoa farm plots.

Census Months	Flower Bud	Open Flower	New Pods	Small Pods	Medium Pods	Large Pods
April	1.6	0.7	0.5	0.8	0.3	0.3
May	3.3	1.7	0.7	0.6	0.4	0.3
June	4.3	2.5	1.1	0.8	0.4	0.3
July	3.6	2.5	2.9	1.2	1.1	0.6
August	2.1	1.6	2.6	1.8	1.3	0.6
September	0.7	0.3	0.8	1.5	1.2	0.6
October	0.8	0.4	1.1	1.6	1.1	0.6
November	0.8	0.4	0.8	1.3	0.8	0.5
December	0.8	0.4	1.2	0.8	0.5	0.5
P-value	0.001	0.001	0.001	0.001	0.001	0.001
LSD(0.05)	0.61	0.51	0.16	0.31	0.2	0.14

Values are average of floral productions per month based on the sum total of sizes of the ten farm plots.

Fruits (small, medium, and large) production started in April and peaked in August. Significant differences were found in the monthly mean floral productions ($P < 0.01$) (Tables 4).The production rate of small fruit ($F=8.97$), medium ($F=14.67$), and large fruits ($F=5.81$) were very significant ($P <0.01$). Fruits found on the tree trunks in the month of April were predominantly the small pods which underscored the cyclical pattern of flowering of the cocoa trees in the study area. The rate of production of diseased fruits and aborted pods (cherelle wilt) followed a similar trend.

Pod sizes varied greatly with the months during the period of study (Fig 6). Small pods, the most abundantly produced reached its peak in August, but sharply reduced in numbers. Medium and large sized pods differed markedly ($P < 0.01$) in their level of production during greater part of the fruiting season. However, more medium pods were produced than the large pods.
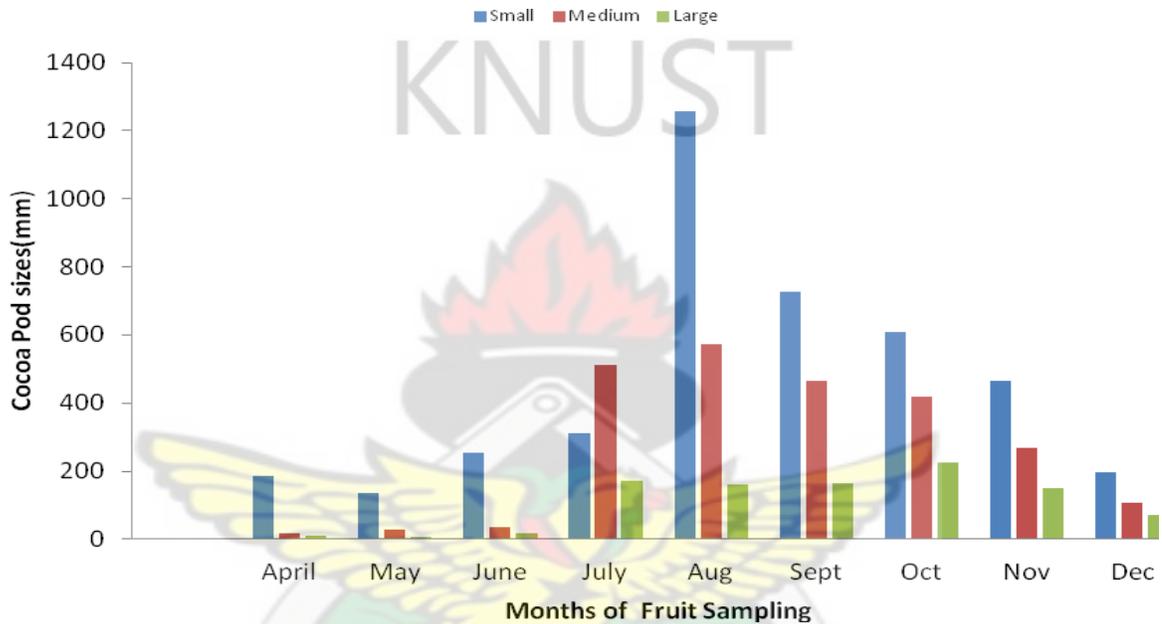
Fig 6: **Monthly changes in pods sizes.**

Fig 7 presents the trend of cherelles production and fruit abortion. The data indicated that the rate of abortion increased with the rate of cherelles production; however, the cherelles were abundantly produced than the aborted fruits. Both the cherelles and the aborted pods were greatly produced during the rainy season (May, June and July), however, the rate of cherelle production declined steeply after the month of August compared to the gradual decline in fruit abortion (Fig 7).
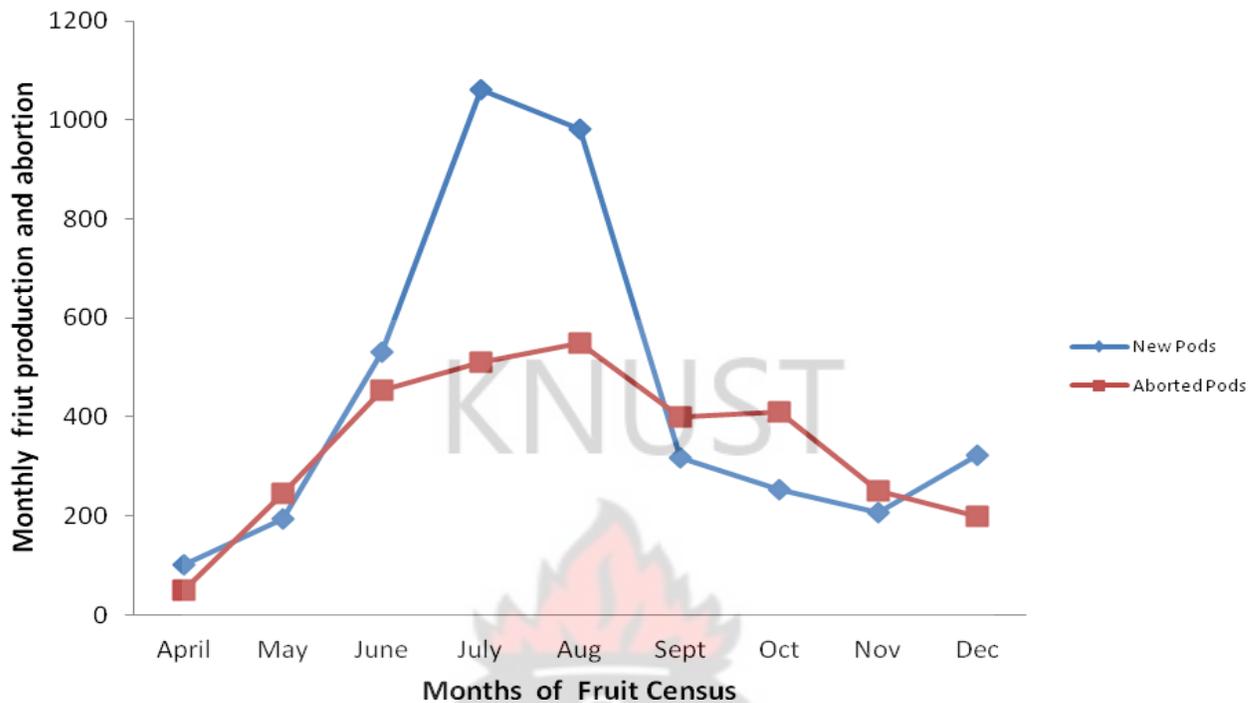
Fig 7: Monthly production of cherelles (new pods) and aborted fruits in the farm plots.

Fig 8 shows that the production of matured fruits commenced during the rainy season. There was a sudden rise in the matured fruit production in the rainiest months starting from June, however, the level of production dropped after the month of August and continued through to December. The diseased pods/fruits followed the same trend, but the level of total fruit production coincided with the level of diseased pods in the month of July. On the whole, more fruits were produced than the diseased pods.
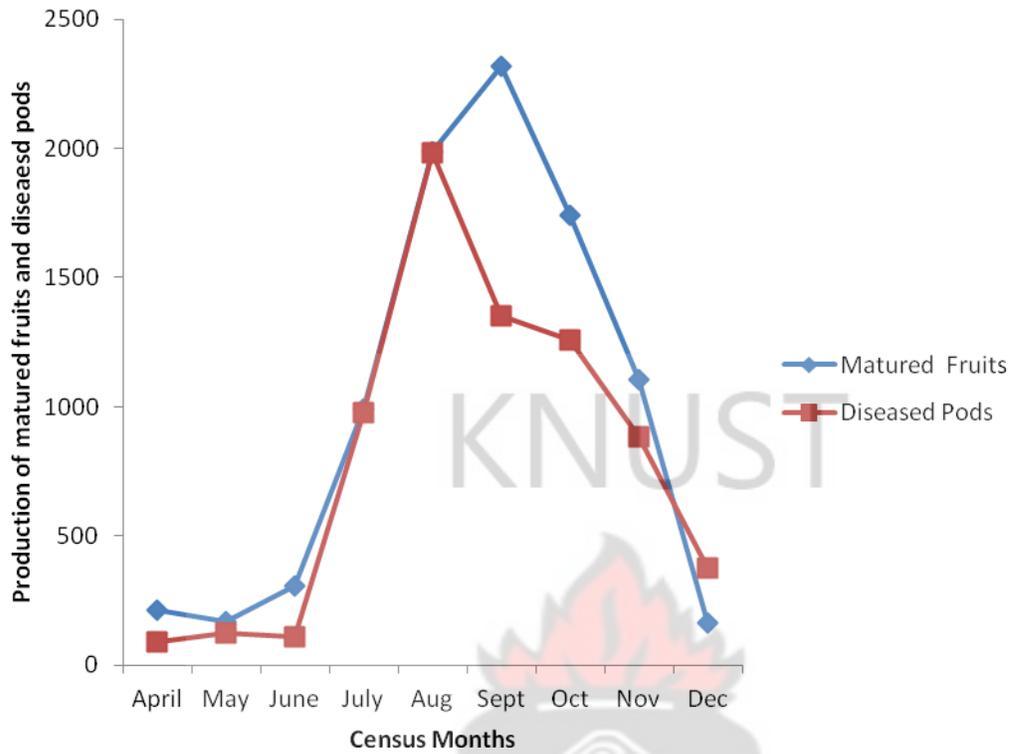
Figure 8: Monthly production of matured and diseased pods.
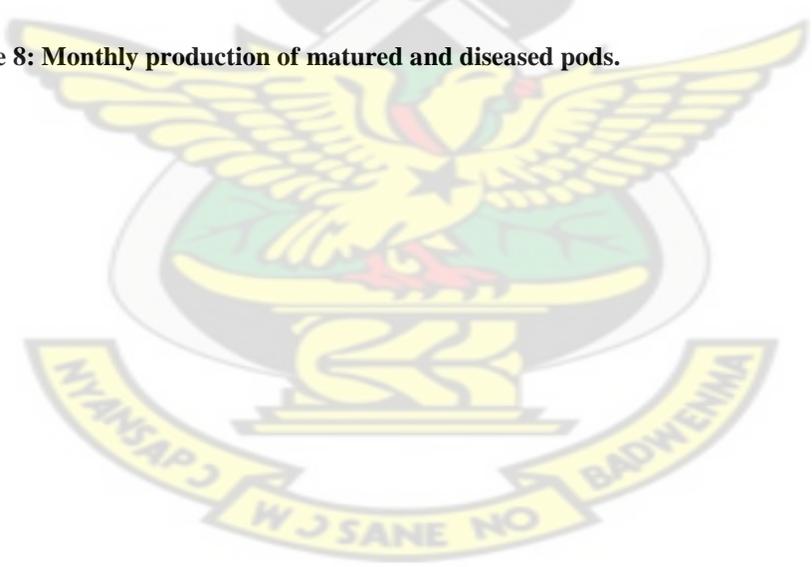
Plate 9: Fruit bearing cocoa tree showing fruitset or Cherelles (arrowed)

4.1.2   *Fruit loss in the cocoa trees*

Table 5 shows that fruit loss was mainly through fruit abortion and incidence of diseases. From April through December more fruits were lost through abortion than through diseases. The estimated ratio of aborted fruits to diseased pods increased with the rains and peaked in July.

Table 5: Fruit loss among cocoa trees (N=100) at Kubease during flowering seasons.

Census Month	Fruit Diseased	Fruit Abortion	Abortion:Disease Ratio
April	11	31	2.82
May	25	92	3.68
June	108	405	3.75
July	88	613	6.96
Aug	215	426	1.98
Sept	175	307	1.75
Oct	386	743	1.92
Nov	103	297	2.88
Dec	109	164	1.5
Total	**1209**	**3047**	
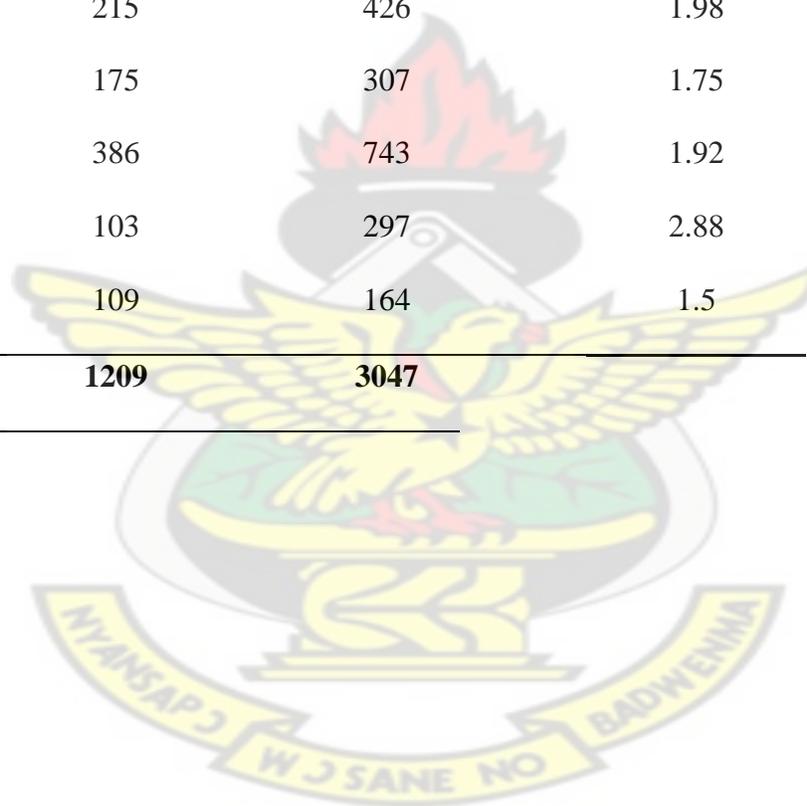
Plate 10: Cocoa tree in a study farm plot with Aborted fruits also called Chrelle wilt

4.1.3 *The effect of climatic conditions in flower production*

The mean temperature recorded during the period of study was 25.82 (± 1.33) °C; relative humidity had a mean of 89.55 (± 2.92) %; mean light intensity was 129.81(±1.33) Fc and rainfall, 144 (±10.5) mm. Stepwise multiple regression analysis (Table 6) showed that the environmental factors exerted varied influence on the individual floral components. Temperature contributed to the production of both the mature floral buds ($R^2 = 0.532$) and open flowers ($R^2 = 0.567$), but made minimum contribution to the production of the new pods ($R^2 = 0.453$). A combination of temperature with light intensity again increased the production of mature floral

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bud and open flower. Conversely, the same combination reduced the production of new pods. The highest level of production of mature floral buds ($R^2 = 0.685$), open flowers ($R^2 = 0.654$), and new pods ($R^2 = 0.501$) occurred when rainfall combined with the other climatic factors. Rainfall was therefore considered critical in the floral productions. Relative humidty appeared not to have impact on the mature floral buds, open flowers, but made great contribution to the production of the new pods.

The data (Table 6) indicated that temperature had positive relationship with the mature floral buds, and the open flowers, however, there was a negative relationship with the new pods. Similar observation was made with the light intensity. Rainfall, however, had positive relationship with all the floral components. Relative humidity had positive relationship with all the floral productions.

Table 6: Stepwise Multiple Regression General Linear Model (GLM) of Environmental variables on Floral production.

Dependent Variables	Model	Multiple Regression Equation	R^2	RMSE
Mature Floral Bud	GLM	= 182.1 + 23.7 TEMP	0.5325	3.3265
	GLM	= 194.3 + 50.8 TEMP + 161.6 LIGHT	0.5443	3.2841
	GLM	= 511.8 + 21.3TEMP + 122.1LIGHT + 34.8RAIN	0.6846	2.9596**
	GLM	= 73.1 + 17.5 TEMP + 213 LIGHT +22. 6 RAIN -18.9REL.HUM	0.4112	3. 451
Open Flower	GLM	= 122.6 + 49.03TEMP	0. 5672	3. 2216
	GLM	= 292.1 + 33.2 TEMP + 112.5LIGHT	0.6366	2.7576
	GLM	= 108.4 + 29.8 TEMP + 157.2 LIGHT + 42.3 RAIN	0.6536	2.6598**
	GLM	= 144.2 + 24.4 TEMP + 281.5 LIGHT + 107. 7RAIN + 129 REL.HUM	0. 5221	2.9621
New Pod (Cherelles)	GLM	= 321.6 - 0.608 TEMP	0. 4532	3. 125
	GLM	= 417.5 - 1.66 TEMP - 128.7LIGHT	0.2546	3. 841
	GLM	= 68.33 – 20.67TEMP - LIGHT + 57.68 RAIN	0.4621	3.0764
	GLM	= 51.18 - 12.37 TEMP - LIGHT + 0.312 RAIN + 47.95 REL.HUM	0.5013	2.2463

** = very significant

Figure 9(a, b, c, d) present the floral productions under the prevailing climatic factors. The optimum temperature for the production of mature floral buds, open flowers, and new pods was 22.5 °C. At this temperature about 7,500 matured buds, 3, 800 open flowers and 2000 new pods were produced per month. Between temperatures 21.5 and 22 °C the production of all the floral components registered low production, and also dropped after 23 °C (Fig 9a); Optimum floral production per month was recorded at 91.8 Fc light intensity (Fig 9b). Similarly, the optimum rainfall for floral productions was 141.1 mm per month (Fig 9c); and optimum relative humidity was 90.2% (Fig 9d).
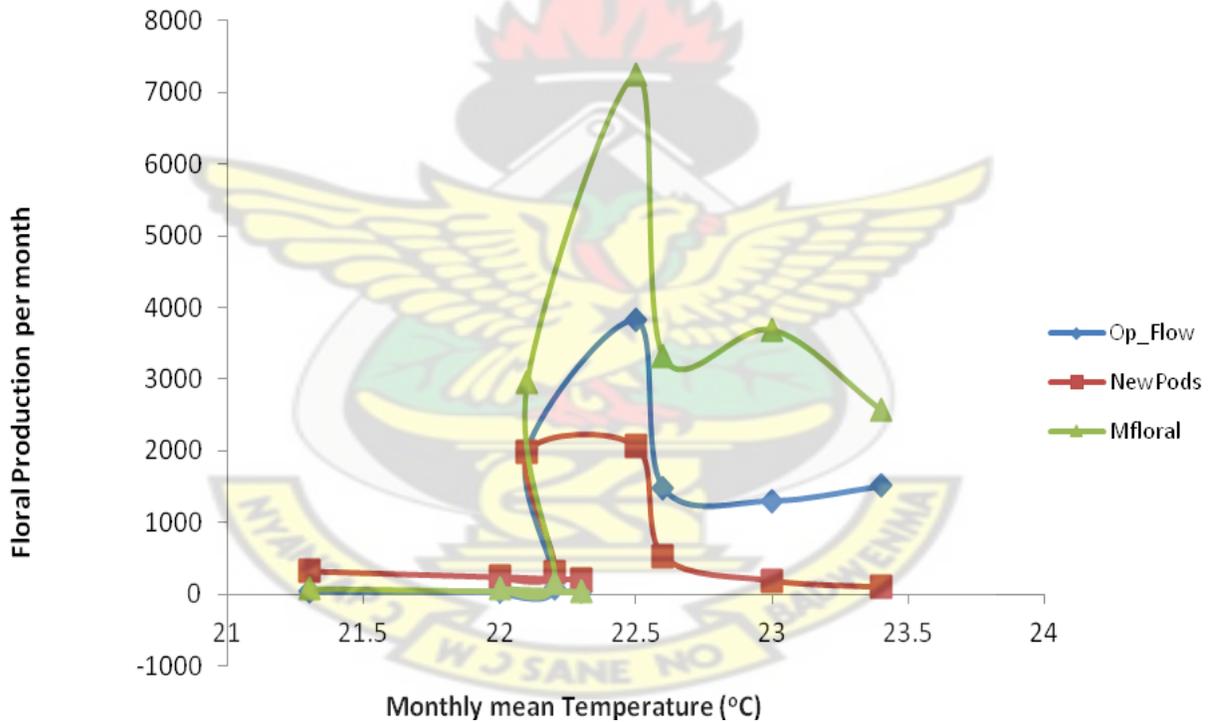
Figure 9a: Effect of temperature on the level of mature floral bud, open flower, and new pod production and the optimum temperature needed[5].

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[5] Data on the floral parts were square root tansformed ($\sqrt{X + 0.1}$).
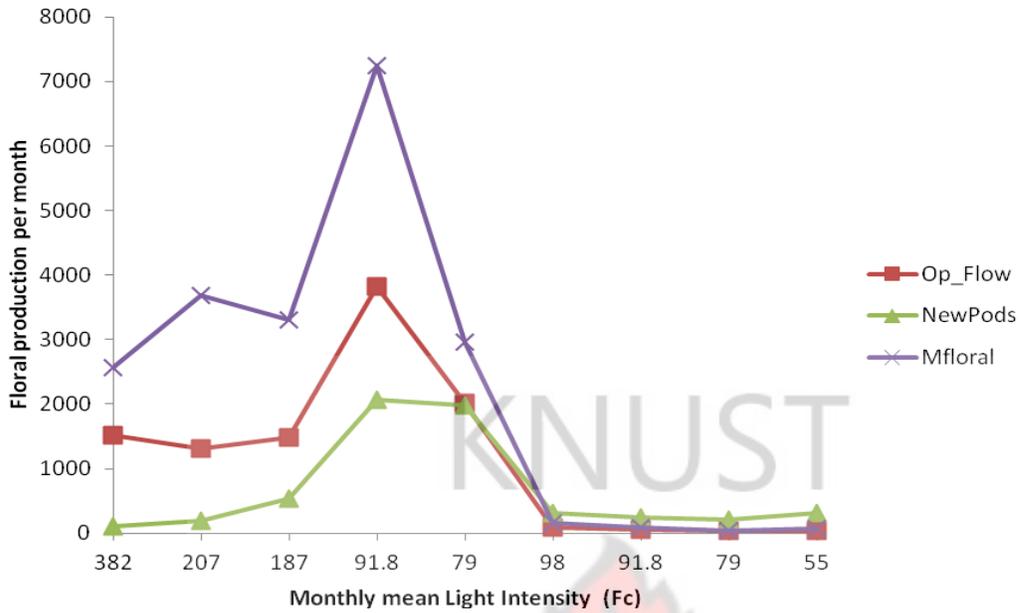
Figure 9b: The effect of light intensity on floral production-mature floral buds, open flowers and new pods[6].
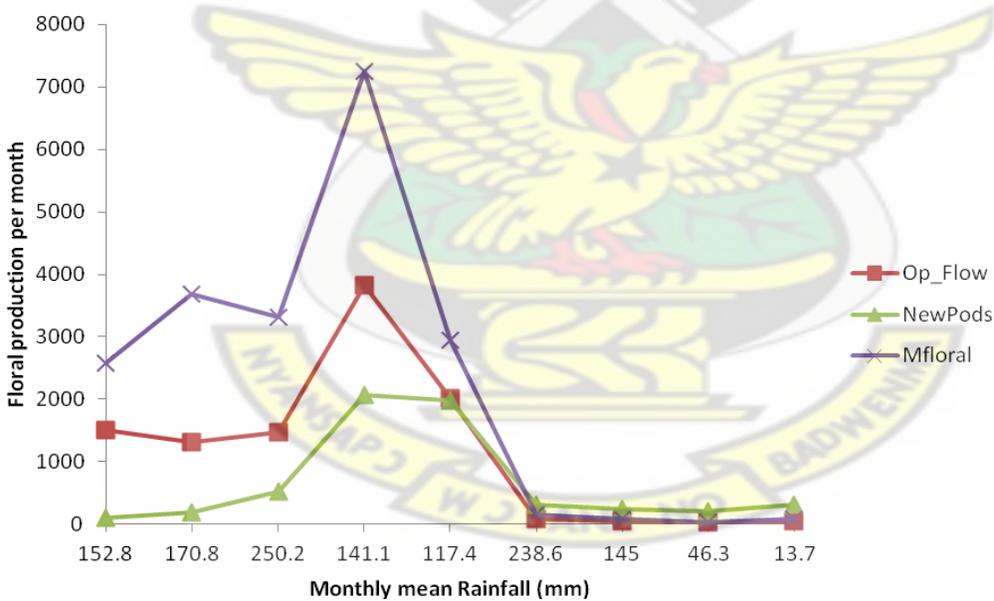
Figure 9c: Effect of Rainfall on production of mature floral buds, new pods and open flowers[7].

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[6] Data on the floral parts were square root tansformed ($\sqrt{X + 0.1}$).

[7] Data on the floral parts were square root tansformed ($\sqrt{X + 0.1}$).
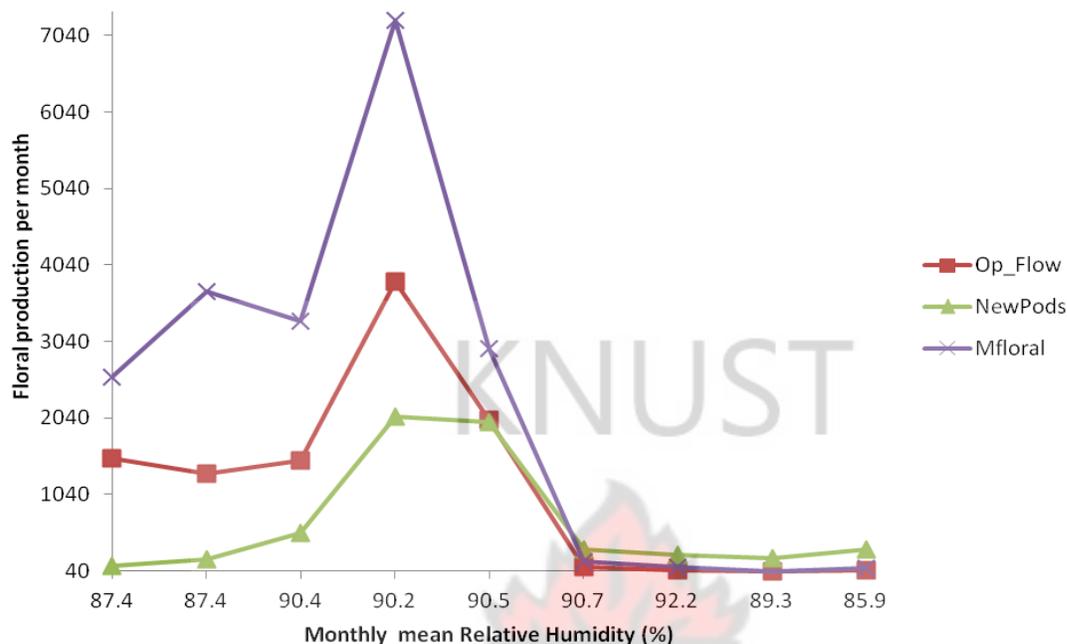
Figure 9 d: Effect of Relative humidity on the floral production levels[8].

4.1.4 *Flower Stability*

The seasons greatly affected the stability of the flowers on the cocoa trees. The number and rate of unpollinated flowers that dropped were higher in the dry season than in the wet. For example, during the dry season a total of 800 flowers (mean 51.2 ± 3.54SE (N= 1110) representing flower-stability ratio of 72%, were available on the second day (Table 7), but there were no un-pollinated flower on the tree after the third day in the dry season. On the other hand the flower-stability ratio of un-pollinated flowers in the wet season was 94% on the second day (mean number of flowers was 73.3 ± 9.71SE (N=1110), and reduced to 45% (52.0 ± 4.43SE (N=1110) by the third day. Whiles the total number of pollinated flowers dropped from 1110 to 721.5 from 2nd to the 3rd day under the dry season conditions, there was no drop during the wet season within the same period.

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[8] Data on the floral parts were square root tansformed ($\sqrt{X+0.1}$).

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Generally, the number of senescent flowers (i.e. pollinated flowers that remained stable on the trees) was higher both during the dry as well as wet seasons i.e 65-100% in the dry season compared to 95-100% in the wet season (Table 7). Daily observations of the mature buds during the studies indicated that mature buds were completely opened between 0500h and 0700h.

Table 7: Floral Stability of Pollinated and Unpollinated Cocoa Flowers during the Dry and Wet seasons at Kubease in the Ejisu-Juabeng District, Ashanti Region.

	Dry Season				Wet Season			
	Pollinated		**Unpollinated**		**Pollinated**		**Unpollinated**	
Day	Flower-Stability		Flower-Stability		Flower-Stability		Flower-Stability	
	Ratio (%)	Total	Ratio (%)	Total	Ratio (%)	Total	Ratio (%)	Total
2	100	1110	72	800	100	1110	94	1043
3	65	721.5	0	0	100	1110	45	500
4	0	0	0	0	98	1088	5	0
5	0	0	0	0	95	1054	0	0

4.1.5 *Vertebrate predation*

Most of the squirrel (*Scuirius* sp) predation /attacks and damage to pods occurred on the branches and not on the trunks and therefore were not reckoned for analysis. All the squirrel damaged pods, however, inspected had turned black and all the seeds were removed. Squirrel damaged pods in the ten farm plots were counted. Farm plot 6 experienced the highest level of predation (*ca*=64 pods) followed by farm plot 9 (*ca*= 30). Farm plots 2 and 3 had the lowest level of squirrel attacks (i.e. 7 damaged pods). Small pods were mostly attacked.

4.2.0   Insect Assemblage in the Cocoa Plantations and their Pollination Status in Relation to the Cocoa Trees

4.2.1   *Insect species in the ten cocoa Farm plots*

A total of 2,721 insects belonging to 36 species and 7 orders were sampled from the ten study farm plots over the two flowering seasons (Appendix I). About 40% of the insect species were crawling insects and were found on cocoa trees (i.e.arboreal), and on the ground among the cocoa leaf litter, and belong to the orders Coleoptera and Hymenoptera. The rest were predominantly aerial or flying insects.

Table 8: Diversity and Abundance of insects in the ten (10) Farm plots

Order	No. of insect species	No. of individuals	% of the total no. of insects
Hymenoptera	17	1264	46.45
Hemiptera	3	21	0.78
Lepidoptera	7	57	2.09
Orthoptera	1	25	0.93
Diptera	4[+]	1266	46.52
Coleoptera	2	37	1.36
Heteroptera	2	51	1.87
Total		**2721**	**100.00**

(+) Due to taxonomic challenges, all midges were grouped under their generic names.

Dipterans constituted 46.52% (N=1,266) of insects resident in the cocoa farms, followed by the hymenopterans 46.45% (N=1,264), with the least abundant insects being the hemipterans 0.78%

(N=21) (Fig 12). Insect species of four of the orders viz: hymenoptera, diptera, orthoptera, and coleoptera were common to all the ten farm plots (Appendix I). More species of insects were sampled in farm plots which were closer to the forest than the other farms (Table 8).

Table 9: Diversity and Species richness[9] of insects according to the cocoa farm plots

Farm plot	Species Richness	Shannon Diversity
1	36	2.7
2	36	2.84
3	26	2.9
4	26	2.94
5	30	2.97
6	26	2.98
7	25	2.99
8	24	3.0
9	26	3.0
10	20	3.0

Multivariate analysis (MANOVA) indicated significant differences in the insect communities according to the farm locations in the four distance blocks and the population (distance block (96-171m) $F =3.799$, $P=0.001$, population $F=2.441$, $P=0.001$; distance block (280-300m) $F = 3.057$, $P=0.001$, population $F= 2.794$; distance block (670-1,340m) $F = 11.6$, $P= 0.001$, population $F =1.54$, $P=0.001$; and distance block (1,710-1,730m) $F = 3.55$, $P= 0.001$, population $F =2.51$, $P=0.001$.

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[9] The richness and/or diversity refer to the number of taxonomic groups and were expressed in absolute terms (Beck and Schulze, 2000).

Some of the insect species, for example, *Anoplocnemis curvipes* (order: Hemiptera) and *Gideona klots* (order: Lepidoptera) were singletons (i.e. species with only one individual collected in a particular farm throughout the sampling periods) (Appendix I). However, most were doubletons or found in their numbers. The hymenopterans sampled during the study consisted mainly of ants, and bees, and was the order with most varied insect species. They were followed by the order Diptera and Lepidoptera (Appendix I).

4.2.2   *Abundance and Distribution*

On species by species basis ants (all species) represented most of the non-pollinating hymenopterans in the ten cocoa farms and constituted *ca* 45.28% when all the insects were pooled together. The ants were higher in numbers and pervasive in distribution (Table 10). The bees (order: Hymenoptera) constituted *ca* 1.06% but were not as ubiquitous as the ants (Table 10; Appendix I). Among the dipterans the *Forcipomyia* spp, (biting midges) the most dominant individuals constituted *ca* 21.24% of all the insect species, followed by cecidomyiids (gall midges) *ca* 17.16%. Butterflies (order: Lepidoptera) made up to 2.09% and were limited to farms close to the intact forest. All the other insect species, viz: coleopterans, hemipterans, and orthopterans were obtained in virtually insignificant numbers (Appendix I). The heteropterans *Distantiella theobroma* and *Bathycoelia thalassina* found on the trunks and pods are known pests of cocoa.

Table 10:   Abundance and Distribution of some key insect species sampled in the ten farmplots at Kubease.

Insect Groups	Order	Number of Individuals	Distribution 10 Farmplots	% of Insects N=2721
Ants	Hymenoptera	1232	Present in all farmplots	45.28
Stingless bee	Hymenoptera	29	Absent in farmplot 10	1.06
Biting midges	Diptera	578	Present in all farm plots	21.24
Gall midges	Diptera	467	Present in all farm plots	17.16
Butterflies	Lepidoptera	57	Present in some farm plots	2.09

4.2.3 *Focal patch observation: Insects Visitation to the Cocoa flowers*

More than half (*ca* 52.81%) of the insects belonging to 26 species sampled from all the orders (except the order: Heteroptera) did not visit the cocoa trees at the time of observation; 10.4% mainly hymenopterans were found on the cocoa trees but not on the flowers (Table 11). The regular visitors observed largely belonged to orders: Hymenoptera and Diptera. The hymenopteran visitors included the stingless bee *Hypotrigona aurajoi* (Michener), *Camponotus acvapimensis* (Mayr), *Cremastogaster depressa* (Emery), *Pheidole megacephala* (Fabricius), while the dipterans were gall midges cecidomyiids spp, and the biting midges, *Forcipomyia* spp. It was noted that the ceratopogonids (order: Diptera) accounted for an estimated 97% (N=2721) of all visitors to the cocoa flowers. Their visit frequency (square-root transformed) showed no effect by location of farm plot (*F*= 22.02; *P* < 0.05).

Table 11: Classification of sampled Insect species based on their visitation to the cocoa trees at Kubease.

Visitation Status	Number of Species	Order(s)	% Total no. insect (N=2721)
Non-visiting insects	26	6 orders*	52.81
Tree visiting insects	2	Hymenoptera	10.4
Flower visiting insects	7	Diptera, Hymenoptera	46.04
Visit of pollinator importance	2	Diptera	38.4

*Orders of some of non-visiting insects: Hymenoptera, Lepidoptera, Orthoptera, Coleoptera, Diptera and Hemptera

There was no significant difference ($t = 44.22$, $P>0.05$) in percentage flower set between the banded and unbanded flowers from t-test obtained (Table 12). It was observed that flowers dried up after 24 hrs and fell in the presence of ants, aphids and other crawling insects.

Table 12: Differences in average fruit set between grease banded flowers and unbanded (control) flowers.

Treatment	Number of Flowers	Fruit set (%)[a]
Grease-banded flowers	1,800	8.7
Unbanded flowers (control)	1,800	7.7
T		1.4
P		0.09

[a] Data was arcsine square-root transformed prior to analysis. Differences were tested with t-test with one-tailed probabilities given.

Generally, there were significant differences in the visitation frequencies of the insect visitors. The ceratopogonids visited ($F_{1,30}= 28.79$, $P <0.05$) more than other visitors including: ants, *Drosophila* spp and *H. araujoi* (Appendix II). However among the ceratopogonids the visitation frequency of

the gall midge *Cecidomyiids* spp was higher (6.5 ±1.1) than that of the biting midge *Forcipomyia* spp (4.2±0.19) per flower per 10 mins, and was significant (P <0.05) (Fig 10). Thus, the *Forcipomyia* spp was lower in abundance. The visitation frequency of the other visitors were not significant (P= 0.05) (Appendix II).

Plate 11: The biting midge *Forcipomyia* spp on a slide. Mag: x 1200
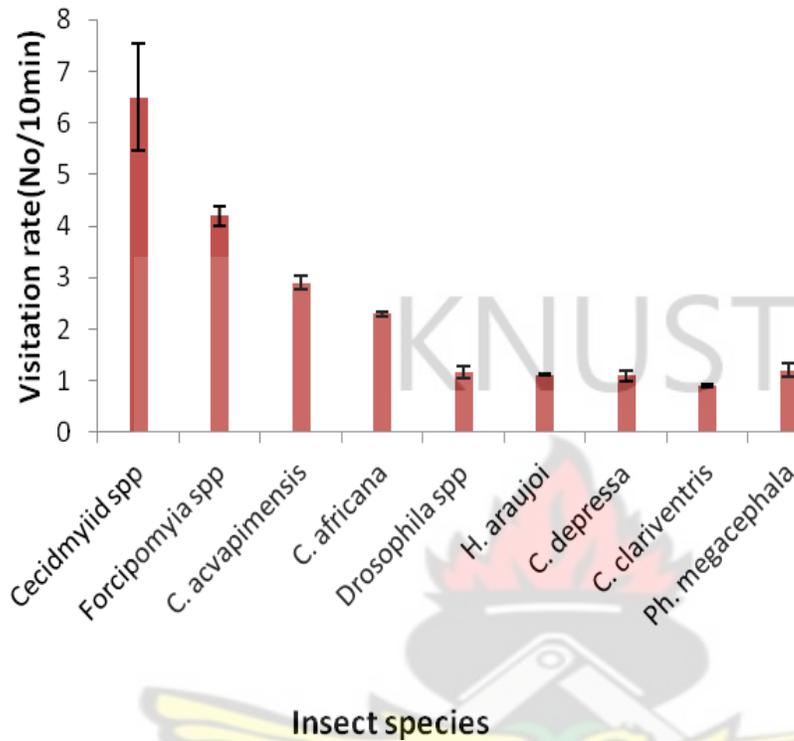
Fig 10: **Visitation Frequency of some insects species sampled on cocoa flowers.**

Visitation frequency, however, varied significantly between species ($F= 95.72, P < 0.05$), between months ($F= 4.35, P<0.05$) and times of the day ($F=2.02, P < 0.001$). While the visitation of the midges increased during the rainy season, and greatly reduced in the dry season, visits by *Drosophila* spp were constant throughout the study period. The numbers of the visiting ants *Cremastogaster acvapimensis* (Mayr)*, Cremastogaster Africana* (Emery)*, Cremastogaster depressa* (Latreille)*, Cremastogaster clariventris* (Mayr)*, Pheidole megacephala* (Fabricius) increased during the minor rainy seasons in September.

Among the insect species the midges and the *H. araujoi* were the 'early birds' that visited the flowers. The number of midges found to visit the flowers between 0600 and 0900 h GMT were high

compared to the visitation activities in the afternoon, between 1500 -1700 h GMT. The other insect visitors to the cocoa flowers commenced their visitation (i.e from 0700h GMT) later than the midges.

4.2.4   *Focal patch observation: Bee Activities on the Cocoa flowers*

Two honeybee species were identified in the ten cocoa farm plots (Appendix I): *Hypotrigona araujoi* Michener (1959) (Hymenoptera: Apidae: Melliponinae) and *Apis mellifera adansonii* L. (Hymenoptera: Apidae: Apininae). While the former was seen on the cocoa tree the latter never visited the trees or the flowers.

A total of 105 stingless bee foraging consisting of 64 visits and 41 hoverings were recorded during the entire period of study (May –June) of the years 2008 and 2009. However, it was observed that number of bees that visited the cocoa flowers at a time was generally low, sporadic and largely unpredictable. The visits per hour was not significantly different (F=18.58, P>0.02) among the cocoa trees. More bees visited in the mornings (F=0.45, P< 0.05) than in the afternoons, with the mean number of visits being 2.5 ($\pm$0.01). The highest number of bees recorded was four (4) at 7 am and 10 am on six different days of observation. The visitation rates ranged from 0.05 to 1.03 (Table 13). On the average the bees landed on 3 flowers per visit. Five percent (N=64) of cases the bee visits were characterized by succession in visits (i.e. one bee following the other). About 1% (N=600) of the flowers were dislodged, when the visit was frequent. The Pearson Chi-square for flower count (17.64), total visits (11.68), duration of visits (35.79) were significant (P<0.05). Mean duration of the bee visits ranged between 1.5 ($\pm$0.71) and 2.9 ($\pm$ 0.42) seconds. Those which hovered over the flowers spent between 1 and 3 seconds per session.

Table 13: Foraging pattern of *Hypotrigona araujoi* on cocoa flowers (N=600)

Visitation Rates Flower/hr	Mean Duration Secs	No. of Flowers Probed
0.51(0.38)	1.5 (±0.71)	24
1.03(0. 02)	2.6 (± 0.43)	60
0.52(0.11)	1.6 (± 0.69)	30
1.5 5(1.01)	1.9 (± 1.58)	40
0.05(0.03)	1.1 (± 1.72)	20
0.05(0.03)	2.9 (± 0.42)	24

All analysis were carried out at P< 0.05)

4.2.5   *Pollinator importance of Insect Visitors*

Laboratory examination of flowers revealed that the presence of the microdipterans i.e. *Forcipomyia* spp., and the *Cecidomyiids,* aided the deposition of mass of pollen on the stigmas of the flowers. Even though fewer number of *Forcipomyia* spp. visited the flowers they appeared to be more important pollinators than the gall midges as they deposited greater number of pollen grains per mm^3 (60.1 ± 13, n = 500) per visit (Fig 11). The rest of the visitors did not deposit any pollen on the stigmas.

Six (6) percent of the 64 stingless bee visits involved pollen collection which lasted between 5 and 10 secs. In such cases the bees worked on 1 or 2 flowers. Bees that visited for less than 5 seconds never picked up any pollen. Of the 60 stigmas selected for examination under the stereomicroscope only one (1) stigma had 4 single grains of pollen. Therefore pollen deposition on stigmas was not

analyzed statistically. Again out of 200 flowers that were tagged after the bee visit, 189 flowers representing 94.5% dropped by the second day, signifying lack of pollination.
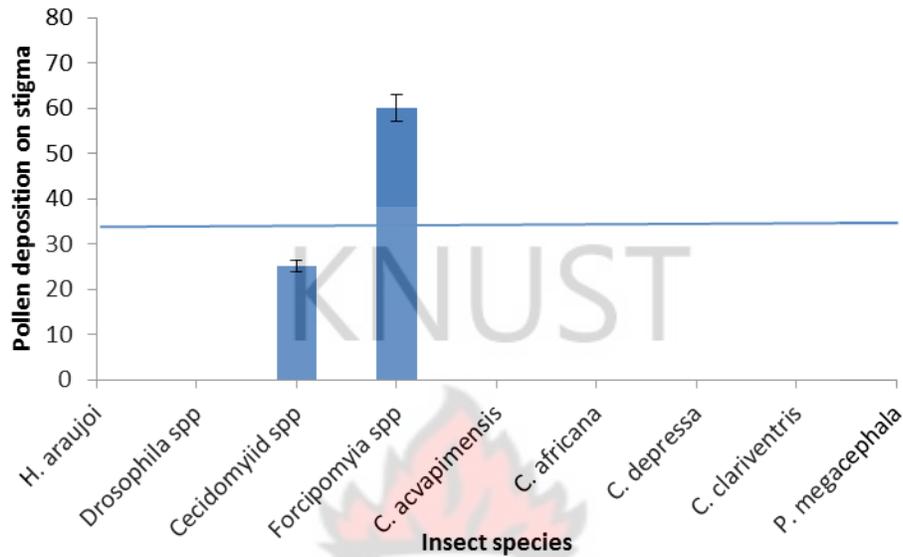
Figure 11: Pollen deposition on pistils as index of pollination importance.

4.3 Comparative Evaluation of Breeding Substrates for Cocoa Pollinator in Cocoa Farms

4.3.1 *Midges preference of the Breeding Substrates*

In both field and laboratory studies, midges' populations were significantly (F= 0.65, P<0.05) greater under rotten banana pseudostem than the other substrates. Midges' populations were 114.9 % greater under the rotten banana pseudostem than the mean of the other two substrates (Table 14). There was a strong and positive correlation (R^2 = 0.948) between the weight of substrates and the midges population in the field.

Table 14: Population of Midges in the Substrates

Substrate Type	Estimated Midge Population per Substrate	
	3kg	160 kg
Banana pseudo stem	144	7,680
Cocoa pod husk	98	5,226
Cocoa leaf litter	36	1,920
Control	27	1,440
LSD 5%	5.35	4.40

Significant differences ($P<0.05$) in mean values existed between the flower set, fruit set, and fruit abortion produced under the substrate types. The flower set was generally higher than the fruit set and fruit abortion ($F_{1,15}$ = 13.64, $P<.0.05$) (Table 15); and the level of pollination correlated (R^2 = 0.835) with the fruit abortion. Flower abortion was highest under the control condition (Table 15).

Table 15: Effect of midge population in substrates on flower set, flower abortion and fruit set.

Substrate Type	Estimated Percentage Increase			
	Flower set	Fruit set	Flower Abortion	Fruit Abortion
Rotten Banana pseudostem	95.78	85.01	11.24	62.69
Rotten cocoa pod husks	89.05	82.08	7.83	58.51
Rotten cocoa leaf litter	68.42	57.58	15.84	31.34
Control	43.42	21.71	50.01	20.05
LSD 5%	4.32	17.51	8.31	11.75

Both the substrates and their weights showed significant effect on the level of pollination and the subsequent fruit set (Appendix IV). The impact of breeding substrates on flower set increased with the weight of substrate types (Figure 12). The pollination level under the banana pseudostem and

cocoa pod husk substrates in the 8th week appeared the highest, while that of the control was the lowest.
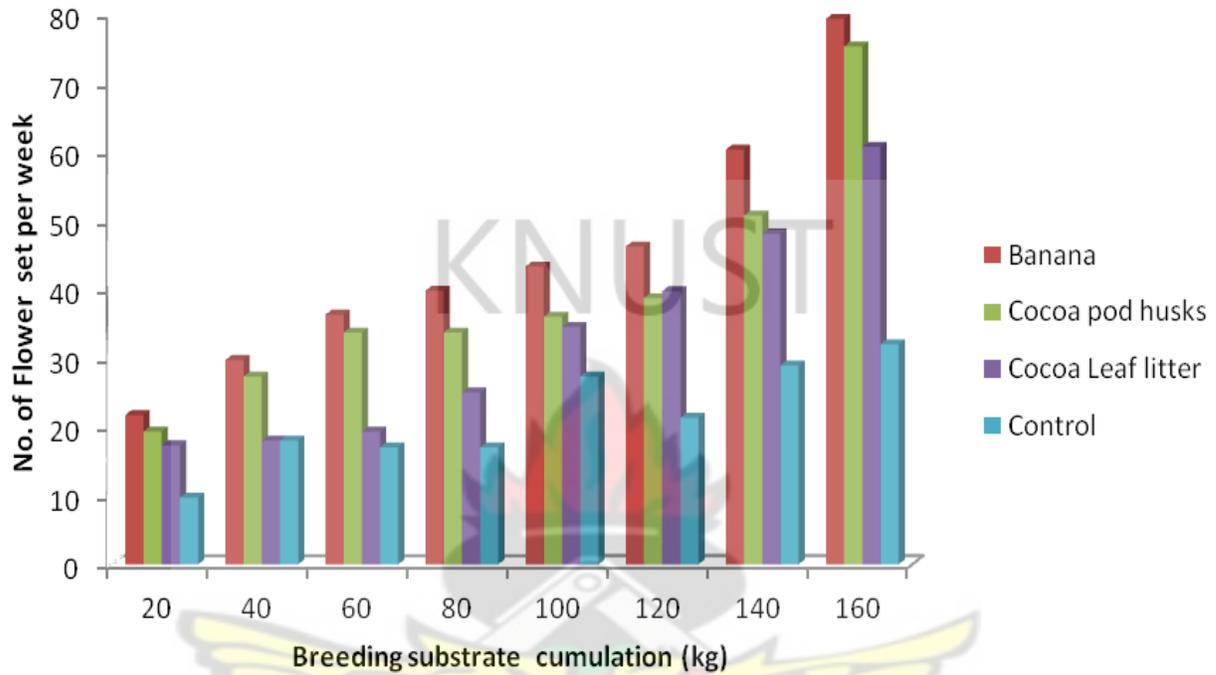
Figure 12: Impact of breeding substrates on cocoa pollination represented by the flower set

There was however, no significant difference between the substrates during the first and second weeks when the weight of each substrate was 20 kg ($F= 2.51$, $P=0.259$) and 40 kg ($F = 1.20$, $P=0.411$) respectively (Table 16). Actual differences showed in the third week between the banana pseudo stem and cocoa pod husk substrates on one hand, and the cocoa leaf litter and the control treatment on the other hand when weight of substrates had been increased to 60 kg. During weeks 4 and 5 significant differences ($F= 1.99$, $P<0.05$) existed among all the substrates, whiles in weeks 6 and 7 only the control was significantly different ($F= 4.89$, $P<0.05$) from the other three treatments. By the eighth week the impact of the banana and cocoa pod husk substrates was highly significant ($F= 7.85$, $P<0.01$) from the others when the weight of substrates were 160 kg per substrate.

Table 16: Actual Differences between the Substrates for each Week[10].

Week 1	
Substrate	**Mean**
Banana	21.67 A
Cocoa Leaf Litter	21.33 A
Cocoa Pod Husk	19.33 A
Control	9.67 A
LSD	12.35 A

Week 2	
Substrate	**Mean**
Banana	29.67A
Cocoa Leaf Litter	18.00A
Cocoa Pod Husk	27.33A
Control	18.00A
LSD	15.06

Week 3	
Substrate	**Mean**
Banana	33.33A
Cocoa Leaf Litter	19.33B
Cocoa Pod Husk	36.67 A
Control	17.00B
LSD	12.18

Week 4	
Substrate	**Mean**
Banana	43.67A
Cocoa Leaf Litter	25.00C
Cocoa Pod Husk	33.67B
Control	17.00D
LSD	7.93

Week 5	
Substrate	**Mean**
Banana	43.33 A
Cocoa Leaf Litter	36.00 BA
Cocoa Pod Husk	33.67B C
Control	27.33 C
LSD	8.27

Week 6	
Substrate	**Mean**
Banana	38.67A
Cocoa Leaf Litter	42.67 A
Cocoa Pod Husk	39.67A
Control	21.33 B
LSD	9.32

Week 7	
Substrate	**Mean**
Banana	50.33A
Cocoa Leaf Litter	50.67A
Cocoa Pod Husk	60.67A
Control	31.00B
LSD	13.39

Week 8	
Substrate	**Mean**
Banana	79.33A
Cocoa Leaf Litter	60.67B
Cocoa Pod Husk	77.33 A
Control	32.33 C
LSD	12.00

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[10] Means with the same letter are not significantly different.

4.3.2   *Impact of Substrates on Cherelle and Fruit Abortion*

Figures 13 (a, b, c, d, e, f, g, h) present the relationships between the increasing weight of substrate types and the resulting fruit set and aborted fruits. Banana substrate positively affected the rate of cherelle production (Y=10.18+15.90X; R^2=0.88; P<0.05) (Fig 13a). There was a sharp increase in the rate of cherelles production after the banana stem was increased to 160kg. A similar trend was noted under that cocoa pod husk substrate (Y=15.57+2.59X; R^2=0.71; P <0.05) (Fig 13c), however, the number of cherelles dropped from total of 33 cherelles (summed from 72 cocoa trees) at 80kg to 25 at 100kg of cocoa pod husk substrate. Under the cocoa leaf litter and the control treatments the rate of cherelle production were also positively affected (Y= 3.68 +8.18X; R^2=0.94; P = 0.05) and (Y= 1.11+3.11X; R^2=0.94; P = 0.05) respectively.   In all cases the the rate of cherelle production was gradual but increased sharply after the 140kg accumulation of substrate.

The accumulation of the substrates also resulted in the corresponding increase in fruit abortion under all the substrates. Banana pseudo stem substrate had a positive relationship with the rate of fruit abortion (Y= 9.49x + 14.18; R^2=0.68; P=0.05) (Fig 13b). The trend was observed under all the other substrates. There was also a drop in number of aborted fruits when the cocoa leaf litter increased to 80 kg.
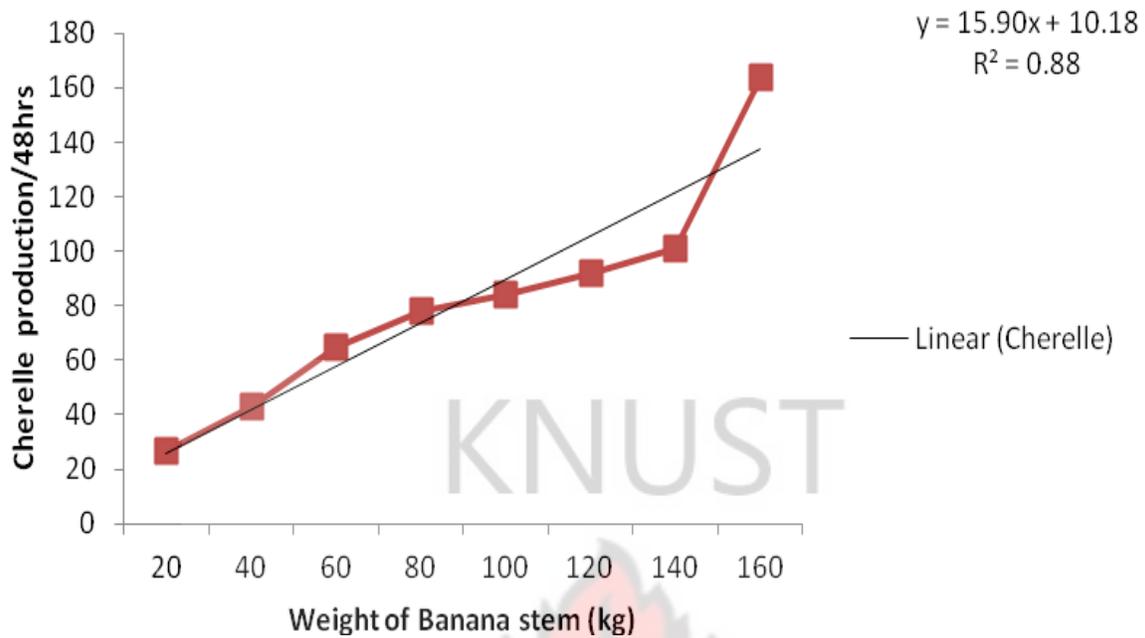
Figure 13a: **Rate of Cherelle production under Banana substrate treatment**
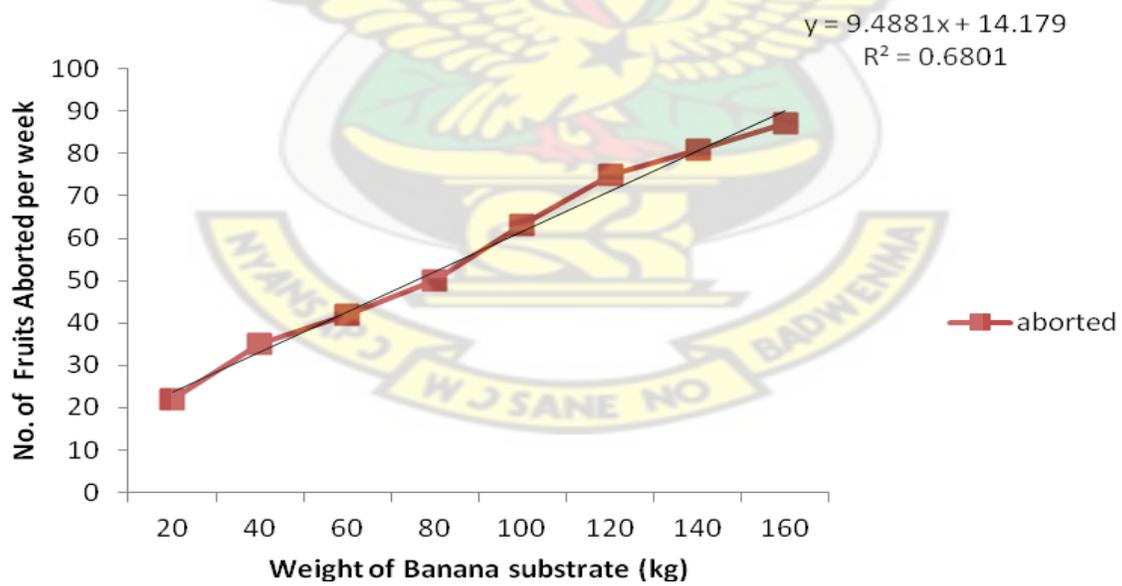
Figure 13b: **Rate of Fruit abortion under Banana pseudostem substrate**

$y = 2.59x + 15.57$
$R^2 = 0.71$
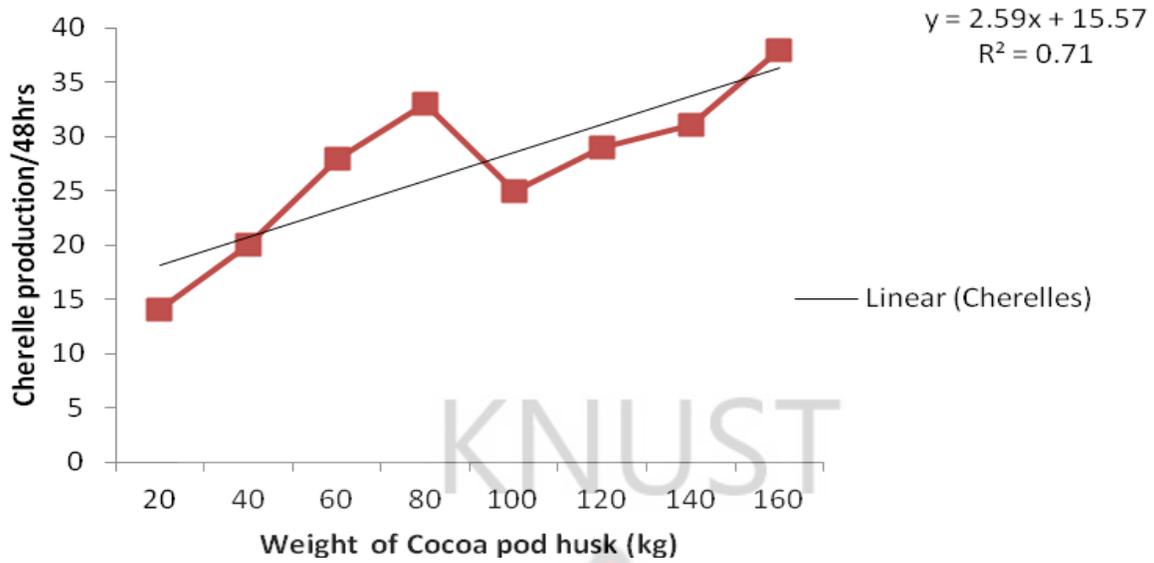
Figure 13c: **Rate of Cherelle production under Cocoa pod husk substrate**

$y = 7.1905x + 0.8929$
$R^2 = 0.6548$
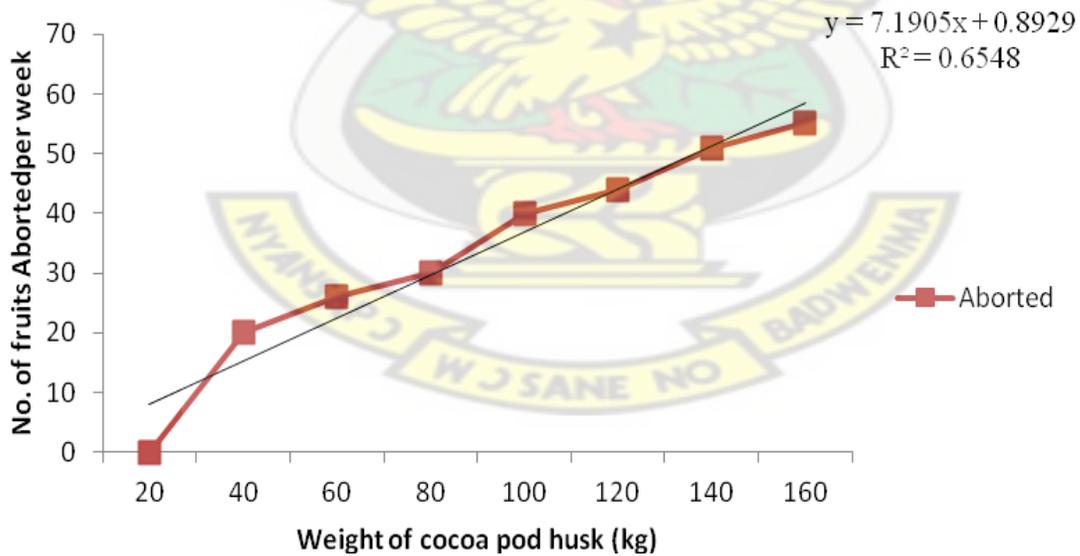
Figure 13d: **Rate of Fruit abortion under the Cocoa pod husk substrate.**
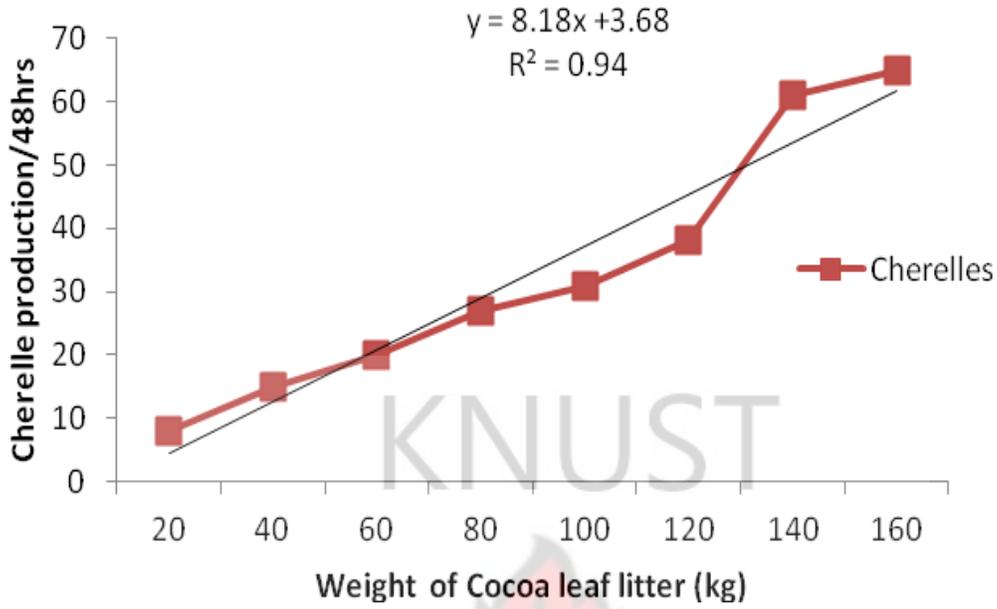
Figure 13e: **Rate of Cherelle production under Cocoa leaf litter treatment**
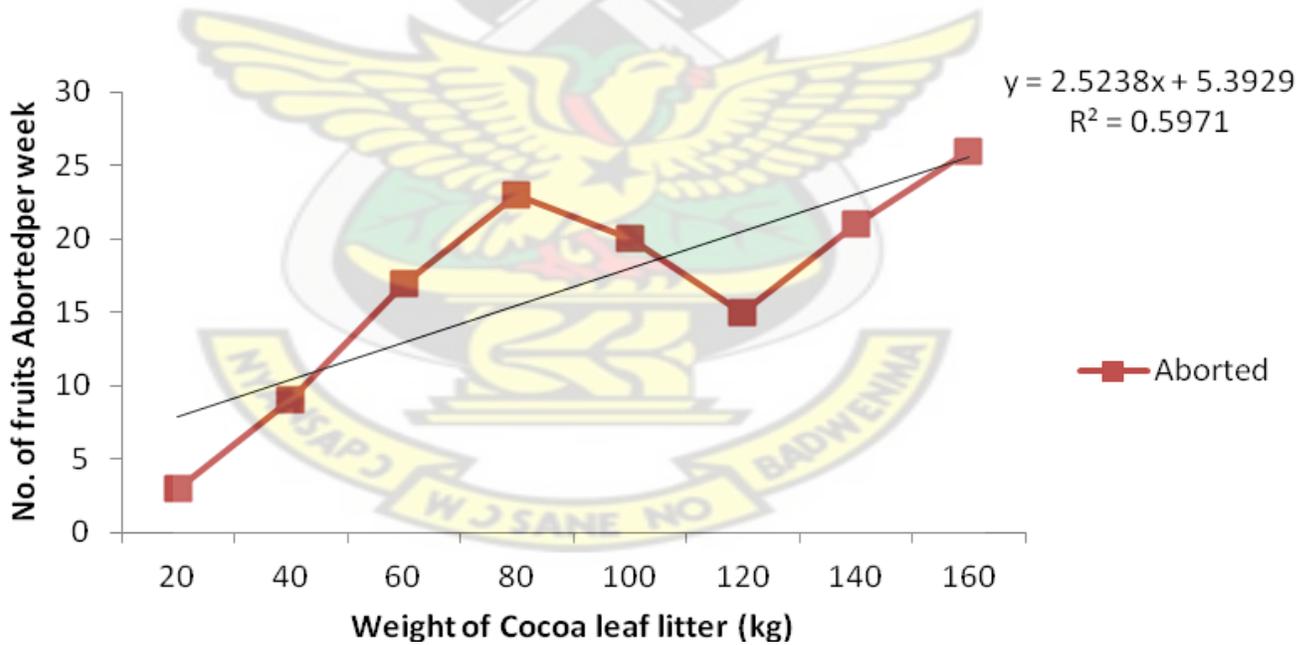
Figure 13f: Rate of Fruit abortion under Cocoa Leaf litter treatment

$$y = 3.1071x + 1.1071$$
$$R^2 = 0.9499$$
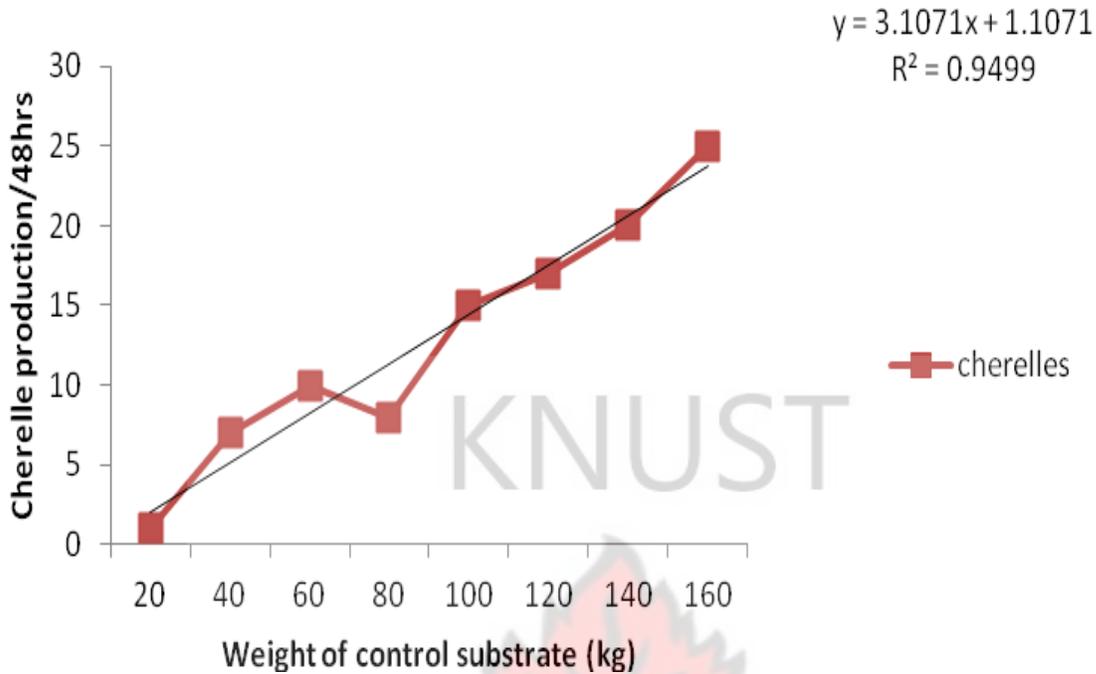
Figure 13g: **Rate of Cherelles production under Control treatment**

$$y = 6.333x + 6.5$$
$$R^2 = 0.659$$
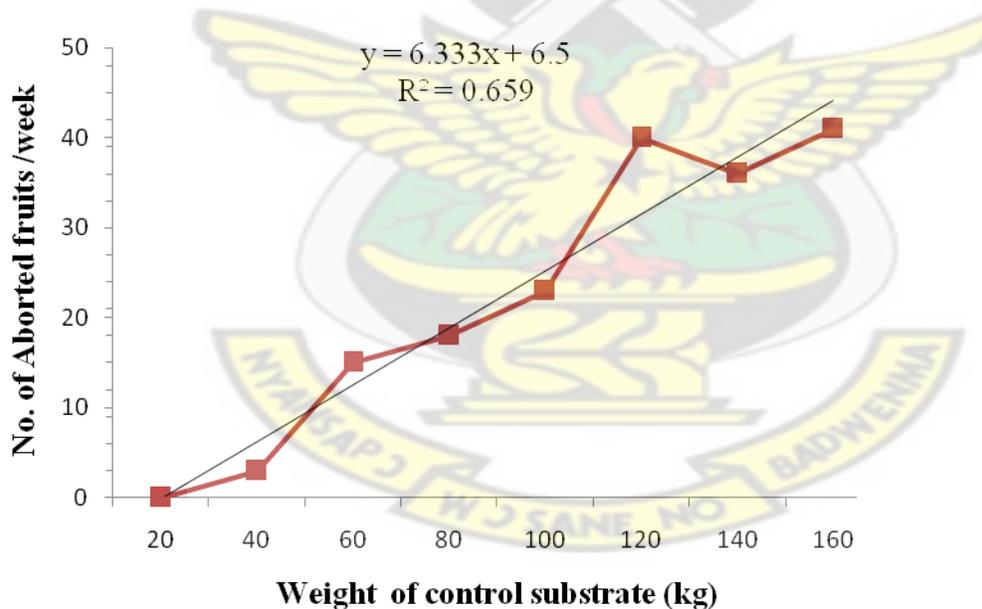
Figure 13h: **Rate of Fruit abortion under Control treatment**

Analysis of the fruits and seeds production in the years, by Pearson's goodness-of-fit statistic of the data obtained under the cocoa pod husk and banana stem substrates treatment indicated that there was no significant inter-year difference in the fruit set (df =1, X^2, =1.43, P= 0.357 or seed set (df =1,

X^2, =210, P = 0.158) of the pollinated flowers. There was significant difference in the fruit proportions produced between the different treatments (t-test =1.82; df =238; *P*=0.05), which shows that all the trees were not under the same pollinating conditions.

4.3.3 *Midge Flights from Substrates*

Daily observation indicated a consistent upsurge of midge flights in the morning (06.00hrs) during the 14-hour daily observation for 7-days. Midge flights reduced at 07.00 hrs and gradually increased till 10.00hrs. There was another decrease between 11.00hrs and 13.00hrs. Increase in the rate of midge flights occurred between 18.00 and 19.00hrs (Fig 17).
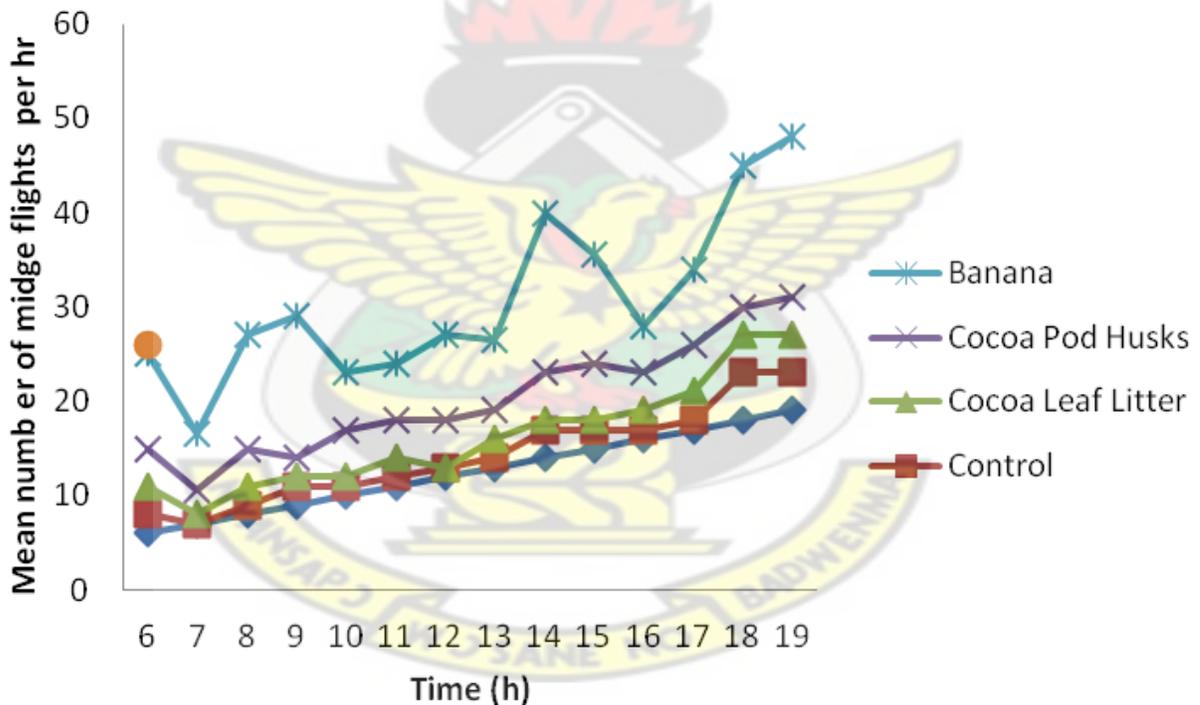
Figure 14: **Mean number of Midges emerging during the 14 hour observations.**

Out of the six emergence boxes containing the three substrates, the banana substrate produced a total of 283 midges with a mean of 7.1 (± 2.2) per day, followed by the cocoa pod husk (Cph) which released 271, mean 6.23 (±1.9). Cocoa leaf litter (Cll) had the lowest of 79 insects with a mean of 2

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(± 1.2) over the 10- day period of observation. There was however, differentials in range of flight events, from as narrow range as 1-5 in the case of cocoa leaf litter to a wide range of 1- 21 for the banana stem (Table 17).

Cocoa leaf litter extract attracted relatively lower number of midges as the water ($t_{4,5}$ = 2.85, $P<0.05$). The cocoa pod husk extract and the banana extracts recorded relatively higher midge upsurge, ($t_{3,16}$ = 3.15, $P< 0.05$) but the banana extract had the highest(Table 17). The total number of midges attracted to the various extracts daily for 10 days varied significantly ($F_{9,15}$ =3.02, $P < 0.026$) with a coefficient of determination value of R^2= 0.69. Differences in the treatment was also significant ($F_{10,7}$ =3.64, $P < 0.05$). The flight rates of the midges showed that the population of midges in the banana substrates was significantly different ($F_{1,2}$ =3.64, $P< 0.05$) from that of the other substrates. The one-way analysis of variance (Appendix V) showed the rate of emergence from the six boxes was significantly different ($P < 0.05$).

Table 17:   Midge emergence from substrates during the 10-day observation

Substrate Type	Daily Rate of Emergence	
	Mean ±SE)	Range
Water (Control)	0.85 (1.12)	1-3
Cocoa Leaf litter	1.95 (1.25)	1-5
Cocoa pod husk	6.23 (1.73)	1-13
Banana pseudostem	7.07 (2.19)	1-21

4.4.0 Natural and Artificial Pollination

4.4.1 *Pollinator exclusion experiment*

The exclusion of pollinators caused a significant decrease in fruit set (df = 2, X^2 =12.5, P = 0.001), flower set (df=2, F = 25.2, P < 0.001) compared to trees that experienced the routine natural and manual pollination. All the flowers that were covered and thus un-pollinated dropped in batches between the second and third days. The calculated value of pollen limitation index [$L=1-(P_o/P_s)$] was L=0.593.

4.4.2 *Natural Pollination (NP) verses Artificial Pollination (AP)*

Table 21 shows that the mean number of seeds in the small pods of naturally pollinated cocoa were higher (36.47 ± 11.22) than those produced through the artificial pollination (28.20 ± 7.94). Also the number of seeds in the medium and large sized cocoa pods of the naturally pollinated cocoa was more than that of the artificially pollinated. There were significant diferences between the number of seeds of naturally pollinated and that of artificially pollinated fruits of small size pods (P< 0.04), medium size pods (P< 0.01) and the large sized pods (P<0.01). The number of seeds per fruit in the small size pods were significantly different (paired t test = 4.08, 29 d. f.; P = 0.01); just as those of the medium and large (paired t test =3.54, 29 d. f., P = 0.01; and paired t test = 1.26, 29 d.f., P = 0.02 respectively) (Table 21). There was a correlation between the sizes of the pods and the number of seeds (Table 21). However, the correlation grew weaker from the small pods (correlation = 0.37; significance P = 0.04) to larger pods (correlation = 0.28; significance P= 0.13).

Table 18: Number of Seeds in Different Cocoa Pod sizes

Mode Of Pollination	Mean ± SD	Correlation	Significance
	Small size cocoa pods (5-10cm)(n=30)		
NP[a]	36.47 ± 11.21		
AP[b]	28.2 ± 7.94		
NP-AP[c]	8.27 ± 2.15	0.37	0.04
	Medium size cocoa pods (11-60cm) (n=30)		
NP	43.97 ± 4.67		
AP	39.60 ± 6.69		
NP-AP	4.37 ± 6.76	0.33	0.01
	Large size cocoa pods(>60cm)(n=30)		
NP	46. 47 ± 5.56		
AP	44.97 ± 5.27		
NP-AP	1.50 ± 6.5	0.28	0.01

$t_{(small)}$ = 4.08; df = 29; significance = 0.001; $t_{(medium)}$= 3.54; df = 29; significance = 0.001
$t_{(large)}$ = 1.26; df = 29; significance = 0.22
[a]NP = Natural Pollination; [b]AP= Artificial Pollination; [c] NP-AP=difference between Natural and Artificial Pollination

A similar trend was found in the weight of cocoa pods produced under the natural and artificial pollination. The mean weight of small pods of naturally pollinated cocoa was higher (4.09 ± 15. 24) than the mean weight of the artificially pollinated (3.87± 12.77); and the weights of medium and large fruits of naturally pollinated fruits were similarly greater than those of the artificially pollinated (Table 22). There were also significant diferences between the weight of naturally pollinated and the artificially pollinated fruits of small size pods ($P< 0.05$), medium size pods ($P < 0.019$) and the large sized pods ($P <0.019$), and the weight per fruit of the small size pods were significantly different

(paired t test = 1.10, 29 d.f.; $P < 0.01$). The medium and large sized pods followed the same trend (paired t test =2.23, 29 d.f., $P = 0.03$; and paired t test = 3.05, 29 d.f., $P = 0.05$ respectively)

Table 19: Weight of Cocoa Pods of Different Pod Sizes

Mode Of Pollination	Mean± SD	Correlation	Significance
	Small size cocoa pods (5-10cm)(n=30)		
NP	4.09 ± 1.24		
AP	3.87 ± 1.77		
NP-AP	2.20 ± 11.05	0.02	0.05
	Medium size cocoa pods (11-60cm) (n=30)		
NP	7.31 ± 5.08		
AP	6.59 ± 1.30		
NP-AP	7.13 ± 13.92	0.01	0.019
	Large size cocoa pods(>60cm)(n=30)		
NP	10.0 ± 6.28		
AP	8.93 ± 2.91		
NP-AP	1.1 ± 6.19	0.014	0.019

$t_{(small)}$ = 1.10; df= 29; significance = 0.01; $t_{(medium)}$ = 2.23; df = 29; significance = 0.03
$t_{(large)}$ = 3.05; df=29; significance = 0.05

There were large variations in the individual fruit weight and quantity of seeds, however, a regression analysis showed a significant linear relationship between weight (y) and seed number (x) of individual fruits: $Y = 18.56 + 0.016x$; $R^2 = 0.45$.

A preliminary proximate analysis, using AOAC (2005) method revealed that the food constituents were common in fruits produced under the two pollination regimes (Table 20). Pod by pod the moisture and fat content of the naturally pollinated pod was lower than the artificially pollinated, but the protein, fibre, ash and carbohydrate of the naturally pollinated fruits were higher. On the other

hand, apart from the moisture content of the naturally pollinated seeds which was higher value all other constituents were lower than the artificially pollinated fruits.

Table 20: Preliminary Proximate Analysis Report sample: cocoa pod and seed

PARAMETER	NATURAL POLLINATION		ARTIFICIAL POLLINATION		METHOD
%	POD	SEED	POD	SEED	
Moisture	73.3	49.17	79.62	40.65	AOAC*
Fat	0.08	5.12	0.11	3.94	AOAC
Protein	1.86	9.8	1.32	8.32	AOAC
Fibre	7.21	22.64	6.48	17.61	AOAC
Ash	1.6	1.5	1.25	19.17	AOAC
Carbohydrate	15.95	19.01	11.22	18.17	AOAC

Association of Official Analytical Chemists (1990). Results quoted on wet basis.

CHAPTER FIVE

DISCUSSION

5.1.0   Floral Phenology of Cocoa Trees

5.1.1   *Flowering patterns and Fruit production*

The data showed that the Amazonia cocoa exhibited seasonally-related phenological patterns of flowering and fruit-set involving overlapping cycles i.e. phenophases of flowering, fruiting, leaf-formation and leaf-fall. Similar findings have been found in other tropical tree species (Alvim, 1977, 1984; Ashton et al., 1988; Young, 1986; Ewusie, 1992) emphasizing the fact that cocoa shares in this characteristics with other tropical tree species. Through segmental flowering pattern, the trees exhibited a longer spread of flowering as the branches and trunks flowered in turns. This phenomenon is akin to the "manifold" (a term first used by Whitmore, 1975) leaf production in some tropical plants. Flowering started on the tree trunk at the beginning of the rainy season, but by the close of the year, the branches were the main flower-bearing part of the tree.

Flowering was highest in the rainiest months (Fig 2; Table 9) which started in April each year. The result agreed with the study by Omolaja et al. (2009) which indicated that more flowers are produced in May among the clones of *Theobroma cacao,* indicating that rainfall promotes flowering intensity. However, it suggested that cocoa is much unlike most tropical trees (Ewusie, 1992) which produce less flowering during the rainy season.

Fruit set is the stage of flower-to-fruit survivorship at which plants match the number of pollinated flowers with resources available for fruit maturation (Shapiro and Addicott, 2004). Its production also followed the rainfall pattern (Fig 3), however, the floral production was inversely proportional

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to the fruit set (Fig 4). This could be a function of both the number of flowers and pollination efficiency as indicated by Efron et al. (2003) in the SG2 cocoa hybrid.

The observation that flowering appeared to have been affected by the level of fruiting in the preceding year suggested a possible inter-year linkage in resource use by leaves and fruit as suggested by Southwick (1984) and Snow and Whigham (1989). Further investigation into this observation might be important. However, it confirms some earlier suggestion that there is competition between pods and flowers for assimilates Alvim, 1977 cited in Valle et al., 1990).

The tree-to tree differences in the production of floral buds, opening of flowers, and cherelles formation (Table 9) seem to coroborate the findings that cocoa flower initiation, shape and morphogenesis though dependent on the environment is regulated endogenously (Mohr and Schopfer, 1994 cited in Omolaja et al., 2009). The observation that cherelles formation and cherelle wilt (fruit abortion) increased during the rainy season and the peak of flowering (Fig 6; Table 12) was observed in earlier study (Valle et al., 1990).

The data suggested that more small pods were produced, followed by the medium (Fig 5). The large pods were least produced. This could be attributed to the characteristics of the Amazon cocoa variety found in the study area. The reduction in the number of cherelles after the month of August (Fig 6), when fruit formation had increased implies that more cherelles might have matured into fruits. The level of new pod production and pod losses through abortion (Fig 7) also reflected the phenological patterns of flowering of the cocoa trees.

From the result more flowers were produced as compared to the fruits. This phenomenon is observed in angiosperms (Larson and Barrett, 2000). Vaughton and Ramsey (1995) had suggested two proximate ecological mechanisms might account for this pattern: insufficient pollen delivery to stigmas and limited resources for maturation of fruits and seeds. Effective pollination and adequate nutrients are therefore critical in the cocoa production. In practice, however, nutrients are often monitored to the neglect of pollination.

5.1.2 *Fruit Loss in the study cocoa trees*

The study showed that pod losses through incidences of diseases were relatively less and that fruit losses occurred more through abortion (Table 12). Earlier studies had shown that conditions, which favoured pod production, coincided with conditions favourable to disease incidence (Meideros, 1976; Holderness, 1992). The study confirmed such findings. Some studies had also correlated the incidence of black pod disease with environmental factors e.g. black pod *P. palmivora* in West Africa in relation to rainfall, temperature, and humidity (Thorold, 1967; Wood, 1974). Efombagn et al. (2004) identified fruit aged 2-3 months as the highly susceptible stage of development in the most susceptible clone. Olaiya et al. (2006) had suggested that pod loss due to biotic factor was of greater importance than cherelle wilt in the overall reduction of pod yield, and is a major factor in cocoa plantations. The observed levels of diseased pods in the area indicated that disease might not be a major determinant of final cocoa yield levels in the area as at the time of study. However, pollination success largely determined the initial levels of fruit-set (Young (1986).

Fruit abortion is a well-known phenomenon in angiosperms (Stephenson, 1981; Sutherland, 1987; Valle et al., 1990; Ehrle´N, 1991; Brown and McNeil, 2006). Daymond and Hadley (2008) described fruit abortion (or cherelle wilt) in cocoa as a fruit thining mechanism, characterized by an

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initial cessation of cherelle growth, after which it turned yellow and then blackened and shriveled whiles remaining on the tree. This was corroborated by Groenveld et al. (2010). According to McKelvie (1956) cited in Daymond and Hadley (2008) cherelle wilt could occur anytime up to around100 days after pollination. It is described as a within-plant condition that creates physiological yield-reducing condition (McKelvie, 1958, 1962; Valle et al., 1990; Brown and McNeil, 2006; Bos et al., 2007). Fruit losses through cherelle wilt had been reported in the range of 19–25% in Trinidad (Pyke, 1933 cited in Humphries, 1943), and 22–84% in Ghana (Hewison and Ababio, 1929).

Some authors have attributed the causes of cherelle wilt to competition for carbohydrates (Nichols, 1964; Valle et al., 1990; Lachenaud, 1991), and greater carbohydrate demand through increased respiration rates (Mossu, 1980; Wood and Lass, 1985; Valle et al., 1990; Hadley et al., 1994). Thus, cherelle wilt could be important in regulating the number of fruit per tree (Wood and Lass, 1985; Lachenaud, 1991). By aborting fruits early in their development, plants conserve many of the necessary resources for flowering, developing the remaining fruits, future reproduction, and vegetative growth (Valle et al., 1990). The above explanations might account for the increase in fruit abortions with higher yield of the cherelles observed in this study. Efron et al (2003) suggested that as rainfall increased the proportion of wilted cherelles decreased. However, this was not found in the present study, and might be attributed to differences in the cocoa varieties.

Earlier studies had demonstrated that flowering and fruit set in some trees occur at the expense of future growth. For example, flower and fruit production led to a decrease in growth in several conifers (Eis et al., 1965; Snow and Whigham, 1989). Many nectar producing flowering plants can invest more energy into flowering than the amount of energy stored in fruits (Southwick, 1984). Cost of high fruit set is avoided to some extent by plant species that abort pollinated flowers or young

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fruits (Lloyd 1980; Stephenson, 1981). The heavy fruit of cocoa can contain over 400 times more energy than is stored in its flowers (Valle et al., 1990). This investment increases the physiological importance of fruit abortion (Bos, 2006).

5.1.3   *Effect of climatic factors on floral production*

The data suggested that flowering of the cocoa trees in the study farms were affected by exogenous factors such as rainfall, temperature, and light (Figs 8-11). The temperatures were stable with slight fluctuations. The wind was almost still in the environment most of the time of data collection unless the occasional thunderstorm accompanied by high wind velocity during rainfall. The contribution of temperature to the production of the flower buds and open flower production (Table 6; Fig 8) confirmed earlier sggestions that fruit developmental rate is affected by temperature in a number of tropical plant species (Alvim, 1966; Adams et al., 2001; Snelgar et al., 2005; Omolaja et al., 2009). Temperature, however, had negative effect on the new pod production. This trend had been observed under field (Alvim et al., 1972), and semi-controlled conditions (End et al., 1988; Daymond and Hadley, 2004). Relative humidity, however, did not make much impact on the flushing process. Light intensity complimented the effects of temperature.

From the result rainfall could be isolated as the most critical factor in the floral phenology since it was observed to play a major role in the phenological cycle of the cocoa plant (Table 6; Fig 10). This was confirmed when flowering occured at the beginning of the raining. It suggested that the cocoa variety studied reacted to stimulus of water implying that water is a major cue for flowering for this crop. During the dry seasons (January through March) and before the onset of the rains in April there were no buds, flowers, or new pods on any of the tree trunks although flowering occured on the inaccessible branches. This could be an evidence of water stress (Dand, 1999). Flowering was

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greater in the humid period of the year than in the drier weather as suggested by the study. The result corroborated earlier claim by Ojo and Sadiq (2010) that rainfall plays vital role in cocoa production. Cocoa therefore could be susceptible to drought and hence the pattern of cropping could be related to rainfall distribution as suggested by Anim-Kwapong and Frimpong (n.d).

The monthly mean variations in the floral and fruit productions as well as fruit losses appeared to be the same during certain times of the year e.g. from September through December the floral production (mature floral buds, open flowers, and cherelles), fruits and fruit losses were similar, and significantly different ($P<0.05$) from April through August. This again underscored the importance of rainfall. The amount of rains largely determined the total floral and fruit production according to this study.

The study has established the role of climatic factors (extrinsic) in the phenology of the Amazonian cocoa tree. Other studies (e.g. Alvim, 1977, 1984; Young, 1986; Ewusie, 1992) however, have also indicated that genetic and physiological (intrinsic) factors appear to contribute to the phenological behavior of the cocoa plant. Ewusie (1992) has proposed that the genetic and physiological factors may be the primary factors responsible for basic timing of flowers while the climatic controls are the modifying factors and has classified these factors as proximal and distal factors respectively. The phenology of flowering and fruiting is seasonally variable among species, and these events do not always correspond to times of greatest photosynthetic activity or resource availability (Snow and Whigham, 1989).

5.1.4 *Flower Stability*

From the study the cocoa trees massively lost their flowers when unpollinated. The implication is that the stability of the flowers on the cocoa trees was greatly influenced by seasons and pollination i.e. abiotic and biotic factors (Table 5). Flowers that dropped in the dry season appeared dry, pinkish and reduced in size. The rate of flower drop was high before the onset of the rains. On the other hand pollinated flowers were stable during the five days of observation even though in the dry period few flowers dropped. According to Young (1983) a significant issue of cocoa pollination biology concerns synchronization (or lack thereof) between pollinator population cycle and the phenology of the flowering cocoa trees, and the adequacy of pollination i.e. the minimum number of seeds per fruit. Earlier studies by Brew (1984) indicated that the numbers of cocoa pollinating midges reduced during the dry season but increased in the wet season. He attributed this to the habit of the midges which live and breed in cool and moist environment.

Vaughton and Ramsey (1995) had earlier suggested that low level of pollination resulting from low numbers of pollinators and reduced accessibility to nutrients and nutrients uptake may account for the great loss through flower drops. These were confirmed in this study during the dry seasons in 2007 and 2008 when cocoa leaves were dried up and unsuitable for breeding of midges (Brew, 1984). It has long been known that tropical forest accumulates plant nutrients in the top few centimeters of soil. When such forest is cleared, the nutrients are rapidly released and give the soil a high fertility for a few years. However, the Amazon cocoa, which is high yielding variety, depletes the soil of its nutrients at a faster rate (Anim-Kwapong et al.*, unpublished*). The relatively higher stability of cocoa flowers in the wet season might be explained in terms of requisite nutrients of cocoa which need water for effective uptake, and physiological cues inherent in the cocoa trees.

During the dry season the reduced moisture in the soil might have affected the amount of nutrients for the cocoa plant. To obtain physiological balance flowers are abscised. The vast number of flowers that abscised might also result from a predisposition of cocoa to limit fruit development as explained above. Consequently, only under the best of circumstances may the necessary physiological signals be able to override the flower abortion signal. Hasenstein and Zavada (2001) had attributed the initiation of the abscission process in mature flower buds to the decline in auxin level. This seems to apply to cocoa flowers. Since flower production represents a considerable investment for the plant (Stephenson, 1981) the number of flowers could affect the allotment of auxin for each flower.

5.1.5 *Vertebrate predation*

The results showed that a few of the mature cocoa pods were attacked by squirrel, *Scuirius* sp. They deplete pods which would otherwise be available to midges as breeding substrates. Also by nibbling the pods they allow pod rotting pathogens to enter. Given the number of pods attacked over the period of study the squirrel attack may not be a major problem in this area. Baksh (2008) has proposed that well shaded and protected cocoa provide an ideal environment for rodents. This study therefore suggests a regular review of squirrel activities by farmers and researchers taken into consideration the shade factor.

5.1. 6 *Ecological Significance of the Phenological pattern*

The flower opening or anthesis at dawn had earlier been suggested by Swanson (2005). The opening of mature flower buds at dawn 0500h-0700h might be an adaptive mechanism to ensure effective pollination by the insect pollinators whose visits are known to start at dawn (Brew, 1988). The significance of this study lies in the fact that cocoa pollinating agents presumably derive some

nutritional benefits from cocoa flowers therefore the breeding cycles of these insects may be similar to the flowering cycles of cocoa trees (Young, 1982).

The intensity of flowering of cocoa coinciding with the rainy season could present some advantage for cocoa reproduction and productivity. The main pollinators which belong to Ceratopogonidae (biting midges) are moisture loving dipterans (Brew, 1984; 1988) and are known to increase in population during the rainy months (May to July) when flower production reaches its peak. Their population declines in the dry seasons (Brew, 1988). Having more flowers in the rainiest months therefore could enhance its reproductive capacity. During the study there were days in the rainy seasons that saw continuous rainfall. The flowering behavior of the cocoa trees as demonstrated in this study could allow for continuous pollination and subsequent fruiting. Harvesting of cocoa therefore may not be a one-time activity for the farmer, but a series of activities which may begin from one year and end in the following year.

An issue of the study is that a whole-tree floral census was not possible, given the height of the trees, however, the total number of trees (N=100) used in the study, spread across the study area made it possible to assess the generality of patterns observed in the area. The results, therefore, could be instructive. Conducting this study at ten different sites over a period of three years could form the basis of examining the spatial and temporal predictability in phenological patterns of cocoa in view of the fact that these sites were less than 2 km apart, and shared the same species, and experienced similar climatic regimes (Young, 1983; Donald, 2004).

5.2.0   Insect Assemblage in the Cocoa Plantations and their Pollination Status in Relation to the Cocoa Trees.

5.2. 1   *Insect species in the Cocoa Farm plots*

The main objective of this study was to establish the insect pollinator assemblage in the cocoa ecosystems. The findings indicated that the cocoa ecosystem is a mosaic of insect species (Table 7; Appendix I), and was consistent with the assertion that "insects dwell within complex ecosystems and interact with other taxonomic groups and the abiotic environment" (Hunter, 2002). Earlier studies had indicated that cocoa farms with their diverse shades have the potential to support high insect species richness equivalent to that of forest ecosystems (Parrish et al., 1998; Zapfack et al., 2002; Bobo et al., 2006).

The insect assemblage in the cocoa farm plots could be attributed to certain favourable factors including microclimate, particularly temperature, humidity and light intensity, interactions with other species. The temperature (range: 20-25^{0}C) and relative humidity (range: 41-98.5%) in the study plots appeared suitable for the insect species. Though the cocoa trees studied were largely unshaded implying their exposure to the full effect of solar light, the inter-tree spacing virtually merged the canopies thus creating self-shade. This might have ameliorated the direct and extreme impact of the solar radiation on the soil surface (Nair, 1984; and Beer, 1987). It was observed that farm plot number 10 which had the lowest canopy cover of 10.92% due to wider spacing of cocoa trees was also lowest in terms of insect abundance and species richness (Table 8; Appendix I). This was because parts were exposed to direct sunlight were drier and warmer than those that were protected by canopy cover. The results agreed with findings of some authors (Levings and Windsor, 1982; Feener and Schupp, 1998; Ofori-Frimpong et al., 2007; Klein et al., 2003) who demonstrated

that microclimate play important role in insect distribution, diversity, abundance, foraging activity, and nesting places.

5.2.2 *Insect Abundance and Distribution in the cocoa farms*

The relative proportions and distribution of insect species (Tables 7 and 9) might represent the response of the various insect communities to the ecological factors in the study farm plots (Kareiva, 1994; Dauber et al., 2003). The results (Table 7) indicated that Diptera, Hymenoptera, and Lepidoptera were more abundant and more specious, compared to Coleoptera, Hemiptera, and Orthoptera. Therefore to discuss the ecological significance of the insect communities in the cocoa ecosystems emphasis was placed on the three i.e. Diptera, Hymenoptera, and Lepidoptera.

Table 7 suggested that the Dipterans, were present in significant proportions (46.52%), and thus dominated the cocoa ecosystem by numbers. The midges together constituted 83.25% of the dipteran population and 38.40 % of the total insect population (Table 9). Cocoa entomologists (Entwistle, 1972; Kaufman, 1975; Brew, 1984; Young, 1994) are unanimous that these ceratopogonids i.e. midges are the accredited pollinators of cocoa; hence their presence could be of high ecological importance. The findings of this study supported this claim.

The order Hymenoptera was dominated by 14 different species of ants, and two species of bees (Appendix I, Table 9). Ants were the most abundant insect species. The sampled ants amounted to 45.28% of the entire insect assemblage in the cocoa ecosystem. The observation corroborated findings by earlier workers that in general, ants tend to dominate the arthropod fauna of many tropical ecosystems, including cocoa plantations. Ants constituted 10-33% of the arboreal arthropod biomass in Brazilian cocoa plantations (Majer et al., 1994) and 89% of the total insect numbers in

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Ghanaian cocoa plantations (Leston, 1973). Arboreal sampling indicated that ant species richness was high in cocoa plantations, with 67 species and 88 species found in Ghanaian and Papua New Guinea cocoa plantations (Room, 1971; Room, 1975). Estimates of species richness increased substantially when ground-foraging ants were included in the samples, rising to 128 species in Ghanaian cacao plantations (Room, 1971). The richness and abundance of ants was not affected by distance of farms to the forest implying the adaptability of ants to diverse ecological conditions. The result suggested that ants could be excellent bio-indicators as they could be sensitive to environmental variables and rapid response to environmental changes (Andersen, 1990). The importance of ants have been well documented (Gomez and Zamora, 1992; Majer, 1993; Garcia et al., 1995; Gomez et al., 1996; Gomez, 2000; Ghazoul, 2001; Ballmer, 2003; Philpott et al., 2006). Though the results indicated that ants did not deposit any pollen on the stigmas of the cocoa flowers, some authors (Brew, 1984; Altshuler, 1999; Philpott et al., 2006) had suggested that ants facilitated pollination success indirectly by attacking pollinators and subsequently increasing their movement and thus pollen transfer between flowers. The presence of some dominant species of ants (*Oecophylla spp, Crematogaster and Pheidole*) might be due to the fact that there is some established and functioning ant mosaics based on nest site selection by the species sampled (Djieto-Lordon and Djean, 1999) implying that ant mosaics can be manipulated to favour one ant species to the detriment of others, as part of a biological control. Bees are an important functional group due to their mutualistic interactions with plants (Allen-Wardell et al., 1998), however, this study could not confirm this assertion.

In relative proportion the lepidopterans (butterflies) sampled in the farms ranked third (2.09%), and were greatly dispersed in the farms. Butterflies are known to be affected by predation and parasitism (Kruess and Tscharntke, 1994; Zabel and Tscharntke, 1998), habitat heterogeneity in terms of

plant/floristic diversity (Steffan-Ingolf and Tscharntke, 2000; Schulze et al., 2001), microhabitat, and generally by habitat fragmentation (Schulze et al., 2001). The cocoa farms studied were by and large a monocultural system and therefore could not be a satisfactory surrogate for the reserve forest in terms of plant or flower diversity. Also the morphology of the cocoa flower and the virtual absence of nectar could not be attractive to the butterflies because lepidopterans have shown that their visitation to flowers positively correlates to the flower size (Thompson, 2001). A combination of factors therefore might be responsible for the presence of the butterflies in the cocoa ecosystem studied. The self-shade due to the canopy cover, coupled with the relatively low wind speed could be conducive to the butterflies (Estrada et al., 1997; Hamer et al., 1997); and flowers of various plants species available in the surrounding farms and vegetation were visited by the butterflies (Mennechez et al., 2003; Schticking and Baguette, 2003).

The low numbers of the coleopterans, hemipterans and orthopterans in the cocoa ecosystem might be due to some agricultural practices. Studies by Wilson et al. (1999) showed that beetles are affected by monocultural practices (as in the cocoa farm plots studied) instead of mixed farming. Orthoptera are known to lay eggs at or near the ground surface. This makes them vulnerable to soil disturbance (Marshall and Heas, 1988; Wilson et al., 1999). Residing in the cocoa farms which is under regular farming activities could affect the fecundity and hence the low numbers. Hemipterans though thrive under intensive farming regimes are known to visit periodically especially during the fruiting period.

5.2.3   *Focal patch observation: Insect Visitation to the Cocoa trees*

The results also indicated that about 52.81% (Table 10) of the insect species resident in the cocoa ecosystem did not visit the cocoa trees at all implying that there was no ecological relationship

between the cocoa trees and that group of insects. Despite the wide range of insect species in the cocoa farms only a small fraction of them visited the flowers (Table 10), and a still lower numbers were effective pollinators (Fig 14 and 15). These findings are consistent with earlier studies by Wilson and Thomson (1999) and Johnson and Steiner (2000). It was noted that the main flower visitors in all farms and in all the years of study were the ceratopogonids (order: Diptera) which accounted for an estimated 97% of the flower visitors. They interacted with the cocoa flowers carrying pollen. These most abundant visitors could be collectively called "the principal visitors". Their visit frequency was not affected by location of farm plot ($P < 0.05$). Contrary to earlier assertion by Kaufmann (1973) none of the crawling insects found on the cocoa trees provided any pollinating service since the flowers dried up after 24 hrs and fell in their presence (Tables 11 and 12). The non-interaction between large number of resident insects and the cocoa plant therefore might be attributed to their lack of requisite morphological structures that could enable them to successfully pollinate the cocoa flowers.

5.2.4   *Focal patch observation: Bee Activities on the Cocoa flowers*

The study revealed that the number of *Hypotrigona araujoi* bees on the cocoa flowers per day of observation was generally very low, and was not as regular as expected. The data demonstrated that the visitation rate of *Hypotrigona araujoi* was very low for only a handful of them visited during the period of observation. The mean number of visits per hour was not statistically significant ($P>0.02$) among the study trees meaning that the level of availability of the bees could not be important to the pollination of the cocoa as suggested by Can-Alonzo et al. (2005) who demonstrated that effective pollinators could be determined by the high density of foragers. The results also corroborated the position of Buide (2006) who established some correlation between visitation rate of pollinators per plant, and mean number of flowers visited per plant, on one hand and the total seed production.

Engel and Irwin (2003) proposed a relationship between pollinator visitation and pollen receipt by flowers. They suggested that increased pollinator visitation should result in increased pollen receipt by stigmas. Given the the low availability and abundance of *Hypotrigona araujoi* on the cocoa flowers it could not be an effective pollinator.

5.2.5   *Pollinator importance of Insect Visitors*

The data showed that the *Forcipomyia spp* midges demonstrated great pollinator importance by their visitation rates and massive deposition of pollen (60.1 ± 13) on the stigmas (Fig 14 and 15). Brew (1984) had noted that cocoa flower needed 35 or more pollen grains deposited on the stigma for effective pollination to occur. The study therefore corroborated assertions by earlier authors (Brew, 1984; Bos et al., 2007; Klein et al., 2007; Frimpong et al., 2011) that they are the true pollinators.

The investigation showed that the stinglessbee scarcely deposited pollen on the stigmas of the cocoa flowers. Of the sixty stigmas selected for examination only one had four (4) grains of pollen. The four pollen grains on one stigma for the period of study (total of 1080 minutes per year of observations) might have been accidentally placed on the stigma. Since pollen deposition is a function of pollinator efficiency i.e. the effectiveness at picking up pollen from anthers and depositing pollen on stigmas (Inouye et al., 1994) it could be inferred that though the bee might have visited the cocoa flowers they did not pollinate the flowers. The perception that the stingless bees pollinate cocoa flowers might have stemmed from the fact that these bees collect some pollen which was presumed to benefit the plant reproduction. This assumption, however, was not confirmed by this present study.

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From the study it could be concluded that contrary to speculations and assertions of earlier authors there were no other cocoa pollinators which remain unknown or undescribed, and that the abundance of insect species did not make them pollinators.

5.2.6 *Ecological significance of the Insect communities in the cocoa farms*

The Amazonia cocoa that was studied was self-incompatible and its reproductive system entirely dependent on insects. Therefore, the non-interaction between some resident insects and the cocoa plant might be as a result of the floral structure of the cocoa plant which requires few specialized insects for reproductive success (Valle et al., 1990; Brew and Boorman, 1993). Cocoa might be among those plants which posseses flowers that restrict access to all but a few pollinator species. The Amazonia cocoa therefore has what could be considered as specialised pollination system, in which pollinators belong to just one pollinator class, and are often just a few species of a single insect family, tribe or even genus as suggested by Goldblatt and Manning (2005). Olsen (1997) observed that an insect's foraging strategy, whether specialist or generalist, reflects selection to maximize exploitation of floral rewards. The observed pollinating behavior of the ceratopogonid midges underscores a specialized association (Bystrak and Wirth, 1978; Brew, 1984). However, the degree to which midges are specialized pollinators of cocoa is undetermined (Young, 1986).

The significance of this study lies in the fact that the specialization in plant-pollinator relationships as in the case of the Upper Amazon cocoa has implications for conservation biology, since pollinator decline has effect on plant fitness making it important to identify the essential factors of the interaction between plants and their pollinators (Bond, 1995; Allen-Wardell et al., 1998; Kearns et al., 1998; Karrenberg and Jensen, 2000). This study therefore is an attempt to contribute to the

understanding of the role of cocoa ecosystems in the protection of tropical diversity, and this, is a promising avenue for research and management of cocoa ecosystem.

5.3.0 Comparative Evaluation of Breeding Substrates for Cocoa Pollinator in Cocoa Farms.

5.3.1 *Midges' Population in the Breeding Substrates and Effect on Cocoa Pollination*

The result demonstrated that all the decomposing substrates accommodated the cocoa pollinators. This abservation agrees with earlier assertion by (Entwistle, 1972) that cocoa pollinators depend on moist rotten plant material, where they breed. The substrates could therefore be described as their breeding sites. The implication is that farm management that controls the availability of nesting habitats for ceratopoginid midges can play an important role in local increases of pollinator abundances as suggested by Young (1982).

Table14 showed that there was a positive correlation between the increased weight of the substrates and the midge population meaning that the accumulation of the substrates might have led to an increase in the population of the pollinators. This observation could be attributed to the fact that each of the substrate type was collected from different parts of the farms, thus enhancing the chances of getting more midges (both adult and younger stages) for each substrate. As the substrates are breeding sites of the midges their gathering as done in this study might have brought along more midges. The results are consistent with an earlier prediction that the provision of suitable breeding sites for cocoa pollinators could increase pollination (Winder, 1977).

Again the data (Table 14) illustrated that the population of midges also differed significantly ($P<0.05$) with different substrate types implying that decaying organic substrates could be limiting factors in the breeding structure of pollinator populations (Young, 1987). Rotten banana pseudostem

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presented the highest output in terms of population of midges and pollination level among the substrate types and, rotten cocoa pod husk substrate had the next higher output as indicated by the result (Tables 14 and 15). As the substrate types were found within the same farm plots the data seem to suggest that the pollinators might have preferred the banana pseudostem substrate to others. This might corroborate earlier observation by Young (1982) that adding discs of rotten banana stems to the ground-litter, on a shaded cocoa farm, increased fruit set on nearby trees. Winder and Silva (1972), however, had earlier on classified rotten cocoa pods as the best substrate with regard to species diversity and number of insects, however, this run counter to the findings of the present study. The observation raises the issue of substrate preference by the different species of midges. Winder, J.A (*Unpublished*) had shown that Ghana has more citations (65%), on individual species of positively identified pollinators of cocoa, followed by Costa Rica (24%) and lastly Brazil (12%). It is probable that different species of midges might have preferences for different substrates. Owing to taxonomic difficulties it was not possible to classify the midges in this study. A further study in this direction is therefore proposed. The results of the control regime as presented in Tables 14 and 15 implies that the non-accumulation of substrate as pertains to the current cultural practices in cocoa cultivation could result in pollination deficit, as pollinator abundance is limited by breeding sites, and fruit set by pollinator abundance (Young, 1982; 1987).

The data also indicated that increase in population of the midges led to increases in pollination levels (Table 15). Accumulation of the substrates might have resulted in higher rates of pollinator visits to the flowers, and also enhanced their pollinator importance (Armbruster, 1988; Olsen, 1997). As the substrates increased leading to increase in the population size of the midges, more midges became available, hence there was increase in the visitation rate and the level of pollination. The increased pollination could also be explained in terms of inverse relationship between time pollinators spend

searching for flowers and the population size of the pollinator as observed by Holland and DeAngelis (2002). The result therefore underscored the earlier proposition that pollinators contribute more toward pollination success of a plant (Jennersten and Morse, 1991; Thomson and Thomson, 1992). Results (Appendix IV) showed that both the increase in substrates weight and substrate type had effect on the level of pollination and the subsequent fruit set thus underscoring assertion by (Young, 1987).

From the data the higher the levels of successful pollination, indicated by flower set, the greater the fruit set (cherelle) and this involved higher fruit abortion (Tables 15). Moreover, the flower set far exceeded the fruit set and fruit abortion. This underscores the findings by Groenveld et al. (2010) that the intensity of flower production and pollination intensity are responsible for the number of fruits. Conversely, substrates which produced more flower sets had less flower abortion (Table 15). Flower abortion has been attributed to low pollination or reduction in auxin level due to delayed pollination (Hasenstein and Zavada, 2001; de Almeida and Valle, 2007). Given the fact that the pollinator population determined the pollination intensity, as indicated above, the flower abortion could be higher under the control conditions where the lowest pollinator population was registered.

The progression in the pollination intensity (Figure 15) observed among the four treatments over the period of observation. The data indicated that there was no significant difference in level of pollination in the first two weeks. This may be attributed to the midges' adaptive response to the moisture availability in the farms. During the period of experiment rainfall started in the second half of April each year, but until June when the experiment was carried out, the rainy season was characterized by intermittent and unpredictable rainfall. The rainfall pattern might have affected the population density of midges as they require continuous and sustained moist conditions where food

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supplies such as bacteria, fungi, and mould thrive (Young, 1984; Brew, 1984; Ruf, 1995). That holding it might have taken sometime for the pollinators to build up their population in order to effect pollination as was observed from the third and fourth weeks. The data also showed differences in substrate impact over the eight week period of observation (Table 16). This may reflect on the population dynamics over the period of experiment. Since the population of pollinators determines the pollination intensity (as explained above), it could be inferred that there were shortages of pollinators among the substrates at a point in time as the demand for pollination services exceeded available pollinator capacity in the substrate (NAS-USA, 2007). The data, however, indicated that for greater part of the time of experiment the banana substrates carried more pollinators.

5.3.2   *Impact of Substrates on Cherelles and Fruit Abortion*

The data showed positive linear relationships between the substrates and the production of cherelles as well as fruit abortion (Figures 16a, b, c, d, e, f, g, h). This implied that the substrates enhanced both the visitation rate and pollinator importance of the ceratopogonids which have been identified as the principal pollinators of cocoa (Armbruster, 1988; Olsen, 1997; Holland and DeAngelis, 2002). This thesis had already demonstrated that under normal conditions the visitation rate of the biting midges *Forcipomyia* spp was low though its pollinator importance was established as high (Figs 13 and 14). The result therefore suggest that deliberate accumulation of substrates under cocoa trees must be encouraged as it could lead to stepping up the visitation rate of pollinators translating into higher level of pollination.

The increase in fruit set and fruit abortion with increase in pollination as indicated in the study is consistent with the observations by Stanton et al. (1986), Valle et al. (1990) and Bos et al. (2007). It may be explained in terms of the energetic budget of the cocoa tree that determines its carrying

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capacity (Bazzaz, 1987; Brown and McNeil, 2006). Since cocoa fruits contain up to 400 times more energy than flowers (Valle et al.1990), more fruit set at a time could place greater demands on the energetic budget of the cocoa plant, disrupting its metabolism and manifesting in greater rates of fruit abortion (Stephenson 1981, Stanton et al., 1987; Brown and McNeil 2006). High abortion of fruit set after the high levels of pollination could therefore decrease the initial benefits derived from increased pollination as suggested by Bos et al. (2007). It implies that there might be an optimum level of pollination for the cocoa plant. A hand pollination study by Groenveld et al (2010) indicated that optimum pollination for cocoa is beyond 40% of the flowers, meaning that under normal conditions natural pollination levels in cocoa determined at 10% of all flowers on a tree (Falque et al., 1995; Bos et al., 2007; de Almeida and Valle, 2007) are likely to fall very short of the pollination optimum. This study has shown that boosting up the population of the pollinators could increase the pollination intensity and hence raise the level of pollination.

A critical issue is the possible balance between the fruit abortion and the final yield of cocoa. From the results above the fruit abortion increased with high level of pollination and corresponding fruit set. This could erode the initial benefits obtained from enhanced pollination as assessed at the fruit-setting stage. Due to time constraints the observation was limited to premature measures of yields. However, study by Groenveld et al. (2010) has indicated that the anticipated shortfall could be only partial in that increases in pollination intensity could result in much higher number of pods and yield compared to pollination under normal conditions where only 10% of the flowers were pollinated.

5.3.3 *Midge Flights from the Substrates in emergence boxes.*

The observation showed that generally the movement of the midges followed a cyclic flight pattern involving an upsurge in flights followed by low flights. This might be an adaptive behaviour by the

pollinating insects for effective foraging, thus ensuring pollinating efficiency. The flight pattern may be determined by the flower opening time (Glendinning, 1972; Brew, 1984), floral reward (Young and Severson, 1994; Seymour et al., 2003) and energetics in pollinator activities (Price, 1997). Brew (1984) has indicated that pollination activities peak between 06.00 and 09.00 hrs. He observed that pollination is at its best from the time of flower opening in the morning till noon, and that the *Forcipomyia* spp were extremely scarce between the hours of 10.00 and 11.00 until between 15.00 and 17.00hrs when pollination was resumed on a smaller scale. Price (1997) has observed that the cost of foraging is high in terms of calories used in flying, and therefore the rewards must be higher to make a net energy gain while foraging. The cocoa plant might be responding to the energy needs of the midges by producing cluster of small flowers that flower synchronously. It could also be suggested that temperature, and other exogenous factors have a profound effect on pollinators, particularly the poikilothermic insects (Adjaloo and Yeboah - Gyan, 2003).

The results again indicated that while the cocoa leaf litter extract and water attracted equal numbers of midges the extracts of banana stem and cocoa pod husks were quite high (Fig 23; Table 17). This implies that rotting cocoa pod husk and the banana stems might possess the right environment for the nurturing of the immature stages of the larvae, and therefore are much more preferred alternative for the pollinating insects. However, among the substrate types banana is most preferred according to the study as already demonstrated in this study. Screening of the extracts for their chemical constituents using standard phytochemical procedures revealed the presence of reducing sugars and tannins in all the substrate extracts, but no alkaloids and saponins. A further chemical analysis of the cocoa pod husk and the banana stems to determine the exact constituents the immature midges thrive on will go a long way to establish the possibility of formulating a feed for these precious beneficial insects.

5.3.4   *Ecological significance of the substrates on the cocoa farms*

Each of the substrate found on the farms had great significance in the cocoa ecosystem as they all harboured the cocoa pollinators. Data indicated that from the 5th to the 6th week the cocoa leaf litter had an impact on the cocoa pollination comparable to that of the cocoa pod husk; however, its capacity was greatly reduced by the 8th week. Since the cocoa was produced in unfertilized farms the litter from cocoa could play a central role in the supply of nutrients to the crop. Therefore its importance in the pollination ecology of cocoa cannot be underrated. The tropical cocoa tree has up to several leaf production cycles (rhythmic, discontinuous flushes) per year (Miyaji et al.*,* 1997), and in Ghana cocoa leaf-litter production follows a definite cycle, with a peak in the dry season, August, November to February and through the wet season, March to July and September (Brew, *unpublished*). Several factors might contribute to their fall. These include fungal infections, raindrops, wind damage (Alvim, 1977; Miyaji and Tagawa, 1979), insect injury, and physiological fall (result of higher rate of transpiration) (Miyaji et al., 1990). However, percent leaf decomposition rather than total leaf-fall has been identified as the important factor in determining the midge population (Brew, A.H. *unpublished*) and that there were significant positive correlations between both immature and adult midge populations and percentage leaf decomposition under both shaded and unshaded regimes (Brew, A.H. *unpublished data*).

Cocoa pod husks, the commonest by-product of cocoa are known to contain phosphorus, a critical element to the fertility of the soil. Leaving the pod husk on the cocoa farms therefore improves the soil nutrients. Some earlier studies also suggested that the pod husk may be the home of many genera of immature ceratopogonids (Winder, J.A. *unpublished*; Young, 1982; Brew, 1988). The data in this study suggested that cocoa pod husk could support the cocoa reproduction more than the cocoa leaf litter. The provision of large amounts of cocoa pod husk near growing cocoa trees may,

however, conflict with current cultural control of black pod disease and cocoa pathologists strongly recommend that such residue should be removed and burnt.

Bananas, a fast growing and high biomass-yielding plant, are rich in both phosphorus and potassium which are important macro-nutrients plants need. It might be possible that these constituents play a role in the ability of the banana pseudo stem substrate in harbouring more midges. The results of this study suggested that the rotten banana pseudostem supported highest population of the pollinators most of the time, and therefore contributes immensely to the pollination ecology of cocoa. It implies that its continuous presence on the cocoa farm could be recommended.

5.3.5   *Implications of study for Cocoa Production and Agroforestry*

The results of the study call for some modification in the current cultural practices in order to boost the pollinator densities by increasing the habitat-carrying capacity. Kaufman (1975) had suggested the need to create conducive environment for the cocoa insect pollinators in the farms. Earlier workers have observed that habitat destruction has been a problem in the pollination of cacao (*Theobroma cacao*), and that the removal of rotting substrate in which the pollinating midges undergo larval development (Winder, 1977; Kevan and Truman, 2001), have resulted in yield reduction. Some authors have therefore suggested placing appropriate plant materials such as banana (Young, 1982) or palm trunks (Ismail and Ibrahim, 1986) to encourage and maintain adequate pollinator forces. The study also corroborates the observation by Frimpong et al. (2011) that the presence of plantain/banana intercrop influenced the abundance of ceratopogonids; and confirms the assertion of Elizondo and Enriquez (1988) that permanent addition of banana to the intercrop system could help boost the population of pollinating ceratopogonids thereby enhancing of cocoa production. The outcome of this study is consistent with these observations and suggestions.

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A cocoa farm with a heterogenous shade cover and/or substrates can be expected to have a larger resident pool of pollinating midge species. The fact that small proportion of cultivated cocoa flowers (ca. 5%) are successfully pollinated indicate that there is obviously a delicate balance between crop success and failure and this combined with huge potential losses later in the cycle viz: failure to set seed (no fertilization), fruit abortion, incidence of diseases means that the level of pollination may be a constraint on the achievement of economic yields in many growing areas of the world. Results of this study therefore are relevant to improving the ultimate yield per ha of cocoa.

Traditionally, most Ghanaian cocoa farms have either plantain or banana trees together with other useful companion crops other crops purposefully grown such as cocoyam, maize, to diversify farm products and income, secure land tenure, and cocoa seedlings and generate early returns increase biophysical advantages for production (Isaac et al., 2007; Somarriba, 2007). However, as they are planted primarily to provide shade for young cocoa plants most cocoa farmers do not maintain the banana or plantain intercrop.

Somarriba (2007) had earlier indicated that banana has higher carbon storage capacity of 20 tC/ha/year and higher carbon fixation rate of 1,5 tC/ha/year compared to plantain (storage: 8tC/ha/year, and fixation: 0,5 tC/ha/year). Though this study did not include plantain substrate it could be inferred that the additional benefit of carbon availability in banana to the environment could serve as an advantage over plantain in cocoa agroforestry.

Observations from the study are probably of general application in view of the worldwide tropical distribution of *Forcipomyia* spp and other closely allied ceratopogonid midges. Moreover, the

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importance of pollinating midges has led to some attempts at its breeding under laboratory conditions with limited success (Winder, 1978; Brew, 1988). Accumulation of substrates which leads to higher population density of the midges under natural farm conditions could be an indirect approach to breeding of pollinators.

5.4.0   Natural and Artificial Pollination

5.4.1 *Pollinator exclusion experiment*

The result indicated that exclusion of pollinators resulted in pollen limitation (pollen limitation index $[L=1-(P_o/P_s)]$ was $L= 0.593$)**.** Data therefore demonstrated that pollination was a limiting factor in cocoa production. Larson and Barrett (2000) had noted that self-incompatible angiosperms generally have higher pollen limitation index ($L= 0.59$).They explained that self-compatibilty and autogamy were associated with reduced pollen limitation, presumably because of the capacity for self-fertilization which decreases reliance on cross-pollination by pollinators. The result confirms earlier findings of insect exclusion experiments which showed that cocoa is strictly entomophilous and obligatorily requires insect pollinators (Cilas, 1988; Ibrahim, 1988; Posnette, 1950). The Amazon cocoa studied was self-incompatible and therefore needed an external agency for the sustenance of its reproductive system. Pollen limitation is an important factor influencing seed set in plants (Ashman et al., 2004). Klein et al. (2007) have categorized cocoa among the 13 leading crops whose production would be reduced by over 90% in the absence of pollinators. Pollination in cocoa has been evaluated to be a higher order limiting factor in cocoa yield than agronomic resources (Groeneveld et al., 2010).

Knight et al. (2005) attributed pollen limitation in plants to consequences of ecological perturbations such as floral phenotype, presence of co-flowering plant species, decreases in plant population size/density and pollinator decline. Plants pollinated by biotic pollinators are usually considered to be more specialized than those pollinated by abiotic vectors (Culley et al., 2002). Such specialization may provide more reliable pollination, resulting in lower levels of pollen limitation. Alternatively, plants (such as cocoa) relying on specialized pollinators may experience greater variation in their pollination success as the abundance of a few specialist pollinators may fluctuate in space and time (Waser, et al., 1996; Knight et al., 2005). The result therefore is instructive as it underscores the need to ensure the presence of adequate pollinators of cocoa.

5.4.2 *Natural verses Artificial Pollination*

The results demonstrated that the natural pollination could contribute to the reproductive success, and hence the productivity of cocoa. Data indicated that generally the number of seeds of naturally pollinated fruits was more than those of the artificially pollinated (Table 21). This might be explained in terms of unrestrained access of pollinators to the cocoa flowers under the natural conditions. The structure of the cocoa flowers retricts the pollinator guild to the ceratopogonid midges, *Forcipomyia* spp, for effective pollination as already suggested in this study, however, the number of midge pollinators which could visit the flowers in a given period and their visitation rate were not regulated by any internal or external mechanism (Bos et al., 2007). Therefore the cocoa flowers in the open farm environment could receive unlimited number of pollinating insects, thus resulting in pollinator effectiveness (Ivey, 2003). All these observations could be explained largely in terms of the pollination intensity (Falque et al., 1995), and hence pollen grains deposited onto the stigma during the pollination process. It could determine the number and size of pollen load deposited per stigma, and hence, the seed number per fruit. Various authors (Quesada et al., 2001;

DaMatta et al., 2008) investigating the reproduction in angiosperms have linked the number of pollen grains per stigma to the production of fruits and seeds. It could be that as the number of compatible microgametophytes (both the pollen grains and tubes) increases, seed set also increases (Quesada et al., 2001); thus, the genetic composition of pollen load could determine the number and size of seeds produced per fruit. The results obtained in the study confirmed this.

From Table 22 the mean weight of the naturally pollinated fruits was also higher than the artificially pollinated ones irrespective of the pod size. Fruit weight has been found to increase with increasing pollen load in crops such as cashew (Soares et al., 2007; Vergara et al., 2010) and coffee (Ricketts, 2004; Vazquez et al., 2005) which was due to an increase in growth rate. The results seem to agree with the proposition that there is some relationship between the number of seed produced and weight of crops (Marcelis and Hofman-Eijer, 1997; Albano et al., 2009). Other authors (Daymond and Hadley, 2008; Groenveld et al., 2010) have also indicated that the weight of individual cocoa fruits might be determined by the number of fruits per tree at a time. They explained that the available nutrients determine the fruits production. Efron et al. (2003) have reported that cocoa production involves several different stages starting with flowering, followed by pollination, cherelle and pod development and ending with mature ripe pods. Each stage is controlled by a different set of genes that may interact differently with the various components of the environment, and may or may not be affected by events that occurred in previous stages. High number of flowers increases the probability of obtaining more pods, however, the number of fruits/pods harvested depends on post-flowering events (pollination, cherelle wilt and pod/fruit losses), and the underlying factor is the availability of nutrients.

The weak correlation between the number of seeds and pod/fruit sizes might imply that there might be some variation in the contributions of pollinators toward pollination of the cocoa flowers (Ivey,

2003). Earlier authors (Picken, 1984; Marcelis and Hofman-Eijer, 1997) have indicated that higher seed numbers could increase the rate of fruit growth at the early stage of its development. This study did not cover this process and is recommended for further investigation in the case of cocoa.

Data suggested that there was no correlation between the weight of the cocoa pods and the the sizes of pods (Table 22). This implies that the weight of cocoa fruit could be dependent on other factors. Though the precise relationship between the fruit weight and the fruit sizes of cocoa may not be known, given the fact that there is some relationship between the weight of fruits of crops and the number of seeds in the fruit, and in view of the fact that the number of seeds per fruit affects the rate of fruit growth it could be inferred that some relationship could exist as in other crops (Marcelis and Hofman-Eijer, 1997; DaMatta et al., 2008).

The preliminary proximate analysis (Table 23) suggests that the food nutrients in fruits produced under the two pollination regimes are the same as expected. The results therefore suggest that natural pollination of cocoa could contribute to the productivity and therefore the overall production of cocoa.

CHAPTER SIX

6.0   CONCLUSION

6.1   *Introduction*

The seminal role of cocoa in the Ghanaian economy is well acknowledged (Dormon et. al., 2004; Vigneri, 2008), however, its production at the expense of the forest is of great concern (Binam et al., 2008). Moreover, the yield per hectare is low compared to the other major producers (MoFA, 2003). Even though the low yield has been attributed to other determinants of yield such as poor farm maintenance against pests and diseases and low yielding varieties (Anon, 1999; Abekoe et al., 2002), Brew (1984) and Mabbett (1989) believe that low pollination due to some cultural practices which affect pollinators. It is against this background that the pollination ecology of cocoa and the breeding substrates of cocoa pollinators were examined with the aim of determining the management of pollination system which is relatively untried (Kearns and Inouye, 1997).

The study commenced with the determination of the floral phenology of the Amazon cocoa which forms the bulk (58.52%) of varieties of cocoa in the country. It was followed by a survey of insects in the cocoa ecosystem and determination of their pollinator status. The relative importance of the breeding substrates in the cocoa ecosystem was also investigated, and the natural pollination was compared with the artificial pollination with regards to yield of cocoa. The following are the conclusions were reached from the studies:

6.2   *Conclusion on Floral Phenology of cocoa trees*

Cocoa like all tropical tree species in general exhibit season-related phenological pat-terns of flowering but unlike most tropical plants, flowering is at the height of the long rainy season. This

was evidenced in the segmental flowering pattern in repated phases. In addition, fruit-set involved overlapping cycles (the phenophases of flowering, fruiting, and leaf-formation and leaf-fall more than once a year) under both intrinsic and extrinsic controls. Rainfall, as an extrinsic factor, was found to be the most critical in the floral phenology. The floral phenological pattern coincided with the activity of the main pollinators of cocoa which resulted in enhanced reproductive capacity for increased production of cocoa. The study showed that cocoa productivity could also be affected by biotic factors such as flower drops due to no or insufficient pollination, fruit abortion, but to very lesser extent squirrel damage.

6.3   *Conclusions on Insect Assemblage in the Cocoa Plantations and their Pollinator Status.*

The cocoa ecosystem supports diverse insect communities. A total of 2721 insects belonging to 36 species and 7 orders were sampled from the ten farms. However, the floral structure restricted access to few insects. A limited range of insects were associated with the cocoa trees. Crawling insects including certain species of ants and of psyllids did not pollinate the cocoa flowers as earlier asserted by Kaufmann (1973). Only the midges and stingless bees could enter the flowers and carry pollen. The non-pollinator status of the bees was also established. The presence of majority of insects enriched the ecosystem but had no direct bearing on the cocoa production. The ceratopogonid midges (order: Diptera) were beneficial to the productivity of cocoa through pollination. The study therefore suggests that contrary to speculations and assertions of earlier authors the cocoa tree has a specialised pollination mechanism, in which pollinators belong to just one pollinator class (Goldblatt and Manning, 2005), and that the abundance of insect species was not necessarily indicative of pollinator status. The species of ceratopogonid midges found in this study suggested a specialized association confirming assertion by earlier authors (Bystrak and Wirth, 1978; and Brew, 1984),

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however, the degree to which midges are specialized pollinators of cocoa is undetermined (Young, 1986). The significance of this study lies in the fact that the specialization in plant-pollinator relationships asin the case of the Upper Amazon cocoa has implications for conservation biology, since pollinator decline has effect on plant fitness it is important to identify the essential factors of the interaction between plants and their pollinators (Bond, 1995; Allen-Wardell et al., 1998; Kearnset al., 1998; Karrenberg and Jensen, 2000). As cocoa pollination is species-specific, there is the need to determine the suitable environment for the sustenance of its optimum population on the cocoa farms (Kaufman, 1975). This study therefore is an attempt to contribute to the understanding of the role of cocoa ecosystems in the protection of tropical diversity, and this, is a promising avenue for research and management of cocoa ecosystem.

6.4 *Conclusions on the Comparative Evaluation of Breeding Substrates for Cocoa Pollinator in Cocoa Farms*

Of the three substrates found in the cocoa farms the decomposing banana pseudo stem was the most preferred substrate as it supported the highest population of the midges. The rotten banana pseudo stem might have provided a more condusive breeding microhabitat for the ceratopogonids. Increase in all substrates led to increase in the level of pollination. This implies that by increasing the habitat-carrying capacity, the population density of *Forcipomyia* spp. is enhanced. Therefore the accumulation of breeding substrates under natural farm conditions could be an indirect approach to breeding of midges in cocoa production systems.

6.5 *Conclusions on Natural Pollination and Artificial Pollination*

Natural pollination increased number of seeds and weight of cocoa pods. Also the food nutrients in fruits produced under the two pollination regimes are the same. Therefore natural pollination

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contributes to the productivity and therefore the overall production of cocoa. Artificial pollination has advantage over natural pollination as it may be employed to develop new varieties of crop. Since farmers do not have the artificial pollination technique, and cannot cope with the cost implications natural pollination should be encouraged in the cocoa cultivation.

6.6   *Significance of the Study*

The study has wide application for the over fifty developing countries across Asia, Africa and Latin America where cocoa is grown. Ghana's cocoa production enjoyed a period of growth, yet her cocoa yields per hectare are still low by international standards. It is expected that with the application of the findings cocoa production in Ghana, West Africa and the world wide, will be increased in terms of yield per ha (intensively), and not by expansion by acreage (extensively). A presentation has been made to personnel from the Cocoa Research Institute of Ghana (CRIG) and has received their commendation. The findings could be employed by the Extension Unit of the Ministry of Food and Agriculture to help cocoa farmers incorporate them in the cocoa cultivation practices.

The application of the findings could lead to sustainable conservation of populations of cocoa pollinators which is the main objective of The African Pollinator Initiative, a wing of the International Pollinator Initiative of the Food and Agriculture Organization (FAO). Finally, the study has added to and updated the body of literature on cocoa pollination.

6. 7   RECOMMENDATIONS

The study has brought to light many more uncharted areas related to the pollination ecology of cocoa and the breeding substrates of cocoa pollinators. The following are therefore being presented as recommendations for possible future research:

1. Lack of identity of the ceratopogonids posed great limitation in this study. Taxonomic services are necessary if further advancement could be made. Some taxononmic studies have been done in Southern America. To date there is no qualified midge taxonomist in Ghana. There is the need to train some individual (s) to facilitate studies.

2. The detailed pollinating activities of the midges need to be studied to enhance scientific understanding of the pollination process. I recommend that a scientific approach using a sharp camera for a 24-hour surveyance be used to follow the pollination process. Future studies should establish how the pollinators enter a new cocoa farm, and what happens prior to the bearing of fruit by the cocoa tree.

3. The study recognizes that the rate of visiting same flower by one or more pollinators has not been assessed. It is therefore recommended that future study of natural pollination should include the frequency of pollinator visitation and fruit set.

4. There is the need to determine the chemical composition of the breeding substrates. The similarity or differences in the chemical composition might assist in formulating a feed for the pollinators to enhance pollination.

5. It is also recommended that the effect of higher seed numbers on the rate of fruit growth at the early stage of cocoa's development should be studied.

6. Demand for scientific information by farmers is high, especially with reference to pollination. Personnel of the Extension Unit, Ministry of Food and Agriculture should be well equipped with the expertise.

Abundance, Diversity and Distribution of Insects sampled in 2006/2007/2008

Flowering Period in the ten Farms. Number of individual insects.

Insect Taxa	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	Total
Order:Hymenoptera											
Family: Formicidae											
Polyrhachis viscose (Mayr)	25	20	15	10	5	12	14	10	5	12	128
Polyrhachis decemdentata (Mayr)	8	5	5	2	2	3	2	1	2	2	32
Polyrhachis weissi (Bolton)	11	3	1	2	2	4	10	5	4	1	43
Polyrhachis militaris (Bolton)	10	12	0	5	5	7	5	4	2	4	54
Polyrhachis laboriosa (Forel)	50	55	20	16	14	45	20	11	11	10	252
Polyrhachis lestoni (Bolton)	4	21	1	2	3	4	10	1	2	7	55
Camponotus acvepimensis (Mayr)	28	23	6	14	2	1	1	4	1	2	82
Bothroponera pachyderma (Santschi)	17	15	4	0	0	10	6	31	22	0	105
Bothroponera silvestrii (Emery)	20	19	11	5	5	11	10	2	4	1	88
*Oecophylla longinoda (*Latreille)	15	13	1	0	1	0	0	0	2	0	32
Cremastogaster africana (Emery)	14	16	0	12	15	0	4	4	3	0	68
Cremastogaster depressa(Latr).	52	45	15	11	3	0	4	6	4	5	145
Cremastogaster clariventris(Mayr.)	44	40	11	5	4	1	0	4	1	0	110
Pheidole megacephala **(**Fabricius)	4	6	0	1	2	1	7	0	9	8	38
Family: Apidae											
Apis mellifera adansonii	2	0	0	1	5	2	1	0	0	0	11
Family: Meliponinae											
Hypotrigona araujoi (Michener)	5	2	1	1	3	2	1	1	2	0	18
Family: Vespidae											
Polistes marginalis	1	1	1	0	0	0	1	0	0	0	3
Order: Hemiptera											
Family: Pentatomidae											
Chlorochroa sayi (Stål)	2	0	1	0	5	0	0	2	1	1	12
Family: Coreidae											
Anoplocnemis curvipes (Nymph)(Fabricius)	1	1	0	0	0	1	1	0	0	0	4
Dysdercus spp	2	2	1	0	2	0	0	0	0	0	5
Order: Lepidoptera											
Bebearia congolensis (male)	5	4	0	1	2	1	0	0	0	0	13
Euphaedra janetta (male)	6	3	1	0	1	1	0	0	0	0	12
Euphaedra medon (male)	3	5	0	0	2	0	0	0	0	0	10
Euphaedra medon medon(female)	2	1	0	0	0	0	1	0	0	0	4
Bebearia absesa abesa(male)	4	2	0	0	1	1	0	0	0	0	8
Gideona klots (moth)	1	1	1	0	0	0	1	1	1	0	6
Pterocarpus spp	2	1	0	0	1	0	0	0	0	0	4
Order: Orthoptera											
Family: Acridae											
Heterachis guineensis	8	4	1	1	1	2	1	2	3	2	25
Order: Diptera											
Drosophila spp	60	11	15	20	11	16	5	25	17	10	190
Cecidomyiid spp	51	37	36	54	33	42	51	56	55	52	467
Forcipomyia spp	70	45	81	50	55	45	91	55	21	65	578
Musci domestica	2	5	1	1	6	7	2	5	1	1	31
Order: Coleoptera											
Carabid sp	3	4	2	2	1	2	3	2	1	1	21
Apogonia sp	2	1	3	1	1	2	2	1	1	2	16
Order: Heteroptera											
Distantiella theobroma Distant	3	8	0	1	0	0	1	0	0	1	14
Bathycoelia thalassina Scumacher	9	4	1	6	0	8	3	2	1	3	37
Grand Total of species											**2721**

APPENDIX II

Statistical analysis of visitation frequency vs pollination efficiency (i.e. Visitor x Flower interaction) (2-way ANOVA) of midges compared with visitation frequency vs pollination efficiency of other visitors.

Source of variation	Df	SS	MS	F
Ceratopogonids				
Visitation frequency	5	102.5	20.5	3.55
Visitor x flower interaction	7	1,210.58	172.94	28.79**
Residual	35	202.17	5.78	
Total	47	1,515.25		
Other visitors				
Visitation frequency	3	3.14	1.05	0.801
Visitor x Flower interaction.	5	31.65	6.33	7.93 ns
Residual	15	19.72	1.31	
Total	23	54.51		

**$p < 0.05$; ns=non-significant

ONE WAY ANOVA FOR EACH WEEK OF SUBSTRATE APPLICATION TO SHOWING DIFFERENCES WITHIN WEEKS.

Week 1: Effect of Substrate Application on Pollination

Source	Df	Sum of Squares	Mean Squares	*F*	P
Substrate	33	332.833	66.567	1.74	0.2586
Error	62	229.167	38.194		
Total	95	562.000			

Week 2: Effect of Substrate Application on Pollination

Source	Df	Sum of Squares	Mean Square	*F*	P
Substrate	33	339.417	67.883	1.20	0.4107
Error	62	340.833	56.805		
Total	95	680.250			

Week 3: Effect of Substrate Application on Pollination

Source	Df	Sum of Squares	Mean Square	*F*	P
Substrate	33	908.0833	181.6167	4.89	0.0395
Error	62	222.8333	37.13888		
Total	95	1130.917			

Week 4: Effect of Substrate Application on Pollination

Source	Df	Sum of Squares	Mean Square	*F*	P
Substrate	33	1343.0000	268.60000	17.02	0.0017
Error	62	94.66667	15.7778		
Total	95	1437.66667			

Week 5: Effect of Substrate Application on Pollination

Source	Df	Sum of Squares	Mean Square	F	P
Substrate	33	400.0833	80.0166	4.67	0.0437
Error	62	102.8333	17.13888		
Total	95	502.91667			

Week 6: Effect of Substrate Application on Pollination

Source	Df	Sum of Squares	Mean Square	F	P
Substrate	33	840.416	168.0833	7.73	0.013
Error	62	130.500	21.75000		
Total	95	970.9167			

Week 7: Effect of Substrate Application on Pollination

Source	Df	Sum of Squares	Mean Square	F	P
Substrate	33	1414.333	282.8666	6.30	0.022
Error	62	269.333	44.889		
Total	95	1683.666			

Week 8: Effect of Substrate Application on Pollination

Source	Df	Sum of Squares	Mean Square	F	P
Substrate	33	27090.84375	820.93466	27.93	0.0001
Error	62	1822.39583	29.39348		
Total	95	28913.23958			

APPENDIX IV

Two Way analysis of variance for the effect of Substrate and weight on Pollination and Fruit set.

Source of Variation	d.f.	S.S.	M.S.	F	P
Substrate	3	6479.364	2159.788	73.48	0.001
Weight	7	17397.989	2485.427	84.56	0.001
Substrate *wt_substrate	21	3071.219	146.248	4.98	0.001
Residual	31	142.271	4.589		
Total	62	27090.843			

APPENDIX V

One-way ANOVA of midges' emergence from the six emergence boxes.

Source	Df	Sum of Squares	Mean Square	F	Pr>F
Model	6	1629.3	271.5	1.50	0.2910
Error	8	1450.4	181.3		
Total	14	3079.7			

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