RELATIVE ABUNDANCE OF THE WILD SILKMOTH, *Argema mimosae* BOISDUVAL ON DIFFERENT HOST PLANTS AND HOST SELECTION BEHAVIOUR OF PARASITOIDS, AT ARABUKO SOKOKE FOREST

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university or any other award.

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DEDICATION

This thesis is dedicated to my family, parents, brothers and sisters for their perseverance, love and understanding which made this task possible.

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ABBREVIATIONS AND ACRONYMS

ASF	Arabuko Sokoke Forest
ASFMT	Arabuko-Sokoke Forest Management Team
ASL	Above Sea Level
ANOVA	Analysis of Variance
CIP	Commercial Insect Programme
DIFAAFA	Dida Forest Adjacent Area Forest Association
FADA	Forest-Adjacent Dwellers Association
GC-MS	Gas Chromatography - Mass spectrometry
GEF	Global Environmental Facility
Hr	Hour
ICBP	International Council for Birds Preservation
Icipe	International Center of Insect Physiology and Ecology
IFAD	International Fund for Agricultural Development
KEFRI	Kenya Forest Research Institute
KFS	Kenya Forest Service
KWS	Kenya Wildlife Service
CQI	Composite Quality Index
MNR	Ministry of Environment and Natural Resources
NK	Nature Kenya
NMK	National Museums of Kenya
PFM	Participatory Forest Management
SAS	Statistical Analysis Software

UNDP	United Nations Development Programme
VOC	Volatile Organic Compounds

ABSTRACT

Sustainable utilization of natural resources and biodiversity conservation form the basis of the livelihood and existence of communities living in Africa and in other developing countries. Overexploitation of natural resources and forest destruction for better livelihood by local communities has led to decline in the African biodiversity. Beneficial insects like wild silkmoths exist in the eco-system, and can be integrated in biodiversity conservation and income generating micro enterprises. Hence, there is need to conserve wild silkmoths and increase their distribution for their economic use in silk industry. The general purpose of this study was to survey the relative abundance of wild silkmoth *Argema mimosae* Boisduval (Lepidoptera: Saturniidae) on different indigenous food plants and explore food plant volatiles responsible for parasitism/predation of the developmental stages of the moth in Arabuko Sokoke forest, Kenya. The distribution of the food plants was studied through Global Position System and mapped with Geographical Information System. Larvae were reared in semi-captivity using net sleeves on the branches of *Lannea schweinfurthii* Engl. (Anacardiaceae) and *Ozoroa obavata* Oliv. (Anacardiaceae). The mass and size of cocoons were measured using weighing scale and vernier calipers (0-15cm) respectively. A scanning electronic microscope was used to examine the grains and the filament structure of the cocoons. The cocoons were boiled in a solution of 5gms/ltr of sodium carbonate at different time intervals to soften them for silk extraction and in distilled water as a control. Chemical volatiles emitted by growing *A.mimosae* food plants viz *L. shweinfurthii* and *O. obovata* were collected using a portable volatile collection system with super Q as the adsorbent trap.Volatiles were collected for 2 hr in the morning between 0900 and 1500 hr with two replication in each set up. Characterization of the volatile compounds was done by Gas Chromatography-Mass Spectrometer and analysis was carried out on a HP 7890A model series GC coupled to a 5975C mass spectrometer and a Triple Axis Detector. Components of the volatiles were identified by comparing their mass spectral data with those in the library of mass spectrometer and by retention time analysis. The distribution of the food plants was significantly different between the forest and the farm land. Among the seven food plants recorded, *O. obovata* has not been reported elsewhere in East Africa as food plant for *A. mimosae*. Most of the cocoons recorded belonged to *A. mimosae* moth and occurred more in the farmland in smaller clusters of 10-20 on the *L. schweinfurthii* food plant while few cocoons were found in *O. obavata* in clusters of 1-5. This shows that *L. schweinfurthii* is probably more preferred by the *A. mimosae* larvae than *O. obovata.* Larvae passed through six instars and the developmental period was not significantly different between the two food plants. The instars expressed cryptic colouration of black, orange and green in different instar as a defense mechanism against natural enemies. Silvery brown floss was extracted from cocoons boiled in sodium carbonate solution but not in those boiled in distilled water only. Several terpenes such as (E)-β-farnesene, (E)-β-Caryophyllene, (E)-β-ocimene were identified. (E) beta-Farnesene was distinctively trapped from *L. shweinfurthii* leaves at higher peaks when the *A. mimosae* larvae were feeding on the leaves. The abundance of the wild silkmoth food plants discussed under this study provides a basic planning strategy to initiate wild silkmoth farming in Arabuko sokoke forest and conserve the forest sustainably.

CHAPTER ONE

1 GENERAL INTRODUCTION

1.1 Background information

Sericulture is the process of rearing silk-producing insects in captivity or collecting their silk in the field for human use, mainly leading to the production of fabrics (Peigler, 1993; Akai, 2000). In the world, there are about 400 -500 species of silk-producing moths, out of which 8-9 are known to produce silk of commercial value (Dingle *et al*., 2005). Natural silk is broadly classified as *B. mori* or domesticated silk from *Bombyx mori* L. (Lepidoptera: Bombycidae), which produces 95-99 % of the silk under commercial use in the world today (Scoble, 1995; Raina, 2000, 2004; Dingle *et al*., 2005; Raina *et al*., 2007), and non-*B. mori* silk (Gongyin and Cui, 1996; Kioko *et al*., 1999a,b, 2000a,b, 2007; Veldtman *et al*., 2007a,b; Fening *et al*., 2008a,b).

The domesticated silkmoth, *B. mori* originated from its wild counterpart, *Bombyx mandarina-moore* by gene duplication and chromosomal fusion mechanism (Botlagunta *et al*., 2006). Wild silkmoths or non-*B. mori* silkmoths are generally those that are not reared in captivity. Instead of rearing the moths, native people collect cocoons from wild populations. In some cases, some semi-captivity rearing is done, often outdoors with little or no protection of the larvae (Dingle *et al*., 2005; Rai, 2005; Kioko *et al*., 2000a,b; Ngoka *et al.*, 2008). Despite great efforts by various National and International Agencies, raw silk production has failed to keep up with the steady rising demand. Some of the leading *B. mori* silk producing countries such as India, Japan and China appear to

have reached saturation point, attributable to the acute scarcity of labour and the increasing cost of production.

This offers developing countries an opportunity, with the enabling environment (surplus labour, land and ideal climate) to raise their silk production for the developed world market. For this reason, the high quality untapped non-*B. mori* silk has drawn the attention of silk users (Jolly *et al*., 1979; Raina *et al*., 1999, 2000; Kioko *et al*., 2000a, 2007; Raina, 2000, 2004; Raje, 2005). In addition, the low volume of wild silk production offers an exclusive niche market where scarcity and naturalness is highly valued, leading to a high price for fabrics made from wild silk (Veldtman *et al*., 2002; Raina, 2000, 2004). In Africa, the development of sericulture technology as a rural cottage industry is needed to enhance the income generation potential of the poor-resource rural communities and to ensure the conservation of the rich biological diversity (Peigler, 1993; Raina *et al*., 1999, 2000, 2007, 2009, 2011; McGeoch, 2002; Raina, 2000, 2004; Rodgers, 2005; Salehe, 2005; Kioko *et al*., 2007; Fening *et al*., 2008a).

Research on the utilization of silkmoths as a source of income for rural communities and conservation of biodiversity in Kenya has been going on for the last ten years at the International Center of Insect Physiology and Ecology (*icipe*) supported by the International Fund for Agricultural Development (IFAD) (Kioko *et al*., 2000a,b; Raina, 2004). The potential of the East African indigenous species for wild silk production has been documented in Kenya (Kioko *et al*., 2000a; Raina, 2004; Raina *et al*., 2009, 2011) and Uganda (Gowdey, 1953; Okelo, 1972). *Gonometa postica* Walker (Lasiocampidae),

Argema mimosae Boisduval (Saturniidae), *Anaphe panda* Boisduval (Thaumetopoeidae) and *Epiphora mythiminia* (Euphorbiaceae) have been reported as potential wild silkmoths species in different regions of Kenya (Kioko *et al.*, 1999, 2000a, b; Ngoka, 2003; Ngoka *et al*., 2008; Mbahin *et al*., 2008).

Previous studies in Kenya by Kioko *et al*. (2000b), and Raina, (2004) have recorded *Lannea schweinfurthii* (Engl.) Engl. (Anacardiaceae), *Sclerocarya birrea* (A. Rich.) Hochst. (Anacardiaceae) and *Spirostachys venenifera* (Pax) Pax (Euphorbiaceae) as food plants for *A. mimosae.* Identification of *Gonometa* species parasitoids and predators and a few for *A. mimosae* have been recorded in Southern Africa and also East Africa but parasitism remains one of the major challenges facing wild silk production (Hartland-Rowe, 1992; Kioko *et al.*, 2007; Veldtman *et al.*, 2004; Raina, 2000; Fening *et al*., 2008a).

Arabuko Sokoke forest cover is about 420 square kilometers and comprises the largest and biologically the most important coastal forest in East Africa (Munir, 1994). The International Council for Birds Preservation (ICBP) lists the forest as the second most important bird center in Africa after Itobwe forest of Democratic Republic of Congo (Collar and Staurt, 1988). More than 230 species of bird has been recorded including nine globally threatened species. *Ploceus golandi* is only found in Arabuko Sokoke. It occurs mainly in *Brachystegia* woodland. Arabuko Sokoke is rich in rare and endemic wildlife, and supports at least 50 globally or nationally rare plant taxa. Six taxa of butterfly endemic to the Eastern Africa coast are present, as well as three rare, near-

endemic mammals. Despite its significant in biodiversity, the forest has been subject to a lot of human disturbance (KIFCON, 1995; Burgess and Clarke, 1999). The over-exploitation of the forest resources, drivemmmn by extreme poverty (Per capita incomes of less than US$50 a year) of the neighbouring communities has been responsible for the degradation of the forest (Gordon and Ayiemba, 2003). Local people use forest products for many purposes, including fuel wood and medicinal plants, and collect water at the seasonal pools. The forest is surrounded by agriculture for all sides. The Mahaji settlement was excised from the eastern edge of the forest after independence, and pressure remains high from some quarters for degazettement and settlement of the southeastern Kararacha - Mpendakula section, despite the fact that the soils are extremely infertile and have unsuitable for agriculture. Demand for agricultural and settlement land as well as utilization of the forest resources for survival is high and has threatened the biodiversity of the forest. Profitable and sustainable conservation initiatives need to be stressed for broader and diversified income sources than agriculture (Raina *et al*., 2009, 2011). Such initiatives have been undertaken by Kipepeo Project based at Gede ruins which has introduced butterfly farming with community participation in forest and biodiversity conservation. In addition to butterfly farming, the project has been exporting *A. mimosae* pupa (Gordon and Ayiemba, 2003; Ugo, 2004). However, the relative abundance of *A. mimosae*, food plants preference and parasitism have not been studied in Arabuko Sokoke area.

1.2 Problem statement

Wild silk moths have their natural enemies and experience economic loss from parasitism compared to the domestic ones. Raina (2000) reported 84% mortality of *G. postica* larvae due to parasitoids, predators and other factors but the incidence of parasitism was reduced from 35 to 55% through the use of semi captive rearing net sleeves. Most of the predators and parasitoids affecting *Gonometa* species wild silk moths such as *Pimelimyia* and *Palexorista* species have been identified in South and East Africa but only a few have been recorded for *A. mimosae* (Raina, 2000; Veldtman *et al.*, 2004; Kioko *et al.*, 2007). Kioko *et al.* (2000b) and Van denberg (1990) recorded *Mesocomys pulchriceps* Cameron (Eulpelmidae), *Pediobius anastati* Crawford (Eulophidae), *Telenomus* sp. (Sclelionidae) and unidentified Encyrtidae as parasitoids of *A. mimosae* eggs. *Hockeria* sp. and *Brachymeria* sp. both of Chalcicidae family have been recorded in South Africa as a parasitoid of both *A. mimosae* and *Gonometa rufobrunnea* larvae (Veldtman *et al.*, 2002; Veldtman *et al.*, 2004). In Asia, parasitoids that have been reported to attack wild sericulture caterpillars are chalcidoid wasps (Jolly *et al*., 1979; Thangavelu *et al*., 1988; Peigler, 1989). Records of natural enemies of wild silkmoths are still scanty although parasitism remains one of the major challenges facing wild silk production (Coffelt and Schultz, 1992, 1993a; Veldtman *et al.*, 2004; Raina, 2000; Kioko *et al.*, 2007; Fening *et al*., 2008a). In order to develop a sustainable *A. mimosae* population, it would be necessary to identity the indigenous natural enemies and to develop control measures to combat them in order to increase the relative abundance of the moth.

The evolutionary strategy of the parasitoid – host relationship is different from that of either the predator or the parasite – host relationship in that the host's future development is of importance only to the parasitoid (Vinson, 1975). The process that results in successful parasitism can be divided into four steps: (a) host habitat location, (b) host location, (c) host acceptance, and (d) host suitability (Salt, 1935; Flanders, 1953; Doult, 1964). A fifth step, host regulation, was added by (Vinson, 1975) in order to adequately describe the factors necessary for successful parasitism. The first three of these steps can be combined as aspects of the selection process. The host selection process may consist of two or three steps in one relationship or many steps in others. Because of this, there is often some overlap in describing and comparing a particular parameter or a behavioral process that leads to host selection.

Although literature exists on the biology and host relationships of parasitoids (Clausen, 1940; Sweetman, 1963), further work is needed on the identification of both contact and volatile chemicals that may be responsible for host habitat and host selection (Vinson, 1976). The hierarchy of cues and the role of physical host factors independent of odors and contact chemicals should be studied further. These studies should lead to a better understanding of host selection and may ultimately provide the means for the manipulation of both the parasitoid and the host. In view of the noted information, the general objective of this study was to survey the relative abundance of wild silkmoth *A. mimosae* Boisduval (Lepidoptera: Saturniidae) on different indigenous food plants and

explore food plant volatiles responsible for parasitism/predation of the developmental stages of the moth in Arabuko Sokoke forest, Kenya.

1.3 Justification

The fundamental goal of biodiversity conservation is to support sustainable development by protecting and using biological resources in ways that do not diminish the world's variety of genes and species or destroy habitats or ecosystems. With the increasing concern for biodiversity, and the mounting evidence of irreversible environmental damage, there is need to involve the local communities in the utilization and conservation of their indigenous biodiversity. A solution to this depletion of the biodiversity lies in introducing economic incentives that integrate conservation with economic development of the rural people (Munthali and Mughogho, 1992; Raina, 2000; Raina *et al*., 2000; Rodgers, 2005; Salehe, 2005). With the rich diversity of wild silkmoths recorded in Africa, wild silk farming has the potential to provide such economic incentives (Gowdey, 1953; Okelo, 1972; Ashiru, 1988; Hartland-Rowe, 1992; Oberprierler, 1995; Kioko *et al*., 1999b; 2000a, b; Raina, 2000; Raina *et al*., 2000; Veldtman *et al*., 2002).

Records in Kenya show that *A. mimosae* silk has not been harnessed for commercial purposes due to lack of awareness and natural enemies (Kioko *et al*., 2000b; Raina, 2000). Consequently, determining its potentiality would increase income for the community adjacent to the Arabuko Sokoke forest. Most of the parasitoids affecting wild silkmoths in the world have been identified but methods to control them to allow

economical production of cocoons need to be studied (Hartland-Rowe, 1992; Kioko, 1998; Ngoka, 2003; Veldtman *et al.*, 2004; Fening *et al*., 2008a).

Generally silkworms are herbivores and plants primarily combat herbivory through the production of several defensive chemicals like terpenes that directly affect herbivores and pathogens. Volatiles that are emitted when such chemicals are mobilized may be secondarily exploited by natural enemies of the herbivores (Turlings *et al.*, 1990; Sabelis and de Jong, 1988**;** Whitman and Eller, 1990). The fact that predators and parasitoids effectively exploit the chemical signals provided by the plants has been the basis for the hypothesis that plants may actively recruit the natural enemies of *A. mimosae* larvae.

Scarce records of *A. mimosae* parasitoids exist and therefore further studies are necessary to explore their natural enemies and control measures in their habitat. The current study on relative the abundance and host selection behaviour by parasitoids is important prerequisite to initiate wild silk farming as a supplementary activity for income generation for rural communities living around arabuko sokoke forest that mainly depend on subsistence farming.

Despite their economic importance for rural household, little research has been carried out on these silkmoths. Hence, the current research was undertaken to provide insight on the host plant diversity and economic potential of *A. mimosae.* It is hoped that this knowledge will be useful for sustainable production of wild *A. mimosae* silk in areas adjacent to the Arabuko sokoke Forest. It is hoped that this will also assist in conserving both the wild silkmoths and the forest. Conserving and utilizing these silkmoths for the

production of silk fibres will generate income for the resource – poor farmers, as well as conserve the threatened natural forests.

1.4 Hypotheses

a) There is no difference in abundance of the wild silkmoth, *Argema mimosae* found on different indigenous food plants in Arabuko Sokoke forest

b) There is no difference between cocoons spun by *A. mimosae* larvae fed on different food plant species and all produce good quality silk.

c) Food plant volatiles do not play a significant role in host-parasitoids interaction in locating different developmental stages of the *A. mimosae* from medium to long distances.

1.5 Objectives

1.5.1 General objective

To determine the relative abundance of *A. mimosae* on different food plants and olfactory cues responsible for parasitoids attack on the developmental stages.

1.5.2 Specific objectives

a) To determine the relative abundance of wild silkmoth *A. mimosae* found on different indigenous food plants in Arabuko Sokoke forest.

b) To determine the developmental period of *A. mimosae* larvae fed on different food plants.

c) To identify volatiles responsible for parasitoid attraction to different developmental stages of *A. mimosae* found on different food plants.

d) To analyze the physical and mechanical properties of wild silk extracted from *A. mimosae* cocoons.

1.6 Limitations

The life cycle of the *A. mimosae* includes a diapause phase between the pupa and the adult. The diapause period, occured during the dry spell and limited the study to be continuous. A drought also affected the study because the host plants lacked enough forage to support larval rearing and volatiles collection during the month of February – March and September – October every year.

1.7 Conceptual frame work

The wild silkmoth population increase would probably depend on food plants distribution and natural enemies among other environmental conditions. The population of wild silkmoths is expected to be high in an area where there are abundant food plants but the populations are checked by the natural enemies. To maintain high natural population of wild silkmoths, a control mechanism in the field can be developed to push or pull these natural enemies away from the rearing area. This can be developed using the concept of volatiles emitted by plants or the wild silkmoth larvae.

Plants exhibit a number of physiological and biochemical responses to physical stimuli such as wounding, and chemical stimuli such as elicitor compounds from pathogens or herbivores. Among these responses is the systemic synthesis and release of volatile organic compounds from leaves following injury by arthropod herbivores (reviewed by Dudareva *et al*., 2004). These volatile compounds can attract natural enemies of the

herbivores (Dicke and Sabelis, 1988; Turlings *et al*., 1990), thereby indirectly protecting the plant from damage through an induced mechanism (Kessler and Baldwin, 2001). Wild silkmoth food plants are not exceptional and are assumed to release volatiles to attract parasitoids against these herbivores. The specific volatiles elicitors if isolated can be synthesized and used in push-pull strategy to control the parasitoids (Figure 1.1).

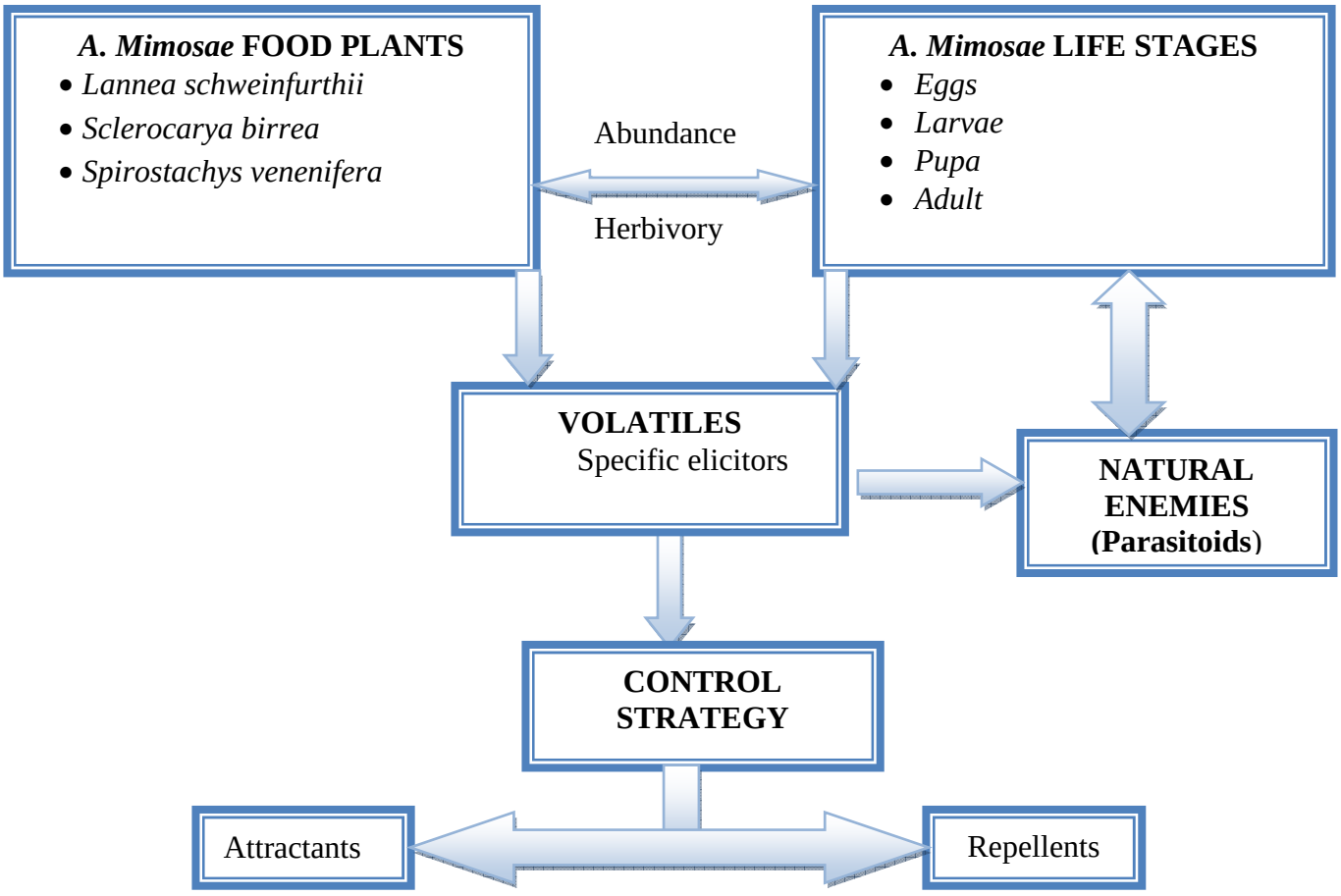


Figure 1.1 Conceptual framework

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Historical perspective of silk and wild silkmoths

The history of silk is as long as that of civilization itself. From its origin in China in about 2,200 B.C, the silk industry has had an adventurous course of evolution, becoming established with time in many parts of the world. Tradition credits His-ling-shi, the 14 year old bride of the China Emperor Huang Ti, with discovery of the potential of the cocoon and the invention of the first silk reel. China successfully guarded the secret until AD 300, when Japan and later India, penetrated the secrecy (Robert, 1997).The superiority of silk as a textile fibre has been recognized from time immemorial, the luxurious look; sleek feel and lustre of silk fabric are unquestionably inimitable.

References in the Old Testament (The Bible, 1971) indicate that silk was known in biblical times in western Asia, from which it was presumably known to the Greek Islands of the Aegean Sea. When Darius III, king of Persia, surrendered to Alexander the Great, he was clothed in such silken splendour that Alexander was completely overshadowed and demanded as spoils the equivalent of US $7 million in silk. Caravans carried silk on camel backs from the heart of Asia to Damascus, Syria, the marketplace where East and West met. Silk became a valuable commodity in both Greece and Rome. The Roman statesman and general Gaius Julius Caesar restricted silk to his exclusive use, the purple Roman stripes and his favoured officials. Despite this restriction, the use of silk in Rome spread in the paroxysm era. Until AD 550 all silk woven in Europe was derived from Asiatic sources. About that time, the Roman emperor Justinian I sent two

Nestorian monks to China, where due to the risk on their lives, they stole B. mori seeds and silkmoth eggs, and brought them to Byzantium (Robert, 1997). Thus, the Chinese and Persian silk monopolies ended. By the 12th and 13th centuries, Italy became the silk centre of the west, but at 17th century France challenged Italy's leadership and the silk looms were established in the Lyons area from that time until today (Robert, 1997).

The "Golden age" of the wild silkworm industry was during the 30 years of the Meiji era 1897. In those days, about fifty-two percent of the seven hundred farmers in the district of Ariake (China) reared wild silkworms in their fields, which occupied an area of about 3,000 ha. During the same era, wild silkworms' industry production was estimated at about 8,500,000 cocoons (Nakajima, 1980).

2.2 Scope and classification of natural silk

According to Raina *et al.* (1999), the natural silk is broadly classified into two types: silk of plant and animal origin. The natural silk of plant origin is obtained from silk cotton tree and floss-silk tree. The natural silk of animal origin is broadly of two types: *B. mori* and non-*B. mori*. *B. mori* silkmoth is a domesticated type, whereas non-*B. mori* is universally known as "wild type", which is also found in semi-domesticated form.

Further, the non-*B. mori* variety of silk is classified as insect and non-insect type. Insect type of silk is named as Eri, Muga, Anaphe, Fagara, Coan and Tasar (Raina *et al*., 1999). The non-insect silk is named as mussel and spider silk and is obtained from mollusca and spider, respectively. The insect type of silk is again classified as commercial and noncommercial.

Eri, Muga, Anaphe, Fagara, Coan and Tasar are commercial type of insect silk; whereas non-commercial type of silk is obtained from the weaver ants and green lacewing fly. The genera *Tasar*, *Eri*, *Muga* and *Anaphe* in the order Lepidoptera, are the principal non-*B. mori* silk producers in the tropical or temperate regions. Other genera include *Fagara*, *Coan*, *Gonometa* and *Argema* (Jolly *et al*., 1979; Raina, 2000; Kioko *et al*., 2000a, b; Raina, 2004). The potential of *A. mimosae* silk has not been documented. Fibres of the wild silkworm are generally golden, green or brown and have a coarse, hard texture. *B. mori* silk produced by the domesticated silkworm is a textile fibre highly appreciated for its outstanding properties (handle, luster, comfort) (Chen *et al.*, 2004). Natural silks achieve a combination of strength and toughness unprecedented in the world of artificial fibres. They offer a unique combination of strength and ductility which is unrivalled by any other natural or man-made fibres (Chen *et al*., 2004).

2.3 Silk production and consumption trends

The demand for silk is constantly increasing in the world market (Table 2.1) providing excellent opportunities for any producer country to diversify and optimize any source of production. There is a steady growth in silk consumption both in producing and consuming countries. Silk consumption in Japan, U.S.A and Europe, the three major silk consuming countries together, have contributed about 50% of raw silk product in the world. Judging from the consumption trends, silk demand is growing annually by about 3 to 5%. However, despite the growth in demand and production, the contribution of silk to the world textile fibres is only about 0.2%, and this situation has remained unaltered since the last three decades (Robert, 1997). This is mainly as a result of insufficient

production of silk in the wild, lack of sufficient research and development, low local know how on silk farming practices, low host plant diversity and distribution, parasitism and predation aspects.

Table 2.1: Details of cocoon (Dry), raw silk import and export (in Tonnes) in the world

Country	1999				2000				2001			
	Cocoon export	Raw silk export	Cocoon import	Raw silk import	Cocoon export	Raw silk export	Cocoon import	Raw silk import	Cocoon export	Raw silk export	Cocoon import	Raw silk import
Brazil	60	1742	-	1	0	1770	40	44	74	1287	2	52
Bulgaria	0	4	0	0	-	-	4	-	0	0	0	0
Cambodia	0	0	4	140	4	-	-	-	0	0	-	-
China	466	8506	572	109	0	10496	-	-	-	-	-	-
Colombia	0	9	0	0	-	-	-	0	0	0	0	0
Egypt	0	0	16	97	-	-	-	23	0	0	0	43
France	0	0	-	228	-	3	-	318	-	-	-	-
India	112	59	-	2824	31	499	25	6936	8	45	6	4713
Indonesia	-	-	-	-	1	3	-	6	1.3	3.3	-	5.8
Iran	-	-	-	-	0	0	50	-	-	-	-	-
Italy	-	-	23	2727	0	93	49	3168	0.3	139	0	2760
Ivory coast	0	0	0	0	0	0	0	0	0	0	0	0
Japan	15	-	672	2504	76	-	675	2360	60	2	402	1849
Malaysia	-	-	-	-	-	6	-	-	-	-	-	-
Philippines	-	-	-	-	-	-	-	24	-	-	-	0.1
Thailand	0.15	396	31	243	0.2	0.4	10	139	0.2	0.2	45	134
Turkey	0	50	-	-	22	0	-	-	0	0	-	-
Uganda	-	-	-	-	0	2	-	-	-	-	-	-
Vietnam	-	-	-	-	-	0	0	-	0	1500	30	450

Source: International silk commission, Lyon, France

The prospect for development of silk industry in Africa is very bright. Sericulture is an agro-based undertaking, which can provide employment to millions of people living in rural areas. African countries can use it as a venture for generating income and employment for rural households and improve their livelihoods by earning supplementary income. The world trend in silk production (Table 2.2) and consumption are also quite favourable. The investment requirements are low and the reproduction period required to generate income is very short. The demand of silk is on the increase and the production in major silk-producing countries is gradually diminishing due to various reasons such as: diversification, affluence and high cost of labor (Raje, 1999). Hence, African countries that enjoy congenital climate for cultivation of *B. mori* and rearing wild silkmoths have great scope and opportunity to promote sericulture. However, efforts to introduce and promote sericulture in the African continent have not been very successful and are riddled with many problems namely; lack of research and development support and suitable infrastructure, low awareness of the potential returns of silkworm farming and inexperience with sericultural techniques (Raina, 2000; 2004).

Table 2.2: Trends in world raw silk production (include all types) in tonnes

Country	1998	1999	2000	2001	2002	2003	2004 (P)	Share (%)
China	57500	56956	61648	64567	68600	94600	102560	81.65
India	15544	15214	15857	17351	16319	15742	16500	13.14
Brazil	1821	1554	1389	1485	1607	1563	1512	1.2
Thailand	900	1000	955	1510	1510	1500	1420	1.13
Uzbekistan	1500	923	1100	1260	1260	950	950	0.76
Vietnam	862	780	780	2035	2200	750	750	0.6
Japan	1080	650	557	431	394	287	263	0.21
Korea S.	210	200	165	157	154	150	150	0.12
Others (Madagascar, Uganda,Egypt, Kenya)	1572	1250	1952	1692	3814	1500	1500	1.19
Total	80989	78530	84403	90488	95858	117042	125605	100

Source: ISC- 2005; P: Provisional

2.4 Diversity of wild silkmoths in Kenya

There are over 60 wild silkmoth species in East Africa and their high potential has not been utilized (Raina, 2000, 2004; Kioko *et al*., 2000a). Despite this richness and abundance, only a few of these species have so far been utilized for wild silk production in East Africa (Table 2.3, 2.4). Studies by Kioko *et al*., (2000a) showed that *Gonometa* sp. occurred in Mwingi District and Sultan Hamud in Makueni District; the host plants being *Acacia elaitor* Brenan and *Acacia senegal* (L) Willd and its host plant diversity and distribution was further studied by Fenning *et al*. (2008a) in the same district. According to Ngoka *et al*. (2008), *Gonometa* sp. was also found to occur in Kamaguti in Uasin Gishu District; the host plants being the indigenous *Acacia hockii* De Wild and exotic wattle tree (*Acacia mearnsii* De Wild). The chorion structure *of A. mimosae* eggs

(Kioko *et al*., 1999b), developmental cycle of *Gonometa postica* (Ngoka *et al*., 2008), and spatial distribution of the silk cocoon nests and egg-clusters of the silkmoth *A. panda* and its host plant *B. micrantha* in the Kakamega Forest (Mbahin *et al*., 2008) has been studied.

Table 2.3: Potential wild silkmoths in Kenya

Species	**Family**	**Locality**	**Host Plant**
Gonometa sp.	Lasiocampidae	Nguni in Mwingi District and Sultan Hamud in Makueni District	*Acacia elatior* *A. Senegal*
Gonometa sp.	Lasiocampidae	Kamaguti in Uasin Gishu District	*Acacia hockii* and Wattle tree
Ceratopacha sp.	Lasiocampidae	Uasin Gishu District Kamaguti	Cocoons found on *Carissa edulis* (Forssk).
Epiphora vacuna	Saturniidae	Kakamega forest	Adult caught in light trap
Anaphe panda	Thaumetopoeidae	Kakamega forest	*Bridelia macrantha*
Lechriolepsis pulchra	Lasiocampidae	Kakamega forest	Unidentified shrubs
Argema mimosae	Saturniidae	Makueni District Wote & S'Hamud	*Sclerocarya birrea, Spirostachys venenifera and Lannea schweinfurthii*
Philotherma sp.	Lasiocampidae	Sultan Hamud	*Sclerocarya birrea*

Source: Kioko *et al*., 2000a.

Table 2.4: Potential wild silkmoths in Africa

Family	Scientific Name	Status of commercial use	Country(ies)
Silkmoths (Lepidoptera)			
Lasiocampidae	*Gonometa regia* Aurivillius	Commercially used	Uganda, Kenya
	Gonometa nysa Druce	Commercially used	Cameroon, Nigeria, Congo, Ivory Coast
	Gonometa rufobrunnea Aurivillius	Commercially used	South Africa, Namibia
	Gonometa nigrottoi	Commercially used	Kenya
	Gonometa podocarpi Aurivillius	Commercially used	Uganda, Kenya
	Gonometa titan Holland	Commercially used	Nigeria
	Leichriolepsis leucostigma Humpner	Commercially used	Uganda, Kenya
	Pachymeta stigma Strand	Commercially used	Uganda
	Pachypasa subfascia Walker	Commercially used	Uganda, Kenya, Tanzania
	Borocera cajani Vinson	Commercially used	Madagascar
Saturniidae	*Argema mimosae* Boisduval	Commercially used	South Africa, Malawi, Mozambique
	Argema besanti Rebel	Has potential for commercial use	Kenya
	Epiphora mythmnia Westwood	Has potential for commercial use	Malawi, South Africa, Mozambique, Kenya
	Epiphora bauhiniae Gu´erin-M´eneville	Commercially used	Ghana, Gambia, Nigeria, Sudan
Thaumetopoeidae	*Anaphe carteri* Walsm	Commercially used	Uganda, Nigeria
	Anaphe moloneyi Druce	Commercially used	Uganda, Nigeria
	Anaphe panda Boisduval	Commercially used	Uganda, Tanzania, Kenya, Nigeria
	Anaphe reticulata Walker	Commercially used	Nigeria, Uganda, Kenya
	Anaphe venata Butler	Commercially used	Uganda
	Anaphe vuillet De Jouan	commercially used	Congo, Sudan, Nigeria

Source: Raina *et al*., 2011.

2.4.1 Benefits of wild silkmoths

Wild silkmoths can produce good quality silk fibre, food to some African and Indian local communities where the edible species are abundant. In tasar silkmoths, dead pupae, which remain after reeling, are very rich in protein (63-65%), oil (20-25%), carbohydrates (10%) and minerals (7-8%) (Agarwal *et al*., 1974). Pupae of silkmoths are an excellent source of proteins for animals and their oil is extracted for making soaps, cosmetics and the remaining powder is used in the baking industry for preparation of protein-rich biscuits in India (Agarwal *et al*., 1974). Wild sericulture helps to generate income for poor-resource communities as well as the conservation of the forest for eco-tourism (Raina *et al*., 2007; Fening *et al*., 2008a).

Wild silkmoths have the potential of playing a role in eco-tourism, providing a focal point for raising international and local awareness of their unique biodiversity and of the real and current threats to their existence (Raina, 2000). This can be done by including these silkmoths in live butterfly house displays both at home and abroad. *A. mimosae* pupae is exported alongside butterflies in Arabuko Sokoke forest earning income for communities adjacent to the forest (Gordon and Ayiemba, 2003).There are many people and firms interested in observing live silkmoths. The prices paid for live pupae range from US $ 1-8, depending on the species. Selling pupae could bring immediate benefits to the community farming and harvesting the silkmoths. It could also provide a chance to farm silkmoth species, which spin silk cocoons of low quality but are the majority of species in Africa (Kioko, 1998; Kioko *et al*., 2000a; Raina, 2000).

Wild silkmoths can be used as ecological indicators of environmental change (Oberprieler, 1995; Kioko, 1998; Raina *et a*l., 2009). This is because they often have a restricted distribution and food plant range. The decline in numbers of silkmoth species in an area may be the first sign of degradation of the environment, whether by pollution, denudation of the natural vegetation, invasion of alien plants or other causes. Similarly, an increase in numbers may also be a signal of change in the environment for example the introduction of a palatable exotic species or an increase of the natural food plants. In the past, wild silkmoth species in East Africa were used to identify areas and ecosystems in need of conservation, and also to monitor such areas for possible decline and change in terms of species composition. Once thorough knowledge is gained on their life cycles and distribution, these species could play a role in the formulation of future research and conservation strategies in the region (Kioko, 1998).

The cocoons of *Gonometa* spp. have had other uses in Africa. The Denver Museum of Natural History has three pairs of ankle rattles made by Bushmen in Botswana; each rattle is a string of numerous cocoons containing tiny chips of stone or ostrich eggshell (Peigler, 1993). Similarly, *A. mimosae* cocoons are much favoured by Zulus, who wear them as anklets giving a satisfying rattle (Pinhey, 1975). In Kenya, at ICIPE, wild silk from *Gonometa postica* has been weaved in combination with *B. mori* silk to develop a cloth of high value (Raina *et al*., 2007). The *kente* silk cloth produced and worn by *Ashanti* traditional chiefs in Ghana is a good example of the prestige and high value attached to silk. The *Ashanti* people price the brilliant orange and conspicuous yellow colours of the cloth as much as they value their gleaming gold sculptures. At present one

does not need to be a member of the royal family to wear *Kente* cloth. Many people wear *kente* cloth in modern societies, including the United States. Its traditional importance as a prestige item worn only by royalty has prompted modern people to wear it as a sign of pride in Africa or African heritage. Currently at many college graduation ceremonies, African- American students wear a strip of *kente* cloth with their graduation gowns (Frank, 1993; Mary, 1999; Shaw-Eagle, 1999; Rosemary, 1999; Christopher, 1999; Fening, 2007). Wild silk powder successfully obtained from the silkmoths *A. pernyi*, *Samia cynthia ricini* and others, has been used to make non-textile products which include high valued cosmetics, food additives and silk-spread materials (Akai, 2000). These products require a large quantity of silk with differing characteristics, thus stimulating small industries in various areas of the world.

2.4.2 Prospects and problems of wild sericulture in Africa

The national and global markets demand higher standards of goods. Various methods are being developed to enhance the aesthetic value of newly introduced *B. mori* silk.

Among these are the introduction of African designs, use of eco-friendly natural dyes to enhance the organic value of silk fibre and fabrics, and application of strict quality control measures to meet international standards. In the case of wild species of silkmoths, the programme has focused on conservation of biodiversity through scientific training of local people (Raina, 2000; Kioko *et al*., 2007; Fening *et al*., 2008a).Various methods are being developed to minimise predators, parasitoids, pests and disease problems in wild silk farming to enhance yield and help rural people. For instance, in attempts to overcome egg parasitism of *A. mimosae* and *G. postica* in the field, cocoons

are protected in enclosures before moths to emerge. The eggs laid, hatch indoors and the larvae are released in the field (Kioko *et al*., 1999b).

2.4.3 Economic potential of *Argema mimosae* silk

In Africa, during the last three decades, there has been a decrease in the production of traditional export crops, as well as the unsustainable exploitation of indigenous forests and agricultural land. Among the many activities that might assist the poor people to escape their vicious cycle of poverty is the raising of bees and silkworms referred to as apiculture and sericulture respectively (Raina *et al.*, 1999; Raina, 2000; 2004). In East Africa biodiversity surveys in the indigenous forests indicate that there are at least 60 identified silkmoth species in the wild.

According to Jolly *et al.* (1979), by applying the available knowledge and filling in the crucial gaps with well-focused research, a large increase in raw silk production can be realized in a number of countries where global potential of non-*B. mori* sericulture exists. As long as human desire for silk garments continues, the demand for sericulture related activities remains. Silk is the “queen” of textiles and a naturally produced animal fiber.The silk industry is having increased demand than natural production can satisfy, hence, the necessity of “seeding”. This involves use of cocoons as seeds and rearing wild silk larvae in semi-captivity using net sleeves (Ngoka *et al*., 2008).

2.4.4 Wild silk farming and forest conservation

Forests not only constitute the basic means of livelihood of millions of people but also serve to regulate precipitation, conserve soil fertility and reduce erosion. Since the

nineteenth century, continuous deforestation due to wood trade demands has been a major threat to forest conservation (Jollys *et al*., 1979). Wild silk farming holds great promise for the world forestry conservation as a supplementary activity that helps to curb forest destruction and biodiversity conservation with gainful utilization of this vast natural wealth. The wild silk farming can be initiated as a supplementary activity for income generation for rural communities that mainly depend on subsistence farming.

2.5 The natural vegetation cover in Arabuko Sokoke forest reserve

Arabuko Sokoke forest covers a total area of 420km^2 and is the largest remaining block of the indigenous natural lowland forest on the East African coast, a habitat that once dominated Kenya's coastal fringe. The natural vegetation cover in Arabuko Sokoke forest reserve is classified into three types: *Brachystegia* woodland, *Cynometra* thicket and *Afzelia* mixed forest (Burgess *et al.*, 1998). The vegetation provides the habitat for a number of endemic and endangered flora and fauna species that make this forest one of the most important biodiversity areas in Africa (Gordon and Ayiemba, 2003).

*Mixed forest***:** The mixed forest covers 7,000 ha in the east, on grey sands. This habitat is relatively dense, tall and undifferentiated, with a diversity of tree species. Characteristic trees include *Combretum schumannii*, *Drypetes reticulata*, *Afzelia quanzensis*, *Dialium orientale*, *Hymenaea verrucosa* and *Manilkara sansibarensis*.

*Brachystegia forest***:** The Brachystegia woodland (7,700 ha) runs in a strip through the approximate centre of the forest, on white, very infertile soil. This relatively open habitat is dominated by *Brachystegia speciformis*.

*Cynometra forest***:** In the west, on red Magarini sands, is Cynometra forest and thicket, dominated by *Cynometra webberi* with *Manilkara sulcata*, *Oldfieldia somalensis* and *Brachylaena huillensis*. This latter tree, much in demand for the carving trade, has been almost logged out from much of the forest. The transition between white and red soil is sudden, and marked by a chain of seasonal ponds. There are two areas of relatively tall *Cynometra* forest, with a canopy height of up to 20 m, in the north (3,300 ha) and the South (6,000 ha) of this zone. Between these is a lower, scrubbier formation of intermediate *Cynometra* (11,300 ha) with a canopy height of 7-8m. The dry north-western part of the Reserve is covered by a low, dense, and often almost impenetrable *Cynometra* thicket (2,300 ha), with the canopy no more than 5 m high (Burgess *et al.*, 1998).

2.5.1 Human population adjacent to the Arabuko Sokoke forest

Arabuko Sokoke forest is all that remains of what was previously a much more extensive forest. Population growth, coupled with increasing demand for timber and land for agriculture, have contributed to a reduction in the extent and condition of the forest. Much of the forest is now degraded, particularly through the removal of commercial timber species for carving and general construction (FitzGibbon *et al.*, 1995; ASFMT Report, 2002).There are approximately 50 villages surrounding the forest, with a total population of about 104,000 (Appendix II). The main ethnic group in the area is the Giriama; they displaced the former Sanya communities, who were originally forest dwellers and hunters. Today the forest-adjacent population is mostly small scale subsistence farmers who utilise the forest for some of their livelihood

requirements. The main crops grown are maize, cassava and beans. Locally grown cash crops include coconut, mango, cashew-nut and sesame. Farmers are increasingly taking up dairy farming, although levels are still low. There are no squatters within the forest. The shamba system has been used for establishing exotic plantations in the past, but it was not very successful due to crop raids by wild animals, mainly elephants and baboons (ASFMT Report, 2002).

2.5.2 Arabuko Sokoke forest conservation issues

Arabuko Sokoke is rich in biodiversity, but of particular importance is the exceptionally high degree of endemism. This, together with the forest's large area of continuous woody vegetation (most remaining coastal forests cover only a few hundreds of hectares, sometimes much less) gives it a very high conservation value. The forest is managed jointly by the Kenya Forest Service (KFS) and the Kenya Wildlife Service (KWS) under a Memorandum of Agreement, through the Arabuko Sokoke Forest Management Team (ASFMT). The arrangement brings together other institutions which include Kenya Forestry Research Institute (KEFRI), National Museums of Kenya (NMK), Nature Kenya (NK) and Birdlife International. Extensive licensed logging has occurred in the past, with noticeable negative effects on wildlife species and those in bird are documented (FitzGibbon *et al.,* 1995). Licensed selective logging continues on a smaller scale, along with the licensed collection of dead wood for fuel. Both of these practices have proven difficult for the police to merge, and regular poaching of the valuable trees continues to be a major problem. *Brachylaena huillensis*, which is preferred for the carving and construction industry, has been severely affected, as have timber species such as *Pleurostylia africana*. Illegal hunting, mainly of duiker and

elephant shrews, is evident (FitzGibbon *et al.,* 1995); other species threatened by poaching are the dikdik and the giant forest Hog, although its impacts have not been determined. Local people use forest products for many purposes, including fuel wood and medicinal plants, and collect water at the seasonal pools (Robertson and Luke 1993; FitzGibbon *et al.,* 1995). The forest is surrounded by agricultural land on all sides.

2.6 Semiochemicals

Chemical compounds that mediate interactions between organisms are called infochemicals or semiochemicals. Those that are transmitted between individuals of different species are called allelochemicals, while those mediating between individuals of the same species are known as pheromones (Gullan and Cranston, 1994; Howse *et al*., 1998). Allelochemicals are subdivided into three classes: allomones, kairomones and synomones (Nordlund and Lewis, 1976; Gullan and Cranston, 1994). Allomones are signals that benefit the emitter and they could be negative or of no significance to the receiver. Kairomones benefit the receiver, either evoking a behavioural or a physiological reaction. Synomones benefit both the emitter and the receiver. Floral odours in general can be classified as synomones became as the insect collects nectar and pollen the flower also becomes pollinated.

2.6.1 The role of semiochemicals in insect behaviour and response to food odours

Many species of insects rely to a high degree on odour cues in their search for food, mating partners, hosts and suitable oviposition sites. A good example of the extreme sensitivity is provided by the noctuid moth (cotton leaf worm) *Spodoptera littoralis* Boisduval (Noctuidae), whose heart frequency is affected by less than 10 molecules of

odorant hitting the antennae (Angioy *et al*., 2003). Insects have fewer receptors (~50) extremely sensitivity to a certain degree of odour (Keller and Vosshall, 2003). The chemical cues which are utilized by parasitoids and the behaviour sequences which they elicit have been discussed by various authors (Vinson, 1976, 1985; Arthur, 1981; Jones, 1981; Weseloh, 1981; Van Alphen and Vet, 1986; Kainoh, 1990; Ngi-Song *et al*., 1996; Ruther *et al*., 2002; Faheem *et al*., 2004; Paul, 2006).

Many species of insects use odours to locate their food. The insect may respond to the commonly occurring chemicals produced by the normal metabolism of the host animal or plant, or the decomposition of organic materials, or to specific chemicals used by many organisms for communications within or between species. The larvae of many phytophagous insects respond to food plant odours. There is evidence that larvae of polyphagous insect species respond mainly to primary metabolites, among which carbon dioxide is known to be important, while monophagous and oligophagous species respond to the secondary chemicals (Jones and Coaker, 1978). Similarly, some parasitoids of phytophagous insects use the secondary compounds to locate the habitats in which the hosts occur (Vinson, 1984).

Some predators and parasitoids locate their prey or hosts by responding to a host pheromone. For example, *Thaneroclerus buqueti* Walker (clerid beetle) and *Campsicnemius charliechaplini* Evenhuis (dolichopodid fly) are attracted by the aggregation pheromones of the bark beetles on which they prey, and a number of species of beetles follow ant trail pheromones to locate the ants brood which they feed on. The

parasitoid *Trichogramma* locates the insect eggs in which it oviposits by responding to the sex pheromone of the female host (Hayness and Birch, 1985).

2.6.2 Herbivore –induced plant volatiles and their role in natural enemies attraction

Almost half of all insect species are herbivorous and are a constant threat to plants (Shoonhoven *et a*l., 2005). Although sessile and vulnerable plants have developed effective defense strategies in order to reduce insect attacks, many plants produce toxic and deterrent phytochemicals as direct defenses against herbivore attack (Wittstock and Gershenzon, 2002). These compounds have anti-herbivory functions that can reduce insect development, interfere with digestion, and may ultimately kill the insect. Plants also use indirect defenses, such as the release of Volatile Organic Compounds (VOC) from the surface of leaves to attract natural enemies of the herbivores (Kessler and Baldwin, 2001; Pichersky *et al*., 2006; Schnee *et al*., 2006). Immediately after wounding, short-chain alcohols and aldehydes derived from a lipoxygenase-based pathway and collectively termed ‘‘green leafy volatiles’’, are released by ruptured cells (Turlings *et al*., 1998). Other VOCs are released only after extensive wounding and their production and release rely on induced de novo synthesis (Pare´ and Tumlinson, 1997). The most common group of induced VOCs are the terpenes. Most of these terpenes are considered secondary metabolites and they are derived from a five-carbon compound, isopentenyl diphosphate (Trapp and Croteau, 2001). Terpenes (or terpenoids) are classified based on the number of five-carbon units. The most common subclasses of terpenes are hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), and diterpenes (C20) (Dudareva *et al*., 2004). These volatile compounds can attract natural

enemies of the herbivores (Turlings *et al*., 1990), thereby indirectly protecting the plant from damage through an induced mechanism (Kessler and Baldwin, 2001).

Nevertheless, whether or not these volatiles are a result of co-evolutionary processes among plants, herbivores, and their natural enemies remains unclear (Holopainen, 2004). This is because most studies have been performed on agriculturally important plant species under greenhouse or laboratory conditions, thus making it difficult to derive direct conclusions on the evolutionary role of herbivore-induced plant volatiles (Arimura *et al*., 2005). Although greenhouse and laboratory studies provide useful information on individual interactions among plants, herbivores, and natural enemies, they often fail to include various biotic and abiotic stresses that influence volatile production under natural conditions. Plants in the field often are attacked by multiple insect herbivores and diseases at the same time, in contrast to the situation in the laboratory where plant damage is carefully controlled and usually restricted to one attacking species. Abiotic stresses, such as high light intersity and drought also have been shown to influence the production of plant VOCs although these are rarely studied in the laboratory (Gouinguene and Turlings, 2002; Blanch *et al*., 2007). There are only a few studies that report the volatile bouquets produced from the vegetative parts of naturally growing plants and very scarce reports specifically related to volatiles emitted by wild silkmoths food plants (Blanch *et al*., 2007).

2.6.3 *Argema mimosae* larvae as herbivores

Larvae of *A. mimosae* feed on the food plant leaves and may cause substantial defoliation that can reduce growth and/or fruit production (Van den berg, 1990; Kioko *et*

al., 2000b). The amount of foliage and the number of co specifics determine the degree of defoliation by the larvae (Floater, 2001; Rhainds *et al.,* 2002). When the primary host tree is defoliated, a secondary food plant has to be selected or the larvae will starve to death. Although larvae may be able to find secondary hosts, dispersal may be extremely costly when food plants are far a part or co-occur with non-food plants (Floater, 2001; Hodar *et al*., 2002). Remaining on the food plant to forage or pupate can guarantee that the right hosts will be oviposited (Bernays and Chapman, 1994). Larval stages found on alternative host can indicate shortage of food due to defoliation or scarce distribution of the main food plant (Floater, 2001; Hodar *et al*., 2002). On the other hand, feeding on non-food plants may also occur as a way of escaping from the natural enemies (Guildford, 1992). As a defense mechanism, the food plants are presumed to emit volatiles that attract natural enemies against the larvae of *A. mimosae*. It is on the basis of the fore information that outlines the purpose of the current study where volatiles can be used to control natural enemies of wild silkmoth.

2.7 Post cocoon analysis

The quality of silk cocoons is determined by a number of characteristics. Each of these characteristics measures a different aspect of the quality. In the absence of a single measure for the overall quality, the price of the cocoons is based on several characteristics, which are deemed to be the most important by either the buyer or the seller.

The important characteristics that define the quality of cocoons are described below:

i. Single Cocoon Weight (SCW); this is simply the average weight of a cocoon.

ii. Shell Weight (SW); this is the average of the single shell weight. The shell is that portion of the cocoon after removing the pupae. The shell yields the raw silk and hence the higher the shell weight, the higher the yield of the raw silk.

iii. Shell Ratio (SR); this is defined as the ratio average shell weight to the average single cocoon weight and expressed as a percentage. This ratio estimates the raw silk content of each cocoon. This means the higher the shell ratio, the better the quality.

iv. Filament Size (FS); this is the thickness of a silk filament. This is also expressed as the denier. The denier is expressed as the weight of the silk filament measured in grams for 9000 meters of the filament. A lower denier implies finer silk filament and hence is more desirable.

v. Raw silk (RS); this is a measure of a raw silk expressed as a percentage. It is the ratio of the number of kilograms of cocoons required to produce one kilogram of raw silk and expressed as a percentage.

vi. Neatness (N); this measures the neatness of the silk filament. This is expressed as a percentage. The number of small knots, loops and the frequency of distribution on raw silk are represented as percentage by comparing a sample of 20 panels taken on a seriplane board, with the standard photographs for neatness defects. This characteristic has impact on the quality of the fabrics woven from the silk.

vii. Boil Loss (BL); boil loss or degumming loss is the loss of sericin that is used as gum for binding silk filaments together in the form of a cocoon.

A methodology is developed to create a Composite Quality Index (CQI), which encompasses all the important characteristics. The cocoons are graded into three categories, Low, Medium and High quality based on the CQI. Discriminate analysis is used to map the individual characteristics directly on the three grades of cocoons (Vishuprasad, 2004).

2.7.1 Silk testing and quality control

The textile industry is becoming an increasingly competitive environment. Differentiating products is therefore important and this can be facilitated through improving quality. Quality control has been found to be the most effective measure to maintain the prerequisite quality and quantity of any textile product either at yarn or fabric stage. Silk quality testing can be used to improve the product and achieve compliance to international, regional or retailer specific standards (Sonwalkar, 1993).

It is well known that silk textile quality control has become of increasing interest and the quality assessment of textiles is of vital importance for both manufacturers and consumers. An increased number of knowledgeable consumers with firm demands for specific performance behaviour and longer life textile goods, in combination with the numerous advances in technology have made essential the better understanding of properties of fibres, yarns and fabrics (Lee, 1999).

Quality raw silk cannot be made just by producing good quality cocoon alone. Appropriate reeling or spinning technique is equally important for the production of good quality silk. Introduction of silk testing and grading is a step in the right direction

for bringing in quality among rearers, reelers or spinners (Lee, 1999).The unique synthesis of strength and fineness has made silk very useful in certain important sectors such as surgical fields and fabrication of precision equipment. Silk fibre is also highly extensible; therefore the determination of strength and elongation of raw silk is an important test. The breaking load, that is the load the thread can with stand just before it breaks is expressed in terms of *grams per tex* or *per denier* and is known as tenacity (Lee, 1999).The tenacity test is carried out on a serigraph strength tester or a serimeter. The tester is capable of recording simultaneously the breaking load and the corresponding elongation of the threads.

CHAPTER THREE

3 GENERAL MATERIALS AND METHODS

3.1 Study site

This study was carried out at Arabuko Sokoke forest which is the largest single block of indigenous coastal forest remaining in East Africa. It is situated in Kenya's coast province and transverses Kilifi and Malindi Districts at a latitude of 3° 20' S and longitude 39° 50' E (Appendix I). The eastern part of the forest lies on a flat coastal plain at an altitude of about 45 m above sea level (ASL). This rises to a plateau of about 60–200 m ASL in the central and western parts of the forest. The total forest area is approximately 41,600 ha (Figure 3.1). The forest was originally declared as Crown Forest in 1932 and gazetted as a forest reserve in 1943. An additional 2,675 ha at Kararacha in the south east was added in 1968. Within the forest area about 4,300 ha was designated as a strict Nature Reserve in 1977. This was extended in 1979 by 1,635 ha. Average annual rainfall ranges from 900 mm (in the relatively dry and scrubby north-west) to 1,100 mm (in the east). The relatively flat eastern section lies on Pleistocene lagoon sands and clays, separated by a wide band of apparently riverine sandy deposits from the ridge of red Magarini sands that forms the western part of the Reserve (Robertson and Luke 1993).Three very distinctive forest types, each with its-own special flora and fauna, correspond to these soil types; *Brachystegia* woodland, *Cynometra* thicket and *Afzelia* mixed forest (Burgess *et al.*, 1998).

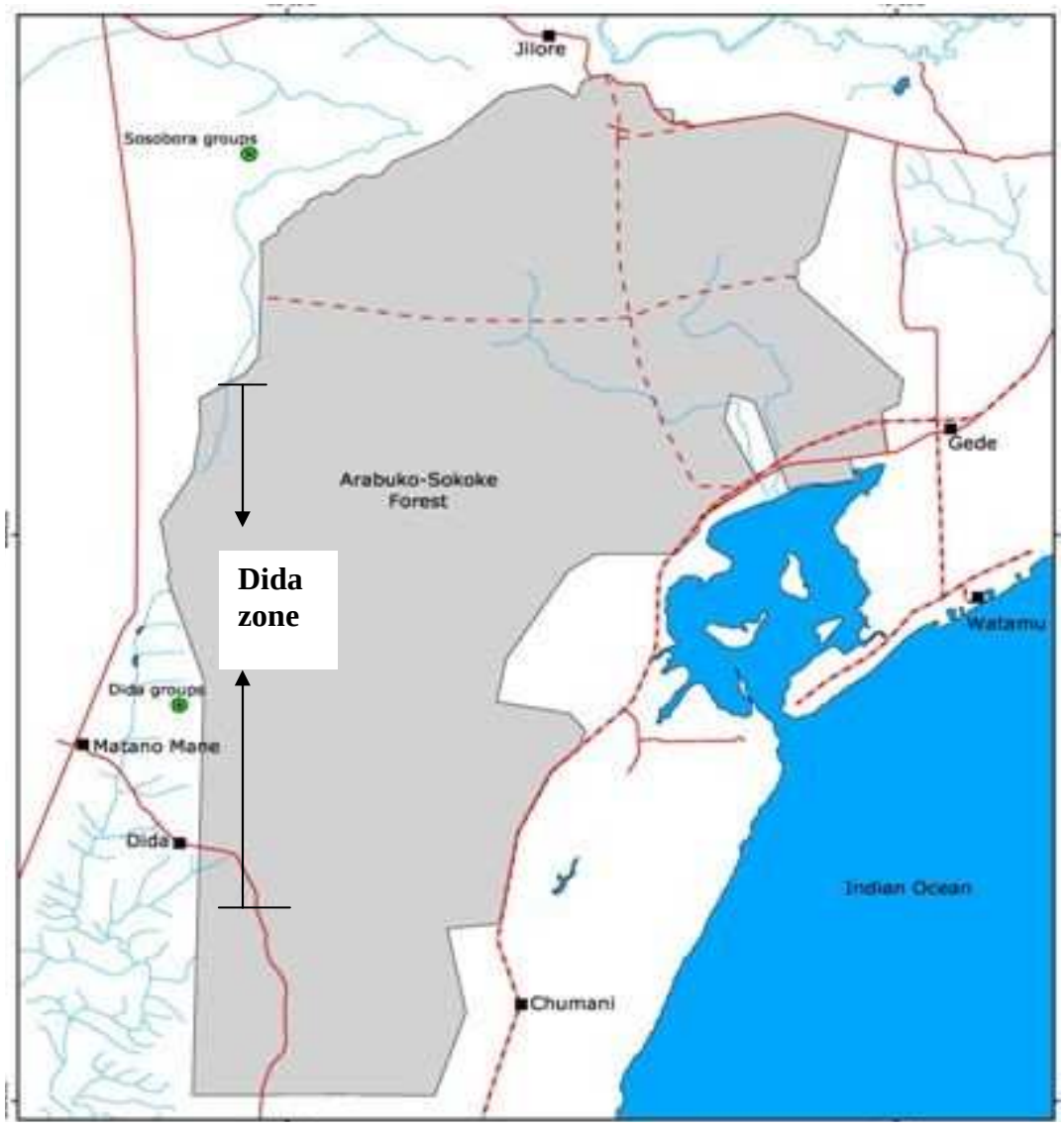


Figure 3.1: Map of the Arabuko Sokoke Forest, at latitude of 3° 20’ S and longitude 39° 50’ E

Arabuko-Sokoke lies a few kilometres inland on the Kenyan coast, between the towns of Kilifi and Malindi and some 110 km North of Mombasa. The coastal forest is interpreted as a vanishing refuge with the endemic species gradually becoming more and more relict (and presumably extinct) due to climatic change and human destruction. Most coastal forest endemic species have a narrow distributional range, often exhibiting single-site endemism or with scattered or disjunct distributional patterns. Arabuko- sokoke is the largest extant fragment of the forests that once covered much of the East African coast, and whose remnants constitute the East African lowland coastal forests. This forest is a globally important forest for biodiversity conservation. In the early 1990s, 54% to 59% of the local community wanted the entire forest cleared for settlement and the forest was invaded by farmers on several occasions. The Kipepeo Project was set up to change community attitudes to the forest by giving them a stake in its conservation. Kipepeo trained farmers living next to the forest to rear forest butterflies and moths including *Argema mimosae*. Butterfly pupae were purchased from the farmers for export to the live butterfly exhibit industry in Europe and the United States. Cumulative community earnings from 1994 to 2001 exceeded $130,000 with significant positive effects on both livelihoods and attitudes (Gordon and Ayiemba, 2003).

3.2 Survey on spatial distribution of *Argema mimosae* food plants and wild silkmoth cocoons

Arabuko Sokoke forest is divided into six Participatory Management zones namely Dida, Gede, Roka, Ngerenya, Malanga and Mongotini. Dida zone is in the south-west site of the forest and it’s sparsely populated with 10-40 people per square Km (Appendix

II). The zone has comparatively little forest encroachment and a pilot project on Participatory Forest Management (PFM) was permitted by the Permanent Secretary, Ministry of Environment and Natural Resources (MENR) in February 2000. This PFM project was run through community associations such as Dida Forest-Adjacent Area Forest Association (DIFAAFA) and Forest-Adjacent Dwellers Association (FADA) (ASFMT, 2002). The sparse population and little forest encroachment were the factors considered to select the zone to study the spartial distribution of the wild silkmoth food plants and cocoons.

A Provisional survey was conducted to identify food plants of *A. mimosae* in forest with reference to previous studies by researchers in other parts of Kenya (Kioko *et al*., 2000a,b). The Larvae and cocoons collected on the food plants were used as essential tools in determining the ideal plants. This was later confirmed by rearing larvae on the identified food plants. Plant samples were collected and preserved by pressing between newspapers supported by hard cartons. The samples were then taken to the Kenya National Herbarium for identification.

3.3 Phenology of developmental stages of *Argema mimosae*

The study on the development of *A. mimosae* larval stage was done on *lannea schweinfurthii* and *Ozoroa obovata* food plants, both of Anacardiacea family. This was done using semi-captive rearing, a method which previously was used successfully to rear *Gonometa postica* larvae (Raina, 2000; Kioko *et al*., 2007; Ngoka *et al*., 2008). The number of instars was determined by observing the exuviae cast-off after each moult (Schmint *et al*., 1977).

After larval period, cocoons formed by the larvae were harvested after seven days and kept in ventilated cartons. The healthy cocoons were selected for physical characteristic analysis and also comparing pupal weight for the larvae fed on *l. schweinfurthii* and *O. obovata* food plants.

3.4 Harnessing natural silk fibre from *Argema mimosae* cocoons

To extract clean *A. mimosae* silk, cocoon shells were boiled in a solution of 5gms/L of sodium carbonate and distilled water for comparison at different time intervals. The silk was extracted and spun by hand spinning wheel. The quality of silk filament; breaking load (tenacity) and elongation were tested in the silk quality control laboratory at the International Center of Insect Physiology and Ecology (*icipe*) (Plate 3.1).

Plate 3.1: Elongation and breaking load testing machine

3.5 Volatiles responsible for parasitism on different developmental stages of *Argema mimosae* on different species of food plant

Volatiles responsible for parasitism were collected in the field from both the food plants and 4th larval stage. After collection, the volatiles were analysed in the behavioural and chemical ecology laboratory at the International Center of Insect Physiology and Ecology (icipe). Chemical odors emitted by *A. mimosae* food plants viz *Lannea schweinfurthii* and *Ozoroa obovata* and the 4th larval stage were collected using a portable push-pull volatile collection system (USDA/ARS-CMAVE, Gainesville, Florida). Characterization of the volatile compounds was done by Gas Chromatography-Mass Spectrometer (GC-MS). GC-MS (Agilent Technologies, Wilmington, DE, USA). The volatile compounds analysis was carried out on a HP 7890A model series GC coupled to a 5975C mass spectrometer and a Triple Axis Detector. These experiments were carried out at International Center of Insect physiology and Ecology.

3.6 Data analysis

Data were analyzed using the PROC GLM analysis of variance (ANOVA) procedure of SAS to compare differences in variables. When ANOVA showed significant differences between the means ($P<0.05$), the post-hoc means were separated using the tukey test (SAS Institute Inc.2003).The physical characteristics of *A. mimosae* larvae and cocoons were summarized using descriptive statistics.

CHAPTER FOUR

4 RELATIVE ABUNDANCE OF *Argema mimosae* (BOISDUVAL) FOOD PLANTS AT ARABUKO SOKOKE FOREST

4.1 Introduction

The African continent is wealthy as regards to insects which provide many useful products. Currently Africans face enormous challenges in both conserving their biological heritage and improving their livelihoods. With the increased globalization of the economy and limited environmental resources, it is imperative for African nations to collaborate with each other and come up with exploiting and sustaining methods of the available natural resource. The development of fragile ecosystems and their sustainability lies in the rational use of the existing biodiversity. In East Africa the biodiversity of wild silkmoths is deteriorating along with their habitats, especially in the forests which act as gene pools. Kenya's loss of forest cover and the associated biodiversity has led to serious environment deterioration, the consequence of which is the marked decrease in food production and rural poverty which is found across these previously rich and abundant lands. Improving management forest resources that includes a strong biodiversity conservation component, requires a strategic mix of law enforcement and local capacity building with community participation based on incentives through the diversification of livelihood options (Gordon and Ayiemba, 2003; Raina *et al*., 2009).

Arabuko Sokoke forest was under increasing pressure from human encroachment, charcoal burning and overuse and until 2003, from 'legal' excision (Gordon and

Ayiemba, 2003). Licensed selective logging continues on a smaller scale, along with the licensed collection of dead wood for fuel. These practices have proven difficulty to control and regular poaching of valuable trees continues to be a major problem. Communities living within five kilometers of the forest boundaries benefit from a whole range of forest resources and there is need to sensitize them and initiate incentives for sustainable use of the resources if biodiversity conservation is to be realized (Gordon and Ayiemba, 2003; Raina *et al*., 2009).

Wild silk farming holds great promise for the forestry as a supplementary activity to curb forest destruction and support biodiversity conservation. On one hand it can help in forest conservation, and on the other hand it permits gainful utilization of this vast natural wealth. Wild silk farming can be initiated as a supplementary activity for income generation for rural communities that mainly depend on subsistence farming (Kioko *et al*., 2007). To develop wild silkmoth farming, survey and identification of their food plants is very important and forms part of the study. Other than providing food for the wild silkworms, the trees have other benefits such as soil erosion control, protection of water catchments areas and improved biodiversity conservation in general.

4.2 Materials and methods

4.2.1 Survey on spatial distribution of *Argema mimosae* food plants

The spatial distribution of the food plants was determined through setting four transect blocks in the forest and farmland. The blocks A, B, C and D were 3 km apart (Plate 4.1). In each block five transects of 50m x 20m with a distance of 50m apart were sampled in

random direction. The entry and end points of the transects were marked with Global Position System (Model Garmin Geko 101) (Appendix III). The transect markings started 200m from the farm and forest boundary to give disturbance allowance in the boundary. Each wild silkmoth food plant identified within the randomly selected transects were marked as a GPS point and recorded in degrees and minutes to map their distribution.

4.2.2 Survey on spatial distribution of *Argema mimosae* and other wild silkmoth cocoons

A. mimosae and other wild silkmoth cocoons observed on the food plants within the transects under study were marked as GPS points and recorded against the food plant species. All cocoons were stored in ventilated cartons and sent to National Museums of Kenya and icipe- Commercial Insects Programme for identification.

4.3 Data analysis

Data on the abundance of food plants and the cocoons of wild silkmoths were analyzed using the PROC GLM analysis of variance (ANOVA) procedure of SAS. When ANOVA showed significant differences between the means ($P<0.05$), post-hoc mean separation was conducted using the Tukey test (SAS Institute Inc.2003). Comparison between the farmland and forest was done using Student's t-test ($P<0.05$). Where the data were not normal, log (x+1) transformation was done before subjecting them to the t-test.

The GPS points were analyzed with a computerized Geographic Information Systems, version 9.2 (GIS) and mapped. The mapping was supported by Google earth mapping system (Data SIO, NOAA, U.S. Navy, NGA, GEBCO, US department of state geographer @ 2010 take atlas (Plate 4.1).

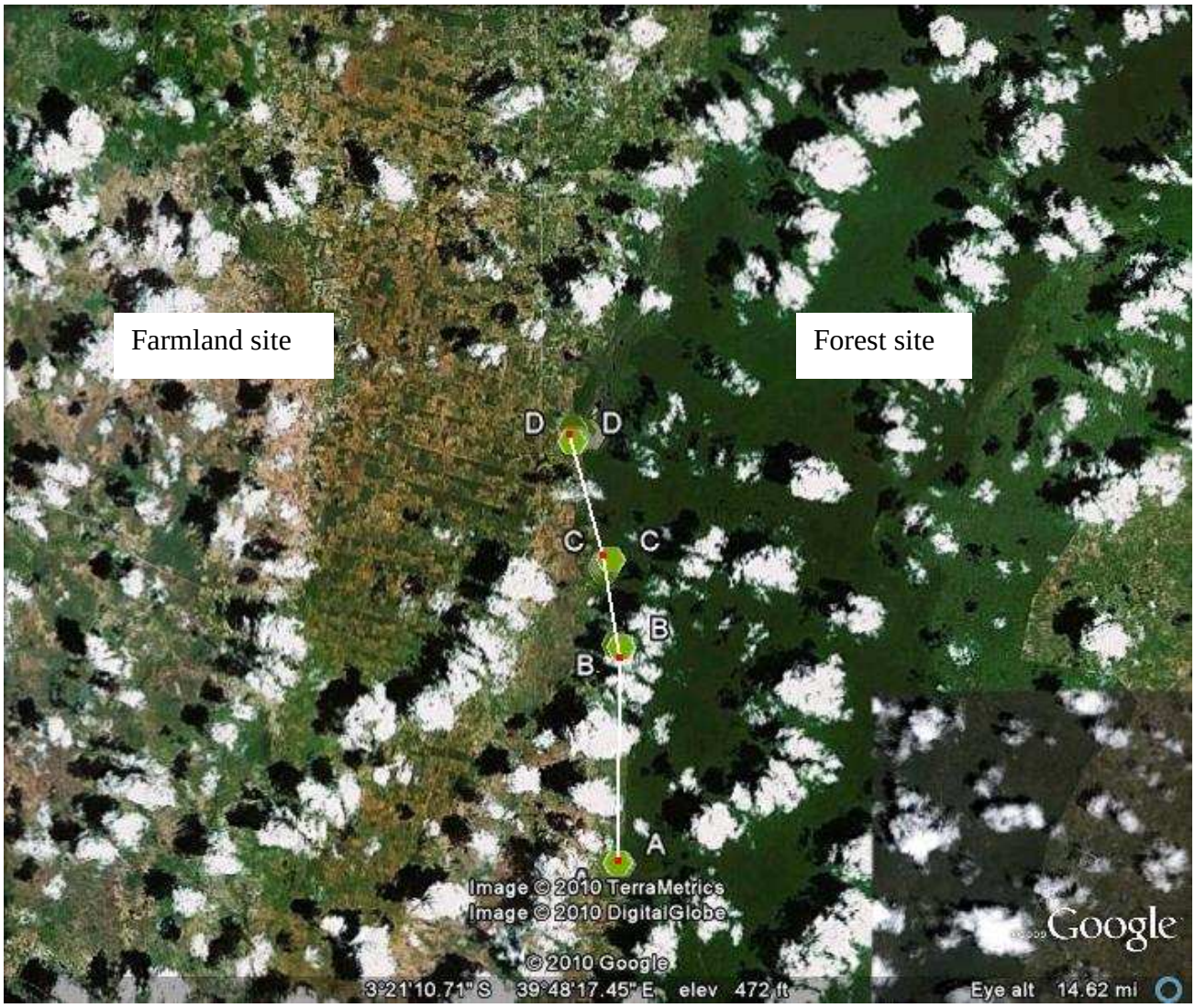


Plate 4.1: Google earth map showing the four blocks in Dida Zone, where the survey of wild silkmoths and their food plants were carried out.

4.4 Results

4.4.1 The common wild silkmoths food plants in the Arabuko Sokoke forest

Three wild silkmoth species were recorded in Arabuko Sokoke Forest with different food plants as listed in table 4.1.

Table 4.1: Wild silkmoths and their food plants in the Arabuko Sokoke forest

Wild silkmoth species	Family	Food plant	Family	Local name (Giriama)
Argema mimosae	Saturniidae	*Lannea schweinfurthii*	Anacardiacea	Mnyumbu
Argema mimosae	Saturniidae	*Ozoroa obovata*	Anacardiacea	*Mukayukayu*
Argema mimosae	Saturniidae	*Sclerocarya birrea*	Anacardiacea	*Mfula*
Gonometa postica	Lasiocampidae	*Brachystegia speciformis*	Fabaceae	*Mrihi*
Gonometa postica	Lasiocampidae	*Acacia reficiens* Wawra	Leguminosae	*Mrerengwa*
Epiphora mythiminia	Lasiocampidae	*Croton macrostachyus* Hochst	Euphorbiaceae	*Mbonokoma*

The abundance of the three plant species in the farmland and the forest showed a significant difference ($P<0.05$). *Brachystegia speciformis* food plant species in the farmland was significantly different compared to those of *O. obovata* and *L. shwenfurthii* species. When *O. obovata* and *L. shwenfurthii* in the farm land were compared, the former had a higher mean abundance of 0.06±0.009 plants while the later had insignificant mean number of plants which was as a result of transforming the data to Log (x + 1) which was necessitated by having many zeros which were recorded during the study (Figure 4.1). *Brachystegia speciformis* was abundant in the forest in

comparison to both *O. obovata* and *L. shwenfurthii*. The abundance of the food plants was significantly different between the forest and the farm land. *Brachystegia speciformis* had a higher mean number of 0.129±0.015 plants in the forest compared to a mean number of 0.027±0.007 in the farm land. On the other hand, *O. obovata* had higher a mean number of 0.06±0.009 plants in the farmland compared to a mean number of 0.006±0.004 plants in the forest (Figure 4.1).

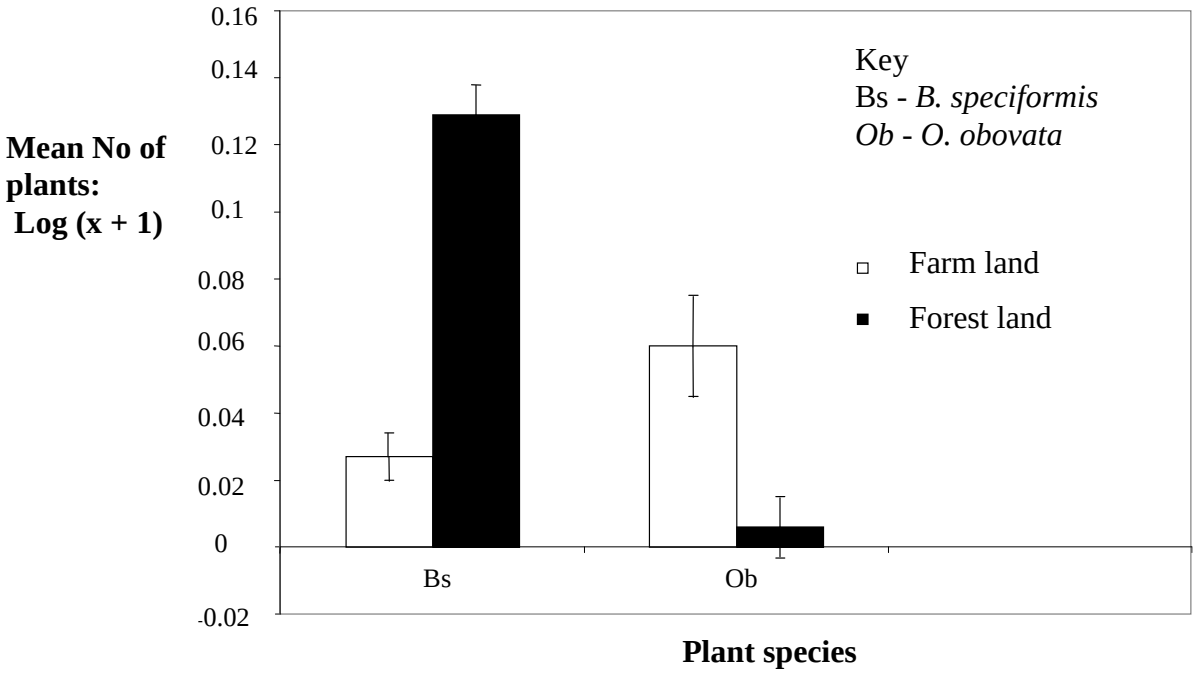


Figure 4.1: Distribution of the three wild silkmoth food plants in Dida Zone

4.4.2 Spatial abundance of wild silkmoth food plants and cocoons in Dida zone, Arabuko Sokoke forest

The abundance of the wild silkmoth food plants in Dida zone was displayed in four blocks namely A, B, C and D (Plate 4.1). *Argema mimosae* cocoons were more on the food plants in the farm land blocks compared to those which were found at the forest site. The cocoon abundance differed from one block to another (Figure 4.2 – 4.5).

In block A, wild silkmoth food plants were sparsely distributed and only two species were recorded namely *L. schweinfurthii* and *O. obovata.* Although the mapping was done both in the farmland and in the forest, the food plants were only recorded in the farmland but not in the forest site. A cluster of *A. mimosae* cocoons 1-5, was collected from *L. schweinfurthii* and *O. obovata*. In block B the food plants were recorded in farmland only with *L. schweinfurthii* being abundant compared to *O. obovata*. *A. mimosae* cocoons were collected not recorded in block B (Figure 4.2 and 4.3).

Unlike in blocks A and B, Block C had a better abundance of the wild silkmoth food plants both in the farmland and in the forest sites. Distribution of the food plants was sparse in the farmland site compared to the distribution in the forest site in block C. Although the three species of food plants namely: *B. speciformis, L. schweinfurthii* and *O. obovata* were recorded in the forest, *L. schweinfurthii* was not found in the farmland site of this block. *Gonometa postica* cocoons and not those of *A. mimosae* were collected from *B. speciformis* in the farmland site and there were no wild silkmoth cocoons observed in the forest (Figure 4.4).

In Block D, the three food plants recorded in blocks A, B and C were also recorded in this block, both in the farmland and forest sites (Figure 4.5*)*. Although the distribution was not uniform, it was relatively better compared to the previous blocks. In the forest site, the food plant species were more abundant than in the farmland. *Brachystegia speciformis* appeared to be more dominant and better distributed in the forest site than the other two food plants which were dominant in the farmland. Wild silkmoth cocoons were more abundant in this block compared to the other three blocks. Most of the cocoons recorded belonged to *A. mimosae* moth and occurred more in the farmland in clusters of 10 – 20 on the *L. schweinfurthii* food plant while few cocoons were found in the *O. obovata* in clusters of 1-5 and few cocoons of *Gonometa postica* were collected on the *B. speciformis* (Figure 4.5).

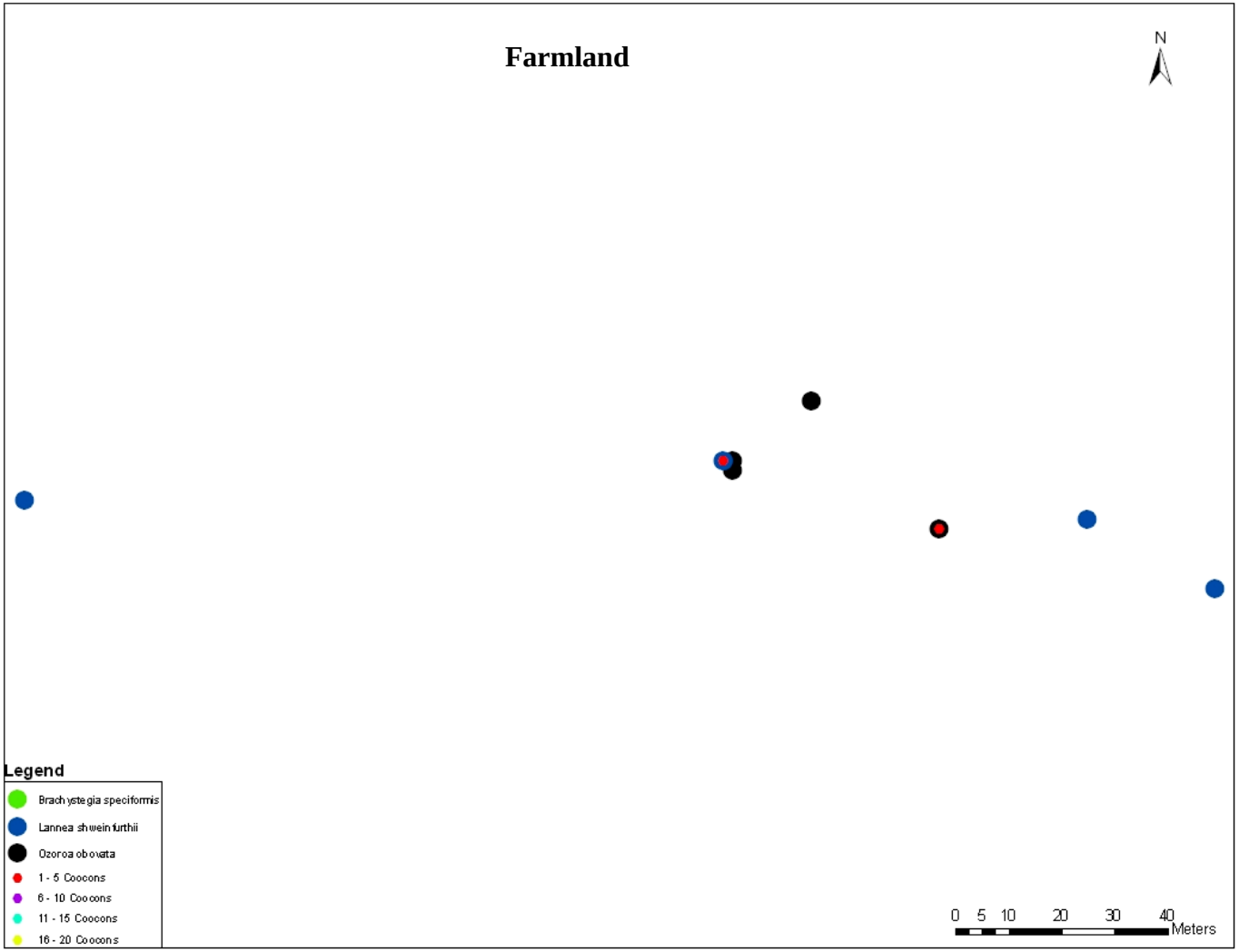


Figure 4.2: Distribution of wild silkmoth food plants and cocoons in Farmland, Block A

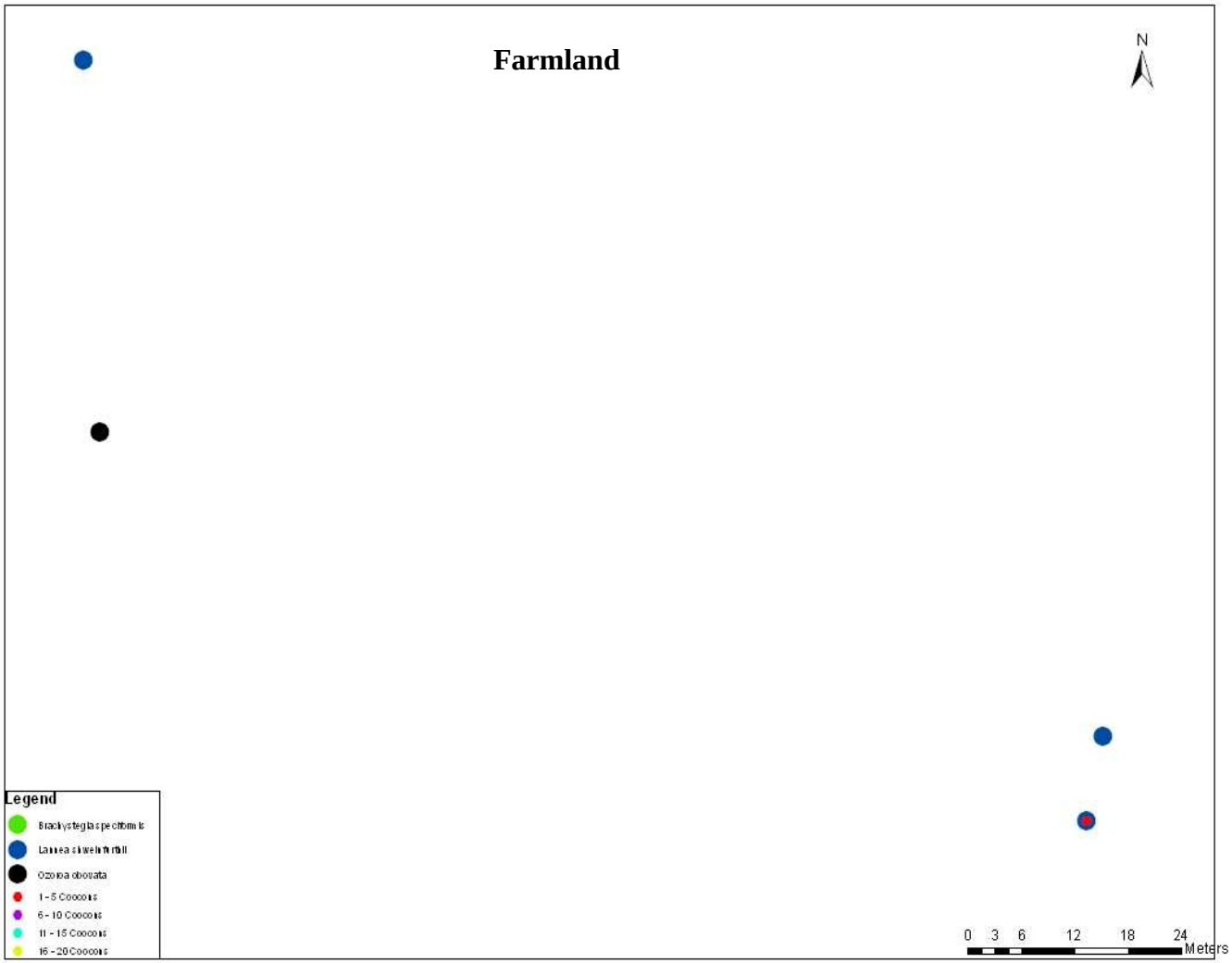


Figure 4.3: Distribution of wild silkmoth food plants and cocoons in Farmland, Block B

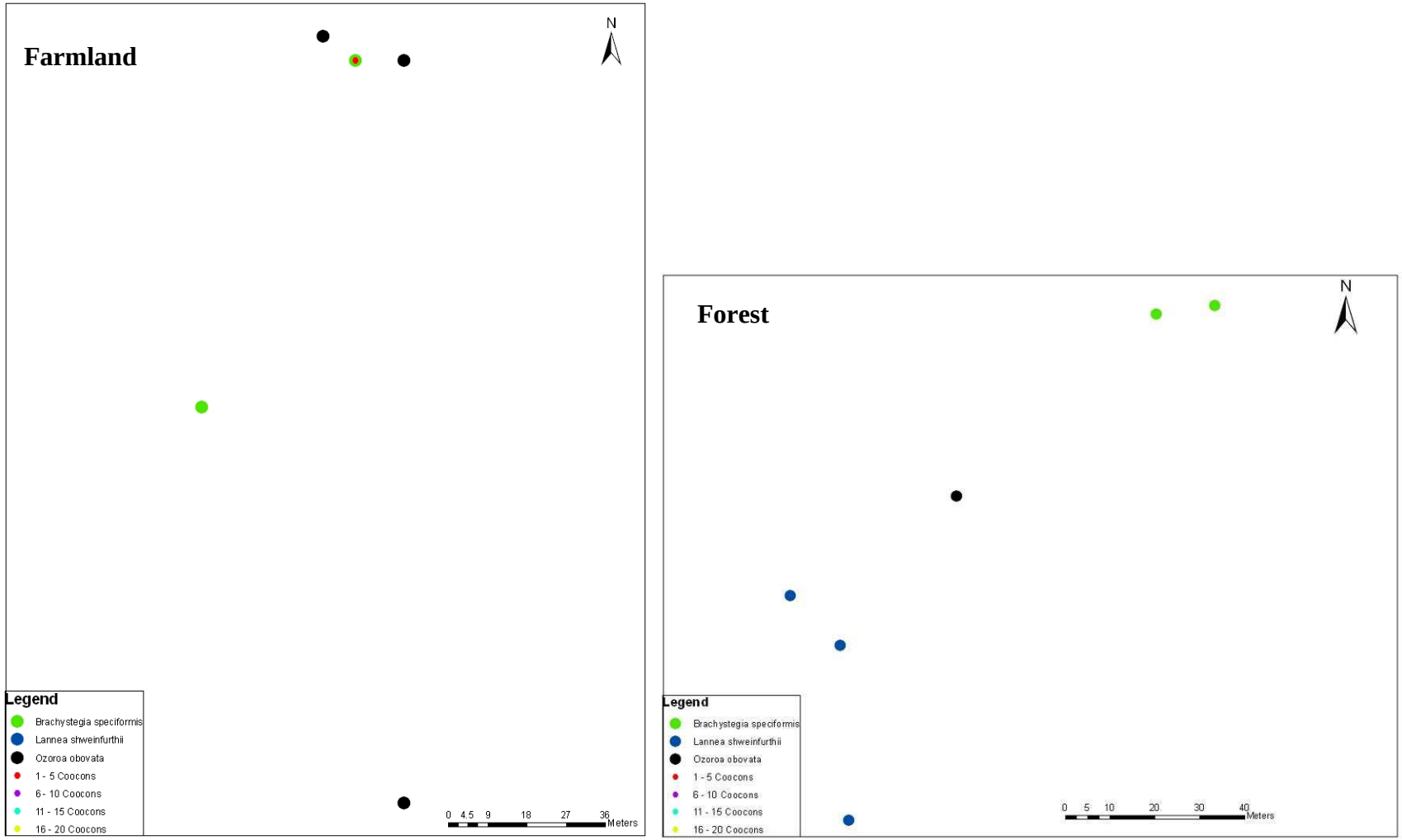


Figure 4.4: Distribution of wild silkmoth food plants and cocoons in Farmland and Forest, Block C

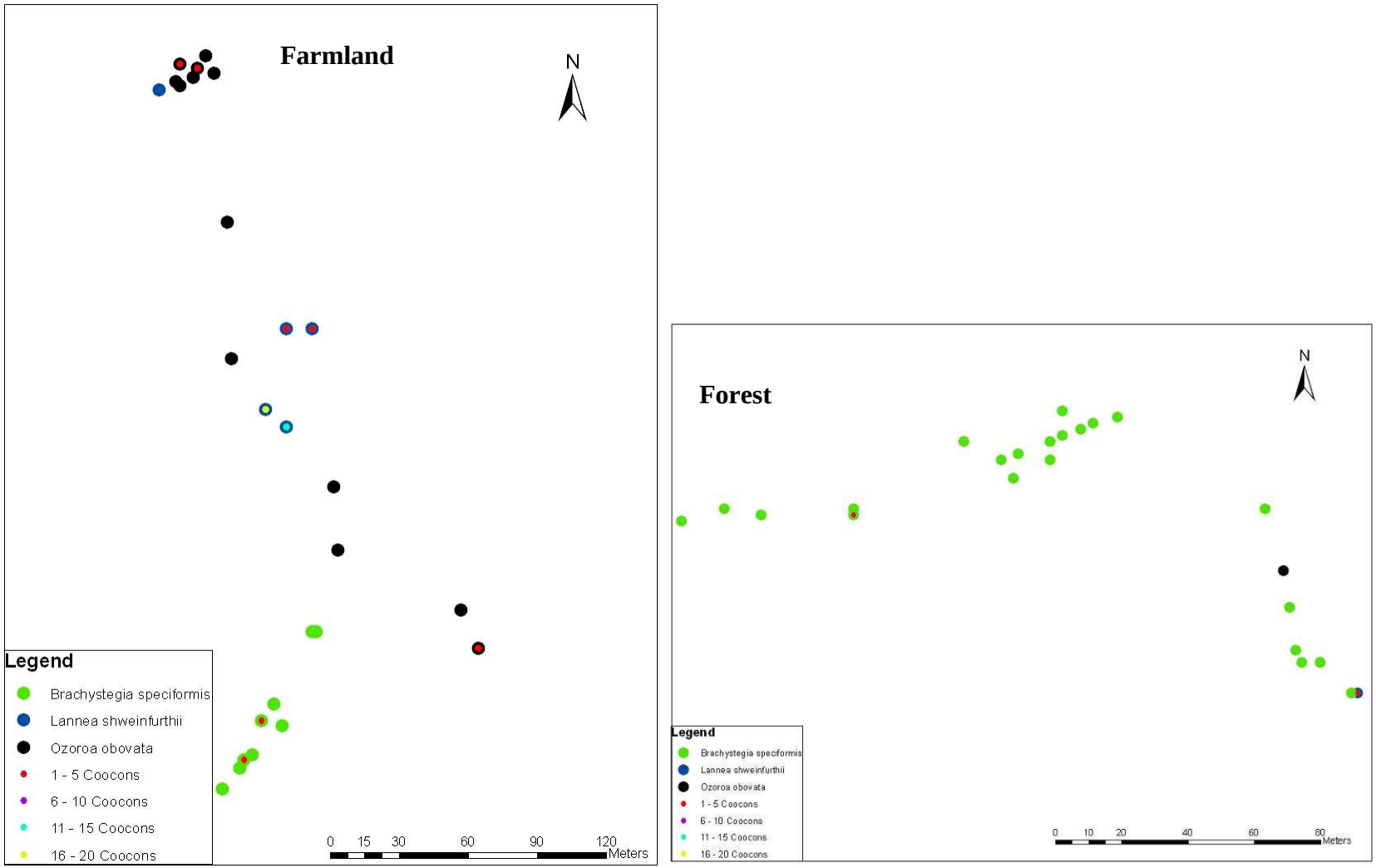


Figure 4.5: Distribution of wild silkmoth food plants and cocoons in Farmland and Forest, Block D

4.5 Discussion

This study identified three (3) food plants namely *Lannea schweinfurthii*, *Sclerocarya birrea* and *Ozoroa obovata* being utilized by the *Argema mimosae* wild silkmoth in the Arabuko Sokoke forest. *Lannea schweinfurthii* and *S. birrea* locally known as Mnyumbu and Mfula respectively which have been reported in this study as food plants for *A. mimosae* have been previously recorded in Sultan Hamud, Makueni District, Kenya (Kioko *et al*., 2000b). However, *O. obovata* locally known as Mukayukayu which was identified and reported in this study has not been reported elsewhere in Kenya and East Africa as wild silkmoth food plant. Other wild silkmoth food plants which were identified in the Arabuko Sokoke forest included *Croton macrostachyus* (for *Epiphora mythimnia* silkmoth*), Brachystegia speciformis* and *Acacia reficiens* (for *Gonometa postica* Walker silkmoth*)*. This survey confirms the findings of other studies carried out in South Africa and East Africa that revealed that, the African wild silkmoth *Gonometa postica* feed mainly on the African *Acacia* and *Brachystegia* spp. (Hartland-rowe, 1992; Kioko *et al*., 2000b; Veldtman *et al*., 2002; Fening *et al*., 2008a).

The geographical information system in this study revealed that, the distribution of the wild silkmoth food plants in the Dida zone of the Arabuko Sokoke forest was uneven. The distribution though discrete in the farmlands, it was abundant than in the forest. In blocks A and B the wild silkmoth food plants were recorded only in the farmland and none was recorded in the forest. In blocks C and D, the wild silkmoth food plants were recorded in both the farmland and the forest. *Lannea schweinfurthii* and *O. obovata* were more abundant in the farmland than in the forest where *B. speciformis* was dominant.

Some of the plants identified as food plants for the wild silkmoths in the forest and its buffer zones such as *S. birrea*, *A. reficiens* and *C. macrostachyus* were not recorded in the surveyed blocks. These mighty have occurred due to their low abundance in the sampled blocks.

The study also showed that *B. speciformis* occured more in the forest than in the farmland while *O. obovata* and *L. schweinfurthii* occured more in the farmland than in the forest. Even distribution of *B. speciformis* in the forest could be related to the previous studies by Burgess *et al.* (1998), who classified Arabuko Sokoke forest into three types of vegetation: *Brachystegia speciformis* L. woodland, *Cynometra webberi* L. thicket and mixed forest, formerly dominated by *Afzelia quanzensis* L. The main threats to the Arabuko Sokoke forest included agricultural encroachment, charcoal production, illegal extraction of timber for wood-carving, building, firewood and illegal unsustainable game meat hunting. Since *B. speciformis* is used for poles and charcoal, its scarcity in the farm land could have been contributed by these factors in comparison to the other silkworm food plants which do not have such diverse uses. Pressure on the forest has been, at least partially, attributed to significant increase in human population density around the forest in the recent past (Gordon and Ayiemba, 2003). Agriculture in the surrounding Dida zone of the Arabuko Sokoke is unproductive because of the poor soils and families around the forest carry out subsistence agriculture. The subsistence farmers plant maize, cassava, and beans, with their income being supplemented by cash crops such as cashew nuts, mangoes, and coconuts. Information is required on spatial distribution of wild silkmoth populations to assist in developing management plans for community-driven conservation initiatives and their sustainable utilisation for income generation to ensure

that the communities can draw a livelihood from these vital ecosystems without destroying them. Global Information System (GIS) can greatly contribute towards conservation and long-term monitoring of biodiversity (Mbahin *et al*., 2008).

This study has also shown that the availability and distribution of food plants as some of the factors that determined the abundance of the wild silkmoths. Wild silkmoths cocoons were abundant in block D which had a better distribution of the food plants. Abundance of more wild silk cocoons in the farmland than in the forest showed that there were more factors such as natural enemies in the forest checking the wild silkmoth population growth. Predators and parasitoids have been reported to cause significant reduction in the abundance of wild silk cocoons and their effect is presumed to be more in the undisturbed forest habitat than in the farmland (Ngoka, 2003; Veldtman *et al.*, 2004; Kioko *et al.*, 2007; Fening *et al*., 2008a). *A. mimosae* cocoons were found more on the *L. schweinfurthii* than they were on *O. obovata* which indicated that the former was probably a more preferred food plant by the silkworms. Kioko *et al*. (1999a, 1999b) collected *Argema mimosae* cocoons on *Lannea schweinfurthii* in sultan Hamud, Makueni, Kenya.

This study revealed for the first time that *O. obovata* was a suitable food plant for the African wild silkmoth *A. mimosae* in the Arabuko Sokoke forest. The pupa of the moth commonly known as moon moth has been utilised along side those of butterflies for export for live exhibits in Eorope by the communities living around the forest through the kipepeo project (Gordon and Ayiemba, 2003; Ugo, 2004). Shells of the pupae can be

utilised for the extraction of natural wild silk fibre, thus providing additional income to the community engaged in the conservation of the forest.

Kenya's closed canopy forests, today, cover less than 1.8% while the woodland covers less than 15% of the total land area, and every year these forests further decrease in size and regeneration capacity (Raina *et al*., 2009). The forest reserves have always been most vulnerable to encroachment, overuse and degazettment due to growing demand for the forest. The new forest policies and legislation as well as the over-arching environment policy and legislation provide for seeking incentives for local communities to accept and actively conserve the natural forest cover, through participatory management processes. Experience at Arabuko Sokoke has shown that even small numbers of farmers benefiting from commercial insects can have significant impacts on forests conservation: demonstrated by 150 butterfly farmers protesting against the excision of 2500 ha of forest (Gordon and Ayiemba, 2003).

The study supports conservation and sustainable use of biodiversity within and outside the protected forests by improving forest management through the involvement of local communities who depend upon them for their livelihoods. This could be done through nursery establishment of the wild silkmoth food plants to enhance tree planting both in the farmland and forest and initiate wild silk farming as an alternative source of income to ease the pressure on the forest. The study also helps to increase the awareness of communities and national institutions of the ecological importance of insects and their forest habitants. Since most of the wild silkmoth races and monotypes are not amenable to human handling ex-situ conservation in the research centres is not feasible, the study

suggests in-situ conservation. Thus, preservation and promotion of the food plants of wild silkmoths in their natural habitats along with preservation of the niche/habitat will promote natural conservation of wild silkmoths which are our heritage and GOD’s gift.

CHAPTER FIVE

5 DEVELOPMENTAL PERIOD OF AFRICAN WILD SILKMOTH *Argema mimosae* (BOISDUVAL) ON DIFFERENT FOOD PLANTS

5.1 Introduction

Environmental factors including the food plant state play an important part on the development and growth of insects (Sinha and Chaudhuri, 1992). It is important to understand the seasonal timing of insects' growth and development i.e. their phenology in order to utilize them. Larvae of the African Lunar moth, *A. mimosae* (Boisduval) have been recorded feeding on the foliage of the *Sclerocarya birrea, Lannea schweinfurthii* and *Spirostachys venenifera* in Kenya (Kioko *et al*., 2000b; Raina, 2004). In South Africa, Van den Berg (1990) reported that larvae cause substantial defoliation on *Sclerocarya caffra* (Marula), which is economically important as a commercial subtropical fruit, resulting in poor plants growth and/or reduction in fruit production (Hortzhausen *et al*., 1986). Marula trees are attacked by *A. mimosae* larvae during the months of October to December and February to March when most of the larvae are in their fourth and fifth instar stages (Van den Berg, 1990). This study was carried out in order to describe the characteristics of the different life stages of *A. mimosae* in the field and record the duration they take. Accurate knowledge on the developmental cycle is essential for proper development of sustainable management strategies of the moth and cocoon production.

5.2 Materials and methods

5.2.1 Study site

The study was carried out at Dida buffer zone of Arabuko Sokoke forest in Edward Mwakombes farm which is two kilometers from the forest (039^o 48'N, 03^o 20'E). Studies and results in chapter four helped in determining and identifying the *A. mimsae* food plants through the survey. These were *Lannea shweinfurthwii* and *Ozoroa obovata* which were found to be abundantly distributed in the farmlands and were selected to carry out the study on the developmental stages of the moth.

5.2.2 Breeding cage for the eggs collected

The initial population of *Argema mimosae* was set up from healthy pupae in the cocoons collected from host plants in the study area. The cocoons were enclosed in 2 x 2 x 2m net sleeve cages for the moths to emerge. Enclosed in each cage were the naturally growing short-trimmed *L. schweinfurthii* or *O.obovata* trees aimed at providing points of attachment for the emerging moths. The cocoons were attached with strings to the tree branches for the moths to emerge freely and mate within the cages. Freshly laid eggs were marked and observed until hatching date.

5.2.3 Larval developmental period

The development of *A. mimosae* life stages was studied through the use of semi - captive method of rearing using net sleeves. In the previous studies, this method has been used successfully to study the life satges of *G. postica* larvae (Raina, 2000; Kioko *et al*., 2007; Ngoka *et al*., 2008; Mbahin *et al*., 2010). The net sleeves were made by local tailors and

measured 58 x 88 x 125cm. They had an opening with a zip of 120cm in length at one side that was used to cover a branch with foliage and was closed up around the stem of the branch with a strong thread. The net sleeve had a capacity of holding a maximum of 50 silkworms at the last instar. Net sleeves with a mesh size of 0.1cm were used for the 3rd, 4th and 5th instar larvae to act as mechanical barrier from predators and parasitoids. Net sleeves with a mesh diameter of less than 0.1cm but larger enough to allow air circulation was used for rearing 1st and 2nd instars to prevent them from escaping through the net because of their tiny size in addition to protecting them from their natural enemies. The experiment was set with three (3) net sleeves holding ten (10) silkworms each and replicated three times in each food plant. Larvae were reared on those food plants till they spun cocoons. The period between the hatching of eggs and the spinning date was recorded as the larval period. The larval period on the two food plants was determined and compared to identify the food plant that could be recommended for wild silk cocoon production in the study area. The number of instars was determined by observing larvae exuviae after each moult through the entire larval stages as described by (Schmint *et al*., 1977).

5.2.4 Pupal developmental period

Larvae formed silken cocoons at the end of the larval period. The harvesting was done seven days after spinning to allow the silken cocoons to become dry and the larvae to transform into pupae. After harvesting, the cocoons were kept under field conditions in the enclosed net sleeves until the moths emerged. The weight of the pupa and the cocoon

shell were measured using a weighing balance after 7 days from the time of spinning (Scout pro spu402, Max. 400g, China).

5.2.5 Data analysis

Data on the developmental period of larvae and pupae, pupal and cocoon shell weights were analysed using Analysis of variance, ANOVA procedure of SAS, 2003. When ANOVA showed significant differences between the means ($P<0.05$), mean separation was conducted using the Tukey's test (SAS Institute Inc., 2003). Comparison between the food plants was done using student *t*- test ($P\leq0.05$).

5.3 Results

5.3.1 Developmental period of larvae

During the April - October 2008 season, the eggs hatched in a period of 10±0.0 days and had a larval mean developmental period of 30.5±0.43 and 30.83±0.4 days for *L. schweinfurthii* and *O. obovata* respectively (Table 5.1). The developmental period of larvae between the food plants was not significantfly different (P>0.05). During the April – October season 2009, the eggs took a mean of 9.7±0.21 and 9.3±0.33 days to hatch for *L. schweinfurthii* and *O. obovata* respectively (Table 5.1). The Larvae took a total of 29.83±0.6 and 29.17±0.54 days on *L. schweinfurthii* and *O. obovata* respectively to pupate and the difference was not significantly different (Table 5.1). The incubation period of the eggs and the developmental period of the larvae were not significantly different between the 2008 and 2009 rearing reasons.

Table 5.1 Mean developmental period in days ± S.E of *A. mimosae* larvae instars reared on *Lannea schweinfurthii* and *Ozoroa Obovata* in April – October 2008 and 2009.

Food plant	Egg incubation period		Larval period (days)	
	2008	*2009*	*2008*	*2009*
Lannea schweinfurthii	10±0aA	9.7±0.21aA	30.5±0.43aB	29.8±0.6aB
Ozoroa Obovata	10±0aA	9.3±0.33aA	30.8±0.4aB	29.2±0.54aB

Means followed by the same lower case letters in the same row and upper case letters in the same column are not significantly different (Tukey test=0.05).

5.3.2 Physical characteristics of larval instars of *Argema mimosae*

Direct observation of the exuviae of the larvae reared throughout the entire larval stage on both *L. schweinfurthii* and *O. obovata* food plants revealed that five (5) moults occurred from the first instar to the time larvae formed a silken cocoon, thus indicating that there are six larval instars (Plate 5.1–5.4). Larvae were solitary and showed distinct form and cryptic colour changes from the first to the fourth instar. The colour changes were the same on all the food plants.

The first instar larvae were black with interior and posterior regions orange in colour and six rows of tubercles. The second instar larvae were orange green with six orange rows of tubercles. The third instar larvae were green with six rows of tubercles whose tips were orange. The fourth to sixth instar larvae were green with only two dorsal rows of green tubercles with long hairs at the tips. The green segments were separated by a pink colour. The green colour also closely matched the colour of mature leaves of the food plants.

The interval between the 1st and 2nd instars varied between 3-4 days and 5-6 days in 3rd, 4th and 5th instars, both on *L. schweinfurthii* and *O. obovata* food plants. The interval

between the 6th instar and cocoon formation varied between 6 -7 days in both food plants (Figures 5.1 and 5.2, Appendix IV and V).

Plate 5.1: First instar larva of *A.mimosae*

Plate 5.2: Second instar larva of *A.mimosae*

Plate 5.3: Third instar larva of *A.mimosae*

Plate 5.4: Fourth instar larva of *A.mimosae*

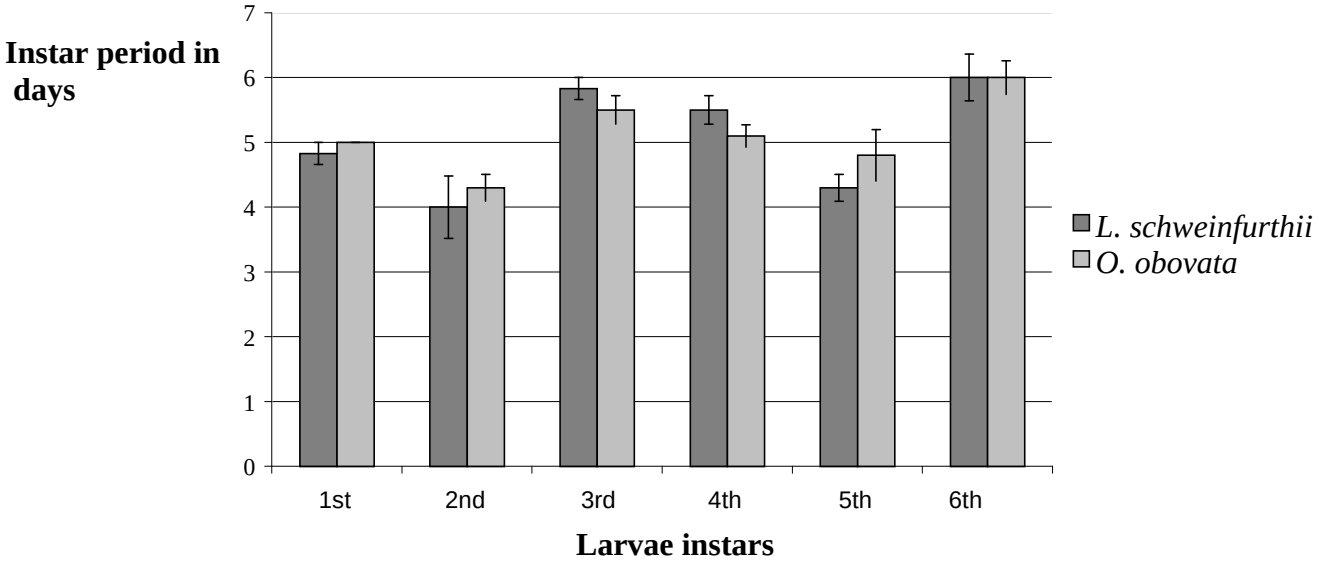


Figure 5.1: Mean larvae instar period of *A. mimosae* in days, reared on *L. schweinfurthii* and *O.obovata* from April - October 2008

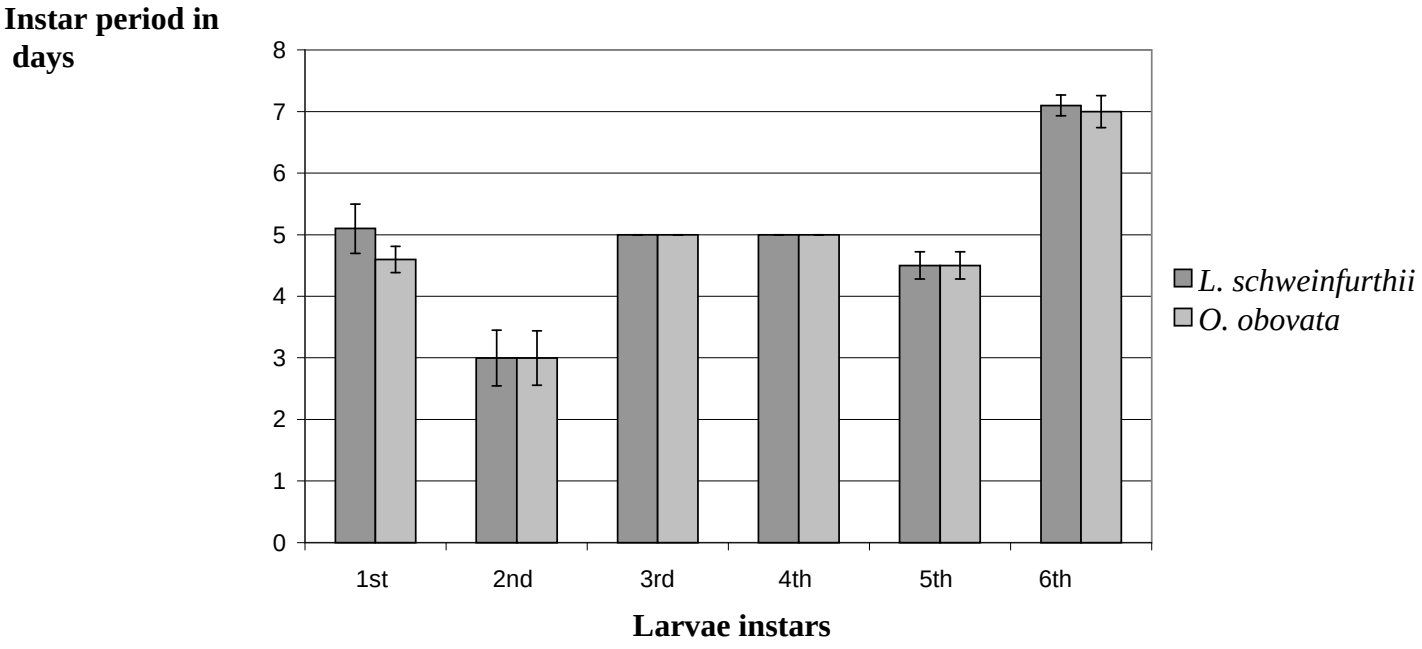


Figure 5.2: Mean larvae instar period of *A. mimosae* in days, reared on *L. schweinfurthii* and *O.obovata* from April - October 2009

5.3.3 Pupa development

Argema mimosae larvae after their sixth instar developmental stage formed a tough silvery cocoons within 24 hours. The cocoons had several perculiar perforations on the surface and brush like openings at the front tip where the moth emerged from. The pupa lied with its mouth parts facing the opening and the undeveloped appendages facing the cocoon point of attachment to the branch (Plate 5.5). The mean pupal weight in *L. schweinfurthii* and *O. obovata* was not significantly different at 5.7±0.17 and 5.1±0.18 grams respectively. The pupa shells from the *L. schweinfurthii* reared larvae had a higher mass of 0.71±0.0 grams which was not significantly different with those from *O. obovata* with a mass of 0.65±0.02 grams but the cocoon weight was significantly different (Table 5.2).

Table 5.2: *Argema mimosae* pupa, shell and total weight

Food plant	Pupa weight	Shell weight	Cocoon weight
Lannea schweinfurthii	5.71±0.17a	0.71±0.0a	6.45±0.02a
Ozoroa Obovata	5.1±0.18a	0.65±0.02a	5.81±0.04b

Means followed by the same letter in the same column are not significantly different (Tukey test=0.05**).**

Plate 5.5: Pupa of *A. mimosae* in its cocoon during metamorphosis

5.3 Discussion

This study recorded six larval instars during the entire developmental period. The results of larval instars is comparable to other wild silkmoths like *Gonometa postica* Walker (Lepidoptera: Lasiocampidae) and the domesticated silkmoth, *Bombxy mori* (Lepidoptera: Bombicidae) (Ngoka *et al.,* 2008). The study recorded an approximated everage of 10 days as egg incubation period and 30 days as the larval period. In South Africa Van der Berg (1990) recorded 28.4 days as the feeding larval period for *A. mimosae* larvae fed on *Sclerocarya caffra*. He further reported that there were five larval instars and this differs with the results obtained from this study which recorded six larval instars. This difference occurs between the 4^{th} – prepupa stage but the days are comparatively the same. Van der Berg (1990) also reported that *A. mimosae* eggs took 10.9±0.06 days to hatch and that the first to fourth instars were completed in periods of 5.6±0.12, 4.2±0.11, 3.4±0.24, and 5.8±0.17 days respectively for the larvae fed on

Sclerocarya caffra. These findigs concur well with the intervals of instars recorded in the current study for the larvae which were fed on *L. schweinfurthii* and *O. ozoroa*. The findings recorded in this study and those reported from South Africa indicate that food plants may not have much effect on the number of days of *A. mimosae* larvae took to develop. Kioko (1998) reported 32.9±3.8 days as the developmental period for *A. mimosae* larvae fed on *Sclerocarya birrea* and S*pirotachys venenifera* in Sultan Hamud, Makueni district, Kenya. Since there was no significant difference on the developmental days of the larvae fed on *L. schweinfurthii* and those fed on *O. obovata*, it implies that the reported plants are actually the preferred food plants of this moth in the Arabuko Sokoke forest.

The larva showed colour variations of black to orange from 1st to 3rd instar. However, the larvae attained green colouration from the 4th to the 6th instar. This green colour matched with the green foliage of the food plants probably serving as a protection mechanism for larvae from predators. Other wild silkmoths for example *G. postica* develop protective spines in addition to cryptic colouration as a defensive mechanism from predators (Ngoka, 2003; Kioko, 1998; Veldtman *et al*., 2004; Fening *et al*., 2008a). In another group of Lepidoptera, the sphingids, Schmidt (1990) also observed larvae cryptic colouration combined with a general tendency to remain relatively sedentary as means of escape from predation.

The pupal stage slightly showed significant difference in shell weight and the total pupal weight between the food plants. The total average cocoon weight of larvae reared on *L. schweinfurthurthii* was significantly higher compared to those of *O. obovata* food plant.

This shows that the pupal development was slightly affected by rearing larvae on the two food plants namely: *L. schweinfurthii* and *O. obovata* but the two food plants can be recommended to farmers who are engaged in wild silkmoth farming. In Madagascar, cocoons are sold by weight (Tsiresy *et al*., 2006). The same author also reported that cocoons produced by the saturniid moth *Argema mittrei* were approximately equal in weight to cocoons produced by *Borocera madagascariensis* from the highlands of Madagascar. Although cocoons produced by *Antherina suraka* weighed half as much, as *A. mittrei, A. suraka* were easier to rear and more common. *A. mittrei* silk fibers, like *A.mittrei* cocoons, are porous. In addition, these authors also noted that cocoons produced by silk worms reared on guava (introduced plant species, *Psidium guajava*, Myrtaceae) were heavier than cocoons produced by Borocera spp. reared on other plants.

In South Africa, Veldtman *et al.* (2002) reported that cocoon sizes of wild silkmoths differed significantly between species, the sexes and localities, but not between generation, and host-plant-specific populations. They added that variability in cocoon sizes found at localities would have implications for silk yields, but sex and species are by far the most important determinants of cocoon size. The information on cocoon shell and pupal weight reported in this study and compared with previous studies and form a the basis of a sustainable harvesting programme for *Argema mimosae*, in Arabuko sokoke and other parts of East Africa.

CHAPTER SIX

6 VOLATILES RESPONSIBLE FOR PARASITISM ON DIFFERENT DEVELOPMENTAL STAGES OF *A. mimosae* ON DIFFERENT SPECIES OF FOOD PLANTS

6.1 Introduction

Plants emit a wide range of volatile organic compounds in response to damage by herbivores, and many of the compounds have been shown to attract natural enemies of insect herbivores or may serve for inter - and intra-plant communication. Parasitic and predatory arthropods often prevent plants from being severely damaged by killing herbivores as they feed on the plants. Recent studies have shown that a variety of plants when injured by herbivores, emit chemical signals that guide natural enemies to the herbivores (Kigathi *et al*., 2009; Uniscker *et al.,* 2009). It is unlikely that herbivore-damaged plants initiate the production of chemicals solely to attract parasitoids and predators. These signals probably develop secondarly from plant responses that produce toxins and deterrents against herbivores and antibiotics against pathogens. To effectively function as signals for natural enemies, the emitted volatiles should be clearly distinguishable from the background odors, specific for prey or host species that feed on the plant, and emitted at times when the natural enemies forage. Studies on the phenomena of herbivore-induced emissions of volatiles conducted by various authors indicate that (i) the clarity of the volatile signals is high, as they are unique for herbivore damage, produced in relatively large amounts, and easily distinguishable from background odors; (ii) specificity is limited when different herbivores feed on the same

plant species but high as far as odors emitted by different plant species and genotypes are concerned;

(iii) the signals are timed so that they are mainly released during daytime, when natural enemies tend to forage, and wane slowly after herbivory stops (Turlings *et al*., 1995). Most studies have focused on volatile emission in the laboratory while little is known about emission patterns in the field.

6.2 Materials and methods

6.2.1 Identification of parasitoids

Cocoons of *A. mimosae* were collected from unprotected rearing food plants and enclosed into net sleeve cages measuring 30 x 30 x 45 cm (Plates 6.1a & b). Parasitoids emerging from the cages were observed on daily basis and collected for preservation and identification. Identification was done in icipe-Biosystematics unit and the National Museums of Kenya, Nairobi.

Plate 6.1a: *Argema mimosae* cocoons after harvesting

Plate 6.1b: *Argema mimosae* cocoons enclosed in net sleeves to trap parasitoids

6.2.2 Collection of chemical odors

Chemical odors emitted by *A. mimosae* food plants viz *L. schweinfurthii* and *O. obovata* were collected using a portable push-pull volatile collection system (USDA/ARS-

CMAVE, Gainesville, Florida). The volatile collection system consisted of an air suction pump, a flow meter, (Cole Palmer Instrument Co., USA), Reynolds® oven bag (Turkey size 482 mm x 596 mm, Reynolds Kitchens, Richmond, VA) and Super Q adsorbent traps (Analytical Research Systems, Gainesville, FL). The bag was cleaned by baking it overnight in an oven at 120° C. The oven-cleaned bag was used to cover a branch with foliage and was closed up around the stem of the branch with a strong PVC thread. Airflow into the sampling bag was provided by two Teflon® tubes. One tube pumped air into the bag over the foliage while the other tube pulled air out of the bag through the Super Q trap at the end and then through the flow meter at a rate of 0.26 L/min (Plate 6.2).

Volatiles were collected from *L. schweinfurthii* and *O. obovata* leaves before disturbing the plant by feeding *A. mimosae* larvae on them. To determine the volatiles released by the food plants because of herbivory which are probably responsible for parasitism and predation of the *A. mimosae* larvae, volatiles were collected when 10 *A. mimosae* larvae in their 4^{th} instar stage were actively feeding on the leaves. These larvae were isolated after feeding and volatiles released by them collected separately as a control. Volatiles were collected for 2 hr in the morning between 0900 and 1500 hr. The Super Q trap was removed, sealed with Teflon® tape and stored in a freezer until use. The experiment was replicated twice, in the two different plants in the three set ups (n = 12). Each trap was eluted with 200 µl of GC/GC-MS grade dichloromethane (Burdick and Jackson, Muskegon, Michigan, USA), and then stored in a freezer prior to analysis by coupled Gas Chromatography-Mass Spectrometry.

Characterization of the volatile compounds was done by Gas Chromatography-Mass Spectrometer (GC-MS). GC-MS (Agilent Technologies, Wilmington, DE, USA). The analysis for the volatile compounds was carried out on a HP 7890A model series GC coupled to a 5975C mass spectrometer and a Triple Axis Detector. The separation was done on a HP5 MS 5% nonpolar methyl silicon capillary column 30 m (length) x 0.25 mm (internal diameter) x 0.25 µm (film thickness). The GC was interfaced to a HP monitor (Dell Optiplex x 520) via 3365 MSD Chemstation software (G1701EA E.02.00.493) onto whose screen individual derived chromatographic fractions were acquired and evaluated. The spectrometer was operated in the electron impact (EI) mode at 70 eV and a temperature at the ion source and interface at 230 and 150°C respectively. Helium was used as a carrier gas at a constant flow rate of 1.2 ml min^{-1}. The chemical components of the volatiles were identified by comparing their mass spectral data with those in the library of mass spectrometer and by retention time analysis.

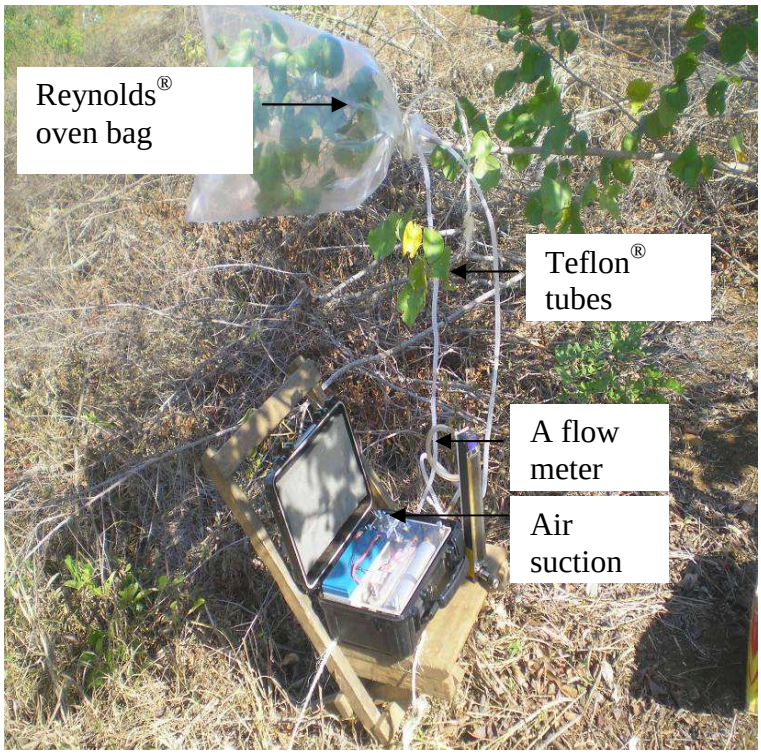


Plate 6.2: Portable volatile collection system

6.3 Results

6.3.1 Parasitoids identified

Since the cocoons have an opening at one end and natural perforations, most of the parasitized cocoons had no apparent exit holes for parasitoids but some were noted to have a single puncture at one point of the cocoon (Plate 6.3a). *Brachymeria* sp. (Hymenoptera: Chalcicidae) was identified as a parasitoid emerging from the infected cocoons (Plate 6.3b).The parasitoids killed the pupa of the infected cocoons as shown in plate 6.3c. *Goryphus* sp. (Hymenoptera: Ichneumonidae) was observed hovering on *A. mimosae* larvae protected with net sleeves and preying on those exposed (Plate 6.3d).

Plate 6.3a: *Argema mimosae* cocoon with parasitoid puncture

Plate 6.3b: *Argema mimosae* parasitoid, *Brachymeria sp.*

Plate 6.3c: Infected *Argema mimosae* cocoon

Plate 6.3d: *Argema mimosae* larvae parasitoid, *Goryphus sp*.

6.3.2 Volatile compounds trapped from *Argema mimosae* larvae food plants; *Lannea schweinfurthii* and *Ozoroa obovata*

Several monoterpenes and sesquiterpenes were trapped from *L. schweinfurthii* and *O. obovata* leaves before and after the *A. mimosae* larvae were allowed to feed on them (Table 6.1). Four monoterpenes and Eight Sesquiterpenes were emitted by *L. schweinfurthii* leaves before they were fed on the *A. mimosae* larvae. After exposing the same leaves to the larvae, linalool and Linalool isovalerate were emitted as additional monoterpenes while the linalool propanoate and (Z)-β-Ocimene were not emitted. At the same time, a sesquiterpene (E)-β-Farnesene was emitted in addition to other previously emitted sesquiterpenes (Table 6.1).

Eight monoterpenes and seven sesquiterpenes were emitted by *O. ozoroa* leaves before they were fed by *A. mimosae* larvae. After feeding the leaves on *A. mimosae*, three monoterpenes and three sesquiterpenes were emitted. Among the sesquiterpenes, identified included, (E)-β-Farnesene, distinctively trapped from *L. schweinfurthii* leaves after the larvae of *A. mimosae* were subjected to feed on them (Table 6.1). This volatile compound was not emitted before the caterpillars were allowed to feed on the food plants leaves.

Before exposing *L. schweinfurthii* leaves to *A. mimosae*, α- Copane, Valencene, α-Humulene and Germacrene were emitted with higher Ion abundance than the other compounds (Figure 6.1, Table 6.2). After feeding the leaves on the larvae (E)-β-Farnesene was distinctively emitted with high ion abundance. α - Humulene was emitted

halftimes in terms of ion abundance after exposing the caterpillars on the *L.schweinfurthii* leaves (Figures 6.2, Tables 6.3).

6.3.3 Volatiles released by the *Argema mimosae* larvae fed on the food plant leaves

The larvae released fewer and quite different compounds in comparison to those produced by the leaves of host plants. The *A. mimosae* larvae which fed on *O. obovata* leaves released the sesquiterpenes cedrene, curcumene and funebrene, while those which fed on *L. schweinfurthii* released the hydrocarbons hexacosane, nonadecane, octadecane, octacosane and eicosane.

Table 6.1: Volatile compounds emitted by *A. mimosae* food plants *Lannea schweinfurthii* and *Ozoroa obovata* before and after herbivory under field conditions

Volatile Compound	*Lannea schweinfurthii*		*Ozoroa obovata*	
	Before larval feeding	larvae feeding on the leaves	Before larval feeding	larvae feeding on the leaves
Monoterpenes				
(E) β - Ocimene	√	√	√	√
(Z) β - Ocimene	√	-	-	-
α – Pinene	√	√	√	√
β - Pinene	-	-	√	-
Linalool	-	√	√	-
Linalool propanoate	√	-	-	-
Linalool isovalerate	-	√	√	-
δ-3-carene	-	-	√	√
Cumene	-	-	√	-
Limonene	-	-	√	-
Sesquiterpenes				
(E) β- Farnesene	-	√	-	√
(E) - Caryophyllene	√	√	√	√
(Z) - Caryophyllene	√	√	√	√
α - Humulene		√	√	-
α-Copaene	√	√	√	-
α-Cubebene	√	√	√	-
B - Cubebene	√	-	-	-
Valencene	√	√	-	-
δ-Cadinene	√	-	-	-
δ-amorphene	√	-	-	-
δ and β - Longipinene	-	-	√	-
Muurola-3-5-diene (cis),	-	-	√	-

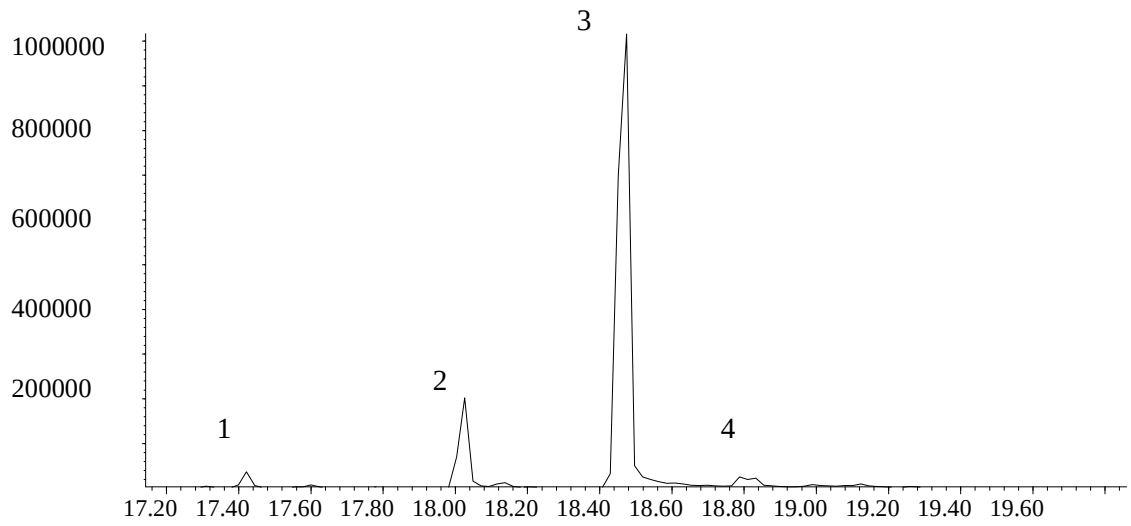


Figure 6.1: Chromatographic profiles of volatiles emitted by *Lannea shweinfurthii* leaves before exposing them to *Argema mimosae* larvae

Table 6.2: Volatiles emitted by *Lannea shweinfurthii* leaves before exposing them to *A. mimosae* larvae

Peaks	Volatile compound	Retention time
1	α - Copaene	17.431
2	Valencene	18.031
3	α - Humulene	18.475
4	GermacreneD	18.803

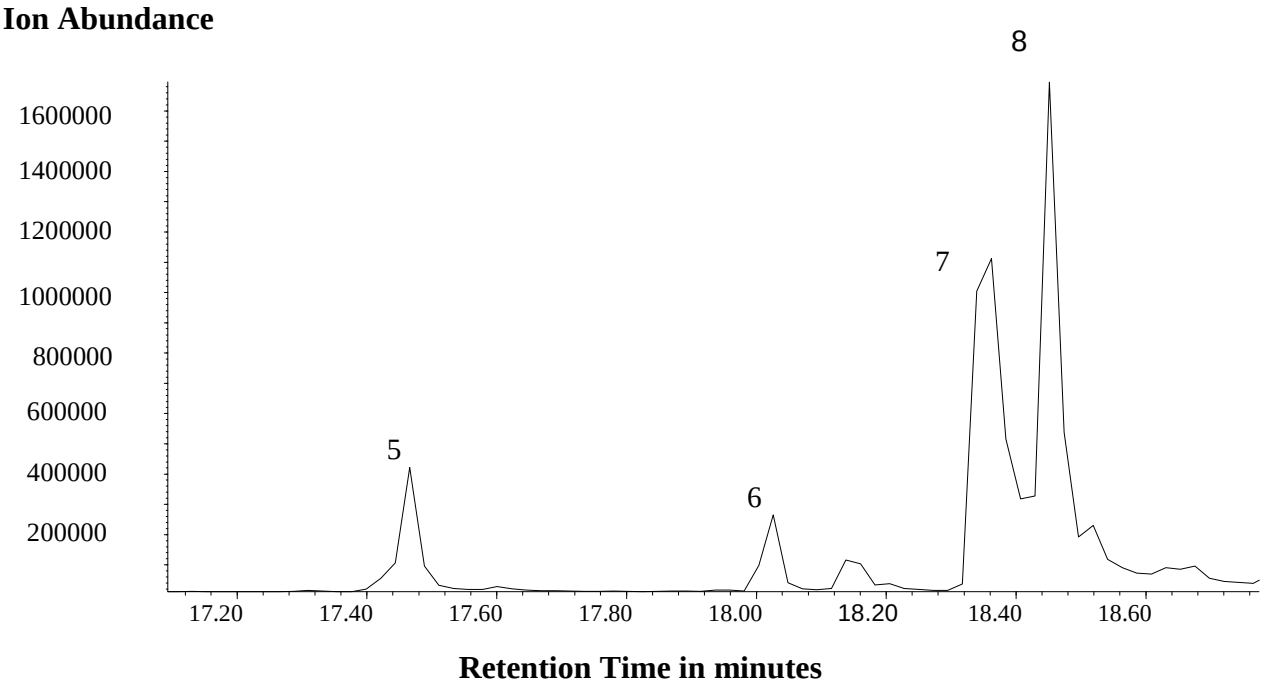
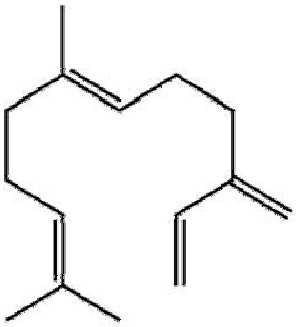


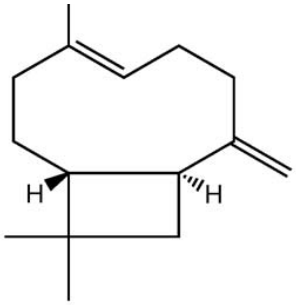
Figure: 6.2 Chromatographic profiles of volatiles emitted by *Lannea schweinfurthii* leaves after exposing them to *A. mimosae* larvae

Table 6.3: Volatiles emitted by *Lannea shweinfurthii* leaves after exposing them to *A. mimosae* larvae

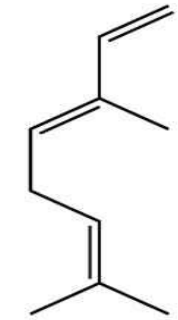
Peaks	Volatile compound	Retention time
5	Dausene	17.473
6	Valencene	18.031
7	(E)- β- Farnesene	18.364
8	α - Humulene	18.461



(*E*)-β-Farnesene



(*E*)-β-Caryophyllene



(*E*)-β-Ocimene

Figure 6.3: Chemicals structure of (E)-b-Farnesene, (E)-b-Caryophyllene and (E)-b-Ocimene

6.4 Discussion

Among the factors that limit commercial wild silk production is attack by parasitoids, which cause a significant reduction in the abundance of cocoons (Hartland-Rowe, 1992; Ngoka, 2003; Veldtman *et al*., 2004; Kioko *et al*., 2007; Fening *et al*., 2008; Raina *et al*., 2011). The exit holes left in cocoons by adult parasitoids render them unsuitable for degumming and spoil the continuity of silk filament during reeling (Kioko, 1998; Veldtman *et al*., 2004). Chalcidid *Brachymeria* sp. and Ichneumonid *Goryphus* were identified as parasitoid of *A. mimosae* larvae. The parasitoid probably lays eggs on the last instars of the larvae and emerges as adults after the host pupates in a cocoon (Veldtman *et al*., 2004). *Brachymeria nr. Albicrus* (Klug) has been reported in Mwingi forests, Kenya as one of the parasitoid attacking *G. postica* larvae and emerging as adults in the host cocoons (Fening *et al*., 2008). *Goryphus* sp. has been reported in Kenya and South Africa as a parasitoid of *G. postica* and *G. rufobrunnea* (Veldtman *et al*., 2004; Fening *et al*., 2008b).

Prospects for developing a wild silk industry could be brighter if silkworm survival under mass production conditions could be improved. A study in Kakamega forest Western Kenya, indicated a survival rate of 62.3% of *Anaphe panda* larvae protected with net sleeves compared to 16.8% for those not protected (Mbahin *et al*., 2010). Generally, this indicates that utilization of volatiles might increase the survival rate of wild silkmoths.

Low-molecular-weight terpenoids, including monoterpenes and sesquiterpenes were released in the undamaged and the damaged *Lannea schweinfurthii* and *Ozoroa obovata* leaves. The volatile monoterpene (E)-β-ocimene was trapped from leaves of both undamaged and larvae-damaged plants and probably could be playing a defense role in plants against herbivores. In other related studies, the red clover, *Trifolium pretense* L. (Fabaceae) under both laboratory and field conditions has been reported to emit (E)- β-ocimene and (Z)- β-ocimene among other compounds after herbivory by *Spodoptera littoralis* caterpillars (Kigathi *et al*., 2009). The monoterpene (E)- β-ocimene is a component of many floral scents (Knudsen *et al*., 1993) and is one of the most common volatiles whose release is induced by herbivory (Pare´ and Tumlinson, 1999). (E)- β-Ocimene is emitted by arthropod feeding on a set of plant species, such as corn *(Zea mays*) (Turlings *et al*.1990), cotton (*Gossypium hirsutum*) (Loughrin *et al*., 1994; Ro¨ se *et al*. 1996; Pare´ and Tumlinson, 1997), cucumber (*Cucumis sativus*) (Takabayashi *et al.* 1994), lima bean (*Phaseolus lunatus*) (Dicke *et al.*, 1999; Horiuchi *et al.*, 2001), potato (*Solanum tuberosum*) (Bolter *et al*., 1997), cultivated tobacco (*Nicotiana tabacum*) (De Moraes *et al.*, 2001) and its wild relative *N. attenuata* (Kessler and Baldwin, 2001). In these plant species, (E)-β-ocimene is a component of the induced volatile blend that is thought to protect plants by attracting natural enemies of invertebrate herbivores, either parasitoids or predators. The emission of this volatile compound by both food plants of *A. mimosae* larvae suggests a defensive mechanism probably to attract predators or parasitoids against these *A. mimosae* and other herbivores.

This study showed that monoterpenes Ocimene and pinene were emitted from *L. schweinfurthii* and *O. obovata* leaves before and after the feeding of *A. mimosae* larvae. On the other hand, Monoterpene δ-3-carene was emitted by *O. obovata* leaves after exposing them to *A. mimosae* but had at low insificant peaks. The sesquiterpenes (E) - Caryophyllene (Z) – Caryophyllene and α- humulene were also released at higher peaks before and after exposing *A. mimosae* larvae on *L. schweinfurthii* and *O. obovata.* This points out that the volatiles are naturally occurring compounds which are not probably induced by damage or herbivory of leaves by *A. mimosae* larvae.

The study also reported that sesquiterpene (E) - β-farnesene was not emitted in considerable peaks before feeding the larvae on the leaves of *L. schweinfurthii* and it was not emitted at all by *O. obovata* leaves. However, after exposing *A. mimosae* larvae to feed on the two food plants leaves, (E)-b-farnesene was trapped in higher peaks in *L. schweinfurthii* and at low peaks in *O. obovata*. These increased emissions of the volatile compound could have been induced as a defense mechanism by the plants to attract parasitoids or predators against the *A. mimosae* larvae. Rodriquez-Saona *et al.,* (2001), indicated that treatment of cotton with exogenous methyl jasmonate (MeJA) can directly and systemically induce emission of volatiles such as (E,E)-a-farnesene, (E)-b-farnesene, (E)-b-ocimene and linalool, that may serve as odor cues in the host-search behavior of natural enemies.

The term farnesene refers to a set of six closely related chemical compounds which are sesquiterpenes. α-farnesene and β-farnesene are Isomers, differing by the location of one

double bond. α-farnesene is 3, 7, 11-trimethy-1, 3, 6, 10-dodecatetraene and β-farnesene is 7,11-dimethyl-3-3-methylene-1,6,10-dodecatriene. The alpha form can exist as four stereoisomers that differ about the geometry of two of its three internal double bonds (the stereoisomers of the third internal double bond are identical). The beta isomer exists as two stereoisomers about the geometry of its central double bond. β-farnesene has one naturally occurring isomer. The E isomer is a constituent of various essential oils. It is also released by aphids as an alarm pheromone upon death to warn away other aphids. Several plants including potato species, have been shown to synthesize this volatile sesquiterpene as a natural insect repellent.

Most of the terpenes including (E)-β-ocimene, α-humulene, linalool and (E)-β-caryophyllene collected under this study are fundamentally important volatiles released by the plants as induced mechanism against herbivores such as the *A. mimosae* larvae. Kigathi *et al*., (2009) reported increased emissions rates of (E)-β-ocimene, (Z)-β-ocimene, linalool, (E)-β-caryophyllene, (E,E)-α-farnesene, 4,8-dimethyl-1,3,7-nonatriene (DMNT),1-octen-3-ol, and methyl salicylate (MeSA) by *Trifolium pratense* (red clover) after herbivory by *S. littoralis* caterpillars.This study is fundamental for further studies to explore the specific volatiles compound which might be responsible for predators and parasitoids attack.

CHAPTER SEVEN

7 PHYSICAL PROPERTIES OF SILK FIBRE OF THE *Argema mimosae* COCOONS

7.1 Introduction

Wild silkworms produce silk with different cocoon filament sizes, microstructures, and chemical compositions (Kato *et al*., 1997; Kato *et al*., 1999). The fine structure of cocoon filaments from various lepidopteran insects can be classified into two types, porous and compact filaments (Akai, 1988; 1998). The porous filament contains numerous tubular structures in all the filaments but the compact filament has no such structure. *Antheraea yamamai*, *A. pernyi*, *A. mylitta* and all other Saturniidae insects spin the porous filament while *B. mori*, *B. mandarina* and all other insect orders except Saturniidae spin the compact type (Akai and Nagashima, 2002). Studies on the structural characteristics of cocoons and cocoon filaments from various silk-spinning insects, including Bombycidae, Saturniidae, Lasiocampidae, Thaumetopoiedae, and Zygaenoidea have shown that only Saturniidae silkmoths in which *A. mimosae* belongs produce the porous cocoon filament (Akai, 1998; Akai and Nagashima, 2001).

Silk has been used as a textile fiber for over 5000 years. Its many highly desirable physical characteristics such as good mechanical properties, brightness, drape ability, comfort, softness, dye ability and the convenience of reeling long (300–1200 m) continuous fibers from cocoons have certainly contributed to its success as a specific fiber (Shenai and Saraf, 1993; Asakura and Kaplan, 1994). Natural raw silk is composed mainly of sericin, fibroin, water and mineral salts. Fibroin is a single protein that is

insoluble in hot water, and roughly 76% of its amino acids have nonpolar side chains. Sericin is primarily amorphous protein and acts as a gum binder to maintain the structural integrity of the cocoon, thus sericin is more water-soluble than fibroin (Karmakar, 1999). This difference makes the gum easily removable from the filaments through various processes without considerable damage to the filaments. Sericin gives a harsh and stiff feeling to the fiber and hides the rich luster. It also prevents the penetration of dye liquor and other solutions during wet processing, so silk degumming is an essential process to obtain an ideal fiber for the textile industry.

During the degumming process, sericin is hydrolyzed, and the amide bonds of the long protein molecules are broken into smaller fractions, which are dispersed and solubilized in degumming agents and media (Karmakar, 1999; Sadov *et al*., 1973). The removal of sericin is accomplished by degumming process involving the boiling of cocoons in water under pressure at 115 ºC (Gulrajan, 1992). However, for this reason this treatment gives a risk of fibroin being damaged when the time of treatment is prolonged. At the same time the process may give incomplete degumming. Sometimes soap or synthetic detergent must be added in order to improve the degumming effect (Gulrajan, 1992). Marseilles soap and olive oil soap are used at 98º C (Brag, 1929) but as a result of high temperature, this process tends to attach both sericin and fibroin. In addition, this process requires soft water in order to avoid the formation of scum. Degumming with acid such as hydrochloric acid and citric acid has not received much attention in silk industry since alkaline solution is safer for fibroin than acids. Unlike the *B. mori* (*Bombyx mori* L.) silk cocoons, most of the wild silk cocoons cannot be satisfactorily softened by boiling in

plain water (Jolly *et al*., 1979). The cocoons have to be softened by more drastic boiling off techniques (Pandey and Goel, 1990). Generally the cocoons are cooked in presence of strong alkali agent like Sodium carbonate or other related chemicals. In this study sodium carbonate solution was used in boiling *A. mimosae* cocoons to make them soft for silk fibre extraction.

7.2 Materials and methods

7.2.1 Sampling for *Argema mimosae* cocoons

Cocoons of *Argema mimosae* wild silkmoth were collected from the Arabuko Sokoke forest, located at Kenya's coast as shown in appendix I (3^0 20'S, 39^0 55'E). The first batch of the cocoon samples were collected between 2005 and 2007. These cocoons are referred to as old cocoons in the text. The second batch was obtained from larvae reared in semi-captivity using net sleeves during the April – October 2008 rearing season and at the same period in 2009. These cocoons are referred to as fresh cocoons in the text.

7.2.2 Physical characteristics of *Argema mimosae* cocoons

The physical characteristics of the cocoons were observed under light microscope, Jeica model, at a magnification of 20x. Holes or perforations on the surface of the cocoons were counted manually and recorded. A sample of 100 cocoons was selected randomly for the counting of the holes and each cocoon was divided into front, mid, end and bottom sides and their distribution means computed and compared. The diameter and mass of the cocoon shells were measured by use of a venier caliper (0-15cm, 0-6 in) and

a weighing balance (SCOUT PRO SPU402) respectively. A scanning electronic microscope (JEOL-JSM T330 A, Gold coater; JEOL JFC-1100E) was used to examine the grains and the filament structure of the cocoons.

7.2.3 Raw silk extraction from *Argema mimosae* cocoons

To extract clean silk, the cocoons were cut open and pupa debris removed. They were boiled in a solution made of 5gms/L of sodium carbonate and distilled water at different time intervals of 60, 90 and 120 minutes. Each treatment had three replicates and was compared with cocoons of *Bombyx mori* making a total of fifty four (54) observations. The cocoons were enclosed in wire mesh cages with a volume of 717 cm^3 to ensure homogeneous boiling. The cocoons could not be reeled because they have an opening at the front tip which breaks continuity of the filament. After boiling, the cocoons were soaked in star soft solution of 50ml/ltr of distilled water for 3 minutes to emulsify them for deflossing. To extract silk floss, deflossing was done manually when the cocoons were semi-dry. The semi-dry floss was spun with wooden hand spinning wheel and transferred into a bobbin (Plate 7.1).

Plate: 7.1: A wooden manual spinning wheel used to spin *A. mimosae* silk floss

7.2.4 Raw silk quality testing

Quality control has been found to be the most effective measure to maintain the requisite quality and quantity of any textile product either in yarn or fabric stage. In this study tenacity and elongation tests of the raw fiber silk extracted from the *A. mimosae* cocoons was carried out.

7.2.5 Tenacity and elongation percentage

Tenacity and elongation is the quantity of weight which a given fibre can support before breaking and a length to which a fibre may be stretched before breaking, respectively. The apparatus for the tenacity test and the degree of elongation consisted of a sizing reel of 1.125m in circumference (400 revolutions equal to 450m) and a constant speed of 300 revolutions per minute was used to prepare the test sample (Plate 7.2) and a serigraph CYC008, a tensile strength tester with an automatic attachment simultaneously recording

the force and elongation of the silk (Plate 7.3 and 7.4). The samples skeins were conditioned in a room maintained at a standard temperature of 20^0C and relative humidity of 65%. The clamp distance on the serigraph was 10cm and the extension speed was 15cm/min. The sample skeins were mounted on the serigraph and testing done. The tenacity and elongation were recorded; elongation was expressed as a percentage of the total stretch of the portion tested, while tenacity was expressed in grams per denier using the formula.

$$\text{Tenacity in grams per denier} = \frac{Z}{N \times D}$$

Z = Breaking load in grams of test skein

N= Number of strand tensioned

D=Denier of test skein

The silk fibre was used as a weft with a *B. mori* silk as a warp to weave a natural silk fabric. This study demonstrated the potential and the role this *A.mimosae* wild silkmoth is capable of playing in the natural fibre production.

Plate: 7.2: A Sizing reel used to prepare elongation and tenacity test samples

Plate: 7. 3: *Argema mimosae* silk fibre fixed on serigraph before elongation and tenacity test

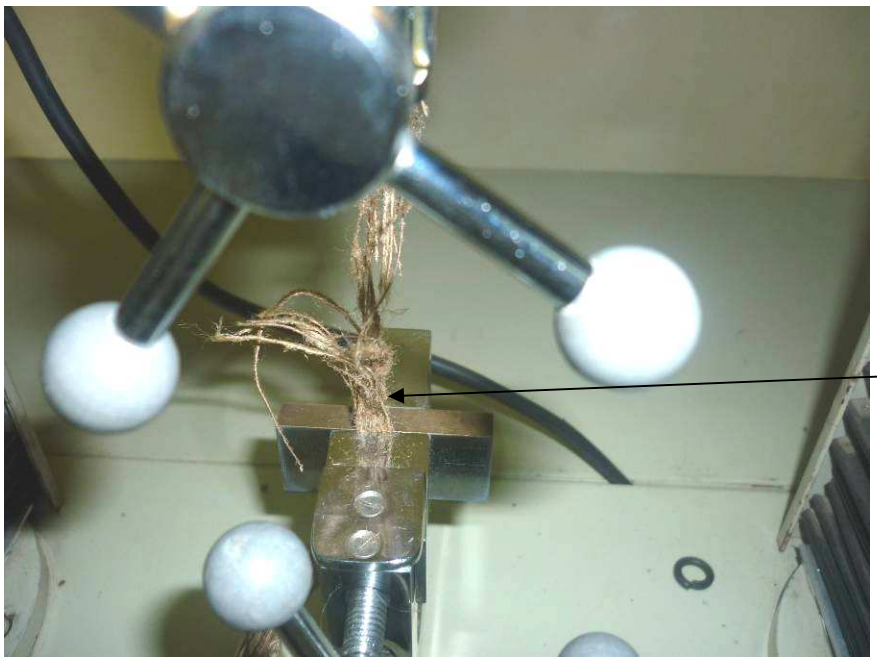


Plate: 7. 4: *Argema mimosae* silk fibre fixed on serigraph after elongation and tenacity test

7.3 Data Analysis

The physical characteristics such as cocoon filament length, cocoon diameter, cocoon weight, number of holes present on cocoon surface were summarized using descriptive statistics. Data analysis to compare the mean of holes and weight of yarn were done in R, statistical software. Linear regression was used to assess the relationship between the boiling time of different cocoon types and the weight of yarn produced at different boiling intervals in minutes. The difference in means was tested using Turkey test.

7.4 Results

7.4.1 Physical characteristics of *Argema mimosae* cocoons

The *A. mimosae* cocoon is silvery in colour, tough and thick with a prominent opening at the anterior end (Plate 7.5). The opening has loose filaments forming a brush-like valve at the tip where the moth emerges. Numerous irregular small holes or perforations were observed on the surface of the cocoons distributed in the open front tip, middle, rear tip and at the point of attachment to the plant (Plates 7.5, 7.6).

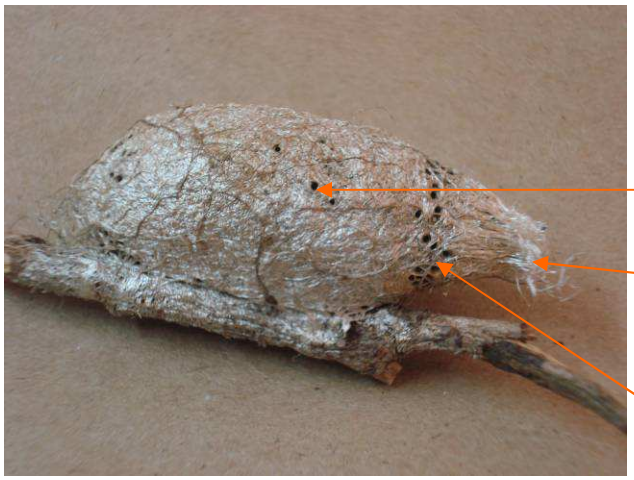


Plate 7.5: *Argema mimosae* cocoons, front tip, magnification: 15x

Plate 7.6: *Argema mimosae* cocoons, rear end, magnification: 15x

The perforations of the cocoons were magnified to show round shaped holes (Plate 7.7a & b). At a higher magnification, the silk filaments interweave across the holes forming round shapes. The tip of the cocoon has loosel brush-like filaments (Plate 7.8a & b).

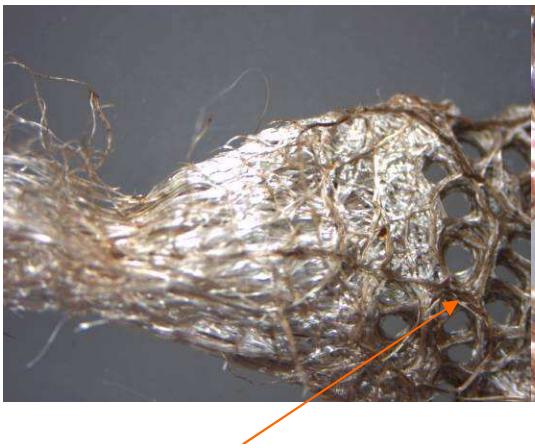


Plate 7.7a: *Argema mimosae* cocoons filament, magnification: 50x

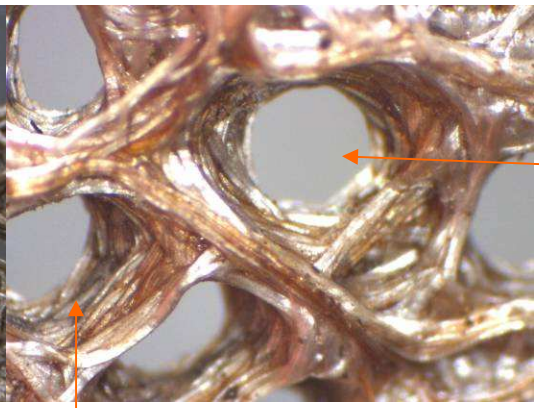


Plate 7.7b: *Argema mimosae* cocoons filament interwoven across the perforations, magnification: 500x

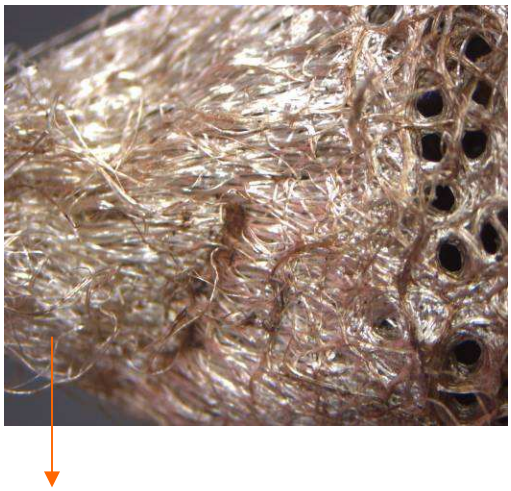


Plate 7.8a: A tip of the *A. mimosae* cocoon, Magnification: 15x

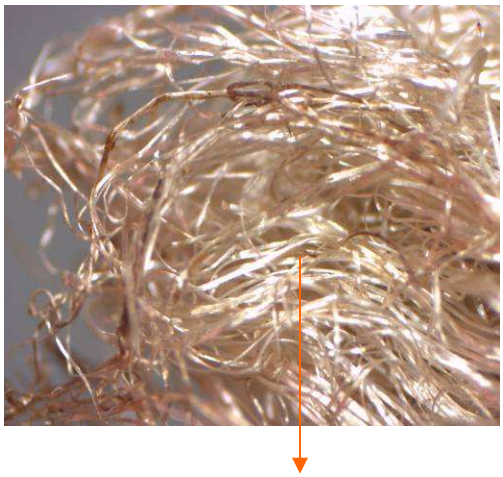


Plate 7.8b: Loose brush-like filaments at the tip of the *A. mimosae* cocoon, Magnification: 15x

The filaments at the main body of the cocoon are intact with scarcely distributed holes and are tightly interwoven (Plate 7.9).

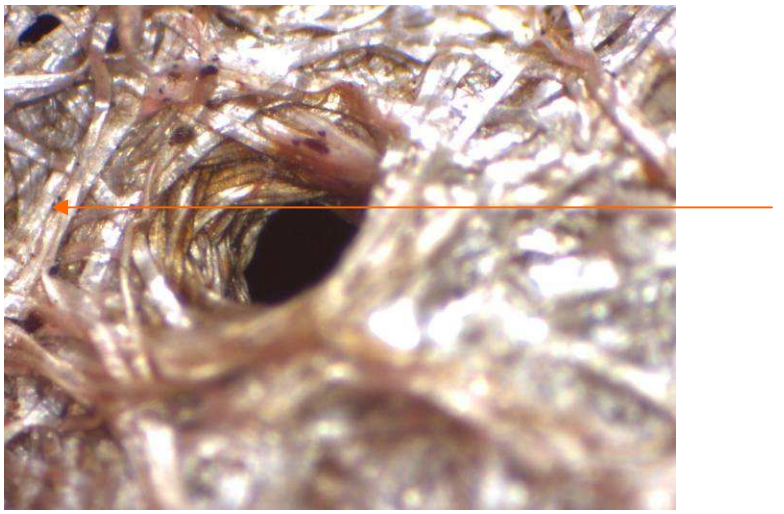


Plate 7.9: Tightly bound filaments at main body of the *A. mimosae* cocoon, magnification: 75x

7.4.2 Diameter of the cocoon and distribution of the holes

Plate 7.5 and 7.6 have shown some holes infront and at the end of the cocoon forming a ring like pattern. Holes at the middle formed irregular pattern while those at the bottom formed continuous lines along the attachment points of the cocoons to the branches of food plants. The total number of holes infront, middle, end and bottom sides differed significantly. The end side had the least mean number of holes with a mean of 28.79±1.45 followed by the middle side with an average of 32.49±3.75 holes (Table 7.1). The highest mean number of holes was recorded at the front side with a mean of 60.45±2.38 and at the bottom side with a mean of 44.03±2.85 holes. The total mean number of holes per cocoon was 170.89±8.16 with a significant minimum and maximum range of 6 and 556 holes respectively. The middle and bottom sides recorded a minimum of 0 holes and with a maximum of 234 holes, which was the highest mean number

recorded per side. This meant that some cocoons were intact at the middle and bottom sides with no perforations.

The tip with brush like filaments had a mean length of 0.94±0.02 cm. The weight and diameter of the cocoon shell at the front, mid, end and bottom sides had no significant linear correlation at prob>: r; under Ho:Rho=0 (Table 6.1). Further observation by Scanning Microscope measured the diameter of the silk fibre and the holes. The diameter of a single silk fibre and a hole ranged between 0.07 - 0.08 and 0.4 – 0.5 mm respectively (Plate 7.10 and 7.11).

Table 7.1: Mean of holes distribution on the surface of *A. mimosae* cocoons

Side	**No of holes**	**Diameter in cm**	**Weight in gms**	**R^2**
Front	60.45±2.38a	1.04±0.02a	-	0.37
Middle	32.49±3.75b	1.84±0.02b	-	0.136
End	28.79±1.45c	0.94±0.02c	-	0.068
Bottom	44.03±2.85d	-	-	-
Shell	-	-	0.72±0.013	-0.033

Means followed by the same letter in the same column are not significantly different (Tukey test =0.05**).**

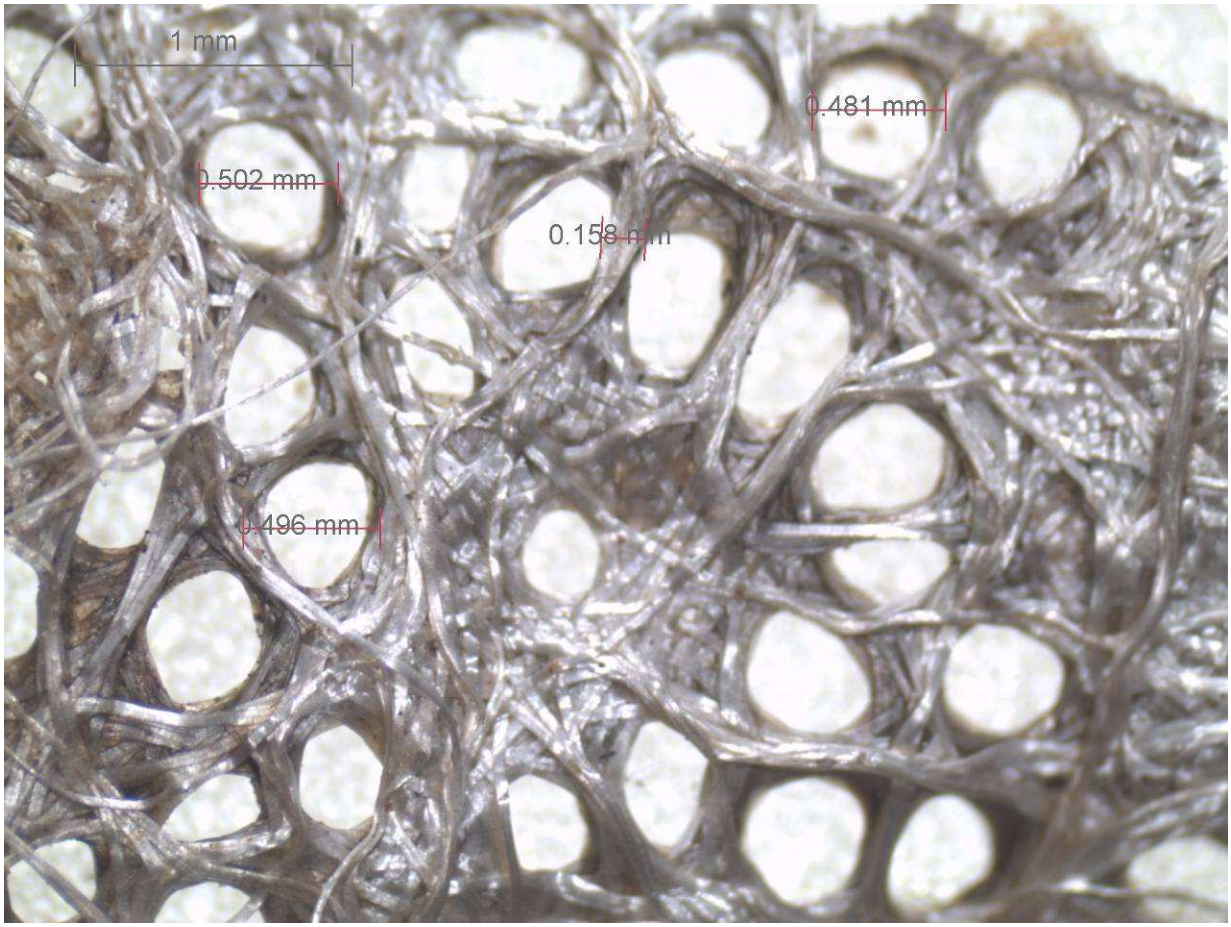


Plate 7.10: A cross section of *A. mimosae* cocoon showing diameter of the holes measured in millimeters, magnification: 350x

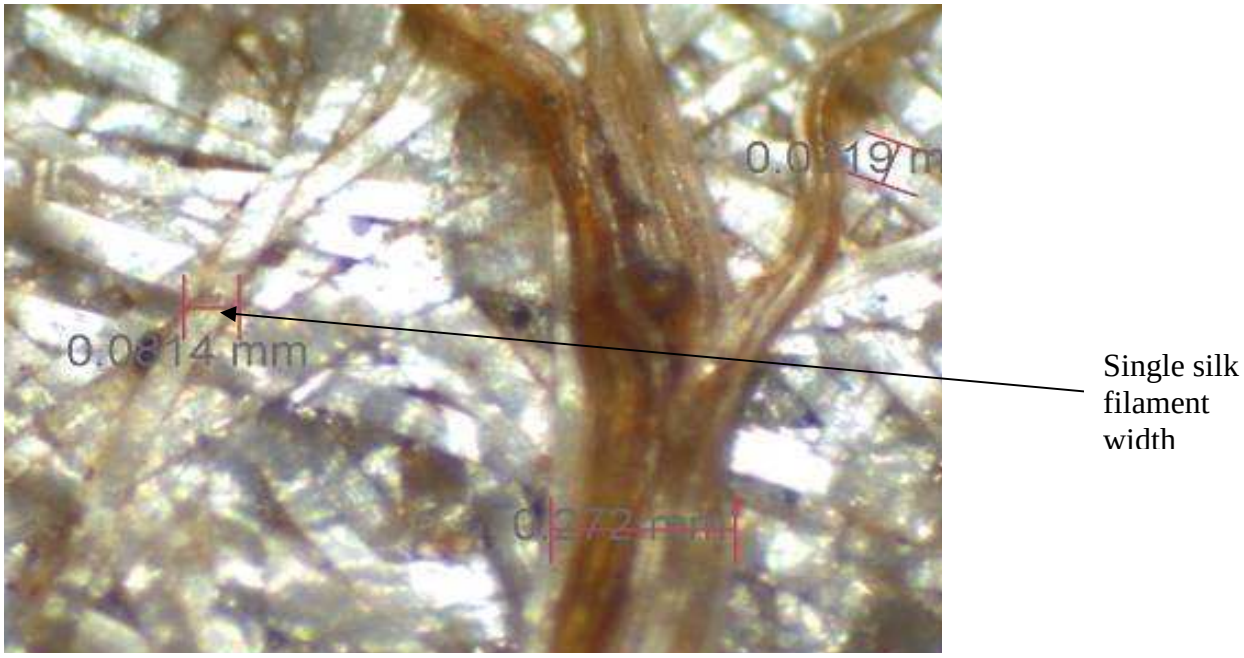


Plate 7.11: A cross section of *A. mimosae* cocoon showing silk filament width in Millimeters, Magnification: 350

7.4.3 Effect of boiling time on the amount of silk yarn produced from *A. mimosae* cocoons

The experiment was based on the number of cocoons which could make 50 grams. The mean number of cocoons which could make 50 grams ranged between 67±1.00 - 89±2.31 and 170±5.57 - 200±3.93 for *A. mimosae* and *B. mori* respectively. After boiling the *A. mimosae* cocoons, silvery brown floss was extracted and spun into silk fibre. For the various treatments, 50 grams of *A. mimosae* cocoons produced significantly varying amounts of silk fibre (Table 6.2). The maximum silk yield of 35.28±2.40 gm recorded in the old cocoons in 120 minutes boiling time was not significantly different from that of the fresh cocoons at the same time. The minimum silk weight (yield) of 7.0±1.22 gm recorded in the old cocoons in 60 minutes boiling time was significantly different

compared to 16.87±0.69 gm which was obtained from the fresh cocoons at the same time (Table 7.2). Boiling time in sodium carbonate solution had no significant effect on the weight of *B. mori* cocoons. The *A. mimosae* silk yarn weight increased with the increase in boiling time while the weight of silk yarn from *B. mori* cocoons decreased with the increase of boiling time (Figure 7.1). The cocoons of *A. mimosae* boiled in distilled water were not deflossable and did not produce yarn regardless of the boiling time. *B. mori* which was used for comparison in the experiment produced relatively low yield when boiled in distilled water. The yield by *B. mori* cocoons boiled in distilled water was not significantly different in 60 and 90 minutes but the difference was significant in 120 minutes (Table 7.2)**.** There was a high significant difference for the *B. mori* cocoons boiled in sodium carbonate solution and those which were boiled in distilled water. Fresh and old *A. mimosae* cocoons boiled in distilled water did not produce floss yield for comparison.

Table 7.2: Mean weight of silk yarn produced from 50grams of cocoons boiled at different time intervals

Cocoons	Boiling time in minutes	Mean number of cocoons		Mean Yarn weight (g)	
		0.5 g of Naco3	Distilled water	0.5 g of Naco3	Distilled water
	120	77.67±2.19b	75.33±0.67a	33.44±2.23a	0
A. mimosae	90	80.33±1.2a	73.67±4.33a	28.89±1.06a	0
Fresh cocoons	60	81±3.21a	73.67±1.2a	16.87±0.69b	0
	120	89±2.31a	86±3.79a	35.28±2.40a	0
A. mimosae	90	70±0.58b	67±1.01b	19.13±3.69b	0
Old Cocoons	60	68.67±4.18b	74±8.08b	7.0±1.22c	0
	120	181.67±1.20a	175±4.51a	32.27±3.24a	15.35±3.88a
B. mori	90	200±3.93b	170±5.57a	34.22±.08a	2.8±0.98b
Cocoons	60	178.33±3.76a	171.33±3.71a	35.95±0.04a	2.7±0.16b

Means followed by the same letter in the same column are not significantly different (Tukey test = 0.05**).**

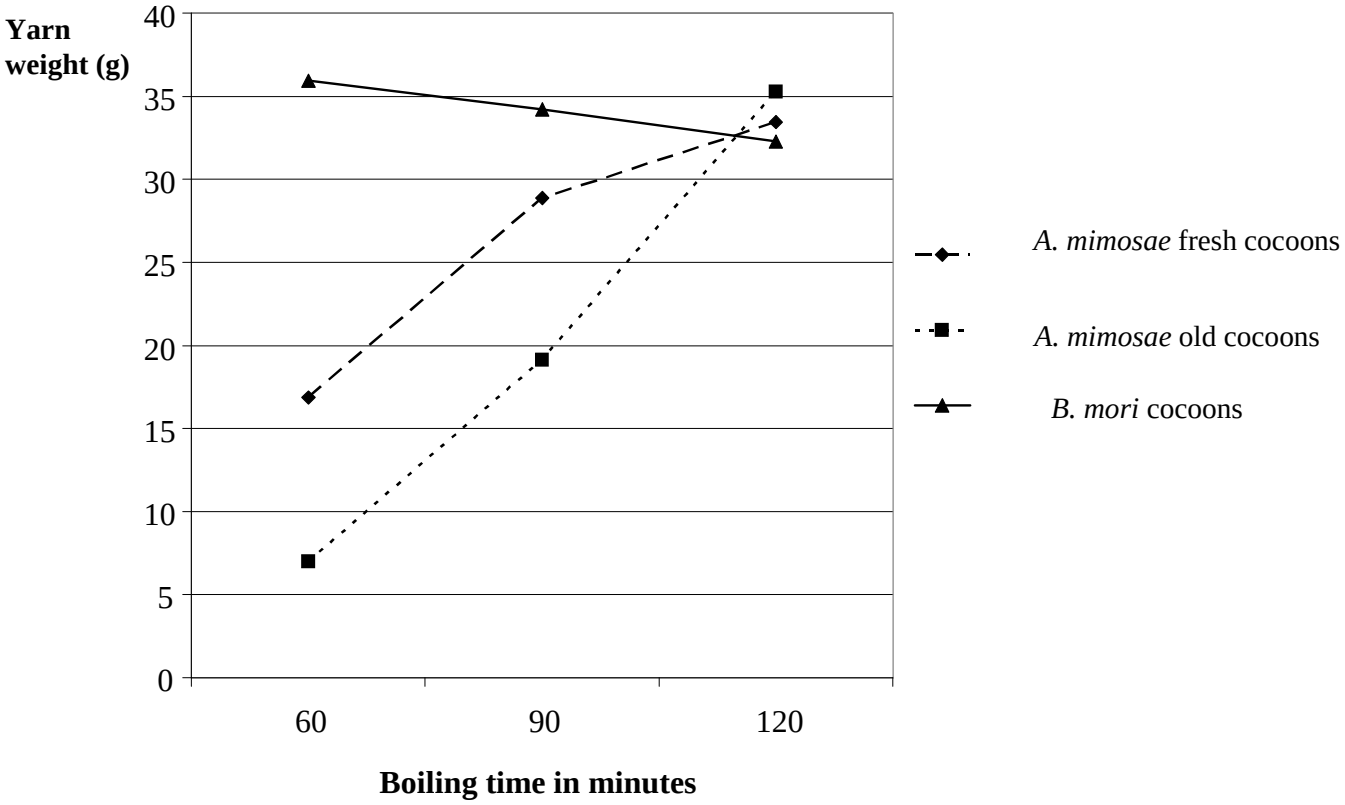


Figure 7.1: Comparison of silk yarn produced by fresh and old cocoons of *Argema mimosae* in different time intervals

7.4.4 The linear (Lin) and Quadratic effects of time on yarn weight and their interaction with cocoons

The cocoons of *A. mimosae* boiled in distilled water were not deflossable and therefore no silk fibre was produced even at the longest boiling time of 120 minutes. However, for the cocoons boiled in the sodium carbonate solution of 5gms/litre, boiling time affected the amount of silk fibre produced as evidenced by the significance of the boiling time in the model; $F_{1,21} = 66.49$, $p < 0.001$. The interaction between boiling time and the cocoon type was also highly significant; $F_{2,21} = 30.75$, $p < 0.001$. Indeed yarn weight increased linearly with the increasing boiling time for the old and fresh *A. mimosae* cocoons where as the yarn weight of the *B. mori* cocoons decreased with the increasing boiling time, though the slope of the line was not significantly different from zero (Figure 7.2). The estimated parameters of the fitted models are shown in table 7.3.

Table 7.3 Linear regression parameter estimates for the regression of yarn weight against the boiling time for the different cocoon types

Parameter	***Estimate***	***Standard error***
Intercept	39.66	4.532
Time	-0.06	0.049
B. mori	Reference	
Fresh cocoon	-38.12	6.409
Old cocoon	-61.61	6.409
Time: *B. mori*	Reference	
Time:New cocoon	0.34	0.069
Time: New cocoon	0.34	0.069

Using the estimates in table 7.2, the relationship between boiling time and the weight of yarn for different types of cocoons was as follows:

Yarn weight = 39.66 – 0.06 (Boiling time)	*B. mori*
Yarn weight = 1.54 + 0.28 (Boiling time)	Fresh cocoon
Yarn weight = -21.95 + 0.47 (Boiling time)	Old cocoon

The fresh cocoons yielded higher weights than the old cocoons below boiling time of 120 minutes. However, the increase in yarn weight per unit increase in time was higher for the old cocoons (slope of line = 0.47) than for the fresh ones (slope of line = 0.28). It is shown that the old and fresh cocoons could produce approximately the same amount of yarn (36g) when the cocoons are boiled for over 120 minutes (Figure 7.2).

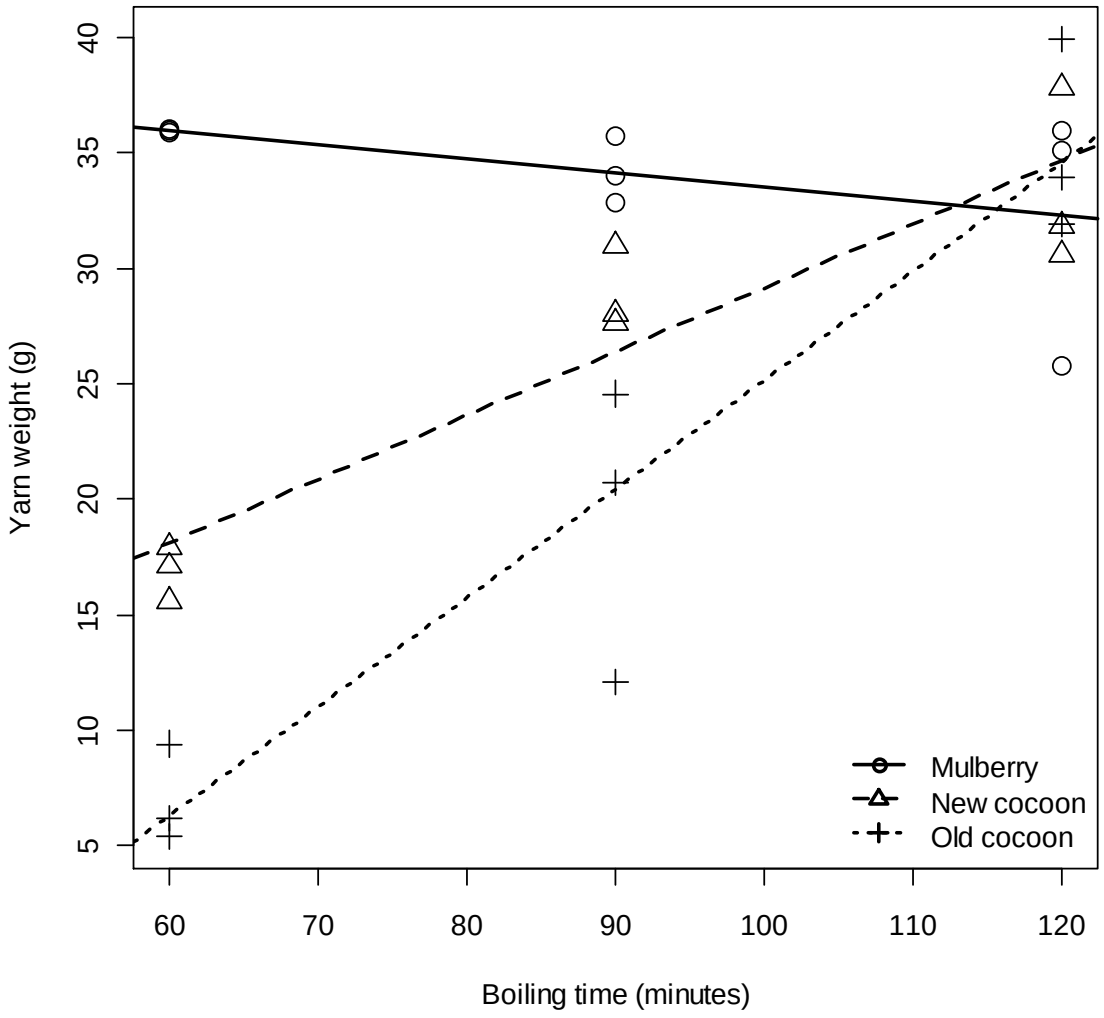


Figure 7.2: Observed relationship and fitted regression lines for the relationship between yarn weight and boiling time for the three cocoon types

7.4.5 Elongation of *Argema mimosae* silk

The length at which the *A.mimosae* silk fibre could extend before breaking differed significantly with the boiling time. The silk fibre from fresh *A. mimosae* cocoons had higher elongation of 7.53±0.62 mm as the marginal mean which was significantly different compared to 5.67±0.41 and 5.77±0.37 marginal means of the old *A. mimosae* and *B. mori* cocoons respectively. The boiling time had a significant effect on the silk fibre from fresh *A. mimosae* cocoons with a higher mean of 9.8±0.9 mm recorded in the 60 minutes and the extension mean length reduced with the increase in the boiling time (Table 7.4). Fibre of the fresh *A. mimosae* cocoons had weak elongation in the 120 minutes. Silk fibre from the old *A. mimosae* cocoons showed no significant difference in elongation when compared to those of the *B. mori* in the different boiling intervals. Silk fibre from the *B. mori* cocoons had no significant difference in elongation in the different boiling time intervals. The results of *B. mori* and old *A. mimosae* cocoons compared well and there were no significant difference between the two types in all the boiling time intervals. In addition, the boiling time did not significantly affect their elongation performance (Table 7.4). It was also noted that all cocoons types recorded higher means of elongation in the 60 minutes boiling time and the lowest mean of 4.6±0.42 mm was recorded in the 90 minutes duration for the old *A. mimosae* cocoons. The boiling time had no significant difference in the old *A. mimosae.* Generally, the marginal means indicated that elongation reduced with increase in boiling time with a significant difference of fresh cocoons when compared with the old ones and those of the *B. mori*. However, elongation was almost the same when the boiling time was increased to 120 minutes (Figure 7.3).

Table 7.4: Elongation of fresh and old *A. mimosae* and *B. mori* cocoons

Cocoon type	**Elongation in mm**			**Marginal means**
Minutes				
	60	90	120	
Fresh *A. mimosae*	9.8±1.09aA	6.6±0.58aB	5.9±0.82aB	**7.43±0.57a**
Alpa, power, Lsn	*0.05, 0.834, 21*			
Old *A. mimosae*	6.3±0.96bA	4.6±0.42bA	5.8±0.61aA	**5.67±0.41b**
Alpa, power, Lsn	*0.05, 0.302, 65*			
B. mori	6.5±0.85abA	5.9±0.48abA	4.9±0.5aA	**5.77±0.37b**
Alpa, power, Lsn	*0.05, 0.313, 63*			
Marginal means	**7.53±0.62A**	**5.7±0.31B**	**5.53±0.37B**	

Means followed by the same, lower case letter in the same column and upper case in the same row are not significantly different (Tukey test=0.05**),** LSN: Least Significant Number

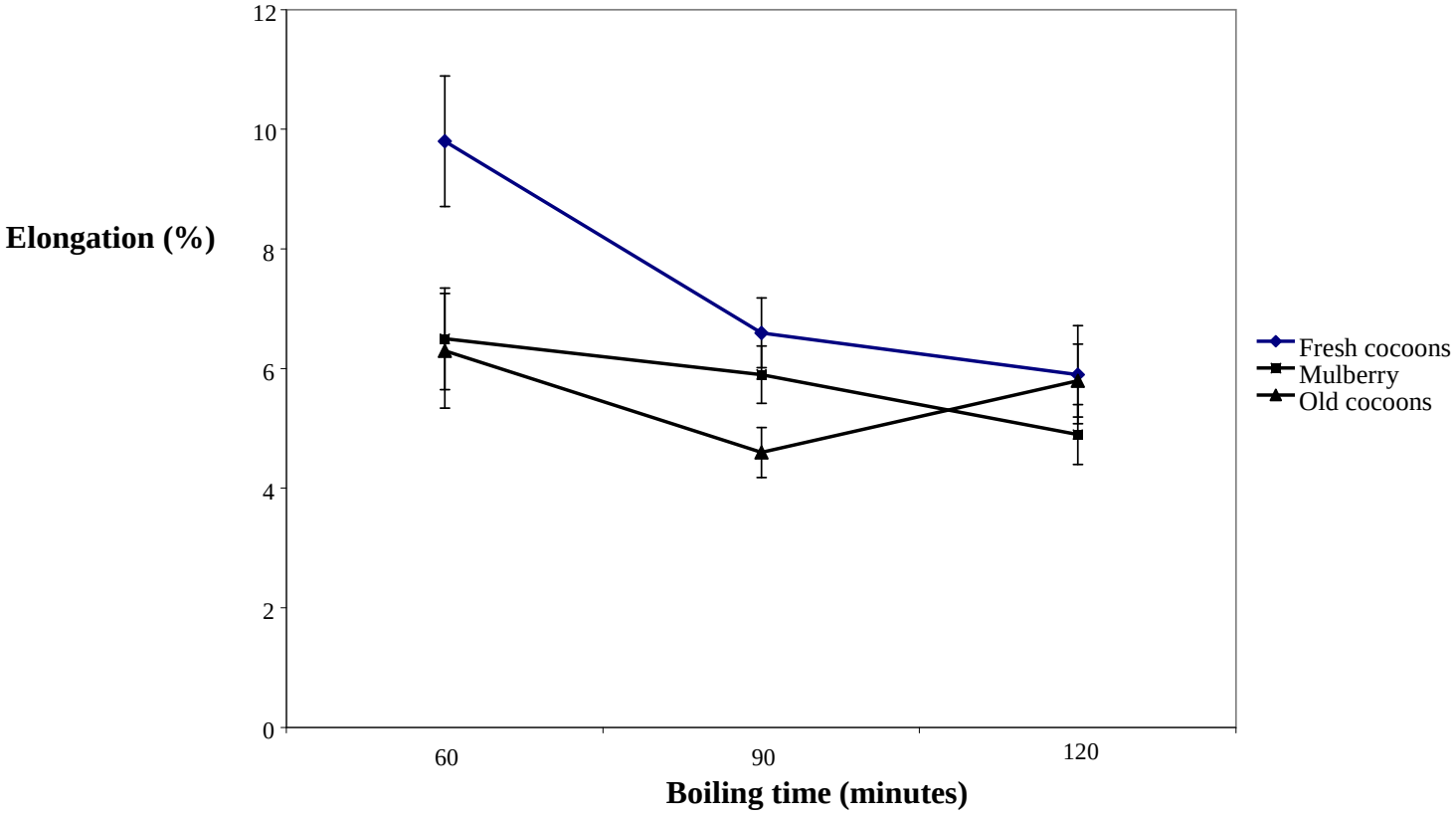


Figure 7.3. Effect of boiling time on elongation in fresh and old A. mimosae cocoons compared with those of *B. mori*

7.4.6 Breaking load or tenacity of *Argema mimosae* silk

Silk fibre from the cocoons boiled for 60 minutes had the highest mean breaking force of 147.93±6.2 N. The breaking load of fresh and old *A. mimosae* cocoons had no significant difference but was significantly different with those of the *B. mori* cocoons (Table 7.5). The *B. mori* silk fibre required a higher mean breaking load of 160.83±6.6 N compared to 117.3±0.57 and 117.88±4.43 of fresh and old *A. mimosae* cocoons respectively. The breaking load of silk fibre from the old *A. mimosae* cocoons in 60, 90 and 120 minutes of boiling time was significantly different. The boiling time had no significant difference on the breaking load of silk fibre from the *B. mori* cocoons (Table 7.5).

Table 7.5: Breaking load of fresh and old *A. mimosae* and *B. mori* cocoons

Cocoon		**Breaking load (Force in Newton)**		**Marginal means**
Minutes				
	60	90	120	
Fresh *A. mimosae*	88.2±2.74aA	125.3±5.52aB	138.4±6.53aB	**117.3±0.57a**
Alpa, power, Lsn	*0.05, 0.999, 9*			
Old *A.* ***mimosae***	92.83±1.62aA	117.3±3.97aB	143.5±5.4aC	**117.88±4.43a**
Alpa, power, Lsn	*0.05, 0.999, 8*			
B. mori	155.8±10.4bA	164.8±7.08bA	161.9±16.33aA	**160.83±6.67b**
Alpa, power, Lsn	*0.05, 071, 646*			
Marginal means	**147.93±6.2A**	**135.8±4.9A**	**112.28±6.71B**	

Means followed by the same letter in the same row (upper case) and column (lower case) are not significantly different (Tukey test=0.05**),** LSN: Least Significant Number

Tenacity of the fresh and old cocoons increased linearly with the boiling the time as evidenced by the significance of the boiling time in the regression model, $F_{1,26} = 61.36$; $p<0.001$. However, the linear increase was not significantly different between the two types of cocoons, $F_{1,26} = 0.58$; $p = 0.453$ and so a common linear regression line was fitted to the data (Figure 7.4).

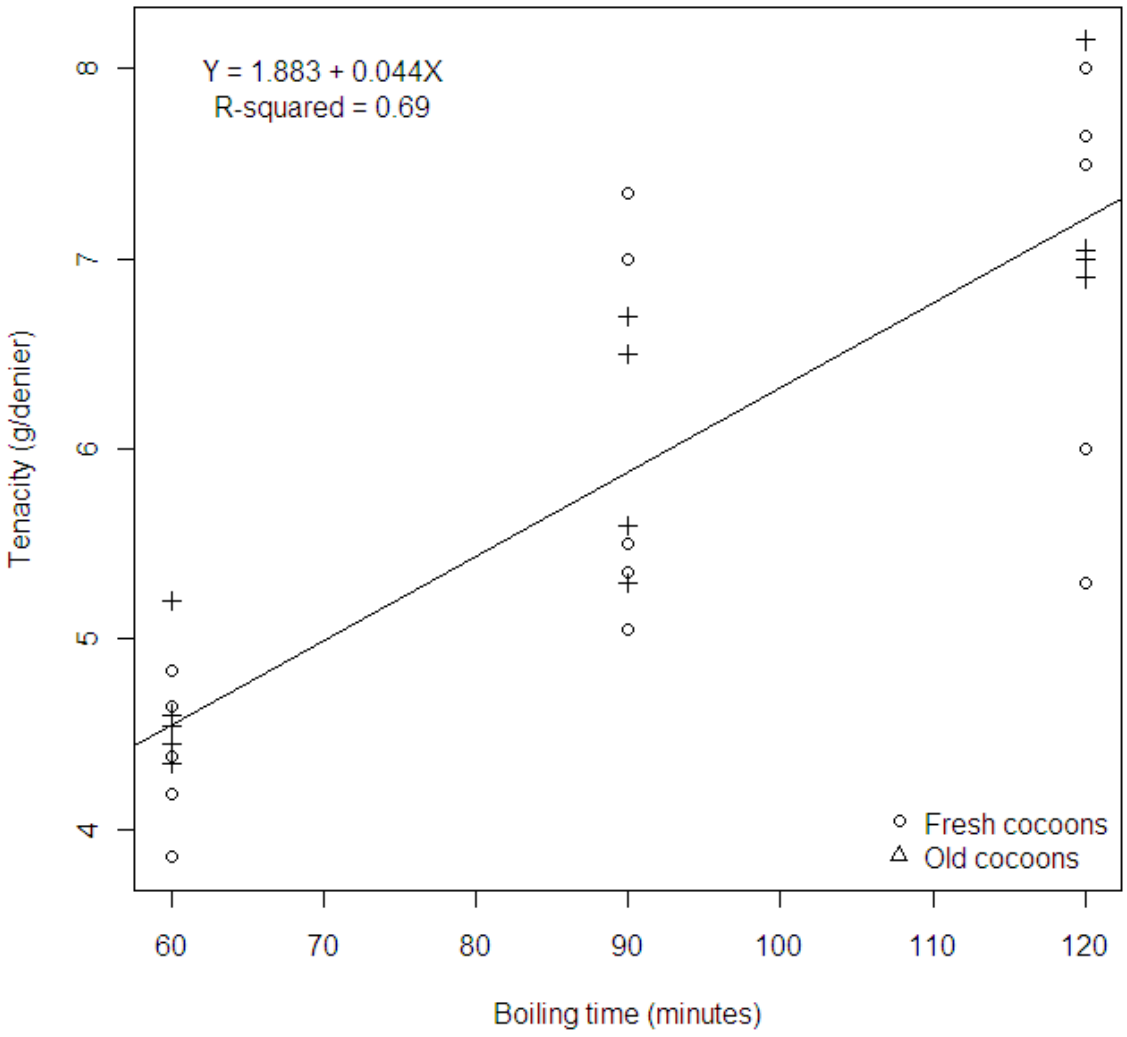


Figure 7.4 Effect of boiling time on elongation in fresh and old *A.* mimosae cocoons

7.5.6 Silk fibre and fabric

The study showed that a mean weight of 13.35±2.78 grams of silk fibre produced a weft of 3.53±0.77 inches of fabric on *B. mori* warp. A silk fibre of 28.3 inches was woven from a silk fibre weight of 106.8 grams (Figure 7.5 and Plate 7.8).

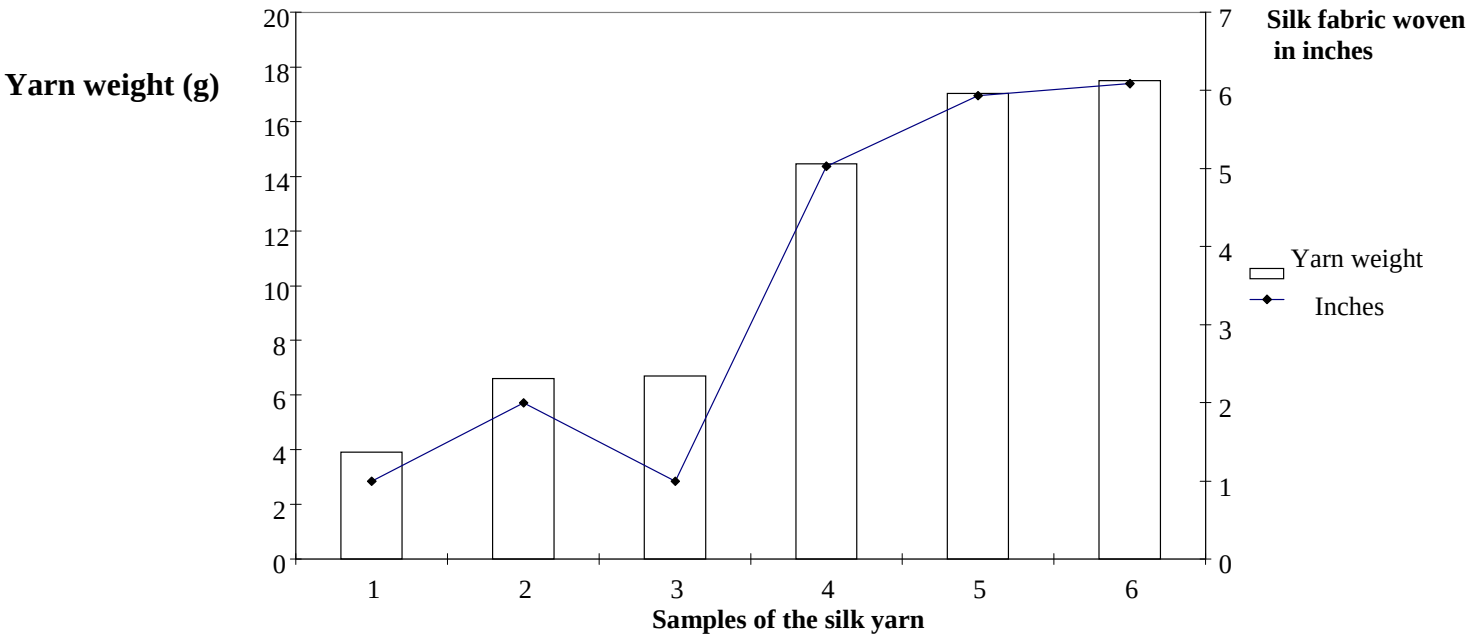


Figure 7.5: Inches of silk fabric against the weight of silk yarn produced from *A. mimosae* cocoons

Plate 7.12: *Argema mimosae* fabric on weaving power loom

7.5 Discussion

The steadily growing demand for silk in the silk consuming countries provides an excellent opportunity for any exporting country to increase its production. To secure this opportunity, it is important to diversify the domesticated *B. mori* silk and wild natural silk producing opportunities like utilizing *A. mimosae* cocoons. In addition, for the success of silk production initiative, there is need to focus on the process of silk production, processing and the quality of the products. The production of raw silk fibre from cocoons is a crucial middle stage in the silk industry. An earlier examination of *A. mimosae* cocoons by researchers indicated that the cocoons could not be reeled but could be spun (Raina 2000; Kioko, 1998, Kioko *et al*., 2007).

The results on the physical characteristics of *A. mimosae* cocoons revealed that there were natural perforations observed, made when larvae are pupating and are not associated with parasitoids (Kioko *et al*., 2000b; Akai and Nagashima, 2002; Veldtman *et al*., 2004; Fening *et al*., 2008a). The perforations on the surface of the cocoons shells penetrate into the cocoon and are probably for ventilation during the development of the pupa. This is evident from the observation that the holes made do not show or suggest any discontinuity in the silk filament. The holes are randomly distributed on the main body of the shell and uniquely form a ring at the open front tip and at the rear end. The front tip had a peculiar opening valve through which the moth emerged. After dissection of the cocoons, it was observed that the pupa lied inside the shell with the mouth parts facing the valve surrounded by the loose filaments. This proved the importance of this opening, which is left when the larva is spinning the cocoon.

Most of the spaces between the filaments in the cocoon shell are filled with sericin thus allowing no air spaces into the shell, having these unique holes in the cocoon shell might be useful during ventilation and allow the entry of fresh air from outside (Kioko, 1998; Akai and Nagashima, 2002). The mean number of peculiar holes observed in this study (170.89±8.16) conforms to the findings of Akai and Nagashima (2002), who reported a range of 100–200 holes in *A. mimosae* cocoons. The current study also revealed that, distribution and the number of holes did not depend on the size or the weight of the shell but they determined both the silk fibre quality and quantity (Raina, 2000).

Silk degumming is an essential process to obtain an ideal fiber for the textile industry. During the degumming process, sericin is hydrolyzed and the amide bonds of the long protein molecules are broken into smaller fractions, which are dispersed and solubilized in the degumming agents and media (Karmakar, 1999; Mbahin *et al*., 2008). *A. mimosae* cocoons probably have strong bonds thus explaining the reason why there was no floss obtained or the softening of cocoons after boiling them with plain water at different time intervals. This was evidenced by comparing its cocoons with those of *Bombxy mori* which produced considerable floss with the same treatment. From these results it can be concluded that cocoons can only be processed by using degumming agents such as sodium carbonate. After degumming, *A. mimosae* cocoons could not be reeled because the tips had opening with brush like filaments where moths emerge. Several filaments ended at the tip individually thus making the cocoon unreleeable. Degumming was done using sodium carbonate and produced silvery floss. The silk had brown silvery colour probably due to the amount of sericin which gave it a harsh and stiff feeling.

This study recorded the ideal time of boiling *A. mimosae* cocoons to be 120 minutes in sodium carbonate solution. Sixty minutes boiling time produced the amount of silk which could not be economically viable.

Silk fibre from the fresh cocoons indicated that it has a higher elongation than the fibre from the old cocoons. This showed that silk fibre from cocoons lost their elongation with time. The elongation was also affected by the increased boiling time because the study showed that the shorter the boiling time the higher the elongation.The breaking load was not affected by the age of the cocoons but the breaking load was affected by the boiling time. Generally, the results showed that the breaking load of silk fibre from all the cocoon types decreased with the increase of boiling time. From the results, it can therefore be deduced that boiling time had no significant difference in the breaking load between 60 and 90 minutes but there was a significant difference in the breaking load in the 120 minutes. Thus, it can be generalized that when the boiling time is increased, the elongation and the breaking force reduce. According to the International Silk Association (ISA) classification of silk category III (Appendix VII), silk fibre with tenacity or breaking load below 3.7 per gram and elongation below 18% is classified as grade B. Thus based on the result in this study *A. mimosae* silk fibre could be categorized in grade B.

The study has also shown that the processing of fresh cocoons would give better results than the old ones. The study provided basic estimates of the amount of silk which could be produced from 50grams of cocoons and this could be used to estimate the number of

cocoons required to produce one kilogram of silk yarn. If the average of 78 fresh cocoons can weigh 50grams, 1,560 grams would be required for 1 kilogram of cocoon. Since 13.35 grams of fibre can produce 3.53 inches of silk fabric, 78 cocoons can produce an average of 33.4 grams of silk fibre. This translates to 152. 52 grams of silk fibre required to produce 1 meter of the fabric, which is equivalent to 356 cocoons. This indicates that 1 kilogram of *A. mimosae* cocoons can produce 668 grams of silk fibre which can produce 4.4 metres of silk fabric.

Using Mwingi area as a case study Raina, (2000), Raina *et al*. (2009), showed that one tree with a canopy of 8 -10 cubic feet could support upto 200 larvae. One thousand trees each supporting 200 cocoons could therefore yield a total of 200,000 cocoons. Assuming a 50% survival rate, this translates to 100,000 cocoons per hectare, which can produce 280 meters of fabric silk. The latter is estimated to produce an income of USD7, 560 at a price of USD27. The results obtained from this study did provide economic baseline information that could help farmers to venture into wild silk farming.The (E)-β-farnesene, (E)-β-Caryophyllene, (E)-β- ocimene and linalool volatiles which were constantly released by the *L. schweinfurthii* and *O. obovata* tree species are recommended for bioassays to establish their role in wild silkworms predator and parasitoids attack.

CHAPTER EIGHT

8 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 General discussion

Climate change is having a significant effect on land-use practices, reducing the viability of vast areas of the African continent for the provision of food and other important livelihood resources. Deepening drought cycles lead to food insecurity. Climate changes, together with increasing population pressure, have already made significant negative impact on the health of land and natural resources. In Kenya, this has been accelerated by deforestation and unsustainable use of forest resources especially by people living around forests (Raina *et al*., 2009). Kenya's loss of forest cover and associated biodiversity, have led to serious environmental deterioration and consequent decreased food production and increase in rural poverty. Closed forests canopy cover, less than 2% of the total land area, yet every year forests in Kenya are under increasing pressure from encroachment, burning and overuse. Thousands of people live within five kilometers of forest boundaries in Kenya and benefit from a whole range of goods and services from the forest (Gordon and Ayiemba, 2003). The forest reserves have always been most vulnerable to encroachment, overuse and degazettement due to growing demand for agricultural land and fuel-wood resources, which override perceived benefits from the forest.

Arabuko Sokoke forest reserve has not been an exception despite being one of the world biodiversity hot spot zones along the East Africa coastal strip (Collar and Stuart, 1988).

The current study focused on addressing some of these challenges through the use of commercial insects such as the African wild silkmoths, *A. mimosae* and community participation in utilizing the forest resources sustainably and in conserving the biodiversity. The identification of plants that were fed on by *A. mimosae* larvae viz *L. schweinfurthii*, *O. obovata* and their distribution in farmland and in the forest provides important basic information useful to plan in establishing wild silkmoth farming. Several factors could have contributed to the low numbers of food plants both in the farm land and also in the forest. The trees are preferred for other purposes like charcoal burning and construction poles. To improve on the conservation of these wild silkmoth food plants, it calls for economic incentives such as wild silkmoth farming that integrates biodiversity conservation with livelihood augmentation. This would enhance community participation in conserving the food plants through several ways which include; establishment of seedling nurseries, planting more trees in their farms and in the forest. Such kind of voluntary changes in behaviour towards insect utilization with a link to forest conservation has been previously reported in the forest (Gordon and Ayiemba, 2003) where people leaving adjacent to the forest have been rearing and exporting live butterflies pupa through Kipepeo project. In Malawi local people have been given the right to harvest saturniid caterpillars for food and sale and establish beehives in exchange for curbing other uses which are incompatible with the objectives of the Kasungu National Park (Martin, 1984). Initiating wild silkmoth farming would support and enhance the current Kenya forest Act which requires community participation through Participatory Forest Management (PFM). The Act requires that, Kenya Forest Services (KFS) should actively involve communities living adjacent to the forest boundaries

through Community Forest Associations (CFAs) in forest sustainable conservation matters. This has been demonstrated by the *icipe* Commercial Insects Programme which has initiated utilization of bees and wild silkmoths as incentives to communities around the forest boundaries to participate in forest conservation (Raina *et al*., 2009).

A survey in East Africa on wild silkmoths and their host plants conducted by Kioko *et al*. (2000a), reported *Spirostachys venenifera, L. schweinfurthii* and *Sclerocarya birrea* as the food plants for *A. mimosae.* The current study has identified *O. obovata* as additional food plants found in the Arabuko Sokoke forest mostly occurring in the farm land but had sparse distribution in the forest site. This food plants provide a wider food choice for the *A. mimosae* caterpillars and to the wild silk farmers for mass rearing of the silk worms. *Argema mimosae* larvae reared on the different food plants did not show any significant difference in terms of developmental days. These shows that *L. schweinfurthii* and *O. obovata* plants were equally prefered by the wild silkworm larvae. The larval stage was completed in 28 – 30 days which is comparable to domesticated to that of the silkworm *B. mori.* Other wild silkmoth species in Kenya have been reported to take more days. For instance, *Gonometa postica* takes 45 - 60 days (Kioko, 1998; Ngoka *et al*., 2008) while *Anaphe panda* takes 80 – 120 days (Mbahin, 2008).When the larval developmental period is shorter for *A. mimosae*, it is advantageous to the wild silk farmers because they would make income in a short period and save on labour and time taken.

Cryptic colour defense mechanisms were prominently exhibited by *A. mimosae* larval instars. The larvae colour progressively changed from black in the first instar, orange green in the second instar, green in the third instar with orange tubercles and then green with green tubercles in the fourth, fifth and sixth instar to match the colour of food plant leaves and the bark. In his review of the caterpillar defense mechanisms, Lederhouse (1990) found that among North America saturniids, the most common primary defense mechanism was inconspicuousness aided by cryptic colouration and reduced movement. In another group of Lepidoptera, the sphingids, Schmidt (1990) also observed cryptic colouration of the larvae which was combined with a general tendency to remain relatively sedentary as means of escape from predation. Tuskes *et al*. (1996) observed that cryptic larvae, such as *Hyalophora* and *Callosamia* species (Saturniidae) had brightly coloured scoli (tubercles) though the adaptive significance of these structures have not been systematically studied. Similarly, it was observed in *A. mimosae* the scoli usually became smaller relative to the body size as the larvae changed from one larval instar to the next.

One remarkable property of silk is its high tensile strength and its fibres that can be easily torn or damaged (Ballenberger, 2007). It also has natural texture and fineness; and can be stretched and will recover to its original size unless stretched beyond 20-25% of its original length. Almost as strong as cotton, it is more elastic than either cotton or linen, and has been used in the past in making ropes to take advantage of this characteristic (Ballenberger, 2007). In the current study, the fresh cocoons had more desirable qualities of yield and elongation compared to the old cocoons. To produce high quality silk,

farmers should be advised not to store cocoons for more than two years. Old *A. mimosae* cocoons which were used in this study had been stored for more than two years, while the fresh ones were less than a year old.

In this study very few volatile compounds were trapped from the *A. mimosae* larvae compared to the food plants. The *A. mimosae* larvae which fed on *O. obovata* leaves emitted only three compounds namely; cedrene, curcumene and funebrene compared to the ten sesquiterpenes and eleven monoterpenes which were reseased by the food plant leaves. Those which were fed on *L. schweinfurthii* emitted the hydrocarbons Hexacosane, Nonadecane, Octadecane, Octacosane and Eicosane compared to the fifteen sesquiterpenes and eight monoterpenes which were emitted by the food plant leaves.

It is expected that the larvae emitted very few odors compared to the plants that can be detected by their natural enemies. On the other hand, the food plants under attack by the *A. mimosae* larvae could have benefited from the presence of the natural enemies which attack and kill the larvae and reduce herbivory. The food plants could have developed some means of chemical signals that could have revealed the presence of the *A. mimosae* larvae. Studies have shown that plants have the ability to respond to herbivore attack by emitting blends of volatiles that are detected and exploited by parasitoids for host location (Turlings *et al*., 1995).

This study revealed terpenes that were released by the wild silkmoth food plants *L. schweinfurthii* and *O. obovata.* Terpenes have many useful applications and have a broad variety of roles within the plant kingdom. They are common components of flavorings and aromas, and can be effective pharmaceuticals (Goff and Klee, 2006). In the plants,

they function in biotic interactions as defensive compounds against herbivores and pathogens (Wittstock and Gershenzon, 2002), and even as attractants to pollinators (Pichersky and Gang, 2000). Volatile terpenes are well-established as acting in indirect defenses as attractants of parasitoids or predators of herbivorous arthropods which feed on many plant species. In corn, volatile terpenes that attract female parasitoid wasps are emitted after the feeding by the beet armyworm, *Spodoptera exigua* or exposure to the insect oral secretions (Turlings *et al*., 1990).

The released volatile blends can be specific to the feeding herbivore and to the damaged plant species (De Moraes *et al*., 1998; Leitner, 2005; Walling, 2000**)**. Furthermore, volatile profiles can vary depending on the developmental stage of a single herbivore species (Takabayashi *et al.,* 1995). Parasitoid wasps are able to distinguish between volatile blends from plants which are damaged by host herbivores or by non-hosts, thus demonstrating that volatile signals are produced with such specificity that they allow parasitoids to attack the preferred prey (De Moraes *et al*., 1998). Emission of odors is well timed; odors are emitted shortly after herbivores start damaging the plants and are mainly produced during daytime when most parasitoids forage.

8.2 Conclusions

1. The study identified *Lannea schweinfurthii*, *Ozoroa obovata*, *Sclerocarya birrea* as common food plants of the African wild silkmoth *Argema mimosae* in Arabuko sokoke forest and its surrounding farmlands. The food plant abundance was

siginificantly different between the forest and the farmland with the farmland having more of *O.obovata* and *lannea schweinfurthii*.

2. The study revealed *O. obovata* for the first time in East Africa as food plant for *A. mimosae* larvae. *Ozoroa obovata* was more abundant in the farmland compared to the forest. On the other hand, *L. schweinfurthii* was more preferred by *A. mimosae* larvae because most of its cocoons were found on it.
3. *Argema mimosae* had a mean developmental larval period of 29.5 days when larvae were reared on *L. schweinfurthii* and *O. obovata* and was not significantly different between the two food plants. The larvae expressed cryptic colouration of black, orange and green as they passed through the six larvae instars.
4. The *A. mimosae* pupae were enclosed in silvery cocoons with unique peculiar perforations and brush-like openings at the front tip where the moths emerged.The cocoon filaments were interwoven across the peculiar perforations.
5. This study illustrated that *A. mimosae* cocoons could be degummed using sodium carbonate in order to produce natural silk fibre. Fresh *A. mimosae* cocoons produced relatively more silk fibre than the old cocoons at the different boiling time intervals.
6. This study has identified important volatiles in host-parasitoid attraction relationship viz **(**E)-β-farnesene, (E)-β-Caryophyllene, (E)-β- ocimene and linalool. The study has also reported a sesquiterpene (E)-(b)-farnesene as one of the potential defensive volatile released against herbivory on *L. schweinfurthii* and *O. obovata* leaves by *A. mimosae* larvae.

8.3 Recommendations

1. The study recommends the planting of the identified wild silkmoth food plants which could serve as a key component in establishing wild silkmoth farming activities. Other than providing food for the wild silkworms, the trees would have other benefits such as source of wood fuel, construction poles, soil erosion control, and protection of water catchment areas and improve biodiversity conservation in general.
2. The study recommends training farmers on the rearing techniques of *A. mimosae* larvae and silk fibre extraction from the cocoons for income generation. It is important to note that for wild silk farming to be sustainable, farmers should be encouraged to do mass rearing as opposed to wild harvesting which depletes the population.

8.4 Future research

1. There is need for further study on the growth and development of *A. mimosae* reared on leaves of different food plants for the comparison with the results of this study.
2. There is need for an investigation study to reveal the specific role played by the volatiles which have been reported in this study viz; (E)-β-farnesene, (E)-β-Caryophyllene, (E)-β- ocimene and linalool in host-parasitoid relationship.
3. Since parasitoids and predators for *A. mimosae* and other wild silkmoths have been identified in this study and other previous studies, biossays are necessary in future research to develop possible control measures of the parasitoids using

volatiles as traps or repellents for the parasitoids as shown in the conceptual frame work (Figure 8.1).

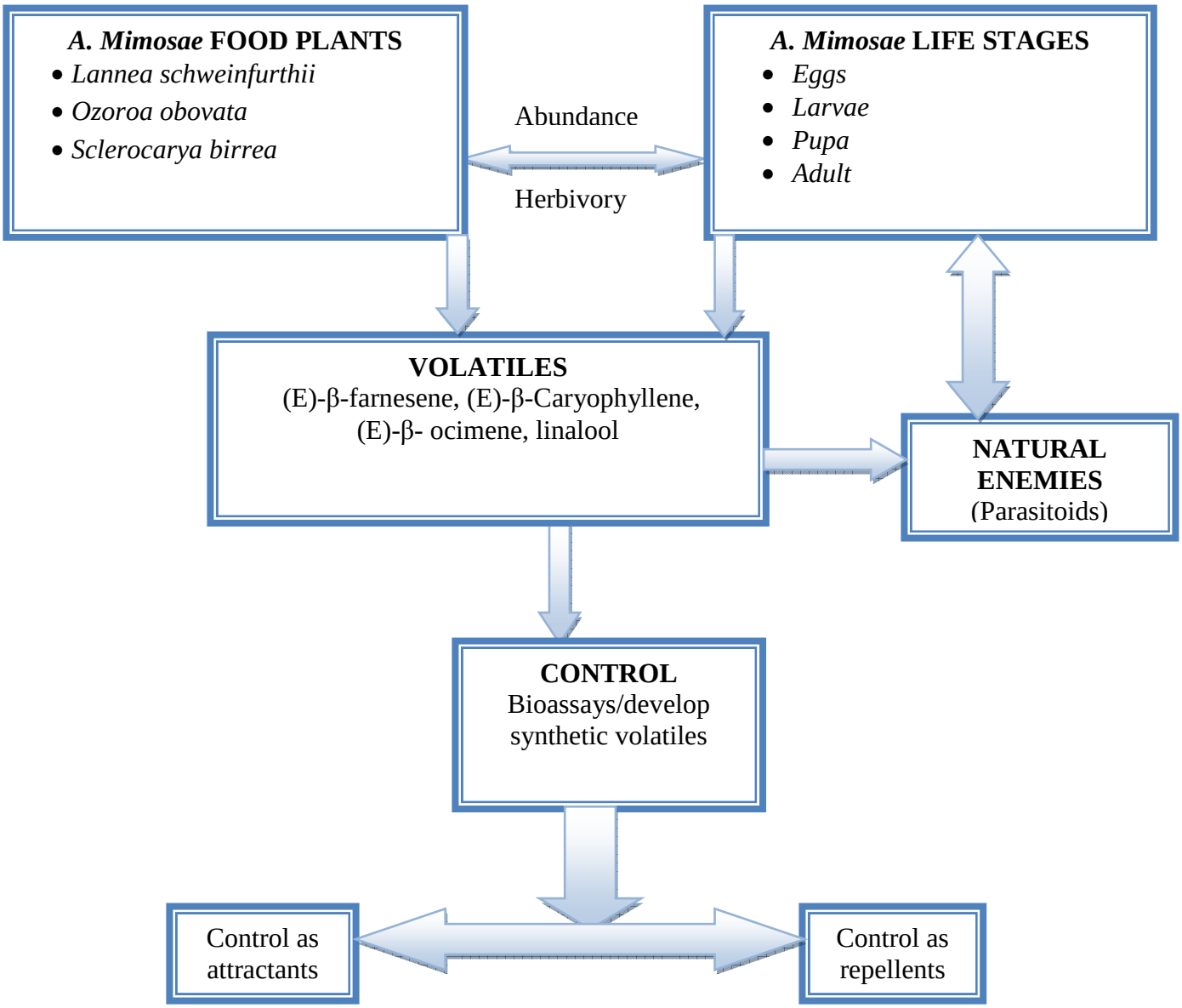


Figure 8.1 Conceptual conclusion

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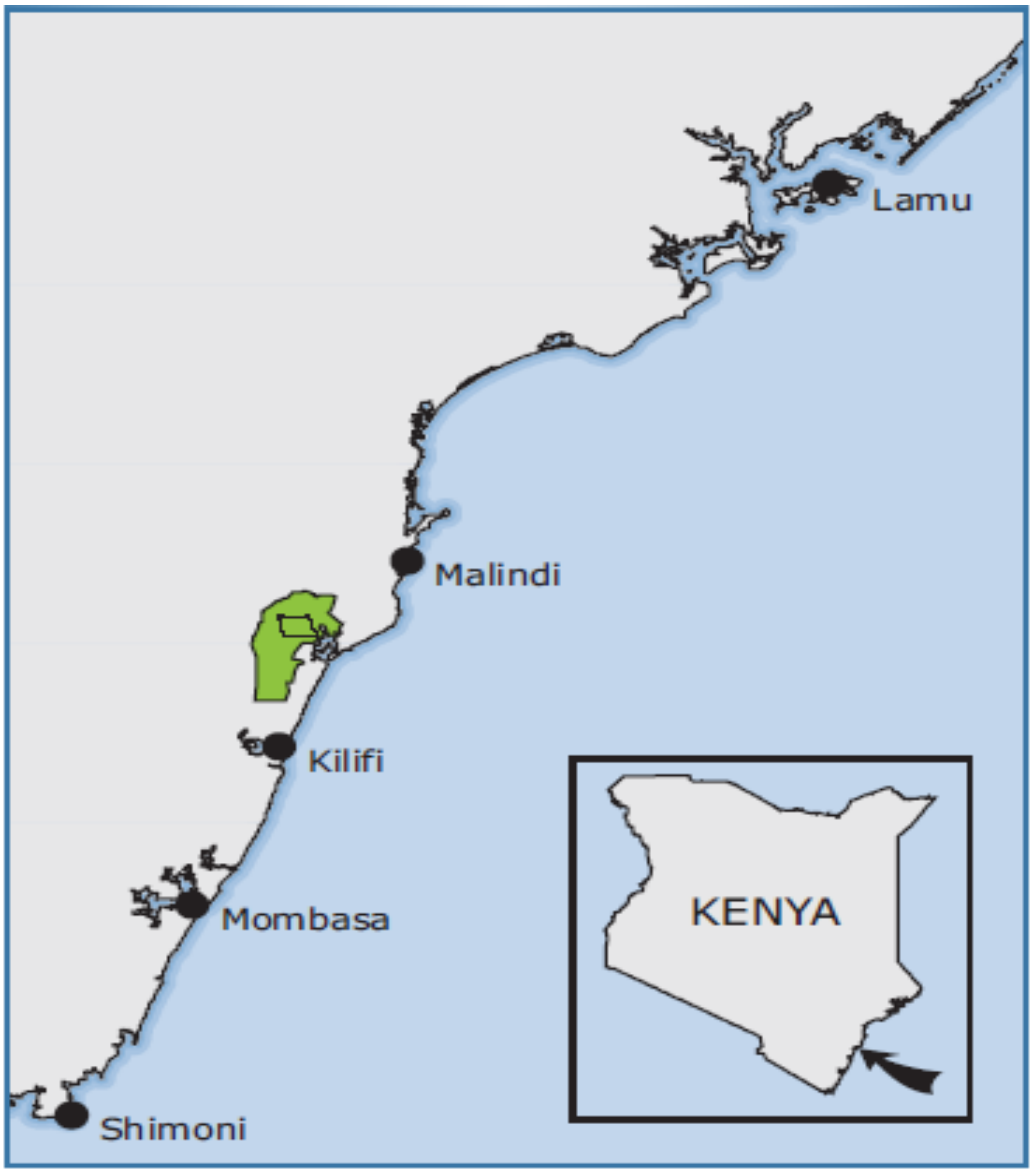
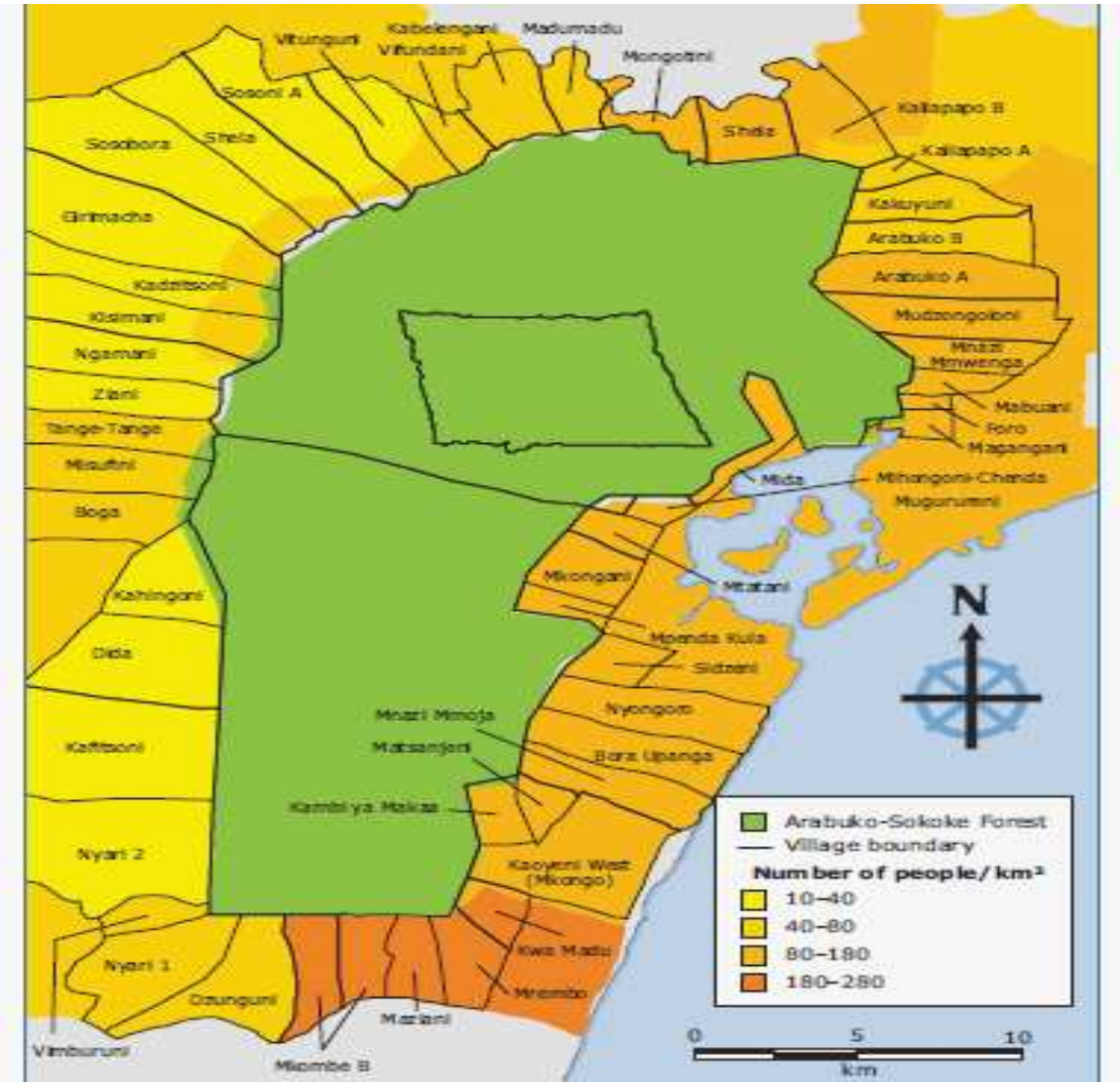
APPENDICES

Appendix I: General Location of Arabuko-Sokoke Forest



Source: Arabuko-sokoke Strategic Forest Management Plan
Janaury 2002, prepared by ASFMT

Appendix II: Population density surrounding Arabuko Sokoke forest



Source: Arabuko Sokoke Strategic Forest Management Plan Janaury 2002, prepared by ASFMT

Appendix III: GPS points recorded in four blocks Dida zone, forest

Transect No.	Transect GPS: Entry		Species /Hosplant GPS		No. of Host plants per species.				
	S	E	S	E	Ls	Ob	Bs	Cocoons	Site
A	39.80975	-3.45336667	39.80961667	-3.4266	1	0	0	0	Farmland
A			39.80781667	-3.426567	3	0	0	0	Farmland
A			39.80936667	-3.426617	0	2	0	1	Farmland
A			39.80915	-3.4264	0	1	0	0	Farmland
A			39.80901667	-3.426517	0	4	0	0	Farmland
A			39.80901667	-3.4265	0	1	0	0	Farmland
A			39.809	-3.4265	2	0	0	2	Farmland
A			39.80983333	-3.426717	1	0	0	0	Farmland
B	39.81168333	-3.38161667	39.80976667	-3.38225	1	0	0	1	Farmland
B			39.80978333	-3.382166667	1	0	0	0	Farmland
B			39.80875	-3.3815	1	0	0	0	Farmland
B			39.808767	-3.381867	0	1	0	0	Farmland
C	39.8072	-3.36431667	39.8076	-3.364383333	0	0	4	0	Forest
C			39.80771667	-3.364366667	0	0	1	0	Forest
C			39.80686667	-3.36495	1	0	0	0	Forest
C			39.8072	-3.36475	0	1	0	0	Forest
C			39.80698333	-3.3654	1	0	0	0	Forest
C			39.80696667	-3.36505	2	0	0	0	Forest
C			39.80636667	-3.365233333	0	2	0	0	Farmland
C			39.80626667	-3.365233333	0	0	1	2	Farmland
C			39.8062	-3.365183333	0	1	0	0	Farmland
C			39.80595	-3.36595	0	0	1	0	Farmland
C			39.80636667	-3.366766667	0	1	0	0	Farmland
D	39.80065	-3.33843333	39.80036667	-3.338483333	0	0	1	0	Forest
D			39.80101667	-3.338433333	0	0	2	0	Forest
D			39.80113333	-3.3384	0	0	3	0	Forest
D			39.80123333	-3.338416667	0	0	2	0	Forest
D			39.80148333	-3.338416667	0	0	1	1	Forest
D			39.80148333	-3.3384	0	0	1	0	Forest
D			39.80178333	-	0	0	1	0	Forest

				3.338216667					
D			39.80188333	-3.338266667	0	0	1	0	Forest
D			39.80193	-3.33825	0	0	4	0	Forest
D			39.80191667	-3.338316667	0	0	1	0	Forest
D			39.80201667	-3.338266667	0	0	3	0	Forest
D			39.80201667	-3.338216667	0	0	2	0	Forest
D			39.80205	-3.33813333	0	0	1	0	Forest
D			39.80205	-3.3382	0	0	1	0	Forest
D			39.8021	-3.338183333	0	0	1	0	Forest
D			39.80213333	-3.338166667	0	0	6	0	Forest
D			39.8022	-3.33815	0	0	3	0	Forest
D			39.8026	-3.3384	0	0	1	0	Forest
D			39.80265	-3.338566667	0	1	0	0	Forest
D			39.80266667	-3.338666667	0	0	1	0	Forest
D			39.80268333	-3.338783333	0	0	1	0	Forest
D			39.8027	-3.338816667	0	0	1	0	Forest
D			39.80275	-3.338816667	0	0	3	0	Forest
D			39.80285	-3.3389	1	0	0	2	Forest
D			39.80283333	-3.3389	0	0	1	0	Forest
D			39.8031	-3.338866667	0	0	38	8	Forest
D			39.80326667	-3.33905	0	0	1	0	Forest
D			39.80323333	-3.339266667	0	0	10	0	Forest
D			39.80323333	-3.339233333	0	0	22	4	Forest
D			39.80326667	-3.33925	0	0	1	0	Forest
D			39.80081667	-3.33955	0	1	0	0	Farmland
D			39.80088333	-3.3397	0	1	0	2	Farmland
D			39.80023333	-3.33845	2	0	0	1	Farmland
D			39.80013333	-3.33845	1	0	0	2	Farmland
D			39.7999	-3.338033333	0	1	0	0	Farmland
D			39.79985	-3.33745	0	1	0	0	Farmland
D			39.79981667	-3.337383333	0	1	0	0	Farmland
D			39.79978333	-3.337433333	0	1	0	1	Farmland
D			39.79976667	-3.337466667	0	1	0	0	Farmland
D			39.79971667	-3.337416667	0	1	0	2	Farmland

D			39.7997	-3.337483333	0	1	0	0	Farmland
D			39.79971667	-3.3375	0	1	0	0	Farmland
D			39.79963333	-3.337516667	1	0	0	0	Farmland
D			39.79991667	-3.338566667	0	1	0	0	Farmland
D			39.80005	-3.338766667	3	0	0	17	Farmland
D			39.80013333	-3.338833333	1	0	0	12	Farmland
D			39.80031667	-3.339066667	0	1	0	0	Farmland
D			39.80033333	-3.339316667	0	1	0	0	Farmland
D			39.80023333	-3.339633333	0	0	1	0	Farmland
D			39.80025	-3.339633333	0	0	1	0	Farmland
D			39.80008333	-3.339916667	0	0	1	0	Farmland
D			39.80011667	-3.34	0	0	1	0	Farmland
D			39.80003333	-3.339983333	0	0	1	1	Farmland
D			39.8	-3.340116667	0	0	1	0	Farmland
D			39.79996667	-3.340133333	0	0	1	1	Farmland
D			39.79995	-3.340166667	0	0	1	0	Farmland
D			39.79988333	-3.34025	0	0	3	0	Farmland

Key

Ls *Lannea schweinfurthii*

Ob *Ozoroa obovata*

Bs *Brachystegia speciformis*

Appendix IV: Larval development 2008

Life Cycle of *Argema mimosae*
Food plant: *Ozoroa obovata* (Mukayukayu)
April- May
08 NET A 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
23/04/08	Hatched	10	0	10	
28/04/08	1st Instar	10	0	5	
3/5/2008	2nd Instar	10	0	5	
9/5/2008	3rd Instar	10	0	6	
14/05/08	4th Instar	10	0	5	
18/05/08	5th Instar	10	0	4	
24/05/08	6th Instar	10	0	6	
	Total			31	

April-May- 08 NET B 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
23/04/08	Hatched	10	0	10	
28/04/08	1st Instar	10	0	5	
2/5/2008	2nd Instar	10	0	4	
7/5/2008	3rd Instar	10	0	5	
11/5/2008	4th Instar	10	0	6	
16/05/08	5th Instar	9	1	5	
23/05/08	6th Instar	9	1	7	
	Total			32	

April-May- 08 NET C 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
23/04/08	Hatched	10	0	10	
28/04/08	1st Instar	10	0	5	
3/5/2008	2nd Instar	10	0	5	
9/5/2008	3rd Instar	8	2	6	
14/05/08	4th Instar	8	0	5	
18/05/08	5th Instar	8	0	4	
24/05/08	6th Instar	8	0	6	
	Total			31	

Life Cycle of Argema mimosae
Food plant: Lannea Schweinfurthii (Mnyumbu)
June - July08 NET A 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
12/6/2008	Hatched	10		10	
16/06/08	1st Instar	10		4	
19/06/08	2nd Instar	10		3	
25/06/08	3rd Instar	9	1	6	
30/6/2008	4th Instar	9	0	5	
5/7/2008	5th Instar	9	0	5	
11/7/2009	6th Instar	9	0	6	
	Total			29	

June - July08 NET B 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
11/6/2008	Hatched	10	0	10	
16/06/08	1st Instar	10	0	5	
19/06/08	2nd Instar	10	0	3	
25/06/08	3rd Instar	10	0	6	
30/6/2008	4th Instar	10	0	5	
4/7/2008	5th Instar	10	0	4	
11/7/2009	6th Instar	10	0	7	
	Total			30	

June - July08 NET C 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
11/6/2008	Hatched	10	0	10	
16/06/08	1st Instar	10	0	5	
19/06/08	2nd Instar	10	0	3	
25/06/08	3rd Instar	10	0	6	
30/6/2008	4th Instar	9	1	5	
4/7/2008	5th Instar	9	0	4	
11/7/2009	6th Instar	9	0	7	
	Total			30	

Life Cycle of *Argema mimosae*
Food plant: Ozoroa obovata (Mukayukayu)
July- Sept 08 NET A 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
9/7/2008	Hatched	10	0	10	
14/07/08	1st Instar	10	0	5	
18/07/08	2nd Instar	10	0	4	
24/07/08	3rd Instar	9	1	6	
29/07/08	4th Instar	9	0	5	
31/07/2008	5th Instar	9	0	4	
5/8/2008	6th Instar	9	0	5	
	Total			29	

July-Sept 08 NET B 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
26/08/08	Hatched	10	0	10	
31/08/08	1st Instar	10	0	5	
6/9/2008	2nd Instar	10	0	4	
11/9/2008	3rd Instar	10	0	5	
16/9/08	4th Instar	10	0	5	
22/9/08	5th Instar	10	0	6	
28/9/08	6th Instar	10	0	6	
	Total			31	

July-Sept08 NET C 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
26/08/08	Hatched	10	0	10	
31/08/08	1st Instar	10	0	5	
6/9/2008	2nd Instar	10	0	4	
11/9/2008	3rd Instar	10	0	5	
16/9/08	4th Instar	10	0	5	
22/9/08	5th Instar	10	0	6	
28/9/08	6th Instar	10	0	6	
	Total			31	

Life Cycle of *Argema mimosae*
Food plant: *Lannea Schweinfurthii*
Sep-Oct 08 NET A 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
12/9/2008	Hatched	10	0	10	
17/9/2008	1st Instar	10	0	5	
22/9/08	2nd Instar	10	0	5	
27/09/08	3rd Instar	10	0	5	
3/10/2008	4th Instar	10	0	6	
8/10/2008	5th Instar	9	1	5	
14/10/08	6th Instar	9	0	6	
				32	

Sept-Oct 08 NET B 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
12/9/2008	Hatched	10	0	10	
17/9/2008	1st Instar	8	2	5	
22/9/08	2nd Instar	8	0	5	
27/09/08	3rd Instar	8	0	6	
3/10/2008	4th Instar	8	0	6	
8/10/2008	5th Instar	8	0	4	
14/10/08	6th Instar	8	0	5	
	Total			31	

Sept-Oct 08 NET C 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
5/10/2009	Hatched	10	10	10	
10/10/2008	1st Instar	10	10	5	
15/10/2008	2nd Instar	10	10	5	
22/10/08	3rd Instar	10	10	6	
28/10/08	4th Instar	10	10	6	
2/11/2008	5th Instar	9	1	4	
7/11/2008	6th Instar	9	0	5	
	Total			31	

Appendix V: Larval development 2009

Life Cycle of *Argema mimosae*
Host Plant: *Ozoroa obovata* (Mukayukayu)
May-09 NET A 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
1/5/2009	Hatched	10	0	9	
6/5/2009	1st Instar	10	0	5	
8/5/2009	2nd Instar	10	0	2	
13/5/2009	3rd Instar	8	2	5	
18/5/2009	4th Instar	7	1	5	
23/5/2009	5th Instar	7	0	5	
31/05/2009	6th Instar	8	0	8	

May- 09 NET B 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
2/5/2009	Hatched	10	0	10	
6/5/2009	1st Instar	10	0	4	
8/5/2009	2nd Instar	10	0	2	
13/05/2009	3rd Instar	7	3	5	
18/05/2009	4th Instar	7	0	5	
23/05/2009	5th Instar	7	0	5	
30/05/2009	6th Instar	7	0	7	

May- 09 NET C 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
2/5/2009	Hatched	10	0	10	
6/5/2009	1st Instar	10	0	4	
8/5/2009	2nd Instar	10	0	2	
13/05/2009	3rd Instar	9	1	5	
18/05/2009	4th Instar	8	2	5	
23/05/2009	5th Instar	8	0	5	
29/05/2009	6th Instar	8	0	6	

Life Cycle of *Argema mimosae*
Host Plant: Ozoroa obovata (Mukayukayu)

Jun-09 NET A 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
4/6/2009	Hatched	10	0	9	
9/6/2009	1st Instar	10	0	5	
13/06/2009	2nd Instar	8	2	4	
17/06/2009	3rd Instar	8	0	5	
22/06/2009	4th Instar	8	0	5	
26/06/2009	5th Instar	8	0	4	
3/7/2009	6th Instar	8	0	7	

Jun-09 NET B 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
4/6/2009	Hatched	10	0	10	
9/6/2009	1st Instar	10	0	5	
13/06/2009	2nd Instar	10	0	4	
17/06/2009	3rd Instar	10	0	5	
22/06/2009	4th Instar	10	0	5	
26/06/2009	5th Instar	10	0	4	
3/7/2009	6th Instar	10	0	7	

Jun-09 NET C 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
4/6/2009	Hatched	10		8	
9/6/2009	1st Instar	10		5	
13/06/2009	2nd Instar	9	1	4	
17/06/2009	3rd Instar	9	0	5	
22/06/2009	4th Instar	9	0	5	
26/06/2009	5th Instar	9	0	4	
3/7/2009	6th Instar	9	0	7	

Life Cycle of *Argema mimosae*
Host Plant: *Lannea Schweinfurthii* (Mnyumbu)

May-09 NET A 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
1/5/2009	Hatched	10		10	
6/5/2009	1st Instar	10		5	
8/5/2009	2nd Instar	10		2	
13/5/2009	3rd Instar	9	1	5	
18/5/2009	4th Instar	7	2	5	
23/5/2009	5th Instar	7	0	5	
31/05/2009	6th Instar	7	0	8	

May-09 NET B 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
2/5/2009	Hatched	10	0	9	
6/5/2009	1st Instar	10	0	4	
8/5/2009	2nd Instar	10	0	2	
13/05/2009	3rd Instar	7	3	5	
18/05/2009	4th Instar	7	0	5	
23/05/2009	5th Instar	7	0	5	
30/05/2009	6th Instar	7	0	7	

May-09 NET C 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
2/5/2009	Hatched	10	0	10	
6/5/2009	1st Instar	10	0	4	
8/5/2009	2nd Instar	10	0	2	
13/05/2009	3rd Instar	10	0	5	
18/05/2009	4th Instar	9	1	5	
23/05/2009	5th Instar	9	0	5	
30/05/2009	6th Instar	9	0	7	

Life Cycle of *Argema mimosae*
Host Plant: Lannea Schweinfurthii
Jun-09 NET A 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
4/6/2009	Hatched	10	0	10	
9/6/2009	1st Instar	10	0	6	
13/06/2009	2nd Instar	10	0	4	
17/06/2009	3rd Instar	8	2	5	
22/06/2009	4th Instar	8	0	5	
26/06/2009	5th Instar	8	0	4	
3/7/2009	6th Instar	8	0	7	

Jun-09 NET B 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
4/6/2009	Hatched	10	0	9	
9/6/2009	1st Instar	7	3	6	
13/06/2009	2nd Instar	7	0	4	
17/06/2009	3rd Instar	7	0	5	
22/06/2009	4th Instar	7	0	5	
26/06/2009	5th Instar	7	0	4	
3/7/2009	6th Instar	7	0	7	

Jun-09 NET C 10 Larvae

Date	Life stage	No life	No dead	Days taken	Remarks
	Eggs Laid				
4/6/2009	Hatched	10	10	10	
9/6/2009	1st Instar	10	10	6	
13/06/2009	2nd Instar	9	1	4	
17/06/2009	3rd Instar	9	0	5	
22/06/2009	4th Instar	9	0	5	
26/06/2009	5th Instar	9	0	4	
3/7/2009	6th Instar	9	0	7	

Appendix VI: Distribution of *A. mimosae* cocoon holes and the corresponding diameter

No	Holes Front	Holes Mid	Holes End	Holes Bottom	Total	Di Front	Di Mid	Di End	Tip Length
1	36	43	29	28	136	0.88	1.89	0.64	0.84
2	24	80	9	43	156	1.3	2.06	0.93	1.07
3	83	117	43	66	309	1.17	2.11	1.09	0.6
4	92	84	62	53	291	1.1	2.76	1	0.7
5	83	4	25	78	190	1.07	1.74	1.06	0.84
6	62	155	44	82	343	0.89	2.09	1.06	0.53
7	80	54	24	71	229	1.18	1.94	0.84	0.7
8	28	39	11	8	86	1.18	1.99	0.93	0.86
9	68	62	43	62	235	1.04	1.84	0.94	0.94
10	43	3	9	52	107	0.94	1.99	0.73	1.04
11	69	43	42	22	176	1.14	1.94	1.01	1.01
12	44	42	22	43	151	1.01	1.98	1.06	1.27
13	107	73	28	65	273	1.11	1.92	0.94	0.53
14	30	30	23	27	110	0.8	1.89	0.83	0.85
15	48	5	18	59	130	0.91	1.96	0.93	0.65
16	57	70	54	116	297	1.29	1.94	0.92	1.11
17	89	37	32	33	191	1.07	1.67	1.06	0.87
18	51	13	15	37	116	1.03	1.58	1.05	1.14
19	44	39	54	50	187	1.04	1.7	1.03	1.73
20	51	40	46	25	162	1.06	1.95	1.3	1.05
21	110	83	54	41	288	0.97	1.77	1.03	0.96
22	90	49	38	71	248	1.24	2.1	0.93	1.06
23	40	0	13	39	92	1.06	1.7	1.26	1.26
24	56	51	11	86	204	1.16	2.04	1.04	1.2
25	112	7	39	48	206	2.1	1.17	0.86	1.18
26	57	0	16	27	100	0.95	1.5	0.96	0.86
27	44	2	21	34	101	0.95	1.63	0.84	0.85
28	56	6	18	14	94	1.06	2.19	0.96	1.04
29	100	17	44	30	191	1.04	2.04	1.1	0.91
30	42	20	18	32	112	0.99	1.83	0.81	0.85
31	64	34	15	15	128	1.2	1.85	0.93	1.04
32	48	9	8	41	106	0.7	1.98	0.86	1.01
33	107	16	24	23	170	1.13	2.02	0.74	1.02
34	104	33	38	50	225	1.08	2.02	1.01	1.26
35	78	0	25	25	128	0.94	1.77	1.03	1.03
36	36	2	32	38	108	0.95	1.99	0.94	1.05
37	58	38	27	106	229	1.04	1.78	0.95	1.06
38	56	5	27	12	100	1.04	1.91	1.09	0.91
39	62	90	50	52	254	0.97	1.71	0.98	0.97
40	38	10	10	8	66	0.75	1.61	1.75	1.09
41	59	76	27	42	204	0.97	2.02	1.06	1.04

42	82	12	17	65	176	0.98	1.96	1.01	1.75
43	73	7	43	38	161	1.2	2.02	1.11	1.08
44	37	0	24	17	78	1.01	1.79	1.02	0.98
45	62	21	20	42	145	1.03	1.81	1.04	1.16
46	97	79	53	21	250	1.16	2.18	1.04	0.91
47	19	5	12	4	40	1.06	1.75	1.02	0.92
48	37	0	13	18	68	1.04	1.94	1.28	1.03
49	50	8	19	16	93	0.93	1.88	1.02	0.94
50	41	0	16	23	80	0.96	2.03	1.07	1.02
51	90	20	32	23	165	0.92	2.21	1.23	0.94
52	105	234	67	150	556	1.09	1.7	0.99	0.93
53	100	44	42	39	278	0.82	1.7	0.97	0.78
54	19	0	9	6	88	0.97	1.72	1.1	1.4
55	81	0	6	29	171	0.99	1.77	0.94	1.04
56	45	6	33	44	184	0.64	1.67	0.93	0.81
57	63	33	32	76	261	0.91	1.04	0.94	1.08
58	29	6	22	39	154	0.95	1.86	1.14	0.8
59	71	15	34	16	195	0.97	2.08	1.03	1.01
60	30	26	47	70	233	0.97	1.67	1.05	0.94
61	38	11	21	55	186	0.98	1.71	0.64	1.06
62	46	75	38	121	280	1.18	1.91	0.81	1.26
63	36	2	13	25	76	0.9	1.82	1.07	1.26
64	42	34	16	65	157	1	1.21	0.86	0.58
65	80	9	20	27	136	1.04	1.79	0.84	0.71
66	50	14	42	12	118	1.08	2.06	1.24	1.29
67	71	9	13	25	118	1.01	1.93	1.07	0.89
68	50	24	43	44	161	1.17	1.87	1.07	0.77
69	97	35	40	20	192	1.28	1.79	1.04	0.86
70	110	100	75	38	323	1.26	1.85	0.95	0.82
71	57	19	34	33	143	1.07	1.66	0.77	0.84
72	43	5	27	15	90	1	1.8	0.81	0.91
73	40	11	18	22	91	1.02	1.93	0.72	0.88
74	48	33	21	49	151	1.08	2.07	0.89	0.84
75	69	9	40	32	150	1	1.86	0.9	1.06
76	67	118	33	100	318	1.02	1.89	0.8	0.74
77	53	0	15	46	114	1.05	1.94	0.75	0.84
78	46	47	29	66	188	1.09	1.92	0.82	1.08
79	37	8	26	30	101	1.03	1.66	1.06	0.98
80	70	16	19	48	153	1.09	1.66	0.67	1.01
81	57	60	28	48	193	1	1.73	0.62	0.54
82	43	20	30	36	129	1	1.87	0.96	0.89
83	79	49	29	57	214	1.03	1.72	0.73	0.97
84	80	24	53	95	252	1.04	1.86	0.81	0.98
85	83	33	34	110	260	1.02	1.71	0.66	0.96
86	56	10	28	32	126	0.87	1.91	1.04	0.92
87	62	3	16	53	134	1.17	1.91	0.72	1.03
88	46	1	17	19	83	1.03	2.07	0.87	1.04
89	46	7	20	14	87	0.84	1.79	0.66	0.98

90	66	12	26	93	197	1.09	1.88	0.82	0.67
91	2	0	4	0	6	0.82	1.61	0.8	0.84
92	41	50	14	24	129	0.98	1.61	0.6	0.76
93	43	1	9	27	80	1.05	1.7	0.65	0.94
94	57	68	40	38	203	0.96	1.7	0.78	0.58
95	59	69	29	46	203	1.07	1.84	0.97	1.08
96	61	74	50	70	255	1.08	1.72	0.76	0.75
97	68	6	39	16	129	1.08	2.01	0.91	0.56
98	44	10	31	17	102	1	1.78	0.51	0.7
99	55	10	21	45	131	0.99	1.91	1.12	1
100	110	66	49	100	325	1.03	1.71	0.82	0.84

Appendix VII: ISA classification table for raw silk of category III (34 denier and coarser)

Major items / Grade		4A	3A	2A	A	B
Size Deviation (denier)	34 d. - 49 d	2.60	3.10	3.65	4.45	above 4.45
	50 d. - 69 d.	3.75	4.40	5.20	6.35	above 6.35
	70 d. and above	4.45	5.25	6.20	7.60	above 7.60
Maximum Deviation (denier)	34 d. - 49 d.	8.0	9.5	11.0	13.5	above 13.5
	50 d. - 69 d.	11.0	13.0	15.5	19.0	above 19.0
	70 d. and above	13.5	16.0	18.5	23.0	above 23.0
Evenness Variation I (count)		150	170	190	210	above 210
Evenness Variation II (count)		10	17	26	37	above 37
Cleanness (%)		97	95	93	88	below 88
Average Neatness (%)		94	92	90	87	below 87
Low Neatness (%)		90	87	83	77	below 77
Auxiliary / Class		(1)	(2)	(3)	(4)	(5)
Evenness Variation III (count)		0	1	2	6	above 6
Auxiliary / Class		(1)	(2)		(3)	(4)
Winding (breaks)	34 d. - 69 d.	1	6		13	above 13
	70 d. and above	0	4		10	above 10
Auxiliary / Class		(1)				(2)
Tenacity (grams)		3.7				below 3.7
Elongation (%)		18				below 18