PLANT-FEEDING BEHAVIOUR AND ITS EFFECTS ON THE FITNESS AND COMPETENCE OF THE MALARIA VECTOR *ANOPHELES GAMBIAE* (DIPTERA: CULICIDAE)

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This 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

I dedicate this thesis to the Almighty GOD, my parents Pauline Ngatsing and Fidele Tatchuwo, and my loving brothers and sisters who gave me immeasurable moral support while I was far away here in Kenya toiling towards this goal.

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Abstract

Malaria remains a serious threat in sub-Saharan Africa. Available control tools are now largely ineffective. Because of the role of vectors in malaria transmission, integrated vector management strategies that are environmentally safe, economically feasible and acceptable to resource-limited communities are one way to achieve effective malaria control. However, adequate knowledge of the biology, behaviour and ecology of the malaria vectors are imperative. Sugar-feeding from plants is a basic requirement for adult mosquitoes, and so far have received little attention. The present study, conducted in Suba district (a malaria endemic area of western Kenya), aimed to investigate the plant-feeding behaviour and its effects on the fitness and competence of *Anopheles gambiae*. Females of two species of *Anopheles gambiae s.l.* namely *Anopheles gambiae s.s.*, and *Anopheles arabiensis* were sampled during both dry and wet seasons. Approximately 12% were fructose-positive at the time of collection, indicating recent plant-feeding. Proportions of plant-fed mosquitoes were similar in all the 2 *Anopheles* species (P=0.90), at all their gonotrophic status (unfed, blood-fed, half-gravid, and gravid) (P=0.69), at all ages (parous and nulliparous) (P=0.98), and during both seasons (P=0.06). However, more mosquitoes had fed on plants when collected far from larval habitats (P=0.001). When offered 13 dominant plant species [*Cassia hirsuta* L., *Senna bicapsularis* L., *Senna didymobotrya* F. (Leguminosae), *Datura stramonium* L. (Solanaceae), *Flaveria trinervia* M., *Parthenium hysterophorus* L., *Psiada punctulata* V., *Tithonia diversifolia* H. (Asteraceae), *Hamelia patens* J. (Rubiaceae), *Ipomea hildebrandtie* V. (Convolvulaceae), *Lantana camara* L. (Verbenaceae), *Ricinus communis* L. (Euphorbiaceae), *Tecoma stans* L. (Bignoniaceae)] growing around human dwellings and mosquito larval habitats in the study area, behavioural responses of mosquitoes to those candidate plants were recorded in the greenhouse, each plant being presented to mosquitoes singly, and concurrently with other plants species in the no-choice and choice bioassays respectively. Gas chromatography analysis was carried out to determine and compare plants and mosquito sugar profiles. Five plants species were found to be most preferred by *Anopheles gambiae*, namely; *Ricinus communis* L., *Senna didymobotrya* F., *Parthenium hysterophorus* L., *Tecoma stans* L., and *Hamelia patens* J (P<0.001). Flowers were most fed on by mosquitoes, but leaves were most preferred in two plants species (*Lantana camara* and *Parthenium hysterophorus*); leaves and stems in *Ricinus communis*. With the exception of one plant species (*Parthenium hysterophorus*), survival (P<0.001) and fecundity (P=0.01) were high in the mosquitoes which fed on most of the preferred plants, compared to those which fed on the less preferred plant. Sugar composition and concentration in the preferred plants were positively correlated to the survival (r=1, P<0.001) and fecundity (r=0.87, P=0.04) of mosquitoes. Four plants species (*Lantana camara, Senna didymobotrya, Ricinus communis* and *Parthenium hysterophorus*) significantly reduced the prevalence of infection in mosquitoes (P<0.01). There was also a drastic decrease on the parasite load (oocyst intensity) in the midguts of the infected mosquitoes which fed on 3 plants species (*Lantana camara, Senna didymobotrya* and *Pathernium hysterophorus*) (P<0.01). The inhibitory effects of those plants were more pronounced when mosquitoes plant-fed throughout (before and after) an infectious blood meal, followed by plant-feeding post-infection. The greatest impact of deleterious plants was found in the transitions macrogamete-ookinete (only in pre-infection plant-feeding) and ookinete-oocyst (only in post-infection plant-feeding). These studies imply that certain plant species preferred by *Anopheles gambiae* in the endemic area significantly decrease their fitness and competence to develop *Plasmodium falciparum*, and therefore can reduce malaria transmission.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Malaria is an important cause of death and illness in children and adults in tropical countries. Mortality, currently estimated at over a million people per year has risen in recent years (WHO, 2006). It is responsible for enormous economic burdens in endemic regions (Gallup and Sachs, 2001). It is caused by a species of protozoan parasite of the genus *Plasmodium* and transmitted by mosquitoes belonging to the genus *Anopheles*.

In many developing countries, the major control measures against malaria are directed to the parasite in human. These measures include the use of drugs to treat, suppress and prevent infections. Chemotherapy is expensive and has drawn a lot of resources from the governments in these developing countries. There are also limited anti-vector measures that have been used. These include personal protection by use of insecticide treated bed nets, mosquito repellents, elimination of adults and larvae by use of insecticides and larvicides. Despite all these strategies, the disease has re-emerged in the last decade due to the emergence of widespread parasite resistance to formerly effective drugs like chloroquine and other antimalarial drugs (Snow *et al.*, 1998; Winstanely, 2000), resistance of mosquitoes to chemical insecticides (Service, 1980; Brooke *et al.*, 2000), population shifts, war-damaged infrastructures, altered meteorological conditions and drastic ecological transformation (WHO, 2000, 2003), and poor organization and funding of control programs (Gwadz and Collins, 1996). These limitations have led to failure in the fight against malaria and the disease has even entered previously unaffected locations.

Today, the WHO`s objective is not the eradication of the disease, but reduction in mortality and morbidity. To accomplish this goal, new strategies that aim at reducing malaria transmission are needed (Killeen *et al.*, 2002). Many efforts are directed towards malaria prevention by the control of its vectors, but these efforts are hampered because the biology of the vector does not seem to be fully understood.

1.2 Statement of the problem

Several vector control strategies have been used in the African malaria endemic countries including indoor residual spraying, insecticide-treated bednets, larval control and environmental management. However, they have been limited to small scales and shown limitations because the biology of mosquitoes and specifically their life in the wild (e.g. nectar-feeding behaviour) is not fully understood. Sugar-feeding is a basic component of the life of male and female mosquitoes (Yuval, 1992). Malaria vectors live and breed in habitats with several plants on which they obtain their sugar meals (McCrae *et al.*, 1976; Laarman, 1968; Foster, 1995). However, to date, little information is available on the plant-feeding behaviour of the African malaria vectors. Studies on the medical implications of the plant-feeding by mosquitoes may be worthwhile. If mosquitoes feed on certain plants in nature, these plants may be of indirect epidemiological importance by affecting lifespan, flight capacity, fecundity of potential *Anopheles* vectors as well as their ability to develop mature infections of human pathogens. These plant juices shape the biology of mosquitoes, their physiology, behaviour, ecology, life history and ultimately their vectorial capacity.

This study was designed to assess the plant-feeding behaviour of the African malaria vector *Anopheles gambiae* Giles and the effects of plants on its fitness and vector competence.

1.3 Justification

In Kenya, malaria is a problem that continues to place an unacceptable burden on health and economic development of the country. A lot of efforts have been focused on the treatment of malaria cases. Due to the fact that many patients do not have access or cannot afford treatment, the control of vectors is therefore an alternative, long lasting and a cheap way to control malaria. However, its success lies on proper knowledge of the biology of vectors especially plant-feeding behaviour. Information acquired from this study will lead to the identification of plants, growing around human communities that affect the fitness and vector competence of *An. gambiae,* thus affect malaria transmission. It is also hoped that understanding plant-feeding behaviour of *An. gambiae* can lead to the possibility of using sugar sources for its biological control. This study will also contribute to new ideas for malaria vector control.

1.4 Hypotheses

a. Female *Anopheles gambiae* feed on plants at all stages of their adult life.

b. *Anopheles gambiae* have high feeding preference on some plant species.

c. Plant juices affect the fitness of *Anopheles gambiae.*

d. Some preferred plants diets affect the development of malaria parasites in the midgut of infected mosquitoes.

1.5 Objectives of the study

1.5.1 Main objective

The main objective of this work was to determine the plant-feeding behaviour of *Anopheles gambiae* and effects of plants on its fitness and competence in transmission of malaria parasite.

1.5.2 Specific objectives

a. To determine plant-feeding practices of wild malaria vector populations;

b. To identify the preferred plants by *An. gambiae*;

c. To identify the type of sugars present in preferred plants;

d. To establish the effect of plant juices on female *An. gambiae* fitness;

e. To establish the effect of preferred plants on the development of *P. falciparum* in *An. gambiae*.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of malaria situation

Malaria is one of the most serious challenges to modern healthcare. Each year, there are hundreds of millions of cases of this disabling disease reported worldwide. According to the World Health Organization report (WHO, 2005), more than 40% of the world's population lives in areas where they are at risk of malaria attack. Estimates for the annual mortality from malaria vary widely. It has been reported that malaria leads to more than 1 million deaths every year, particularly children, with 75% in Africa sub-Sahara, and a global burden of 350 million to 500 million cases per year (WHO, 2004, 2006). But we do not know the true extent of the disease. The figures for the reported cases vary widely and suggest that only a small fraction of those in need of treatment ever get access to it. Furthermore, Breman (2001) predicted that the malaria burden will double by 2020 if effective control interventions are not implemented.

2.2 Global distribution of malaria transmission risk

Malaria continues to place an unacceptable burden on health and economic development in over 100 countries across the world. Anyone living or traveling to an area with endemic malaria is at risk for infection. These areas include Africa, Latin America and the Caribbean, South East Asia, the Eastern Mediterranean, the western Pacific and parts of Europe (WHO, 2005) (Fig. 2.1). Risk varies both seasonally and diurnally. In areas with seasonal climatic variations, it is highest during the rainy season when mosquito populations increase. In regions with stable, warm, humid climate, the risk is throughout the year. Activity peaks for

Anopheles mosquitoes occur from dusk to dawn, although this is variable because these insects have highly adaptable behaviour patterns.

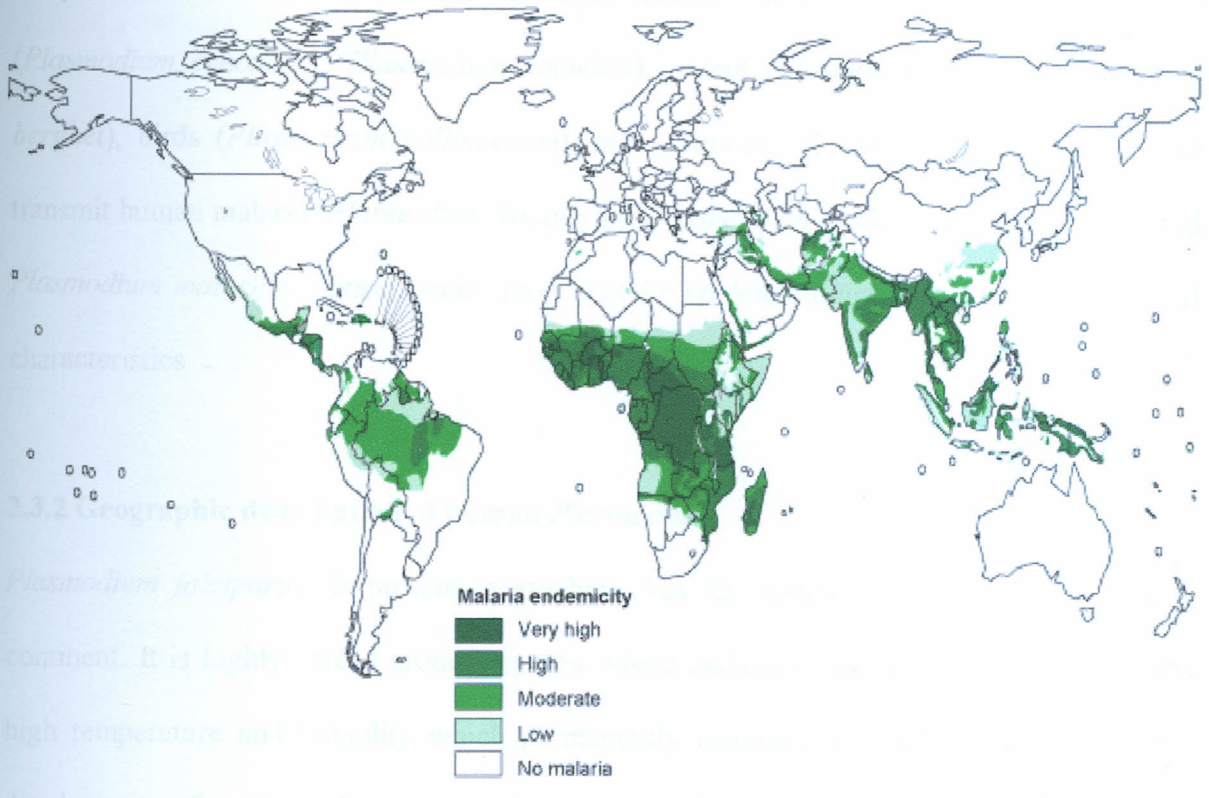


Fig. 2.1 Map of global distribution of malaria transmission. World Health Report (2005)

2.3 Malaria parasites

2.3.1 Different species

Plasmodium is a protozoa from the kingdom of protista, phylum of apicomplexa, class of sporozoa, order of Haemosporideae or Eucoccideae and family of Plasmodiidae. Apart from its parasitism on human, *Plasmodium* also infects many animals such as: monkeys (*Plasmodium cynomolgi*, *Plasmodium knowlesi*), rodent (*Plasmodium yoelii*, *Plasmodium berghei*), birds (*Plasmodium gallinaceum*), etc... Among all, only 4 species are able to transmit human malaria: *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium vivax*, and *Plasmodium malariae*. Those species have specific epidemiological, biological and clinical characteristics.

2.3.2 Geographic distribution of human *Plasmodium*

Plasmodium falciparum is present everywhere, but its relative importance varies among continent. It is highly spread around equator where malaria is endemic. This is favored by high temperature and humidity which permanently maintain favorable conditions for the development of vector and parasite in the mosquito. *Plasmodium vivax*, formerly present in Europe is also present in tropical Africa (Ethiopia, western Kenya), and very common in Asia. *Plasmodium malariae* has an irregular distribution which has not yet found definitive explanations. Its sporogonic cycle within the mosquito is long compared to *P. falciparum*, suggesting its transmission by only vectors with high longevity present in micro-ecological sites. The fouth species, *P. ovale* is relatively less frequent.

2.3.3 Life cycle of *Plasmodium*

Plasmodium parasites exhibit a complex life cycle involving development through two host species: an insect vector (definitive host) and a vertebrate species (intermediate host). The life cycle of *Plasmodium* has four phases, one sexual and three asexual. The sexual and first asexual phases take place in an *Anopheles* mosquito, the second and third asexual phases occur in a vertebrate host (Fig. 2.2).

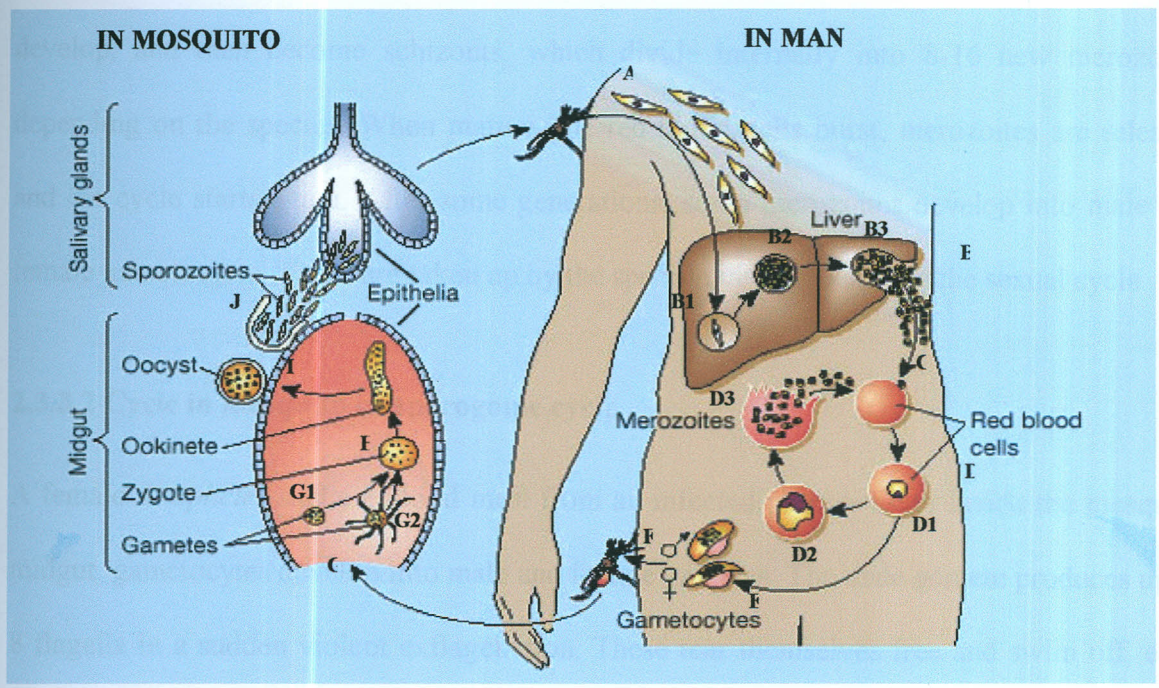


Fig. 2.2 Biological cycle of *Plasmodium falciparum*

Legend: A- Injection of sporozoites into the bloodstream during *Anopheles* bite; B - Liver schizogony: Invasion of liver cells by sporozoites (B1), formation of hepatic schizonts (B2), liberation of thousand of invasive merozoites (B3) which invade red blood cells (C); D - Erythrocytic schizogony: Formation of erythrocytic trophozoites and schizonts (D1, 2) which divide into merozoites and burst to reinfect red blood cells (D3). E - Sexual differenciation of merozoites into male (♂) and female (♀) gametocytes. F - Ingestion of gametocytes by the mosquito. G - Process of activation of macrogametocytes (G1) and exflagellation of microgametocytes (G2) to produce gametes. H - Fertilized macrogamete or zygote which develops into ookinete. I - Penetration of ookinete trough the stomack wall to become oocyst which grows and mature. J - The mature oocyst burst and frees sporozoites that migrate through the body of the mosquito and invade her salivary glands.

2.3.3.1 Cycle in a vertebrate host or asexual development

When the mosquito feeds on vertebrate host, sporozoites are injected into the blood. They invade liver cells and become hepatic trophozoites. These develop, then, divide to produce thousands of invasive liver merozoites. The infected liver cells burst, releasing the merozoites into the blood. In *P. vivax*, some sporozoites become hypnozoites (Krotoski, 1985), which lie dormant in liver cells, to develop months or years later and cause the illness to relapse. Merozoites invade red blood cells and become erythrocytic trophozoites. These develop, and then become schizonts, which divide internally into 8-16 new merozoites depending on the species. When mature, the red blood cells burst, merozoites are released and the cycle starts again. After some generations, some merozoites develop into male and female gametocytes. These are taken up by the specific vector to initiate the sexual cycle

2.3.3.2 Cycle in mosquito or sporogonic cycle

A female *Anopheles* sucks a blood meal from an infected person. Once inside the mosquito midgut, gametocytes develop into male and female gametes. The male gamete produces up to 8 flagella in a sudden violent exflagellation. These tear themselves free and swim off, each with a male nucleus attached. Fertilization takes place and a zygote is produced. This develops into a motile ookinete, which bores into the outer stomach wall, rounds up and becomes an oocyst. The oocyst develops, then, divides to produce thousands of invasive sporozoites. The mature oocyst bursts and the free sporozoites migrate through the body of the mosquito and invade their salivary glands. When mosquito feed again on a human being, sporozoites will be injected. This cycle takes approximately 10-20 days.

2.3.3.3 Factors controling the sporogonic cycle of *Plasmodium*

The sexual development of *Plasmodium* is a dynamic process which involves components of different natures. These components included environmental conditions, the parasite, the vertebrate host and the mosquito vector, and their interactions give a functional environment for the parasite. It is obvious that the parasite passing from human to the mosquito faces a change of environment and undergoes some important morphological and structural modifications. These processes are susceptible to favor or inhibit parasite development.

a. Environmental factors

Environmental conditions play a major role on spatial distribution and epidemiology of malaria. They affect transmission by three mechanisms (Boudin and Robert, 2003).

(i) Climate affects distribution and abondance of *Anopheles* vectors, success of a sporogonic cycle of the parasite within a mosquito, and modulation of man-mosquito contact.

(ii) Temperature affects the length of sporogonic development of the parasite. The sporogonic development of the parasite is optimal between 18°C and 35°C.

(iii) Relatively high humidity (70-80%) increases the success of the sporogonic development of the parasite.

b. Parasite's factors affecting the sporogonic cycle

(i) Gametocyte density: Only mature gametocytes are able to infect mosquitoes and that infectivity depends more or less on the gametocyte density (Bruce-Chwatt, 1980). But the implications of gametocyte density as controlling infection have been controversial. Muirhead-Thomsom (1957) and Ponnudurai *et al.* (1989a) realised that it was imposible to

predict the outcome of an infection based on the gametocyte density. But these observations were partially rejected by the observations from Graves (1980), Boudin *et al.* (1989) and Tchuinkam *et al.* (1993). These authors found that there was a relation between high gametocyte densities and success of infection.

(ii) Variation in the sex ratio: Population of gametocytes present an important heterogeneity based on the sex ratio (Read *et al.*, 1992). Important disparity between sexes will negatively influence an infection (Boyd, 1949). In the normal sex ratio, females are dominant. This compensates biologically the production of 8 microgametes from one gametocyte male. An optimal condition of infectivity will be one gametocyte male for 8 female gametocytes, specifically when a mosquito ingests a low number of gametocytes and the number of gametocytes males is critical (Rel *et al.*, 2002).

(iii) Age of gametocytes: There is a maximal infectivity period for gametocytes. Very young and very old gametocytes are less or not infective (Hogh *et al.*, 1998)

(iv) Aggregation of gametocytes: This phenomenon favors the infectivity of parasites. It reduces the probability of a gametocyte to be isolated in a blood meal (Pichon *et al.*, 2000)

(v) High asexual parasitemia: High asexual parasitemia sometimes reduces the infectivity of gametocytes. The mechanism is unknown. But it is probably due to inhibitory role of some cytokines, for instance TNF (Naotune *et al.*, 1993; Lensen *et al.*, 1997), highly produced when asexual parasites increased in human. But this phenomenon is rare in *P. falciparum* infection due to the delay in gametocytes maturation in relation to high asexual parasites from where they evolved in human.

(vi) Apoptosis: This is the last parasite's factor recently proven to play a role in the reduction of the midgut stages of the sporogonic development of the parasite. More than 50% of the

midgut stages of *P. berghei* commit suicide before crossing the midgut membrane of *An. stephensi* (Al-Olayan *et al.*, 2002).

c. Human's factors affecting the sporogonic cycle

During a blood meal, a mosquito ingests together with gametocytes, blood cells which can play a role in the development of the parasite. Among these factors susceptible to inhibit parasite development, some are found naturally in a human living in an endemic area and these factors are commonly named transmission blocking immunity (antibodies, cytokines, immuno-competent cells, etc.) (Gouagna *et al.*, 1998), others can be acquired artificially through vaccination (antibody against Pfs 25, antibodies against mosquito tissues) (Lensen *et al.*, 1992). The hemoglobine type of the gametocyte carrier also affects the sporogonic cycle. A carrier with sickle cells trait will facilitate sporogonic cycle (Robert *et al.*, 1996). Drugs taken by human can also affect transmission directly as some drugs can affect the infectivity of gametocytes due to their sporonticidal role. For instance sulfadoxine-pyrimethamine is highly gametocytogenic and sporonticidal (Hogh *et al.*, 1998: Robert *et al.*, 2000).

d. Mosquito's factors affecting the sporogonic cycle

All the mosquitoes are not able to transmit malaria. Other species have a biological incompetence to develop the parasite. There is also physiological blockage by toxicity or by mechanical factors as mid-gut membrane (Shahabuddin and Kaslow, 1994) and by chemical nature of stomach cells (Huber *et al.*, 1991; Sieber *et al.*, 1991; Shahabuddin *et al.*, 1993). Digestive enzymes (Billingsley and Hecker, 1991) and mosquito immune system (Dimopoulos *et al.*, 1998) also play a role in the success of a sporogonic cycle. Many other factors like life history of mosquito, for instance age (Boudin *et al.*, 1989), speed of blood

digestion (Ponnudurai *et al.*, 1989a), quantity of blood meal, body size and anthropophilic features of mosquito (Lyimo and Koella, 1992) also affect the success of a sporogonic cycle. Body size of adult mosquitoes and their consequent vectorial capacity have been linked to resources available to larvae (Briegel, 1990b; Kitthawee *et al.*, 1995). Adult nutrition (sugar-feeding) may also affect the sporogonic cycle of the parasite in the mosquito. Lectins are substances that can be found in plant tissues and can be very toxic for parasites. For instance, defensin, a lectin molecule has a lytic activity on *Plasmodium* (Richman *et al.*, 1996). Plant juices taken by mosquitoes are directed to the mosquito gut where the parasite develops.

2.4 Malaria vectors

2.4.1 Classification

The exclusive vectors of malaria are mosquitoes (Order Diptera: Family Culicidae) of the subfamily of Anophelinae and genera of *Anopheles*. Although there are about 400 species of *Anopheles* mosquitoes in the world, only 60 of them transmit malaria under natural conditions, and only 30 are of major importance (Bruce-Chwatt, 1985). In Africa, the main vectors for malaria are species of the *Anopheles gambiae sensu lato* and *Anopheles funestus* complex Giles (Gillies and De Meillon, 1968). Mosquitoes of the *An. gambiae* complex consist of seven morphologically indistinguishable species with six of them transmitting human malaria (Service, 1985). Four species are freshwater-breedings, *An. gambiae* [the nominal taxon, also referred to as *An. gambiae sensu stricto* (*s.s.*)], *An. arabiensis* (these two are the most important vector species in the complex) (White, 1974), *An. bwambae*, *An. quadriannulatus* A (not a vector) and *An. quadriannulatus* B (Hunt *et al.*, 1998). Two other

members of the complex namely *An. melas* and *An. merus* are salt-water species, localized vectors depending on their levels of contact with people.

2.4.2 Distribution of malaria vectors

Malaria vectors are different from one region to another. Each *Anopheles* species has characteristics of longevity, adaptation to parasite, host preference (human being or animal), frequency of bloodmeals and choice of resting and oviposition sites that are specific to it. Mosquito-man contact is the principal condition for malaria transmission. It depends on the ecology of mosquitoes and human. It is directly affected by human activities and indirectly by environmental modifications. All these factors modulate the geographical distribution of *Anopheles* in the world.

In Asia, major vectors include *An. culicifacies, An. minimus, An. fluviatilis, An. dirus, An. anthropophagus, An. stephensi;* in South America, *An. darlingi, An. nuneztovari*; in Eastern Europe, *An. sacharovi*; in Central America, *An. albimanus, An. darlingi*; In Australasian area, *An. punctulatus* complex. *Anopheles claviger* is mostly found in the Middle East countries. In Africa south of the Sahara, two major vectors are found, *An. gambiae s.l.*, and *An. funestus s.l.* The occurrence of 80% of the world`s malaria in tropical Africa is due to *An. gambiae s.s.*, *An. arabiensis* and *An. funestus* (WHO, 1993), which are widespread in tropical Africa where malaria occurs. The other species of the *An. gambiae* complex are localized vectors which are strongly attached with specific habitats. *Anopheles melas* is found in West Africa and *An. merus* in East Africa. *Anopheles bwambae* is responsible for localized malaria transmission among the Bambute pygmies of Bwamba in Uganda. *Anopheles quadriannulatus* B is found in South Africa and is distinct from its Ethiopian Counterpart *An.*

quadriannulatus A (Hunt *et al.*, 1998). *Anopheles nili*, *An. moucheti*, *An. hargreavesi* and *An. hancocki* are also malaria vectors of minor importance in certain localities, the first two are found in forest areas of Central Africa.

2.4.3 Biology of *Anopheles gambiae*

2.4.3.1 Life cycle of *Anopheles gambiae*

The development of *An. gambiae* is characterized by a succession of aquatic stages (eggs, larvae and pupae), and an air stage (adult). In tropical climates, development is rapid and therefore the egg-adult cycle may be completed in 6 days (Gillies and De Meillon, 1968).

After mating and blood feeding, a gravid mosquito lays eggs on the second day after she has digested blood (Clements, 1992; Service, 1980). The eggs hatch into larvae approximately 2 days after oviposition. The larvae are filter feeders and take less than 7 days to develop through four instars. Larval development is dependant on the temperature, the ecological conditions and the availability of food. Lack of food may prolong the larval stages (Mouchet and Carnevale, 1991). The pupal stage duration varies from 1 to 3 days depending on the environmental conditions. Most pupation occurs during the late morning and afternoon (Goma, 1959). Pupae do not feed but they breathe.

From pupae will emerge adult mosquitoes, which live, eat and mate relatively near the water where they have emerged from. Mosquitoes take at least one day and rest near the breeding sites to reach sexual maturity. Sugar-feeding and sexual reproduction also occur during this stage. Females are mated once. In nature, males and females feed on nectars and only females are hematophageous. Mated females seek blood meals from vertebrate hosts. Blood

is needed for egg development, which takes about 2 days (Clements, 1992). The female then oviposits and seeks another blood meal. The lifespan of an adult mosquito is generally 3-4 weeks, but vary with environmental conditions (Cheesbrough, 1987).

2.4.3.2 Mating behaviour of *Anopheles*

Males tend to swarm near both the breeding waters and floral food sources, which usually go hand and hand, and probably never travel further one kilometer from that site. In a group, the swarming males are thought to attract females to the swarm by their characteristic pitch created by wing beats. Mating is associated with swarming (Charlwood *et al.*, 2002). Most Anophelines mate in crepuscular swarms, including members of the *An. gambiae* complex (Charlwood and Jones, 1980; Marchand, 1984; Charlwood *et al.*, 2002). The male *An. gambiae* beats his wings about twice the rate of the female of the species. This results in a clearly audible difference between the sounds of the sexes, an obvious adaptation to the use of sound of the females to attract males (Belto, 1994). However, there is extensive overlap between harmonic amplitudes of the wing beat of members of the *Anopheles gambiae* group (Tripet *et al.*, 2004; Wekesa *et al.*, 1998). Thus other mechanisms such as volatiles (Takken and Knols, 1999) or contact pheromones (Nijhout and Craig, 1971; Polerstock *et al.*, 2002) must be invoked as final recognition cues before copulation. Detecting the lower wing beat of the approaching female, a male may fly towards and clasp on to her in mid-air. Copulation takes only a few seconds and they are released from their nuptial flight. As a fairly general rule, mating takes place before the female has had its first blood meal, and one male can fertilize more than one female. The single mating will provide sufficient sperm for the life of the female. Because the female will have several gonotrophic cycles, the insemination

ordinarily takes place before the cyclic activity begins or after the first blood meal (Githeko *et al.*, 1993).

2.4.3.3 Blood -feeding behaviour of *Anopheles gambiae*

Once the female *Anopheles* has been inseminated, she will seek out a blood meal necessary for the development of her eggs. It has been accepted that the most successful vectors of malaria do preferentially feed on humans, and secondarily on cattle and other domestic animals depending on host availability (Garrett-Jones *et al.*, 1980; Mbogo *et al.*, 1993). Blood feeding behaviour is controlled by different sensory stimuli that represent the host to the host-seeking insect. At long distance, the process is mediated by olfactory cues (Knols *et al.*, 1995), whereas visual and physical cues are important in the close vicinity of the host (Gillies and Wilkes, 1969). Long distance olfactory information includes volatile chemicals of the skin and breath produced by the potential host (Takken, 1991). Carbon dioxide is active at medium range (Brady *et al.*, 1997) and body heat and moisture at short-range (Gillies and Wilkes, 1969, Shofield and Sutcliffe, 1997). This characteristic smell of human odor is likely to be a principle determinant in preferential host selection. Body odour is responsible for more than 90% of the attractiveness of humans to *An. gambiae* (Mboera *et al.*, 1997), in particular carbon dioxide from expired breath (Brady *et al.*, 1997).

2.4.3.4 Resting behaviour of *Anopheles gambiae*

Both before and after blood-feeding, some species of African malaria vectors will rest in houses (endophilic) whereas others will rest outdoors (exophilic) in a variety of natural shelters such as vegetation, in rodent burrows, in cracks and crevices in trees, under bridges,

in culverts, in termite mounds, in caves, and among rock fissures and cracks in the ground. *Anopheles gambiae* routinely rest within houses after a blood meal, and the choice of resting sites can be influenced by microclimate (Fialho and Schall, 1995). They usually aggregate at particular sites within houses such as dark crevices, the inside of the roof and under furniture (Gillies and De Meillon, 1968).

2.4.3.5 Oviposition behaviour of *Anopheles gambiae*

Generally after blood feeding, the female *Anopheles* usually produces a brood of eggs, the process known as gonotrophic concordance. It has been found that the smaller females require multiple blood meals in order to produce their first batch of eggs (Lyimo and Takken, 1993). *Anopheles gambiae* is discriminative in its oviposition behaviour (Muirhead-Thomsom, 1945). Its preferred larval habitats are fresh water pools that are generally small, transient and sunlit, devoid of vegetation and often turbid with moderate temperature (Service, 1993). Oviposition tendency might therefore be related to location and availability of such sites. Oviposition behaviour can be both complex and highly specific, and various physical and semiochemical factors affect the choice of oviposition site (Sumba, 2004).

2.4.3.6 Aestivation

A number of *Anopheles* mosquitoes have a resting phase known as aestivation. This is similar to hibernation which enables them to survive adverse seasonal climatic conditions. In the case of *An. gambiae,* this important malaria vector and members of this complex can survive during adverse conditions by aestivating indoors for several months (Gillies and De Meillon, 1968; Omer and Cloudsley-Thompson, 1970). It is normally the fertilized females

that are found in this phase. The site selected is usually a dark and cool place, like caves (Gillies and De Meillon, 1968). The female becomes immobile and does not blood-feed, her oviposition activity is completely suspended during this time. This situation would help to explain the fast reappearance of *An. gambiae* after the start of the rains. In Africa, females in this state are quite able to survive throughout the adverse dry season.

2.4.3.7 Foraging behaviour

2.4.3.7.1 Sugar feeding

a. Dependence on sugar

The extensive studies on blood-feeding should not obscure the fact that feeding on sugar rich foods is an integral part of mosquito life history. Sugar is the basic food of adult mosquitoes (Foster, 1995). It is the only source of nutrition for males and contributes substantially to energy requirements of female mosquitoes. In general, in many mosquito species, both male and female mosquitoes sugar-feed soon after emergence and continue this behaviour throughout adulthood (El-Akad, 1989; Haramis and Foster, 1990; Foster, 1995; Foster and Takken, 2004). In the case of *An. gambiae* females, sugar-feeding practices in that species have been controversial. Some authors have reported that they feed exclusively on blood because no females appeared to contain any fluid in the oesophageal diverticulum at the time of biting or when resting (Gillies, 1968), and that *An. gambiae* must rarely take sugar meals in the wild although it would do so freely in the laboratory (Muirhead-Thomsom, 1951). But McCrae (1968) and Laarman (1968), from field observations concluded that sugar feeding was part of their life and strongly supported the view that sugar-feeding activity in mosquitoes resulted from purposive appetitive behaviour showing distinct circadian rhythms and was not simply the result of chance encounter of nectar sources in the course of random

flight activity. Wild females *An. gambiae s.l.* and *An. funestus* were found containing sugar in their crop in western Kenya (Beier, 1996). Field collected *An. freeborni* was also found with sugar (Holliday-Hanson, 1997).

b. Sugar sources

Adult mosquitoes can obtain sugar from floral and extra-floral nectars, sap, honeydew, rotting or damaged fruits and leaves and even discarded plant materials such as sugar cane trash, but the most commonly recorded sugar sources for mosquitoes are floral nectars (Foster, 1995). Some mosquito species were also reported to probe plant tissues for nutrients (Abdel-Malek, 1964; Schlein and Müller, 1995; Müller and Schlein, 2005). Laboratory experiments have identified possible nectar sources of mosquitoes using radioisotopes-labeled and unlabeled plants (Abdel-Malek, 1964; Patterson *et al.*, 1969), but natural mosquito-plant relationship is validated exclusively by direct observation in the field, where the mosquito species usually visits the nectar producing species (McCrae *et al.*, 1969, 1976; McCrae, 1968, 1989; Magnarelli, 1977, 1978; Anderson and Jaenson, 1987). However, for *Anopheles gambiae*, specific plants where they feed on are still unknown.

c. Host finding

Sugar feeding on plants by mosquitoes can be diurnal, nocturnal or crepuscular and occurs with a characteristic, often bimodal, diet periodicity (Andersson and Jaenson, 1987, Yee and Foster, 1992). Whether feeding activity involves a distinct nectar-seeking period is not known, but sugar is not contacted by chance. It has been shown that some mosquitoes of the genus *Anopheles*, *Culex* and *Aedes* have nonspecific appetitive feeding periods (Yee and Foster, 1992), and that the order in which sugar and blood are sought largely may be a

function of factors other than differences in diel rythms. But *Ae. aegypti* exhibited a biphasic diel cycle of nectar-feeding behaviour and *An. arabiensis* a monophasic diel cycle (Healy and Jepson, 1988). These observations show that host finding is specific to mosquito species.

d. Source preference

The reasons of nectar-host preferences are speculative. The available research strongly suggests that a combination of visual and olfactory cues likely mediate mosquito plant - feeding behaviour. It is therefore clear that most of the plants utilized by mosquitoes have attractive characteristics. Among conceivable benefits of feeding on certain plants are sugars concentration and composition in the nectar, and floral structure. Female mosquitoes prefer flowers with hexose-rich nectar, whereas males prefer sucrose-rich nectar (Hancock and Foster, 1989). Certain types of plants may also provide architectural advantages for mosquitoes. Small flowers in large clusters create an easily visible landing and feeding platform (Foster, 1995). Nectar-host flowers of mosquitoes are pale colored or white (Gadawski and Smith, 1992). Mosquitoes also respond to extracts and odours of flowers, honey, synthetic fragrances, and raw fruit in various static air and wind tunnel olfactometers (Bowen, 1992; Healy and Jepson, 1988; Hancock and Foster, 2000), in bagged flowers in the field (Brantjes and Leemans, 1976), and in field traps (Reisen *et al.*, 1986, Kline *et al.*, 1990). Strongly fragrant flowers often have more mosquitoes feeding on them (Sandholm and Price, 1962). The rules by which females make food-choice decisions have been inadequately explored, and there is still lack of convincing evidence that sugar availability in nature varies sufficiently to affect mosquito populations (Foster, 1995). Whereas the role and chemical nature of plant volatiles in the sugar-feeding behaviour of various mosquito species is well

understood, it is less clear to what extend the malaria vector *An. gambiae* use nectar-derived cues.

2.4.3.7.2 Sugar feeding and mosquito fitness

a. Survival

Newly emerged adult mosquitoes provided with only water can live for only a few days on their energy reserves (Andersson, 1992; Briegel, 1990a; Briegel, 1990b). Each sugar meal or blood meal extends life by days or even weeks, depending on its total caloric value and the amount allocated to reserves. Naturally occurring, nutritive sugars supporting long life spans include glucose, fructose, sucrose, maltose and melezitose; other sugars support shorter life spans or have no effect (Nayar and Sauerman, 1971). Laboratory studies (Gary and Foster, 2001; Okech *et al.*, 2003, 2004) found that *An. gambiae* females live longer when sugar is available. Even other mosquito species have been shown to survive longer when sugar is available (Day *et al.*, 1994). The survival of *An. gambiae* was also high when they fed on specific plants (Gary and Foster, 2004, Impoinvil *et al.*, 2004). It is hypothesized that *An. gambiae* selects plants which it feeds on in nature. But, if true, whether selective feeding affects mosquito survival is still unknown.

b. Fecundity

The direct effect of sugar and energy reserves on male reproduction has not been investigated. For females however, numerous studies have examined direct effects on the reproductive system: the smaller the energy reserve, the larger the blood meal required to initiate egg development (Edman and Lynn, 1975). *Anopheles gambiae* females without access to sugar have a higher daily fecundity (Gary and Foster, 2001), which is similar to the

findings of Scott *et al.* (1997) and Costero *et al.* (1998) in *Ae. aegypti*, another anthropophilic and endophilic species. But the question whether sugars are essential for egg maturation in these species remains to be solved. Sugar availability in the laboratory often delays the onset or completion of oviposition in gravid females, effectively reducing fecundity (Klowden and Dutro, 1990; Loubinos and Conn, 1991). But the relevance of this effect in natural conditions is still unknown. The reasons for the sugar inducing delay are speculative. Up to date, there is no information about the role of natural sugar meals on the fecundity of *Anopheles* mosquitoes, even for the important malaria vectors. Therefore, the effect of plant sugars on the reproductive fitness of malaria vector *An. gambiae* need to be investigated.

c. Vector competence

In malaria vectors, energy depletion could come about either as a result of a direct competition for resources between *Plasmodium* and the infected mosquito (Maier *et al.*, 1987) or because infected mosquitoes require extra nutrients to compensate for parasite damage (example tissue repair) or to fuel the mounting of a costly immune response (Ahmed *et al.*, 2000). Rivero and Ferguson (2003) found that *An. stephensi* infected with the oocyst stages of the virulent genotype of *P. chabaudi* contained up to 50% more sugar (glucose) than the control or *Anopheles* infected with avirulent genotype of the same parasite. The high level of sugar found in these mosquitoes arose from increased sugar intake subsequent to infection, in response to parasitism. Whether the increased intake benefited the mosquito or the parasite is unclear. By increasing the consumption of sugar, which are important precursors of all carbon-based chemical compound and an essential resource for maintenance in mosquitoes (Clements, 1999), infected mosquitoes could minimise or compensate for the harm caused by the parasite, such as damage to midgut epithelial cells, and competition for

host metabolic products (Maier *et al.*, 1987). However, in nature, *Anopheles* mosquitoes acquire sugars from plants. When feeding on nectar or any other plant juice, they do not only take sugars, but other components of the plants as well. There is therefore an urgent need to investigate the effects of various plant substrates *Anopheles* mosquitoes feed on in the wild on the parasite development in their midgut. This seems important because infected mosquitoes may feed on certain plants to relieve themselves from parasite, especially as *Plasmodium* infection reduces the fitness of anophelines (Hurd, 2003).

2.5 Malaria control

In general, the malaria situation is deteriorating worldwide, largely as a result of rapid selection for parasite resistance to drugs and vector resistance to insecticides (Snow *et al.*, 1998; Winstanely, 2000; Service, 1980; Brooke *et al.*, 2000). Malaria control can be defined as all the measures supposed to reduce morbidity and mortality due to malaria. Following failure of eradication campaign launched by WHO in 1957, WHO (1993) proposed the following key basic technical elements for the global malaria control strategy for reducing malaria mortality and morbidity.

2.5.1 Chemotherapy

Prompt diagnosis and treatment is recommended in the management of malaria. Chloroquine was the drug of choice for many years for the treatment of acute attack of malaria of all species (Kreier, 1980). However, due to widespread of chloroquine resistant *falciparum* malaria, and emerging resistance to sulfadoxine-pyrimethamine, combination of two or more antimalarials with different mechanisms of action is now recommended in many countries

(WHO, 2006). Artemisinin-based combination therapy (ACTs) are the recommended treatments for uncomplicated *falciparum* malaria, artesunate, artemether and quinine for severe malaria (WHO, 2006). But these new treatments are substancially more expensive and not accessible to patients from many endemic countries.

2.5.2 Vector control

The vector control options that are currently available are: indoor residual and space spraying, biological control, larviciding, environmental management, and personnal protection (WHO, 1995).

2.5.2.1 Indoor residual house and space spraying

In the past, the use of chemical insecticides such as DDT and organophosphate insecticides for indoor residual house spraying or space spraying was the method of choice for mosquito control. However, because of the appearance of mosquitoes resistant to insecticides (Roberts and Andre, 1994; Chandre *et al.*, 1999; Hargreaves *et al.*, 2000), coupled with long-term detrimental effects of chemical insecticides to non-target organisms and the environment (Attaran and Maharaj, 2000; Zaim and Guillet, 2002), the past successes are now eroded.

2.5.2.2 Larviciding and biological control

Both larvicides and biological control are usually used for larval control. Larval control with either chemicals such as temephos, phenitrothion, chlopyrifos or biological agents such as *Bacillus thuringiensis var israelensis* de Berjac and *Bacillus sphaericus* Neide is a relevant method of vector control if high proportion of breeding sites are accessible and of

manageable sizes (WHO, 1995). It has a major advantage in the control of mosquitoes before they disperse and transmit diseases (Killeen *et al.*, 2002).

2.5.2.3 Environmental management

Environmental management approaches to vector control aim at modifying the environment to deprive the target vector population of its requirements for survival. This reduces human-vector contact and renders the conditions less conducive for disease transmission (WHO, 1995). Anti-larval measures of control are of particular value when employed in conjunction with other means such as environmental management (Bruce-Chwatt, 1985). The chance of rolling back malaria could be substancially increased if environmental management is integrated with pharmacological, insecticidal and bednet interventions (Utzinger *et al.*, 2001).

2.5.2.4 Personnal protection: Insecticide treated bednets, Repellents, Protecting clothings

Insecticide-treated bednets protect individuals by either diverting host-seeking vectors to look for a blood meal elsewhere or by killing those that attempt to feed on that person (Killeen *et al.*, 2000). Treated nets can therefore also prevent malaria in unprotected individuals by suppressing vector numbers (Killeen *et al.*, 2002). At present, insecticide treated bednets have been widely advocated as a means of personal protection against malaria vectors in Africa, but give no protection against anopheline mosquitoes in the evening before people go to bed. The results of individual studies often differ and although some trials with African vectors have demonstrated substancial reductions of vector density, survival and sporozoites prevalence (Carnevale *et al.*, 1988; Magesa *et al.*, 1991), others have

found little or no effects on the vector population as a whole (Lindsay *et al.*, 1993; Quinones *et al.*, 1998). One reason of the limited efficacy of insecticide treated bednets may be due to infective bites received outside the nets (Lindsay *et al.*, 1998). The use of insect repellents has been considered as an alternative method where other conventional vector control methods are not feasible (Gupta and Rutledge, 1994). Insect repellents have an important role in regions where mosquito vectors bite in the early evening since people are often not yet sleeping under bednet at this time. Physical barriers such as long clothing are unpopular in the tropics since the evenings are warm.

Despite the application of all these control methods listed above, malaria situation is worsening. There is therefore an urgent need to improve, modify or integrete the existing tools and develop new ones. This will only be possible by a thorough study of the biology and all the basic behaviours of malaria vectors, and knowledge acquired will be used to fight them.

CHAPTER THREE: GENERAL MATERIALS AND METHODS

3.1 Study area

The study was carried out in Suba District, located along the shores of Lake Victoria in western Kenya, Nyanza Province, a region where malaria is holoendemic. The residents are fishermen and/or traditional farmers who live adjacent to the lake due to accessibility of fresh water. Communities belong to the "Luo" ethnic group, and the predominant economic activities are fishing and farming with the main crops being maize, sorghum and millet. Most of the houses are made of mud and wattle walls roofed with corrugated iron sheets, with 4 to 5 occupants per house. The major mosquito vectors are, besides *An. gambiae s.s.* Giles, *An. arabiensis* Patton, and *An. funestus* Giles (Petrarca *et al.*, 1991; Minakawa *et al.*, 1999; Mutero *et al.*, 1998) which yield an exposure rate ranging between 0 and 53.8 infectious bites per person per month (Shililu *et al.*, 2003). In Suba, *P. falciparum* malaria is a leading cause of morbidity, accounting for 50% of all illness clinically diagnosed at the local health centre (Gouagna *et al.*, 2004). There are two rainy seasons at Suba, the long rains (March to May) and the short rains (October to November). Mean annual rainfall is 700-1200 mm and, means minimum and maximum daily temperature are 17°C and 34°C respectively. Permanent, semi-permanent and temporary breeding sites are widespread. Vegetation consists of a wide variety of indigenous and introduced plants, flowers, shrubs, trees and bushes growing especially along the Lake shores, the roads and human habitations. Around houses, an average of 10 very common flowering plants is found. These included herbacious plants *Ipomoea hildebrandtie*, *Senna didymobotrya*, *Datura stramonium*, *Psiadia punctulata*, *Senna bicapsularis*, *Cassia hirsuta*, *Flaveria trinervia*, and *Pathernium hysterophorus*. Two

common tropical/subtropical shrubs were also found: *Lantana camara*, and *Ricinus communis*. In addition, four ubiquituous plants are found around larval habitats: *Pathernium hysterophorus*, *Thitonia diversifolia*, *Senna bicapsularis* and *Senna didymobotrya*. These plants might serve as nectar sources for local mosquitoes. One major advantage of this locality for studies of plant feeding is that the mosquito breeding continues along the lake shores and low area of seepage inland throughout the dry season, where vegetation remains verdant.

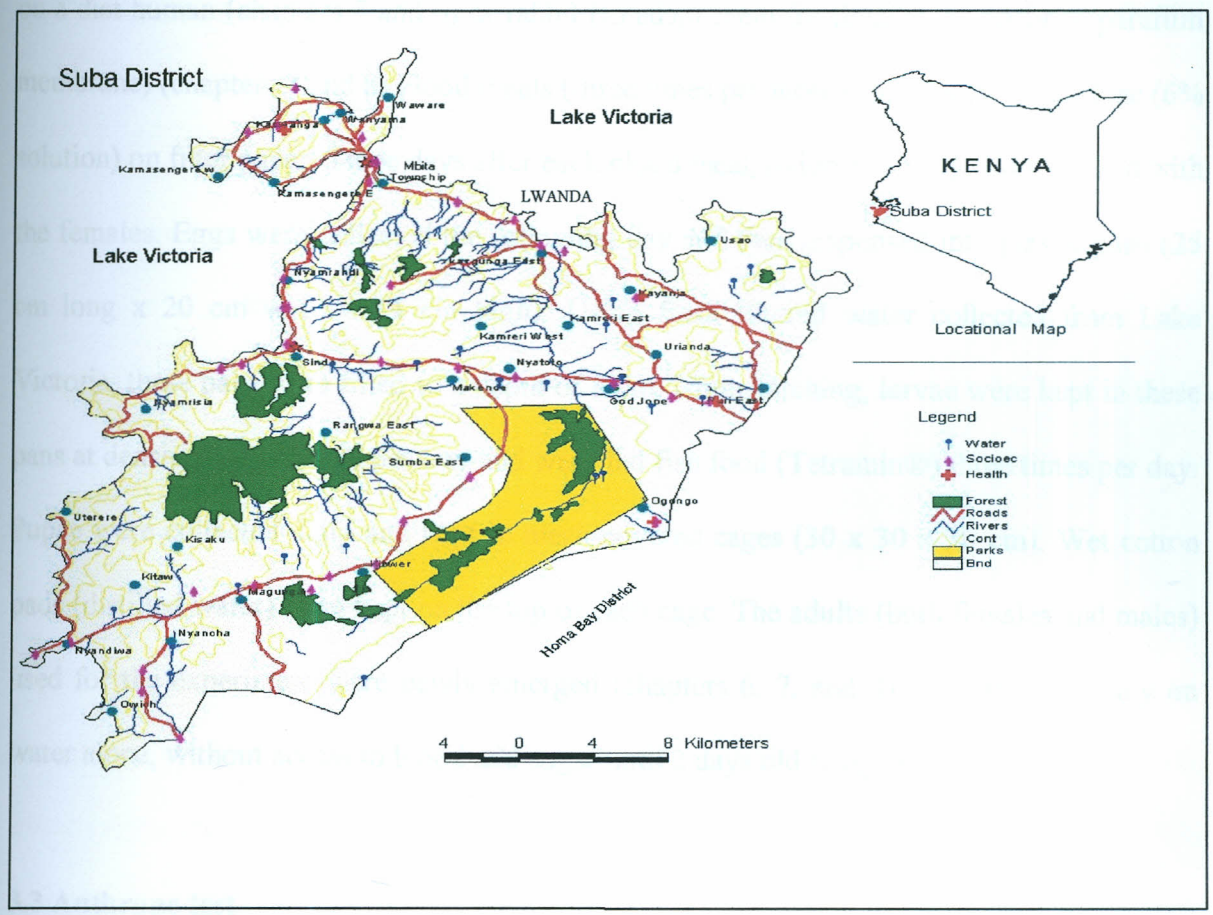


Fig. 3.1 Study area: Suba District (inset: map of Kenya showing position of Suba District)

3.2 Mosquito colony and rearing

All laboratory and semi-field tests were carried out with the laboratory-reared *An. gambiae s.s* maintained at the Thomas Odhiambo Campus of the International Centre of Insect Physiology and Ecology (ICIPE), Mbita Point (00°25'S, 34°13'E). The colony was originally initiated from the adults collected in 1996 at Njage village (70 km from Ifakara) southeast Tanzania (chapter 5) and from adults collected from Mbita Point (Chapters 6, 7 and 8). All individuals were reared at ambient temperature and humidity, with adults being maintained on a diet human (chapters 5 and 6) or rabbit (to adapt them for feeding on artificial parafilm membrane) (chapters 7 and 8) blood meals (three times per week), and continual glucose (6% solution) on filter paper. Three days after each blood meal, oviposition cups were placed with the females. Eggs were collected the following day and then dispensed into plastic pans (25 cm long x 20 cm wide x 14 cm high). Using fresh filtered water collected from Lake Victoria, these pans were filled to a depth of 8 cm. Upon hatching, larvae were kept in these pans at densities of 100-150 per tray and were fed fish food (Tetramin®) three times per day. Pupae were collected daily and kept in mesh-covered cages (30 x 30 x 30 cm). Wet cotton pads (distilled water) were kept on the top of each cage. The adults (both females and males) used for the experiment were newly emerged (chapters 6, 7, and 8) or maintained only on water alone, without access to blood and sugar until 2 days old (chapter 5).

3.3 Anthrone test

Cold Anthrone test (Van Handel, 1972) was used in Chapters 4, and 5 to determine the presence of fructose (main monosaccharide unique to plant sugar) in the mosquito crop. Therefore, 380 ml of concentrated sulfuric acid was carefully poured into 150 ml distilled

water. Then 150 mg of anthrone powder was mixed with 100 ml of the diluted sulfuric acid (fructose reagent). Mosquitoes were individually crushed in a 10 x 75 mm test tube, each containing 0.5 ml of the fructose reagent (Chapter 4) or a portion of the mosquito homogenate crushed in distilled water was put in a 10 x 75 mm test tube plus 0.5 ml of the fructose reagent (Chapter 5) and incubated for 60 min at room temperature (25°C). The positivity was assessed by visual inspection at the end of the incubation to observe the change of color to green or blue. The test results were categorized into light, medium and heavy, depending on the intensity of reaction, hence the amount of nectar. If the reagent color remains unchanged in about an hour, the test was considered negative.

3.4 Gas chromatography (GC) analysis of plant and mosquito extracts

In order to understand the specificity of *An. gambiae*/plant interactions (Chapter 5) and determine the composition and concentration of sugars in plants (Chapters 5 and 6), GC screening of plant carbohydrate-components including mono-, di-, and trisaccharides was performed on candidate plants tested and mosquitoes.

3.4.1 Preparation of plants for gas chromatography (GC)

Samples of leaves, flowers and stems were individually incubated in darkness in small cryo-preservation vials containing 200 µl 50% EDTA solution for 2 h, after which the plant material was removed and the extracts remaining in the vials were allowed to dry out completely under gaseous nitrogen.

3.4.2 Standards

Nine standards, α-D-glucose, D(+) raffinose, D(+) galactose, D(+) mannose, β D(-) fructose, sucrose, D-gulose, D-altrose, D-allose were prepared. In addition, α-lactose was included as a check of the analytical procedure. They were purchased from Sigma-Aldrich Co. Ltd (Poole, Dorset, UK).

3.4.3 Trimethylsilylation of standards, plant sugars and mosquito extract.

The technique adopted has been previously described by Hamilton and El Naiem (2000). In brief, sugar standards, plant and mosquito extracts were trimethylsilylated to allow their analysis by gas chromatography (GC). This was essential because GC analysis of sugars requires initial silylation to derive the highly polar carboxyl and hydroxyl groups. Using a clean 2-ml reacti-vial, the sugar standards were trimethylsilylated by dissolving 1 mg of each in 100 µl of dry pyridine. An equal volume of the derivitizing reagent N-methyl-N-trimethylsilyltrifluoroacetanamide (MSTFA) was then added. The mixture was placed in an oven set at 60°C for 60 min, after which the sample was removed from the oven and stored at room temperature until analysis. Analysis was carried out on sugar extracts from each plant, prepared in the same way as the standards, except that plant extracts were allowed to dry out completely under a flow of nitrogen before a volume of 100 µl of pyridine and MSTFA was added to 1-2 mg (dry weight) of tissue extract. Extracts from individual mosquitoes were transferred in a 2-ml reacti-vial and macerated with 50µl pyridine. The samples were then placed in a fume hood and allowed 12 hr to dry completely at room temperature. Dry pyridine (2 µl) and MSTFA (2 µl) were then added in each vial and the reaction allowed to proceed at 60°C for 60 min.

3.4.4 Sample analysis

After trimethylsilylation, analyses were performed on a Hewlett Packerd (HP) 5890 series II gas chromatograph, equipped with a split-less injector system, a 50 m x 0.2 mm (i.d.) crossed-linked methylsilicone (0.33 µm film thickness) capillary column, and FID coupled to HP 3393 A Series II integrator. The carrier gas was nitrogen, with flow set at 0.005 ml/min. The initial temperature (100°C) was increased 30°C/min until 170°C and then 2°C/min until 210°C, followed by 50°C/min until a final isothermal temperature of 280°C that was maintained for 30 min.

The derivitized sugar standards were diluted by a factor of 40 with dichloromethane (DCM 99.9+ %, PRA grade) and the derivitized plant sugars were diluted by the same solvent (3 parts of the reactive mixture and 1 part of the solvent). Derivitized extracts from mosquitoes were analyzed without dilution. One microlitre of each sample was injected into the gas chromatograph. DCM was used as solvent to clean syringes between samples. It was concluded that the sugar meal of the mosquito had been identified when the profiles of extracts from mosquitoes matched the sugar profile of extracts from particular plant species. Plant-derived sugars were identified by comparing retention times of sugar standards with those present in the plant extracts. Quantification of different plant sugars (Chapter 6) was done by using the peak area of each sugar standard with peak area of plant sugars and all the dilution factors into the following formula: (Peak area of sample/Peak area of standard) x (quantity of sugar standard injected).

3.5 Experimental infection of females *An. gambiae*

To study the effects of plants on the vector competence of *An. gambiae* for *P. falciparum*, mosquitoes held on different plant species were experimentally infected with gametocyte positive blood (Chapters 7 and 8).

3.5.1 Detection of gametocyte carriers

Plasmodium falciparum gametocyte carriers were detected during survey of apparently healthy children attending nursery and primary schools in the communities in Suba District. Prior to the survey, the school head teachers and the community leaders were informed of the purpose of the study and communicated the information to the parents through a meeting, a letter and through their children. During cross-sectional parasitological surveys, finger-prick blood samples were taken from which thick blood films were made. Blood smears were Giemsa-stained and examined microscopically for the presence of asexual and sexual stages of the malaria parasite *P. falciparum*. All individuals with asexual parasitemia (>1000 parasites/µl) were treated with Sulfadoxine-pyrimethamine (Fansidar®). *Plasmodium falciparum* gametocytes were counted in microscopical fields that cumulatively contained 500 leucocytes and an estimate of gametocyte density was obtained by assuming a standard number of 8000 leukocytes/µl of blood. Although gametocyte densities in the venous blood and the peripheral finger-prick blood might differ, potential differences were assumed to be proportional among the carriers.

3.5.2 Exclusion criteria

Exclusion criteria from the study included mixed-species infections, any symptoms indicating severe clinical malaria or other concomitant diseases requiring hospitalization or follow-up, anaemia, and children less than 3 years old because of concerns of the vulnerability of these patients to treatment delay.

3.5.3 Ethical consideration

The Kenyan, United States National Institutes of Health (NIH) and University of Miami ethical review committees approved the recruitment procedures reported in this study. Volunteer *P. falciparum* gametocyte carriers were enrolled in the study upon signing an informed consent form (appendix 1). All children unable to give consent were volunteered to participate by parents or guardians. Each volunteer was taken to the health centre for clinical check up and given appropriate treatments.

3.5.4 Membrane feeding procedure

Three-day-old mosquitoes were aspirated from each cage and put in paper cups labeled according to the plant or food regime. Mosquitoes were starved at 07:00 hrs in the morning for 3-8h (depending on ambient relative humidity) prior to feeding on gametocytaemic blood. For experimental infections, the clinical officer or health technician withdrew 4 ml of venous blood from each volunteer and transferred it into heparinized tube. This blood sample was then dispensed into glass mini-feeders (fig. 3.2) wrapped in Parafilm® membrane, pre-warmed to 37°C (to prevent premature exflagellation of gametocytes) and immediately given to mosquitoes as previously described (Fig. 3.3) (Tchuinkam *et al.* 1993). Mosquitoes were

allowed to feed for 15 min after which the fed and unfed mosquitoes were sorted. The fed mosquitoes were released in their respective cages and offered their respective plants. The whole process from blood draw, infection and release of mosquitoes to their respective plants took approximately 1h.

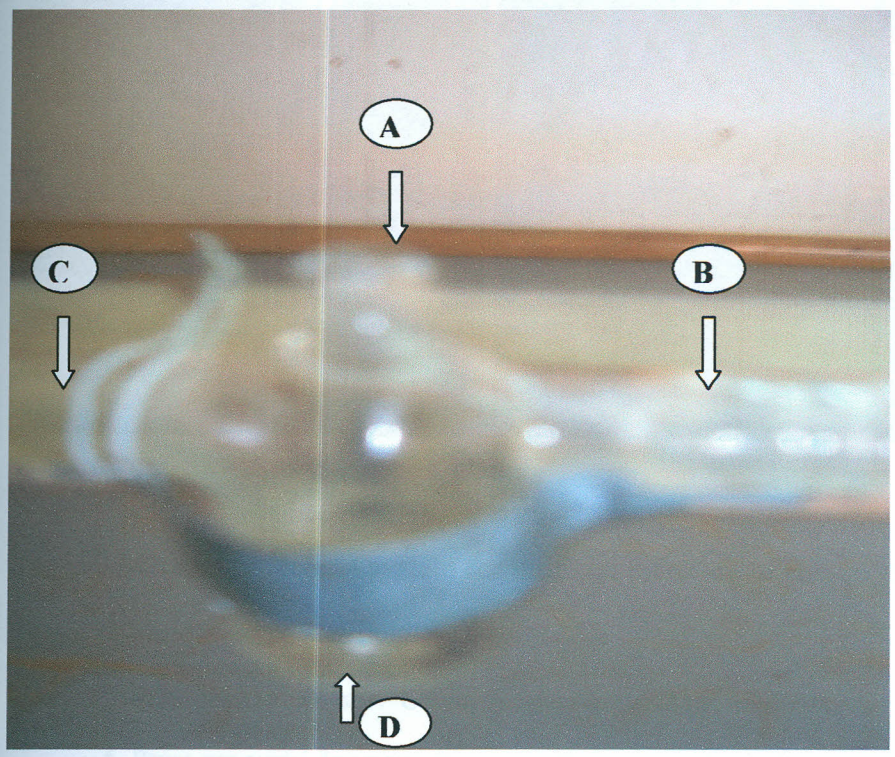


Fig. 3.2 Mini-glass feeder for artificial infection of mosquitoes

Legend A: Entry point for blood
B: Entry of water at 37°C
C: Exit of water at 37°C
D: Blood retention chamber

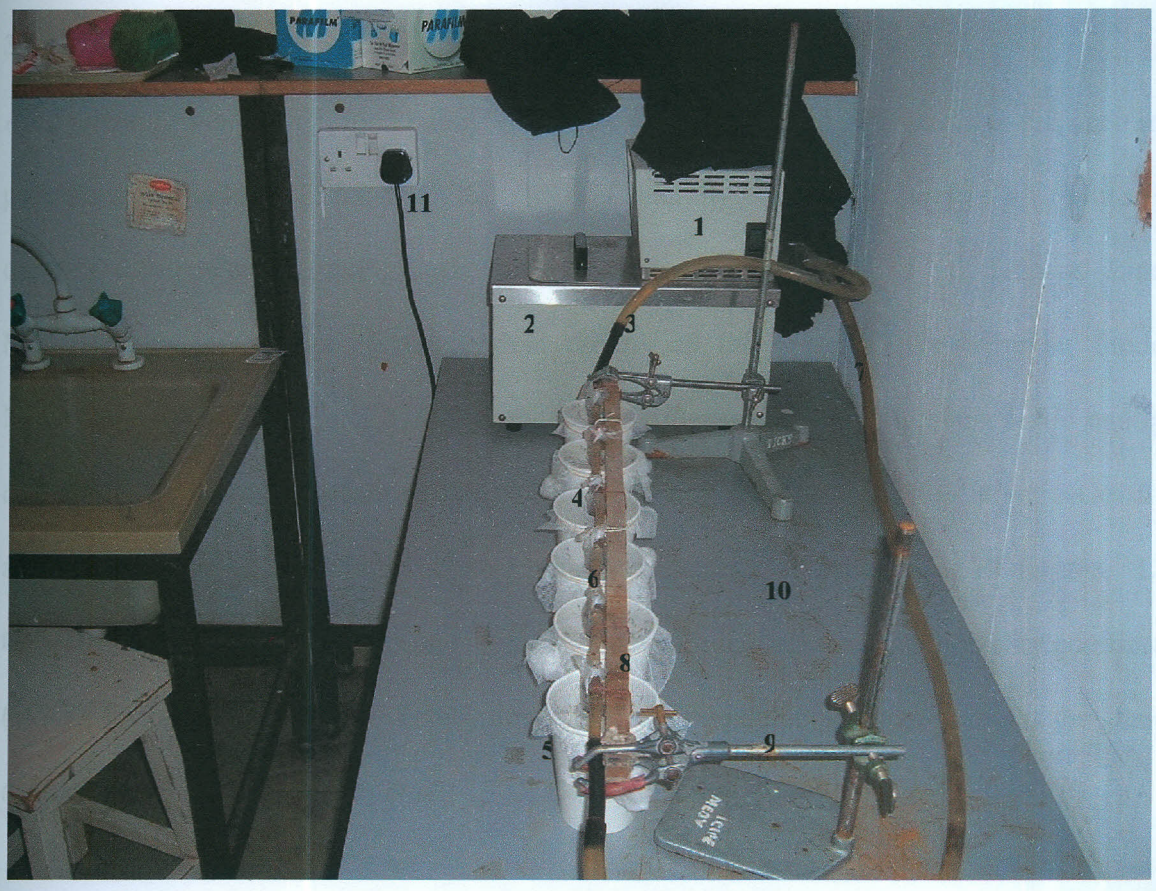


Fig. 3.3 Membrane feeding system for artificial infection of mosquitoes

Legend: 1- Electric temperature controller
2- Electric water bath
3- Pipe for water exit at 37°C from the glass-feeder to the water batch
4- Mini-glass feeder
5- Paper cup containing mosquitoes
6- Pipe connecting glass-feeders to allow continuous water flow in the feeders
7- Pipe for water entry at 37°C to the glass-feeder from the water bath
8- Wooden stick for support of feeders
9- Metallic ramp for suspension of feeders
10- Table
11- Connection to electric power

3.6 Data analysis

All processing were performed using Microsoft Excel 2000 and SAS version 8.2 computer package for Windows (SAS Institute, 1999). Generally, Chi-square analysis was used to quantify difference between groups, analysis of variance (ANOVA) and General Linear Model to test the impact of independent factors on the output, Tukey-Kramer, Student Newmans Keuls (SNK) and Least Significant Difference (LSD) were used for multiple comparisons of means. Spearman and Pearson correlations were used to determine level of association between factors and to determine the linearity of dependance of factors. Life table analysis was used to determine inter-stage parasite mortalities. The mortality coefficient (k) for parasites was computed as they developed from gametocytes to oocysts via the gamete and ookinete stages (Vaughan *et al.*, 1992; Gouagna *et al.*, 1998).

CHAPTER FOUR: PLANT-FEEDING PRACTICES OF WILD *ANOPHELES GAMBIAE* POPULATIONS IN A MALARIA ENDEMIC AREA OF WESTERN KENYA

4.1 Introduction

Adult mosquitoes of both sexes require sugar which provides energy for somatic functions, flight, and reproduction (Yuval, 1992; Foster, 1995). For males of nearly all species, sugar appears to be essential for them to achieve more than minimal mating activity and early death. For females, many anautogenous species survive longer and/or produce more eggs with sugar as well as vertebrate blood in their diet (Nayar and Pierce 1980, Gary and Foster 2001, Okech *et al.*, 2003). They also use sugar as a ready source of flight energy (Clements, 1955; Nayar and Van Handel, 1971) and as nutrients to allow the ovarian follicles of small females to reach the gonoactive resting stage (Feinsod and Spielman, 1980; El-Akad and Humphreys, 1990). But the frequency with which these sugars are taken varies greatly among mosquitoes species, within sexes of the same species, on availability of specific sugar sources in nature (O`Meara, 1987; Yuval, 1992; Foster, 1995; Martinez-Ibarra *et al.*, 1997), and may also depend on mosquito physiology and environmental factors. The main source of sugar for mosquitoes is generally believed to be floral nectar, followed by extrafloral nectaries, with honeydew, tree sap, rotting fruits, and sugar cane utilized only rarely (Foster, 1995).

In females of some mosquito species, sugar feeding commences soon after emergence, before the first blood meal, and continues after blood feeding during eggs maturation (Vargo and Foster, 1984; Andersson, 1990; Bowen *et al.*, 1994). *Aedes aegypti* (L.) seems to have

developed an accomodative behaviour in relation to plant-feeding. Females feed on plant sugar, but their intake varies under different environmental conditions (Martinez-Ibarra *et al.*, 1997).

Anopheles gambiae s.l., an important malaria vectors in Africa, feed on plant sugar in nature (Beier, 1996), but factors influencing their plant-feeding behaviour are unknown. Although much research has been done on the reproductive physiology of mosquitoes in the genera *Culex* and *Aedes* in relation to sugar feeding, relatively little is known about *Anopheles* species, even the major African malaria vectors. To understand the plant-feeding practices in wild *An. gambiae* populations, the plant-sugar contents of the field collected *Anopheles gambiae s.l.* was assessed in a malaria endemic area of western Kenya. Specifically, (1) the proportion of fructose-fed females, and (2) the relationship between mosquito dispersal, seasons, mosquito physiology and plant-feeding occurrence were determined.

4.2 Material and Methods

4.2.1 Study area

The study was conducted in Lwanda village (S 00° 28.621' and E 034° 17.331'), located at 12 Km from Thomas Odhiambo Campus of the International Centre of Insect Physiology and Ecology (ICIPE-TOC), Mbita Point; the field station of ICIPE, at the shores of Lake Victoria, Suba District, Nyanza Province, western Kenya (Fig. 3.1). The study village has an altitude of 1169 m above sea level. Malaria vector populations, environmental and climatic conditions are similar to those described in the study area of chapter 3.

4.2.2 Mosquito sampling

The sampling of mosquitoes was carried out during the dry season (January-March 2003) when plant growth and flowering around human habitations were supposed to be sparse, and during the rainy season (April-May 2003), when plants were in growth and flowering phases, so that the sampling could incorporate the variety of conditions which would be expected to influence the plant-feeding behaviour. Sampling was done using aspiration catch (WHO, 1975). Resting mosquitoes were collected in the morning (7.00 am to 9.00 am) inside houses in Lwanda village. Mosquitoes were collected using mouth aspirators with the aid of flash light and kept in each separate labelled paper cups for each house group. Eight sentinel houses were selected randomly across the village and the distance to the nearest larval habitats estimated. Candidate houses were then divided in 2 groups: four located near the larval habitats (< 200 m) and four far from the larval habitats (> 200m). After collection, mosquitoes were immediately killed with chloroform, and put into a cold chest containing frozen ice pack, to avoid digestion of sugar, and brought to the laboratory for processing.

4.2.3 Laboratory processing of mosquitoes

Mosquitoes collected were morphologically identified into species using the Gillies and De Meillon (1968) key. Only females *An. gambiae s.l.* were considered for the scope of this study. For *An. gambiae s.l.* identified, wings and legs of individual mosquitoes were removed and preserved in eppendorf tube filled with silcagel, labelled according to each mosquito number and kept in the freezer (-20°C) for later polymerase chain reaction (PCR) identification of species of the *An. gambiae* complex. The gonotrophic status of each mosquito was recorded by visual assessment under a dissecting microscope into 4 groups: unfed, blood fed, half-gravid and gravid (Bruce-Chwatt, 1985) and recorded in data sheets.

Their parity rate was also recorded by dissection of ovaries for appearance of coiled tracheoles (skeins) and presence of dilatations (Detinova, 1962). Mosquitoes were then individually tested the same day for the presence of fructose (the main monosaccharide unique to plant sugar) in the crop, which indicates recent plant feeding using a Cold Anthrone test (Van Handel, 1972) as described in Chapter 2.

4.2.4 PCR analysis of *An. gambiae s.l.*

Samples of the *An. gambiae* complex collected were identified to species using PCR following the protocol developed by Scott *et al.* (1993).

4.2.4.1 DNA Extraction

Wings and legs of each mosquito sample were homogenized using pestles in 1.5 ml eppendorf tube containing 100µl of grinding buffer (0.08 M NaCl, 0.06 M EDTA, 0.10 M Tris-HCl, 0.05% SDS, distilled water), and incubated in water bath at 65 °C for 30 minutes. In each sample 14 µl of 8 M potassium acetate was added and incubated on ice for 20 minutes. The samples were then centrifuged for 10 minutes at 13000 rpm. The supernatants were then pipetted out and transferred to corresponding 1.5 ml tubes containing 95% ethanol, and incubated at - 70°C for 2-3 hours or at -20 °C overnight. The samples were span at 13000 rpm for 20 min, the supernatant decanted and washed with 200 µl of 70 and 95 % ethanol and spinned for 10 and 5 min, respectively. The supernatants were discarded and the DNA samples were air dried and suspended in 100 µl of Tris-EDTA buffer pH 8.0 (Scott *et al.*, 1993).

4.2.4.2 DNA amplification and Electrophoresis

Extracted DNA template was suspended into 100 µl of Tris-EDTA. The PCR reaction mix was prepared in a total volume of 14 µl as follow: 10 x PCR buffer (1.5 µl), 5mM of dNTP-mix (0.6 µl), 2.5 mM $MgCL_2$ (1.8 µl), Taq^{TM} (0.075 µl), 12.5 ng ml^{-1} universal primer (0.6 µl), 6.25 ng ml^{-1} *An. gambiae* primer (0.6 µl), 18.75 ng ml^{-1} *An. arabiensis* primer (0.6 µl), 12.5 ng ml^{-1} *An. merus* primer (0.6 µl), 25 ng ml^{-1} *An. quadriannulatus* primer (0.6 µl); 1 mg ml^{-1} BSA (1.5 µl), plus ddH_2O (5.525 µl).

PCR reaction mix (14 µl) was distributed into PCR tubes. DNA template of 1 µl was added to the corresponding PCR mix and labelled. The samples were loaded into PCR machine with the controls. The PCR products (15 µl of PCR product containing 3 µl of agarose dye) were electrophorosed at 180 v in an electrophoresis unit containing TAE (running buffer), and stained with ethidium bromide for 20 minutes. The lanes were visualized and scored.

4.2.5 Data analysis

Data were analyzed using Statistical Analysis System (SAS, version 8.2). Chi-square test was used to compare the proportions of mosquitoes containing fructose among the different species, sites of collection, seasons and physiological status and age. For all tests, α was 0.05.

4.3 Results

4.3.1 Proportion of plant-feeding *Anopheles*

A total of 870 *Anopheles s.l.* was collected from January-May 2003 in the study area, in which 307 (35.3%) were *An. gambiae s.s.*, and 563 (64.7%) were *An. arabiensis*. Out of the *Anopheles* collected, 109 (12.5%) were positive for fructose. There was no significant difference in the proportion of fructose positive females for all the two species ($\chi^2 = 0.01$, df = 1, P = 0.90); 12.7% (39/307) fructose positive *An. gambiae s.s.* and 12.4% (70/563) fructose positive *An. arabiensis*.

4.3.2 Seasonal variation in plant-feeding

A total of 657 mosquitoes were collected during the wet season and 213 during the dry season. The proportion of fructose-positive females (i.e., species total) was similar between the 2 seasons ($\chi^2 = 3.71$, df = 1, P = 0.06). Figure 4.1 shows the proportion of fructose-positive females of each *Anopheles* species per season. Females of *An. gambiae s.s.* sugar-fed equally on plants during the dry and wet seasons ($\chi^2 = 0.04$, df = 1, P = 0.84). The same was found for *An. arabiensis* ($\chi^2 = 1.93$, df = 1, P = 0.16).

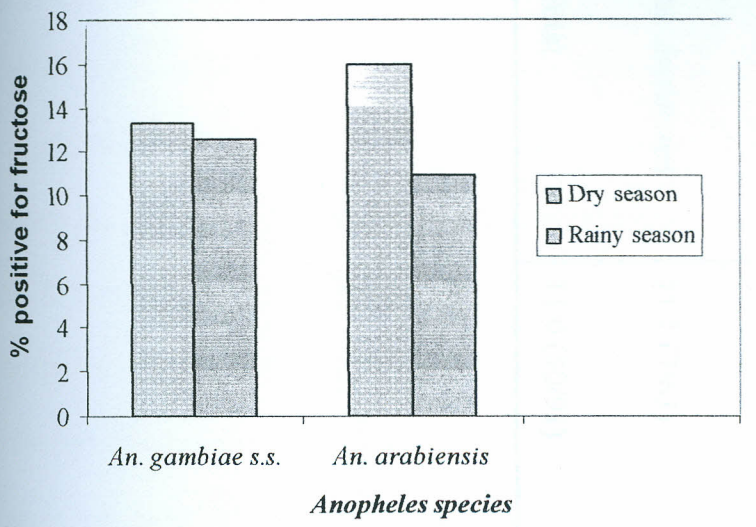


Fig. 4.1 Percentages of mosquitoes positive for fructose per *Anopheles* species during dry and rainy seasons.

4.3.3 Effect of *Anopheles* dispersal on plant-feeding

A total of 615 mosquitoes were collected in houses located less than 200 m from the larval habitats and 255 mosquitoes were collected in houses located more than 200m from larval habitats. The percentages of fructose positive females per species and per position are presented in Table 4.1. The proportion of fructose-positive females was significantly higher when mosquitoes were sampled in houses located more than 200 m from larval habitats ($\chi^2 =$ 9.99, df = 1, P = 0.001). Similarly, for each of the mosquito species, the percentage of fructose positive females was significantly higher in mosquitoes collected more than 200m from larval sites compared to those collected less than 200 m ($\chi^2 = 4.12$, df = 1, P = 0.01; $\chi^2 =$ 7.98, df = 1, P = 0.004) for *An. gambiae s.s.* and *An. Arabiensis* respectively. The same trend was observed during both seasons in all the two *Anopheles* species (Table 4.1).

Table 4.1 Percentages of mosquitoes positive for fructose in each *Anopheles* species, collected less than 200m from larval habitats and more than 200m from larval habitats during dry and wet seasons.

Anopheles *species*	*Percentage fructose-positive mosquitoes (%)*					
	Dry season		Wet season		Dry +Wet seasons	
	< 200m	> 200m	< 200m	> 200m	< 200m	> 200m
An. gambiae s.s.	10.7 (3/28)	18.7 (3/16)	11.1 (22/199)	17.1 (11/64)	11.0 (25/227)	17.5 (14/80)
An. Arabiensis	11.1 (7/63)	17.9 (19/106)	9.5 (31/325)	18.8 (13/69)	9.8 (38/388)	18.3 (32/175)
Total	11.0 (10/91)	18.0 (22/122)	10.1 (53/524)	18.0 (24/133)	10.2 (63/615)	18.0 (46/255)

4.3.4 Relationship between gonotrophic status, physiological age and plant-feeding

Table 4.2 shows the percentages of mosquito positive for fructose at each gonotrophic status and physiological age for the two *Anopheles* species. The proportion of fructose positive female *An. gambiae s.l.* collected was similar between the 4 gonotrophic status (unfed, blood fed, half-gravid, and gravid) ($\chi^2 = 1.44$, df = 3, P = 0.69) and the 2 parity status (nulliparous and parous) ($\chi^2 = 0.0002$, df = 1, P = 0.98). For each of the two species of *Anopheles* collected, the percentages of fructose positive females did not differ according to gonotrophic status: *An. gambiae s.s.* ($\chi^2 = 0.26$, df = 3, P = 0.96) and *An. arabiensis* ($\chi^2 = 1.23$, df = 3, P = 0.74). Also, there was no significant difference in the percentages of mosquitoes positive for fructose in parous and nulliparous groups in each of the two *Anopheles* species: *An. gambiae s.s.* ($\chi^2 = 0.85$, df = 1, P = 0.35) and *An. arabiensis* ($\chi^2 = 0.02$, df = 1, P = 0.88).

Table 4.2 Percentages of fructose positive mosquitoes in each of the gonotrophic status and two ages per *Anopheles* species.

Anopheles *species*	*Percentage positive for sugar (%)*					
	Gonotrophic status				Physiological ages	
	Blood fed	Unfed	Gravid	Halfgravid	Nulliparous	Parous
An. gambiae s.s.	12.4 (16/129)	14.1 (13/92)	11.7 (7/60)	11.5 (3/26)	14.4 (22/152)	10.9 (17/155)
An. arabiensis	12.1 (23/190)	14.4 (27/187)	10.3 (12/116)	11.4 (8/70)	12.6 (39/309)	12.2 (31/254)
Total	12.2 (39/319)	14.3 (40/279)	10.8 (19/176)	11.4 (11/96)	13.2 (61/461)	11.7 (48/409)

4.4 Discussion

This study evaluated plant-feeding practices in wild populations of *Anopheles s.l.* in Lwanda village, western Kenya. Results obtained from this study showed that some indoor resting females *An. gambiae s.s* and *An. arabiensis* contained fructose in their crops, indicating recent plant-feeding. The frequency of plant-feeding was similar in the two species of the *An. gambiae* complex collected during both the dry and wet seasons, and in mosquitoes at all physiological stages and ages. However, the proportion of fructose-positive of the indoor resting *An. gambiae s.l.* collected (12.5%) in Lwanda, western Kenya was low and similar to that found by Beier (1996) in Kisian, western Kenya (10.4%), but greater than that observed by Beier (1996) in Saradidi, western Kenya (4.3%). This difference of fructose-positive in mosquitoes between Lwanda and Saradidi areas may reflect a number of factors that may influence mosquito plant-feeding in the wild, such as climate, availability of flowering plants (Martinez-Ibarra *et al.*, 1997), and availability of vertebrate hosts (Van Handel *et al.*, 1994). In the present and previous studies on *An. gambiae* species, the low percentage of fructose-positive females obtained could be explained by the fact that, mosquitoes were tested for fructose, a plant sugar that is completely converted to other carbohydrates and lipids after it leaves the crop. Therefore, the method used only detects recently plant-fed mosquitoes, and can not give a real picture of the frequency or the rate of sugar feeding by female mosquitoes in nature. Secondly, the collection time may also have had an influence because mosquitoes were collected in the morning when resting indoor, and most of them may had spent their night inside houses and may already had digested their sugar.

In the present study, the percentages of plant-fed mosquitoes were similar in the two *Anopheles* species (P = 0.91), suggesting that the propensity of feeding on sugar may be similar in all mosquito species when sharing the same ecosystem. These two *Anopheles* species have probably adapted similar plant-feeding behaviours. There may be a case of parallel evolution, of the sugar-feeding strategies, given the two species are sympatric in this area of Kenya. Similar results were reported by Beier (1996) on *An. gambiae s.l.* and *An. funestus* in Kisian and Saradidi, also in western Kenya. Russel and Hunter (2002) also reported similar results on other mosquito species *Ochlerotatus canadensis* and *Ochlerotatus provocans* feeding on nectar and honeydew in Algonquin Provincial park, Ontario, Canada.

The frequency of plant-feeding was similar during both seasons in all the two *Anopheles* species tested (P = 0.06), indicating that there is probably no sugar-feeding appetitive season. However, Van Handel *et al.* (1994), and Costero *et al.* (1998) in their studies on *Aedes,* found seasonal differences in sugar feeding and or digestion of sugar meals. Martinez-Ibarra *et al.* (1997) on *Ae. aegypti*, and Schlein and Jacobson (1999) on the sand fly *Phlebotomus papatasi*, a non mosquito species, indicated that sugar feeding was strongly influenced by the availability of specific flowering plants. The presence of plants may increase the incidence of sugar feeding in nature. Although plant growth is always negligible during the dry season, in this study area, which is located on the shores of Lake Victoria, plants remain verdant during the dry season, therefore available as sugar sources to mosquitoes. This can probably explain the results obtained in this study.

Most females found containing fructose in the two species tested were those collected more than 200m from the larval habitats (P = 0.002). This may indicate that, like other mosquito species (Magnarelli, 1977, 1978; Andersson, 1990; Bowen *et al.*, 1994; Martinez-Ibarra *et al.*, 1997), and sand flies (Yuval and Schlein, 1986), *An. gambiae* and *An. arabiensis* feed on nectar to provide fuel for flight, and this behaviour is more pronounced when they move.

Anopheles gambiae s.s. and *An. arabiensis* females were fructose-positive equally at all the gonotrophic status (unfed, blood fed, half-gravid, and gravid) (P = 0.69). These observations are in agreement with previous studies which found that, in some species of *Culiseta* and *Culex*, blood-fed females seek sugar meals throughout the gonotrophic cycle (Nasci and Edman, 1984; Reisen *et al.*, 1986; Andersson and Jaenson, 1987). However, other studies noted that most *Aedes* and *Anopheles* do not imbibe nectar during the blood-fed or gravid stages and derive all their nutrients from blood meal (Edman *et al.*, 1992; Yee and Foster, 1992; Yee *et al.*, 1992). The results of this study differed from those found by Holliday-Hanson *et al.* (1997) in *Anopheles freeborni,* and by Yuval *et al.* (1988) in sand flies, where gravid females contained more nectar sugar. These differences support the hypothesis that the frequency by which sugars are taken varies greatly among species.

The proportions of parous and nulliparous females that were fructose positive were similar (P = 0.98), indicating that the two *Anopheles* species feed similarly on plant nectar at the two physiological ages of their adult life. These findings are similar to those of Martinez-Ibarra *et al.* (1997) in *Ae. aegypti*, another anthropophilic mosquito vector. Similar results were also found on other mosquito species, *Psorophora ferox* Humbold (Magnarelli, 1980), *Ae.*

communis De Geer (Andersson, 1990), and *Ae. triseriatus* say (Haramis and Foster, 1990). However, in other mosquitoes, *Ae. sollicitans* (Walker) (Magnarelli, 1977), *Ae. vexans* Meigen, *Cx. restuans* Theobald (Vargo and Foster, 1984), *Cx. pipiens* L., and *Cx. torrentium* Martini (Andersson and Jaenson, 1987), sugar feeding appears to be more common in nulliparous than in parous females, indicating that younger individuals require additional sources of energy for survival. These different observations indicate that sugar-feeding depends on energy requirements at a particular time and these may vary among species.

In conclusion, *An. gambiae s.s.* and *An. arabiensis* feed on plant sugar in the wild, even when the human hosts are readily available. The present study showed that in western Kenya, this behaviour is not affected by the species difference and by seasons. The probability of feeding on sugar meals increases when mosquitoes move far from the larval habitats. The plant-feeding occurrence in these females is independent of their gonotrophic status and physiological ages. From these results, it is assumed that any vector control strategy using plant-feeding will probably target mosquitoes at any season, physiological status and age.

CHAPTER FIVE: IDENTIFICATION OF PREFERRED PLANTS OF *ANOPHELES GAMBIAE* IN A MALARIA ENDEMIC AREA OF WESTERN KENYA

5.1 Introduction

Plant-derived sugars appear to be the primary food source for male mosquitoes (Clements, 1999). Emphasis is usually placed on the female mosquito's avidity for blood meals (Gillett, 1971; Klowden, 1995), because it is this habit that mediates disease transmission. Nevertheless, plant-derived sugars also feature in the diet of female mosquitoes (Yuval, 1992; Foster, 1995), because it provides for their immediate energy needs. The role of plant-derived sugars in the biology of the mosquito *An. gambiae* Giles (Diptera, Culicidae) is of particular interest because females of this mosquito are the major vectors of *Plasmodium falciparum* in tropical Africa, the parasite that causes the most severe form of human malaria (White, 1974; Service, 1980; Collins and Paskewitz, 1995).

Adult mosquitoes may take sugar meals from various sources, including floral and extrafloral nectaries, honeydew, sap, rotting or damaged fruits and leaves and discarded plant materials such as sugar-cane trash (Foster, 1995). However, the primary source of plant-derived sugars for most mosquitoes is assumed to be nectar from flowers and extra-floral nectaries, with numerous plant species having been implicated (Sandholm and Price, 1962; Grimstad and DeFoliart, 1974; Magnarelli, 1977, 1978). Other than the observations of McCrae *et al.* (1969, 1976) and McCrae (1968, 1989), nothing is known of the preference, if any, mosquitoes may have for feeding from particular plant species common in malarious areas of Africa. Given that mosquitoes are dependant on nutrients from plants, an important question is how the mosquito-

plant association shapes the biology of mosquitoes, and specifically their physiology, ecology, life history, and vectorial capacity.

The objective of this part of the study was to establish the behaviour and feeding preferences of *An. gambiae s.s.* exposed to 13 commonly occurring plants in Suba District, western Kenya in choice and no-choice situations.

5.2 Materials and Methods

5.2.1 Study area

The study was carried out at Mbita Point, located along the shores of Lake Victoria in Suba District, western Kenya, as described in Chapter 3.

5.2.2 Experimental plants

Because of the broad variety of native plants that occur in the ecosystems of the study site in Suba, only dominant flowering plant species were selected for testing on the basis of 1) their occurence around human dwellings and mosquito aquatic habitats, 2) their distribution over a wider ecological range within 30 km of the study site, from the lake shore to high-elevation mountain habitats, and 3) their presence in the habitat year round. Thirteen plant species were selected (Appendix 2): *Cassia hirsuta* L., *Senna bicapsularis* L., *Senna didymobotrya* Fresen (Leguminosae), *Datura stramonium* L. (Solanaceae), *Flaveria trinervia* Mohr, *Parthenium hysterophorus* L., *Psiada punctulata* L., *Tithonia diversifolia* Hemsl. (Asteraceae), *Hamelia patens* Jacq (Rubiaceae), *Ipomea hildebrandtie* Vahl (Convolvulaceae), *Lantana camara* L. (Verbenaceae), *Ricinus communis* L. (Euphorbiaceae), *Tecoma stans* L. (Bignoniaceae). When

needed, cuttings (branches with inflorescences) were obtained and taken to the laboratory for testing with mosquitoes, care being taken not to damage the inflorescences.

5.2.3 Mosquitoes

Laboratory colony of *An. gambiae s.s.* mosquito Ifakara strain reared as described in Chapter 3 was used. Newly emerged adult female and male mosquitoes used for experiments were maintained only on water (they were not given blood meals and they had no access to sugar) and exposed to plants when they were 2-days old.

5.2.4 Choice arena bioassay

The arena was a large (3.5 x 3.5 x 2.0 m high) mesh-covered cage placed in a screen-walled hazard-proof greenhouse (11.5 m x 7.1 m) (glass roof with a layer of reed mats to prevent temperature extremes). Cuttings (weight 45 – 50g) from each of the 13 plant species were used. Each was being held in a 500-ml Erlenmeyer flask filled with distilled water, plugged with cotton wool and, sealed with Parafilm® to deny the mosquito access to the water.

Using a Latin square design (13 x 13), mosquitoes inside the cage were tested during each trial with all 13 plant species present in the cage at the same night and equidistant one another (95 cm). Trial done each individual night was a single replicate and for each replicate, we used fresh cutting of the same plant. In addition, the experimental set up of each replicate was varied by randomly changing the position of the plant material in the cage every night to avoid any positional or neighbouring bias. The experiment was replicated 39 nights (3 blocks). In each block, a combination of 13 plants was presented in random order per night over 13 nights. Thus each plant was assigned 3 times to all the 13 positions inside the cage, providing 39

exposures for each plant during the entire study. These arrangements of plants were made to ensure that mosquitoes received equal exposure at candidate plants. Trials were carried out weekly, with minimum intervening periods of six nights to avoid potential residual effects and to allow the death by starvation of any mosquitoes not collected in the previous trial.

Testing started by releasing 200 two-day-old adult mosquitoes (100 females and 100 males) into the centre of the cage at 19.00 hrs. Using a hand-held electric torch (60-watt red bulb) and placing a transparent perspex glass (150 x 60 cm) between the observer and the cage to minimise perception by mosquitoes of volatiles emanating from the human, the number of mosquitoes that were perching (resting on a plant, without contact of the proboscis with the plant), and feeding (applying the tip of the proboscis to the plant, with intermittent or sustained ingestion of fluid) on each cutting during four 60-min intervals (between 20:00 and 23:30) was counted. Although in some instances, probing mosquitoes (intermittently applying their proboscis to the plant) became noticeably distended with clear evidence of ingestion, the level of distention was difficult to determine during observation, so both intermittent and sustained ingestion were designated as feeding.

Plants were removed from the cage immediately after each trial at 23:30, and at least 80-90% of mosquitoes were collected from the cage and immobilized with chloroform and then stored at -20 °C until the time of testing. Each mosquito was crushed in distilled water and tested for fructose (the main monosaccharide constituent of plant sugar), using cold-anthrone tests (Van Handel, 1972) (procedure described in chapter 3). A portion of the homogenate from the mosquitoes that tested positive for fructose was frozen (-20°C) for later analysis using gas

chromatography. Mosquitoes that remained in the cage were allowed to starve to death during the inter-trial period.

5.2.5 No-choice bioassay

The objective of these tests was to determine whether mosquitoes show the same pattern of plant preference as in the choice arena experiments. Thus, the mosquito's predisposition to ingest sugars from each plant species in isolation, when lacking a choice of alternative plant species from which to feed on was measured. The basic testing procedure was similar to the choice arena except that mosquitoes were given access to only one plant species per test in smaller cages (30 x 30 x 30 cm) and only 100 individuals of *An. gambiae* (50 males and 50 females) were released into the cage. These were left for 24 h and then collected for the cold-anthrone assay (described in chapter 3), and the number of mosquitoes that tested positive was recorded. Mosquitoes that escaped from the cage during collection were allowed to starve to death inside the screen-walled hazard-proof greenhouse. Each test was replicated three times, using different batches of mosquitoes and plant associations.

5.2.6 Gas chromatography (GC) analyses

Aliquots of homogenates from individual mosquitoes that tested positive for fructose (cold-anthrone tests) in the choice assays and extracts of specific parts of all 13 plant species were analysed by GC. The objective was to determine the specific plant species from which the mosquitoes acquired sugars and the type of sugars acquired (mono-, di- and trisaccharides).

5.2.6.1 Preparation of mosquito and plant samples

Homogenates from a sub-sample of 80 randomly picked mosquitoes (40 males and 40 females) from the last experimental block were taken for GC analysis. Newly emerged mosquitoes (10 males and 10 females from the same rearing batches with tested mosquitoes) that had no previous access to blood or sugar meals were used as controls.

For each candidate plant, plant parts (including leaves and flowers) that were exposed to mosquitoes and they were observed probing or feeding on them in the choice assay were extracted for analyses. The flowers included floral nectar and other floral structures. In addition, the stems of *R. communis* were analyzed because mosquitoes were observed feeding on them in the choice assay. Samples of plants and mosquitoes were prepared following protocol detailed in Chapter 3.

5.2.6.2 Trimethylsilylation of standards and samples and analyses

Sugar standards, mosquito and plant homogenates were trimethylsilylated and analyzed by gas chromatography following protocol detailed in Chapter 3. Plant-derived sugars were identified by comparing retention times of sugar standards with those present in the plant extracts. The sugar meal of the mosquito was identified when the profiles of extracts from mosquitoes matched the sugar profile of extracts from a particular plant species.

5.2.7 Data analysis

Using Tukey-Kramer tests for multiple comparison of means and chi-square tests of independence, differences between groups (candidate plants, plant parts and sex of mosquitoes) in the mosquito's activity per trial and during the entire period of the experiments were

determined. Repeated-measures analysis of variance and simple ANOVA were used to determine whether there was any association of independent factors (plants, observation time, replicate) with the behavioural responses of mosquitoes to the candidate plants. Prior to analysis (except chi-square), percentages of mosquitoes resting, feeding (choice assay), and positive for sugar (no-choice assay) were arcsin-transformed to increase their fit to the normal distribution. All statistics were carried out using Excel 2000® and SAS version 8.2 for Windows®.

5.3 Results

5.3.1 Choice-arena bioassay

Three types of mosquito behaviours on plants were observed: perching, probing and feeding (sustained ingestion). In 76.9 % of the candidate plants, these behaviours were observed mostly on flowers. Perching ($F = 10.09$, $df = 2$, $P < 0.001$) and feeding ($F = 16.42$, $df = 2$, $P < 0.001$) were observed more often on flowers than on other plant parts. The percentages of mosquitoes perching were 5 ± 0.0%, 4 ± 0.0% and 2 ± 0.0% and those feeding were 60 ± 0.1%, 4 ± 0.1% and 8 ± 0.0% respectively on flowers, leaves and stems. (Initially, a distinction was made between probing and sustained ingestion. However, subsequent examination of mosquito homogenates indicated that probing was associated with sugar intake. This is because the percentages of mosquitoes that tested positive for fructose (56 ± 2.6%) were closer to the percentages of mosquitoes seen both probing and becoming distended (enlarged) (72 ± 0.1%) with sugar than to the percentage of mosquitoes seen only becoming distended (10 ± 0.0%). Accordingly, these two behaviours were treated as a continuum between intermittent and sustained ingestion, and were referred to as "feeding". Figures 5.1 and 5.2 show the percentage

of mosquitoes perching and feeding (intermittent or sustained), respectively, on each candidate plant per night. Despite the low percentages of mosquitoes observed perching, the mean percentage of mosquitoes perching ($F = 2.19$, $df = 12$, $P = 0.01$) and feeding ($F = 6.47$, $df = 12$, $P < 0.001$) varied significantly among plant species. There was no direct correlation between the two behaviours. However, of the five plants with the highest percentage of feeding mosquitoes, four species (*H. patens*, *T. stans*, *R. communis* and *S. didymobotrya*) were also among the seven plants that elicited high perching rates. On the other hand, *P. hysterophorus* was among plants that elicited least perching, yet it was among those most preferred for feeding.

The average percentages of mosquitoes that perched and fed on all plants (in 39 replicates) on each of the four 1-hour observation periods are summarized in Fig. 5.3. The percentages of mosquitoes perching ($F = 12.82$, $df = 3$, $P < 0.001$) and feeding ($F = 7.28$, $df = 3$, $P < 0.001$) varied significantly during the observation times, with more mosquitoes perching closer to midnight, but more feeding during the early hours of the night. These trends were similar in all experimental nights, although there was also a significant variation in the proportion of mosquitoes perching ($F = 3.19$, $df = 38$, $P < 0.001$) or feeding ($F = 2.73$, $df = 38$, $P < 0.001$) on different nights. There was a significant plant-time interaction for the proportion of mosquitoes observed feeding ($F = 2.43$, $df = 36$, $P < 0.001$), but not for the proportion observed perching ($F = 0.86$, $df = 36$, $P = 0.8$).

An average of 169 of the 200 mosquitoes released per trial were recovered, comprising 58% females and 42% males, and giving a total of 6591 mosquitoes recovered for all trials

combined. The average percentage positive for fructose per replicate was 56 ± 2.6%. Significantly more females (68 ± 2.6%) than males (44 ± 3.2%) tested positive for fructose (χ^2 = 45.92, df = 1, P < 0.001) per trial.

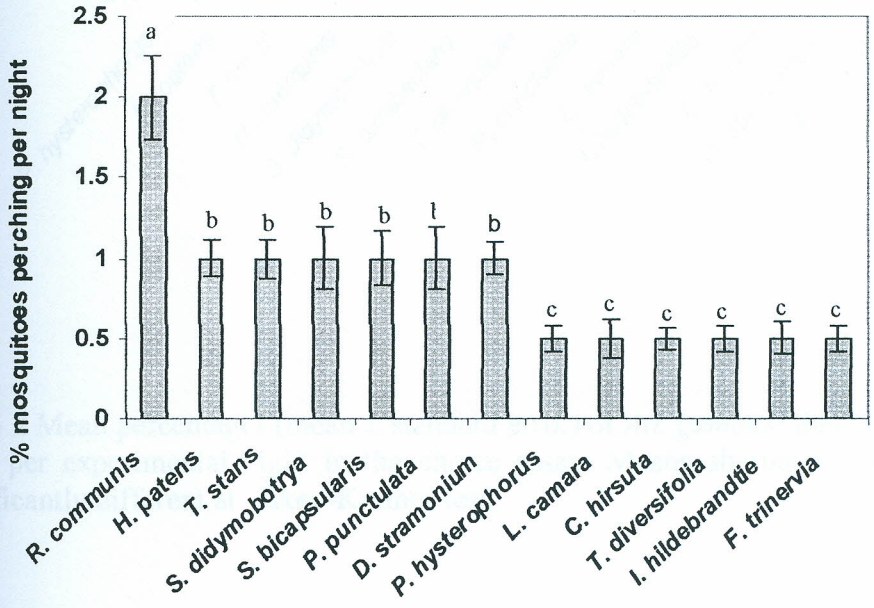


Fig. 5.1 Mean percentages (mean ± standard error) of *An. gambiae* that were perching (resting on plant) per experimental night in the choice assay. Means sharing the same letter are not significantly different at Tukey-Kramer test.

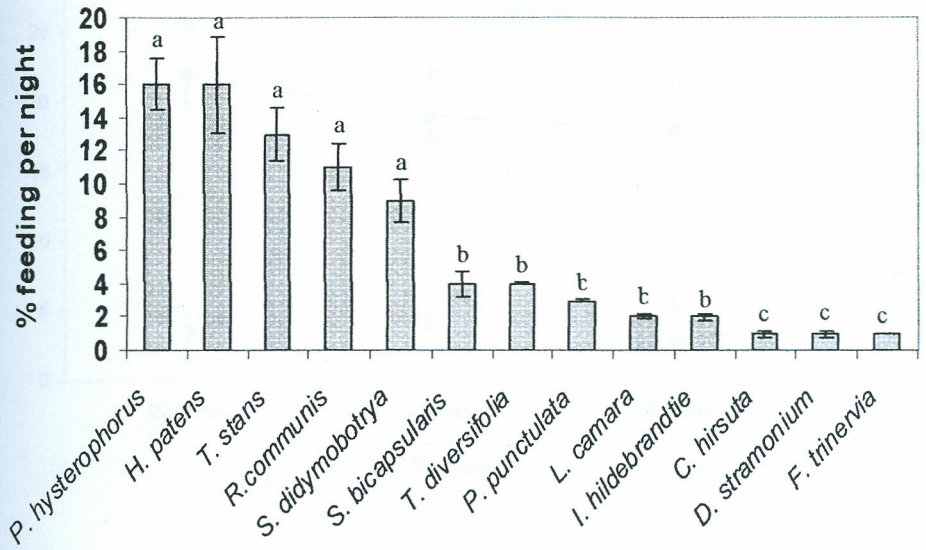


Fig. 5.2 Mean percentages (mean ± standard error) of *An. gambiae* that were feeding on each plant per experimental night in the choice assay. Means sharing the same letter are not significantly different at Tukey-Kramer test.

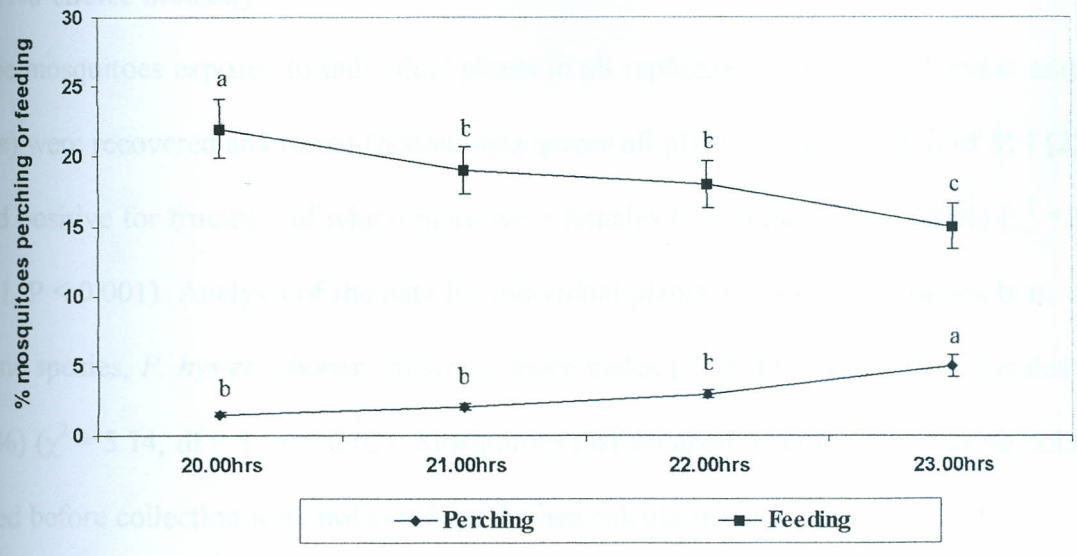


Fig. 5.3 Mean percentages (mean ± standard error) of mosquitoes perching and feeding on plants per observation time (20:00 hrs, 21:00 hrs, 22:00 hrs and 23:00 hrs) per experimental night. Means with different letters are significantly different at $\alpha = 0.05$ Tukey-Kramer multiple comparison test.

5.3.2 No-choice bioassay

Of the mosquitoes exposed to individual plants in all replicates, 3832 (1773 females and 2059 males) were recovered and tested (pooled data across all plant species). A total of 859 (22.5%) tested positive for fructose, of which more were females (25%) than males (20%) ($\chi^2 = 16.58$, df = 1, $P < 0.001$). Analysis of the data for individual plants showed the same sex bias, except for one species, *P. hysterophorus*, on which more males (47 ± 4.03%) fed than females (35 ± 12.3%) ($\chi^2 = 5.14$, df = 1, $P = 0.02$). Mosquitoes that escaped from the cage during collection or died before collection were not considered when calculating these percentages.

Figure 5.4 provides the average percentage of mosquitoes that tested positive for fructose the in no-choice tests from the 3 replicates combined. Comparison of these results with those obtained from the choice tests shows that four of the plants (*P. hysterophorus, T. stans, S. didymobotrya and R. communis*) that were most preferred for feeding by mosquitoes in the choice arrangement also were the most often fed on in the no-choice set up. Two plants (*D. stramonia* and *F. trinervia*) were among the least preferred in both arrangements.

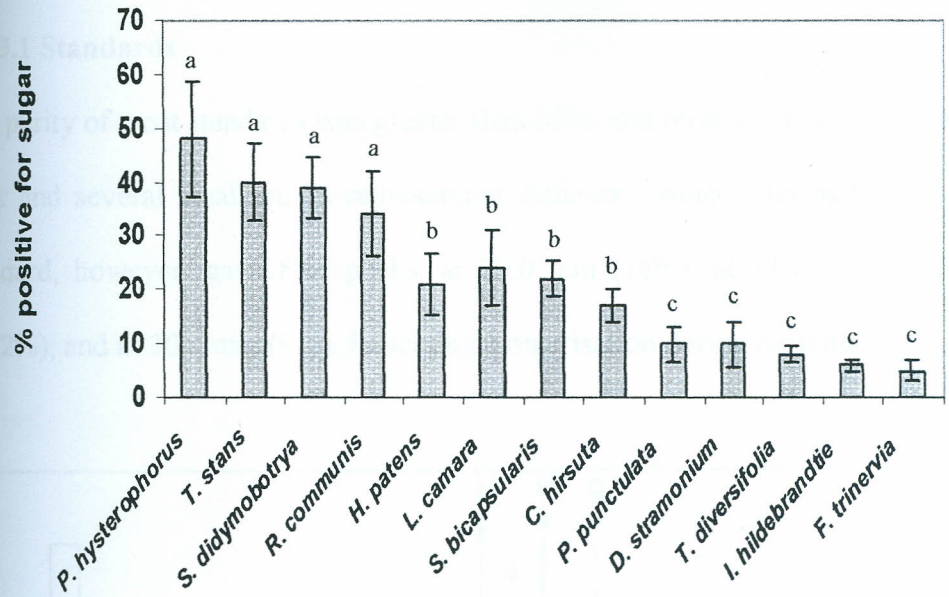


Fig. 5.4 Mean percentages (mean ± standard error) of *An. gambiae* that were positive for fructose in a no-choice assay per plant species. Means sharing the same letter are not significantly different at Tukey-Kramer test.

5.3.3 Gas-chromatography analysis

5.3.3.1 Standards

The purity of most standards was greater than 95%, and most standard sugars had a single main peak and several small peaks representing different isomeric forms (Fig. 5.5). The fructose standard, however, gave four peaks, at 29.0 min (16%), at 31.2 min (24.0%), at 31.5 min (49.2%), and at 30.4 min (9%), indicating isomerisation during derivitization.

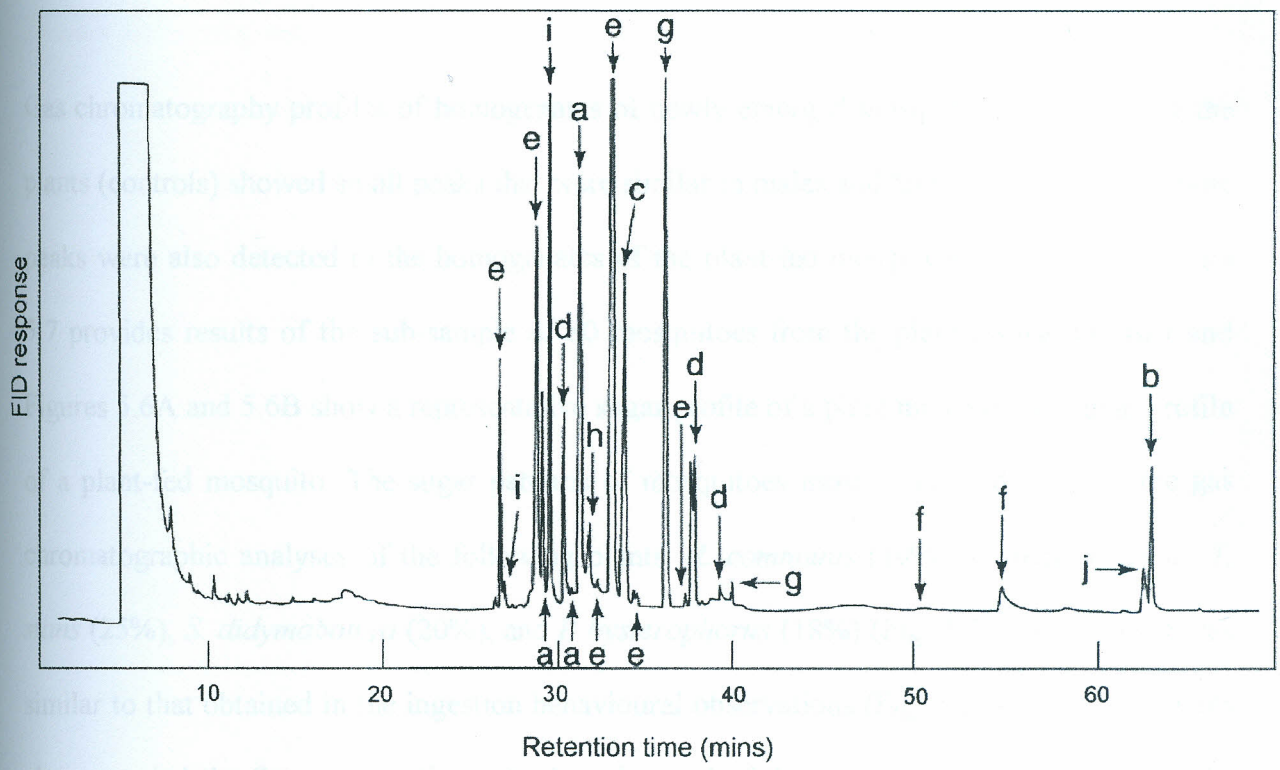


Fig. 5.5 Chromatogram of MSTFA sugar derivatives from mixed sugar standards. Letters on top or down of peaks represent different types of sugars.
Legend a= Fructose, b= Sucrose, c= Galactose, d= Mannose, e= Gulose, f= Raffinose, g= Glucose, h= Allose, i= Altrose, j= Lactose
FID = Flame ionization detector

5.3.3.2 Identification of sugar from plant extracts and mosquito homogenates

Table 5.1 shows the different sugars identified in plants. Glucose, fructose, sucrose, mannose, and gulose were the most common sugars from whole flowers with galactose, raffinose, and altrose also being present in some plants (Table 5.1). Leaves contained fewer types of sugars than flowers. None of the peaks from the leaves of *H. patens*, *I. hildebrandtie*, *C. hirsuta*, *P. punctulata* and from flowers of *P. hysterophorus* corresponded to the retention times of the derivatized standard sugars (Table 5.1).

Gas chromatography profiles of homogenates of newly emerged mosquitoes unexposed to the plants (controls) showed small peaks that were similar in males and females (Fig. 5.6C). These peaks were also detected in the homogenates of the plant-fed mosquitoes (Fig. 5.6B). Figure 5.7 provides results of the sub-sample of 80 mosquitoes from the plant-choice bioassay and Figures 5.6A and 5.6B show a representative sugar profile of a plant matching the sugar profile of a plant-fed mosquito. The sugar patterns of mosquitoes mostly matched those of the gas chromatographic analyses of the following plants: *R. communis* (39%), *H. patens* (26%), *T. stans* (25%), *S. didymobotrya* (20%), and *P hysterophorus* (18%) (Fig. 5.7). This ranking was similar to that obtained in the ingestion behavioural observations (Fig. 5.2) where these plants also occupied the five top positions. At the other end of the mosquito preference profile, the number of mosquitoes feeding on *D. stramonia* and *F. trinervia*, as revealed by gas chromatographic analyses, also matched the behavioural observations on feeding. Except for *H. patens*, the same plants that ranked among the more preferred when all plant species were present simultaneously also ranked high when fructose ingestion was assessed with only one plant species available at a time. *F. trinervia* consistently ranked the lowest. In 44% of the

mosquitoes, sugar profiles matched the profiles of more than one plant species, accounting for the total plant use values exceeding 100%.

As expected, most feeding occurred on flowers ($\chi^2 = 21.45$, df = 1, $P < 0.001$) (Table 5.2). However, in 27.5% of mosquitoes, the composition of homogenates suggested that they may have fed on both the leaves and flowers of the same plant species. In the case of mosquitoes that had fed on *R. communis*, 21%, 26% and 35% were identified as having fed on the leaves, flowers and stems, respectively. Of those that had fed on *L. camara*, 11% and 3% mosquitoes gave profiles that matched those of leaves and flowers, respectively. Of particular interest was an exclusive match between the profiles of the 18% mosquitoes that were identified as having fed on *P. hysterophorus* and the profiles of the leaves of this plant.

Table 5.1 Sugar contents of the plant parts of each candidate plant obtained from gas chromatography analysis

Candidate plants	*Plant parts*	*Glucose*	*Fructose*	*Sucrose*	*Mannose*	*Gulose*	*Galactose*	*Raffinose*	*Altrose*	*Allose*	*Lactose*
C. hirsuta	Flower	+	+	-	-	+	-	+	-	-	-
	Leaf	-	-	-	-	-	-	-	-	-	-
D. stramonium	Flower	+	+	-	-	-	-	-	-	-	-
	Leaf	+	-	-	-	-	-	+	-	-	-
F. Trinervia	Flower	+	-	-	+	+	-	-	-	-	-
	Leaf	-	-	-	-	-	-	-	-	-	-
H. Patens	Flower	+	-	+	+	-	-	-	-	-	-
	Leaf	-	-	-	-	-	-	-	-	-	-
I. hildebrandtie	Flower	+	-	-	+	-	-	-	-	-	-
	Leaf	-	-	-	-	-	-	-	-	-	-
L. camara	Flower	+	+	+	+	+	-	-	-	-	-
	Leaf	+	-	-	-	-	-	-	-	-	-
P. hysterophorus	Flower	-	-	-	-	-	-	-	-	-	-
	Leaf	-	-	+	-	-	+	-	-	-	-
P. punctulata	Flower	+	-	-	+	+	-	-	-	-	-
	Leaf	-	-	-	-	-	-	-	-	-	-
R. communis	Flower	+	-	-	+	-	-	-	-	-	-
	Leaf	-	-	-	+	+	-	-	-	-	-
	Stem	+	+	-	-	-	-	-	-	-	-
S. bicapsularis	Flower	+	+	+	-	-	+	-	-	-	-
	Leaf	+	+	+	+	+	-	+	-	-	-
S. didymobotrya	Flower	+	+	+	+	+	+	-	-	-	-
	Leaf	+	+	+	-	+	-	-	-	+	-
T. stans	Flower	+	+	+	+	+	-	+	+	-	-
	Leaf	+	-	-	-	-	-	-	-	-	-
T. diversifolia	Flower	+	+	+	+	+	+	-	-	-	-
	Leaf	+	-	-	+	+	+	-	+	-	-

+ sugar type found in the plant, - sugar type not found in the plant.

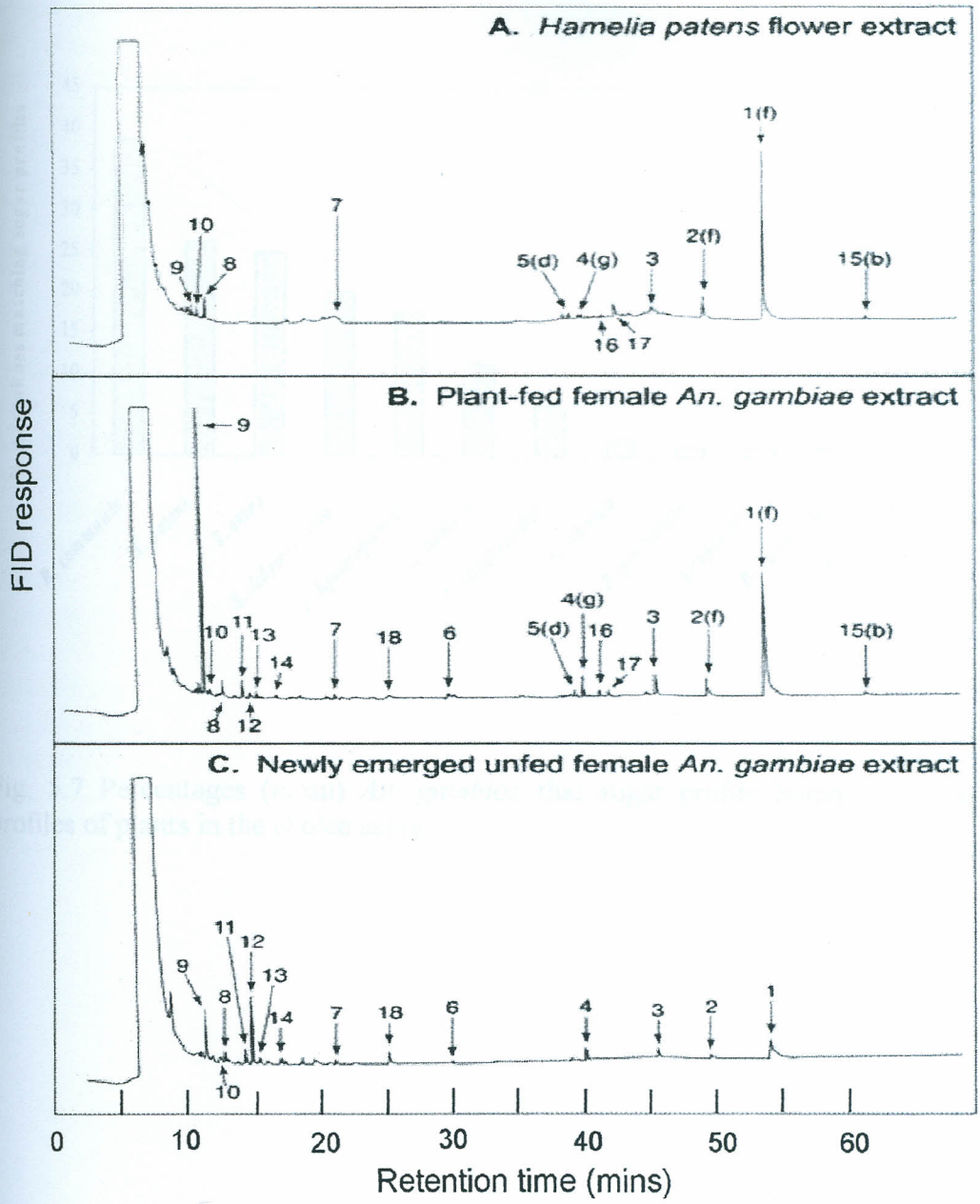


Fig. 5.6 Chromatograms of MSTFA sugar derivatives from extracts of, (A) *Hamelia patens* flower, (B) plant-fed female of *An. gambiae,* (C) newly emerged female *An. gambiae*. Since all the chromatograms could not be presented, A and B are randomly picked chromatograms illustrating sugar profiles of extracts of plant-fed mosquitoes matching the sugar profile of *H. patens* flower. Peaks with the same number in B and C represent the same type or sugars (metabolic sugars carried on from larval feeding). Extra peaks in B represent sugars acquired from plants. Letters on top of peaks represent identified sugars as indicated in Fig. 5.5.

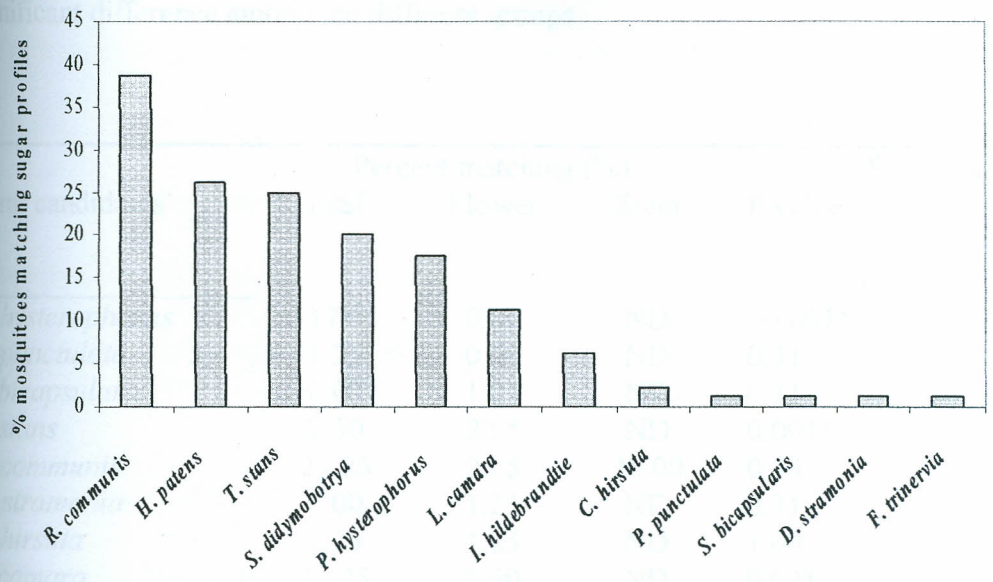


Fig. 5.7 Percentages (mean) *An. gambiae* that sugar profile homogenate matched sugar profiles of plants in the choice assay.

Table 5.2 Percentages of *An. gambiae* (pooled males and females) with sugar profiles matching the sugar profiles of the plant parts of each candidate plant by gas chromatography analysis. N=80 (40 males + 40 females) is the total number of mosquitoes tested. ND = Not done, * Significant difference among the different groups.

Plant candidates	Percent matching (%)			Statistics	
	Leaf	Flower	Stem	P value	Chi-square (χ^2)
P. hysterophorus	17.5	0.00	ND	<0.001*	15.34
P. punctulata	1.25	0.00	ND	0.31	1.00
S. bicapsularis	0.00	1,25	ND	0.31	1.00
T. stans	2.50	22.5	ND	0.001*	14.62
R. communis	21.25	26.5	35.00	0.14	3.88
D. stramonia	0.00	1.25	ND	0.31	1.00
C. hirsuta	1.25	1.25	ND	1.00	0.00
L. camara	11.25	2.50	ND	0.02*	4.78
S. didymobotrya	2.50	17.5	ND	0.001*	10.00
T. diversifolia	0.00	2.50	ND	0.15	2.02
I. hildebrandtie	3.75	6.25	ND	0.46	0,52
H. patens	0.00	26.2	ND	<0.001*	15.3
F. trinervia	0.00	1.25	ND	0.31	1.00

5.4 Discussion

Randomly selected plants have previously been investigated to demonstrate the role of plant-feeding in vector survival (Gary and Foster, 2004; Impoinvil *et al.*, 2004). Although these studies demonstrated significant feeding on plant materials, other than preliminary observations by McCrae *et al.* (1969, 1976) and McCrae (1968, 1989), no study has been undertaken to explore the possibility of preferences for different plant species by mosquitoes in a specific environment in Africa.

In the present study, plant preferences of *An. gambiae* were investigated under choice and no-choice bioassays in the laboratory. There was clear evidence of gradation of choices as indicated by specific mosquito behaviours, profiles of sugars ingested by mosquitoes in the choice bioassay and sugar intake in the no-choice bioassay. Significantly, plants selected in the choice design also were selected in the no-choice situation. Whether these choices are reflected in the general fitness of *An. gambiae* remains to be investigated. Mediating factors, such as physical characteristics and phytochemical composition of plants, may play a role in host-plant selection and location by mosquitoes (Sandholm and Price, 1962; Healy and Jepson, 1988; Gadawski and Smith, 1992; Foster and Handcock, 1994).

More female than male mosquitoes tested positive for fructose after exposure to the plants ($P < 0.001$). The basis for this male-female variation is unclear. Females may require more energy than males and may therefore be intrisically more active in sugar consumption from plants. Alternatively, males may digest sugars more rapidly than females, or particular plant

species that are more attractive to females than to males may have been inadvertently chosen for this study.

In this study, the testing period was only 4h during the night (20:00hrs to 23:000hrs). However, there was a tendency for mosquito feeding activity on plants to decrease and perching to increase over this period, suggesting that *An. gambiae* prefer to feed early in the night. Other mosquito species including *An. quadrimaculatus*, *Cx. quinquefasciatus*, *Ae. triseriatus*, *Ae. aegypti* and *Ae. albopictus,* also showed some evidence for diel sugar-feeding periods (Yee and Foster, 1992, Yee *et al.*, 1992). Feeding experiments with *An. gambiae* for longer periods will shed more light on its diel feeding activity.

GC analyses showed that, for almost all the plants investigated in the present study, flowers typically contained more types of sugars than leaves and stems. This may account for greater preference for feeding on flowers by this mosquito. However, evidence of preferential feeding on leaves and stems of three plant species (*R. communis*, *L. camara* and *P. hysterophorus*) was found. GC data showed that the leaves and stems of *R. communis* and *P. hysterophorus* contained more sugars than flowers ($P < 0.001$). As in an earlier study (Impoinvil *et al.*, 2004), *An. gambiae* that fed on *R. communis* concentrated on the extra-floral nectaries that are abundant on the stems and leaves of this plant. On *P. hysterophorus,* mosquitoes appeared to take sugar only from the leaves. Of the mosquitoes that were exposed to *L. camara,* more fed on the leaves than on the flowers ($P = 0.02$), despite higher sugar levels in the latter and the presence of only one monosaccharide (glucose) in the leaves. This is perhaps a consequence of the relatively long corollas of the flowers of this plant,

making their nectar inaccessible to *An. gambiae*. More feeding on leaves might also explain why survival of this mosquito was low when restricted to feed only on this plant (Gary and Foster, 2004; Impoinvil *et al.*, 2004).

In this study, only the ingestion of sugars from plants was considered, but recent studies on another mosquito species, *Cx. pipiens molestus* Forskal (Diptera: Culicidae) (Schlein and Muller 1995), and on sandflies (Schlein and Jacobson, 1994; Schlein *et al.*, 2001), suggest that these insects may also ingest other constituents from plants. A possible implication of this is the effect of the ingested secondary compounds on the vector's susceptibility to parasites.

In summary, the present study demonstrated a preferential feeding pattern of *An. gambiae* on plants. It also showed that when all parts of a plant are available, most feeding occurs in or around flowers, although feeding on extra-floral nectar from stems and leaves also was observed. Plant-feeding by these mosquitoes may play an important part in their survival, reproductive success and population dynamics, and their vectorial capacity (Okech *et al.*, 2003; Gary and Foster, 2001, 2004; Impoinvil *et al.*, 2004).

CHAPTER SIX: EFFECTS OF PLANT SUBSTRATES ON THE SURVIVAL AND FECUNDITY OF *ANOPHELES GAMBIAE*

6.1 Introduction

Besides blood meals, female mosquitoes are known to feed on sugar-rich foods in nature (Laarman, 1968; Magnarelli, 1977, 1978; Anderson and Jaenson, 1987; Holliday-Hanson *et al.*, 1997; Beier, 1996). Under laboratory conditions, it is known that sugar meals influence the flight performance, survival, and fecundity of adult mosquitoes (Nayar and Van Handel, 1971; Van Handel, 1972; Reisen and Emory, 1976). In the case of *An. gambiae s.s.*, laboratory studies have shown that females may live longer when sugar is available (Straif and Beier, 1996; Gary and Foster, 2001; Okech *et al.*, 2003, 2004), but females without access to sugar actually have higher daily fecundity (Gary and Foster, 2001). Sugar availability delays the onset or completion of oviposition in gravid females, effectively reducing fecundity (Klowden and Dutro, 1990; Loubinos and Conn, 1991). However, the relevance of this effect in natural conditions is still unknown.

In nature, the primary sources of sugar meals for most mosquitoes are floral nectars and extra-floral fluids (Foster 1995). In the previous chapter, where *An. gambiae s.s.* were offered 13 dominant plants species in a choice arena bioassay with all plants present, and in a no-choice bioassay involving individual plant, it was established that *An. gambiae* adults discriminate between plants, showing a tendancy to feed more on certain plants. The same study also showed that *An. gambiae* express preferences for feeding on particular plant parts. Contrary to general assumption of exclusive feeding on flowers, in some plants species, mosquitoes preferably fed on leaves and stems. Specifically, the question of whether

mosquitoes that exhibit selective plant-feeding increase their fitness by doing so is not yet understood. Little is known about the fitness consequences to the mosquito feeding differentially on plants. Important questions remain also unresolved regarding how the nutrients content and concentrations in the nectar or fluids of particular plant species affect survival and egg production.

In previous studies, *An. gambiae*'s survival was determined when given access in experiments to arbitrarily chosen plant species (Gary and Foster, 2004, Impoinvil *et al.*, 2004). This chapter attempts to establish the adaptive significance of feeding on particular plant species for which *An. gambiae* has known levels of preference, and also to determine whether sugar quality and quantity in plants is correlated with mosquito's fitness.

6.2 Materials and Methods

6.2.1 Study area

Semi-field tests were conducted in the greenhouse in the Thomas Odhiambo Campus (TOC) of the International Centre of Insect Physiology and Ecology (ICIPE) Mbita Point. Plants were collected in Suba district, described in chapter 3.

6.22 Mosquitoes used in the study

Mosquito species used in this study was *An. gambiae s.s* females and males obtained from an already established *An. gambiae s.s* (Mbita strain) mosquito colony at ICIPE-TOC and reared as described in chapter 3.

6.2.3 Plants used in the study

The plants tested in this study were selected among the 13 predominant flowering plants species occurring in ecosystems of the study site as described in chapter 5. Six plants species including *Ricinus communis* L. (Euphorbiaceae), *Senna didymobotrya* F. (Leguminosaceae), *Pathernium hysterophorus* L. (Asteraceae), *Tecoma stans* L. (Bignomiaceae), *Hamelia patens* J. (Rubiaceae), and *Lantana camara* L. (Verbenaceae) were used in the study. The first five of these plants were identified as most preferred ones by *An. gambiae* in the previous study (Chapter 5). As needed, cuttings (branches with inflorescences) were made and plant species parts were taken to the laboratory for testing with mosquitoes.

6.2.4 Survival assay

To test the influence of plant-feeding on survival, mosquitoes were grouped into nine nutritional regimes (six treatment groups and three control groups). For each treatment group, a single cutting with flowers from each of the six plants species was used, each being held in a 250 ml Erlenmeyer flask filled with Lake Victoria water, plugged with cotton wool and, sealed with Parafilm® to prevent the mosquito access to the water in which the plant was held. Fifty mosquitoes (25 males and 25 females) were released in 30 x 30 x 30 cm netting cages and held in a screen-walled greenhouse (11.5 x 7.1 m) exposed to ambient climatic conditions (temperature ranging from 18 to 31°C). The greenhouse was roofed with glass with a layer of reed mats adjoined under roof to prevent temperature extremes. Mosquitoes in each cage had access to one of the six plant nutrition sources and cotton pads wet with distilled water. In addition, two mosquito groups were maintained with continuous access to 6% (wt/vol) glucose solution in filter-paper wicks or distilled water on cotton pads,

representing positive and negative controls respectively. As an additional negative control, a third mosquito group (starved) was kept without plants, water or glucose. All plants were cleared of any potential predators (e.g., ants and spiders) before testing began. Plant materials and the cotton pads with distilled water were changed every two days. Glucose solution was also replaced every 2 days. In each test of a treatment or a control group, dead mosquitoes were recorded and removed daily until all mosquitoes die. All testing were replicated six times and were carried out under hazard proof, controlled semi-natural conditions.

6.2.5 Fecundity assay

Batches of 100 newly emerged *An. gambiae* (50 females + 50 males) each were released in 30 x 30 x 30 cm cages containing each plant species. They were allowed to feed from the plants, while in two groups mosquitoes had continuous access to 6% (wt/vol) glucose solution in filter-paper wicks or distilled water on cotton pads, both representing positive and negative controls, respectively. On the evening of the third day, all mosquito groups were allowed to blood feed on a human arm (from the same volunteer) for 15 minutes and for three consecutive days. Unfed mosquitoes were discarded only after the first blood-feeding. During this time and for the entire period of the assay, mosquitoes were held in cages with their respective diet regime. Prior to blood-feeding, it was impossible to identify a plant-fed from a non-plant-fed mosquito. It was assumed that all mosquitoes still alive by the evening of the third day from the start of the experiment may have fed at least once on the plant.

Multiple bloodmeals were offered, as this has been shown to increase the chance of oviposition by females (Briegel and Hörler, 1993). Mosquitoes that did not feed during the

first feeding opportunity with blood were discarded. Despite that, only replicates with sufficient (at least 15) mosquitoes per batch were taken into account. During the blood-feeding period, there was no oviposition because the cups used as oviposition sites were not yet in the cages. The second evening after their last blood meal, gravid and hypergravid (delayed onset of oviposition) female mosquitoes were individually put in a 15 x 15 x 15 cm netting cages with their respective food regime to allow them continued feeding. Plant cuttings were put in a 100 ml Erlenmeyer flask, filled with fresh lake water (access to the water in the flask prevented as above). Another experiment was performed similar with that described above, but mosquitoes were offered only one blood-meal.

For both type of experiments, individual mosquitoes inside the cages were supplied with one plastic oviposition cup (2 cm depth, 4 cm diameter), filled with 15 ml distilled water and lined with white filter paper to allow oviposition. Mosquitoes were also allowed to continue ovipositing for 7 days. Every morning (from 09.00 h) after the beginning of the oviposition assay, the number of eggs oviposited on the filter papers by each individual mosquito was counted under a dissection microscrope. Plants inside the cages were also checked for the presence of eggs. Oviposition cups in each cage were changed daily, providing mosquitoes with fresh substrates. Food regimes were also refreshed every two days. New batches of mosquitoes were used for each experimental replicate.

Dead mosquitoes within the oviposition period were individually dissected under a dissection microscope, their insemination status was checked, and their ovaries examined for the presence of retained eggs and their numbers. After the oviposition period, remaining

mosquitoes were killed and dissected to determine and quantify retained eggs. Eggs collected every morning from all mosquitoes that were previously exposed to the same nutritional regime were pooled and dispensed into plastic trays (25 cm long × 20 cm wide ×14 cm high). These were filled to a depth of 8 cm with filtered lake water. First instars larvae were counted upon hatching. Experiment involving each plant species was replicated seven times when mosquitoes were offered 3 blood meals and 4 times when they were offered only one blood meal. Only inseminated mosquitoes were considered and included in the analysis.

6.2.6 Characterization and quantitation of sugars present in each plant

To determine and quantify different types of sugars present in each plant, GC was performed on each plant tested as described in Chapter 3. Quantification of different plant sugars was done by using the peak area of each sugar standard with peak area of plant sugars and all the dilution factors into the following formula:

(Peak area of sample/Peak area of standard) x (quantity of sugar standard injected).

6.2.7 Data analysis

Comparisons between replicate groups of pooled mosquitoes indicated an unexplained, but uniform difference in the survivorship of mosquitoes in replicates 3 and 4. Although longevity of mosquitoes was higher in these replicates, the pattern of survival in different nutrition group was the same as in other replicates, and they were included in the analysis. In addition, a preliminary analysis excluding these replicates did not affect the significance of difference among groups. For fecundity assay, there was no statistical difference in the dependant factors between replicates. Therefore, there was no statistical interaction between

the effect of plants on mosquito fitness and replicates, consequently, results of replicates of all nutrition groups were pooled and analyzed as single data sets. Survival analysis techniques (PROC LIFETEST) including log-rank test were used to obtain and compare survival curves and test whether the probability of survival was different according to the nutrition regime and sex of mosquitoes. The effect of feeding treatment on the survivorship was estimated using analysis of variance (ANOVA), with data Log_{10} transformed. Mean survival times were compared between groups using Student Newman Keuls (SNK). For fecundity assay, fecundity was measured as the average percentage of mosquitoes that oviposited, the number of eggs developed, retained, laid per female fed on each nutritional group, and the viability of their eggs. Data on the number of eggs laid, retained and developed were subjected to log_{10} transformation, and percentage of eggs hacthing and mosquitoes ovipositing were arcsin transformed to normalise their distribution. ANOVA was used to determine the effect of the sugar source on the number of eggs developed, retained, laid and the proportion hatching into larvae. Means of these variables were separated using Student Newman Keuls (SNK) test. Spearman correlation was used to test for a linear relationship between sugar composition and concentration in plants and mosquito survival and fecundity. t-test was used to compare sugar concentration in flowers and leaves. Differences were considered significant at $P<0.05$. All statistics were carried out using Excel 2000® and SAS version 8.2 for Windows®.

6.3 Results

6.3.1 Effects of plants on survival

Table 6.1 shows the means and median survival times of mosquito *An. gambiae* fed on each nutritional regime. Mean survival times varied significantly among the groups ($F = 202.81$, $df = 8$, $P < 0.001$). Mosquitoes from the positive control group (given 6% glucose solution) survived significantly longer than mosquitoes from any other group. When data from groups provided with plants were compared, mosquitoes generally survived better on plants that were more preferred. The longest mean survival was on *T. stans* (range, 2-55 days), followed by survival on *R. communis* and *S. didymobotrya* and with mosquitoes fed on *H. patens* being next. However, despite *P. hysterophorus* being one of the more preferred plants, mosquitoes exposed to this plant had mean survival significantly shorter than that of mosquitoes in any other treatment, being comparable to on water alone.

On the least preferred (*L. camara*) of the six plants that were investigated, survival was significantly higher than that of mosquitoes on *P. hysterophorus* ($F = 79.78$, $df = 1$, $P < 0.001$), and survival was comparable to that of mosquitoes on *H. patens* ($F = 1.62$, $df = 1$, $P = 0.2$). However, survival on *L. camara* was significantly lower than that of mosquitoes on any of the other plants and the control glucose ($F = 74.13$, $df = 4$, $P < 0.001$).

When looking at the average effect of each treatment relative to the control glucose on the survival of mosquitoes, *T. stans*, *R. communis,* and *S. didymobotrya* had a reducing effect on the survival of mosquitoes, being at the magnitude of 18%, 30% and 32% (Table 6.1) respectively below the control. In the other hand, *L. camara* and *P. hysterophorus* had a

much higher reducing effect on mosquito's survival, being at more than 50% below the control (Table 6.1).

Table 6.1 Average longevity (median and mean ± SE), and range in days of adult mosquito *An. gambiae* when provided with various nutrition sources under semi-field conditions: pooled data for 6 replicates of approximately 50 mosquitoes.

Nutrition regime	*N*	*Median (days)*	*Mean[§] (days)*	*±SE*	*Range (days)*	*(C-T)/C*	*P*
Glucose (6%)	300	16.0	17.1 a	0.4	2.0 – 35.0	0.00*	NA
T. stans	294	12.0	13.4 b	0.5	2.0 – 55.0	0.18[a]	0.06
S. didymobotrya	278	11.0	11.6 c	0.3	2.0 – 34.0	0.30[a]	0.03
R. communis	293	10.0	11.4 c	0.4	2.0 – 37.0	0.32[a]	0.01
H. patens	288	7.0	7.7 d	0.3	2.0 – 32.0	0.50[b]	0.001
L. camara	286	6.0	7.2 d	0.2	2.0 – 26.0	0.58[b]	<0.001
P. hysterophorus	290	4.0	4.7 e	0.2	2.0 – 26.0	0.72[c]	<0.001
Water	297	3.0	3.8 e	0.1	2.0 – 12.0	0.77[c]	<0.001
Starved	296	3.0	3.0 e	0.0	2.0 – 6.0	0.80[c]	<0.001

N, total number of mosquitoes tested; SE, Standard error.
(C-T)/C is [(Control glucose-Treatment)/Control glucose] and represents the average effect of each treatment on survival of mosquitoes relative to the control, glucose.
* Reference group
[§]Means with different letters are significantly different as determined by SNK test for multiple comparison at $\alpha = 0.05$.

The probability of survival was influenced by the feeding regime ($\chi^2 = 1633.25$, df = 8, $P < 0.001$) (Fig. 6.1). There were especially rapid declines in the numbers of surviving mosquitoes in the starved, water-only and *P. hysterophorus* groups, with numbers dropping dramatically during the first 5 days of the experiment. Only 10% of mosquitoes fed on *P. hysterophorus* survived more than 7 days. There was also a rapid decline in the mosquitoes fed on *L. camara*, with 80% being dead by day 10 (Fig. 6.1). Mosquitoes on 6% glucose solution, and mosquitoes on *T. stans*, *R. communis* and *S. didymobotrya* had consistently greater probability of survival than mosquitoes in other groups.

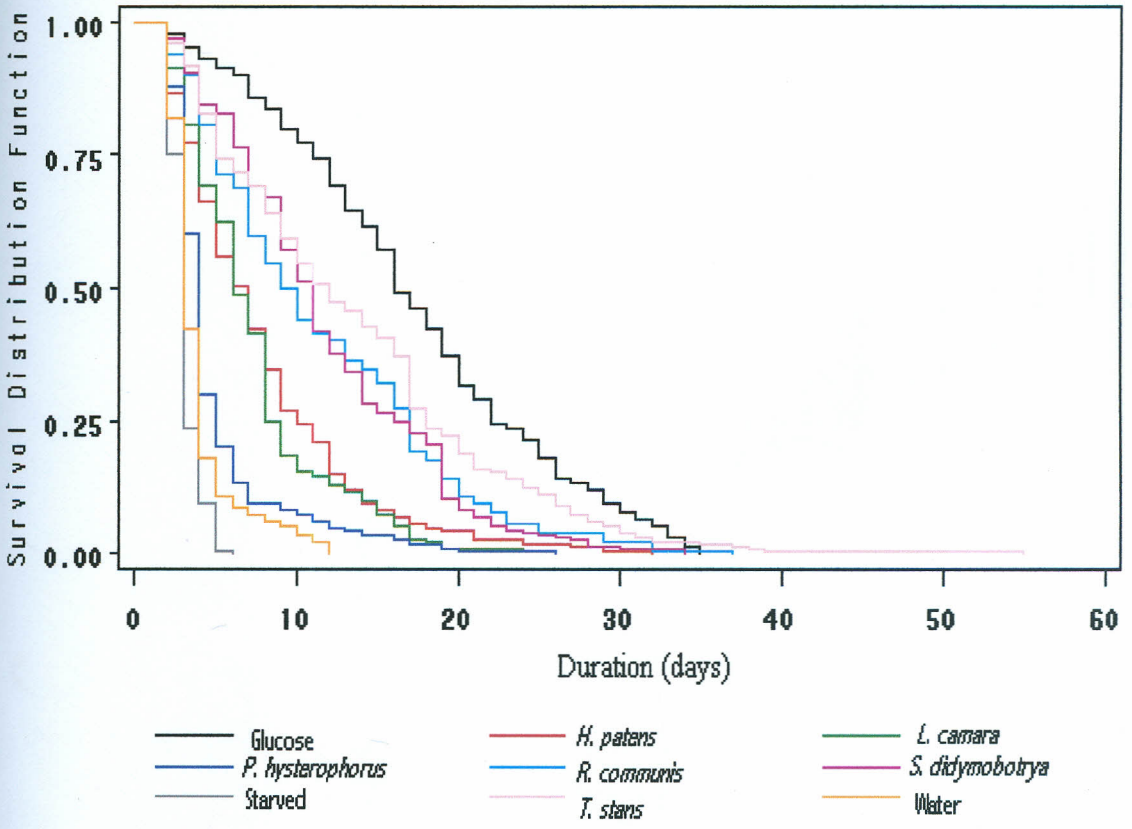


Fig. 6.1 Probability of survival over time of mosquito *An. gambiae* when given different food regimes.
Duration is time in days mosquitoes survived.

Survivorship curves for pooled males and females (PROC LIFETEST) and log-rank tests of pooled replicates and pooled plants; $\chi^2 = 0.28$, df = 1, P = 0.63) were not significantly different. Table 6.2 shows the mean survival time of male and female mosquitoes per plant species tested. With exception of *R. communis* where the survival function of females was better than males ($\chi^2 = 3.07$, df = 1, P = 0.07), and *L. camara* where males survived better than females ($\chi^2 = 2.93$, df = 1, P = 0.08), although the difference was not statistically different in both cases, no difference was found on the survival curves of males and females mosquitoes in all the other plant species (Table 6.2).

Table 6.2 Means (± SE) survival in days and sex differences between survival curves of adult male and female *An. gambiae* mosquitoes placed in cages with various nectar sources in semi-field conditions: pooled data for 6 replicates of approximately 25 males and 25 females.

Nutrition regime	*Males (N) Means ± SE*	*Females (N) Means ± SE*	*(χ^2)**	*P*	*Longer survival*
Glucose (6%)	(148) 17.3±0.6	(151) 17.2±0.6	0.004	0.94	No difference
T. stans	(145) 14.2±0.8	(154) 13.2±0.6	0.89	0.34	No difference
S. didymobotrya	(131) 11.9±0.5	(142) 11.4±0.5	0.01	0.88	No difference
R. communis	(143) 10.9±0.5	(142) 12.6±0.6	3.07	0.07	No difference
H. patens	(136) 8.1±0.4	(150) 7.4±0.4	0.88	0.34	No difference
L. camara	(149) 7.6±0.3	(145) 6.7±0.3	2.93	0.08	No difference
P. hysterophorus	(138) 4.7±0.3	(141) 4.7±0.3	0.002	0.96	No difference
Water	(148) 3.8±0.1	(149) 3.8±0.1	0.01	0.90	No difference
Starved	(144) 2.9±0.07	(143) 3.1±0.07	2.58	0.10	No difference

* Test statistic for log-rank analysis: survival distribution comparison between male and female mosquitoes in each respective nutrition regime. P is the probability indicating the level of significance of the difference between the survival distribution of both sexes. N is number of mosquitoes tested, SE is standard error.

6.3.2 Fecundity of *An. gambiae* fed on different plants

The number of eggs developed by individual females was obtained by counting the number of eggs oviposited plus the number of eggs retained. Table 6.3 shows the average number of eggs developed, oviposited, retained by mosquitoes and the proportion of eggs hatching into larvae per plant species and controls when mosquitoes were offered three consecutive blood meals. Average number of eggs oviposited, retained and developed by mosquitoes on *P. hysterophorus* were significantly lower than on *R. communis*. Otherwise, except for the average number of eggs developed ($F = 2.36$, $df = 6$, $P = 0.02$), eggs retained ($F = 1.48$, $df = 7$, $P = 0.17$) and laid ($F = 1.22$, $df = 6$, $P = 0.29$) per female was statistically comparable across plant species and controls, although the lowest number of eggs laid was on *P. hysterophorus*. Although there was no significant variation on the proportion of mosquitoes which developed eggs per group ($F = 1.33$, $df = 7$, $P = 0.25$), there was a significant variation on the proportion of mosquitoes that oviposited ($F = 3.38$, $df = 7$, $P = 0.006$) and the proportion of mosquitoes that had retained ($F = 2.90$, $df = 7$, $P = 0.014$) eggs. Especially few mosquitoes maintained on *P. hysterophorus, L. camara* and water oviposited and more than 95% of the mosquitoes in these three groups delayed onset of oviposition by a day longer than mosquitoes in the other groups. When looking at the effect of each treatment relative to the control glucose on the proportion of females ovipositing, only water, *L. camara* and *P. hysterophorus* groups highly reduced the chance of mosquitoes to oviposit, being at more than 40% (Table 6.3) below the control glucose. In the other hand, all the other plant species had an effect almost similar to the effect of glucose on mosquitoes ovipositing.

Like with other fitness parameters, egg hatchability was lower on the *P. hysterophorus* group compared to other groups. However, it did not vary significantly among groups ($F = 1.68$, $df = 7$, $P = 0.14$).

When mosquitoes were offered only one blood meal, a significant variation on the average number of eggs laid per mosquito fed on each plant species was observed ($F = 2.82$, $df = 7$, $P = 0.01$). Mosquitoes fed on *P. hysterophorus* and *L. camara* laid a significant low number of eggs (Table 6.4).

Table 6.3 Means number of eggs developed, retained and laid by individual laboratory-reared female *An. gambiae* that were offered various nutritional regime and three consecutive blood meals, average percentage of eggs hatching into larvae per group, and percentage of mosquitoes that oviposited per group: pooled data for 7 replicates of approximately 15 females each.

Nutritional regime	*N*	*Eggs developed Mean ± SE*	*Eggs retained Mean ± SE (per female retaining)*	*Eggs laid Mean ± SE (per female laying)*	*% eggs hatched Mean ± SE*	*% mosquitoes ovipositing Mean± SE*	*% mosquitoes ovipositing (C-T)/C*	*P*
Glucose (6%)	117	76.5 ± 3.5ab	60.4 ± 5.2a	77.0 ± 4.6ab	67.4 ± 1.2a	71.2± 6.7a	0*	NA
T. stans	115	63.4 ± 3.1ab	50.1 ± 4.0a	67.4 ± 3.9ab	69.0 ± 6.8a	69.2± 6.7a	0.02a	0.85
S. didymobotrya	115	70.1 ± 3.2ab	63.1 ± 5.0a	68.0 ± 4.2ab	61.9 ± 3.3a	64.5± 6.8a	0.09a	0.78
R. communis	90	78.2 ± 3.7a	67.4 ± 5.5a	79.3 ± 4.7a	58.3 ± 7.7a	60.5± 7.3a	0.15a	0.75
H. patens	95	66.0 ± 3.5ab	60.5 ± 4.5a	67.7 ± 5.8ab	67.3 ± 9.1a	46.8± 8.0ab	0.34b	0.46
L. camara	82	70.6 ± 3.8ab	65.0 ± 5.8a	75.6 ± 5.1ab	67.8 ± 4.1a	40.2± 7.6b	0.43b	0.03
P. hysterophorus	80	60.4 ± 3.6b	53.7 ± 6.1a	57.8 ± 4.1b	34.4 ± 4.9a	39.1± 10.7b	0.45b	0.02
Water	69	60.7 ± 3.9b	67.2 ± 4.8a	58.5 ± 6.1b	61.6 ± 17.4a	27.5± 6.8b	0.60c	0.007

N, number of mosquitoes tested. SE, standard error.
(C-T)/C is [(Control glucose-Treatment)/Control glucose] and represent the effect of each treatment on the percentage of mosquitoes ovipositing relative to the control glucose.
* Reference group
Any two means sharing a letter in common are not significant at 5% level of significance (SNK test).

Table 6.4 Means number of eggs laid by individual laboratory-reared female *An. gambiae* that were offered only one blood meal and various nutritional regimes. Pooled data for 4 replicates of approximately 15 females tested each.

Nutritional Regime	*N*	*Eggs laid* *Mean ± SE (female laying)*
Glucose (6%)	44	35.7 ± 2.1a
T. stans	40	34.8± 2.0a
S. didymobotrya	46	33.1 ± 1.9a
R. communis	39	33.0 ± 2.3a
H. patens	48	39.1 ± 2.6a
L. camara	46	21.2 ± 2.5b
P. hysterophorus	38	15.1 ± 2.0b
Water	16	15.5± 3.2b

N, number of mosquitoes that oviposited. SE, standard error.
Any two means sharing a letter in common are not significant at 5% level of significance (SNK test).

6.3.3 Quantification of essential sugars present in each candidate plant extract and correlation with mosquito fitness (survival, fecundity).

Plant species tested had different sugar composition and concentration (Table 6.5). The flower of *T. stans* was the richest in sugar composition (77.7% of sugars tested) and more concentrated in sugars (506µg/mg of dry extract), while the leaf of *P. hysterophorus* was one of the poorest in sugar composition (22.2% of sugars tested), with the lowest concentration (2µg/mg of dry extract) and the flower having no identifiable sugars. The flower of the least preferred plant (*L. camara*) was among the richest in sugar composition (55.5% of sugars tested) and concentration (220µg/mg of dry extract) (Table 6.5). Among all the sugars tested, α-D-glucose, β-D-fructose, sucrose, D(+)mannose, and D-gulose were dominant in all species. With the exception of *P. hysterophorus*, sugars tended to be more concentrated in flowers than in leaves of all plant species ($t = 2.48$, $P = 0.01$ for pooled plants).

There was a positive correlation between the total amount of sugars present on each *An. gambiae* preferred plant (and plant part) and median survival time of mosquitoes on those plants (Spearman correlation: $r = 1$, $P < 0.0001$). Table 6.5 also shows the correlation of each sugar type with median survival time of mosquitoes. Three types of sugars (glucose, fructose, and gulose) and in higher concentration significantly increased the survival of mosquitoes. However, two types of sugars (sucrose and galactose) had a negative but non significant correlation with the longevity of mosquitoes. Except lactose, the other four types of sugars were also positively correlated with the longevity of mosquitoes. When sugars composition and concentrations of *L. camara* flower (least preferred) was included in the analysis, the Spearman coefficient of correlation was insignificant between total (Spearman correlation: r

= 0.71, P = 0.11) and between individual sugar concentrations and survival of mosquitoes on the same plants.

In the case of mosquito fecundity, when offered three consecutive blood meals, there was no significant association between the total sugar concentration on plants (preferred by *An. gambiae*) and the number of eggs developed and oviposited (r = 0.70, P = 0.18 and r = 0.30, P = 0.62 respectively). Similar results were obtained when *L. camara* (least preferred) was included in the analysis (r = 0.60, P = 0.20 and r = 0.20, P = 0.7 respectively). However, the proportion of mosquitoes ovipositing was correlated with sugar concentrations in the plants (r = 0.82, P = 0.04). Delay in oviposition was also found in groups with low sugar concentration. There was also an association between the total sugar concentration on plants (preferred by *An. gambiae*) and the number of eggs oviposited (r = 0.87, P = 0.04) when mosquitoes were offered only one blood meal.

Table 6.5 Sugars present in plant species and quantity in 1 mg of dried plant parts extracts, and correlation between each sugar, total amount of sugars in the preferred plants and median survival times of mosquitoes.

Plants	*Plant parts*	*α-D-Glucose* (μg)	*β-D-Fructose* (μg)	*Sucrose* (μg)	*D-Mannose* (μg)	*D-Gulose* (μg)	*D-Galactose* (μg)	*D-Raffinose* (μg)	*D-Altrose* (μg)	*D-Allose* (μg)	*Total* (μg)	*% sugar types*
H. patens	Flower	5.1	0	12.8	3.0	0	0	0	0	0	20.9	33.3
	Leaf	0	0	0	0	0	0	0	0	0	0	0
L. camara	Flower	43.8	30.6	2.4	8.6	134.6	0	0	0	0	220	55.5
	Leaf	4.6	0	0	0	0	0	0	0	0	4.6	11.1
R. communis	Flower	23.3	0	0	2.2	0	0	0	0	0	25.5	22.5
	Stem	7.3	15.2	0	0	0	0	0	0	0	22.5	22.5
	Leaf	0	0	0	13.4	12.4	0	0	0	0	25.8	22.5
S. didymobotrya	Flower	78.8	5.4	2.5	14.8	110.8	7.3	0	0	0	219.6	66.6
	Leaf	2.4	10.5	1.1	0	38.9	0	0	0	0.9	53.8	55.5
P.	Flower	0	0	0	0	0	0	0	0	0	0	0
hysterophorus	Leaf	0	0	1.2	0	0	0.8	0	0	0	2	22.2
T. stans	Flower	58.5	161.7	0.3	3.9	263.3	0	3.95	14.5	0	506.2	77.7
	Leaf	2.0	0	0	0	0	0	0	0	0	2.0	11.1
r		0.94	0.97	-0.30	0.60	0.97	-0.11	0.70	0.70	0.35	1	
Probability		0.003*	0.04*	0.62	0.28	0.004*	0.85	0.18	0.18	0.55	<0.0001*	

r is Spearman coefficient of correlation, * probability significant at $\alpha = 0.05$ (significant association of sugar with survival)

6.4 Discussion

The findings in this study suggest that, for adults of *An. gambiae*, there are fitness consequences of the plant species to which there is access for feeding. On the whole, survival data matched closely the findings from the earlier study on plant choice (Chapter 5). *Anopheles gambiae* adults survived poorly on *L. camara*, a plant that the previous study showed to be less often chosen under competitive testing, but survived well on four of the plant species that were often chosen in the previous study. Although *An. gambiae* adults often chose *P. hysterophorus*, their survival on this plant was poor compared to all other plant species.

The results of the present study also matched those of chapter 5 where, for all the preferred plants, *An. gambiae* often fed on plant parts rich in sugars. The present study indicated a positive association between: 1) total sugar concentration and 2) individual sugar concentration in the preferred plants (and plant parts) and median survival time of mosquitoes in those plants ($P < 0.0001$). Among 10 sugar types tested, monosaccharides glucose ($P = 0.003$), fructose ($P = 0.04$), and gulose ($P = 0.004$) were significantly associated with high survival, while sucrose, though common in most plants, had a negative, but no significant ($P = 0.62$) correlation with survival. These results justify the hypothesis that the quality of sugar meals may be correlated with mosquito longevity. Nayar and Sauerman (1971) also indicated physiological effects of carbohydrates on the survival of female *Ae. taeniorhynchus* adults. The difference on the impact of those sugar types on the survival of *An. gambiae* is still unclear.

In the present study, the high concentration of sugars in *T. stans*, *S. didymobotrya*, and *R. communis* with high amount of monosaccharides glucose, fructose and gulose may have enabled mosquitoes to consume greater amount of these sugars, thereby offsetting the possible costs of less nutritive components. On the contrary, the main sugar in *P. hysterophorus*, being the disaccharide sucrose, and in low quantity could only provide less benefit to mosquitoes. On the same line of sugar concentration on plants affecting survival, a previous study by Gary and Foster (2004) found that mosquito survival was longer in plants where the amount of fructose ingested was high. Similar results were reported on an insect other than mosquito, a parasitoid wasp, *Bathyplectes curculionis* that feeds on carbohydrate-rich food (Spafford and Evans, 2004). Impoinvil *et al.* (2004) also found that the lifespan of mosquitoes feeding on plants was associated with color intensity of anthrone test, indicating the concentration of fructose ingested by mosquitoes from plants. However, the present study confirms these previous findings while having the merit of identifying different sugars in plant offering a higher benefit to mosquitoes.

The poor survival of *An. gambiae* on *P. hysterophorus* in this study goes against the prediction on the evolutionary basis of preference. The reasons of the high attractiveness of *An. gambiae* by that plant despite the poor benefit in term of survival are still unknown. That plant might be offering a different advantage to mosquitoes that need to be investigated. Poor survival on *P. hysterophorus* may be attributed to the inadequate production of sugars by nectaries of the plant. The results of the previous study (Chapter 5) showed that *An. gambiae* fed preferably on the leaves of *P. hysterophorus.* Components other than sugars can also play

a role in reducing survival, because insects that feed on floral and extrafloral nectaries, do ingest food containing multiple sugars and other compounds (Gardener and Gillman, 2002).

When sugar composition and concentration of *L. camara* flower was included in the correlation analysis, the coefficient of correlation dropped. The survival of mosquitoes fed on *L. camara* was low despite the flower part of the later being rich in sugars. Gary and Foster (2004) and Impoinvil *et al.* (2004), when they fed mosquitoes on arbitraly chosen plants, also found a low survival of mosquitoes fed on *L. camara*. The poor survival of *An. gambiae* on *L. camara* may be a consequence of this plant being originally an unattractive food source for *An. gambiae*. It has been shown that mosquitoes commonly acquire sugar meals on the leaves (Chapter 5) and the present study showed that *L. camara*'s flowers are rich in sugars compared to leaves. Consequently, it is likely that nectar from *L. camara*'s flower is an unavailable food source, and nectar from the leaves is nutritionally inadequate for mosquito survival. *Lantana camara* leaves are known to contain a series of secondary compounds deleterous to some insects and other organisms (Deka *et al.*, 1998; Fatope *et al.*, 2002).

The present study did not show any significant effect of plants on eggs developed and oviposited by *An. gambiae*. Gary and Foster (2001) found that the total fecundity of *An. gambiae* was not affected by sugar availability, but daily fecundity was higher in mosquitoes fed blood alone compared to those fed blood plus sugar. This was because female *An. gambiae* could replace sugar with the increased blood feeding without suppressing their reproductive fitness. These observations were similar to the findings of Scott *et al.* (1997) and Costero *et al.* (1998) in *Ae. aegypti*, another anthropophilic species. In the present study,

the three consecutive blood meals given to mosquitoes may have replaced sugars in mosquitoes feeding on low sugar-content plants and the least preferred plant without supressing their reproductive fitness. This was confirmed in the correlation analysis, where no significant association was found between the total and individual sugar on plants and fecundity. Therefore, sugar gradient (energy potential) from plants did not increase or reduce the ability of mosquitoes to develop and lay a high number of eggs. Despite that, the number of eggs laid on *P. hysterophorus* was the lowest. But when given only one blood meal, fecundity was poor on the mosquitoes fed on the least preferred plant and on the plant poor in sugar. The availability of certain plants, especially those least preferred by mosquitoes may have an epidemiological consequence, because mosquitoes will have to seek for several blood meals to compensate for the energy shortage in order to develop, mature and lay their eggs (Straif and Beier, 1996; Gary and Foster, 2001). Despite all these, delay in the onset of oviposition, with low percentage of mosquitoes ovipositing was observed in mosquitoes fed on water, *L. camara* and *P. hysterophorus*. The reasons for the delay are unclear. Perhaps the delay was a response to low energy reserves. The first blood meal may have been used to increase the energy level, then the second and third forwarded for reproduction, prolonging therefore the gonotrophic cycle. The low proportion of mosquitoes that oviposited could be explained by the fact that for most of the mosquitoes fed on the three nutrition groups, due to low energy for oviposition, retention would then allow females to enhance their fitness (Foster, 1995).

The findings of the present study suggest that most plants commonly fed on by *An. gambiae* enhance their longevity and fecundity, while mosquito's fitness was reduced when feeding

on a least preferred plant. Those plants being indigenous in the study area, and survival being an important factor on the vectorial capacity, these results suggest that malaria transmission may be substantially enhanced by the availability of certain sugar sources to wild vector population in endemic area. But one plant species was of particular attention: *P. hysterophorus*, though very abundant in the study area, and attractive to *An. gambiae*, considerably reduced their fitness. This particular plant might be offering different advantages to mosquitoes which are still unknown. Attractiveness and feeding of mosquito on *P. hysterophorus* despite its low nutritional benefit might explain multiple-feeding behaviour of mosquitoes on different plants (Chapter 5). Sugar composition and concentration in plants play an important role in the fitness of mosquitoes because of their nutritional value (Clements, 1999). But other unidentified components may also affect mosquito fitness.

CHAPTER SEVEN: EFFECT OF SELECTED PLANT SPECIES ON THE DEVELOPMENT OF *PLASMODIUM FALCIPARUM* IN *ANOPHELES GAMBIAE* IN WESTERN KENYA

7.1 Introduction

Anopheles mosquitoes acquire *Plasmodium* infections by bite, and in the ingested blood meal, the parasites multiply and transform from gametocytes to motile ookinetes in the midgut. Ookinetes traverse the midgut and lodge between the basal lamina and midgut epithelia, where they encyst and develop into sporozoites. However, besides blood meal, females *Anopheles* also feed plant-derived foods termed sugar meals (Beier, 1996) which are directed to the mosquito gut. It is clear that the environment for *P. falciparum* development in the gut of plant–fed mosquitoes comprises two different nutrient media and their respective digestive enzymes. The establishment of the infection and development from one stage to another probably depend on the components of the mosquito meals.

It was previously demonstrated that *An. gambiae* feeds selectively on plants in the laboratory (Chapter 5) and in most cases, selective feeding increases their fitness (survival and fecundity) (Chapter 6). An additional important aspect of feeding from plants is the possible effect of the ingested secondary compounds of plants on the susceptibility of vectors to parasites. The effect of various plants on the vector competence of *An. gambiae* for *P. falciparum* should be assessed, especially as *Plasmodium* infection reduces the fitness of anophelines (Hurd, 2003). The presence of sugar in *An. gambiae* crops have been demonstrated (Beier, 1996; Chapter 4), but Schlein and Müller (1995) and Müller and Schlein (2005) reported the presence of plant tissues in the midguts of *Cx. pipiens molestus*

Forskal, *An. sergentii* Theobald, *An. claviger* Meigen and *Ae. caspius* Pallas, using the stain calcofluor in field-tested mosquitoes. Ingested tissues of some plants caused mortality of *Leishmania major* parasites in the gut lumen of *P. papatasi* (Schlein and Jacobson, 1994), and similar meals may have similar effects in *Plasmodium* ingested by mosquitoes. Pathogens that are ingested with blood usually have to penetrate the midgut to establish infections. An initial requirement for penetration is the presence of appropriate receptors and ligands on the epithelial cells of the midgut (Leake, 1992; Billingsley, 1994). Sugar meals contain carbohydrates, lectins, and proteins that may compete with the pathogens on binding sites or destroy them, and thereby affect the establishment and outcome of infections.

It is therefore hypothesized that some plant diets of *An. gambiae* have an adverse effect on the development of the malaria parasite in their midgut. These assumptions are investigated in this objective.

7.2 Materials and Methods

7.2.1 Study area

The study area was Suba District, described in Chapter 3.

7.2.2 Mosquitoes

Mosquitoes used in the experiments were females *An. gambiae s.s.* Mbita strain, maintained at the insectary of ICIPE-TOC, Mbita Point and adapted for the ability to feed on artificial Parafilm® membrane. The rearing procedure is described in Chapter 3.

7.2.3 Effects of individual plant species on the parasite development in the mosquito midgut

7.2.3.1 Plants

Six plants species were used in the experiment: *Hamelia patens* J. (Rubiaceae), *Lantana camara* L. (Verbenaceae), *Ricinus communis* L. (Euphorbiaceae), *Senna didymobotrya* F. (Leguminosae), *Pathernium hysterophorus* L. (Asteraceae) and *Tecoma stans* L. (Bignoniaceae). They were selected from the previous study (Chapter 5) where five were observed to be most preferred by *An. gambiae,* and *L. camara* one of the least preferred plants among 13 dominant flowering plants growing around larval habitats and human dwellings in Suba District, western Kenya.

7.2.3.2 Experimental procedure and infection

7.2.3.2.1 Selection of gametocyte carriers

Plasmodium falciparum gametocyte carriers were selected following procedure described in Chapter 3.

7.2.3.2.2 Experimental infection of mosquitoes

Six batches of 100 newly emerged *An. gambiae* s.s. females were exposed each to different plants species in 60 x 60 x 60 cm netting cages. Another group was exposed to glucose 6% and used as control. Three-day-old mosquitoes were aspirated from each cage and put in paper cups labeled according to the plant or food regime, then starved for 3-8h (depending on ambient relative humidity). Mosquitoes were experimentally infected with gametocytaemic blood as described in Chapter 3. Blood fed mosquitoes were released in their respective

cages, offered their respective plants and held in the hazard-proof screen-walled greenhouse (glass roof with a layer of reed mats to prevent temperature extremes), with temperature ranging between 19-31°C. Mosquito exposed to all plants were reared in the same manner, infected with the same blood and held in the same conditions.

7.2.3.2.3 Estimation of gametocyte densities ingested by mosquitoes

The mean gametocyte density in the mosquito midgut was estimated by multiplying the mean blood meal volume of the mosquitoes ingested through membrane by the mean number of gametocytes per microliter of blood. The mean blood meal volume (1.6 µl ± 0.46) was quantified by a spectrophotometric analysis of the total hemoglobin content of the blood meal in the midguts of freshly engorged mosquitoes.

7.2.3.2.4 Mosquito dissection and parasite detection

On day 7-post infection, midguts of mosquitoes were dissected and oocysts detected by staining in 2% mercurochrome and examining under light miscroscope at 10x. For determination of oocyst infections, which tend to be highly aggregated, a minimum of 15 mosquitoes were examined individually per group per infection. The oocyst prevalence was calculated by dividing the number of oocyst infected mosquitoes by the total number of mosquitoes dissected per group. The mean oocyst intensity, expressed as number of oocysts per mosquito midgut, was described as the total number of oocysts counted divided by the number of positive mosquitoes. The wing length of the mosquitoes, measured from the tip (excluding the fringe) to the distal end of the allula with a precision of 0.02 mm, was used as an indication of body size (Koella and Lyimo, 1996).

7.2.4 Effect of high gametocytaemia on the inhibition of parasite development by plants

One plant species (*P. hysterophorus*), selected based on the results of the previous section of this chapter (effects of individual plant species on the parasite development in the mosquito midgut) as completely inhibiting parasite development in the midgut of *An. gambiae* was used against a control glucose. A range of gametocyte carriers with low and high gametocyte densities (range: 15-1454 gametocytes/µl of blood) were used to feed mosquitoes. Mosquitoes were experimentally infected, held, their body size determined, their midgut dissected and parasites determined as described above.

7.2.5 Effect of time of feeding on plants on parasite development in *An. gambiae* midgut.

Based on the results obtained in the previous section of this chapter (effects of individual plant species on the parasite development in the mosquito midgut), 4 plant species with the following properties were used in this objective. One plant reducing infection rate, but with no effect on parasite load: *R. communis,* three plant species reducing infection rate and parasite intensity: *P. hysterophorus, L. camara* and *S. didymobotrya*. For each plant, mosquitoes were divided in 4 groups. (1) One group fed on plant before infection, then on glucose after infection (plant-glucose), (2) a second group fed on glucose before infection, then on plant after infection (glucose-plant), (3) the third group fed on plant before and after infection (plant-plant), and (4) an additional group fed on glucose before and after infection and used as control (glucose-glucose). The experimental infection, holding conditions, mosquito's dissection and parasite's detection were done as described above.

7.2.6 Data analysis

Only infections resulting to at least one infected mosquito were considered for analysis. Infection variables included oocyst infection rate or oocyst prevalence and oocyst intensity. Prior to analysis (except chi-square), infection rates data were arcsin transformed, and all parasite densities were log transformed to increase their fit to the normal distribution. Infection prevalences were compared with chi-square (χ^2) analyses and oocyst intensities were compared using ANOVA. Means were separated using Student Newman Keuls (SNK). Pearson correlation was used to determine if there was an association between gametocyte densities and infection outcome in mosquitoes. Because there were considerable differences in infectiousness among the gametocyte carriers (Muirhead-Thomsom, 1954; Githeko *et al.*, 1992), the analysis was done per individual gametocyte carrier (or replicate). The effects of plants within each replicate were determined and because the magnitude of each effect was consistent among replicates, data of all replicates for each plant were pooled and analyzed as a single data set. The effect of wing length (size of the mosquito) was also included in the analysis as a potential confounder (Lyimo and Koella, 1992). All statistics were carried out using Excel 2000® and SAS version 8.2 for Windows®.

7.3 Results

A total of 97 gametocyte carriers aged between 3 and 14 years were successfully enrolled in the study and donated blood for mosquito infection. Thirty one carriers were used in the first part of the study, 21 in the second and 45 in the third one. A total of 17,064 mosquitoes were dissected for detection of oocysts with an average batch size of 28 mosquitoes dissected per replicate and per plant treatment.

7.3.1 Effects of individual plant species on the infection of *An. gambiae* by *P. falciparum*

Out of the 31 gametocyte carriers recruited to experimentally infect mosquitoes, 21 yielded at least one oocyst positive mosquito. Details of the number of carriers who successfully infected mosquitoes exposed to each nutritional group are presented in Table 7.1. The highest number of oocyst positive infections was observed on the glucose group [90.47% (19/21)] and the lowest was on *P. hysterophorus* group [0% (0/21)]. The average gametocyte density ingested by mosquitoes was 105.42 gametocytes/µl of blood.

Results on the oocyst prevalence and oocyst intensity of mosquitoes fed on each food regime are presented in Table 7.1. Different food regimes influenced the proportion of infected mosquitoes ($\chi^2 = 107.7$, df = 6, $P < 0.01$) as well as the mean number of oocysts produce per midgut ($F = 58.0$, df = 6, $P < 0.0001$). When ranking the oocyst prevalences of mosquitoes exposed to different plant species, it was substancially higher on *H. patens* and lower on *P. hysterophorus,* followed by *R. communis*, with a magnitude of 36% above, 100 and 53% below the control respectively. Oocyst prevalence was also significantly low on mosquitoes fed on *L. camara* and *S. didymobotrya* (Table 7.1).

A multiple comparison analysis of oocyst intensities in different groups showed that the average numbers of oocysts produced per infected mosquito were significantly high on *H. patens*, *T. stans*, and *R. communis* groups, and these were comparable to the glucose group. On the contrary, infected mosquitoes exposed to *L. camara*, *S. didymobotrya* and *P. hysterophorus* had significant fewer oocysts produced per infected midgut (Table 7.1). The sizes of mosquitoes were comparable in each group and therefore did not influenced the outcome of the infection ($F = 1.42$, $df = 6$, $P = 0.20$) (Table 7.1).

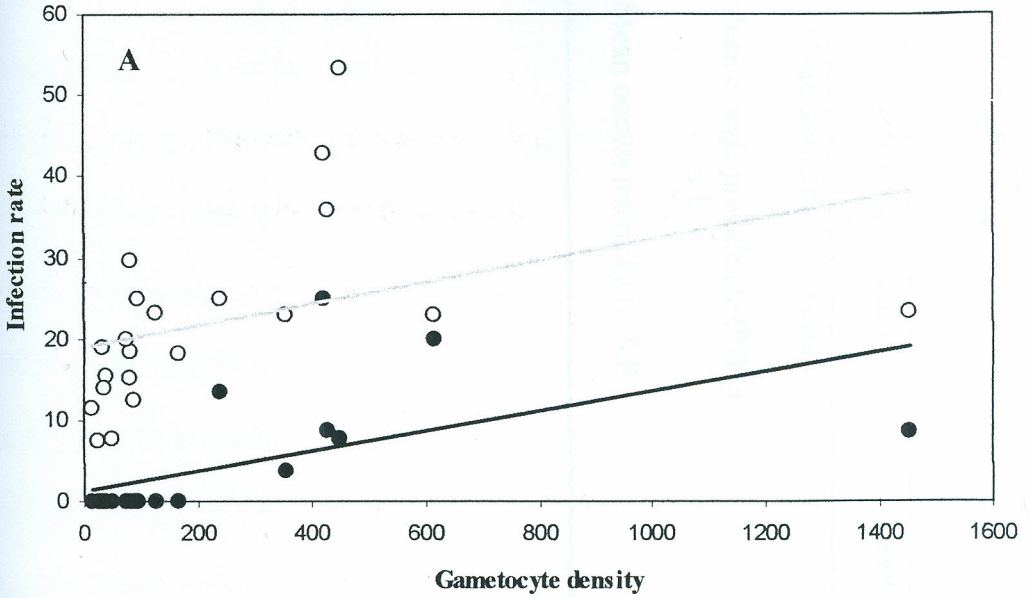
Table 7.1 Proportions of *P. falciparum* gametocyte carriers yielding positive infections, mean oocyst prevalences and mean oocyst intensities in infected mosquitoes allowed to feed on the control glucose, each plant species and their mean body size.

Parameter of infection	*Control Glucose 6%*	H. patens	T. stans	R. communis	L. camara	S. didymobotrya	P. hysterophorus
Positive infection (%)[i]	90.47 (19/21)	76.19 (16/21)	76.19 (16/21)	66.66 (14/21)	80.95 (17/21)	76.19 (16/21)	0.00 (0/21)
Number mosquitoes dissected	727	467	604	523	410	548	349
Oocyst prevalence (%) ± S.E.[ii]	15.21±2.85[a]	20.61±4.68[a]	13.43±3.30[a]	7.18±2.00[b]	9.22±2.23[b]	10.20±2.96[b]	0.00±0.00[c]
Mean oocyst intensity ± S.E.[iii]	9.01±0.98[a]	7.62±0.65[a]	7.67±0.76[a]	7.13±1.22[a]	1.67±0.21[b]	1.30±0.06[b]	0.00±0.00[c]
Mean body size ± S.E.[iv]	3.00±0.01[a]	3.02±0.01[a]	2.99±0.008[a]	2.98±0.009[a]	3.01±0.09[a]	2.99±0.01[a]	2.99±0.01[a]

[i] Proportion of gametocyte carriers used in the experimental feeds yielding positive oocyst infections.
[ii] Mean proportion (± Standard Error) of mosquitoes found with oocyst infections in their midguts expressed as a percentage.
[iii] Mean (± Standard Error) number of oocysts counted in the midguts of positive mosquitoes.
[iv] Mean (± Standard Error) body size of mosquitoes
Means with different letters in superscript in the same row are significantly different in SNK multiple mean comparison test (α=0.05).

7.3.2 Effect of the plant species *P. hysterophorus* on parasite development in mosquito infected with high gametocyte densities

Another set of infections was done on mosquitoes which were allowed to feed on *P. hysterophorus* to determine if the observed total inhibition of the parasite development in the mosquito by that plant persists if mosquitoes were infected with high gametocyte densities. Whereas the 21 gametocyte carriers [mean: 235.52 (range 15-1454) gametocytes/µl blood] infected mosquitoes fed on the control glucose, only 33.33 % (7/21) gametocyte carriers with a mean of 566.57 (range 238-1454) gametocytes/µl of blood yielded to at least one infected mosquito on the *P. hysterophorus* group. There was a positive association between gametocyte densities and mosquito infection rates in the *P. hysterophorus* group ($r= 0.77$; $P<0.01$) and glucose group ($r=0.71$; $P<0.01$). However, infection of mosquitoes which were allowed to feed on *P. hysterophorus* occurred only when mosquitoes were infected with gametocyte densities higher than 200 gametocytes/µl of blood (Figures 7. 1A and 1B). When considering only positive infections occurring on mosquitoes which were allowed to feed on *P. hysterophorus*, the mean prevalence of infection ($\chi^2 = 20.39$, $df = 1$, $P < 0.01$) and mean oocyst intensity ($F = 87.1$, $df = 1$, $P < 0.01$) were significantly lower when mosquitoes were allowed to feed on *P. hysterophorus* compared to glucose (Table 7.2).



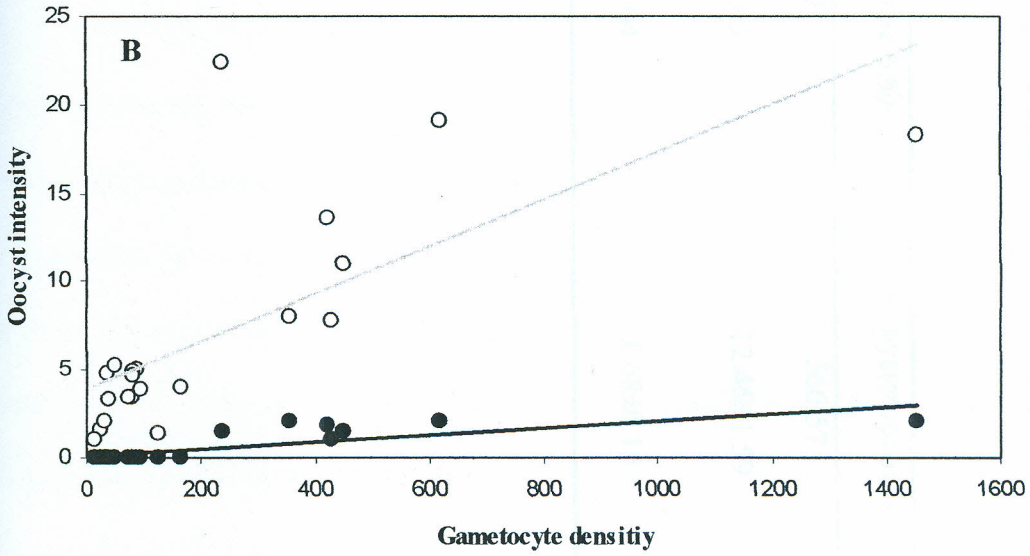


Fig. 7.1 Relationship between gametocyte densities ingested and (A) infection rates, and (B) parasite intensities in mosquitoes allowed to feed on the control glucose and *P. hysterophorus*. (○, ·····) Data from mosquitoes exposed to glucose; (●,——) mosquitoes exposed to *P. hysterophorus.*

Table 7.2 Proportions of mosquitoes infected with oocysts and mean oocyst intensities in infected mosquitoes allowed to feed on glucose and on *P. hysterophorus* when infected with high gametocyte densities yielding positive oocyst infections in mosquitoes allowed to feed on *P. hysterophorus*.

Parameter of infection	*Control (Glucose 6%)*	P. hysterophorus	*Probability*
Mean gametocyte ingested	566.57	566.57	1.00
Mean oocyst prevalence (%) (± S.E.)	32.31±2.43	12.46±1.59	<0.0001
Mean oocyst intensity (± S.E.)	14.30±0.64	1.68±0.15	<0.0001

7.3.3 Effect of time mosquitoes were allowed to feed on plants on the parasite development in their midgut

For the experimental infections carried out on mosquitoes when they were allowed to feed on each of these three plants (*R. communis, L. camara, S. didymobotrya*), (1) only before infection (plant-glucose), (2) only after infection (glucose-plant), (3) before and after infection (plant-plant) and (4) on glucose before and after infection (glucose-glucose), 82.22% (37/45) human volunteers successfully infected mosquitoes, with an average gametocytemia of 119.13 gametocytes/µl of blood. For the mosquitoes which fed on *P. hysterophorus* at different times as mentioned above, 21 carriers infected mosquitoes with a mean gametocytemia of 235.52 gametocytes/µl of blood.

When comparing different times of feeding on the plant within each plant species tested, there was a significant variation on the means prevalence of infection in the treatments within *R. communis* ($\chi^2 = 76.0$, df = 3, $P < 0.01$), and significant variations on the means prevalence of infection and oocyst intensity in the treatments within each of these plant species: *L. camara* ($\chi^2 = 27.5$, df = 3, $P = 0.009$; $F = 48.1$, df = 3, $P < 0.001$ respectively), *S. didymobotrya* ($\chi^2 = 25.3$, df = 3, $P = 0.014$; $F = 71.68$, df = 3, $P < 0.01$ respectively) and *P. hysterophorus* ($\chi^2 = 80.1$, df = 3, $P < 0.01$; $F = 31.86$, df = 3, $P < 0.01$ respectively). Results on the means prevalence of infected mosquitoes and oocyst intensity per infected mosquito per treatment of *R. communis*, *L. camara*, *S. didymobotrya* and *P. hysterophorus* are presented in Fig. (7.2A and 7.2B), (7.3A and 7.3B), (7.4A and 7.4B) and (7.5A and 7.5B) respectively. The mean infection rate was significantly lower when mosquitoes were allowed to feed on the plant either post-infection or throughout (before and after infection) in *R.*

communis and *P. hysterophorus* groups (Fig. 7.2A, 7.5A). However, it was comparable at all the plant-feeding times in *L. camara* and *S. didymobotrya* groups (7.3A, 7.4A), although it was much higher when they fed on plant pre-infection only, but all were significantly lower than the oocyst prevalence of mosquitoes which fed on glucose throughout. Concerning the mean oocyst intensity per infected mosquito, it was only significantly lower when mosquitoes were allowed to feed on the plant throughout (before and after infection). The mean oocyst intensity was not statistically different when mosquitoes were allowed to feed on the plant either prior to or post infection, but it was much lower when they fed on the plant after infection, and all were significantly lower than in mosquitoes which fed on the control glucose throughout. The trend was observed in all plants tested, except on *R. communis* where there was no statistical difference ($F = 3.7$, $df = 3$, $P = 0.1$) on the means oocyst intensitiy at all the plant-feeding times (Fig. 7.2 B, 7.3B, 7.4B, 7.5B).

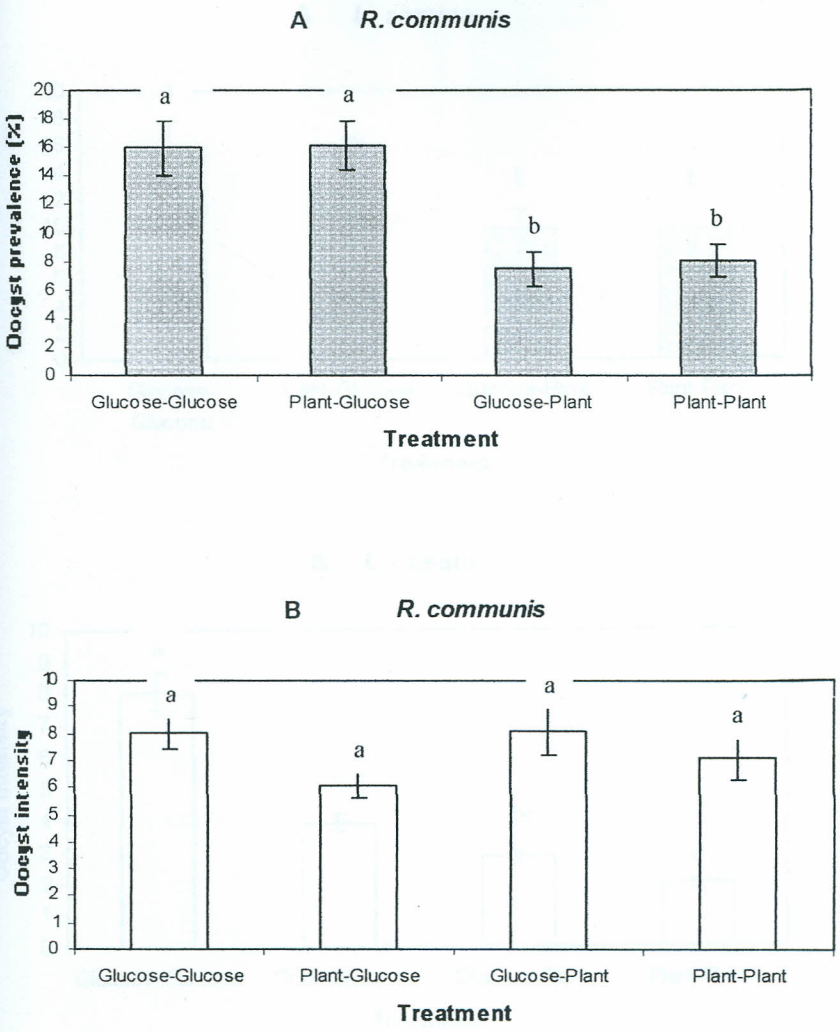


Fig. 7.2 Oocyst prevalences (A) and oocyst intensities (B) of mosquitoes allowed to feed on *R. communis* at different times. Food regime before the dash (-) was given to mosquitoes before infection and food regime after the dash was given after infection.

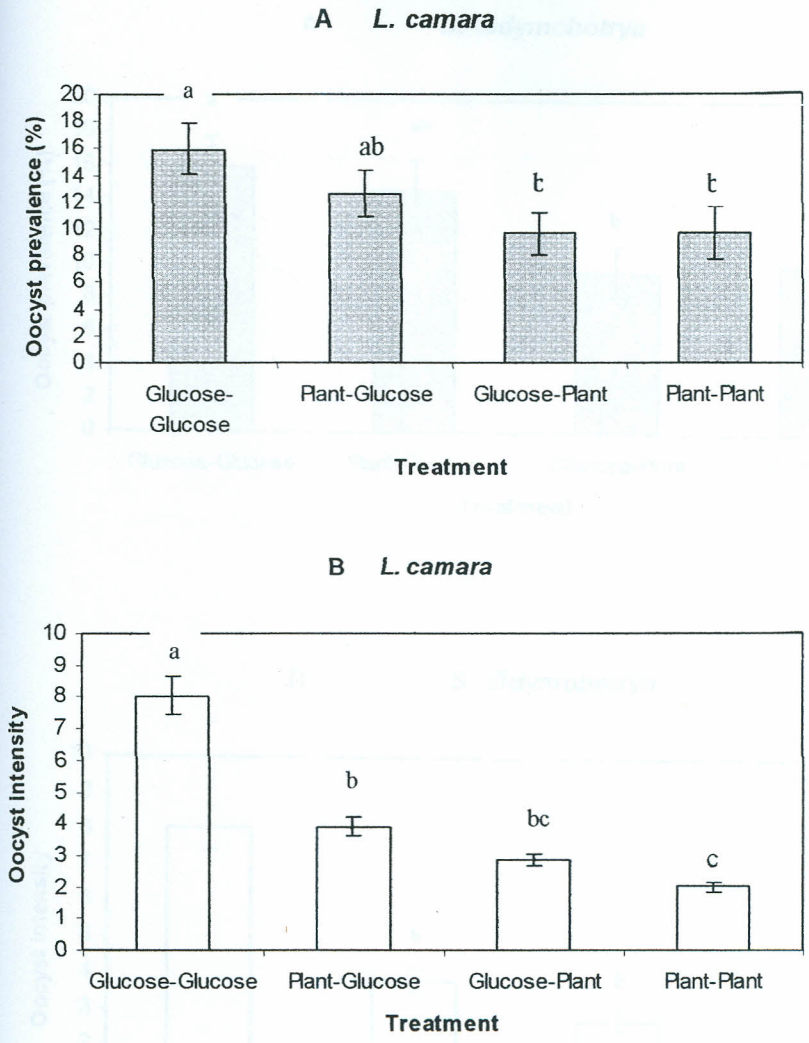


Fig. 7.3 Oocyst prevalences (A) and oocyst intensities (B) of mosquitoes allowed to feed on *L. camara* at different times. Food regime before the dash (-) was given to mosquitoes before infection and food regime after the dash was given after infection.

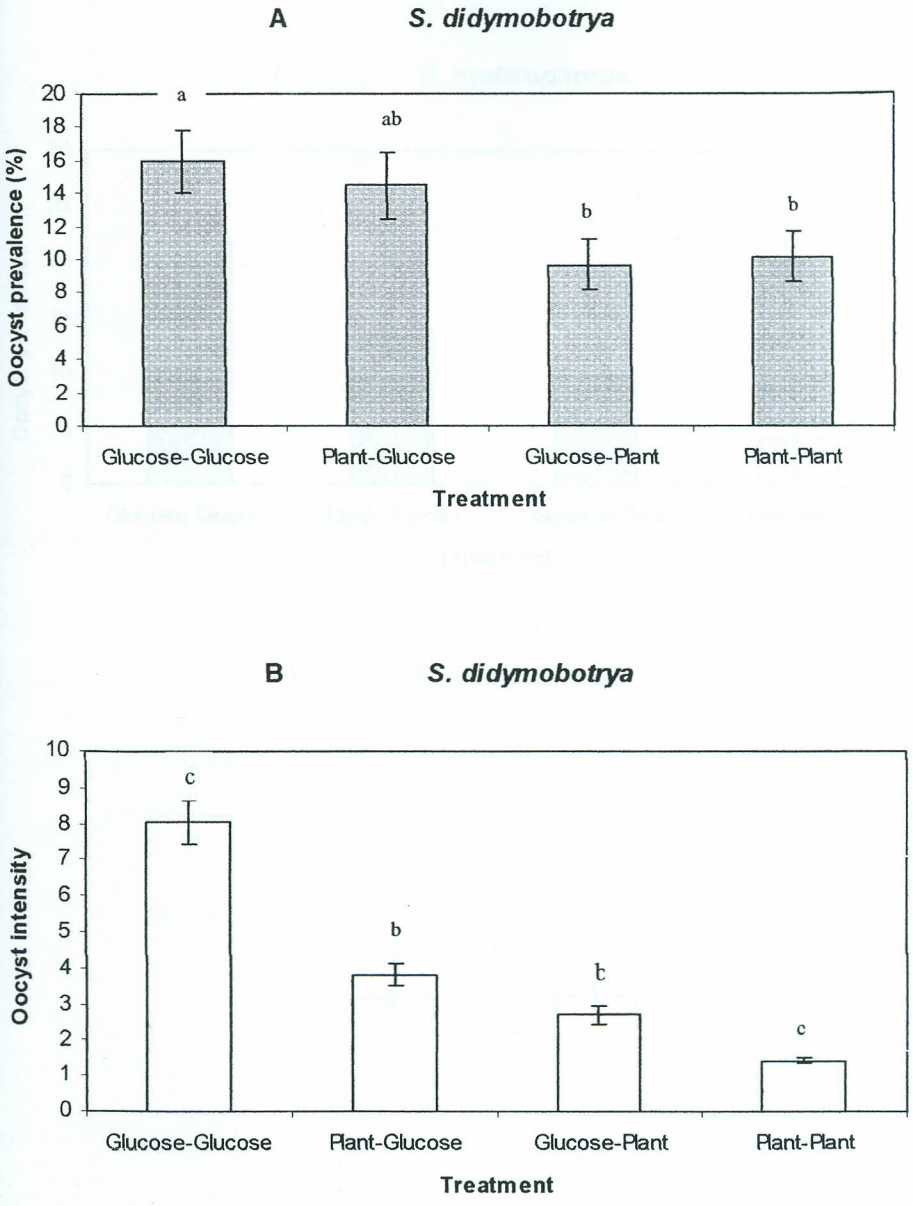


Fig. 7.4 Oocyst prevalences (A) and oocyst intensities (B) of mosquitoes allowed to feed on *S. didymobotrya* at different times. Food regime before the dash (-) was given to mosquitoes before infection and food regime after the dash was given after infection.

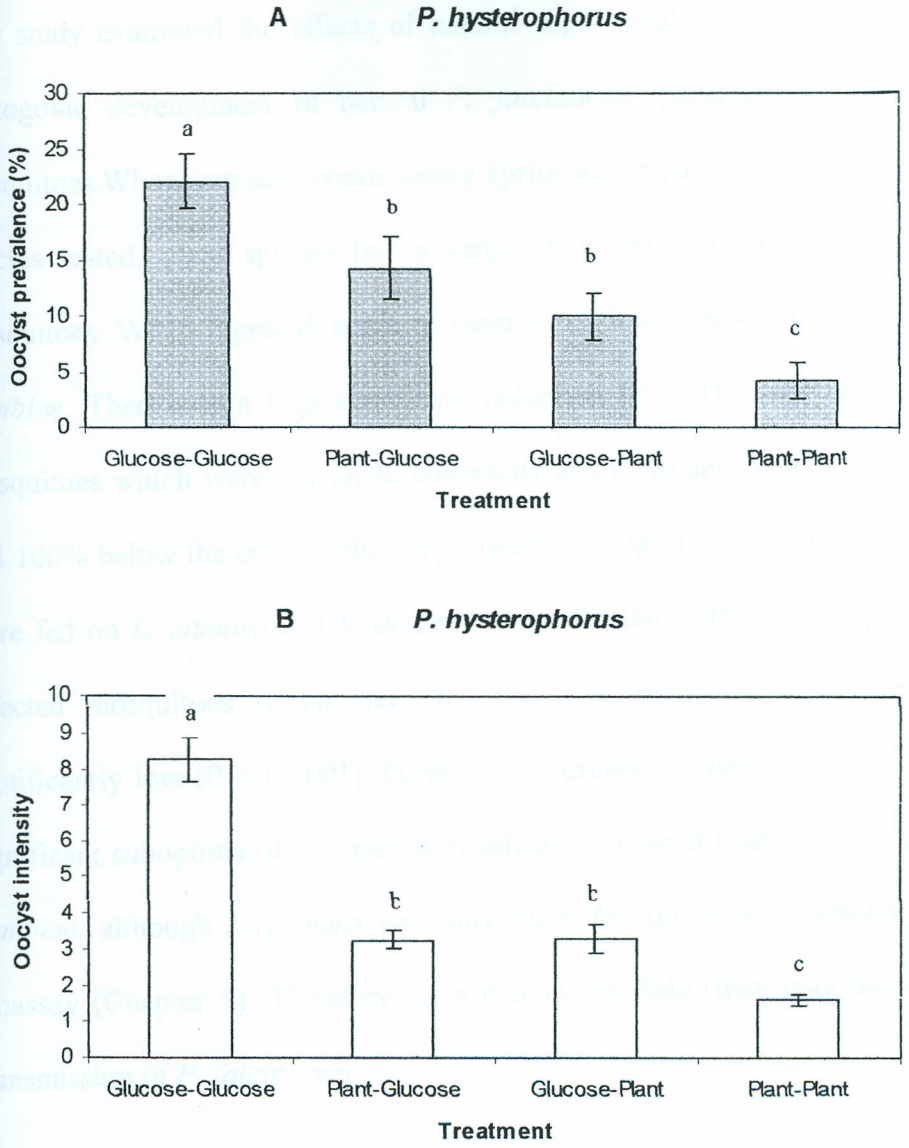


Fig. 7.5 Oocyst prevalences (A) and oocyst intensities (B) of mosquitoes allowed to feed on *P. hysterophorus* at different times. Food regime before the dash (-) was given to mosquitoes before infection and food regime after the dash was given after infection.

7.4 Discussion

This study examined the effects of natural sugar meals of *An. gambiae* mosquito on the sporogonic development of natural *P. falciparum* parasites in experimentally infected mosquitoes.When exposed continuously (prior and post infection) on the individual plant species tested, plant species had a range of effects on the *P. falciparum* infection in mosquitoes. When ingested, some of them were deleterious to the malaria parasites in *An. gambiae*. There was a high significant reduction ($P < 0.01$) on the oocyst prevalences of mosquitoes which were fed on *R. communis* and *P. hysterophorus* with a magnitude of 53 and 100% below the control glucose, as well as a significant reduction in mosquitoes which were fed on *L. camara* and *S. didymobotrya*. Similarly, the oocyst intensities produced by infected mosquitoes which were fed on *L. camara* and *S. didymobotrya* were also significantly low ($P < 0.0001$). Furthermore, except *L. camara*, all these plants that caused significant suboptimal development of infections ranked high in feeding preferences of *An. gambiae,* although *L. camara* was only often fed on by *An. gambiae* in non-competitive bioassay (Chapter 5). Therefore, as a diet in the field, they may decrease the chances of transmission of *P. falciparum*.

Mortality of *Leishmania major* in *Phlebotomus papatasi*, a non mosquito vector was also caused by plant-feeding of the sand flies (Schlein, 1986; Schlein and Jacobson, 1994). However, it is not known which of the plant substances are detrimental to *P. falciparum*. But lectins that are prevalent in plants (Etzler, 1986) are possibly involved. Most plants possess lectins and more than a thousand have already been found in sap, leaves, stems, flowers, fruits and seeds (Doyle, 1994). In *R. communis*, two lectins have been isolated from the

leaves, ricine and N-dimethyl-ricine (Visen *et al.*, 1992). The well-known lectins from the seeds of this plant species, the toxin ricin (RCA_{60}) and the agglutinin (RCA_{120}) have been used in several studies on *Leishmania*. RCA_{60} is known to be cytotoxic for promastigotes (Jacobson *et al.*, 1982). Ricine is also lethal on aphids *Myzus persicae* Suzler fed on castor bean plants (Olaifa *et al.*, 1991). The same toxin may have been the raison of low oocyst prevalence of mosquitoes which were fed on *R. communis*. In *L. camara*, leaves are known to contain a series of secondary compounds deleterous to some insects and other organisms (Deka *et al.*, 1998; Fatope *et al.*, 2002). This can explain the low oocyst prevalence and intensity of mosquitoes fed on that plant, especially as most feeding occur on its leaves (Chapter 5). Similar components in *P. hysterophorus* and *S. didymobotrya* may have also been responsible of the deleterous effects of those plants on *P. falciparum*.

Of all the four plants that showed adverse effects on the parasites in the mosquito, three plant species, *P. hysterophorus, L. camara* and *S. didymobotrya* had a consistent effect on both the oocyst prevalence and intensity. *R. communis* however only significantly reduced the oocyst prevalence. The reason for this inconsistency is unclear and studies on the mechanisms of inhibition of *R. communis* and its sporogonic-stages specific targets can provide a better understanding. It is also noteworthy that the plants that were deleterous to the parasites were edible and innocuous to the vector, since on a previous study, the minimum average survival time of mosquitoes on the plants tested (without any other source of nutrition) was 7 days, except on *P. hysterophorus* (4.7 days) (Chapter 5). Nevertheless, in the context of this study, mosquitoes which were dissected survived for 9 days with only one infectious blood meal and each plant as the only source of food.

The present study also showed that the effect of *P. hysterophorus* on the malaria parasite varied with the gametocyte densities of the feeds. A total inhibition of the parasite development was obtained only when mosquitoes were infected with less than 200 gametocytes /µl of blood, and a partial inhibition with higher gametocyte densities. Although the implication of the gametocyte densities as controlling infection have been controversial, Graves (1980), Boudin *et al.* (1989) and Tchuinkam *et al.* (1993) found that there was a relation between high gametocyte densities and success of infection. The present study also showed a positive correlation between gametocyte densities and oocyst prevalence ($P < 0.01$). The lethal effect of *P. hysterophorus* may therefore be parasite density dependant in the mosquito midgut.

In the present study, significant variation in oocyst prevalences and intensities was observed during intermittent plant/glucose exposure, each given at different times in relation to infection, suggesting that the deleterous effect of a plant species on *P. falciparum* varies depending on the time mosquitoes were allowed to feed on it. In all cases, the deleterous effects of plants on *P. falciparum* was optimal when mosquitoes were allowed to feed on plants throughout, but reduced when glucose was also part of the mosquitoes meal, given either before infection or after. However, in such cases, the lethal effect was higher when mosquitoes were exposed to plants after infection compared to before infection. The same tendency of plant effects was observed in a study on sandfly when post infection plant diet caused high impaired infection compared to preinfection feeding (Schlein and Jacobson, 1994). These results suggest that the possible harmful components of the sugar meals may affect specific stages of the parasite sporogonic cycle. Alternatively, the long duration of the

infected mosquitoes with plants (when allowed to feed on plants throughout or after infection) may have allowed a cumulative influence of lethal factors that were diminished within the relative short (2 days) period mosquitoes were allowed to feed on plants before infection only. Another explanation is that, Jacobson and Schlein (1999) reported a glucose affinity for lectins, which can therefore inhibit their activity. The lectin inhibitory effect of glucose may have not happened in the treatment where mosquitoes were allowed to feed on glucose preinfection, then on plant postinfection. This is because digestion in the gut may have diminished the amount of glucose present when plants were ingested.

In conclusion, the present study has shown that some plants preferred by *An. gambiae* reduce their vector competence, and this is apparently the consequence of plant feeding in nature. The lethal effects of plants in *P. falciparum* are only optimal when feeding occurs throughout on the same plant, and reduces in the case of intermittent feeding with another food. These observations probably explain what happens in nature since continuous feeding on the same plant is rare. The transmission of malaria will probably be affected by the availability of some plant diets of vectors and this may play a significant role in nature.

CHAPTER EIGHT: IDENTIFICATION OF STAGE-SPECIFIC TARGETS OF PLANT SUBSTANCES ON *PLASMODIUM FALCIPARUM* DEVELOPMENT IN *ANOPHELES GAMBIAE.*

8.1 Introduction

Vector competence is an important element in malaria epidemiology. Only mosquitoes carrying sporozoites in their salivary glands are biologically capable of transmitting the parasites during the blood-feeding process. Sporogonic development of malaria parasite in anopheline mosquitoes involves sequential series of developmental steps starting from female and male gametocytes being taken up by the mosquito through a blood meal. They will develop into gametes, which fuse to form a zygote in the mid gut. The zygotes further develop into ookinetes which penetrate the midgut wall to form oocysts. The oocysts develop into sporozoites that migrate forward and invade the salivary glands. These are subsequently injected into the next victim during another feed. Failure of the parasite to complete any of the essential steps in development prevents transmission in the vertebrate host. Mosquito species innately vary in their vector competence for human malaria parasites due to both genetic and environmental factors.

Several studies with rodent and human malaria parasites have revealed that the early stages of the midgut sporogonic cycle; from gametes to ookinetes and the ookinete-to-oocyst transitions are the most vulnerable stages and links in sporogonic development of malaria parasites (Vaughan *et al.*, 1992, 1994; Gouagna *et al.*, 1998). These stages in the midgut are exposed to various attacks including transmission blocking immunity (TBI) (Gouagna *et al.*, 1998), transmission blocking activity (Mulder *et al.*, 1994) and other environmental factors

such as temperature, humidity (Okech *et al.*, 2004). Other important factors which can affect these stages in the midgut are sugar meals ingested by mosquitoes from plants. These plant substrates are also directed to the mosquito midgut (Schlein and Muller, 1995), and may either compete with the parasites in any stage of their development for various receptors or simply lyse or agglutinate them. However, knowledge is lacking on the stage-specific targets of these plants on the early *P. falciparum* sporogonic development in the mosquito.

8.2 Materials and Methods

8.2.1 Study area

The study was conducted in Suba District. The study area is described in Chapter 3.

8.2.2 Mosquitoes

Mosquitoes used in this study were female *An. gambiae* s.s. mosquitoes Mbita strain, reared as described in Chapter 3 and adapted for the ability to feed through Parafilm® membrane.

8.2.3 Plants

Four plant species were used in this study: *Ricinus communis* L., *Lantana camara* L., *Senna didymobotrya* F., and *Pathernium hysterophorus* L. They were identified in Chapter 7 as reducing infection rate and parasite intensity (*L. camara*, *S. didymobotrya* and *P. hysterophorus*).

8.2.4 Experimental infection of mosquitoes

For each plant, mosquitoes were divided in 4 groups: (1) one group allowed to feed on plant pre-infection, then on glucose post-infection (Plant-Glucose), (2) a second group fed on glucose pre-infection, then on plant post-infection (Glucose-Plant), (3) the third group allowed to feed on plant pre and post infection (Plant-Plant), (4) an additional group fed on glucose pre and post infection and used as control (Glucose-Glucose). Batches of 100-200 newly emerged female mosquitoes were selected and assigned randomly to each group, thereby avoiding a size bias that might have affected the infection outcome (Lyimo and Koella, 1992). They were fed as described above and infected when 3 days old. Screening and recruitment of *P. falciparum* gametocyte carriers, experimental infection of mosquitoes were done as described in Chapter 3. After infection, blood fed mosquitoes were returned to their respective cages, fed as described above and all the groups held in the hazard-proof screen-walled greenhouse (glass roof with a layer of reed mats to prevent temperature extremes) under similar conditions (temperature ranging from 19-31°C).

8.2.5 Estimation of macrogametocyte densities ingested by mosquitoes

The mean macrogametocyte density in the mosquito midgut was estimated by multiplying the mean blood meal volume of the mosquitoes ingested through membrane by the mean number of macrogametocytes per microliter of blood. The number of macrogametocytes per microliter of blood was estimated by multiplying the gametocyte density from the thick blood smear by the gametocyte sex ratio. The mean blood meal volume (1.6 µl ± 0.46) was quantified by a spectrophotometric analysis of the total hemoglobin content of the blood

meal in the midguts of freshly engorged mosquitoes. The average ratio of macrogametocytes to microgametocytes was found to be 2:1.

8.2.6 Determination of mosquito infections

The most direct method for measuring parasite efficiency in the vector involves direct quantification of parasite numbers at successive stages as they develop from gametocyte to oocyst within the vector (Vaughan *et al.*, 1992). Mosquito dissections to detect and quantify macrogametes and ookinetes followed the method previously described by Gouagna *et al.* (1998, 1999). Subsequent to each blood feed, a subsample of 5 fed mosquitoes per treatment were dissected in phosphate buffer saline (PBS) after 30 min and 18-24 h post feeding (Noden *et al.*, 1995; Robert *et al.*, 1995) to detect macrogametes (i.e. evidence of parasite ingestion by the mosquito) and ookinetes (indication of parasite development) respectively. Their entire midguts were disrupted and suspended in a vial containing 50 µl of specific fluorescein isothiocyanate (FITC)-labelled monoclonal antibodies against 48/45 kDa and against 25 kDa (for macrogametes and ookinetes respectively) gamete stage antigens in 0.05% Evans blue (Robert *et al.*, 1995). After 30 min incubation in darkness at room temperature, the suspension was centrifuged in 1,000 µl PBS at 2000g for 2 min to remove excess Evans blue stain. The resulting pellet was then resuspended in 50 µl PBS and homogenized. The preparation was then placed between slide and coverslip and the entire slide screened for macrogametes or ookinetes using a fluorescent light microscope at a magnification of x500 using the x50 oil immersion objective lens. On day 7 post-infection, the remaining mosquitoes from each group were individually dissected. Their midgut were individually stained in 2% mercurochrome and examined under light microscope at a

magnification of x100 using the x10 objective lens. Because pool mosquitoes were used for macrogametes and ookinetes enumeration, macrogamete and ookinete prevalences could not be estimated. Macrogamete and ookinete intensities were calculated as the total of macrogametes and the total of ookinetes divided by the number of mosquitoes in the pool. The intensity of oocyst infection was defined as the total number of oocysts counted on infected midguts. The average oocyst intensity was calculated by dividing the total number of oocysts on the infected mosquito midguts by the total number of infected mosquitoes in each experimental group and is expressed as the number of oocyst per mosquito midgut.

8.2.7 Data analysis

Infection outcome variables included macrogamete intensity, ookinete intensity, oocyst intensity and the overall mortality coefficient (k) for parasites as they developed from gametocytes to oocysts via the gamete and ookinete stages (Vaughan *et al.*, 1992; Gouagna *et al.*, 1998).

Comparisons of the mean intensities of macrogametes, ookinetes and oocysts were calculated using analysis of variance (ANOVA) in SAS version 8.2 for Windows® and means were separated using LSD (least significant difference). The loss in parasite numbers was estimated by a population mortality coefficient (k), representing the differences between intensities at 2 consecutive stages. These were calculated for the transitions in early parasite development from macrogametocytes to macrogametes (k-1), macrogametes to ookinetes (k-2) and ookinetes to oocyts (k-3), as previously described (Vaughan *et al.*, 1992; Gouagna *et al.*, 1998), where k-1 represents $\log_{10}$ (macrogametocytes) minus $\log_{10}$ (macrogametes), k-2

represents $\log_{10}$ (macrogametes) minus $\log_{10}$ (ookinetes), k-3 represents $\log_{10}$ (ookinetes) minus $\log_{10}$ (oocysts), whereas overall mortality , K, is the sum of k-1, k-2 and k-3. The antilog of K gives a quantitative measure of the magnitude of that loss (Vaughan *et al.*, 1992, 1994). However, this may sometimes be represented by parasite yield, Y, which is the inverse of the antilog K, expressed as a percentage.

8.3 Results

For this study, 22 gametocyte carriers successfully infected mosquitoes. A total of 11946 mosquitoes were dissected, oocyst detection was done on an average batch size of 32 mosquitoes dissected per replicate and per experimental treatment, and batches of 5 mosquitoes were dissected for macrogametes and ookinetes per replicate and per treatment. The average gametocyte density per carrier was 275.80 gametocytes/µl of blood.

Data on the mean parasite intensities (macrogametes, ookinetes and oocysts) per infected mosquito in all treatments (feeding time) of each plant tested are presented in Fig. 8.1, 8.2, 8.3 and 8.4 respectively for *R. communis*, *L. camara*, *S. didymobotrya* and *P. hysterophorus*. When mosquitoes were allowed to feed on *R. communis* at different times in relation to infection, and on the control glucose, there was no variation on the macrogamete ($F = 2.15$, $df = 3$, $P = 0.09$), ookinete ($F = 1.89$, $df = 3$, $P = 0.13$) and oocyst ($F = 1.86$, $df = 3$, $P = 0.13$) intensities per infected mosquito (Fig. 8.1).

In the case of *L. camara*, macrogamete, ookinete and oocyst intensities varied significantly across treatments. Macrogamete and ookinete intensities were significantly low when mosquitoes fed on the plant preinfection ($F = 3.45$, $df = 3$, $P = 0.01$) and ($F = 11.3$, $df = 3$, P

< 0.001) for macrogametes and ookinetes respectively. But oocyst intensity was only significantly low when mosquitoes fed on plant throughout, and was comparable when they fed either pre or post infection, but all were significantly lower than on the control glucose (F = 19.37, df = 3, P < 0.001) (Fig. 8.2).

As in the case of *L. camara*, similar results were obtained in *S. didymobotrya*. The intensities of macrogametes, ookinetes and oocysts differed significantly in all the treatments. Macrogamete and ookinete intensities were significantly lower when mosquitoes fed on plant preinfection (F = 3.36, df = 3, P = 0.02) and (F = 10.69, df = 3, P < 0.001) for macrogametes and ookinetes respectively. But the oocyst intensity was only significantly lower when mosquitoes fed on the plant throughout, and was comparable when they fed either pre or post infection, but all were significantly lower than on the control glucose (F = 33.70, df = 3, P < 0.001) (Fig. 8.3).

In the case of *P. hysterophorus*, macrogamete intensities were comparable in all the treatments and the control (F = 1.23, df = 3, P = 0.2). Ookinete intensity was significantly low when mosquitoes fed on plant preinfection (F = 37.67, df = 3, P < 0.001). Oocyst intensities were comparable when they fed either pre or post infection, but significantly low when mosquitoes fed on the plant throughout, and significantly higher on the control glucose (F = 54.88, df = 3, P < 0.001) (Fig. 8.4).

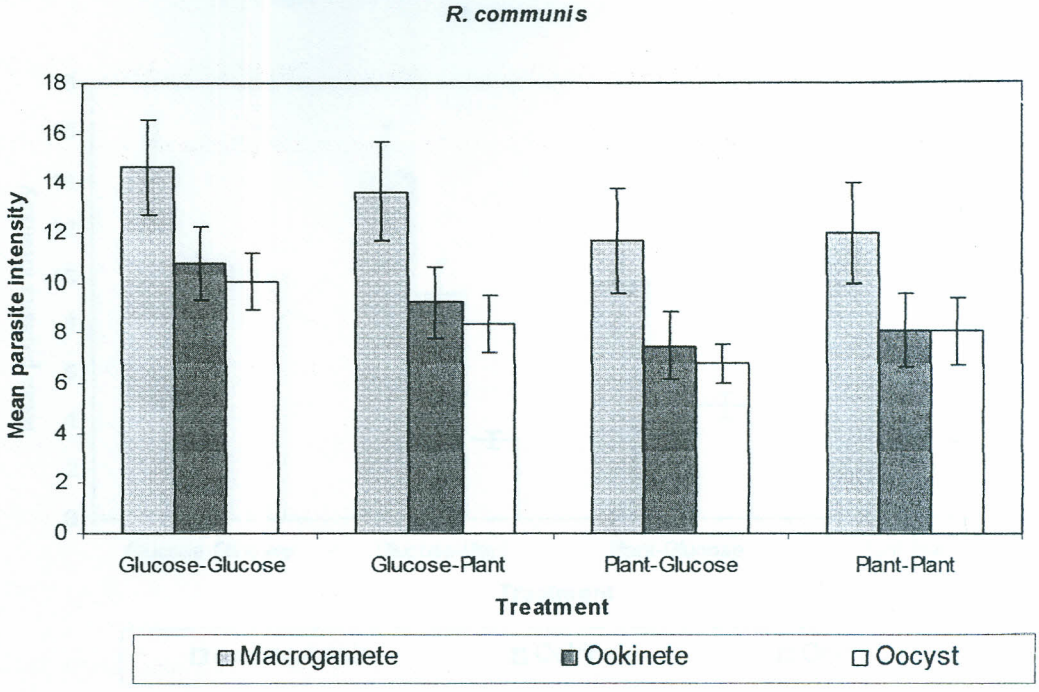


Fig. 8.1 Mean parasite intensities ± SEM of macrogamete (grey bars), ookinete (dotted bars) and oocyst (clear bars) in the midgut of experimentally infected *An. gambiae* dissected 30 min, 24h and 7 days post infection, respectively, and fed on glucose throughout (Glucose-Glucose), on *Ricinus communis* preinfection only (Plant-Glucose), postinfection only (Glucose-Plant) and throughout (Plant-Plant).

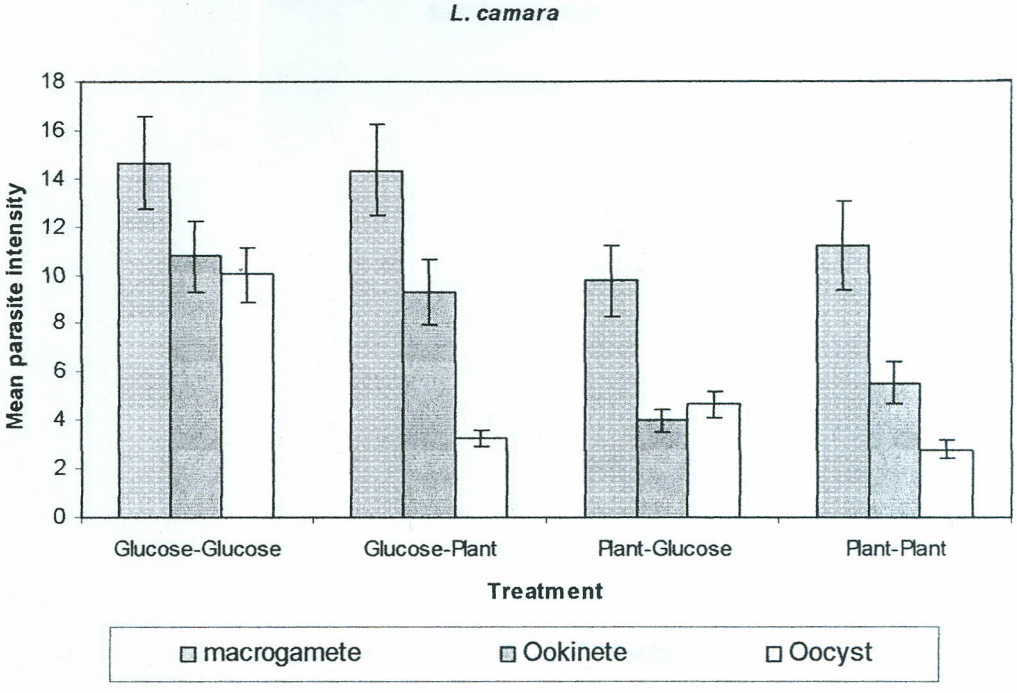


Fig. 8.2 Mean parasite intensities ± SEM of macrogamete (grey bars), ookinete (dotted bars) and oocyst (clear bars) in the midgut of experimentally infected *An. gambiae* dissected 30 min, 24h and 7 days post infection, respectively, and fed on glucose throughout (Glucose-Glucose), on *Lantana camara* preinfection only (Plant-Glucose), postinfection only (Glucose-Plant) and throughout (Plant-Plant).

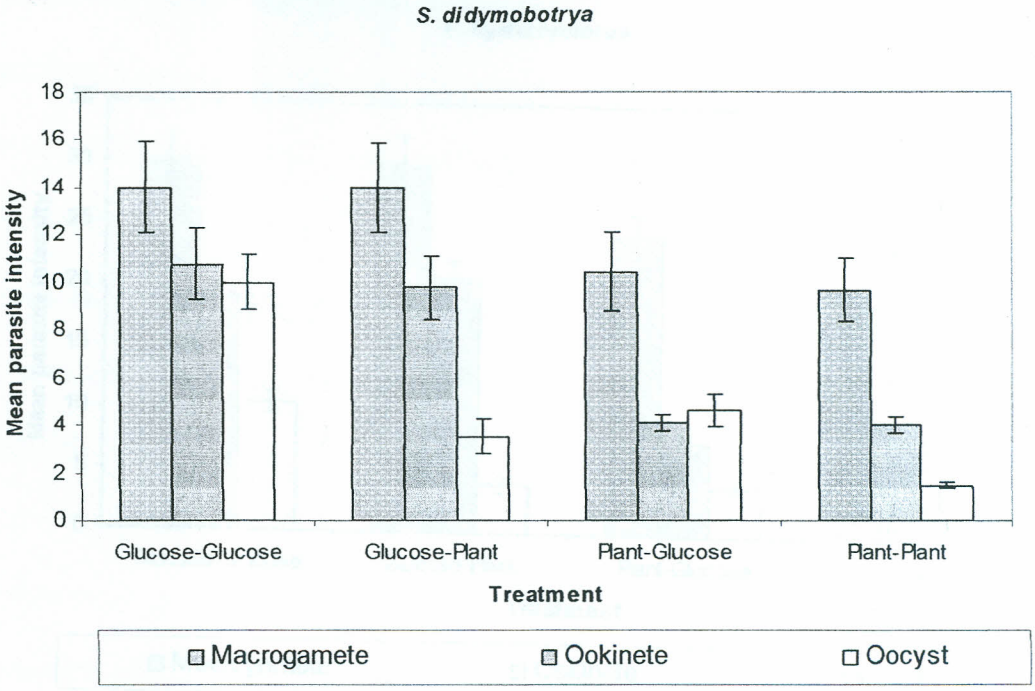


Fig. 8.3 Mean parasite intensities ± SEM of macrogamete (grey bars), ookinete (dotted bars) and oocyst (clear bars) in the midgut of experimentally infected *An. gambiae* dissected 30 min, 24h and 7 days post infection, respectively, and fed on glucose throughout (Glucose-Glucose), on *Senna didymobotrya* preinfection only (Plant-Glucose), postinfection only (Glucose-Plant) and throughout (Plant-Plant).

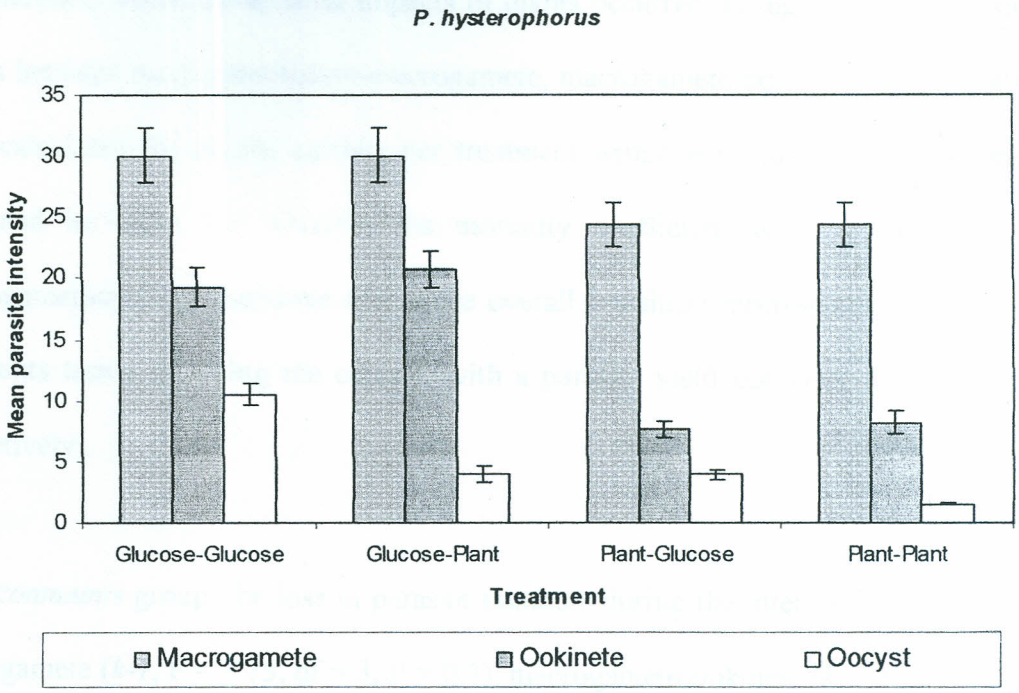


Fig. 8.4 Mean parasite intensities ± SEM of macrogamete (grey bars), ookinete (dotted bars) and oocyst (clear bars) in the midgut of experimentally infected *An. gambiae* dissected 30 min, 24h and 7 days post infection, respectively, and fed on glucose throughout (Glucose-Glucose), on *Pathernium hysterophorus* preinfection only (Plant-Glucose), postinfection only (Glucose-Plant) and throughout (Plant-Plant).

To determine where the greatest impacts of plants occurred during parasite development, *K* values between macrogametocyte-macrogamete, macrogamete-ookinete and ookinete-oocyst were calculated for all the carriers per treatment within each plant tested and means are presented in Table 8.1. Overall, the mortality coefficient was high in the transition macrogametocyte-macrogamete and in the overall transition macrogametocyte-oocyst in all the plants tested including the control, with a parasite yield not more than 15% and 10% respectively.

In *R. communis* group, the loss in parasite numbers during the interstage macrogametocyte-macrogamete (*k-1*, $F = 4.75$, $df = 3$, $P = 0.3$), macrogamete-ookinete (*k-2*, $F = 7.80$, $df = 3$, $P = 0.1$) and ookinete-oocyst (*k-3*, $F = 4.26$, $df = 3$, $P = 0.06$), did not vary whether mosquitoes fed on glucose, on plant pre, post, pre and post infection. Therefore, the overall losses from macrogametocytes to oocyts were also comparable (*K*, $F = 3.75$, $df = 3$, $P = 0.12$).

In *L. camara*, parasite loss in the transition macrogametocyte-macrogamete (*k*-1) was similar in all the treatments ($F = 8.35$, $df = 3$, $P = 0.1$). The parasite loss in the transition macrogamete-ookinete (*k*-2) was significantly high on mosquitoes exposed to the plant preinfection ($F = 9.98$, $df = 3$, $P = 0.01$). A significant lost was obtained on the transition ookinete-oocyst (*k-3*) when mosquitoes were exposed to the plant postinfection ($F = 29.66$, $df = 3$, $P < 0.001$). But the overall lost (*K*) was comparable when mosquitoes were exposed to the plant at any particular time, but significantly higher than when mosquitoes fed on the control glucose throughout ($F = 18.29$, $df = 3$, $P < 0.001$).

In *S. didymobotrya*, parasite loss in the transition macrogametocyte-macrogamete (*k*-1) was statistically comparable in all the groups ($F = 5.45$, $df = 3$, $P = 0.13$). A significant loss in the transition macrogamete-ookinete (*k*-2) ($F = 5.81$, $df = 3$, $P = 0.008$) was obtained in all the treatments where mosquitoes were exposed to the plant preinfection. A significant loss was obtained on the transition ookinete-oocyst (*k-3*) when mosquitoes were exposed to the plant postinfection ($F = 87.07$, $df = 3$, $P < 0.001$). However, the highest overall lost was obtained when the plant was given to mosquitoes throughout (pre and post infection) and the lowest was obtained on mosquitoes which fed on the control glucose throughout ($F = 11.76$, $df = 3$, $P < 0.001$).

In *P. hysterophorus*, parasite loss in the transition macrogametocyte-macrogamete (*k*-1) was statistically comparable in all the groups ($F = 1.85$, $df = 3$, $P = 0.13$). A significant loss in the transition macrogamete-ookinete (*k*-2) ($F = 62.34$, $df = 3$, $P < 0.001$) was obtained in all the treatments where mosquitoes were exposed to the plant prior to infection. A significant loss was also obtained on the transition ookinete-oocyst (*k-3*) when mosquitoes were exposed to the plant postinfection ($F = 56.78$, $df = 3$, $P < 0.001$). However, the highest overall lost was found on mosquitoes which were exposed to the plant throughout the experiment and the lowest was obtained on mosquitoes which fed on the control glucose throughout ($F = 41.59$, $df = 3$, $P < 0.001$).

Table 8.1 Interstage parasite mortalities during the early *P. falciparum* parasite development in *An. gambiae* fed at different times on *R. communis*, *L. camara*, *S. didymobotrya* and *P. hysterophorus* (values in parenthesis are the parasite yield in percentage).

Plants	*Treatments*	*Macrogametocyte-Macrogamete (k-1)*	*Macrogamete-Ookinete (k-2)*	*Ookinete-Oocyst (k-3)*	*Total (K)*
Ricinus communis	Glucose-Glucose	0.99 (10.23)	0.12 (75.85)	0.03 (93.32)	1.14 (7.24)
	Glucose- *R. communis*	1.02 (9.54)	0.16 (69.18)	0.03 (93.32)	1.21 (6.16)
	R. communis-Glucose	1.08 (8.31)	0.18 (66.06)	0.03 (93.32)	1.29 (5.12)
	R. communis-R. communis	1.07 (8.51)	0.16 (69.18)	0.001 (99.77)	1.23 (5.87)
Lantana camara	Glucose-Glucose	0.99 (10.23)	0.12 (75.85)	0.03 (93.32)	1.14 (7.24)
	Glucose-*L. camara*	1 (10)	0.17 (67.6)	0.38 (41.61	1.55 (2.81)
	L. camara-Glucose	1.15 (7.07)	0.34 (45.7)	0.07 (85.11)	1.56 (2.75)
	L. camara-L. camara	1.1 (7.94)	0.27 (53.7)	0.24 (57.54)	1.61 (2.45)
Senna didymobotrya	Glucose-Glucose	0.99 (10.23)	0.12 (75.85)	0.03 (93.32)	1.14 (7.24)
	Glucose-*S. didymobotrya*	1.01 (9.77)	0.14 (72.44)	0.38 (41.68)	1.53 (2.95)
	S. didymobotrya-Glucose	1.13 (7.41	0.35 (44.66)	0.002 (99.54)	1.48 (3.29)
	S. didymobotrya-S. didymobotrya	1.16 (6.91)	0.32 (47.86)	0.31 (48.97)	1.79 (1.62)
Pathernium hysterophorus	Glucose-Glucose	0.83 (14.79)	0.19 (64.56)	0.24 (57.54)	1.26 (5.49)
	Glucose-*P. hysterophorus*	0.83 (14.79)	0.16 (69.18)	0.64 (22.9)	1.63 (2.34)
	P. hysterophorus-Glucose	0.92 (12.02)	0.47 (33.88)	0.24 (57.54)	1.63 (2.34)
	P. hysterophorus-P. hysterophorus	0.92 (12.02)	0.44 (36.3)	0.56 (27.54)	1.92 (1.2)

K is the total mortality from the stage macrogametocyte to oocyst stage.

8.4 Discussion

This field study used the direct membrane-feeding assay to investigate the stage-specific targets of plants, fed by *An. gambiae* on the early sporogonic development of *P. falciparum*. It was observed that mosquitoes which fed continuously on three plant species (*S. didymobotrya*, *L. camara* and *P. hysterophorus*) developed fewer oocysts, and parasite losses were significant in the transition macrogamete-ookinete and ookinete-oocyst. These two transitions have been previously identified as the most vulnerable links of the sporogonic development of the malaria parasite (Vaughan *et al.*, 1992, 1994; Mulder *et al.*, 1994; Gouagna *et al.*, 1998; Okech *et al.*, 2004).

In the present study, parasite loss was very high in the transition macrogametocyte-macrogamete, in all the plants tested including the control, with a parasite yield of not more than 15%. Just how low these parasite yields on the transition macrogametocyte-macrogamete are, depend on underlying conditions. The work was done with natural parasite populations, although exposure of mosquitoes involved an artificial membrane feeding approach. One possibility is damage to gametocytes during blood drawing, heparin, or the process of placing blood into glass feeders during the *ex vivo* feeding procedure (Rutledge *et al.*, 1964), might account for the high loss. The *in vitro* feeding procedure used in this study is known to result in lower mosquito infections compared with direct feeds (Bonnet *et al.*, 2000; Awono-Ambene *et al.*, 2001). The results of this study are therefore conservative and the use of direct feeds, if ethically acceptable, may not change the reported results.

Restricting analysis to the effect of plants and time of plant-feeding on the different stages of the sporogonic cycle of *P. falciparum* in *An. gambiae*; this study has for the first time quantitatively examined against which transition stages, during the mosquito phases of parasite development , plant substrates exert their action. The targets of plant substrates and time of plant-feeding on parasite development efficiency were assessed directly by quantifying the number of parasites at successive stages when they develop from gametocytes to oocysts within the vector. Apart of physiological and immunological factors from mosquitoes and human host that have been reported to affect the transition macrogamete-ookinete (Quakyi *et al.*, 1987; Healer *et al.*, 1997; Lensen *et al.*, 1997), the identification of the transition macrogamete-ookinete as first target of plant substrates, when mosquito fed on plants before infection, suggests the presence of compounds from plants that suppress the macrogametes fertilization, prevent ookinetes formation, agglutinate or lyse them.

The identification of ookinete-oocyst transition as second target of plant substrates when mosquito fed on plants after infection appeared evidenced. Naturally, the development from ookinetes to oocysts can be strongly impaired by the necessity for ookinetes to cross the peritrophic matrix and the midgut wall (Huber *et al.*, 1991; Sieber *et al.*, 1991; Billingsley and Rudin, 1992; Shahabuddin, 1998). In the case of this study, it is possible that plant substrates might have prevented effective crossing of the peritrophic membrane by ookinetes. An initial requirement for penetration is the presence of appropriate receptors and ligands on the membrane cells (Leake, 1992; Billingsley, 1994). Compounds from plant substrates may

therefore compete with the parasites on biding sites, thereby affecting their penetration into the peritrophic membrane.

The present study shows that significant parasite losses occurred at specific plant-feeding times, and the transitions affected depend on the time mosquitoes fed on plants. In the macrogamete-ookinete transition, significant loss occurred when mosquitoes plant-fed before infection or throughout. On the ookinete-oocyst transition, significant loss occurred when they plant-fed after infection or throughout. These results implied that the presence of plant substrates in the gut during a specific midgut parasite development stage can impaire the sporogonic development of the parasite.

Despite the negative effects that some plant substrates impose on the sexual stages of the parasite, the results of the present study indicate that other factors, probably associated with the vector and human host, have regulatory effects on parasite populations because the effective number of parasites that successfully developed from macrogametocyte to oocyst in glucose–fed mosquitoes (control) was also low. In total, only a maximum of 6 % of parasite loss could indeed be attributed to plant substrates. The parasite mortalities observed in the absence of plant substrates have added evidence of integrated vector, parasite and environmental factors controlling the infection by *P. falciparum* (Beier, 1998; Shahabuddin and Costero, 2001; Sinden, 2002).

In conclusion, the present study has demonstrated that the development of *P. falciparum* is impeded in the mosquito midgut when mosquitoes feed on specific plant species, and two

transitions are particularly most affected, macrogamete-ookinete and ookinete-oocyst. It has also shown that the parasite transitions affected depend on the time mosquitoes feed on the plant. The availabity of certain plants in malaria endemic areas may to a limited degree influence malaria transmission dynamics, depending on the specific times mosquitoes feed on them in relation to the ingestion of an infectious bloodmeal.

CHAPTER NINE: GENERAL DISCUSSION

Female *Anopheles gambiae* mosquitoes are best known as blood feeders for it is this habit that mediates disease transmission. Nevertheless, they seldom live on blood alone. To provide for their immediate energy needs, adults require carbohydrates that both males and females acquire direct or indirectly from plants (Foster, 1995). Previous studies on *An. gambiae* sugar-feeding behaviour have concentrated on evaluating their fitness on experimental sugar (Straif and Beier, 1996; Gary and Foster, 2001; Okech *et al.*, 2003) or on the arbitrary selected plant species (Gary and Foster, 2004; Impoinvil *et al.*, 2004). Relatively little has been done about the plant-feeding behaviour of *An. gambiae*. This study has focused mainly on determining the feeding practices of *An. gambiae* in nature, their responses to different plant species and the effects on their fitness and vector competence in a semi-field set up. The plants tested in this study showed varying degree of attractiveness to mosquitoes, the majority of those preferred by mosquitoes offered them a fitness advantage, depending on their sugar content, but showed varying degree of effects on *P. falciparum* development in the mosquito midgut, and the more promising ones can easily be adopted by the rural communities in western Kenya.

In Lwanda village, indoor resting females *An. gambiae* were found with fructose in the guts (Chapter 4), fructose does not occur in the hemolymph of insects (Van Handel, 1984), but is a common component of nectar sugars (Van Handel *et al.*, 1972) and honeydew (Wäckers, 2001). Therefore, detecting fructose in *An. gambiae* females indicates that they recently fed on nectar or honewdew. Previous reports (Muirhead-Thomsom, 1951; Gillies, 1968; Beier, 1996) suggested that females sugar-feed rarely, if at all. These conclusions were based

largely on collections of indoor resting and biting females that either contained no fluid in their crops (Gillies, 1968) or mostly tested negative for fructose (Beier, 1996), a plant sugar that is completely converted to other carbohydrates and lipids after it leaves the crop. However, the present study indicates that sugar-feeding is probably a natural behaviour of females of this mosquito species in the wild, because females fed on plants even when the human host was readily available. There was no seasonal variation on the proportion of fructose positive females, but Van Handel *et al.* (1994) and Costero *et al.* (1998) found season difference on sugar-feeding on *Aedes*. The proportion of fructose-positive females was similar at all the gonotrophic status. These results confirmed those of previous studies in some *Culex* and *Aedes* species (Nasci and Edman, 1984; Reisen *et al.*, 1986; Anderson and Jaenson, 1987), and are opposed to others (Edman *et al.*, 1992; Yee and Foster, 1992; Yee *et al.*, 1992; Holliday-Hanson *et al.*, 1997). Similar results were obtained in nulliparous and parous mosquitoes, similar to those reported on species of *Culex* and *Aedes* (Martinez-Ibarra *et al.*, 1997; Anderson, 1990; Haramis and Foster, 1990) but opposed to other studies on some species of these genera (Magnarelli, 1977; Vargo and Foster, 1984; Anderson and Jaenson, 1987). However, in this study *An. gambiae* plant-fed more when they moved far from larval habitats. This study therefore suggests that plant-feeding is undertaken by mosquitoes to satisfy their energy requirements at any particular time. Its expression may be species specific, molded by the environment in which a species evolved and modulated by hierachical interactions with other physiological and behavioural processes.

Differences on the feeding responses of *An. gambiae* on the 13 candidate plants tested were observed in this study. Both choice and no-choice arena bioassays coupled to GC analysis of

sugar signatures of plant-fed mosquitoes and plants extracts showed a preferential feeding pattern of *An. gambiae* on plants, with 4 plants species (*P. hysterophorus, R. communis, T. stans,* and *S. didymobotrya*) consistently ranking high and *H. patens* in two out of the three methods. These results indicated that mosquitoes are selective in choosing a host plant for feeding, and confirmed previous observations on other mosquito species (Sandholm and Price, 1962; McCrae *et al.*, 1969, 1976); McCrae, 1968, 1989; Andersson and Jaenson, 1987). Mosquitoes fed primarily on flowers of the plant tested, but feeding was also observed on the leaves and stems of some plant species (*P. hysterophorus, L. camara* and *R. communis* respectively), confirming previous observation by Impoinvil *et al.* (2004) on *An. gambiae* feeding on droplets (extrafloral nectar) from the stem of *R. communis*. Except for one plant species (*P. hysterophorus*), flowers of all the plant tested contained more sugar types identified than leaves. This probably explained why flowers were more preferred for feeding than leaves. However, in *L. camara*, leaves were mostly fed on despite the floral part being rich in sugar, probably because of the long corolla of the flower which could prevent the mosquitoes to access the nectar.

Apart from one preferred plant species (*P. hysterophorus*), fitness benefits in terms of survival and fecundity were observed on *An. gambiae* fed on the preferred plants as opposed to those which fed on one of the least preferred plant (*L. camara*). These observations support the evolutionary theory which predicts that those that are preferred should provide better fitness than those that are less preferred. The study also showed a positive correlation between sugar quality and quantity in preferred plants and survival and fecundity, with 3 sugar types (glucose, fructose and gulose) particularly important. Previous studies (Gary and

Foster, 2004; Impoinvil *et al.*, 2004) also found that survival of *An. gambiae* in plants was correlated with the intensity of anthrone coloration, indicating the amount of fructose in the plant. However, this study has the merit of identifying particular sugars important for mosquito survival. The results of this study also indicated that if offered less preferred plant, mosquitoes could replace sugars with the increased blood meals without suppressing their reproductive fitness. This will consequently increase human biting rate. Survival and biting rate being important coefficients of the vectorial capacity equation, the availability of some plant species in nature will therefore impact on the malaria transmission.

This study also investigated the effect of plants on *P. falciparum* development in *An. gambiae*. When mosquitoes infected with *P. falciparum* gametocytes fed continuously on different plant species, 4 plants species (*(L. camara, S. didymobotrya, R. communis* and *P. hysterophorus*) significantly reduced the prevalence of mosquitoes becoming infected, with the last two having the highest effects. Studies on sandfly, a non mosquito vector also observed mortality of *Leishmania major* in *Phlebotomus papatasi* caused by plant-feeding of the sand flies (Schlein and Jacobson, 1994; Jacobson and Schlein, 1999). Three *(L. camara, S. didymobotrya*, and *P. hysterophorus*) out of the four plant species which reduced infection prevalences in mosquitoes also significantly reduced the intensities of parasites in the midgut of the infected mosquitoes. Apart of the deleterious property on *P. falciparum* development in the mosquito, the whole plant of *L. camara* also showed antimalarial activity (Oliver-Bever, 1982). The highly significant adverse effect of *P. hysterophorus* on the parasite development in *An. gambiae* may justify why this plant is preferred by that mosquito species (Chapter 5) despite the poor fitness benefit (Chapter 6), and this may also justify feeding on

multiple plants by a single mosquito (Chapter 5). The effect of *P. hysterophorus* on the parasite was gametocyte density dependant, and for all these plants, the effects on the parasite increased on post-infection plant-feeding, but were higher when mosquitoes fed continuously on them.

Two of the *P. falciparum* sporogonic development interstages were the targets of plant substrates: macrogamete-ookinete and ookinete-oocyst, and the effect of plants depended on the plant-feeding time. The first transition (macrogamete-ookinete) occurs in the mosquito midgut, and the second concerns the crossing of the peritrophic membrane before the parasite leaves the midgut to an environment free of attacks. Since a deleterious effect in one stage of the parasite development can impact on the infection outcome, this study showed that plant-feeding occurred at all stages of the gonotrophic cycle (Chapter 4), therefore there is a great chance for the parasite development to be affected when still in the mosquito midgut if the mosquito feeds on one of those deleterious plants. It is possible that light infections resulting from plant-feeding decrease the vector competence on infected mosquitoes, and this is apparently the consequence of plant-feeding in nature. However, the decrease on the vector competence is optimal when mosquitoes feed continuously on inhibiting plants, and since continuous feeding on the same plant is rare in nature, this decrease is probably not expressed or very little in Suba District because the exposure rate is still high (up to 53.8 infective bites/person/month) (Shililu *et al.*, 2003). Thus it appears that allowing selective plants species to grow abundantly around human dwellings and mosquito larval habitats, in order to target the most vulnerables stages of *P. falciparum* sporogonic cycle in the mosquito will have an effect on the transmission of malaria. This approach may be efficient especially if other control methods like use of bed nets or repellents are also applied, in order to prevent

An. gambiae females from getting blood meals and giving them no choice than to plant-feed in order to survive.

CHAPTER TEN: CONCLUSIONS AND RECOMMENDATIONS

10.1 Conclusions

1. Females *An. gambiae* do feed on plants in nature, and this behaviour is independent of seasons, gonotrophic status and age of the mosquitoes, but the frequency increase as they disperse. Any malaria control measures using plant-feeding is likely to target *Anopheles* mosquito at any seasons, physiological status and age in Suba district, western Kenya.

2. *Anopheles gambiae* exhibits a preferential feeding pattern on host plants. Five plants species (*P. hysterophorus*, *R. communis*, *T. stans*, *S. didymobotrya* and *H. patens*) are most preferred by *An. gambiae* among 13 candidate plant species tested. Flowers are often chosen for feeding by mosquitoes, but leaves and stems are preferred in some plant species.

3. Except for *P. hysterophorus*, *An. gambiae* have a fitness advantage (better survival and fecundity) when fed on the preferred plants, and sugar composition and concentration on those plants positively correlated with mosquito fitness.

4. Except in one plant *P. hysterophorus*, flowers are richer in sugars than leaves. Five types of sugars are predominant in the preferred plants (glucose, fructose, sucrose, mannose and gulose) and three (glucose, fructose, gulose) are positively correlated with *An. gambiae* survival.

5. Among the preferred plants of *An. gambiae* tested, four plant species (*L. camara*, *S. didymobotrya*, *R. communis* and *P. hysterophorus*) reduced the proportion of *An. gambiae* becoming infected with *P. falciparum* oocysts, while *L. camara*, *S. didymobotrya* and *P. hysterophorus* reduced the intensity of parasite in the infected mosquitoes. The inhibitory effect of *P. hysterophorus* is total when mosquitoes are infected with low gametocyte densities, but reduced with high gametocytemia. Two transition stages in the sporogonic cycle of *P. falciparum* in *An. gambiae* are affected by plant substrates: macrogamete-ookinete and ookinete-oocysts. The deleterious effect of plants increase during postinfection plant-feeding. However, the best effect is observed when mosquitoes plant-feed on plants continuously (pre and postinfection). However, the transition affected depends on the time mosquitoes feed on plants.

The results presented in this thesis demonstrate the scientific rationale and objective basis for future incorporation of mosquito plant-feeding and selected plant species with known effects on the mosquito fitness and vector competence into integrated vector management programmes in rural communities where malaria is a major public health problem and transmission is mediated by the major malaria vector *An. gambiae*.

10.2 Recommendations

The following recommendations are made from this study:

1. Studies should be conducted to establish the frequency of plant-feeding by *An. gambiae* in the wild, and establish the impact of the availability of specific plant species assessed in this study on mosquito population dynamics, human-vector contact and malaria transmission.

2. Studies should be conducted to determine how the survival and mating performance of males are affected by the availability of plants in Suba district and elsewhere.

3. Studies should be conducted on the cues used by mosquitoes to select specific plants for feeding, and use them for trapping.

4. The potential of plant-feeding as a "pull" agent, and repellent and insecticide treated nets as a "push" agent can be studied in controlled field experiments and in appropriate ecological settings to develop a "push-pull" tactic to reduce man-mosquito contact and reduce the vector competence of mosquitoes.

5. Some plants evaluated in this study have shown a significant effect on the vector competence of mosquitoes. Therefore, the possibility of planting them in large quantity in endemic area should be studied for greater efficacy and significant impact on the entomological inoculation rate.

6. Screening of larger profile of plants selected from the microenvironment of mosquitoes in endemic areas may help to identify more sugar sources for mosquitoes with adverse effects on their fitness and vector competence.

REFERENCES

Abdel-Malek, A. A. (1964) Study of the feeding habits of *Anopheles sergenti* Theo. At Siwa Oasis using radiophosphorous. *Bull WHO*, 30: 137-139.

Ahmed, A. N.; Baggott, S. L.; Maingon, R. and Hurd, H. (2000) The costs of mounting an immune response are reflected in the reproductive fitness of the mosquito *Anopheles gambiae*. *Oikos* 97: 371-377.

Al-Olayan, E. M.; Beetsma, A. L.; Butcher, G. A.; Sinden, R. E. and Hurd, H. (2002) Complete development of mosquito phases of the malaria parasite in vitro. *Sci.*, 295: 677-679.

Andersson, I. H. (1990) Nectar feeding activity of *Aedes* mosquitoes, with special reference to *Aedes communis* females. *J. Am. Mosq. Contr. Assoc.*, 6: 482-489.

Andersson, I. H. (1992) The effect of sugar meals and body size on fecundity and longevity of female *Aedes communis* (Diptera: Culicidae). *Physiol. Entomol.*, 17: 203-7.

Andersson, I. H. and Jaenson, T. G. T. (1987) Nectar feeding by mosquitoes in Sweden, with special reference to *Culex pipiens* and *Culex torrentium*. *Med. Vet. Entomol.*, 1: 59-64.

Attaran, A. and Maharaj, R. (2000) Doctoring malaria badly: the global campaign to ban DDT. *British Med. J.*, 321: 1403-1405.

Awono-Ambene, H. P.; Diawara, L. and Robert, V. (2001) Comparison of direct and membrane feeding methods to infect *Anopheles arabiensis* with *Plasmodium falciparum*. *Am. J. Trop. Med. Hyg.*, 64: 32-34.

Beier, J. C. (1996) Frequent blood-feeding and restrictive sugar-feeding behavior enhance the malaria vector potential of *Anopheles gambiae s.l.* and *Anopheles funestus* (Diptera: Culicidae) in Western Kenya. *J. Med. Entomol.*, 33: 613-8.

Beier, J. C. (1998) Malaria parasite development in mosquitoes. *Ann. Rev. Entomol.*, 43: 519-543.

Belto, P. (1994) Attraction of male mosquitoes to sound. *J. Am. Mosq. Contr. Assoc.*, 10(2): 297-301

Billingsley, P. F. (1994) Vector-parasite interactions for vaccine development. *Int. J. parasitol.*, 24: 53-58.

Billingsley, P. F. and Hecker, H. (1991) Blood digestion in the mosquito, *Anopheles stephensi*: activity and distribution of trypsin, aminopeptidase and alpha-glucosidase in the midgut. *J. Med. Entomol.*, 28: 865-871.

REFERENCES

Abdel-Malek, A. A. (1964) Study of the feeding habits of *Anopheles sergenti* Theo. At Siwa Oasis using radiophosphorous. *Bull WHO*, 30: 137-139.

Ahmed, A. N.; Baggott, S. L.; Maingon, R. and Hurd, H. (2000) The costs of mounting an immune response are reflected in the reproductive fitness of the mosquito *Anopheles gambiae*. *Oikos* 97: 371-377.

Al-Olayan, E. M.; Beetsma, A. L.; Butcher, G. A.; Sinden, R. E. and Hurd, H. (2002) Complete development of mosquito phases of the malaria parasite in vitro. *Sci.*, 295: 677-679.

Andersson, I. H. (1990) Nectar feeding activity of *Aedes* mosquitoes, with special reference to *Aedes communis* females. *J. Am. Mosq. Contr. Assoc.*, 6: 482-489.

Andersson, I. H. (1992) The effect of sugar meals and body size on fecundity and longevity of female *Aedes communis* (Diptera: Culicidae). *Physiol. Entomol.*, 17: 203-7.

Andersson, I. H. and Jaenson, T. G. T. (1987) Nectar feeding by mosquitoes in Sweden, with special reference to *Culex pipiens* and *Culex torrentium*. *Med. Vet. Entomol.*, 1: 59-64.

Attaran, A. and Maharaj, R. (2000) Doctoring malaria badly: the global campaign to ban DDT. *British Med. J.*, 321: 1403-1405.

Awono-Ambene, H. P.; Diawara, L. and Robert, V. (2001) Comparison of direct and membrane feeding methods to infect *Anopheles arabiensis* with *Plasmodium falciparum*. *Am. J. Trop. Med. Hyg.*, 64: 32-34.

Beier, J. C. (1996) Frequent blood-feeding and restrictive sugar-feeding behavior enhance the malaria vector potential of *Anopheles gambiae s.l.* and *Anopheles funestus* (Diptera: Culicidae) in Western Kenya. *J. Med. Entomol.*, 33: 613-8.

Beier, J. C. (1998) Malaria parasite development in mosquitoes. *Ann. Rev. Entomol.*, 43: 519-543.

Belto, P. (1994) Attraction of male mosquitoes to sound. *J. Am. Mosq. Contr. Assoc.*, 10(2): 297-301

Billingsley, P. F. (1994) Vector-parasite interactions for vaccine development. *Int. J. parasitol.*, 24: 53-58.

Billingsley, P. F. and Hecker, H. (1991) Blood digestion in the mosquito, *Anopheles stephensi*: activity and distribution of trypsin, aminopeptidase and alpha-glucosidase in the midgut. *J. Med. Entomol.*, 28: 865-871.

Billingsley, P. F. and Rudin, W. (1992) The role of the mosquito peritrophic membrane in bloodmeal digestion and infectivity of *Plasmodium* species. *J. Parasitol.*, 78:430-440.

Bonnet, S.; Gouagna, L. C.; Safeukui, I.; Meunier, J. Y. and Boudin, C. (2000) Comparison of artificial membrane feeding with direct skin feeding to estimate infectiousness of *Plasmodium falciparum* gametocyte carriers to mosquitoes. *Trans. R. Soc. Trop. Med. Hyg.* 94: 103-106.

Boudin, C.; Lyannaz, J.; Bosseno, M. F.; Chaize, J. and Carnevale, P. (1989) Production de sporozoïtes de *Plasmodium* humains á Bobo-Dioulasso, Burkina Faso. *Ann. Soc. Belge Méd. Trop.*, 69: 3-23.

Boudin, C. and Vincent, R. (2003) Biologie de la transmission homme-moustique du *Plasmodium*. *Bull. Soc. Pathol. Exot.*, 96(1): 6-20.

Bowen, M. F. (1992) Patterns of sugar feeding in diapausing and nondiapausing *Culex pipiens* (Diptera: Culicidae) females. *J. Med. Entomol.*, 29: 843-849.

Bowen, M. F.; Davis, E. E.; Haggart, D. and Romo, J. (1994) Host-seeking behavior in the autogenous mosquito *Aedes atropalpus*. *J. Ins. Physiol.*, 40: 511-517.

Boyd, M. F. (1949) Malariology. W. B. Sanders Co., Philadelphia, Penn-sylvania, P.608-697.

Brady, J.; Constantini, C.; Sagnon, N.; Gibson, G. and Coluzzi, M. (1997) The role of body odours in the relative attractiveness of different men to malarial vectors in Burkina Faso. *Ann. Trop. Med. Parasitol.*, 91 (Suppl. 1): 121-122.

Brantjes, N. B. M. and Leemans, J. A. A. M. (1976) Silene otites (Caryophyllacea) pollinated by nocturnal Lepidoptera and mosquitoes. *Acta Bot. Neerl.* 25: 281-295.

Breeman, J. G. (2001) The ears of hippopotamus: manifestations, determinants, and estimates of the malaria burden. *Am. J. Trop. Med. Hyg., Suppl.*, 64; 1-11.

Briegel, H. (1990a) Fecundity, metabolism, and body size in *Anopheles* (Diptera: Culicidae), vectors of malaria. *J. Med. Entomol.*, 27: 839-50.

Briegel, H. (1990b) Metabolic relationship between female body size, reserves and fecundity of *Aedes aegypti*. *J. Ins. Physiol.*, 36: 165-72.

Briegel, H. and Hörler, E. (1993) Multiple blood meals as a reproductive strategy in *Anopheles* (Diptera: Culicidae). *J. Med. Entomol.*, 30: 975-985.

Brooke, B. D.; Hunt, R. H. and Coetzee, M. (2000) Resistance to dieldrin + fipronil assorts with chromosome inversion 2La in the malaria vector *Anopheles gambiae*. *Med. Vet. Entomol.*, 14: 190-4.

Bruce-Chwatt, L. J. (1980) Essential Malariology. William hainemann Medical Books Ltd. London.

Bruce-Chwatt, L. J. (1985) Essential Malariology 2nd Ed. John Wiley and Sons, New York.

Carnevale, P.; Robert, V.; Boudin, C.; Halna, J. M.; Pazart, L.; Gazin, P.; Richard, A. and Mouchet, J. (1988) La lutte contre le paludisme par des moustiquaires impregnées de pyréthroides au Burkina Faso. *Bull. Soc. Path. Exot.*, 81: 832-846.

Chandre, F.; Darrier, F.; Manga, L.; Akogbeto, M.; Faye, O.; Mouchet, J. and Guillet, P. (1999) Status of pyrethroid resistance in *Anopheles gambiae sensu lato*. *Bull. WHO*, 77: 230-234.

Chapman, R. F. (1983) The Insects Structure and function. Third Ed. Macmillan India Ltd, Bangalore. Pp. 3.

Charlwood, J. D. and Jones, M. D. R. (1980) Mating in the mosquito *Anopheles gambiae s.l.* II. Swarming behaviour. *Physiol. Entomol.*, 5: 315-320.

Charlwood, J. D.; Pinto, J.; Sousa, C. A.; Madsen, H.; Ferreira, C. And Do Kosario, V. E. (2002) The swarming and mating behaviour of *Anopheles gambiae s.s.* (Diptera: Culicidae) from Sao Tome Island. *J. Vector Ecol.*, 27: 178-183.

Cheesbrough, M. (1987) Medical Laboratory Manual for Tropical Countries. Volume 1. Butterworth-Heineman Ltd. Halley Court, Jordan Hill, Oxford OX2 8EJ. pp 398.

Clements, A. N. (1955) The sources of energy for flight in mosquitoes. *J. Exp. Biol.* 32: 547-54.

Clements, A. (1992) The biology of Mosquitoes. Vol I, Chapman & Hall, UK; pp. XVI.

Clements, A. N. (1999) The biology of mosquitoes. Wallingford, England: CABI Publishing.

Collins, F. H. and Paskewitz, S. M. (1995) Malaria: current and future prospects for control. *Ann. Rev. Entomol.*, 40: 195-219.

Coluzzi, M. (1984) Heterogeneities of malaria vectorial system in the tropical Africa and their significance in malaria epidemiology and control. *Bull. WHO*, 62: 107-113.

Costero, A.; Attardo, G. M.; Scott, T. W. and Edman, J. D. (1998) An experimental study on the detection of fructose in *Aedes aegypti*. *J. Am. Mosq. Contr. Assoc.*, 14(3): 234-242.

Costero, A.; Edman, J. D.; Clark, G. G. and Scott, T. W. (1998) Life–table study of *Aedes aegypti* (Diptera: Culicidae) in Puerto Rico fed only human blood versus blood plus sugar. *J. Med. Entomol.*, 35: 809-813.

Day, J. F.; Edman, J. D. and Scott, T. W. (1994) Reproductive fitness and survivorship of *Aedes aegypti* (Diptera: Culicidae) maintained on blood, with field observations from thailand. *J. Med. Entomol.*, 31: 611-17.

Deka, M. K.; Singh, K.; Handique, R. and Singh, K. (1998) Antifeedant and repellent effects of pongam (Pongamic pinnata) and wild sage (*Lantana camara*) on tea mosquito bug (Helopeltis theivora). *Indian J. Agricul. Sci.*, 68(5), 274-276.

Detinova, T. S. (1962) Age-grouping methods in Diptera of medical importance. *WHO Monogr.*, 47.

Dimopoulos, G.; Seeley, D.; Wolf, A. and Kafatos, F. C. (1998) Malariainfection of the mosquito *Anopheles gambiae* activates immune-responsive genes during critical transition stages of the parasite life cycle. *EMBO J.*, 17: 6115-6123.

Doyle, R. J. (1994) Introduction and function of lectins and their interactions with microorganisms. In Lectin microorganism interactions, eds **Doyle, R. J. and Slifkin, M.** pp. 1-65. New York: Marcel Dekker.

Edman, J. D. and Lynn, H. C. (1975) Relationship between blood meal volume and ovarian development in *Culex nigripalpus* (Diptera: Culicidae). *Entomol. Exp. Appl.*, 18: 492-96.

Edman, J. D.; Strickman, D.; Kittayapong, P. and Scott, T. W. (1992) Female *Aedes aegypti* (Diptera: Culicidae) in Thailand rarely feed on sugar. *J. Med. Entomol.*, 29: 1035-38.

El-Akad, A. S.; Humphreys, J. G. and Winstead, R. L. (1989) Observations on sugar feeding by anopheline females (Diptera: Culicidae). *Melsheimer Entomol. Ser.*, 37: 13-20.

El-Akad, A. S. and Humphreys, J. G. (1990) Effects of larval and adult diet plus mating/insemination upon ovarian development of laboratory reared *Anopheles pharoensis* in Egypt. *J. Am. Mosq. Contr. Assoc.*, 6:96-98.

Etzler, M. E. (1986) Distribution and function of plant lectins and their interactions with microorganisms. In The lectins. Properties, functions and applications in biology and medicine, eds **Liener, I. E.; Sharon, N. and Goldstein, I. J.** pp. 371-435. Orlando, FL: Academic Press.

Fatope, M.O.; Salihu, L.; Asante, S. K. and Taheda, Y. (2002) Larvicidal activity of extracts and triterpenoids from *Lantana camara*. *Pharm. Biol.*, 40(8): 564-567.

Feinsod, F. M, and Spielman, A. (1980) Nutrient-mediated juvenile hormone secretion in mosquitoes. *J. Ins. Physiol.*, 26: 113-117.

Fialho, R. F. and Schall, J. J. (1995) Thermal ecology of a malaria parasite and its insect vector: consequences for the parasite`s transmission success. *J. Anim. Ecol.*, 64: 553-562.

Foster, W. A. (1995) Mosquito sugar feeding and reproductive energetics. *Ann. Rev. Entomol.*, 40, 443-74.

Foster, W. A. and Handcock, R. G. (1994) Nectar-related olfactory and visual attractants for mosquitoes. *J. Am. Mosq. Contr. Assoc. Inc.*, 10 (2): 288-296.

Foster, W. A. and Takken, W. (2004) Nectar-related vs. human-related volatiles: behavioural response and choice by female and male *Anopheles gambiae* between emergence and first feeding. *Bull. Entomol. Res.*, 94: 145-157.

Gadawski, R. M. and Smith, S. M. (1992) Nectar sources and age structure in a population of *Aedes provocans* (Diptera : Culicidae). *J. Med. Entomol.*, 29: 879-86.

Gallup, J. I. and Sachs, J. D. (2001) The economic burden of malaria. *Am. J. Trop. Med. Hyg., Suppl.*, 64: 85-96.

Gardener, M. C. and Gillman, M. P. (2002) The taste of nectar a neglected area of pollination ecology. Oikos. 98: 552-557.

Garrett-Jones, C.; Boreham, F. L. and Pant, C. P. (1980) Feeding habits of anophelines (Diptera: Culicidae). *Bull. Entomol. Res.*, 70: 165-185.

Gary, R. E. Jr. and Foster, W. A. (2001) Effects of available sugar on the reproductive fitness and vectorial capacity of the malaria vector *Anopheles gambiae* (Diptera: Culicidae). *J. Med. Entomol.*, 38: 22-28.

Gary, R. E. Jr. and Foster, W. A. (2004) *Anopheles gambiae* feeding and survival on honeydew and extra-floral nectar of peridomestic plants. *Med. Vet. Entomol.*, 18: 102-107.

Gillett, J. D. (1971) *Mosquitoes*. London: Weidenfeld & Nicholson.

Gillies, M. T. (1968) Journal of meetings. *Proc. R. Entomol. Soc. Lond.*, 33, 27.

Gillies, M. T. and De Meillon, B. (1968) The anopheline of Africa, south of Sahara (Ethiopian zoogeographical region). 2nd South African Institute for Medical Research, Johannesburg, South Africa.

Gillies, M. T. and Wilkes, T. J. (1969) A comparison of the range of attraction of animal baits and carbon dioxide for some West African mosquitoes. *Bull. Entomol. Res.*, 59: 441-456.

Githeko, A. K.; Brandling-Bennett, A. D.; Beier, M.; Atieli, F.; Owaga, M. And Collins, F. H. (1992) The reservoir of *Plasmodium falciparum* malaria in a holoendemic area of western Kenya. *Trans. R. Soc. Trop. Med. Hyg.*, 86: 371-372.

Githeko, A. K.; Service, M. W.; Atieli, F. K.; Hill, S. M.; Crampton, J. M. (1993) Field testing an enzyme-linked synthetic oligonucleotide probe for identification of Anopheles gambiae s.s. and Anopheles arabiensis. *Ann. Trop. Med. Parasitol.* 87: 595-601.

Goma, L. K. H. (1959) Periodic pupation in *Anopheles gambiae* Giles. *J. Ent. Soc. S. Afri.*, 22 : 275.

Gouagna, L. C.; Mulder, B.; Noubissi, E.; Tchuinkam, T.; Verhave, J. P. and Boudin, C. (1998) The early sporogonic cycle of *Plasmodium falciparum* in laboratory-infected *Anopheles gambiae*: an estimate of parasite efficacy. *Trop. Med. Int. Health*, 3: 21-28.

Gouagna, L. C.; Bonnet, R.; Gounoue, R.; Tchuinkam, T.; Safeukui, I.; Verhave, J. P.; Eling, W. and Boudin, C. (1999) The use of anti-Pfs 25 monoclonal antibody for early determination of *Plasmodium falciparum* oocyst infections in *Anopheles gambiae*: Comparison with the current technique of direct microscopic diagnosis. *Exp. Parasitol.*, 92: 209-214.

Gouagna, L. C.; Ferguson, H. M.; Okech, B. A.; Killeen, G. F.; Kabiru, E. W.; Beier, J. C.; Githure, J. I. and Yan, G. (2004) *Plasmodium falciparum* malaria disease manifestations in humans and transmission to *Anopheles gambiae*: a field study in Western Kenya. *Parasitol.*, 128: 235-243.

Graves, P. M. (1980) Studies on the use of membrane feeding technique for infecting *Anopheles gambiae* with *Plasmodium falciparum*. *Trans. R. Soc. Trop. Med. Hyg.*, 74: 738-742.

Grimstad, P. R. and DeFoliart, G. R. (1974) Nectar sources of Wisconsin mosquitoes. *J. Med. Entomol.*, 11: 331-341.

Gupta, R. K. and Rutledge, L. C. (1994) Role of repellents in vector control and disease prevention. *Am. J. Trop. Med. Hyg.*, 50: 82-86.

Gwadz, R. and Collins, F. H. (1996) In: **Beaty, B. J. and Marquardt, W. C.** (Editors). The biology of disease vectors. University Press of Colorado, U.S.A. pp. 73-83.

Hamilton, J. G. C. and El Naiem, D. A. (2000) Sugars in the gut of the sandfly *Phlebotomus orientalis* from Dinder National Park, Eastern Sudan. *Med. Vet. Entomol.*, 14: 64-70.

Hankock, R. G. and Foster, W. A. (1989) In: **Foster, W. A. (1995)** Mosquito sugar feeding and reproductive energetics. *Ann. Rev. Entomol.*, 40: 443-74.

Hancock, R. G. and Foster, W. A. (1993) Nectar or blood? A study of juvenile hormone, energy reserves, and odor preference in *Culex nigripalpus* mosquitoes. In: **Borovsky, D. and Spielman, A. (1993)** Host regulated Developmental mechanism in vector Arthropods. *Proc. 3rd Symp.* Vero Beach : Univ. Fla-IFAS.

Hanckock, R. G. and Foster, W. A. (2000) Exogenous juvenile hormone and methoprene, but not male accessory gland substances or ovariectomy, affect the blood/nectar choice of female *Culex nigripalpus* mosquitoes. *Med. Vet. Entomol.*, 14: 376-382.

Haramis, L. D. and Foster, W. A. (1990) Gonotrophic cycle duration, population age structure, and onset of sugar feeding and insemination of *Aedes triseriatus* (Diptera: Culicidae). *J. Med. Entomol.*, 27: 421-428.

Hargreaves, K.; Koekemoer, L. L.; Brooke, B. D.; Hunt, R. H.; Mthembu, J. and Coetzee, M. (2000) *Anophles funestus* resistant to pyrethroid insecticides in South Africa. *Med. Vet. Entomol.*, 14: 181-189.

Healer, J.; McGuinness, D.; Hopcroft, P.; Haley, S.; Carter, R. and Riley, E. (1997) Complement-mediated lysis of *Plasmodium falciparum* gametes by malaria-immune human sera is associated with antibodies to the gamete surface antigen Pfs230. *Infect. Immun.*, 65: 3017-3023.

Healy, T. P. and Jepson, P. C. (1988) The location of floral sources by mosquitoes: the long range responses of *Anopheles arabiensis* Patton (Diptera: Culicidae) to *Achillea millefolium* flowers and isolated floral odour. *Bull. Entomol. Res.*, 78, 651-657.

Hogh, B.; Gamage-Mendis, A.; Butcher, G. A.; Thompson, R. and Begtrup, K. (1998) The different impact of chloroquine and pyrimethamine/sulfadoxine upon the infectivity of malaria species to the mosquito vector. *Am. J. Trop. Med. Hyg.*, 58: 176-182.

Holliday-Hanson, M. L.; Yuval, B. and Washino, R. K. (1997) Energetics and sugar-feeding of field-collected anopheline females. *J. Vector Ecol.*, 22: 83-89.

Huber, M.; Cabib, E. and Miller, L. H. (1991) Malaria parasite chitinase and penetration of the mosquito peritrophic membrane. *Proc. Nat. Acad. Sci. USA.*, 88: 2807-2810.

Hunt, R. H.; Coetzee, M. and Fettene, M. (1998) The *Anopheles gambiae* complex: a new species from Ethiopia. *Trans. R. Soc. Trop. Med. Hyg.*, 92: 231-235.

Hurd, H. (2003) Manipulation of medically important insect vectors by their parasites. *Ann. Rev. Entomol.*, 48: 141-161.

Impoinvil, D. E.; Kongere, J. O.; Foster, W. A.; Njiru, B. N.; Killeen, G. F.; Githure, J. I.; Beier, J. C; Hassanali, A. and Knols, B. G. J. (2004) Feeding and survival of the malaria vector *Anopheles gambiae* on plants growing in Kenya. *Med. Vet. Entomol.*, 18: 108-115.

Jacobson, R. L.; Slutsky, G. M.; Greenblatt, C. L. and Schnur, L. F. (1982) Surface reactions of leishmania. Lectin mediated agglutination. *Ann. Trop. Med. Parasitol.*, 76: 45-52.

Jacobson, R. L. and Schlein, Y. (1999) Lectins and toxins in the plant diet of *Phlebotomus papatasi* (Diptera: Psychodidae) can kill *Leishmania major* promastigotes in the sandfly and in culture. *Ann. Trop. Med. Parasitol.*, 93(4): 351-356.

Killeen, F. G.; Fillinger, U. and Knols, B. G. J. (2002) Advantages of larval control for African malaria vectors: low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. *Malaria J.*, 1: 8.

Killeen, G. F.; McKenzie, F. E.; Foy, B. D.; Schieffelin, C.; Billingsley, P. F. and Beier, J. C. (2000) The potential impacts of integrated malaria transmission control on entomological inoculation rate in highly endemic areas. *Am. J. Trop. Med. Hyg.*, 62: 545-551.

Kitthawee, S.; Edman, J. D. and Upatham, E. S. (1995) Effect of seasonal variation in the aquatic habitat of immature *Anopheles dirus* (Diptera : Culicidae) on the size and survivorship of emerging adults. *Southeast Asian J. Trop. Med. Public Health.* 26: 573-581.

Kline, D. L.; Takken, W.; Wood, J. R. and Carlson, D. A. (1990) Field studies of the potential of butanone, carbon dioxide, honey extract, 1-octen-3-ol, L-Lactic acid and phenols as attractants for mosquitoes. *Med. Vet. Entomol.*, 4: 383-391.

Klowden, M. J. (1995) Blood, sex, and the mosquito. *BioSci.*, 45, 326-331.

Klowden, M. J. and Dutro, S. M. (1990) Effects of carbohydrate ingestion on the pre-oviposition behavior of the mosquito *Aedes aegypti* (L.). *Bull. Soc. Vec. Ecol.* 15: 59-62.

Knols, B. G. J.; De Jong, R. and Takken, W. (1995) Differential attractiveness of isolated humans to mosquitoes in Tanzania. *Trans. R. Soc. Trop. Med. Hyg.*, 89: 604-606.

Koella, J. C. and Lyimo, F. O. (1996) Variability in the relationship between weight and wing length of the *Anopheles gambiae* (Diptera: Culicidae). *J. Med. Entomol.*, 33: 261-264.

Kreier, J. P. (1980) Immunology and immunization. Malaria Tome 3: 111-162.

Krotoski, W. A. (1985) Discovery of the hypnozoite and a new theory of malaria relapse. *Trans. R. Soc. Med. Hyg.*, 79: 1-11.

Laarman, J. J. (1968) The intake of sugars by females of wild *Anopheles gambiae* and *Anopheles funestus*. *Acta Leiden.* 36:137-44.

Leake, C. J. (1992) Arbovirus-mosquito interaction and vector specificity. *Parasitol. Today* 8: 123-128.

Lensen, A. H.; Van-Gemert, G. J.; Bolmer, M. G.; Meis, J. F. and Kaslow, D. (1992) Transmission-blocking antibody of the *Plasmodium falciparum* zygote ookinete surface protein Pf 25 also influence sporozoïte development. *Parasite Immunol.*, 14: 471-479.

Lensen, A. H.; Bolmer, M.; Van Gemert, G. J.; Eling, W. M. and Sauerwein, R. W. (1997) Leukocytes in a *Plasmodium falciparum*-infected blood meal reduce transmission of malaria to *Anopheles mosquito. Infect. Immun.*, 65: 3834-3837.

Lindsay, S. W.; Alonso, P. L.; Armstrong Schellenberg, J. R. M.; Hemingway, J.; Adiamah, J. H.; Shenton, F. C.; Jawa, M. and Greenwood, B. M. (1993) A malaria control trial using insecticide treated bednets and targeted chemoprophylaxis in a rural area of the Gambia, west Africa. 7. Impact of permethrin-impregnated bednets on malaria vectors. *Trans. R. soc. Trop. Med. Hyg., Suppl 2.*, 87: 45-51.

Lindsay, S. W.; Ewland, J. A.; Samung, Y.; Apiwathnasorn, C. and Nosten, F. (1998) Thanka (Limonia acidissima) and deet (dimethyl benzamide) mixture as mosquito repellent for use by Karen women. *Med. Vet. Entomol.*, 12: 325-327.

Loubinos, L. P. and Conn, J. (1991) Fecundity, parity, and adult feeding relationships among Nyssorhynchus malaria vectors from Venezuela. *Mem. Inst. Oswaldo Cruz.* 86: 57-66.

Lyimo, E. O. and Koella, J. C. (1992) Relationship between body size of adult *Anopheles gambiae s.l.* and infection with the malaria parasite *Plasmodium falciparum. Parasitol.*, 104: 233-237.

Lyimo, E. C. and Takken, W. (1993) Effects of adult body size of fecundity and the pregravid rate of *Anopheles gambiae* in Tanzania. *Med. Vet. Entomol.*, 7: 328-332.

Magesa, S. M.; Wikes, T. J.; Mnzava, A. E. P.; Njunwa, K. J.; Myamba, J.; Kivuyo, M. D. P.; Hill, N.; Lines, J. D. and Curtis, C. F. (1991) Trials of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria. Part 2 Effects on the malaria vector population. *Acta Trop.*, 49: 97-108.

Magnarelli, L. A. (1977) Nectar-feeding by *Aedes sollicitans* and its relation to gonotrophic activity. *Environ. Entomol.*, 6: 237-42.

Magnarelli, L. A. (1978) Nectar-feeding by female mosquitoes and its relation to follicular development and parity. *J. Med. Entomol.*, 14: 527-30.

Magnarelli, L. A. (1980) Bionomics of *Psorophora ferox* (Diptera: Culicidae): seasonal occurrence and acquisition of sugars. *J. Med. Entomol.*, 17: 328-332.

Maier, W. A.; Becker-Feldman, H. and Seitz, H. M. (1987) Pathology of malaria infected mosquitoes. *Parasitol. Today,* 3: 216-218.

Marchand, R. P. (1984) Field observations on swarming and mating in *Anopheles gambiae* mosquitoes in Tanzania. *Neth. J. Zool.*, 34: 367-387.

Martinez-Ibarra, J. A.; Rodriguez, M. H.; Arredondo-Jimenez; J. I. and Yuval, B. (1997) Influence of plant abundance on nectar feeding by *Aedes aegypti* (diptera: Culicidae) in southern Mexico. *J. Med. Entomol.*, 34: 589-593.

Mboera, L. E. G.; Knols, B. G. J.; Della Torre, A. and Takken, W. (1997) The response of *Anopheles gambiae s.l.* and *Anopheles funestus* (Diptera: Culicidae) to tents baited with odour or carbon dioxide in South-East Tanzania. *Bull. Entomol. Res.*, 87: 173-178.

Mbogo, C. N. M.; Kabiru, E. W.; Muiruri, S. K.; Nzovu, J. M.; Ouma, J. H.; Githure, J. I. and Beier, J. C. (1993) Blood feeding behaviour of *Anopheles gambiae s.l.* and *Anopheles funestus* in Kilifi District, Kenya. *J. Am. Mosq. Contr. Assoc.*, 9(2): 225-228.

McCrae, A. W. R. (1968) Activity of tropical mosquitoes at plant sources. *Journal of meetings. Proc. R. Ent. Soc. Lond. (C.)* 33: 27.

McCrae, A. W. R. (1989) Differences in sugar-feeding activity between tropical and temperate mosquitoes: field observations and their implications. *Vec. Ecol. Newsletter*, 20: 16.

McCrae, A. W. R.; Ssenkubuge, Y.; Manuma, P.; Mawejje, C. and Kitama, A. (1969) Mosquito and tabanid activity at plant sugar sources. East African Virus research Republique 1968, (18): 96-102.

McCrae, A. W. R.; Boreham, P. F. L. and Ssenkubuge, Y. (1976) The behavioural ecology of host selection in *Anopheles implexus* (Theobald) (Diptera: Culicidae). *Bull. Entomol. Res.*, 66: 587-631.

Minakawa, N.; Mutero, C. M.; Githure, J. I.; Beier, J. C. and Yan, G. (1999) Spatial distribution and habitat characterization of anopheline mosquito larvae in western Kenya. *Am. J. Trop. Med. Hyg.*, 61: 1010-1016.

Mouchet, J. and Carnevale, P. (1991) Les vecteurs et la transmission in: `Paludisme`. Ellipse, Paris: 34-59.

Muirhead-Thomson, R. C. (1945) Studies on the breeding places and control of *Anopheles gambiae* and *Anopheles gambiae var melas* in the coastal district of Sierra Leone. *Bull. Entomol. Res.*, 36 : 185-252.

Muirhead-Thomson, R. C. (1951) Mosquito Behavior in relation to malaria transmission and Control in the tropics. London: E. Arnold. 219 pp.

Muirhead-Thomsom, R. C. (1954) Factors determining the true reservoir of infection of *Plasmodium falciparum* and Wuchereria bancrofti in a west African village. *Trans. R. Soc. Trop. Med. Hyg.*, 48: 208-224.

Muirhead-Thomson, R. C. (1957) The malarial infectivity of an African village population to mosquitoes (*Anopheles gambiae*): A random xenodiagnostic survey. *Am. J. Trop. Med. Hyg.*, 6: 971-979.

Mulder, B.; Tchuinkam, T. and Dechering, K. (1994) Malaria transmission-blocking activity in experimental infections of *Anopheles gambiae* from naturally infected *Plasmodium falciparum* gametocyte carriers. *Trans. R. Soc. Trop. Med. Hyg.*, 88: 121-125.

Müller, G. and Schlein, Y. (2005) The frugal diet of mosquitoes in adverse conditions. *Med. Vet. Entomol.*, 19, 413-422.

Mutero, C. M.; Ouma, J. H.; Agak, B. K.; Wanderi, J. A. and Copeland, R. S. (1998) Malaria prevalence and use of self–protection measures against mosquitoes in Suba District, Kenya. *East Afr. Med. J.*, 75: 11-16.

Naotune, T. S.; Karunaweera, N. D.; Mendis K. N. and Carter R. (1993) Cytokine-mediated inactivation of malarial gametocytes is dependent on the presence of white blood cells and involves reactive nitrogen intermediates. *Immunol.*, 78: 555-562.

Nasci, R. S. and Edman, J. D. (1984) *Culiseta melanura* (Diptera: Culicidae): population structure and nectar feeding in a freshwater swamp and surrounding areas in southeastern Massachusetts, USA. *J. Med. Entomol.*, 21: 567-572.

Nayar, J. K. and Pierce, P. A. (1980) The effect of diet on the survival, insemination and oviposition of culex nigripalpus Theobald. *Mosq. News* , 40: 210-17.

Nayar, J. K. and Sauerman, D. M. Jr. (1971) Physiological effects of carbohydrates on survival, metabolism, and flight potential of female *Aedes taeniorhynchus*. *J. Ins. Physiol.*, 17: 2221-2233.

Nayar, J. K. and Van Handel, E. (1971) The fuel for sustained mosquito flight. *J. Ins. Physiol.*, 17: 471-481.

Nijhout, H. F. and Craig, G. B. Jr. (1971) Reproductive isolation in Stegomyia mosquitoes. III. Evidence for a sexual pheromone. *Entomol. Exp. Appl.*, 14: 399-412.

Noden, B. H.; Kent, M. D. and Beier, J. C. (1995) The impact of variations in temperature on early *Plasmodium falciparum* development in *Anopheles stephensi*. *Parasitol.*, 111: 539-545.

Okech, B. A.; Gouagna, L. C.; Knols, B. G. J.; Killeen, G. F.; Kabiru E. W.; Beier J. C.; Yan G. and Githure J. I. (2003) Influence of sugar availability and indoor microclimate on survival of *Anopheles gambiae* (Diptera: Culicidae) under semifield conditions in western Kenya. *J. Med. Entomol.*, 40: 657-663.

Okech, B. A.; Gouagna, L. C.; Kabiru, E. W.; Walczak, E.; Beier, J. C., Yan, G. and Githure, J. (2004) Resistance of early midgut stages of natural *Plasmodium falciparum* parasites to high temperatures in experimentally infected *Anopheles gambiae* (Diptera: Culicidae). *J. Parasitol.*, 90(4): 764-768.

Okech, B. A.; Gouagna, L. C.; Knols, B. G. J.; Kabiru, E. W.; Killeen, G. F.; Beier, J. C.; Yan, G. and Githure, J. I. (2004) Influence of indoor microclimate and diet on survival of *Anopheles gambiae* s.s. (Diptera: Culicidae) in village house conditions in western Kenya. *Int. J. Trop. Ins. Science*, 24 (3): 207-212.

Olaifa, J. I.; Matsumura, F.; Zeevaat, J. A. D. and Charalambouse, P. (1991) Lethal amounts of ricine in green peach aphids *Myzus persicae* Suzler fed on castor bean plants. *Pl. Sci. (Limerick)*, 73: 253-256.

Oliver-Bever, B. (1982) Medicinal plants in West Africa. *Cambridge Univers. Press, Lond.*, PP. 118.

O`Meara, G. F. (1987). Nutritional ecology of blood feeding Diptera. Nutritional Ecology of Insects, Mites and Spiders (ed. By Slansky F.and Rodriguez J. G.), pp. 742-764. John Wiley and Sons, New York, Chichester, Brisbane, Toronto.

Omer, S. M. and Cloudsley-Thompson, J. L. (1970) Survival of female *Anopheles gambiae* Giles through nine month dry season in Sudan. *Bull. WHO*, 42: 319-330.

Patterson, R. S.; Smittle, B. J. and De Neve, R. T. (1969) Feeding habits of male southern house mosquitoes on P32-labelled and unlabeled plants. *J. Econ. Entomol.*, 62: 1455-58.

Petrarca, V.; Beier, J. C.; Onyango, F.; Koros, J.; Asiago, C.; Koech, D. K. and Roberts, C. R. (1991) Species composition of the *Anopheles gambiae* complex (Diptera: Culicidae) at two sites in western Kenya. *J. Med. Entomol.*, 28: 307-313.

Pichon, G.; Awono-Ambene, H. P. and Robert, V. (2000) High heterogeneity in the number of *Plasmodium falciparum* gametocytes in the bloodmeal of mosquitoes fed on the same host. *Parasitol.*, 121: 115-120.

Polerstock, A. R.; Eigenbrode, S. D. and Klowden, M. J. (2002) Mating alters the cuticular hydrocarbons of female *Anopheles gambiae* sensu stricto and *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.*, 39: 545-552.

Ponnudurai, T.; Lensen, A. H. W.; Van Gemert, G. J. A.; Bensick, T.; Bolmer, M. and Meuwissen, J. H. E. TH. (1989a) Sporozoite load of mosquitoes infected with *Plasmodium falciparum*. *Trans. R. Soc. Trop. Med. Hyg.*, **83**: 67-70.

Quakyi, I. A.; Carter, R.; Rener, J.; Kumar, N.; Good, M. F. and Miller, L. H. (1987) The 230-kDa gamete surface protein of *Plasmodium falciparum* is also a target for transmission-blocking antibodies. *J. Immunol.*, 139: 4213-4217.

Quinones, M. L.; Lines, J. D.; Thomson, M.; Jawara, M. and Greenwood, B. M. (1998) Permethrin-treated bednets do not have a "mass killing effect" on village populations of *Anopheles gambiae*. *Trans. R. Soc. Trop. Med. Hyg.*, 92: 373-378.

Read, A. F.; Narara, A.; Nee, S.; Keymer, A. E. and Day, K. P. (1992) Gametocyte sex ratios as indirect measures of outcrossing rates in malaria. *Parasitol.*, 104: 387-395.

Reisen, W. K. and Emory, R. W. (1976) Blood-feeding of *Anopheles stephensi*. *Ann. Entomol. Soc. Am.*, 69: 293-298.

Reisen, W. K.; Meyer, R. P. and Milby, M. M. (1986) Patterns of fructose feeding by *Culex tarsalis* (Diptera: Culicidae). *J. Med. Entomol.*, 23: 366-376.

Rèi, F.; Brey, P. T. and Vincent, R. (2002) *Plasmodium* sex determination and transmission to mosquitoes. *Trends Parasitol.*, 18: 32-38.

Richman, A. M.; Bulet, P.; Hetru, C.; Barillas-Mury, C.; Hoffmann, J. A. and Kafatos, F. C. (1996) Inducible immune factors of the vector mosquito *Anopheles gambiae* biochemical purification of a defensin antibacterial peptide and molecular cloning of prodefensin cDNA. *Ins. Mol. Biol.*, 5: 203-210.

Rivero, A. and Ferguson, H. M. (2003) The energetic budget of *Anopheles stephensi* infected with *Plasmodium chabaudi*: is energy depletion a mechanism for virulence? *Proc. R. Soc. Lond. B.*, 270: 1365-1371.

Roberts, D. R. and Andre, R. G. (1994) Insecticide resistant issues in vector-borne disease control. *Am. J. Trop. Med. Hyg.*, 50: 21-34.

Robert, V.; Le Goff, G.; Essong, J.; Tchuinkam, T.; Faas, B. and verhave, J. P. (1995) Detection of *falciparum* malarial forms in naturally infected anophelines in Cameroon using a fluorescent anti-25-kDa monoclonal antibody. *Am. J. Trop. Med. Hyg.*, 52: 366-369.

Robert, V.; Tchuinkam, T.; Mulder, B.; Bodo, J. M. and Verhave, J. P. (1996) Effect of sickle cell trait status of gametocyte carriers of *Plasmodium falciparum* on infectivity to anophelines. *Am. J. Trop. Med. Hyg.*, 54: 111-113.

Robert, V.; Awono-Ambene, H. P.; Le Hesran, J. Y. and Trape, J. F. (2000) Gametocytemia and infectivity to mosquitoes of patients with uncomplicated *Plasmodium*

falciparum malaria attacks treated with chloroquine or sulfadoxine plus pyrimethamine. *Am. J. Trop. Med. Hyg.*, 62: 210-216.

Russel, C. B. and Hunter, F. F. (2002) Analysis of nectar and honeydew feeding in *Aedes* and *Ochlerotatus* mosquitoes. *J. Am. Mosq. Contr. Assoc.*, 18: 86-90.

Rutledge, L.; Ward, R. and Gould, D. (1964) Studies on the feeding response of mosquitoes to nutritive solutions in a new membrane feeder. *Mosq. News*, 24: 407-419.

Sandholm, H. A. and Price, R. D. (1962) Field observations on the nectar- feeding habits of some Minnesota mosquitoes. *Mosq. News*, 22: 346-349.

SAS Institute Inc. (1999). SAS/STAT Software: Version 8, *SAS Institute Inc.*, Cary, NC, USA.

Schlein, Y. (1986) Sandfly diet and Leishmania. *Parasitol. Today* 2: 175-177.

Schlein, Y. and Jacobson, R. L. (1994) Mortality of *Leishmania Major* in *Phlebotomus papatasi* caused by plant-feeding of the Sandflies. *Am. J. Trop. Med. Hyg.*, 50: 20-27.

Schlein, Y. and Muller, G. (1995) Assessment of plant tissue feeding by sand flies (Diptera: Psychodidae) and mosquitoes (Diptera: Culicidae). *J. Med. Entomol.*, 32(6), 882-887.

Schlein, Y. and Jacobson, R. L. (1999) Sugar meals and longevity of the sandfly *Phlebotomus papatasi* in an arid focus of Leishmania in the Jordan Valley. *Med. Vet. Entomol.*, 13: 65-71.

Schlein, Y.; Jacobson, R. L. and Muller, G. C. (2001) Sandfly feeding on noxious plants: a potential method for the control of leishmaniasis. *Am. J. Trop. Med. Hyg.*, 65: 300-303.

Scott, J. A.; Brogdon, W. G. and Collins, F. H. (1993) Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *Am. J. Trop. Med. Hyg.*, 49: 520-529.

Scott, T. W.; Naksathit, A.; Day, J. F.; Kittayapong, P. and Edman, J. D. (1997) A fitness advantage for *Aedes aegypti* and viruses it transmits when females feed only on human blood. *Am. J. Trop. Med. Hyg.*, 57: 235-239.

Service, M. W. (1980) A guide to medical entomology. The Macmillan press Ltd pp. 44.

Service, M. W. (1985) *Anopheles gambiae*: Africa's principal malaria vector, 1902-1984. *Bull Entomol. Soc. Am.*, 31: 8-12.

Service, M. W. (1993) Mosquito ecology: Field sampling methods. 2nd Edition, Elseviers *Appl. Sci., Lond.* New York.

Shahabuddin, M.; Toyoshima, T.; Aikawa, M. and Kaslow, D. C. (1993) Transmission blocking activity of a chitinase inhibitor and activation of malarial parasite chitinase by mosquito protease. *Proc. Natl. Acad. Sci. U.S.A.*, 90: 4266-4272.

Shahabuddin, M. and Kaslow, D. C. (1994) Biology of the development of *Plasmodium* in the midgut: a molecular and cellular view. *Bull. Inst. Pasteur*, 92: 119-132.

Shahabuddin, M. (1998) *Plasmodium* ookinete development in the mosquito midgut: a case of reciprocal manipulation. *Parasitol.*, 116: 583-593.

Shahabuddin, M. and Costero, A. (2001) Spatial distribution of factors and determine sporogonic development of malaria parasites in mosquitoes. *Ins. Bioch. Mol. Biol.*, 31: 231-240.

Shililu, J.; Mbogo, C.; Mutero, C.; Gunter, J.; Swalm, C.; Regens, J.; Keating, J.; Yan, G.; Githure, J. and Beier, J. (2003) Spatial distribution of *Anopheles gambiae* and *Anopheles funestus* and malaria transmission in Suba District, western Kenya. *Ins. Sci. Appl.*, 23: 187-196.

Shofield, S. W. and Sutcliffe, J. F. (1997) Humans vary in their ability to elicit biting responses from *Simulium venustum* (Diptera: Simulidae). *J. Med. Entomol.*, 34: 64-67.

Sieber, K. P.; Huber, M.; Kaslow, D.; Banks, S. M.; Torii, M.; Aikawa, M. and Miller, L. H. (1991) The peritrophic membrane as a barrier: It`s penetration by *Plasmodium gallinaceum* and the effect of a monoclonal antibody to ookinetes. *Exp. Parasitol.*, 72: 145-156.

Sinden, R. E. (2002) Molecular interactions between *Plasmodium* and its insect vectors. *Cell Microbiol.*, 4: 713-724.

Snow, B.; Mwenesi, H. and Rapuoda, B. (1998) Malaria: A situation analysis for Kenya. MoH, Kenya.

Spafford, H. J. and Evans, E. W. (2004) Influence of different sugars on the longevity of Bathyplectes curculionis (Hym., Ichneumonidae). JEN. 128 (4): 316-320.

Straif, S. C. and Beier, J. C. (1996) Effects of sugar availability on the blood feeding behavior of *Anopheles gambiae* (Diptera: Culicidae). *J. Med. Entomol.*, 33: 608-612.

Sumba, L. (2004) Oviposition behaviour of the African malaria mosquito *Anopheles gambiae* Giles (Diptera: Culicidae): influence of external factors on oviposition site selection. PhD. Thesis, Egerton University, Kenya.

Takken, W. (1991) The role of olfaction in host-seeking of mosquitoes: a review. *Ins. Sci. Appl.*, 12: 287-295.

Takken, W. and Knols, B. G. J. (1999) Odor-mediated behaviour of afrotropical malaria mosquitoes. *Ann. Rev. Entomol.*, 44: 131-157.

Tchuinkam, T.; Mulder, B.; Dechering, K.; Stoffels, H.; Verhave, J. P.; Cot, M.; Carnevale, P.; Meuwissen, J. H. E. Th. and Vincent, R. (1993) Experimental infections of *Anopheles gambiae* with *Plasmodium falciparum* of naturally infected gametocyte carriers in Cameroon: factors influencing the infectivity of mosquitoes. *Trop. Med. Parasitol.*, 44: 271-276.

Tripet, F.; Dolo, G.; Traore, S. and Lanzaro, G. C. (2004) The "wingbeat hypothesis" of reproductive isolation between members of the *Anopheles gambiae* complex (Diptera: Culicidae) does not fly. *J. Med. Entomol.* 41: 375-384.

Utzinger, J.; Tozan, Y. and Singer, B. H. (2001) Efficacy and effectiveness of environmental management for malaria control. *Trop. Med. Int. Health*, 6: 677-687.

Van Handel, E. (1972) The detection of nectar in mosquitoes. *Mosq. News* 32: 458.

Van Handel, E. (1984) Metabolism of nutrients in the adult mosquito. *Mosq. News* 44: 573-579.

Van Handel, E.; Haeger, J. S.; Hansen, C. W. (1972) The sugars of some Florida nectars. *Am. J. Bot.*, 59: 1030-1032.

Van Handel, E.; Edman, J. D.; Day, J. F.; Scott, T. W.; Clark, G. G.; Reiter, P. and Lynn, H. C. (1994) Plant-sugar, glycogen, and lipid assay of *Aedes aegypti* collected in urban Puerto Rico and rural Florida. *J. Am. Mosq. Contr. Assoc.*, 10: 149-153.

Vargo, A. M. and Foster, W. A. (1984) Gonotrophic state and parity of nectar feeding mosquito. *Mosq. News,* 44: 6-10.

Vaughan, J. A.; Noden, B. H. and Beier, J. C. (1992) Population dynamics of *Plasmodium falciparum* sporogony in laboratory-infected *Anopheles gambiae*. *J. Parasitol.*, 78: 716-724.

Vaughan, J. A.; Noden, B. H. and Beier, J. C. (1994) Sporogonic development of cultured *Plasmodium falciparum* in six species of laboratory-reared *Anopheles gambiae* mosquitoes. *Am. J. Trop. Med. Hyg.*, 5: 233-243.

Visen, P. K. S.; Shukla, B.; Patnak, G. K.; Tripathi, S. C.; Kulshreshtha, D. K.; Srimal R. C. and Dhawan B. N. (1992) Hepatoprotective activity of *Ricinus communis* leaves. *Int. J. Pharmacog.*, 30: 241-250.

Wäckers, F. L. (2001) A comparision of nectar and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. *J. Ins. Physiol.*, 47: 1077-1084.

Wekesa, J. W.; Brogdon, W. G.; Hawley, W. A. and Besansky, N. J. (1998) Flight tone of field collected populations of *Anopheles gambiae* and *An. arabiensis* (Diptera: Culicidae). *Physiol. Entomol.*, 23: 289-294.

White, G. B. (1974) *Anopheles gambiae* complex and disease transmission in Africa. *Trans. R. Soc. Trop. Med. Hyg.*, 68: 278-301.

WHO, (1975) Methods and techniques. In: manual on practical entomology in malaria. Part II. 13, 1-45.

WHO, (1993) A global strategy for malaria control. WHO, Geneva.

WHO, (1995) Vector control for malaria and other mosquito-borne diseases.

WHO, (2000) 20th WHO expert committee report on malaria. Tech. Rep. Ser., No. 892: i-v, 1-74. Geneva.

WHO, (2003) Africa Malaria Report. 1-69.

WHO, (2004) RBM MERG Task force on malaria morbidity: Malaria incidence estimates at country level for the year 2004.

WHO, (2005) World Malaria Report 2005, Geneva. *http://rbm.who.int/wmr2005*.

WHO, (2006) Guidelines for the treatment of malaria. WHO Press

Winstanley, P. A. (2000) Chemotherapy for *falciparum* malaria: The armoury, the problems and the prospects. *Parasitol. today*,16 (4): 146-153.

Yee, W. L. and Foster, W. A. (1992) Diel sugar-feeding and host seeking rhythms in mosquitoes (Diptera: Culicidae) under laboratory conditions. *J. Med. Entomol.*, 29: 784-791.

Yee, W. L.; Foster, W. A.; Howe, M. J. and Hancock, R. G. (1992) Simultaneous field comparison of evening temporal distribution of nectar and blood feeding by *Aedes vexans* and *Ae. Trivittatus* (Diptera: Culicidae) in Ohio. *J. Med. Entomol.*, 29: 356-360.

Yuval, B. (1992) The other habit: sugar feeding by mosquitoes. *Bull. Soc. Vec. Ecol., 17*: 150-56.

Yuval, B. and Schlein, Y. (1986) Leishmaniasis in the Jordan Valley III. Nocturnal activity of *Phlebotomus papatasi* (Diptera: Psychodidae) in relation to nutrition and ovarian development. *J. Med. Entomol.*, 23: 411-415.

Yuval, B.; Warburg, A. and Schlein, Y. (1988) Dispersal characteristics of the sandfly *Phlebotomus papatasi*. *Med. Vet. Entomol.*, 2: 391-395.

Zaim, M. and Guillet, P. (2002) Alternative insecticides: An urgent need. *Trends Parasitol.*, 18: 161-163.

APPENDICES

APPENDIX 1: RECRUITMENT OF GAMETOCYTE CARRIERS

INDIVIDUAL RECRUITMENT IFORMATION

Site/Location ---------- Date of screening: ---------- Serial Number ----------

Identification code: ----------

IDENTIFICATION

First Name: ---------- Surname: ----------

Sex: --------- Age: ---------- or Date of Birth ----------

District: ---------- Locality: ----------

Parent/Guardian: ----------

CLINICAL PRESENTATION

ID number: ---------- Weight: ---------- Body temperature ----------

I-Major complains and symptoms: (yes = 1, No = 0)

Shiver ------- Abdominal pain -------- Vomiting -------- Dhiarrea -------- Headache --------

Constipation ------- Convulsion ------- Aching pains -------- Cough ------- Asthnia ---------

Fever --------- Initial date of feverache ---------- Anemia ----------

Previous druf history: date ---------- type of drugs ---------- dosage ----------
(within the last two weeks)

Other complains and signs ----------

II- Parasitological presentation

Thick blood film (Positive = +, Negative = 0) ---------- Species ----------

Asexual parasitemia (relative) ---------- (absolute) ---------- parasite/mm^3

Gametocytemia (relative) ---------- (absolute) ----------gcts / mm^3

TREATMENT

Drug: 1) ---------- dosage ----------

2) ---------- dosage ----------

Rendez-vous: ----------

LABORATORY EXPERIMENTS

Blood collection (yes = ok, No) ---------- Volume collected: ----------ml

Experimental infections ----------

Observations ----------

Appendix 1 continued

INFORMED CONSENT AGREEMENT

Title of Study: Vector Competence of African Malaria Vectors

The doctors in charge of this research study are: Dr. John Beier of the University of Miami USA, Dr. John Githure and Dr. Louis Gouagna of ICIPE, Kenya.

Purpose: We are conducting a research study to better understand the mechanism in which the parasites taken by a mosquito from an infected person develop inside the mosquito and the factors that contribute to malaria transmission.

Screening for parasites: . We are asking you/ your child to participate in this research study. We would like to take a small drop of blood from your finger, by finger-prick, to examine for malaria parasites. If malaria parasites are seen on the slide, you/your child will be treated whether or not you/ your child agree to participate in this study and you/your child will be followed up by the medical doctor until all the malaria parasites are cleared from the blood.

Recruitment of volunteers

If you/your child are found to have the form of the parasites (gametocytes) that infect mosquitoes, we will request you/your child to volunteer to participate in the study by allowing us to take a small amount of blood by venipuncture (6 ml which is about a teaspoonful) from you/your child's arm. You/your child will be required to come to our ICIPE Centre where your blood will be taken by a clinical officer associated to our project. We will provide transport and the time taken to and from here will be about 45-60 minutes. The blood taken will be given to mosquitoes to feed on to see whether they become infected with malaria under different environmental conditions. The rest of the blood collected will be used to characterize the type of the malaria parasite present.

Risks: The taking of blood from you/your child's arm may cause a little discomfort from the stick of the needle, and may cause a small bruise. After receiving medication from the Hospital staff, we will take you/your child home or back to the school. We may request you/your child to come a week later or we would contact you/your child at home where we shall take a small drop of blood from your finger to check whether parasites are still present. If malaria parasites are seen on the slide, the Hospital staff will provide you/your child with another treatment that works better. In case you/your child have to come back during appointment visit, a transport fee will be given to you/your child. If any problems develop as a result of drawing blood, the same Hospital staff will provide prompt and appropriate medical attention.

Benefits: You/your child will receive no direct benefit from participating in this study. The results of the study, however, may help the scientific community learn about malaria in mosquitoes and how malaria transmission can be prevented.

I.R.B.
APPROVAL DATE: 7/2/03
EXP. DATE: 6/22/04
INITIALS: _____

Appendix 1 continued

INFORMED CONSENT FORM FOR MINORS

Confidentiality: The medical information will be kept private and your blood sample that is fed to the mosquitoes will have no bearing to you/your child' name

Payment: You/your child will not receive any payment for your participation in this study. We may however provide some money for your transportation costs and for meals if necessary.

I, ...(name of parent/guardian) being the lawful

parent/guardian for my child do hereby consent for the child

named.....to participate in the research project titled:

'Vector Competence of African Malaria Vectors'.

I have been given the opportunity to ask questions concerning this project. Any such questions have been answered to my full satisfaction. Should any further questions arise concerning the right of this child, I may contact Dr. John Githure, the Major Foreign Collaborator, ICIPE, P.O. Box 30772, Telephone: 861680, Nairobi.

I also understand that I may revoke this consent at any time without penalty or loss of benefits, if any.

Parent/Guardian signature and date.....

ID No.....

Village address .....

Witness name, signature and date.....

Investigator's name, signature and date.....

Appendix 1 continued

INFORMED CONSENT FORM FOR ADULTS

I, ...(name of volunteer) do hereby consent to participate in the research project titled: 'Vector Competence of African Malaria Vectors'.

I have been given the opportunity to ask questions concerning this project. Any such questions have been answered to my full satisfaction. Should any further questions arise concerning the right of this child, I may contact Dr. John Githure, the Major Foreign Collaborator, ICIPE, P.O. Box 30772, Telephone: 861680, Nairobi.

I also understand that I may revoke this consent at any time without penalty or loss of benefits, if any.

Volunteer's signature and date.....

ID No.....

Village address .....
.....

Witness name, signature and date.....

Investigator's name, signature and date.....

Revised on 27 June 2003

I.R.B.
APPROVAL DATE: 7/2/03
EXP. DATE: 6/22/04
INITIALS: BM

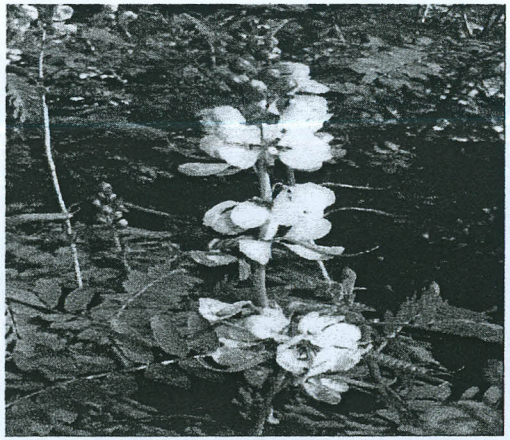
APPENDIX 2: CANDITATE PLANT SPECIES TESTED

Tecoma stans L.

Ricinus communis L.

Pathernium hysterophorus L.

Hamelia patens J.



Senna didymobotrya F.

Lantana camara L.

Appendix 2 continued

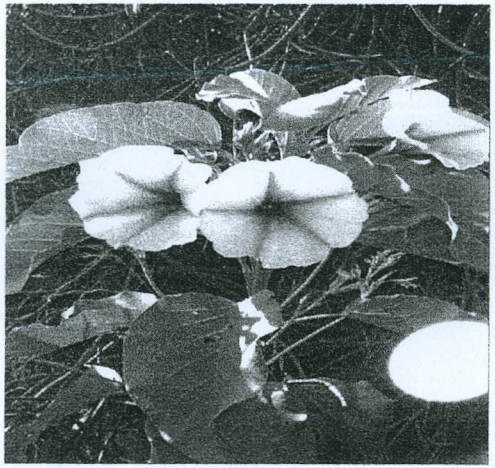
Psiada punctulata V.

Senna bicapsularis L.

Cassia hirsuta L.

Datura stramonium L.

Tithonia diversifolia H.



Ipomoea hildebrandtie V.

Appendix 2 continued

Flaveria trinervia M.