The role of floral and fruit scent compounds as mosquito attractants: developing new methods for monitoring mosquito populations

by

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Preface

The research contained in this dissertation was completed by the candidate while based in the Discipline of Biology, School of Life Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The research was financially supported by National Research Foundation (NRF).

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

Signed: Prof A. Jürgens

Date: 24 May 2016

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Date: 24 May 2016

Declaration

I, Priyanka Pachuwah, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

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a) their words have been re-written but the general information attributed to them has been referenced;

b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

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Dissertation abstract

Mosquitoes are known for their association with various pathogens and transmit diseases such as malaria, dengue fever, rift valley fever and other arboviral diseases. Therefore mosquitoes pose a significant threat to human health. For instance, in 2013 an estimated 584000 deaths worldwide, with very high mortality rates among African children, were the result of malaria. Female mosquitoes require blood meals in order to obtain protein to develop their eggs and transmit diseases in the process. However, it is also known that female mosquitoes also require carbohydrate sources, such as nectar which serves as an energy source and for possible egg production. Mosquitoes are able to locate these resources by means of olfactory cues. Studies have demonstrated the use of fruits and flowers as successful mosquito attractants. Furthermore, some floral volatiles have been reported to attract mosquitoes; and female mosquitoes prefer floral volatiles in the early part of the night (and early part of their life stage) and only later go in search of blood meals. These results suggest that floral and fruit volatiles could be used as possible attractants in mosquito trap/monitoring systems. One advantage of using floral/fruit scent to attract female mosquitoes in the early part of the night (or early part of their life stage) is that this is the time prior to their blood-sucking infectious phase. The aims of the study were to: (1) review the literature regarding mosquito control/monitoring strategies; (2) characterize the potential of selected flowering plants species and fruits as attractants for mosquitoes; and (3) to determine the effectiveness of a floral scent mixture (using synthetic compounds) for attracting mosquitoes in the field.

The first part of the study was conducted to identify flowering plants growing in South Africa which are visited by mosquitoes, and to assess their preference for these fruits and flowers. Flower and fruit volatiles were collected using a dynamic headspace technique and then identified using gas-chromatography coupled to mass-spectrometry (GC-MS). Five fruit species were selected based on previous information in the literature; these were banana (*Musa acuminata* L.), guava (*Psidium guajava*), spanspek (*Cucumis melo*), honey melon (*Cucumis melo inodorus*) and fig (*Ficus sur*). Additionally, four flowering plant species were selected based on field observations of mosquitoes observed feeding on the flowers; these were *Acacia nilotica*, *Gymnosporia buxifolia*, *Bulbine natalensis* and *Bulbine frutescens*. The dominant scent compounds emitted by the fruit were 3-methylbutyl acetate, isobutyl butyrate,

isopentyl 2-methylpropanoate, isobutyl acetate, ethylbenzene, ethanol, acetoin and limonene. The dominant scent compounds emitted from the flowers were an unidentified compound, pentan-1-ol and ethylbenzene which are not typically dominant volatiles from flowers. Mosquitoes regardless of their sex or genera (*Aedes* or *Culex*) showed a strong preference for all the fruits and flowers during choice tests (Y-maze analyses). Our results suggest that fruits and flowers can be used as successful mosquito attractants.

The second part of the study was conducted to compare the efficiency of floral volatiles to other commercial lures with regard to different mosquito species. Field trials were conducted using two olfactory lures: (1) a combination of three floral attractants (phenylacetaldehyde, 2-phenyl ethanol and linalool oxide), and (2) a commercially available lure (Sweetscent™; Biogents). The floral scent mix attracted significantly higher numbers of mosquitoes than the commercially available lure, and also higher numbers of females were trapped that showed no sign of a blood meal. Both male and female mosquitoes were trapped which has implications for controlling mosquito populations effectively. Furthermore, *Aedes* and *Culex* mosquitoes were the only genera trapped.

Future studies are needed to test additional single scent compounds and compound mixtures identified in the present study in the lab and field to evaluate their potential for attracting mosquitoes. It will also be important to test the efficiency of floral volatiles in areas where mosquito-borne diseases are rife and with a particular focus on other disease-carrying genera, such as *Anopheles*, that are vectors of malaria.

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Dedication

To my mother, for her love and undying faith in my abilities

And

To my late father, for paving the pathway to my success from the heavens above

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Introduction to thesis

The importance of mosquitoes

Mosquitoes are known for their association with a large number of pathogens such as malaria. There is therefore an urgent need to develop efficient methods for monitoring and and/or controlling mosquito populations. Although many people would encourage the complete eradication of mosquitoes, as millions of people are affected and/or infected by the diseases they transmit, the ecological importance of mosquitoes must be considered when attempting to eradicate them. With some 3400 species of mosquitoes, only a few hundred require human blood or pester humans (Fang, 2010; Rana, 2010). However, there are two possible scenarios that could follow complete eradication of mosquito populations: While some researchers believe that other organisms could fill the ecological role/niche of mosquitoes in ecosystems others think that a complete ecosystem collapse could be the result (see Fang, 2010; and references therein for the debate). In the last 10 years, mosquitoes have played manifold ecological roles, not only as a food source for other animals, but for pollination aspects as well. For instance, there are some plant species for which mosquitoes play a role as pollinators, such as the blunt-leaved bog orchid (*Habenaria obtusata*) and *Silene otites* (Jhumur et al., 2008; Jürgens et al., 2002). Since mosquitoes may act as pollinators, detrivores and food sources, assuming that other organisms will take on their roles and serve as a buffer for the ecosystem may be improbable (Fang, 2010; Rana, 2010). Therefore, instead of complete eradication controlling mosquito populations, particularly of selected species which carry diseases, seems to be a more appropriate approach with a lower risk for ecosystem function.

The use of fruit and flowers in mosquito attraction

Many mosquitoes require carbohydrate sources in order for their survival and fecundity (Klowden, 1986; Nayar and Sauerman, 1975). Several studies have documented a wide range of flowers and fruits that mosquitoes are known to visit for nectaring (Dötterl and Jürgens, 2005; Jhumur et al., 2006; Jhumur et al., 2008; Müller et al 2010a; Müller et al 2010c). Foster and Takken (2004) demonstrated that *Anopheles gambiae* mosquitoes are strongly attracted by volatiles from honey, which were used as surrogates for nectar-related volatiles. The use of fruits and flowers provides insight into the sources mosquitoes use and scent profiles of these could reveal possible scent compounds or blends thereof which could be used in conjunction with other methods of mosquito control (Müller and Schlein, 2006). It has therefore been suggested by several authors that scent compounds derived from fruits and flowers could be used in attractive toxic sugar bait stations, thereby reducing mosquito populations (e.g. Müller et al., 2008; Müller et al., 2010b).

Attraction of mosquitoes to scent

It has been shown that female mosquitoes are attracted to floral scents to feed on nectar as a carbohydrate food source during the early part of their life cycle before they take up a blood meal (Foster and Takken, 2004; Müller and Schlein, 2006; Nayar and Sauerman, 1975). Carbohydrates are vital resources for adult male and female mosquitoes, and it has been shown that sugar ingestion plays a critical role in longevity, fecundity, flight capacity, and host-seeking behaviour (Klowden, 1986; Nayar and Sauerman, 1975). Female *A. gambiae* individuals strongly prefer nectar-related volatiles over human-related volatiles in specific times of their life cycle, while males of this species generally preferred nectar-related volatiles (Foster and Takken, 2004). Thus, one possible strategy that has been suggested is to attract and capture mosquitoes with floral volatiles during the earlier part of the night, when females prefer to take a nectar meal before a blood meal (see Dötterl and Jürgens, 2005; Jhumur et al., 2008). This might be a strategy for preventing disease transmission (see Dötterl and Jürgens, 2005; Jhumur et al., 2008).

One approach to the problem is to develop an integrated monitoring and control strategy that catches mosquitoes in their early life stage, before they enter their reproductive and thus infectious phase, and start to take up blood meals. To do this I propose to use floral volatiles to attract nectar seeking mosquitoes to monitoring or eradication traps. A strategy based on floral volatiles has two advantages: (i) population increase is already discovered in an early phase, and (ii) female as well as male mosquitoes are targeted.

The aim of the proposed study was to study the effectiveness of different flower and fruit material and mixtures of synthetic floral scent compounds for attracting mosquitoes.

Research Objectives

- To identify flowering plants growing in South Africa which are visited by mosquitoes
- To identify the flower volatiles using GC-MS and to understand which are the key floral signals for attracting mosquitoes
- To compare the attractiveness of floral volatiles to other commercial lures with regard to different mosquito species

Dissertation structure

The dissertation comprises four chapters. Chapter 1 is a literature review which focuses on the biology of mosquito and control methods associated with their various life stages; control strategies which have been used to date with advantages and disadvantages; the need for integrated control strategies and the use of scent in mosquito control. Chapter 2 addresses the attraction of mosquitoes to fruits and flowers, and the analysis of the scent profiles of these fruits and flowers. Chapter 3 focuses on the use of floral scent as mosquito attractants in comparison with a commercial lure. Chapter 4 is an overview of the major findings with recommendations for future research.

References

Dötterl, S., & Jürgens, A., 2005. Spatial fragrance patterns in flowers of *Silene latifolia*: Lilac compounds as olfactory nectar guides? Plant Systematics and Evolution 25, 99-109.

Fang, J., 2010. A World without Mosquitoes. Nature 466, 432-34.

Foster, W. A., Takken, W., 2004. Nectar-related vs. human-related volatiles: behavioural response and choice by female and male *Anopheles gambiae* (Diptera: Culicidae) between emergence and first feeding. Bulletin of Entomological Research 94, 145-157.

Jhumur, U. S., Dötterl, S., & Jürgens, A., 2006. Naive and conditioned responses of *Culex pipiens pipiens* biotype molestus (Diptera: Culicidae) to flower odors. Journal of medical entomology 43, 1164-1170.

Jhumur, U. S., Dötterl, S., Jürgens, A., 2008. Floral odors of *Silene otites*: their variability and attractiveness to mosquitoes. Journal of Chemical Ecology 34, 14-52.

Jürgens, A., Witt, T., Gottsberger, G., 2002. Flower scent composition in night-flowering *Silene* species (Caryophyllaceae). Biochemical Systematics and Ecology 30, 383-397.

Klowden, M. J., 1986. Effects of sugar deprivation on the host-seeking behaviour of gravid *Aedes aegypti* mosquitoes. Journal of Insect Physiology 32, 479-483.

Müller, G. C., Beier, J. C., Traore, S. F., Toure, M. B., Traore, M. M., Bah, S., Doumbia, S., Schlein, Y., 2010a. Field experiments of *Anopheles gambiae* attraction to local fruits/seedpods and flowering plants in Mali to optimize strategies for malaria vector control in Africa using attractive toxic sugar bait methods. Malaria Journal 9, 1-11.

Müller, G. C., Junnila, A., Schlein, Y., 2010b. Effective control of adult *Culex pipiens* by spraying an attractive toxic sugar bait solution in the vegetation near larval habitats. Journal of Medical Entomology 47, 63-6.

Müller, G. C., Kravchenko, V. D., Schlein, Y., 2008. Decline of *Anopheles sergentii* and *Aedes caspius* populations following presentation of attractive toxic (Spinosad) sugar bait stations in an oasis. Journal of the American Mosquito Control Association 24, 147-149.

Müller, G. C., Schlein, Y., 2006. Sugar questing mosquitoes in arid areas gather on scarce blossoms that can be used for control. International Journal for Parasitology 36, 1077-1080.

Müller, G. C., Xue, R., Schlein, Y., 2010c. Seed pods of the carob tree *Ceratonia siliqua* are a favored sugar source for the mosquito *Aedes albopictus* in coastal Israel. Acta Tropica 116, 235-239.

Nayar, J.K., Sauerman, D.M., 1975. The effects of nutrition on survival and fecundity in Florida mosquitoes. Journal of Medical Entomology 12, 92-98.

Rana, F., 2010. Why would God create mosquitoes? Reasons to believe. http://www.reasons.org/articles/why-would-god-create-mosquitoes. Date accessed: 07 October 2014.

Chapter 1

Literature Review

1.1. Introduction

Mosquitoes pose a significant threat to human and animal health. Mosquitoes are vectors of malaria, dengue fever, lymphatic filariasis and other arboviral diseases (Fang, 2010; WHO, 1982). For instance, in 2013 an estimate by the World Health Organization revealed 584 000 deaths as a result of malaria worldwide, with very high mortality rates among African children (WHO, 2014a). Malaria has been described as an ancient disease, dating back to as early as 1700 BC, having possibly originated in Africa (American Mosquito Control Association, 2014). Ronald Ross discovered that mosquitoes were the vectors of malaria in 1897, which led to extensive research on vector control programs (Ramirez et al., 2009). Yellow fever, another mosquito transmitted disease, affects 200 000 people across the world per year resulting in approximately 30 000 deaths of which 90% occurs in Africa (WHO, 2014b; Ramirez et al., 2009).

Mosquito-borne diseases do not solely affect humans but may have serious implications for livestock and pets (WHO, 1982). For example, the West Nile Virus (WNV) affects horses and may result in the loss of pets and profits (horses are used for racing and other sporting events). Rift Valley Fever (RVF) affects both humans and animals (WHO, 2010). Livestock infected with RVF, such as cattle, may produce less milk and calves might be aborted (WHO, 1982; WHO, 2010). Transmission rates can vary from low to high among animals and may pose serious implications for farmers. Therefore, improving the strategies to monitor or control mosquitoes and mosquito-borne diseases are imperative. When considering the ecological importance of mosquitoes complete eradication may not be a suitable way forward (see Fang, 2010; Ostera and Gostin, 2011). Mosquitoes are pollinators and serve as a food source for many organisms such as birds, fish, frogs and other small animals (Fang, 2010). Mosquito larvae are detrivores feeding on algae and other dead/decaying matter in the water therefore cleaning the water and preventing other infections (Fang, 2010).

Many mosquito-borne disease outbreaks have been recorded to date and many existing monitoring and control strategies have been put in place to prevent such outbreaks with varying levels of success. Biological, chemical and/or non-chemical approaches are different control strategies that have been implemented in order to combat mosquito-borne diseases (Ramirez et al., 2009; Ostera and Gostin, 2011; WHO, 1982). A wide variety of products and devices exist in order to protect hosts from mosquitoes and to keep mosquito populations under control or away from hosts. Control strategies targeting specific mosquito stages are used to eradicate a larger number of mosquitoes before the virulent adult mosquito stage (Stanczyk, 2011). In addition, many control strategies target adult mosquitoes, such as the use of adulticides and natural enemies to decrease mosquito abundance. However, due to the unique dietary needs of mosquitoes, alternative methods of control can be used. Interestingly, both newly emerged male and female mosquitoes require carbohydrate meals in the form of floral nectar for longevity, fecundity, flight capacity, and host-seeking behaviour (Lothrop et al., 2012; Müller et al., 2010c). Only after mating, female mosquitoes require blood meals to develop their eggs (Foster and Takken, 2004; Müller and Schlein, 2006). Therefore, recent studies have been interested in finding ways to capture or treat female mosquitoes prior to their reproductive and infectious phase with the goal to prevent disease transmission. Since nectar-seeking mosquitoes are attracted to flowers by floral scents, integrated control methods that use floral olfactory signals may be used to capture and control female mosquitoes before they need blood meals, thus combating mosquito-borne diseases efficiently.

The aim of this review is: to (1) briefly summarize the biology of mosquitoes as vectors for diseases; to (2) review existing management and control strategies, to (3) evaluate the use of scents as mosquito attractants in mosquito traps; and to (4) discuss integrated approaches for mosquito control and monitoring strategies.

1.2. Mosquito Biology

With almost 3400 species, and present on earth for approximately 100 million years, mosquitoes (Culicidae) are a diverse group of dipterans (Backer, 1989; Fang, 2010; Stanczyk, 2011). The life cycle of a mosquito is in most cases incomplete without water as the first three stages of the mosquito life cycle (egg, larvae, and pupae) typically require water for development (Jupp, 1996; Snow, 1990; WHO, 1982). In most species, such as those in the genera *Anopheles* and *Culex*, the eggs are laid on the surface of stagnant water; although some variation among species exists (Jupp, 1996; Snow, 1990). Mosquito eggs may hatch into mosquito larvae approximately 48 hours later depending on environmental conditions (Snow, 1990). The larvae stay just beneath the surface of the water and occasionally come to the surface in order to breathe using a siphon, however, this may vary among species (Jupp, 1996; Snow, 1990). Larvae feed on algae, detritus and micro-organisms in the water. The final stage of development is the non-feeding pupae. Metamorphosis is complete once the pupa develops and the adult mosquito emerges. After emergence from pupae, young male and female adult mosquitoes gain energy mainly from carbohydrate sources such as floral nectar (Foster and Takken, 2004). It has been reported that carbohydrates may promote longevity, fecundity, flight capacity, and egg production (Lothrop et al., 2012; Müller et al., 2010c; Otienoburu et al., 2012). After mating, female mosquitoes seek mostly blood meals to obtain protein that is needed for egg development, but some mosquitoes do not need a blood meal (Foster and Takken, 2004; Bond et al., 2005; Müller et al., 2008; Müller et al., 2010a; Xue et al., 2008). Accordingly, Foster and Takken (2004) and Müller et al. (2010a) found that in the early parts of the night, female mosquitoes are more attracted to nectar sources and floral scents, whereas towards the later part of the night human volatile odours are more attractive. In this stage, female mosquitoes contribute significantly as vectors of many diseases, such as lymphatic filariasis, malaria and other arboviral diseases (Müller et al., 2010a; WHO, 1982; Xue et al., 2008). However, not all mosquito species are attracted to human hosts. Although some are attracted by specific hosts, i.e. humans (anthropophilic), or other animals (zoophilic), most mosquitoes obtain blood from a wide range of hosts without discriminating between human and animal hosts (Stanczyk, 2011). Unfortunately, this means that even if mosquito diseases are controlled among human populations, animal hosts serve as a reservoir for mosquito-borne diseases (Jupp, 2005; Stanczyk, 2011). For example, yellow fever, a mosquito transmitted disease has monkeys as an animal reservoir host (WHO, 1982).

Female mosquitoes are always in search of the perfect environment to lay their eggs. These environments can be manipulated in order to prevent mosquito egg-laying. Control methods include:

- Biological
  - Natural enemies
- Non-chemical
  - Habitat modification

Adult mosquitoes feed and reproduce during this life stage; this governs their feeding ecology and behaviour. Only female mosquitoes require blood meals, resulting in pathogen transmission. Control methods include:

- Biological
  - Natural enemies
  - Genetically modified mosquitoes (GMMs)
- Non-chemical
  - Habitat modification
  - Barriers
  - Personal protection
- Chemical
  - Attractants
  - Insecticides
  - Repellents
- Integrated control methods

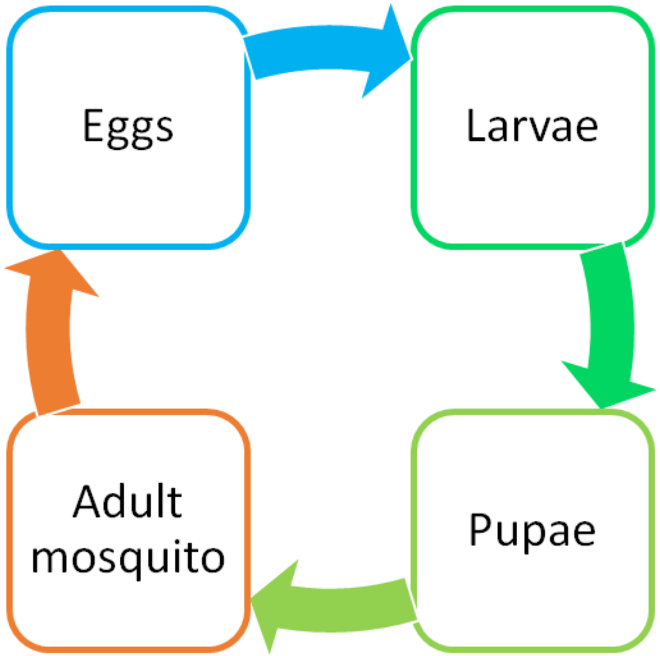

Figure 1.1. Control strategies at different mosquito life stages.

Mosquito larvae ("wrigglers") stay just beneath the water's surface, use a siphon to breathe and feed on algae and other microscopic organisms. Control methods include:

- Biological
  - Natural enemies
  - Microbial larvicides
- Non-chemical
  - Habitat modification
- Chemical
  - Oils and films
- Integrated control methods

Mosquito pupae ("tumblers") are "comma-shaped", do not feed and live just beneath the surface of the water. Control methods include:

- Biological
  - Natural enemies
  - Microbial larvicides
- Non-chemical
  - Habitat modification
- Chemical
  - Oils and films
- Integrated control methods

1.3. Control strategies

A number of different strategies exist to monitor and/or control mosquito populations in order to combat the diseases which they transmit (Figure 1.2). Controlling hosts (such as animals and humans) or the disease-causing agents themselves (viruses, parasites) seems impossible, therefore research focuses on controlling the vectors of the disease – the mosquitoes. Unique environmental conditions are required by certain mosquito species or stages in order to complete their life cycle; which may help to target particular species and diseases, and could indicate specific methods of control (Jupp, 1996) (Figure 1.1). Targeting a certain species of mosquito has implications for controlling the diseases which are transmitted by that specific species. Many applications for controlling mosquito populations have been implemented at earlier water-bound stages of their life cycle to combat mosquitoes before they reach the reproductive and highly mobile adult stage during which females need blood meals (Figure 1.1). Early control strategies aim at removal of breeding sites, but also films and oils have been applied to typical breeding sites, thereby hindering the larvae to take in oxygen at the water surface so they suffocate. Generally non-chemical methods of mosquito eradication are preferred as they are more environmentally friendly as well as less harmful to human or animal health. Biological control strategies have been implemented in many cases, for example using biological control agents such as Bti (*Bacillus thuringiensis israeliensis*), larvivorous fish, and odonata larvae that are used to kill off mosquito larvae in order to reduce the numbers of pupae and adult mosquitoes (Jupp, 1996; Snow, 1990).

However, many control methods focus on adult mosquitoes, for example, the release of sterile male mosquitoes and the application of adulticides in order to reduce or completely eradicate mosquitoes in an area (Benedict and Robinson, 2003; Ramirez et al., 2009). Many chemical strategies, either insecticides or repellents, are in widespread use to control or at least repel mosquitoes (Collier, 2004; Patel et al., 2012); usually they have only temporary success (Collier, 2004) or severe side-effects. For example, the burning of mosquito coils with pyrethrin to repel mosquitoes was widespread and successful in temporarily and locally reducing the number of biting mosquitoes; however it often resulted in allergic reactions and other negative consequences for the environment (see Collier, 2004).

Some studies have focused on attracting and trapping mosquitoes without poisonous substances and to find alternative methods for mosquito control. Müller et al. (2010a) showed that mosquitoes were significantly attracted by fruits and flowers and they could be trapped in a “black-box” approach using flower and fruit material without knowing what the active components are. Recent studies have focused on the attraction of adult mosquitoes to actual scent compounds (or blends of scent compounds) identified from scent bouquets of fruits and flowers that mosquitoes find attractive (e.g. Jhumur, 2007; Jhumur et al., 2006, 2007, 2008). Although many approaches to combat mosquito-borne diseases exist more integrated approaches for monitoring and controlling adult mosquito populations are needed. One possibility would be to use their unique dietary requirements and innate preferences for carbohydrate sources and corresponding scents in combination with other control strategies (Müller et al. 2010a).

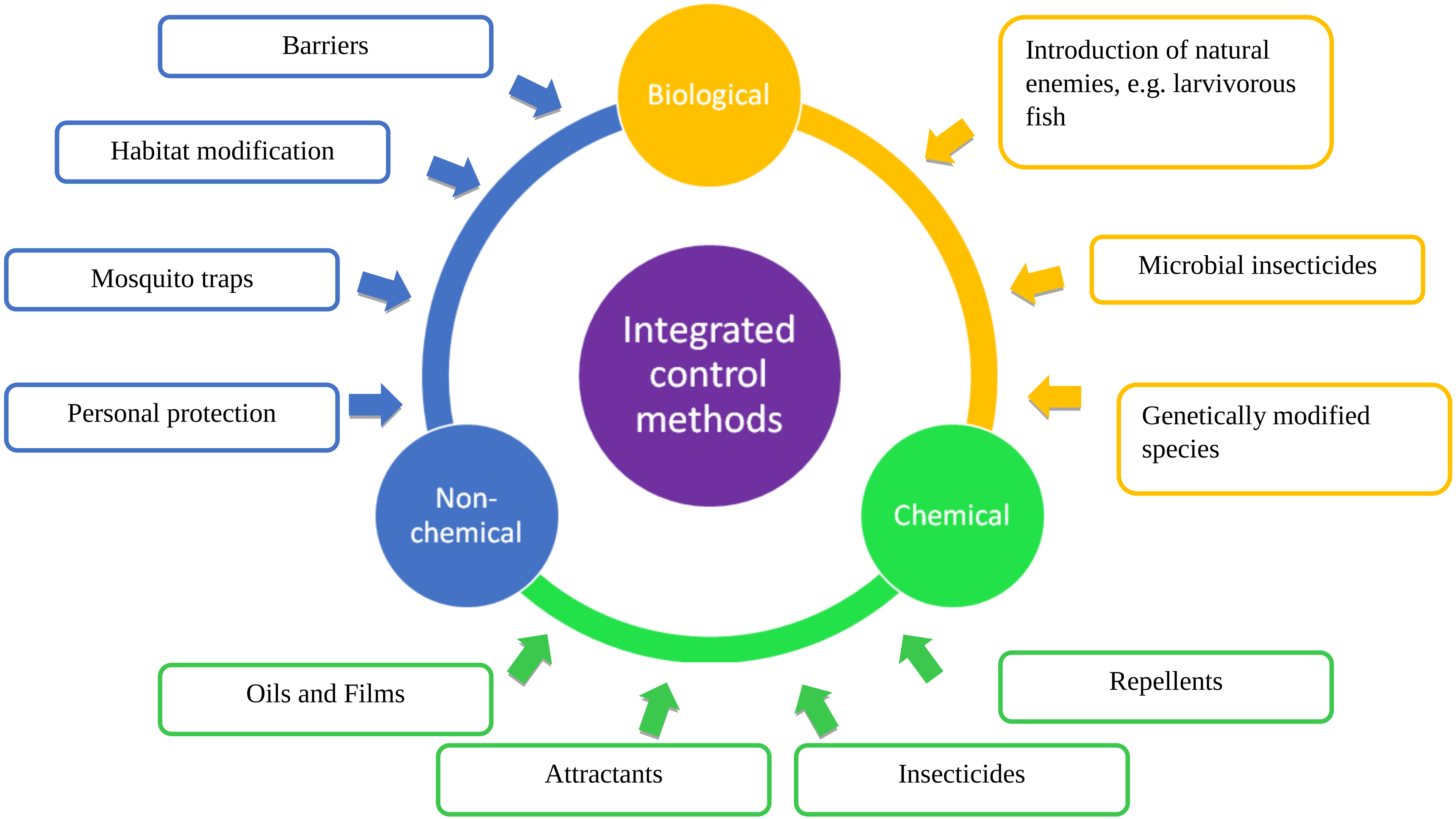

Figure 1.2. Different approaches to mosquito control.

1.3.1. Biological control

1.3.1.1. Introduction of natural enemies and parasites

Biological control methods are natural means of controlling an organism. The concept dates back many years (Kumar and Hwang, 2006). A natural enemy of the organism which needs to be controlled is used in order to significantly reduce or rid areas from the organism creating a problem. These methods of control are often preferred due to the less harmful effects on the environment and those directly involved in implementing the control strategy (Humphries, 2013; Kumar and Hwang, 2006). For mosquitoes, a number of natural predators exist (Kumar and Hwang, 2006; Ramirez et al., 2009). Natural predators for mosquitoes range from amphibian tadpoles to odonate larvae (Kumar and Hwang, 2006). For example, the western and eastern mosquito fish (*Gambusia affinis* and *G. holbrooki*, respectively) are natural predators of mosquitoes and prey on both juvenile and adult mosquitoes (Kumar and Hwang, 2006; Ramirez et al., 2009). Microbial larvicides specifically target the larval stage of mosquitoes (Kumar and Hwang, 2006). Different microbial larvicides have shown to be significant in reducing the number of disease-transmitting mosquitoes (Kumar and Hwang, 2006). Biological larvicides include *Bacillus sphaericus* (Bs), *Bacillus thurningiensis israelensis* (Bti) and *Lagenidium giganteum* (Kumar and Hwang, 2006; Ramirez et al., 2009). For example, *Bacillus thuringiensis israelensis* (Bti) is a bacteria found in soil (see Kumar and Hwang, 2006 and reference therein). Feeding mosquito larvae ingest a dormant spore form of the bacterium (Kumar and Hwang, 2006). The bacteria produce a toxin which affects the gastrointestinal tract of the larvae which results in death. The use of Bti is highly specific and ecofriendly since other organisms and even predators of the mosquito larvae are not affected (Lacey and Undeen, 1986). Another method of biological control is the use of genetically modified mosquitoes (GMMs).

1.3.1.2. Genetic modification and population control

Genetically modified mosquitoes (GMMs) are considered to be a very promising mosquito control strategy (Marshall and Taylor, 2009). Mosquitoes capable of spreading diseases have genes that are necessary for the uptake, transport and transmission of the disease-causing agent. Interestingly, not all individuals belonging to the same mosquito genus (or species) are pathogenic, which allows for the possible identification, replacement or manipulation of those genes relevant for disease transmission (Marshall and Taylor, 2009). Genetically modified mosquitoes with reduced or eliminated disease carrying traits are bred and then released into the wild, reproducing with other mosquitoes. As a result, subsequent generations are replaced by vectors incapable of spreading the disease or results in the reduction of mosquito population thereby reducing the number of human infections (Lavery et al., 2008; Marshall and Taylor, 2009; Scott et al., 2002).

A related technique where manipulated mosquitoes are released in the wild, the sterile insect technique (SIT), has been shown to be better method than gene introgression techniques (Benedict and Robinson, 2003). The sterile insect technique entails the mass rearing and release of genetically modified sterile males over a large area allowing mating between GMMs and wild mosquitoes. The production of viable eggs, and thus offspring, is prevented (Benedict and Robinson, 2003; Ramirez et al., 2009). Mass rearing, the transfer of transgenes and the survival of GMMs are factors which need to be taken into account when releasing laboratory mosquitoes into the natural environment (Scott et al., 2002).

Another technique that aims to reduce the lifespan of mosquitoes and to make them less likely to transmit diseases before they die (Stanczyk, 2011) uses *Wolbachia pipientis* a bacteria found within mosquitoes and other invertebrates (Bourtzis, 2008; Kambris et al., 2010). The bacterium, *Wolbachia pipientis*, was discovered initially in the ovaries of mosquitoes and can be used to suppress or "naturally modify" mosquito populations (Bourtzis, 2008). Females which have mated with males carrying the bacterium are unable to produce viable eggs (Kambris et al., 2010). Moreover, Kambris et al. (2010) found that *Wolbachia* can inhibit the development of *Plasmodium* in *Anopheles* mosquitoes and can possibly reduce the malarial vector frequency of the affected female mosquitoes.

Despite their great potential to control mosquito populations genetically modified organisms (GMOs) are often not well received due to the cultural, social and personal beliefs of many people. This is a challenge in terms of using GMMs in the field (Lavery et al., 2008; Marshall et al., 2010). However, mosquitoes have developed resistance against insecticides and the side-effects of the use of antimalarial and other drugs suggest that alternative methods for mosquito control, such as GMMs, are needed (Scott et al., 2002). Even with biological control strategies, the degree of effectiveness may vary from one place to another, for different species of mosquitoes or even seasonally (Kumar and Hwang, 2006). Mosquitoes may also adopt evasion behaviour when certain biological control strategies are in place (see Kumar and Hwang, 2006 and references therein).

1.3.2. Non-chemical control

Apart from biological control methods, non-chemical methods the preferred second best choice of “eco-friendly” control methods, due to the non- toxic effects on the environment and human health. Prevention is better than cure; therefore physical methods preventing mosquito development are a means of doing so. However, constant application, physical labour and short term effectiveness may not be desirable. By using basic methods such as personal protection, the attraction of mosquitoes to humans or surroundings can be reduced. Creating an unfavourable environment for mosquitoes may also result in the reduction or eradication of mosquitoes in a given area. Other methods of non-chemical control, which use mechanical means, involve specially designed equipment (Collier, 2004; Patel et al., 2012). The various methods can be used together to provide better protection from mosquitoes especially in areas of high disease transmittance. It is imperative that basic methods of personal protection are employed to reduce blood-seeking female mosquitoes.

1.3.2.1. Personal protection

The use of protective clothing (e.g. long-sleeved shirts and long trousers) is highly recommended at dusk and dawn (especially in areas of high disease transmittance) as most mosquitoes are crepuscular (Collier, 2004; Patel et al., 2012). However, not all mosquitoes are crepuscular and caution should be exercised at all times in those areas where mosquito-borne diseases are rife. For example, *Aedes* mosquitoes which carry dengue fever are active during the day (WHO, 1982). A number of synthetic and naturally developed repellents are commercially available. These should be used as an addition to protective clothing in areas of high disease transmittance as a means of personal protection. The use of bed nets and the burning of pyrethrin coils may also help keep the area free from or reduce the number of mosquitoes (Campbell, 2003). Basic sanitary measures should be carried out in order to reduce attractiveness and further transmission of the disease. For example, allowing sweat and other body fluids to accumulate on your body will make you highly attractive to mosquitoes as the sweet stench of lactic acid and other compounds are very appealing- to mosquitoes.

1.3.2.2. Habitat modification

The aspect of habitat modification involves the physical removal of possible breeding/oviposition sites of mosquitoes (Stanczyk, 2011). Stagnant water serves as the perfect breeding environment for mosquitoes. Thus water collecting in old containers, flower pots and tires must be emptied, especially after storms (Collier, 2004). Water in bird baths and water features need to be changed frequently in order to prevent adult mosquito development (Patel et al., 2012). Any stagnant water that can be dried up after heavy rains reduces the possibility of mosquito egg-laying. By modifying the species of plant in the garden, an unsuitable environment for mosquitoes is created. Many plants are known to deter mosquitoes and including these in the garden will result in a decreased number of mosquitoes. One such example is that of *Ocimum* species (Dekker et al., 2011). Physical barriers, such as mosquito nets, have been used in order to prevent mosquitoes from obtaining blood from human hosts and as a result prevent disease transmission.

Barriers

Mosquito nets have been shown to be highly efficient in keeping mosquitoes away from humans (Stanczyk, 2011). This serves as a physical barrier to protect people from biting mosquitoes and prevents disease transmission as a result. Two forms are available, i.e. non-medicated and medicated mosquito nets (Githinji et al., 2010; Patel et al., 2012). Both non-medicated and medicated mosquito nets may vary in size, shape and material (Patel et al., 2012). These nets can be placed over your bed or porch, thus preventing mosquitoes from entering (Gemade and Earland, 2013; Patel et al., 2012). Medicated mosquito nets are the same as non-medicated nets, except they are soaked in K-O (a 25% solution of deltamethrin) and aids in keeping mosquitoes away (Patel et al., 2012). Medicated nets have been approved by the WHO and may cause skin irritation for some people. In areas of high disease transmittance, mosquito nets are highly recommended and are sometimes made available by certain governments in Africa (Gemade and Earland, 2013; Githinji et al., 2010). However, in poverty stricken areas mosquito nets are often misused, for example as fishing nets or bridal veils in Zambia (Brieger, 2010; Gemade and Earland, 2013). Therefore, providing mosquito nets may not always be successful. It also needs to be taken into account that, as with any chemical control agent, mosquitoes can become resistant to various chemicals used on medicated nets (Chalannavar et al., 2013). Mosquito traps on the other hand have been researched as another method for mosquito control and prevention.

Mosquito traps

A variety of mosquito traps have been designed for commercial usage, each controlling mosquito and other arthropod populations (Collier, 2004; Patel et al., 2012). Mosquito traps are designed to lure mosquitoes (both male and female, or female only) and then entrap or capture them (Campbell, 2003; Collier, 2004; Patel et al., 2012). The mosquito trap can be used with or without (in some cases) scents which mosquitoes find attractive (Campbell, 2003; Collier, 2004; Patel et al., 2012). Many traps exist today, with the earliest being recorded in the early 1900s (see Campbell, 2003 and reference therein). Some mosquito traps have been researched for a period of at least 15 years and proved effective in numerous studies, such as the Biogents™ Sentinel professional mosquito trap (Hapairai et al., 2013). Attractants and repellents can be used to more effectively trap mosquitoes, especially those derived from fruits or flowers known to attract or deter them. Some mosquito traps may be treated with adulticides or other chemical forms of insecticides which kill trapped mosquitoes. Along with mosquito traps, alternative methods of control do exist such as electric mosquito zappers or mosquito magnets (Patel et al., 2012).

1.3.2.3. Mechanical methods

Mechanical methods of mosquito eradication involve the use of specially designed equipment which attracts mosquitoes and then kills them. There are many commercially available products which are successful at reducing the number of mosquito ‘bites’ in a given area. For example, the electric mosquito zapper is highly effective at reducing the number of mosquito ‘bites’ (Patel et al., 2012). The concept of the electric mosquito zapper is based on the use of ultraviolet light which attracts mosquitoes and kills them on contact by producing an electric charge. Patel et al (2012) reported that white light is more attractive to mosquitoes than yellow light. Another form of mechanical control is the mosquito magnet, which has similar properties to the host of a mosquito (Collier, 2004; Patel et al., 2012). Carbon dioxide, heat and moisture are given off by the mosquito magnet which the mosquito finds highly attractive and as it approaches the magnet, gets sucked in and is dehydrated till it dies (Patel et al., 2012). Some communities or populations in poor countries with high disease transmittance may disregard this form of control, as it requires electricity and they may not have or cannot afford to maintain this method. For some, chemical methods of control are preferred due to the easy application and high effectiveness of such products.

1.3.3. Chemical control

Chemical methods have been very effective in controlling many disease vectors, especially in the case of mosquitoes (Collier, 2004; Patel et al., 2012; Stanczyk, 2011). However, the use of chemical treatments is subject to an ongoing debate due to the possible environmental and health risks associated with using it. For example, DDT (dichlorodiphenyltrichloroethane), a highly successful chemical used to kill off mosquitoes and associated pests, in the early 1900s had toxic effects on humans and animals (Channa et al., 2012). DDT was banned due to the detrimental effects, however, in the recent years more people are opting to use this chemical. Mosquito attractants, repellents and even insecticides are different forms of chemical methods used to control mosquito-borne diseases. Essentially, mosquito attractants will lure mosquitoes (in most cases these are used in mosquito traps), mosquito repellents will create a barrier driving away mosquitoes which encounter the repellent (available in many forms such as creams and sprays) and insecticides are used to treat immature stages preventing adult development.

1.3.3.1. Attractants

Mosquitoes have well-developed olfactory senses, which allow host detection from 25-30 meters away (see Jhumur, 2007 and references therein). Mosquitoes thus are able to detect scents which they prefer and may relate these to food sources, oviposition sites, resting places and possible mates (Bernier et al., 2000). The behaviour of mosquitoes in relation to specific scents has led to extensive research in terms of control techniques. Human body odours or the specific volatile compounds associated are highly attractive to female mosquitoes in search of a blood meal (to obtain protein for egg development) (Geier and Boeckh, 1999). Studies such as Bernier et al. (2000) focused on analyzing compounds present which are attractive to mosquito species known for their disease transmission. Techniques such as GC-EAD (gas chromatography mass spectrometry coupled with electroantennogram detection) allow for the identification of specific volatile compounds present in human odours or other samples which mosquitoes find attractive (or repulsive) and display responsive behaviour toward (Bernier et al., 2000; Costantini et al., 2001).

Further behavioural tests with regards to preference of specific scent compounds can be conducted in wind tunnel experiments, before being tested in field trials (Costantini et al., 2001). Female mosquitoes are known for their blood-sucking behaviour; however, both male and female mosquitoes require carbohydrate sources such as nectar. In the case of female mosquitoes, carbohydrate sources must be obtained before a blood meal (in most cases) in order to obtain energy, which is significant for the use of attractive scents in mosquito control. Earlier studies focused on monitoring or controlling mosquito populations using fruits and flowers found to be attractive by mosquitoes. Using the GC-EAD (gas-chromatography coupled to electroantennogram detection) technique, compound mixtures can be screened for compounds which show a physiological response in the insect antenna. These compounds can then be tested in bio-assays to identify mosquito repellents or attractants. If mosquitoes can be controlled before obtaining a blood meal using attractants, it has possible implications for the control or possible eradication of mosquito-borne diseases. This can be accomplished by treating attracted mosquitoes with insecticides or introducing disease to them before releasing them.

1.3.3.2. Repellents

Repellents cause a change in mosquito behaviour, resulting in the avoidance of hosts or areas where repellents are being utilized (Stanczyk, 2011). Essentially, the repellent masks scents (or odours) produced by warm blooded mammals which mosquitoes (and possibly other arthropods) find attractive, deterring them from the host (e.g. humans) (Patel et al., 2012). These masked scents (or odours) include carbon dioxide, lactic acid and other compounds (Patel et al., 2012). In addition, a scent (or combination of scents) which mosquitoes do not find attractive can be used. Chemical repellents may come in different forms, for example, lotions or aerosols (Stanczyk, 2011). These are mainly used by humans (but are also used to protect livestock and pets) in order to deter pests and disease vectors. Repellents come in the form of natural or synthetic repellents (Patel et al., 2012). One of the most successful synthetic repellents is DEET (*N*, *N*-diethyl-*m*-toluamide) which masks volatiles expelled from humans during respiration and sweating (Chalannavar et al., 2013; Govere et al., 2001;

Patel et al., 2012; Stanczyk, 2011). Thus mosquitoes cannot find their way to the host to obtain a blood meal which has implications for the reduction of mosquito-borne disease transmission (Dekker et al., 2011). Advantages of synthetic repellents include higher effectiveness and persist for a longer period before reapplication compared to natural repellents (Patel et al., 2012; Stanczyk, 2011). However, disadvantages include allergies (such as skin and eye irritation) and possible brain swelling. Brain swelling has been reported in children and a few cases of death have been documented (Collier, 2004; Patel et al., 2012). Many people may prefer natural repellents due to their less harmful effects, both on human health and the environment. Natural products are generally preferred to synthetic ones because there is the general perception that these products are more "environmental-friendly" or healthy with minimal or no effects as a result of application on humans (Collier, 2004; Dekker et al., 2011; Patel et al., 2012; Humphries, 2013). To repel mosquitoes, natural products like citronella and neem oil (a vegetable oil from *Azadirachta indica* species) are available in different forms and have been demonstrated to be effective (Patel et al., 2012). One great disadvantage is that mosquitoes may no longer respond to chemicals used (Chalannavar et al., 2013; Stanczyk, 2011).

1.3.3.3. Insecticides

Mosquitoes have a complete life cycle, allowing for the targeted control of one or more stages during the life cycle. Insecticides target egg, larval and pupal stages of development, preventing adult development (adults having the ability to spread disease) (Chalannavar et al., 2013). This results in a reduced adult population which may in turn decrease the frequencies of infection. Insecticides are generally applied to water bodies in which mosquitoes develop and may have growth regulators such as methoprene (in the presence of which pupae are unable to develop into adult mosquitoes). Other insecticides include Carbaryl, Malathion and Resmethrin (Collier, 2004). Other forms of insecticides which act on mosquitoes in the final developmental stage are known as adulticides. Adulticides may reduce adult mosquito populations temporarily. Insecticides are a form of pesticide and must therefore be in accordance with environmental regulations outlined by the relevant authorities. One of biggest concerns of using insecticides is, however, their often high toxicity to non-target animals and humans. This is the reason why in some places they are only used in extreme cases of a disease outbreak. Furthermore, mosquitoes have been reported to build resistance to various insecticides (WHO, 1982). Even though there are other naturally derived or synthetic insecticides which are constantly being developed, mosquitoes may become resistant to those at a later stage. DDT, an insecticide which successfully killed off mosquitoes in the past was banned in many countries due to its toxic nature, accumulative effect in the food chain and harmful effects on human health (Channa et al., 2012). However, due to the significant threat posed by mosquitoes in recent years, DDT has become more popular. In rural or poorly developed areas, DDT is sprayed onto the walls of huts in an attempt to control mosquito populations but also to reduce the spread into the environment. Even so, mosquitoes can develop resistance to this chemical, needless to mention the effects on human health (Channa et al., 2012). As a result, alternative methods of control or more integrated approaches are necessary, especially to prevent disease transmission in areas where diseases are prevalent.

1.4. Integrated control strategies

Many approaches to combat mosquito-borne diseases exist. However, more integrated approaches are needed to improve monitoring and controlling of mosquito populations. Even though there are many existing control strategies which target the water dependent stage of mosquitoes, adult mosquitoes develop in large numbers infecting large numbers of people. Attempting to destroy or treat all possible habitats and breeding sites of mosquitoes is a difficult task and may neither economically nor ecologically be a feasible option. However, integrated approaches could be used in order to more effectively control mosquito populations.

Integration refers to the combination or incorporation of two or more strategies. Mosquito populations continue to fluctuate through the different seasons usually with peaks during seasons with high rainfall and temperature. During these periods, the perfect breeding environments for mosquitoes are formed, such as water collecting in old tyres and containers (Collier, 2004). As these are the primary source for swarms of mosquitoes, integrated control methods should start with the removal of these breeding sites. Basic sanitation and personal protection must be in place at all times. In areas of high disease transmittance, physical barriers should be used. Larvivorous fish, odonate larvae and other biological agents should be introduced to ponds and other large or open water bodies housing mosquito eggs and larvae. For larval and pupal stages microbial larvicides, other forms of larvicides, oil films, etc. need to be employed. The use of insecticides/ adulticides should be used in mosquito disease areas as often as required. Other control strategies include the use of attractants and repellents in conjunction with mosquito traps and the use of mechanical methods. Mosquito repellence has been widely researched and is used in various forms to deter mosquitoes. A study conducted by Collier (2004) indicated that when a repellent device was placed with the ABC Mosquito Magnet® traps, the number of mosquitoes caught were reduced.

The feeding ecology of mosquitoes is an important aspect for the use of attractants in conjunction with mosquito traps. Only after mating, mature females prefer blood meals, prior to which they would often require (and prefer) a carbohydrate source such as nectar (Müller et al., 2010b). This is significant as they are in search of flowers containing nectar or fruit. The volatile compounds found in these flowers and fruit responsible for attracting these mosquitoes can be determined and used as effective attractants in mosquito traps. Other methods of control should be used simultaneously such as GMMs. Since genetic modification is effective at reducing mosquito populations, target species should therefore be focused on. Mosquito control is not only essential for human protection, but animal health as well. Even if mosquito diseases are controlled among human populations, animal hosts serve as the perfect reservoir hosts for mosquito-borne diseases (Jupp, 2005; Stanczyk, 2011). For example, yellow fever, a mosquito transmitted disease has an animal reservoir host which is forest monkeys (WHO, 1982). Due to the various effects of climate changes, mosquito populations could at any point, rapidly increase. Integrated approaches could help reduce their impact when spontaneous and random events may occur (WHO, 1982). Therefore, in order to combat the disease vectors such as mosquitoes, a number of different control strategies are implemented or need to be used in conjunction with one another to provide a much more efficient means of mosquito eradication. By using scent in conjunction with mosquito traps and other methods of control, a reduction in the number of mosquitoes will be possible.

1.5. The use of scent in mosquito control

Many existing control strategies focus on using attractants or repellents in order to reduce mosquito populations. By using attractive measures, mosquitoes can be attracted and drawn away from hosts. Carbon dioxide, lactic acid and other human odours have been identified and used as successful mosquito attractants (Pandey and Kim, 2011). The mosquitoes which are trapped are generally those females in search of a blood meal. By exploiting the feeding ecology and manipulating the sensory cues of the mosquito, those which are attracted have not yet went in search of a blood meal. Capturing female mosquitoes at this point further prevents disease transmission. Foster and Takken (2004) found that mosquitoes would go in search for nectar related sources prior to blood. This has implications which mean capturing mosquitoes using nectar or nectar related volatiles would prevent the blood-sucking stage.

Research in the recent years focused on the use of fruits and flowers which were observed to be attractive to mosquitoes as a means of attracting and trapping mosquitoes (Müller et al., 2010a). Fruits or flowers were placed in the cages with the exterior covered in glue to trap mosquitoes. It was found that mosquitoes were highly attracted to the fruits and flowers with varying attractiveness. This was in the form of a "black-box" approach as the exact scent compounds (in the fruit and flowers) known to attract mosquitoes were not known. Problems associated include the use of perishables. The fruits and flowers used have a limited time span before producing different scents (scent emissions change over a period of time), wilting and dying. Therefore identifying actual scent compounds and using these will be able to provide a more effective means of attracting mosquitoes.

A study conducted by Jhumur (2007) assessed the scent compounds found in a flowering plant species pollinated by mosquitoes, *Silene otites* (Caryophyllaceae), using gas chromatography mass spectrometry (GC-MS). Phenylactealdehyde and linalool oxide were among the many scent compounds found in the floral scent. These compounds were also potent attractants in lab wind tunnel experiments (Jhumur et al., 2008). It would therefore be interesting to test scent compounds from fruits and flowers which have been reported to be attractive to mosquitoes in field trials with mosquito traps that are normally used for monitoring mosquito populations. Using fruit and flower scent could be an interesting alternative to attractants based on human skin odours. Mosquito traps, such as Biogents Sentinel™ (a product of Biogents, Regensburg, Germany), have been developed and tested over many years and are mostly based on mosquito attractants based on human skin odours and CO_2 (Crepeau et al., 2013). Various disease carrying mosquitoes have been caught to date using the Biogents Sentinel™ traps. An advantage of using floral/fruit volatiles could be that both male and female mosquitoes will be captured. Nectar bait stations could also be used to trap mosquitoes and to treat them e.g. with biocontrol agents or chemicals. This could either be a way to control the mosquito populations or to control the disease in the mosquitoes.

1.6. References

American Mosquito Control Association (AMCA), 2014. Mosquito-Borne Diseases. http://www.mosquito.org/mosquito-borne-diseases Date accessed: 30 June 2015.

Backer, N., 1989. Life strategies of mosquitoes as an adaptation to their habitats. Bulletin for the Society of Vector Ecology 14, 6-25.

Benedict, M., Q., Robinson, A. S., 2003. The first releases of transgenic mosquitoes: an argument for the sterile insect technique. TRENDS in Parasitology 19, 349-355.

Bernier, U. R., Kline, D. L., Barnard, D. R., Schreck, C. E., Yost, R. A., 2000. Analysis of human kin emanations by gas chromatography/mass spectrometry.2.Identification of volatile compounds that are candidate attractants for the yellow fever mosquito (*Aedes aegypti*). Analytical Chemistry 72, 747-756.

Bond, J. G., Arredondo- Jiménez, J. I., Rodríguez, M. H., Quiroz- Martínez, H., Williams, T., 2005. Oviposition habitat selection for a predator refuge and food source in a mosquito. Ecological Entomology 30, 255–263.

Bourtzis, K., 2008. *Wolbachia*-based technologies for insect pest population control, in: Aksoy, S. (Eds.), Transgenesis and the management of vectore-borne disease. Landes Bioscience and Springer Science and Business Media, New York, pp. 104-113.

Brieger, W., 2010. Millions of nets- but are they being used? Africa Health, 15-19.

Campbell, C. B., 2003. Evaluation of five mosquito traps and a horse for West Nile vectors on a north Florida equine facility. University of Florida. Florida, United States of America.

Chalannavar, R. K., Hurinanthan, V., Singh, A., Venugopala, K. N., Gleiser, R., M., Baijnath, H., Odhav, B., 2013. The antimosquito properties of extracts from flowering plants in South Africa. Tropical Biomedicine 30, 559-569.

Channa, K., Röllin, H. B., Nøst, T. H., Odland, J. Ø., Sandanger, T. M., 2012. Prenatal exposure to DDT in malaria endemic region following indoor residual spraying and in non-malaria coastal regions of South Africa. Science of the Total Environment 429, 183-190.

Collier, B. W., 2004. Field evaluation of mosquito control and repellent devices in southern Louisiana. MSc Thesis. University of Wisconsin-Superior. Louisiana, United States of America.

Costantini, C., Birkett, M. A., Gibson, G., Ziesmann, J., Sagnon, N. F., Mohammed, H. A., Coluzzi, M., Pickett, J. A., 2001. Electroantennogram and behavioural responses of the malaria vector *Anopheles gambiae* to human-specific sweat compounds. Medical and Veterinary Entomology 15, 259-266.

Crepeau, T. N., Unlu, I., Healy, S. P., Farajollahi, A., Fonseca, D. M., 2013. Experiences with the large-scale operation of the Biogents Sentinel™ Trap. Journal of the American Mosquito Control Association 29, 177-180.

Dekker, T., Ignell, R., Ghebru, M., Glinwood, R., Hopkins, R., 2011. Identification of mosquito repellent odours from *Ocimum forskolei*. Parasites and Vectors 4, 183-190.

Fang, J., 2010. A World without Mosquitoes. Nature 466, 432–34.

Foster, W. A., Takken, W., 2004. Nectar-related vs. human-related volatiles: behavioural response and choice by female and male *Anopheles gambiae* (Diptera: Culicidae) between emergence and first feeding. Bulletin of Entomological Research 94, 145-157.

Geier, M., Boeckh J., 1999. A new Y-tube olfactometer for mosquitoes to measure the attractiveness of host odours. Entomologia Experimentalis et Applicata 92, 9-19.

Gemade, E. I., Earland, J., 2013. Long lasting insecticidal nets (LLINs) ownership and use: a qualitative study to explore why people in Kuje area Council of Federal Capital Territory of Nigeria are not sleeping under the LLINs for malaria prevention. The Journal of Macro Trends in Applied Science 1, 26-41.

Githinji, S., Herbst, S., Kistemann, T., Noor, A. M., 2010. Mosquito nets in a rural area of Western Kenya: ownership, use and quality. Malaria Journal 9, 1-6.

Govere, J., Braak, L. E. O., Durrheim, R. H., Coetzee, M., 2001. Repellent effects on *Anopheles arabiensis* biting humans in Kruger Park, South Africa. Medical and Veterinary Entomology 15, 287-292.

Hapairai, L. K., Joseph, H, Cheong Sang, M. A., Melrose, W., Ritchie, S. A., Burkot, T. A., Sinkins, S. P., Bossin, H. C., 2013. Field evaluation of selected traps and lures for monitoring the filarial and arbovirus vector, *Aedes polynesiensis* (Diptera: Culicidae), in French Polynesia. Journal of Medical Entomology 50, 731-739.

Humphries, M. S., 2013. DDT residue contamination in sediments from Lake Sibaya in northern KwaZulu-Natal, South Africa: Implications for conservation in a World Heritage Site. Chemosphere 93, 1494-1499.

Jhumur US, Dötterl S, Jürgens A. 2006. Naïve and conditioned responses of *Culex pipiens pipiens* biotype molestus (Diptera, Culicidae) to flower odors. Journal of Medical Entomology 43, 1164-1170.

Jhumur US, Dötterl S, Jürgens A. 2007. Electrophysiological and behavioural responses of mosquitoes to volatiles of *Silene otites* (Caryophyllaceae). Arthropod–Plant Interactions 1, 245-254.

Jhumur US, Dötterl S, Jürgens A. 2008. Floral odours of *Silene otites*: their variability and attractiveness to mosquitoes. Journal of Chemical Ecology 34, 14-25.

Jhumur, U. S., 2007. *Silene otites* (Caryophyllaceae): Attraction of nectar-seeking mosquitoes to inflorescence odours, and temporal variation of flower scent and flower visitors. Dissertation, 2007, Universität Bayreuth, Fakultät für Biologie, Chemie und Geowissenschaften, 145.

Jupp, P. G., 1996. Mosquitoes of Southern Africa, Ekogilde Publishers, South Africa.

Jupp, P. G., 2005. Mosquitoes as vectors for disease in South Africa. South African Family Practice 47, 68-72.

Kambris, Z., Blagborough, A. M., Pinto, S. B., Blagrove, M. S. C., Godfray, H. C. J., Sinden, R. E., Sinkins, S. P., 2010. *Wolbachia* stimulates immune gene expression and inhibits *plasmodium* development in *Anopheles gambiae*. PLoS Pathogens 6, 1-9.

Kumar, R., Hwang, J., 2006. Larvacidal efficiency of aquatic predators: a perspective for mosquito biocontrol. Zoological Studies 45, 447-466.

Lacey, L. A., Undeen, A. H., 1986. Microbial control of black flies and mosquitoes. Annual Review of Entomology 31, 265-296.

Lavery, J. V., Harrington, L. C., Scott, T. W., 2008. Ethical, social and cultural considerations for site selection for research with genetically modified mosquitoes. The American Journal of Tropical Medicine and Hygiene 79, 312-318.

Lothrop, H. D., Wheeler, S. S., Fang, Y., Reisen, W. K., 2012. Use of scented sugar bait stations to track mosquito-borne arbovirus transmission in California. Journal of Medical Entomology 49, 1466-1472.

Marshall, J. M., Taylor, C. E., 2009. Malaria control with transgenic mosquitoes. PLoS Medicine 6, 164-168.

Marshall, J. M., Touré, M. B., Traore, M. M., Famenini, S., Taylor, C. E., 2010. Perspectives of people in Mali toward genetically-modified mosquitoes for malaria control. Malaria Journal 9, 128-139.

Müller, G. C., Beier, J. C., Traore, S. F., Toure, M. B., Traore, M. M., Bah, S., Doumbia, S., Schlein, Y., 2010a. Field experiments of *Anopheles gambiae* attraction to local fruits/seedpods and flowering plants in Mali to optimize strategies for malaria vector control in Africa using attractive toxic sugar bait methods. Malaria Journal 9, 1-11.

Müller, G. C., Junnila, A., Schlein, Y., 2010b. Effective control of adult *Culex pipiens* by spraying an attractive toxic sugar bait solution in the vegetation near larval habitats. Journal of Medical Entomology 47, 63-6.

Müller, G. C., Kravchenko, V. D., Schlein, Y., 2008. Decline of *Anopheles sergentii* and *Aedes caspius* populations following presentation of attractive toxic (Spinosad) sugar bait stations in an oasis. Journal of the American Mosquito Control Association 24, 147-149.

Müller, G. C., Schlein, Y., 2006. Sugar questing mosquitoes in arid areas gather on scarce blossoms that can be used for control. International Journal for Parasitology 36, 1077-1080.

Müller, G. C., Xue, R., Schlein, Y., 2010c. Seed pods of the carob tree *Ceratonia siliqua* are a favored sugar source for the mosquito *Aedes albopictus* in coastal Israel. Acta Tropica 116, 235-239.

Ostera, G. R., Gostin, L. O., 2011. Biosafety concerns involving genetically modified mosquitoes to combat malaria and dengue in developing countries. The Journal of the American Medical Association 305, 930-931.

Otienoburu, P. E., Ebrahimi, B., Phelan, P. L., Foster, W. A., 2012. Analysis and optimization of a synthetic milkweed floral attractant for mosquitoes. Journal of Chemical Ecology 38, 873-881.

Pandey, S. K., Kim, K., 2011. Human body-odor components and their determination. Trends in Analytical Chemistry 30, 784-796.

Patel, E. K., Gupta, A., Oswal, R. J., 2012. A review on: Mosquito repellent methods. International Journal of Pharmaceutical, Chemical and Biological Sciences 2, 310-317.

Ramirez, J. L., Garver, L. S., Dimopoulos, G., 2009. Challenges and approaches for mosquito targeted malaria control. Current Molecular Medicine 9, 116-130.

Scott, T. W., Takken, W., Knols, B. G. J., Boëte, C., 2002. The ecology of genetically modified mosquitoes. Science 298, 117-119.

Snow, K. R., 1990. Mosquitoes, Richmond Publishing Co. Ltd., England.

Stanczyk, N., 2011. An investigation of DEET-insensitivity in *Aedes aegypti*. PhD thesis. University of Nottingham.

WHO, 1982. Manual on environmental management for mosquito control: with special emphasis on malaria vectors. WHO Geneva.

WHO, 2010. Rift Valley Fever, Fact Sheet. http://www.who.int/mediacentre/factsheets/fs207/en/. Date accessed: 30 June 2015

WHO, 2014a. Malaria, Fact Sheet. http://www.who.int/mediacentre/factsheets/fs094/en/ Date accessed: 30 June 2015.

WHO, 2014b. Yellow fever, Fact Sheet. http://www.who.int/mediacentre/factsheets/fs100/en/ Date accessed: 04 April 2014.

Xue, R., Ali, A., Barnard, D. R., 2008. Host species diversity and post-blood feeding carbohydrate availability enhance survival of females and fecundity in *Aedes albopictus* (Diptera: Culicidae). Experimental Parasitology 119, 225-228.

Chapter 2

A sweeter taste than blood? Attraction of *Aedes* and *Culex* mosquitoes to fruits and flowers and analysis of scent profiles

Abstract

Mosquitoes are important disease vectors and transmit these when taking up a blood meal. Prior to the blood-sucking infectious phase, however, most mosquitoes go in search of carbohydrate sources such as nectar and are able to locate these resources by means of olfactory cues. By exploiting the feeding ecology and manipulating the sensory cues of mosquitoes, it would be possible to lure and possibly control mosquitoes before they go in search of a blood meal. Therefore, the aim of the study was to determine the attraction of *Aedes* and *Culex* mosquitoes to different fruits and flowers and to analyze the scent composition of these fruit and flowers. In order to determine the attractiveness of fruits and flowers to mosquitoes, choice tests were conducted using a y-maze to determine mosquito attraction. Scent was collected using dynamic headspace methods and analysed using Gas Chromatography- Mass Spectrometry (GC-MS). *Musa acuminata* L. and *Ficus sur* were among the most attractive fruits to *Aedes* and *Culex* mosquitoes. Of the flowers tested, *Gymnosporia buxifolia* and *Acacia nilotica* were the most attractive to both *Aedes* and *Culex* mosquitoes. The genera or sex of mosquitoes did not influence their response in choice tests. The tested fruits produced mostly benzenoids and hydrocarbon esters, while the flowers emitted mostly benzenoids, hydrocarbon alcohols and hydrocarbon esters. The dominant scent compounds for *Gymnosporia buxifolia* was an unidentified compound, pentan-1-ol and ethylbenzene; while for *Acacia nilotica* ethylbenzene was the most dominant scent. The dominant scent compounds for *Musa acuminata* were the hydrocarbon esters: 3-methylbutyl acetate, isobutyl butyrate, isopentyl 2-methylpropanoate and isobutyl acetate. Ethylbenzene, ethanol, acetoin and limonene were the most dominant compounds for *Ficus sur*. Based on our results further studies are needed to test which of the

compounds found in fruits and flowers are responsible for mosquito attraction. Such information could be used to develop scent blends which could be used to lure mosquitoes to trap systems.

Keywords: Mosquitoes, Rural, Control, Local, Scent profiles

2.1. Introduction

Mosquitoes are known for their association with various life threatening diseases. Some mosquitoes of the genus *Aedes* have invaded America, Africa and Europe and transmit diseases such as Dengue, Rift Valley fever and Yellow fever (Alto et al., 2008; Eldridge, 2008; Martínez-de la Puente et al., 2015; WHO, 2010; WHO, 2014a). Mosquitoes belonging to the genus *Culex* transmit diseases such as the West Nile virus, filariasis, Japanese encephalitis and avian malaria (Gubler, 2002; Kovendan et al., 2012; Martínez-de la Puente et al., 2015; WHO, 2014b). Mosquitoes have unique dietary requirements. Male mosquitoes require only carbohydrate sources which they obtain from a range of fruit or flowers (in the form of nectar) (Eldridge, 2008; Lothrop et al., 2012; Otienoburu et al., 2012). Female mosquitoes have a more complex diet. Juvenile females mainly focus on sugar feeding after emergence (see Foster and Takken and reference therein, 2004; Müller et al., 2010). Sugar obtained serves as energy and contributes to egg production, which has implications for manipulating and possibly controlling mosquito populations (Eldridge, 2008). After mating, mature females then seek blood meals in order to obtain protein to develop their eggs (Lothrop et al., 2012; Otienoburu et al., 2012). During this phase, diseases are transmitted. Chemical cues play a vital role for mosquitoes in order to detect food sources and blood meals (Eldridge, 2008; Jhumur, 2007). Mosquitoes not only use these fruit and flowers as food sources but, could possibly use these as potential resting sites and a place to find mates which has possible implications for mosquito control.

Previous studies focused on the use of fruits and flowers which were observed and known to attract sugar-seeking mosquitoes. A study conducted by Müller et al. (2010) looked at local fruits and flowers in Mali. A range of those fruits and flowers proved to be successful in field experiments such as figs, guavas and melons. Moreover, Müller et al. (2010) found that female mosquitoes were more attracted to flowers in the early part of the evening (between 19h00 and

22h00), after which they responded more to human related volatiles. In other studies, scent compounds have been identified from flowers known to attract mosquitoes (Jhumur, 2007; Jürgens et al., 2002). Jhumur et al. (2008) analyzed the scent of a flower species pollinated by mosquitoes and reported the attraction of mosquitoes to specific scent compounds in lab assays. The scent compounds were also reported in other studies (Jürgens et al., 2002). Scent compounds can be used as potential attractants and in trapping systems to reduce or rid an area of mosquitoes. It has therefore been suggested that attracting mosquitoes when they are in search of carbohydrate sources (before they seek blood meals), has the potential to control mosquito-borne diseases by exploiting the feeding ecology and manipulating the chemical cues which mosquitoes respond to. The sugary scent of fruit and flowers are highly attractive for mosquitoes which could provide useful knowledge on how to lure them prior to their infectious blood-sucking phase.

Mosquitoes find food and blood sources by means of olfactory cues (Jhumur, 2007). By identifying which fruit and flowers attract and encourage mosquito sugar-feeding behaviour, possible scent blends can be used as attractants instead of fruits and flowers due to their perishable nature. It is important to identify fruits and flowers which are found locally. In addition, the scent compounds in these fruit and flowers can be identified and tested on mosquitoes to determine which specific scents attract them. The aim of study was to assess the attractiveness of mosquitoes to fruits and flower species found locally and to analyze the scent compounds. The objectives were (i) to observe mosquito behaviour on various locally found fruit and flower species, (ii) to perform choice tests and (iii) to analyze scent compounds of these fruits and flowers.

2.2. Methods and Materials

2.2.1. Mosquito rearing

Larvae and pupae were obtained from various bird baths, containers of water and ponds in the Pietermaritzburg region. Larvae and pupae were then placed in a fish tank with the water they were collected in. The fish tank was placed in an insect dome and the temperature of the room was between 25-27°C. Larvae were fed with TetraMin (Tetra Werke, Melle, Germany) fish flakes. Adult mosquitoes were provided with a continuous 10% sucrose solution (on filter paper) and were offered a human hand twice weekly as a blood source. Adult mosquitoes were identified to be those of the genera *Aedes* and *Culex* using the identification key in Snow (1990).

2.2.2. Fruit and flower species

Five fruits were selected: Banana (*Musa acuminata* L.), guava (*Psidium guajava*), spanspek (*Cucumis melo*), honey melon (*Cucumis melo inodorus*) and fig (*Ficus sur*). These fruits were selected based on the previous study conducted by Müller et al. (2010). The fruits were bought from a local perishables dealer. The flowers of four species were selected: *Acacia nilotica*, *Gymnosporia buxifolia*, *Bulbine natalensis* and *Bulbine frutescens*. Flowers indigenous to the area were selected based on field observations of mosquitoes feeding on the flowers of these species (Pietermaritzburg, South Africa).

2.2.3. Observations

Naïve mosquitoes (2-3 day old) were offered a fruit (or flower) in a separate insect dome in order to observe the sugar feeding behaviour. A petri-dish containing a 10% sucrose solution was also available to the mosquitoes. Sugar feeding behaviour was observed continuously between 08h00-10h00 and again between 16h00-18h00 as the most favoured feeding periods. Between the two time periods, mosquito behaviour was observed hourly.

2.2.4. Choice tests

Both *Aedes* and *Culex*, mosquitoes were starved for 36 hours prior to choice tests. Naïve mosquitoes of ages 2-3 days were used. Each fruit or flower was tested for attraction in a Y-maze analysis. A glass Y-maze was used with a scent pump which streamed air carrying the scent of the fruit or flower to the mosquito. Samples were alternated between the right and left arm of the maze to ensure there was no bias when mosquitoes made a choice. Each mosquito was tested twice on each arm of the Y-maze. A total of 240 females and 171 males of the genus *Aedes* were tested. In addition, 101 females and 61 males were tested of the genus *Culex*.

2.2.5. Statistical analysis

The statistical program SPSS version 23 (IBM SPSS Statistics 2011) was used to analyze the data. Responses made by mosquitoes during the choice tests were analyzed using generalized linear models (GLMs) that incorporated a binary sampling distribution and logit link function. Differences between mosquito sex and genera were tested in the same way. Means with 95% confidence intervals that did not overlap were considered to represent significant choices.

2.2.6. Volatile collection and chemical analysis

Scent was collected using the dynamic headspace technique. Each sample was enclosed in a polyacetate oven bag and scent was sampled by inserting an absorbent tube which contained 1.5mg Tenax TA® and Carbotrap® (in between 2 plugs of glasswool). Scent was sampled for 10 minutes with a flow rate of 200 ml/min. Scent samples of the air around were collected simultaneously to distinguish between floral/fruit and ambient contaminants. Thermal desorption (TD) samples were analyzed on a Bruker 450 GC with an Alltech Carbowax column (inner diameter: 30 m x 0.25 mm, film thickness: 0.25 µm), connected to an 11 m Bruker DB1 column (inner diameter: 0.25 mm, film thickness: 0.25 µm) coupled to a Bruker 300 quadrupole MS in electron-impact ionization mode at 70 eV. TD-samples were placed in a Varian 1079 injector equipped with a Chromatoprobe thermal desorption device (Amirav and Dagan, 1997). The flow of helium carrier gas was 1.0 ml/min. The injector was held at an initial temperature of 40°C for 2 min with a 20:1 split and then increased to 200°C at 200°C/min in splitless mode for thermal desorption. After a 3 min hold at 40°C, the temperature of the GC oven was ramped up to 240°C at 10°C/min and held for 12 min. Processing of data was performed using Varian Workstation Software. The identification of scent compounds was carried out using the NIST 11 data base system.

2.3. Results

Mosquitoes of different genera had similar feeding preferences; however they differed in feeding patterns. Mosquitoes which fed on the flowers or fruit were easily distinguished by their distended abdomens. Mosquitoes of the genus *Aedes* were active and fed throughout the day, however, feeding bouts occurred during 08h00-10h00 and 16h00-18h00 for both flowers and fruit (Table 2.1, Table 2.2). Mosquitoes were observed feeding in larger numbers on the flowers of *Gymnosporia buxifolia* and *Acacia nilotica* (Table 2.1). *Aedes* mosquitoes preferred riper fruits and seem to be deterred by overripe fruits. The overripe fruits of *Psidium guajava* (guava) seemed to deter *Aedes* mosquitoes (Table 2.2). *Aedes* mosquitoes fed mostly on the fruits of *Musa acuminata* (banana) and *Ficus sur* (fig) (Table 2.2). Mosquitoes of the genus *Culex* were mostly active and fed at particular times of the day, i.e. feeding bouts occurred between 08h00-10h00 and 16h00-18-00 on both flowers and fruit (Table 2.1, Table 2.2). *Culex* mosquitoes fed mostly on the flowers of *Gymnosporia buxifolia* (fed in large groups), *Acacia nilotica* and *Bulbine natalensis* (Table 2.1, Figure 2.1). Mosquitoes feeding on *Gymnosporia buxifolia* had fully distended abdomens before flying off the flowers. *Culex* mosquitoes also showed a strong preference for *Musa acuminata* (banana) and *Ficus sur* (fig) (Table 2.2, Figure 2.2). Mosquitoes of both *Aedes* and *Culex* were observed feeding on sucrose solution, but in small numbers or when offered a fruit or flower that they did not particularly respond to.

Mosquitoes did not respond differently based on their genus ($\chi^2_{1,\ 2288}$= 1.4, p = 0.238). In addition, the sex of mosquitoes did not influence the choice made, as no significant difference was observed ($\chi^2_{1,\ 2288}$= 0.003, p = 0.959). There was a significant difference in the response of *Aedes* ($\chi^2_{3,\ 125}$ = 41.7, p = 0.0001) and *Culex* ($\chi^2_{3,\ 116}$ = 34.4, p = 0.0001) mosquitoes to the different flower species (Figure 2.3, Figure 2.5). Mosquitoes were more attracted to flowers of *Gymnosporia buxifolia* and *Acacia nilotica* (Figure 2.3, Figure 2.5). *Bulbine natalensis* and *Bulbine frutescens* attracted significantly less mosquitoes (Figure 2.3, Figure 2.5). There was a significant difference in the response of *Aedes* ($\chi^2_{4,\ 157}$ = 14.6, p = 0.006) and *Culex* ($\chi^2_{4,\ 156}$ = 19.3, p = 0.001) mosquitoes to the different fruit species (Figure 2.4, Figure 2.6). *Musa acuminata* and *Ficus sur* attracted significantly more *Aedes* mosquitoes than the other three fruits (*Psidium guajava*, *Cucumis melo inodorus* and *Cucumis melo*) (Figure 2.4). *Culex* mosquitoes responded differently to the fruit species (Figure 2.6). *Musa acuminata* and *Ficus sur* attracted significantly more *Culex* mosquitoes (Figure 2.6). However, mosquitoes responded similarly to *Ficus sur*, *Psidium guajava*, *Cucumis melo inodorus* and *Cucumis melo* (Figure 2.6).

The chemical composition of the flower and fruit scents that mosquitoes found attractive are presented in Table 2.3 and Table 2.4, respectively. A total of 42 compounds were detected from the flowers and 39 volatiles were identified. The most dominant compound classes found among the flower species were benzenoids, hydrocarbon alcohols and hydrocarbon esters (Table 2.3). In addition, a few monoterpenes were found (Table 2.3). *Gymnosporia buxifolia* had 21 detected compounds of which 19 volatiles were identified. The most dominant compounds found were an unidentified compound (Retention time = 5.969), pentan-1-ol and ethylbenzene (Table 2.3). *Acacia nilotica* had a total of 18 detected and identified compounds with the most dominant compound being ethylbenzene (Table 2.3*). Bulbine natalensis* and *Bulbine frutescens* had a similar scent composition, with 19 and 20 identified compounds, respectively. The most dominant scent compounds for *Bulbine natalensis* were ethylbenzene, ethanol, acetone and (3Z)-3-hexenyl acetate (Table 3). The dominant compounds found for *Bulbine frutescens* were ethanol, ethylbenzene, acetone and phenylethyl alcohol (Table 2.3).

There were 51 compounds detected among the fruit and 50 volatiles were identified. The dominant compound classes among the fruits were benzenoids and hydrocarbon esters, with other compounds such as hydrocarbon alcohols and monoterpenes (Table 2.4). *Musa acuminata* had a total of 14 detected and identified scent compounds. The dominant compounds were 3-methylbutyl acetate, isobutyl butyrate, isopentyl 2-methylpropanoate and isobutyl acetate, all of which are hydrocarbon esters (Table 2.4). A total of 17 compounds were identified for *Ficus sur* with the most dominant compounds being ethylbenzene, ethanol, acetoin and limonene (Table 2.4). In addition, other scent compounds were found in smaller quantities. A large number of compounds (27) were found for *Psidium guajava* with the most dominant being ethyl acetate, ethyl hexanoate and ethyl butanoate (Table 2.4). Most of the scent compounds found were hydrocarbon esters. A total of 13 compounds were identified for *Cucumis melo inodorus* with the most dominant scent compounds 2-methybutyl acetate, 2-pentyl acetate and ethyl butanoate (Table 2.4). Only four compounds were detected and identified for *Cucumis melo*. The most dominant compound was ethyl acetate (Table 2.4).

Table 2.1. Mosquito feeding behaviour on flowers of different species.

Flower species	Observation: *Aedes*	Observation: *Culex*
Acacia nilotica	- Both male and female mosquitoes landed and fed on the flowers. However, more males were observed than females. Mosquitoes rarely fed on sucrose solution. - Most mosquitoes fed between 08h00-10h00 and 16h00-18h00. Those mosquitoes feeding on sucrose solution were observed randomly throughout the day.	- Mosquitoes rested and fed on the flowers. Male mosquitoes preferred sucrose solution. - Mosquitoes fed throughout the day, however most mosquitoes were observed feeding during 08h00-10h00 and 16h00-18h00. Males fed on sucrose throughout the day.
Bulbine frutescens	- Male and female mosquitoes fed on the flowers, however, preferred feeding on the sucrose solution. - Mosquitoes fed were observed feeding throughout the day and not at a specific time period.	- Mosquitoes fed on flowers, however females preferred sucrose solution. - Mosquitoes mostly fed between 08h00-10h00 and 16h00-18h00, both for those mosquitoes which fed on the flowers and sucrose solution.
Bulbine natalensis	- Both male and female mosquitoes fed on flowers. More females than males were observed feeding. Mosquitoes were observed feeding on the sucrose solution as well. - Mosquitoes were observed feeding at random times throughout the day.	- Male and female mosquitoes fed on flowers. More mosquitoes of both sexes were observed feeding on sucrose solution. - Mosquitoes were observed feeding throughout the day. Those mosquitoes feeding on sucrose solution were during 16h00-18h00.
Gymnosporia buxifolia	- Male and female mosquitoes rest and feed on flowers until abdomens fully distended. Mosquitoes rarely feed on sucrose solution. - Mosquitoes fed throughout the day. Most female mosquitoes were observed feeding between 08h00-10h00 and 16h00-18h00.	- Both male and female mosquitoes were resting and feeing on the flowers. Few, if any, mosquitoes fed on sucrose solution. - Mosquitoes fed throughout the day. However, most female mosquitoes were observed during 08h00-10h00 and 16h00-18h00 during which they fed till their abdomens were fully distended.

Table 2.2. Mosquito feeding behaviour on different fruit species.

Fruit species	Observation	
	Aedes	***Culex***
Cucumis melo (spanspek)	- Mosquitoes rarely fed on semi ripe fruit. However, both sexes fed on ripe and overripe fruit. Mosquitoes probed through the skin of the fruit to feed even though fruit was cut exposing inside. - Feeding bouts occurred during 08h00-10h00 and 16h00-18h00 mostly. Mosquitoes fed on sucrose solution throughout the day.	- Mosquitoes fed on fruit when it was ripe. More female mosquitoes were observed than males. Both male and females were observed probing through the skin of the fruit in order to feed. - Mosquitoes fed between 08h00-10h00 and 16h00-18h00 mostly, for those feeding on the fruit and even those which fed on the sucrose solution.
Cucumis melo inodorus (honey melon)	- A similar pattern was observed as for spanspek. - Mosquitoes fed throughout the day. In addition, most mosquitoes fed on sucrose solution.	- A similar pattern was observed for the behaviour and feeding times on honey melon as the spanspek.
Ficus sur (fig)	- Both male and female mosquitoes fed on the fig fruit, probing through the skin. Mosquitoes preferred riper figs. - Mosquitoes fed between 08h00-10h00 and 16h00-18h00. However, mosquitoes were observed feeding between 10h00 and 16h00. A few mosquitoes were observed feeding on sucrose solution.	- Mosquitoes fed on the fruit. Mosquitoes probed through the skin of the fruit and even fed on the inside of cut figs. Riper figs were preferred to green. Males and females were observed feeding. - Mosquitoes fed between 08h00-10h00 and 16h00-18h00. A few mosquitoes were observed feeding throughout the day. Some mosquitoes fed on sucrose solution.
Musa acuminata (banana)	- Both male and female mosquitoes fed on banana. Most female mosquitoes were observed feeding. A few mosquitoes fed by probing through the skin of the banana while others fed on the inside of cut bananas. - Mosquitoes mostly fed during 08h00-10h00 and 16h00-18h00. A few mosquitoes were observed feeding on sucrose solution.	- Mosquitoes fed on banana. Both male and female mosquitoes fed, however more females fed on the fruit. Mosquitoes probed through the skin or just fed on the inside of the cut fruit. - Mosquitoes fed between 08h00-10h00 and 16h00-18h00; and rarely fed on sucrose solution.

Fruit species	**Observation**	
	Aedes	***Culex***
Psidium guajava (guava)	- Mosquitoes fed on the fruit. However, were repelled when it was green or overripe. - Mosquitoes fed on fruit between 08h00-10h00 and 16h00-18h00. Between these times, mosquitoes were observed feeding on sucrose solution.	- Mosquitoes rarely fed on fruit. If ripe, a few mosquitoes were observed feeding. Most guavas were overripe at the time. - Mosquitoes fed between 08h00-10h00, however, mosquitoes were observed feeding on sucrose solution.

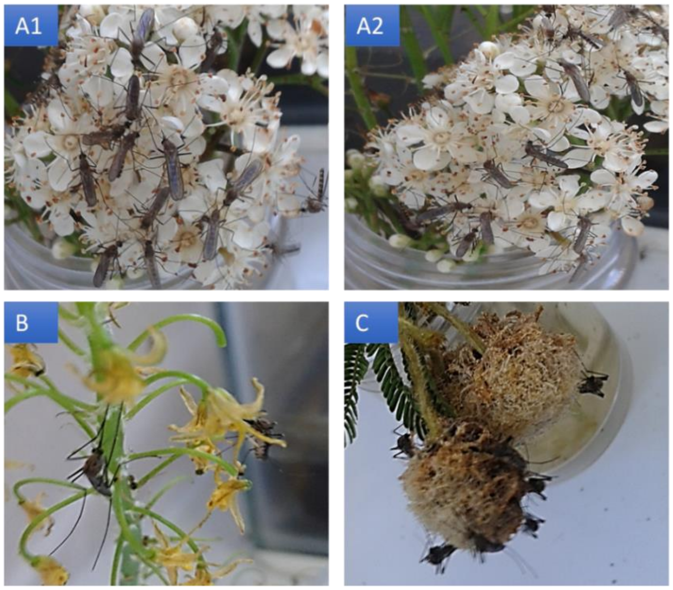

Figure 2.1. Mosquito sugar feeding on flowers. A1 and A2 show mosquito feeding on flowers of *Gymnosporia buxifolia*, B shows female mosquito feeding on flowers *Bulbine natalensis*, C shows mosquito feeding on flowers of *Acacia nilotica*.

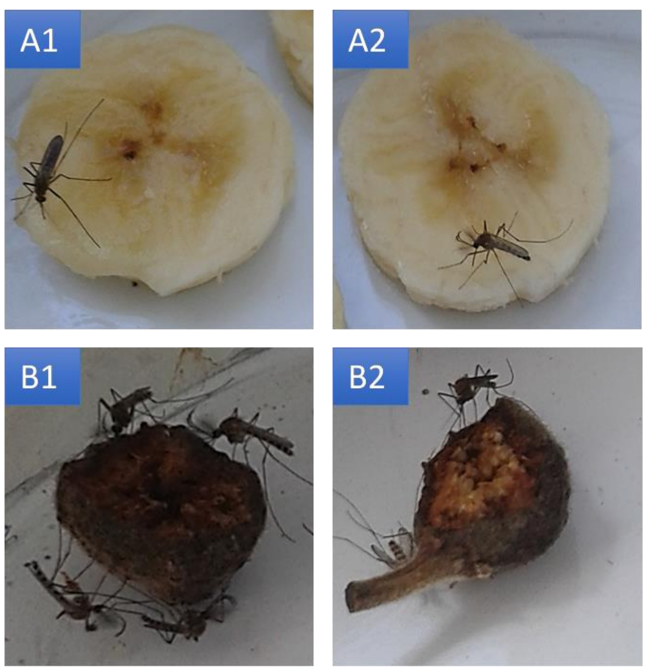

Figure 2.2. Mosquito sugar feeding on fruit. A1 and A2 represent a female and male mosquito, respectively, feeding on the fruit of *Musa acuminata*. B1 and B2 represent male and a female mosquito feeding on the fruit of *Ficus sur*.

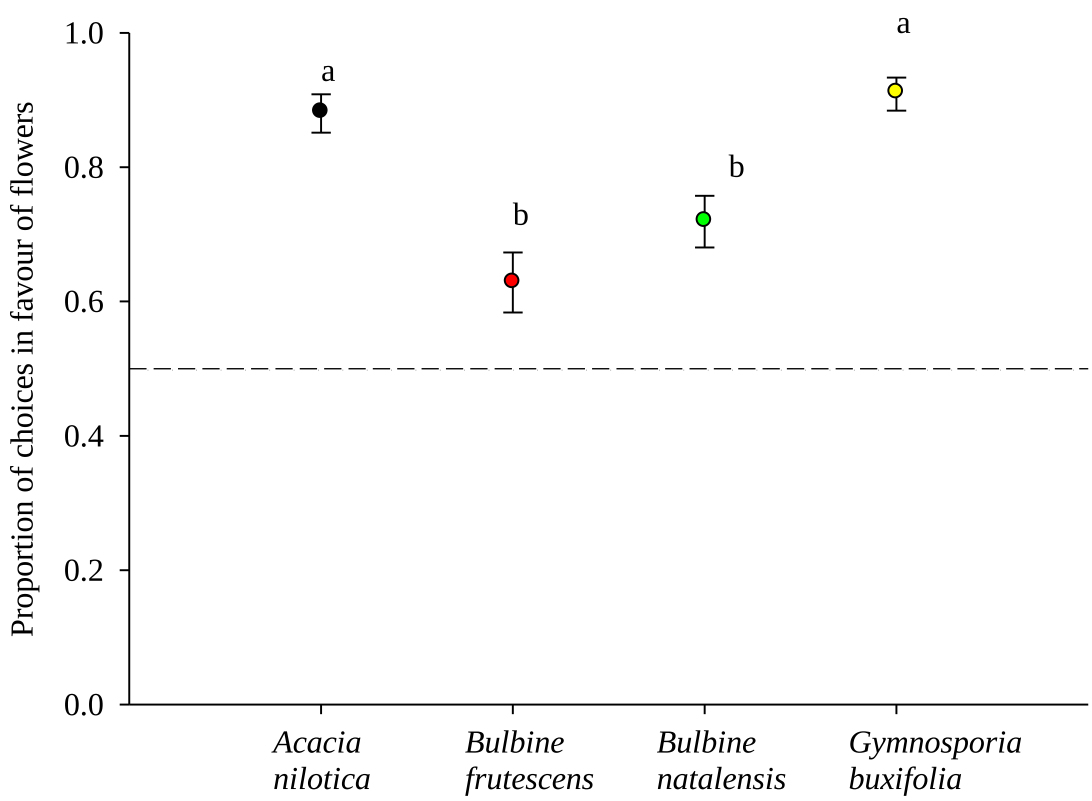

Figure 2.3. Response of *Aedes* mosquitoes to different flower species ($\chi^2_{3, 125} = 41.7$, $p = 0.0001$). Means (± SE) that share letters are not significantly different.

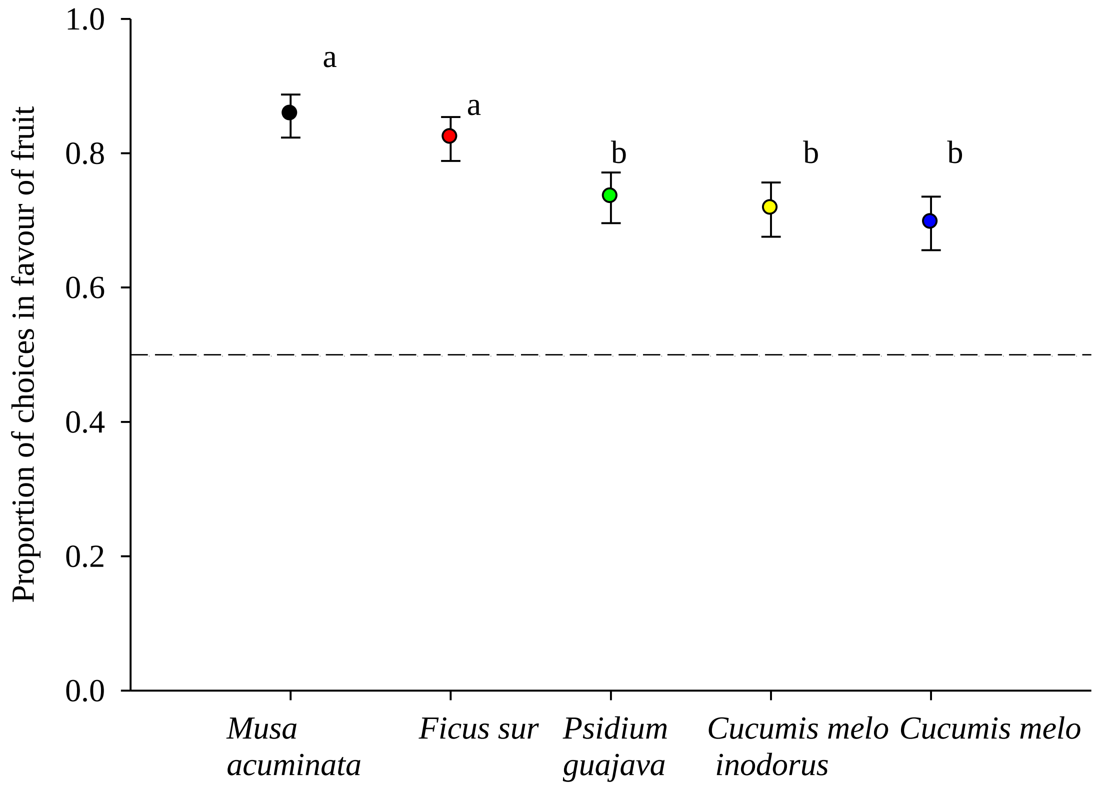

Figure 2.4. Response of *Aedes* mosquitoes to different fruit species ($\chi^2_{4, 157} = 14.6$, $p = 0.006$). Means (± SE) that share letters are not significantly different.

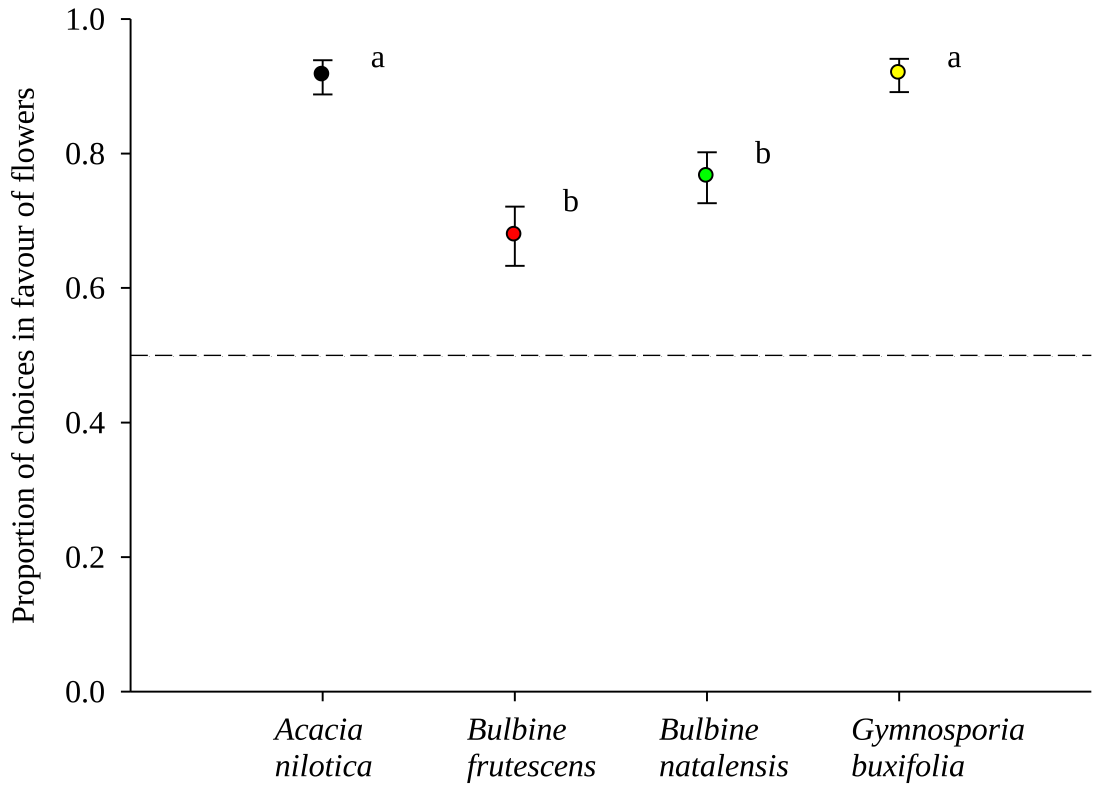

Figure 2.5. Response of *Culex* mosquitoes to different flower species ($\chi^2_{3, 116} = 34.4$, p = 0.0001). Means (± SE) that share letters are not significantly different.

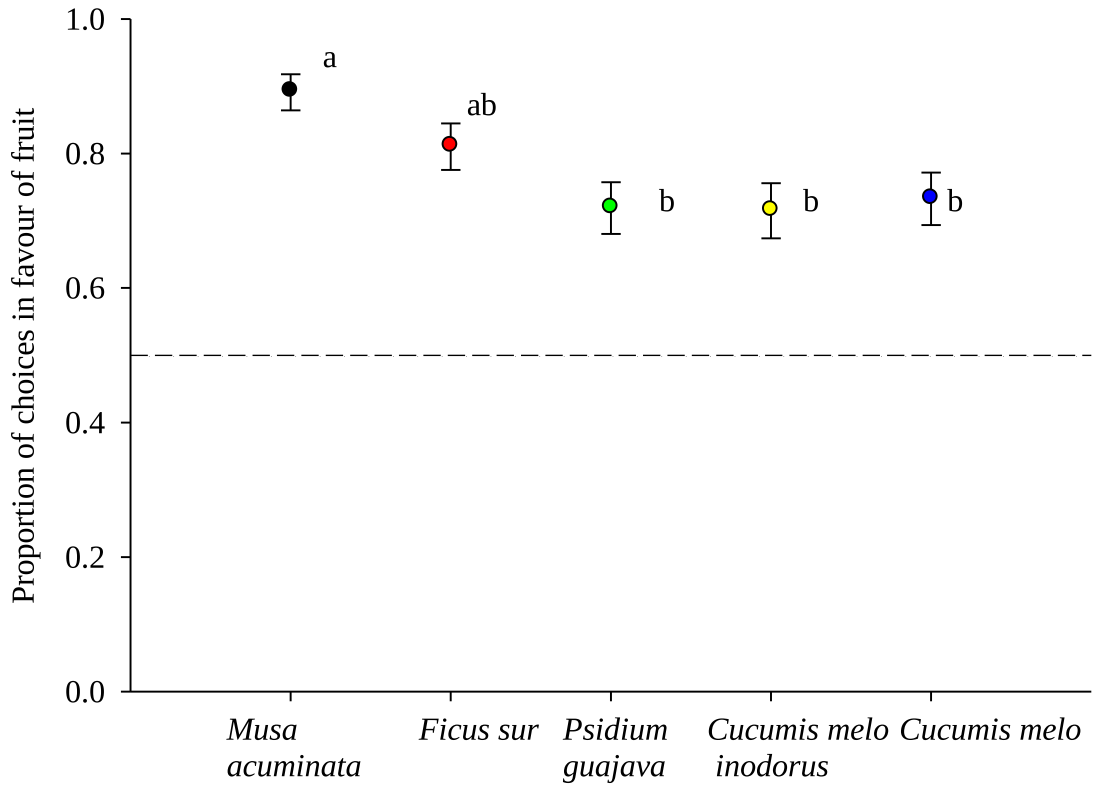

Figure 2.6. Response of *Culex* mosquitoes to different fruit species ($\chi^2_{4, 156} = 19.3$, p = 0.001). Means (± SE) that share letters are not significantly different.

Table 2.3. Scent composition and percentage contribution of flowers found to be attractive to mosquitoes. Chemical Abstract Service number = CAS; Retention time = RT; Relative retention time index = RRT.

					Percentage contribution			
					Gymnospria buxifolia	***Bulbine natalensis***	***Bulbine frutescens***	***Acacia nilotica***
Compound	**Compound class**	**CAS**	**RT**	**RRT**				
Ethanol	Hydrocarbon alcohol	64-17-5	3.320	883	0.91	23.43	1.37	5.09
Pentan-1-ol	Hydrocarbon alcohol	71-41-0	5.050		32.30	-	-	-
Acetone	Hydrocarbon ketone	67-64-1	5.329	863	-	20.34	17.00	3.06
Ethyl Acetate	Hydrocarbon ester	141-78-6	5.722	899	-	-	-	-
Ethanol	Hydrocarbon alcohol	64-17-5	5.901	934	-	-	27.48	-
Unidentified			5.969	1000	43.63	-	-	-
Ethylbenzene	Benzenoid	100-41-4	8.171	1111	13.58	25.27	17.24	53.31
Unidentified Benzene	Benzenoid		8.840		1.00	-	-	-
p-Xylene	Benzenoid	106-42-3	8.866	1155	-	1.47	0.92	4.34
Limonene	Monoterpene	138-86-3	9.359	1207	0.93	2.26	1.73	4.08
Styrene	Benzenoid	100-42-5	9.702	1243	0.77	-	0.50	0.78
Cyclooctatetraene		629-20-9	9.709	1261	-	0.73	-	-

Compound	Compound class	CAS	RT	RRT	Percentage contribution			
					Gymnospria buxifolia	*Bulbine natalensis*	*Bulbine frutescens*	*Acacia nilotica*
γ-Terpinene	Monoterpene	99-85-4	9.885	1244	-	-	-	2.26
Isoamyl butyrate	Hydrocarbon ester	106-27-4	10.016	1255	0.10	-	-	-
3-Methylbutyl butyrate	Hydrocarbon ester	106-27-4	10.063	1283	-	-	1.43	2.56
Isopentyl 2-methylpropanoate	Hydrocarbon ester	1/3/2050	10.082	1256	-	1.24	-	-
(4E)-4-Hexenyl acetate	Hydrocarbon ester	72237-36-6	10.477		-	-	-	0.94
(3Z)-3-Hexenyl acetate	Hydrocarbon ester	3681-71-8	10.517	1315	-	13.49	-	-
Ethyl 2-hydroxypropanoate	Hydrocarbon ester	687-47-8	10.655	1342	1.24	-	-	2.01
1-Hexanol	Hydrocarbon alcohol	111-27-3	10.795	1305	-	-	-	-
6-Methyl-5-hepten-2-one (Sulcatone)	Irregular terpene	110-93-0	10.813	1365	-	2.25	1.44	2.49
(3E)-3-Hexenyl acetate	Hydrocarbon ester	3681-82-1	10.863	1315	-	-	7.22	-
(3E)-3-Hexen-1-ol	Hydrocarbon alcohol	928-97-2	11.131	1326	-	-	0.35	-

Compound	Compound class	CAS	RT	RRT	Percentage contribution			
					Gymnospria buxifolia	*Bulbine natalensis*	*Bulbine frutescens*	*Acacia nilotica*
3-Hexenol	Hydrocarbon alcohol	544-12-7	11.138	1345	-	-	-	1.08
(3Z)-3-Hexen-1-ol	Hydrocarbon alcohol	928-96-1	11.183	1335	-	1.39	-	-
2-Butoxyethanol	Hydrocarbon ether	111-76-2	11.444	1407	-	1.23	0.72	3.78
Nonanal	Hydrocarbon aldehyde	124-19-6	11.637	1405	0.16	0.34	0.53	-
Unidentified			11.754		0.03	-	-	-
Unidentified			11.892		0.09	-	-	-
Acetic acid	Hydrocarbon acid	64-19-7	12.029	1468	0.51	4.29	1.08	1.57
Linalool oxide (furanoid)	Monoterpene	5989-33-3	12.127	1423	0.51	-	0.70	-
Linalool oxide (furanoid)	Monoterpene	5989-33-3	12.470	1423	0.10	0.25	0.27	3.74
2-Ethylhexanol	Hydrocarbon alcohol	104-76-7	12.566	1518	-	0.44	0.40	0.61
Decanal	Hydrocarbon aldehyde	112-31-2	13.005	1502	0.14	0.17	-	-
Benzaldehyde	Benzenoid	100-52-7	13.123	1530	0.47	0.93	0.65	1.69

					Percentage contribution			
					Gymnospria buxifolia	***Bulbine natalensis***	***Bulbine frutescens***	***Acacia nilotica***
Compound	**Compound class**	**CAS**	**RT**	**RRT**				
4-Oxoisophorone	Irregular terpene	1125-21-9	15.089	1675	0.48	-	-	-
Phenylethyl Alcohol	Benzenoid	60-12-8	17.364	1929	-	0.11	12.69	-
Phenol	Benzenoid	108-95-2	18.227	2004	-	0.40	0.26	-
Tetracosane		646-31-1	22.681		0.73	-	-	-
Pentacosane		629-99-2	26.224		-	-	-	4.32
Hexadecanoic acid	Hydrocarbon acid	57-10-3	27.164	2880	1.95	-	-	-
9-Hexadecenoic acid	Hydrocarbon acid	2091-29-4	27.724	2957	0.38	-	-	-

Table 2.4. Scent composition and percentage contribution of fruit found to be attractive to mosquitoes. Chemical Abstract Service number = CAS; Retention time = RT; Relative retention time index = RRT.

					Percentage contribution				
Compound	**Compound class**	**CAS**	**RT**	**RRT**	***Psidium guajava***	***Cucumis melo***	***Cucumis melo inodorus***	***Musa acuminata***	***Ficus sur***
Ethanol	Hydrocarbon alcohol	64-17-5	3.320	883	-	-	-	-	14.24
Ethyl Acetate	Hydrocarbon ester	141-78-6	5.722	899	51.52	80.09	7.41	0.67	-
Ethyl propanoate	Hydrocarbon ester	105-37-3	6.324	957	-	-	0.95	-	-
Isobutyl acetate	Hydrocarbon ester	110-19-0	6.915	1017	-	9.53	-	11.73	-
Ethyl butanoate	Hydrocarbon ester	105-54-4	7.112	1028	16.15	-	10.50	-	-
Hexanal	Hydrocarbon aldehyde	66-25-1	7.559	1048	-	-	-	-	5.31
2-Pentyl acetate	Hydrocarbon ester	626-38-0	7.564		-	-	12.93	5.66	-
2-Methybutyl acetate	Hydrocarbon ester	624-41-9	8.127	1191	2.19	9.28	61.86	-	-
Ethylbenzene	Benzenoid	100-41-4	8.171	1111	-	-	-	-	28.77
3-Methylbutyl acetate	Hydrocarbon ester	123-92-2	8.176	1139	-	-	-	43.11	-
Isobutyl butyrate	Hydrocarbon ester	539-90-2	8.768	1152	1.18	-	-	19.67	-
3-Methyl-1-butanol	Hydrocarbon alcohol	123-51-3	8.876	1206	-	1.10	-	-	-

					Percentage contribution				
Compound	Compound class	CAS	RT	RRT	*Psidium guajava*	*Cucumis melo*	*Cucumis melo inodorus*	*Musa acuminata*	*Ficus sur*
Amyl isovalerate	Hydrocarbon ester	25415-62-7	9.272	1303	-	-	0.81	1.72	-
Limonene	Monoterpene	138-86-3	9.359	1207	-	-	-	-	7.96
1-Methylbutyl butyrate	Hydrocarbon ester	60415-61-4	9.488	1216	1.11	-	0.70	0.76	-
Ethyl hexanoate	Hydrocarbon ester	123-66-0	9.588	1223	18.19	-	1.33	-	-
Acetoin	Hydrocarbon ketone	513-86-0	9.925	1259	0.16	-	-	-	13.67
Pentyl butanoate	Hydrocarbon ester	540-18-1	10.081	1304	3.04	-	-	-	-
Isopentyl 2-methylpropanoate	Hydrocarbon ester	# 2050-01-3	10.171	1214	-	-	-	14.13	-
Ethyl 3-hexenoate	Hydrocarbon ester	64187-83-3	10.349	1290	0.34	-	-	-	-
3-Methyl-4-pentenyl acetate	Hydrocarbon ester	71487-16-6	10.546		0.83	-	-	-	-
Isopentyl 3-methylbutanoate	Hydrocarbon ester	659-70-1	10.566	1296	-	-	1.84	2.17	-
Ethyl 2-hydroxypropanoate	Hydrocarbon ester	687-47-8	10.655	1342	-	-	-	-	1.33
1-Hexanol	Hydrocarbon alcohol	111-27-3	10.795	1305	0.41	-	-	0.18	-
6-Methyl-5-hepten-2-one (Sulcatone)	Irregular terpene	110-93-0	10.813	1365	-	-	-	-	2.14

Compound	Compound class	CAS	RT	RRT	Percentage contribution				
					Psidium guajava	***Cucumis melo***	***Cucumis melo inodorus***	***Musa acuminata***	***Ficus sur***
(3Z)-3-Hexen-1-ol	Hydrocarbon alcohol	928-96-1	11.183	1335	0.11	-	0.31	0.11	-
2-Butoxyethanol	Hydrocarbon ether	111-76-2	11.444	1407	-	-	-	-	3.03
Nonanal	Hydrocarbon aldehyde	124-19-6	11.637	1405	0.04	-	0.16	-	1.18
1-Methylhexyl butyrate	Hydrocarbon ester	39026-94-3	11.955	1401	-	-	-	0.05	-
Hexyl butanoate	Hydrocarbon ester	2639-63-6	12.054	1419	-	-	-	0.03	-
Linalool oxide (furanoid)	Monoterpene	5989-33-3	12.127	1423	-	-	-	-	0.77
Ethyl octanoate	Hydrocarbon ester	106-32-1	12.251	1432	0.11	-	-	-	-
2-Ethylhexanol	Hydrocarbon alcohol	104-76-7	12.566	1518	-	-	-	-	1.13
Isopentyl hexanoate	Hydrocarbon ester	2198-61-0	12.680	1464	-	-	-	0.03	-
Decanal	Hydrocarbon aldehyde	112-31-2	13.005	1502	0.07	-	-	-	-
Benzaldehyde	Benzenoid aldehyde	100-52-7	13.123	1530	0.08	-	0.16	-	4.14
Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, cis-	Monoterpene	7299-41-4	13.419	1616	0.05	-	-	-	-
Terpinen-4-ol	Monoterpene alcohol	562-74-3	14.122	1627	0.13	-	-	-	-
Methyl benzoate	Benzenoid ester	93-58-3	14.303	1638	0.09	-	-	-	-

					Percentage contribution				
					Psidium guajava	***Cucumis melo***	***Cucumis melo inodorus***	***Musa acuminata***	***Ficus sur***
Compound	**Compound class**	**CAS**	**RT**	**RRT**					
Ethyl benzoate	Benzenoid	93-89-0	14.832	1651	2.75	-	-	-	-
Unidentified Benzene	Benzenoid	91-16-7	15.359	1706	-	-	-	-	0.35
Benzyl ethanoate	Benzenoid ester	140-11-4	15.466	1738	0.04	-	1.04	-	-
Naphthalene	Aromatic hydrocarbon	91-20-3	15.734	1744	-	-	-	-	0.48
Benzyl alcohol	Benzenoid alcohol	100-51-6	16.994	1823	0.04	-	-	-	0.88
Phenylethyl Alcohol	Benzenoid alcohol	60-12-8	17.364	1929	-	-	-	-	0.76
Phenol	Benzenoid	108-95-2	18.227	2004	-	-	-	-	0.52
3-Phenylpropanol	Benzenoid alcohol	122-97-4	18.750	2058	0.08	-	-	-	-
3-Phenyl-1-propanol, acetate	Benzenoid ester	122-72-5	17.821	1930	0.86	-	-	-	-
3-Phenyl-2-propenal	Benzenoid aldehyde	104-55-2	18.872	2019	0.06	-	-	-	-
Ethyl 3-phenyl-2-propenoate	Benzenoid ester	103-36-6	19.709	2108	0.07	-	-	-	-
Cinnamyl acetate	Phenylpropanoid ester	103-54-8	19.840	2153	0.04	-	-	-	-

2.4. Discussion

The sugar feeding behaviour of mosquitoes has been widely documented and is important for mosquito survival and daily activities (Foster and Takken and references therein, 2004; Jhumur, 2007; Müller et al., 2010). Sugar can be obtained from various fruit and flower sources. Studies such as Müller et al. (2010) observed the attraction of mosquitoes to various fruits found locally. In this study we used fruits which were also reported in Müller et al. (2010) that were found in the vicinity. In addition, we observed mosquito behaviour on flowers which were indigenous to the area. Mosquitoes of different genera had different feeding bouts. Most *Aedes* mosquitoes fed during the observation periods but were active throughout the day, whereas *Culex* mosquitoes mostly fed in the morning or early afternoon periods. These differences are expected as mosquitoes belonging to the genus *Aedes* are active throughout the day, whereas *Culex* mosquitoes are crepuscular (Eldridge, 2008). Initial observations of mosquito sugar feeding showed that mosquitoes were attracted to the flowers of *Gymnosporia buxifolia* and *Acacia nilotica* which are found in the same habitats as the mosquito larvae. It is very likely that flowers of *Gymnosporia buxifolia* and *Acacia* serve as a carbohydrate source for the adults. *Culex* mosquitoes would feed in large groups on flowers or fruits which they preferred (Figure 2.1). It has been already reported that *Culex* mosquitoes have a greater demand for carbohydrate sources than *Aedes* (Eldridge, 2008) and these behavioural differences in sugar feeding were confirmed by the choice tests of the current study.

Mosquitoes were highly attracted to the flowers of *Gymnosporia buxifolia* and *Acacia nilotica* (Figure 2.3, Figure 2.5) which are widely distributed throughout South Africa and surrounding countries (Behr, 2005; Khan and Ndlovu, 2015). *Gymnosporia buxifolia* was just as attractive as *Acacia nilotica*. However, we observed that mosquitoes were feeding for a longer periods on the flowers of *Gymnosporia buxifolia* than *Acacia nilotica*. *Gymnosporia buxifolia* has a sickly-sweet scent and is generally grown as a hedge, for traditional medicine, as tool handles and is known to attract various types of insects (Khan and Ndlovu, 2015).

Mosquitoes were not as attracted to *Bulbine natalensis* and *Bulbine frutescens* (Figure 2.3, Figure 2.5), which could be due to the small flowers which may not produce enough nectar resources. However, our results show that these two plants could be used as an additional food source if necessary. *Musa acuminata* and *Ficus sur* were among those fruit which were the most attractive to mosquitoes (Figure 2.4, Figure 2.6). Mosquitoes would pierce through the skin if present, which is how the fruit would naturally occur. These fruits are available throughout South Africa and serve as food not only for humans and other animals but for a large number of insects, such as mosquitoes. Mosquitoes fed more on these fruits than the guava (*Psidium guajava*), spanspek (*Cucumis melo*), and honey melon (*Cucumis melo inodorus*) (Figure 2.4, Figure 2.6). Mosquitoes did not respond well to these fruits, possibly due to their scent profiles or even the skin of *Cucumis* species as it is very thick and hard to pierce. Fruits and flowers found to be attractive that are locally occurring provides an understanding of the carbohydrate sources used by the mosquito genera found in the area. Some of the fruits and flowers have been tested in previous experiments showing various levels of attractiveness (Müller et al., 2010). However, a better look at the scent composition of the fruit and flowers could provide insight into the compounds responsible for attracting or deterring the mosquitoes.

The scent profiles of the flowers revealed that ethylbenzene and hydrocarbon alcohols were the most dominant scent compounds found in *Gymnosporia buxifolia* and *Acacia nilotica*. In many instances, mosquitoes are said to be more attracted to human beings when consuming alcoholic beverages (Shirai et al., 2002). There may be a link between alcohol and what mosquitoes find attractive, therefore further testing of such compounds is necessary. *Bulbine natalensis* and *Bulbine frutescens* had similar scent profiles also containing ethylbenzene and hydrocarbon alcohols, however were not as attractive. This could be due to the amount of scent or presence of other scent compounds. The scent profiles of the fruit were mostly made up of benzenoids and hydrocarbon esters. In *Musa acuminata* hydrocarbon esters were the dominant compounds. However, in *Ficus sur* the scent compounds were similar to those emitted from the flowers, which were ethylbenzene and ethanol. These scent compounds could be attractive to mosquitoes. Further testing, e.g. in bioassays or field trials, are however, needed to confirm their role as attractants. It is likely that some of the compounds that were only in small relative amounts in the scent were also attractive to mosquitoes. The role of one of the scent compounds, 6-Methyl-5-hepten-2-one (sulcatone), has been discussed more recently. It has been suggested to use sulcatone as a repellent, e.g. in combination with other repellent compounds such as DEET (Pickett et al., 2007) because sulcatone was one of the compounds found in skin odours of humans that are unattractive to mosquitoes (Logan et al., 2010). However, results of other studies suggest that sulcatone in small quantities could also be attractive to mosquitoes (see Logan et al., 2010 and references therein). Thus, the response of mosquitoes to this compound might be concentration dependent.

Scent profiles of attractive fruits and flowers could provide information on the exact scent or blend thereof (responsible for the attraction) which could be used in field applications. These scent compounds are useful as they could be used in attractive toxic sugar bait stations (ATSB). ATSB uses a scent lure which mosquitoes find attractive encouraging the feeding of mosquitoes on the sugar bait (generally a sucrose solution) laced with a toxic substance which would kill the mosquito after ingestion (Lothrop et al., 2012; Müller et al., 2008; Müller and Schlein, 2006). Jhumur (2007) found that mosquitoes which had been conditioned (or exposed to volatiles) responded to volatiles much more efficiently than those which are naïve. This has implications for the use of scents derived from known carbohydrate sources, allowing mosquitoes to locate or respond to these scents much more efficiently as they are able to recognize these from their food source. One advantage is that scent compounds could be used rather than actual fruits and flowers as they are perishable (scent compounds last longer). Further work should include single compound choice tests, as well as coupled gas-chromatography-electroantennography (GC-EAG) in order to determine exactly what compounds mosquitoes are responding to. These compounds could be then used in field trials to determine how effective they are as lures to control mosquito populations. Methods of controlling mosquitoes (which have been captured) could include treatments of insecticides or the introduction of disease or the use of these scents in sugar bait stations. The application of these scents in sugar bait stations has promising prospects in rural and poor areas which are plagued by mosquito diseases.

2.5. Conclusions

Mosquitoes prefer certain fruits and flowers over others. *Gymnosporia buxifolia* and *Acacia nilotica* were the most attractive flowers in this study. *Musa acuminata* and *Ficus sur* were the most attractive fruits in this study. The flower of *Gymnosporia buxifolia* has a very sickly, sweet scent and was highly attractive to mosquitoes and should be looked at in greater detail in the future. The scent profiles of the investigated species provide some insight into the scent compounds emitted by flowers and fruits. Based on these results the role of single scent compounds or mixtures of scent compounds could be tested to produce an artificial blend that can be used in mosquito traps, similar to the compound mixtures based on human skin odour (e.g. Bosch et al., 2000, Dekker et al., 2002; Geier et al., 2004).

2.6. References

Alto, B.W., Lounibos, L. P., Mores, C. N., Reiskind, M. H., 2008. Larval competition alters susceptibility of adult *Aedes* mosquitoes to dengue infection. Proceedings of the Royal Society B 275, 463-471.

Amirav, A., Dagan, S., 1997. A direct sample introduction device for mass spectrometry studies and gas chromatography mass spectrometry analyses. European Journal for Mass Spectrometry. 3, 105-111.

Behr, K., 2005. Pretoria National Botanical Garden, SANBI.http://www.plantzafrica.com/plantab/acacianilot.htm. Date Accessed: 10 May 2016.

Bosch, J.O., Geier, M., Boeckh, J., 2000. Contribution of fatty acids to olfactory host finding of female *Aedes aegypti*. Chemical Senses 25, 323-330.

Dekker, T., Cardé, R., Geier, M., 2002. L-lactic acid: a human-signifying host cue for the anthropophilic mosquito *Anopheles gambiae*. Medical and Veterinary Entomology 16, 91-98.

Eldridge, B. F., 2008. Biology and control of mosquitoes in California. Vector-Borne Disease Section. Center for Infectious Diseases California.

Foster, W. A., Takken, W., 2004. Nectar-related vs. human-related volatiles: behavioural response and choice by female and male *Anopheles gambiae* (Diptera: Culicidae) between emergence and first feeding. Bulletin of Entomological Research 94, 145-157.

Geier, M., Rose A. & Eiras Á.E., 2004. A new lure for host-seeking anthropophilic mosquitoes and a novel type of a simple, non-CO_2 mosquito trap. International Journal of Medical Microbiology 293, 38-50.

Gubler, D. J., 2002. The global emergence/resurgence of arboviral diseases as public health problems. Archives of Medical Research 33, 330-342.

Jhumur, U. S., 2007. *Silene otites* (Caryophyllaceae): Attraction of nectar-seeking mosquitoes to inflorescence odours, and temporal variation of flower scent and flower visitors. Journal of Chemical Ecology, 42-76.

Jhumur, U. S., Dötterl, S., Jürgens, A., 2008. Floral odors of *Silene otites*: their variability and attractiveness to mosquitoes 34, 14-52.

Jürgens, A., Witt, T., Gottsberger, G., 2002. Flower scent composition in night-flowering *Silene* species (Caryophyllaceae). Biochemical Systematics and Ecology 30, 383-397.

Khan, S., Ndlovu, B., 2015. KwaZulu-Natal National Botanical Garden, SANBI. http://pza.sanbi.org/gymnosporia-buxifolia. Date Accessed: 10 May 2016.

Kovendan, K., Arivoli, S., Maheshwaran, R., Baskar, K., Vincent, S., 2012. Larvicidal efficacy of *Sphaeranthus indicus*, *Cleistanthus collinus* and *Murraya koenigii* leaf extracts against filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitology Research 111, 1025-1035.

Logan, J. G., Stanczyk, N. M., Hassanali, A., Kemei, J., Santana, A. E. G., Ribeiro, K. A. L., Pickett, J. A., Mordue (Luntz), A. J., 2010. Arm-in-cage testing of natural human-derived mosquito repellents. Malaria Journal 9, 239 – 248.

Lothrop, H. D., Wheeler, S. S., Fang, Y., Reisen, W. K., 2012. Use of scented sugar bait stations to track mosquito-borne arbovirus transmission in California. Journal of Medical Entomology 49, 1466-1472.

Martínez-de la Puente, J., Muñoz, J, Capelli, G., Montarsi, F., Soriguer, R., Arnoldi, D., Rizzoli, A., Figuerola, J., 2015.Avian malaria parasites in the last supper: identifying encounters between parasites and the invasive Asian tiger mosquito and native mosquito species in Italy. Malaria Journal 14, 32-38.

Müller, G. C., Beier, J. C., Traore, S. F., Toure, M. B., Traore, M. M., Bah, S., Doumbia, S., Schlein, Y., 2010. Field experiments of *Anopheles gambiae* attraction to local fruits/seedpods and flowering plants in Mali to optimize strategies for malaria vector control in Africa using attractive toxic sugar bait methods. Malaria Journal 9, 1-11.

Müller, G. C., Kravchenko, V. D., Schlein, Y., 2008. Decline of *Anopheles sergentii* and *Aedes caspius* populations following presentation of attractive toxic (Spinosad) sugar bait stations in an oasis. Journal of the American Mosquito Control Association 24, 147-149.

Müller, G. C., Schlein, Y., 2006. Sugar questing mosquitoes in arid areas gather on scarce blossoms that can be used for control. International Journal for Parasitology 36, 1077-1080.

Otienoburu, P. E., Ebrahimi, B., Phelan, P. L., Foster, W. A., 2012. Analysis and optimization of a synthetic milkweed floral attractant for mosquitoes. Journal of Chemical Ecology 38, 873-881.

Pickett, J.A., Logan, J.G., Birkett, M.A., Mordue, A.J., 2007. Pest repellent comprising geranylacetone. Patent WO2007099347 A2, issued date September 7, 2007.

Shirai, Y., Tsuda, T., Kitagawa, A., Naitoh, K., Seki, T., Kamimura, K., Morohashi, M., 2002. Alcohol ingestion stimulates mosquito attraction. Journal of the American Mosquito Control Association 18, 91 – 96.

WHO, 2010. Rift Valley Fever, Fact Sheet. http://www.who.int/mediacentre/factsheets/fs207/en/ Date accessed: 30 June 2015.

WHO, 2014a. Yellow fever, Fact Sheet. http://www.who.int/mediacentre/factsheets/fs100/en/ Date accessed: 04 April 2014.

WHO, 2014b. West Nile Virus, Fact Sheet. http://www.who.int/mediacentre/factsheets/fs354/en/ Date accessed: 30 June 2015.

Chapter 3

Using floral scent as a mosquito attractant

Abstract

Mosquitoes are vectors of pathogens such as malaria and other arboviral diseases. During the blood-sucking process, female mosquitoes transmit these diseases. However, sugar feeding by female mosquitoes may occur prior to and also between blood meals. Female mosquitoes are attracted to floral scents because they drink nectar (carbohydrate sources) during the early part of the night. It has therefore been suggested that luring mosquitoes with floral volatiles during the earlier part of the night might be a strategy for monitoring mosquito populations and also for preventing disease transmission. The potential of floral volatiles has been successfully established in several laboratory assays; however synthetic mixtures of floral scent compounds have not been tested in field experiments. Therefore, the aim of this study was to use the existing information on floral scent compounds that attract nectar seeking mosquitoes and to validate their effectiveness in field experiments. To evaluate the effectiveness of floral scent compounds, field trials were conducted with two olfactory lures: (1) a combination of three floral attractants (phenylacetaldehyde, 2- phenyl ethanol and linalool oxide), and (2) a commercially available lure (Sweetscent™; Biogents). Unscented paraffin oil was used as a control. The floral scent mix trapped significantly higher numbers of mosquitoes than the Biogents Sweetscent™ and the control. Although more female mosquitoes were captured in comparison to males, a significantly higher number of females devoid of a blood meal were trapped using both scent treatments. Furthermore, *Aedes* and *Culex* were the only two genera of mosquito trapped. The data suggest that floral scents can serve as potent attractants to trap females before they take up a blood meal, thus it has the potential for reducing the rate of disease transmission Further studies are needed to test whether this method can be scaled up in areas where mosquitoes are disease vectors. Cost effective lures can be developed using the floral compounds used in this study to reduce the incidence and spread of fatal disease.

Keywords: Mosquitoes, Disease transmission, Floral scent, In-field experiments, Research mosquito traps, *Aedes*, *Culex*

3.1. Introduction

Mosquitoes comprise a diverse group of approximately 3400 species, belonging to the family Culicidae in the order Diptera (Backer, 1989). After emergence, young males and females focus mainly on carbohydrate sources such as floral nectar as an energy source to carry out daily functioning and processes (Lothrop et al., 2012; Müller et al., 2010c; Otienoburu et al., 2012). After mating, mature females prefer mostly blood meals, which provide a source of protein for egg development (see Foster and Takken, 2004 and reference therein; Bond et al., 2005; Müller et al., 2008; Müller et al., 2010a; Xue et al., 2008). Mosquitoes obtain blood from a wide range of hosts, including humans and animals, and they contribute significantly as vectors of lymphatic filariasis, malaria and other arboviral diseases such as Dengue, Rift Valley Fever and West Nile Fever (Müller et al., 2010a; Xue et al., 2008). As a result, extensive research has been initiated to develop methods of monitoring and controlling mosquito populations (Jhumur, 2007; Jupp, 2005; Lothrop et al., 2012; Müller et al., 2008; Otienoburu et al., 2012).

The remarkable olfactory senses of mosquitoes allow them to detect hosts from 25-30 metres away (Jhumur, 2007). Using olfactory cues has been the focus of many studies in order to find ways to monitor or control mosquito populations. *Acacia* species, African fig, guava and sugar cane are some of the plants which been reported to attract mosquito populations in search of a carbohydrate source (Müller et al., 2008; Müller et al., 2010b). Scent profiles of the fruits and flowers which mosquitoes have been attracted to have been analyzed, and particular compounds have been shown to be potent mosquito attractants in wind-tunnel assays (Jhumur, 2007). Scent profiles of *Silene otitis* (family Caryophyllaceae), which is one of the few flowering plant species that is actually pollinated by nectar-seeking mosquitoes (Jhumur, 2007 and references therein), was analyzed. The scent profile comprised phenylacetaldehyde (most dominant) followed by lilac aldehyde, (Z)-3-hexenyl acetate, linalool oxide lilac and hexanol among many other compounds (Jhumur, 2007; Jürgens et al., 2002). It was also shown, using gas chromatographic–electroantennographic detection (GC-EAD), that mosquitoes are more sensitive to some compounds than to others (Jhumur, 2007).

Strategies to monitor and control mosquito populations initially focused on the attraction of mosquitoes to sweet scents (carbohydrate sources) and human volatiles (blood hosts). According to Foster and Takken (2004), female mosquitoes showed a strong preference to nectar related sources at juvenile stages and prior to blood meals. More recently, studies have focused on the response and attraction of mosquitoes to floral and fruit volatiles. Müller et al. (2008) used sugar bait stations laced with an attractive toxic solution (Spinosad) as a lethal attractant able to efficiently reduce the number of female mosquitoes. In addition, Lothrop et al. (2012) tested specific scent compounds, which were highly attractive in previous studies (Müller et al., 2010b; Schlein, and Müller, 2008), on mosquitoes both in the field and laboratory (incorporating sugar bait stations) showing positive results. However, to our knowledge identified floral and fruit volatiles have not been tested in situ. So far fruit and floral volatiles are still used in a "black-box" approach not knowing which compounds are responsible for attracting mosquitoes. The aim of this study was therefore to use the existing information on scent compounds that attract sugar seeking mosquitoes and to monitor their effectiveness as mosquito attractants in assays. The objectives of this study are: (i) to compare the attractiveness of the experimental sweet scent to a commercial product; (ii) to analyze the sex ratios of the attracted mosquitoes; (iii) to assess how many females have had a blood meal prior to being captured, and (iv) to assess the genera of trapped mosquitoes. The study will contribute to the development of effective control methods to curb the density of mosquito populations that transmit vector-borne diseases.

3.2. Materials and Methods

3.2.1. **Study site**

The study was conducted at the University of KwaZulu-Natal Botanical gardens (29°37′S 30°23'E) in South Africa. Three sites where chosen at this location (near a pond, a small stream and under a fig tree).

3.2.2. **Study organisms**

The genera of trapped mosquitoes were characterized according to the mosquito identification key in Snow (1990). The sex ratio of each mosquito was determined using a stereo microscope. Males have feathery antennae, while those of the females appear smoother (Göpfert et al., 1999). The swollen red abdomen in female mosquitoes is characteristic of a blood meal.

3.2.3. **Experimental design**

Experimentation was conducted during the summer periods from March 2013 to January 2015. Field trials were conducted using nine research mosquito traps (BG-Sentinel: Biogents, Regensburg, Germany), three traps were placed at each location (Figure 1). Three treatments were tested. The first treatment was a mixture of three floral scent compounds (phenylacetaldehyde, 2-phenyl ethanol and linalool oxide; mixture of different isomers, Sigma Aldrich) in a 10% v/v dilution in unscented paraffin oil); the second treatment was a commercially available lure (Sweetscent™; Biogents, Germany) and the third was unscented paraffin oil which served as a control.

Figure 3.1. Biogents mosquito research traps.

Phenylacetaldehyde, 2-phenyl ethanol and linalool oxide were selected for this study as they have been found in night-flowering species (including *S. otitis*) known to attract mosquitoes. The Biogents Sweetscent™ is a three component mix including lactic acid, an attractant emitted from human skin (Crepeau et al., 2013). The Sweetscent™ volatile mix is used in a permeable dispenser to guarantee an "optimally timed release of the scent into the surrounding area for up to 2 months" (see Crepeau et al., 2013 and references therein).

At each site, three traps were set up in a triangular orientation (approximately 2-3m apart) - one for each scent treatment was used (Figure 3.1). The scent compounds (1 ml floral scent mix in paraffin oil and Biogents Sweetscent™) were placed in the traps on site. Each trial was run for five days; captured mosquitoes and by catch were placed in petri dishes, dated and then stored in the freezer for further identification and analysis. After mosquitoes and by-catch were removed the traps were randomly rearranged in the triangular orientation. Scent treatments were replaced once a week during each trail. A total of 26 trials were conducted.

3.2.4. **Statistical analysis**

Data were analyzed using MS Excel 2007 and the statistical program SPSS version 23 (IBM SPSS Statistics 2011). To measure the independent and interactive effects count data from all mosquito traps were analyzed using generalized linear models (GLM) and generalized estimating equations (GEE) in conjunction with a negative binomial sampling distribution and log link function (IBM SPSS Statistics 2011). The effect of two different lures (Sweetscent™; Biogents; versus Floral scent; and a control with paraffin oil only) on mosquito capture was analyzed with likelihood-ratio (type 3) tests of generalized linear models (McCullagh and Nelder 1989).

Differences between the treatments used to trap male and female mosquitoes, females which had a blood meal prior to capture or not, and mosquito genera were tested in the same way. In addition, GLMs were performed for the dominant insect orders that were found during the trials.

3.3. Results

Eleven different insect orders were found (Blattodea, Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, Odonata, Orthoptera, Phasmatodea and Thysanoptera). In addition, Arachnids were also found during the study. A total of 7124 insects were trapped: the control (1803), Biogents Sweetscent™ (2668) and Floral scent (phenylacetaldehyde, 2-phenyl ethanol and linalool oxide) (2653). The dominant orders attracted by the control (paraffin oil) were Diptera (excluding mosquitoes) (55.3%) followed by Hymenoptera (21.5%) and Hemiptera (10.6%) (Table 3.1). The most dominant orders attracted by the Biogents Sweetscent™ were Diptera (53.4%), Hymenoptera (21.6%) and Hemiptera (12.7%) (Table 3.1). Similarly, the Floral scent treatment comprised Diptera (58.3%), Hymenoptera (19.6%), and Hemiptera (10.9%) (Table 3.1).

A total of 3291 mosquitoes were trapped during the study (Table 3.2). Most of the mosquitoes trapped using the unscented paraffin oil treatment were females which did not have a blood meal (80.1%), followed by male mosquitoes which contributed 19.3% to the total number and the least number of mosquitoes found were females which had a blood meal (0.6%) (Table 3.2). Similar results were obtained for the Biogents Sweetscent™ treatment with 77.9% females that showed no indication of a previous blood meal; 21.2% males; and 0.9% females that had a blood meal. The experimental floral scent treatment was very similar with 78.7% females with no indication of a blood meal; 20.7% males; and 0.6% females with a previous blood meal. The most prevalent mosquito genera were *Culex* (66.5%), followed by *Aedes* (33.5%) (Table 3.2). Additional data are provided in the supplementary material (Appendix A).

A significantly higher number of mosquitoes were trapped using the Floral scent and Biogents Sweetscent™ in comparison to the control ($\chi^2_{2,\ 234}$= 84.9, p = 0.001) (Figure 3.2). Significantly more mosquitoes were trapped using the Floral scent than the Biogents Sweetscent™ ($p_{2,\ 234}$< 0.001) (Figure 3.2). Furthermore, females mosquitoes which did not have a blood meal were more abundant in the Floral scent and Biogents Sweetscent™ treatments than the control, both of which were significantly different from the control ($\chi^2_{2,234}$= 179.2, p= 0.001) (Figure 3.3). Significantly more females (which did not have a blood meal) were attracted to the Floral scent than the Biogents Sweetscent™ ($p_{2,\ 234}$< 0.001) (Figure 3.3). The Biogents Sweetscent™ and experimental floral scent trapped a significantly higher average number of female mosquitoes (with a blood meal) than the control ($p_{2,\ 234}$ = 0.041) but did not significantly differ from one another ($p_{2,\ 234}$ = 0.221)(Figure 3.4). For both, the floral scent and Biogents Sweetscent™ significantly higher average numbers of male mosquitoes were trapped than in the control traps ($\chi^2_{2,234}$ = 36.1, p= 0.001) (Figure 3.5). No significant difference in males trapped was observed between the floral scent and Biogents Sweetscent™ ($p_{2,\ 234}$ = 0.272) (Figure 3.5).

Significantly more females (both with and without a blood meal) were caught than males ($\chi^2_{1,\ 468}$ = 41.1, p = 0.001) (Table 3.2, Figure 3.5). The gender of mosquitoes trapped was significantly different for each treatment ($\chi^2_{2,\ 234}$ = 50.1, p = 0.001) (Figure 3.5). There was no interaction between the treatments and gender of trapped mosquitoes ($p_{2,\ 234}$ = 0.098) (Figure 3.5). There were significantly more *Culex* mosquitoes caught than *Aedes* ($\chi^2_{1,\ 468}$ = 196.1, p = 0.001) (Table 3.2, Figure 3.6). The average number of mosquitoes for each genus was significantly different for each treatment ($\chi^2_{2, 234}$ = 194.7, p = 0.001) (Figure 3.6). The two mosquito genera shared a preference for the same treatments ($\chi^2_{2, 234}$ = 31.98, p = 0.001) (Figure 3.6).

Diptera (excluding mosquitoes) was the dominant order of insects found in the traps, followed by Hymenoptera (Table 3.1). There were significantly more Diptera caught when using the floral scent treatment than the control ($p_{2, 234}$ = 0.001) (Figure 3.7). No significant difference was observed between the control and Biogents Sweetscent™ ($p_{2, 234}$ = 0.097) (Figure 3.7). No significant difference was observed between the Biogents Sweetscent™ and the floral scent ($p_{2, 234}$ = 0.451) (Figure 3.7). Both the Biogents Sweetscent™ and floral scent mixture trapped a higher number of hymenopterans than the control ($\chi^2_{2, 234}$= 11.99, p = 0.002) (Figure 3.8). No significant difference in the number of Hymenoptera trapped was observed between the Biogents Sweetscent™ and the floral scent mixture (Figure 3.8).

Table 3.1. Total number of individuals trapped (N) and percentage contribution of insect orders trapped with Biogents mosquito traps and different types of lure (Control= paraffin oil), Biogents Sweetscent™, and Floral scent). The Floral scent compounds consisted of phenylacetaldehyde, 2-phenyl ethanol and linalool oxide. Bla = Blattodea, Col = Coleoptera, Dip = Diptera, Hem = Hemiptera, Hym = Hymenoptera, Lep = Lepidoptera, Neu = Neuroptera, Odo = Odonata, Ort = Orthoptera, and Thy = Thysanoptera.

		Percentage contribution									
	N	**Bla**	**Col**	**Dip**	**Hem**	**Hym**	**Lep**	**Neu**	**Odo**	**Ort**	**Thy**
Control	1803	0.2	4.9	55.3	10.6	21.5	6.2	0.2	0.6	0.2	0.3
Biogents Sweetscent™	2668	0.1	4.6	53.4	12.7	21.6	6.5	0.2	0.5	0.0	0.3
Floral scent	2653	0.3	4.0	58.3	10.9	19.6	5.6	0.2	1.1	0.0	0.0
Total	7124	0.2	4.4	55.7	11.5	20.8	6.1	0.2	0.7	0.1	0.2

*Order Diptera numbers are exclusive of mosquitoes

Table 3.2. Total number of individuals trapped (N) and percentage contribution of mosquitoes trapped with Biogents mosquito traps and different types of lure (Control= paraffin oil, Biogents Sweetscent™, and Floral scent). The Floral scent compounds consisted of phenylacetaldehyde, 2-phenyl ethanol and linalool oxide.

		Percentage contribution				
	N	**Female**		**Male**	**Genera**	
		Blood meal	**No blood meal**		***Aedes***	***Culex***
Control	720	0.6	80.1	19.3	37.2	62.8
Sweetscent™	1173	0.9	77.9	21.2	33.1	66.9
Floral scent	1398	0.6	78.7	20.7	31.9	68.1
Total	3291	0.7	78.7	20.6	33.5	66.5

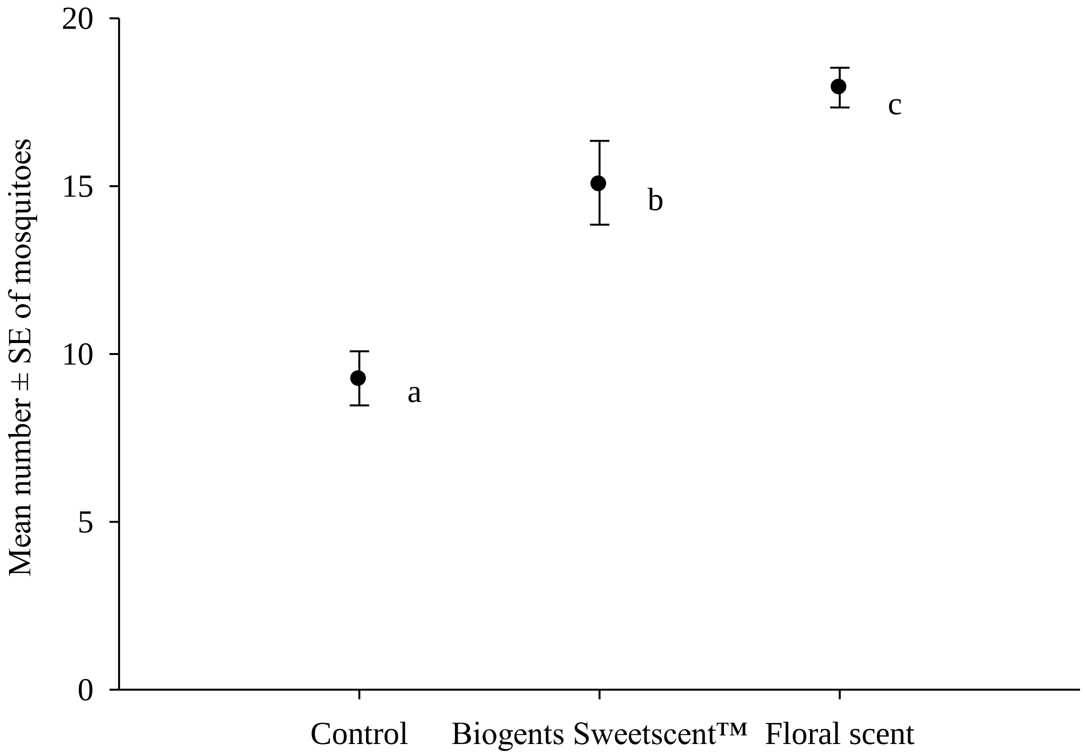

Figure 3.2. The effect of different types of lure (Control (paraffin oil), Biogents Sweetscent™, and Floral scent) on trapping mosquitoes ($\chi^2_{2, 234}$ = 84.9, p = 0.001). Different letters indicate significant differences between scent treatments.

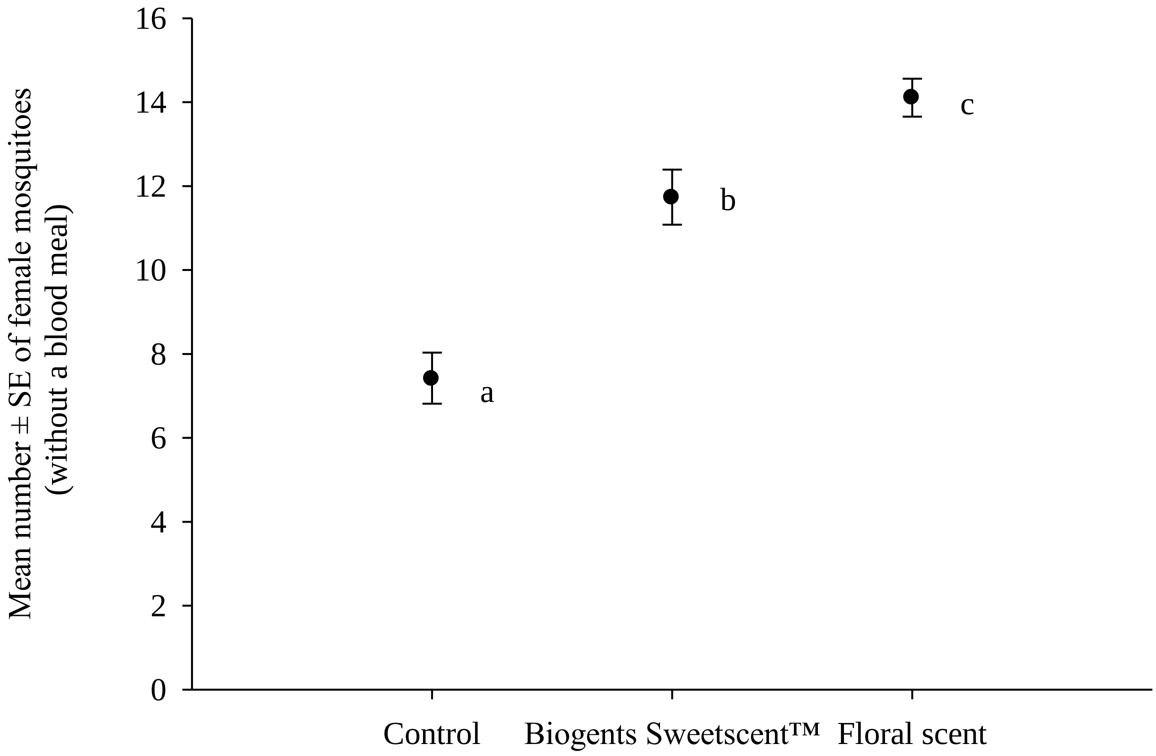

Figure 3.3. The effect of different types of lure (Control = paraffin oil, Biogents Sweetscent™, and Floral scent) on trapping female mosquitoes (without a blood meal) ($\chi^2_{2, 234}$= 179.2, p = 0.001). Means that share letters are not significantly different.

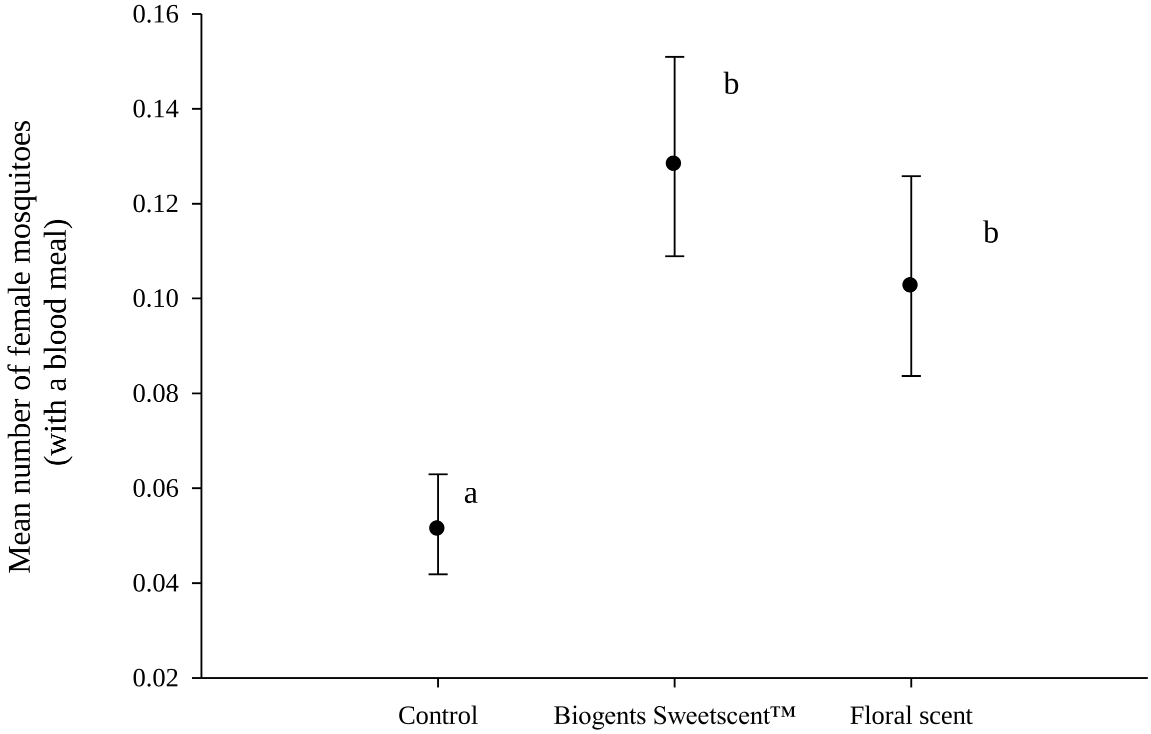

Figure 3.4. The effect of different types of lure (Control =paraffin oil, Biogents Sweetscent™, and Floral scent) on trapping female mosquitoes (with a blood meal) ($\chi^2_{2,\,234}$ = 6.4, p = 0.041). Means that share letters are not significantly different.

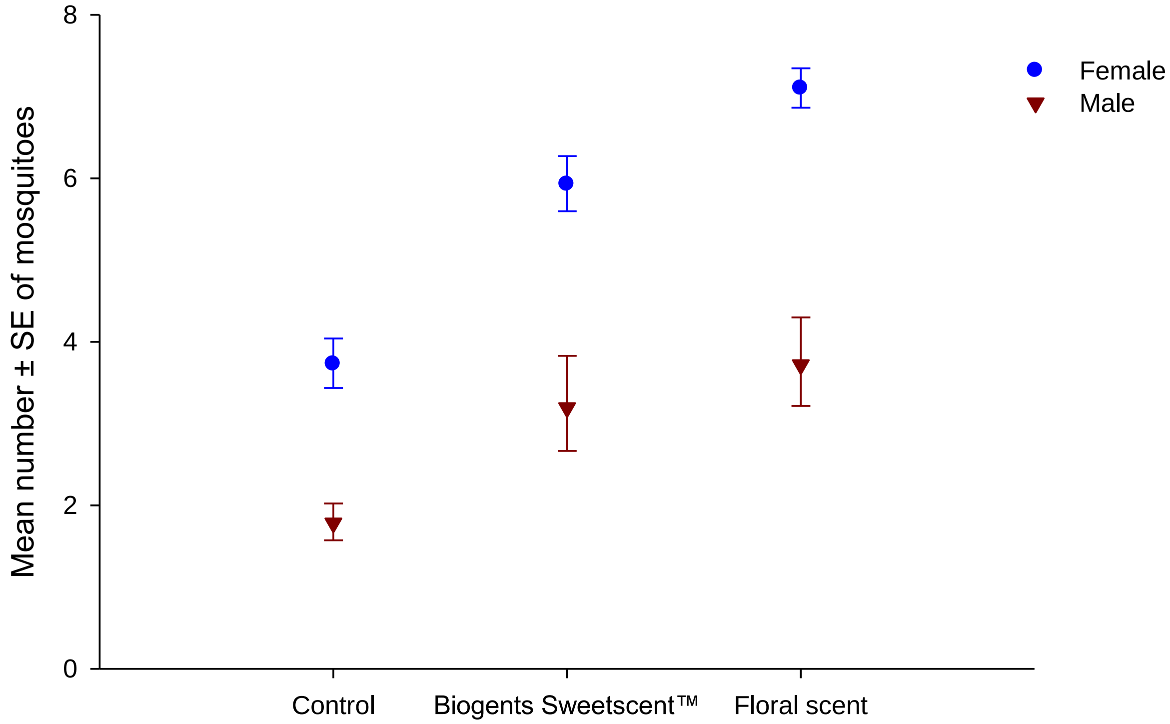

Figure 3.5. The effect of different types of lure (Control = paraffin oil, Biogents Sweetscent™, and Floral scent) on trapping male and female mosquitoes ($\chi^2_{1, 468}$ = 41.1, p = 0.001) and per treatment ($\chi^2_{2, 234}$ = 50.1, p = 0.001). No interaction effect was observed ($\chi^2_{2, 234}$ = 4.7, p = 0.098).

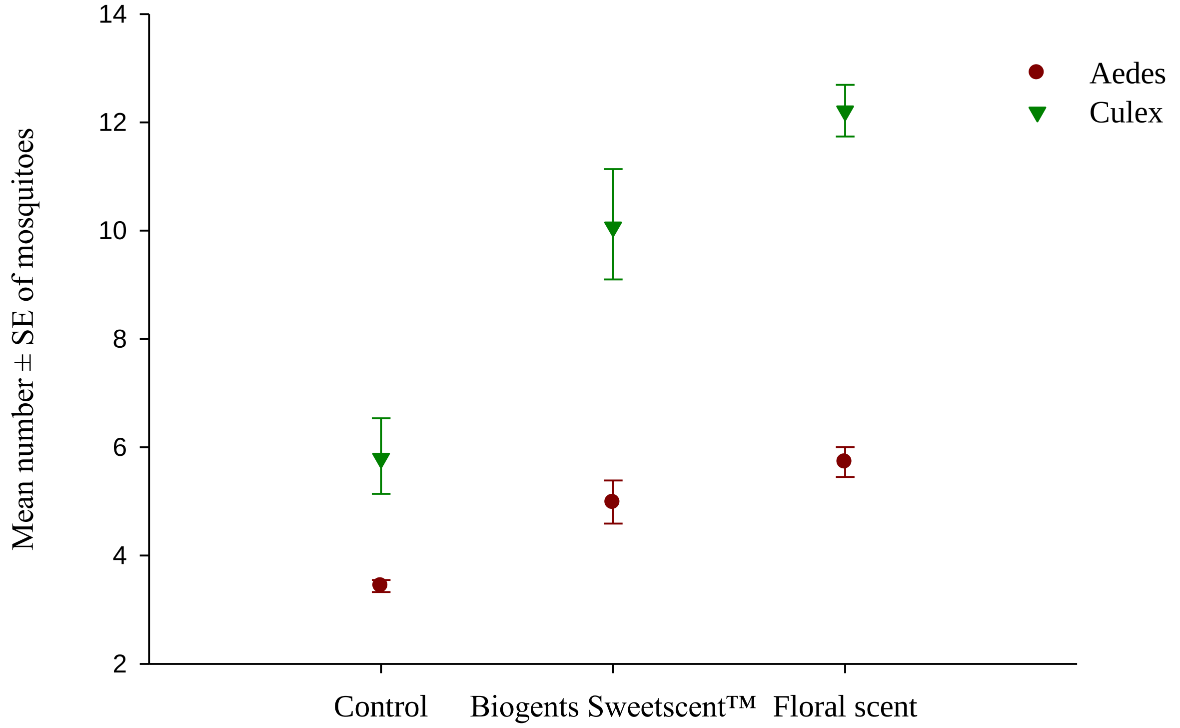

Figure 3.6. The effect of different types of lure (Control (paraffin oil), Biogents Sweetscent™, and Floral scent) on trapping mosquitoes of the two genera, *Culex* and *Aedes*, in general ($\chi^2_{1,\ 468}$ = 196.1, p = 0.001) and per treatment ($\chi^2_{2,\ 234}$ = 194.7, p = 0.001). An interaction effect was observed ($\chi^2_{2,\ 234}$ = 31.98, p = 0.001).

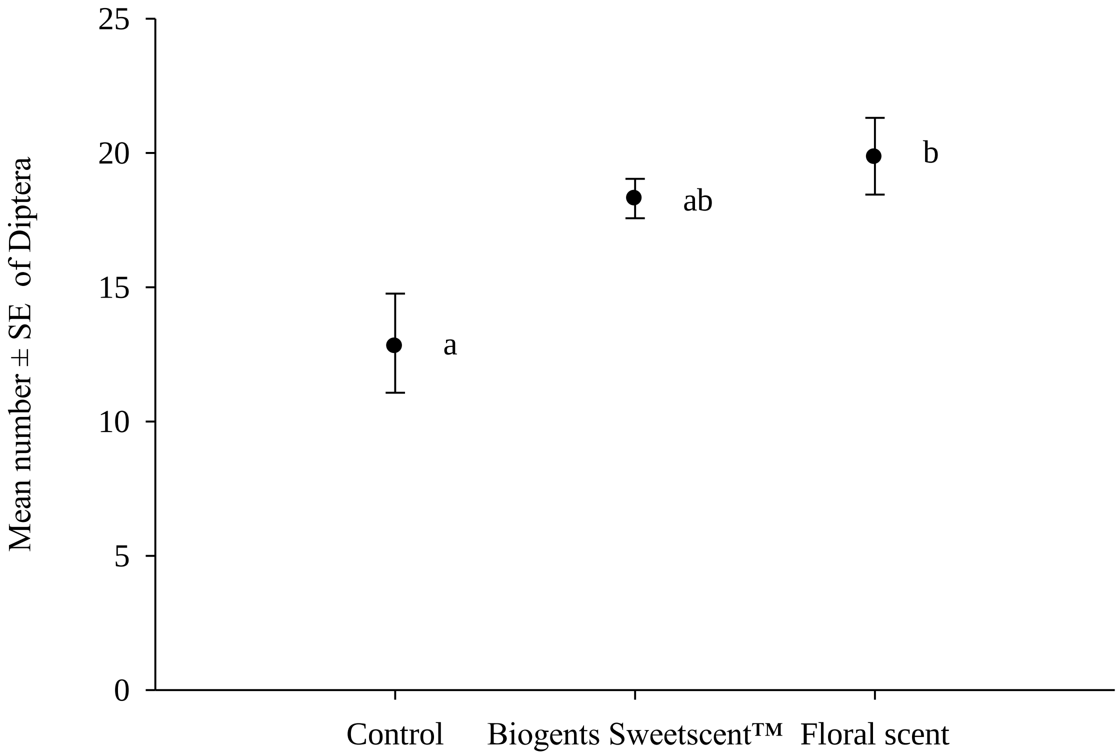

Figure 3.7. The effect of different types of lure (Control (paraffin oil), Biogents Sweetscent™, and a mixture of three floral scent compounds) on trapping Diptera (excluding mosquitoes) ($\chi^2_{2,\ 234}$ = 1056.5, p = 0.001). Means that share letters are not significantly different.

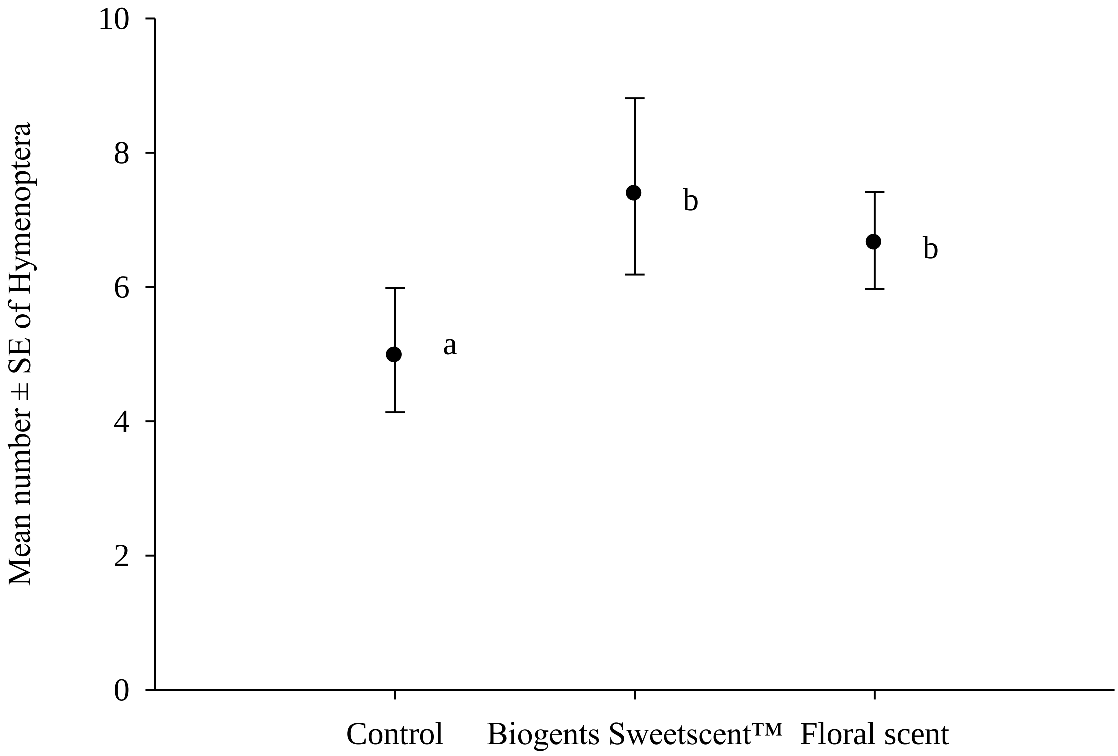

Figure 3.8. The effect of different types of lure (Control (paraffin oil), Biogents Sweetscent™, and a mixture of three floral scent compounds) on trapping Hymenoptera ($\chi^2_{2, 234}$= 11.99, p = 0.002). Means that share letters are not significantly different.

3.4. Discussion

Phenyl acetaldehyde, 2-phenyl ethanol and linalool oxide were identified in previous studies as highly effective mosquito attractants in laboratory assays (Jhumur, 2007). Jhumur (2007) reported the response of mosquitoes to individual scent compounds. However, in our study the three scent compounds were combined as a treatment to imitate a more complex floral scent mixture while at the same time combining known mosquito attractants. Therefore, a combination of these scent compounds may further improve the formula of commercially available lures. Although specifically designed mosquito research traps (Crepeau et al., 2013) were used during the field trials, eleven insect orders were captured despite their specificity. The most dominant orders found as by-catch were Diptera (excluding mosquitoes), Hymenoptera and Hemiptera (found in all treatments) (Table 3.1). It is known that certain species of Hymenoptera (e.g. bees) and Hemiptera are attracted to floral scents (Bosch and Kemp, 2002). Some Lepidoptera (e.g. noctuid moths) are attracted to sweet scents such as phenylacetaldehyde (Eby et al., 2013). Odonata, Orthoptera, Phasmatodea and Thysanoptera were also trapped in relatively small numbers (Table 3.1). Dragon flies and damsel flies (Odonata) were mostly found at the site near the pond.

The experimental floral scent mixture and Biogents Sweetscent™ treatments were effective mosquito lures, with the floral scent trapping significantly more mosquitoes (Figure 3.2). This suggests that the potential of floral scent compounds for attracting mosquitoes can be adopted to monitor or control mosquito populations. More females than males were captured which plays a significant role in vector-borne disease management (Table 3.2, Figure 3.5). Generally, female mosquitoes are in search of nectar related sources (carbohydrates) prior to protein sources (Foster and Takken, 2004). Most female mosquitoes captured had partially distended abdomens indicative of nectar feeding which suggests that they were in search of a carbohydrate source. This might explain why more females were captured when using floral scent compounds. The effect of different treatments on the gender of mosquitoes caught was significant; a higher average number of female mosquitoes were found for both treatments (Figure 3.5). Since female mosquitoes are responsible for transmitting diseases our results suggest that floral scent lures might be deployed for attracting disease-carrying female mosquitoes prior to their infectious phase. Moreover, male mosquitoes were also captured during the trials, which is also necessary to reduce the overall population density of mosquitoes in a particular area (Müller et al., 2008).

During the study, two genera of mosquito were found, .i.e. *Aedes* and *Culex* (Table 3.1, 3.2; Figure 3.6). Some mosquitoes of the genus *Aedes* have invaded America, Africa and Europe and transmit diseases such as Dengue, Rift Valley fever and Yellow fever (Alto et al., 2015; Martínez-de la Puente et al., 2015; WHO, 2010; WHO, 2014a). Mosquitoes belonging to the genus *Culex* transmit diseases such as the West Nile virus, filariasis, Japanese encephalitis and avian malaria (Gubler, 2002; Kovendan et al., 2012; Martínez-de la Puente et al., 2015; WHO, 2014b). The ability to capture these genera of mosquitoes allows for the possible control of the diseases which they transmit. Other mosquito genera, such as *Anopheles*, have also been trapped using the Biogents mosquito traps (Hoel et al., 2014). *Aedes* and *Culex* are the only known genera of mosquitoes found around the study site. The use of scent traps to capture other genera in disease prone regions of the world can help to reduce the incidence and spread of mosquito-borne pathogens. It is therefore useful to conduct trials in affected regions to assess the potential of floral scent compounds as a measure of control.

The use of a relatively simple three component mixture makes the preparation of the lure quite easy. It is likely that environmental factors such as temperature, humidity and availability of water may influence the abundance of mosquitoes in a given locality (WHO, 1982). However, I found no evidence that they affected the results. No site effect was observed in our study, and regardless of where the treatments where placed, mosquitoes were attracted in similar numbers. More detailed identification of the trapped mosquitoes species (e.g. whether those species are pathogenic) may be useful to establish if the floral scent mix is suitable for targeting mosquitoes that transmit diseases such as malaria. The use of plant volatiles as alternatives that preclude mosquito resistance to chemicals such as pyrethrin is an avenue for great opportunity. The identification of compounds used in combination may demonstrate stronger impacts on mosquito populations. Unlike the attractants derived from animal odours, floral scents have the advantage of attracting both sexes of mosquitoes, thereby achieving significantly higher levels of control. Since mosquitoes are attracted to a wide range of flowers from which they obtain nectar, it is without doubt that many compounds remain to be identified. A better understanding of the chemical ecology of mosquito populations may lead to the production of more effective blends, optimal concentrations, release rate and delivery systems. Moreover, it is important to assess the effect of floral scent combinations on different mosquito species.

3.5. Conclusions

Our study suggests floral scent compounds are as effective as a lure developed by Biogents (Sweetscent™) that is based on skin odours, for attracting mosquitoes in the field. Male mosquitoes and females that had not had a blood meal were caught showing the potential of floral scent for monitoring or controlling mosquito populations. Further research is needed in areas where diseases such as malaria and other arboviral ailments are rife. The use of floral scent mixtures in the field should be incorporated as an integrated management strategy together with other techniques, including lures that use skin based volatiles.

3.6. References

Alto, B.W., Lounibos, L. P., Mores, C. N., Reiskind, M. H., 2008. Larval competition alters susceptibility of adult *Aedes* mosquitoes to dengue infection. Proceedings of the Royal Society B 275, 463-471.

Backer, N., 1989. Life strategies of mosquitoes as an adaptation to their habitats. Bulletin for the Society of Vector Ecology 14, 6-25.

Bond, J. G., Arredondo- Jiménez, J. I., Rodríguez, M. H., Quiroz- Martínez, H., Williams, T., 2005. Oviposition habitat selection for a predator refuge and food source in a mosquito. Ecological Entomology 30, 255–263.

Bosch, J., Kemp, W. P., 2002. Developing and establishing bee species as crop pollinators: the example of *Osmia* spp. (Hymenoptera: Megachilidae) and fruit trees. Bulletin of Entomological Research, 92, 3-16.

Crepeau, T. N., Unlu, I., Healy, S. P., Farajollahi, A., Fonseca, D. M., 2013. Experiences with the large-scale operation of the Biogents Sentinel™ Trap. Journal of the American Mosquito Control Association 29, 177-180.

Eby, C., Gardiner, M. G. T., Gries, R., Judd, G. J. R., Khaskin, G., Gries, G., 2013. Phenylacetaldehyde attracts male and female appleclearwing moths, *Synanthedon myopaeformis*, to inflorescences of showy milkweed, *Asclepias speciosa*. Entomologia Experimentalis et Applicata 147, 82–92.

Foster, W. A., Takken, W., 2004. Nectar-related vs. human-related volatiles: behavioural response and choice by female and male *Anopheles gambiae* (Diptera: Culicidae) between emergence and first feeding. Bulletin of Entomological Research 94, 145-157.

Göpfert, M. C., Briegel, H., Robert, D., 1999. Mosquito hearing: sound-induced antennal vibrations in male and female *Aedes aegypti*. The Journal of Experimental Biology 202, 2727-2738.

Gubler, D. J., 2002. The global emergence/resurgence of arboviral diseases as public health problems. Archives of Medical Research 33, 330-342.

Hoel, D. F., Marika, J. A., Dunford, J. C., Irish, S.R., Geier, M., Obermayr, U., Wirtz, R. A., 2014. Optimizing Collection of *Anopheles gambiae* s.s. (Diptera: Culicidae) in Biogents Sentinel Traps. Journal of Medical Entomology 51, 1268-75.

Jhumur, U. S., 2007. *Silene otites* (Caryophyllaceae): Attraction of nectar-seeking mosquitoes to inflorescence odours, and temporal variation of flower scent and flower visitors. Journal of Chemical Ecology, 42-76.

Jupp, P. G., 2005. Mosquitoes as vectors for disease in South Africa. South African Family Practice 47, 68-72.

Jürgens, A., Witt, T., Gottsberger, G., 2002.Flower scent composition in night-flowering *Silene* species (Caryophyllaceae). Biochemical Systematics and Ecology 30, 383-397.

Kovendan, K., Arivoli, S., Maheshwaran, R., Baskar, K., Vincent, S., 2012. Larvicidal efficacy of *Sphaeranthus indicus, Cleistanthus collinus* and *Murraya koenigii* leaf extracts against filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitology Research 111, 1025-1035.

Lothrop, H. D., Wheeler, S. S., Fang, Y., Reisen, W. K., 2012. Use of scented sugar bait stations to track mosquito-borne arbovirus transmission in California. Journal of Medical Entomology 49, 1466-1472.

Martínez-de la Puente, J., Muñoz, J, Capelli, G., Montarsi, F., Soriguer, R., Arnoldi, D., Rizzoli, A., Figuerola, J., 2015. Avian malaria parasites in the last supper: identifying encounters between parasites and the invasive Asian tiger mosquito and native mosquito species in Italy. Malaria Journal 14, 32-38.

McCullagh, P., Nelder, J. A., 1989. Generalized linear models (Monographs on statistics and applied probability 37). Chapman Hall, London.

Müller, G. C., Beier, J. C., Traore, S. F., Toure, M. B., Traore, M. M., Bah, S., Doumbia, S., Schlein, Y., 2010a. Field experiments of *Anopheles gambiae* attraction to local fruits/seedpods and flowering plants in Mali to optimize strategies for malaria vector control in Africa using attractive toxic sugar bait methods. Malaria Journal 9, 1-11.

Müller, G. C., Junnila, A., Schlein, Y., 2010b. Effective control of adult *Culex pipiens* by spraying an attractive toxic sugar bait solution in the vegetation near larval habitats. Journal of Medical Entomology 47, 63-66.

Müller, G. C., Kravchenko, V. D., Schlein, Y., 2008. Decline of *Anopheles sergentii* and *Aedes caspius* populations following presentation of attractive toxic (Spinosad) sugar bait stations in an oasis. Journal of the American Mosquito Control Association 24, 147-149.

Müller, G. C., Xue, R., Schlein, Y., 2010c. Seed pods of the carob tree *Ceratonia siliqua* are a favored sugar source for the mosquito *Aedes albopictus* in coastal Israel. Acta Tropica 116, 235-239.

Otienoburu, P. E., Ebrahimi, B., Phelan, P. L., Foster, W. A., 2012. Analysis and optimization of a synthetic milkweed floral attractant for mosquitoes. Journal of Chemical Ecology 38, 873-881.

Schlein, Y., Müller, G. C., 2008. An approach to mosquito control: using the dominant attraction of flowering *Tamarix jordanis* trees against *Culex pipiens*. Journal of Medical Entomology 45, 384-390.

Snow, K. R., 1990. Mosquitoes, Richmond Publishing Co. Ltd., England.

WHO, 1982. Manual on environmental management for mosquito control: with special emphasis on malaria vectors. WHO Geneva.

WHO, 2010. Rift Valley Fever, Fact Sheet. http://www.who.int/mediacentre/factsheets/fs207/en/ Date accessed: 30 June 2015.

WHO, 2014a. Yellow fever, Fact Sheet. http://www.who.int/mediacentre/factsheets/fs100/en/ Date accessed: 04 April 2014.

WHO, 2014b. West Nile Virus, Fact Sheet. http://www.who.int/mediacentre/factsheets/fs354/en/. Date accessed: 30 June 2015.

Xue, R., Ali, A., Barnard, D. R., 2008. Host species diversity and post-blood feeding carbohydrate availability enhance survival of females and fecundity in *Aedes albopictus* (Diptera: Culicidae). Experimental Parasitology 119, 225-228.

Chapter 4

Conclusions and recommendations

Mosquitoes pose a significant threat to human health transmitting diseases to humans and animals. Many efforts to control mosquito transmitted diseases and the spread thereof have been implemented. An effective option for controlling mosquito-borne diseases seems to be to control the vector instead of treating the hosts (humans and animals) after the infection has happened. Mosquitoes are successful insects which are able to inhabit and reproduce provided the environment is appropriate. Various control methods have been used for many years (biological, chemical or even mechanical) targeting specific life stages. With changing climates providing more conducive environments for mosquito breeding, new control/monitoring methods are urgently needed. While efforts to focus on the control of mosquito eggs, larval and pupal stages have been widely documented, there are still large numbers of adult mosquitoes which emerge. It seems therefore reasonable to believe that integrated management strategies, that combine many different methods, are the best approach for a sustainable control of mosquito populations.

One promising approach presented here in the current study is to exploit the adult feeding behaviour of mosquitoes. While males only feed on carbohydrate sources (sugar or nectar), females have a more complex diet. Young females are interested in carbohydrate sources mostly, after emergence. Thereafter, females are in search of blood meals, transmitting diseases in the process. Mosquitoes are able to locate carbohydrate and blood sources by means of highly developed sensory systems. The use of scents produced by flowers or fruits and sweat, for example, can be used to lure mosquitoes to the relevant source. Thus, by exploiting the feeding behaviour of female mosquitoes searching for a carbohydrate sources, prior to taking up a blood meal, the disease transmission could be prevented. Few studies in South Africa have highlighted the attraction of mosquitoes to fruits and flowers or identified the scent compounds emitted by these odour sources. The aim of the present study was therefore to investigate the effectiveness of different flowers and fruits and artificial mixtures of floral scent compounds for attracting mosquitoes.

In Chapter 2, flowering plants growing in South Africa which were visited by mosquitoes were identified. Furthermore fruits which mosquitoes found attractive in previous studies but are easily available locally were selected. It was found that mosquitoes found all fruits and flowers attractive and as carbohydrate sources, which was evident from their feeding bouts. The most attractive flowers were *Gymnosporia buxifolia* and *Acacia nilotica* which are distributed across most of South Africa. Fruits which mosquitoes found highly attractive were those of *Musa acuminata* and *Ficus sur* which are locally available. In addition, scent profiles revealed the most dominant scent compounds which could be used in future analyses. The most dominant scent compounds from the flowers were: an unidentified compound, pentan-1-ol and ethylbenzene. The most dominant scent compounds from the fruit were: 3-methylbutyl acetate, isobutyl butyrate, isopentyl 2-methylpropanoate, isobutyl acetate, ethylbenzene, ethanol, acetoin and limonene. The role of these scent compounds for locating the carbohydrate sources by mosquitoes is discussed.

In Chapter 3, the efficiency of floral volatiles was compared to another commercial lure with regard to different mosquito species. Floral volatiles phenylacetaldehyde, 2-phenyl ethanol and linalool oxide were used as a blend in field trails against a commercial lure, Sweetscent™ (Biogents, Germany). The floral scent attracted significantly higher numbers of mosquitoes than the commercial lure. More females than males were attracted; and interestingly more females which did not have a blood meal. The potential of using floral/fruit scent compounds as attractants for controlling and monitoring mosquito populations is discussed.

In conclusion, the present study demonstrated that fruit and flower volatiles can be used as efficient mosquito attractants in the field. Further screening of a wider range of naturally occurring carbohydrate food sources is needed, however. Such data could add to a more complete understanding of how the availability flowers and fruits as food sources may affect mosquito populations. Based on the results of the present study the next step could be to develop toxic sugar bait stations to attract and kill mosquitoes. These traps could also be used indoors because the floral scent compounds used in this study have a sweet and pleasant aroma (to the human nose).

Appendix A

Number of spiders (Ara) and insect orders trapped with Biogents mosquito traps and different types of lure (C = control (paraffin oil), BSS = Biogents Sweetscent™, FS = mixture of three floral scent compounds). The three component mixture of floral scent compounds consisted of phenylacetaldehyde, 2-phenyl ethanol and linalool oxide. Number of mosquitoes (sex and blood meal or not), Aranea, insect orders and individuals for each treatment during the different trials (dates). Bla = Blattodea, Col = Coleoptera, Dip = Diptera, Hem = Hemiptera, Hym = Hymenoptera, Lep = Lepidoptera, Neu = Neuroptera, Odo = Odonata, Ort = Orthoptera, Pha = Phasmatodea, and Thy = Thysanoptera.

*Order Diptera numbers are exclusive of mosquitoes

Date	**Treatment**	**Female**		**Male**	**Total mosquitoes**	**Genera**		**Ara**	**Bla**	**Col**	**Dip**	**Hem**	**Hym**	**Lep**	**Neu**	**Odo**	**Ort**	**Pha**	**Thy**
		Blood meal	**No blood meal**			***Aedes***	***Culex***												
21-Mar-13	**C1**	0	7	3	**10**	**4**	**6**	0	0	0	6	0	1	1	0	0	0	0	0
	C2	0	13	0	**13**	**6**	**7**	0	0	0	9	1	2	0	0	1	0	0	0
	C3	0	0	0	**0**	**0**	**0**	0	0	0	0	0	0	0	0	0	0	0	0
	BSS1	1	11	3	**15**	**8**	**7**	0	0	0	23	1	4	0	0	0	0	0	0
	BSS2	0	9	0	**9**	**6**	**3**	2	0	0	17	0	5	3	0	0	0	0	0
	BSS3	0	14	5	**19**	**1**	**18**	0	0	0	67	0	4	0	0	3	0	0	0
	FS1	0	4	2	**6**	**3**	**3**	0	0	1	14	0	7	1	0	0	0	0	0
	FS2	2	37	3	**42**	**14**	**28**	0	0	0	63	0	6	7	0	0	0	0	0
	FS3	1	34	5	**40**	**16**	**24**	0	0	2	56	29	2	2	0	0	0	0	0
23-Mar-13	**C1**	0	14	8	**22**	**8**	**14**	1	0	0	23	0	4	0	0	0	0	0	0

Date	Treatment	Female		Male	Total mosquitoes	Genera		Ara	Bla	Col	Dip	Hem	Hym	Lep	Neu	Odo	Ort	Pha	Thy
		Blood meal	No blood meal			*Aedes*	*Culex*												
	C2	0	6	1	**7**	**3**	**4**	1	1	2	63	26	16	6	0	0	0	0	0
	C3	0	32	6	**38**	**18**	**20**	2	0	0	21	0	3	1	0	0	0	0	0
	BSS1	0	20	7	**27**	**11**	**16**	3	0	5	74	47	7	1	0	0	0	0	0
	BSS2	3	25	19	**47**	**17**	**30**	0	0	17	38	33	11	5	0	0	0	0	0
	BSS3	1	28	6	**35**	**13**	**22**	1	1	1	22	14	8	0	0	1	0	0	0
	FS1	0	22	4	**26**	**7**	**19**	0	0	1	54	11	18	2	0	1	0	0	0
	FS2	0	32	6	**38**	**14**	**24**	3	0	13	27	4	17	1	0	0	0	0	0
	FS3	0	22	5	**27**	**9**	**18**	0	0	1	29	10	12	3	0	2	0	0	0
25-Mar-13	**C1**	1	12	0	**13**	**3**	**10**	0	0	0	1	2	2	0	0	0	1	0	0
	C2	0	34	4	**38**	**12**	**26**	2	0	0	14	10	5	1	0	1	2	0	0
	C3	0	13	6	**19**	**8**	**11**	1	0	0	4	1	4	0	0	0	0	0	0
	BSS1	1	35	3	**39**	**17**	**22**	0	0	10	32	1	10	1	0	0	0	0	0
	BSS2	0	22	5	**27**	**8**	**19**	0	0	0	6	4	7	2	0	0	0	0	0
	BSS3	1	32	9	**42**	**13**	**29**	0	0	1	8	6	5	0	0	1	0	0	0
	FS1	0	41	3	**44**	**15**	**29**	7	0	2	8	8	2	0	0	0	0	0	0
	FS2	1	45	4	**50**	**12**	**38**	1	0	1	4	0	4	1	0	0	0	0	0
	FS3	0	34	1	**35**	**8**	**27**	0	0	6	18	4	6	1	0	0	0	0	0
27-Mar-13	**C1**	0	12	0	**12**	**8**	**4**	0	0	2	13	8	3	0	0	0	0	0	0
	C2	0	6	1	**7**	**2**	**5**	0	0	1	24	12	2	0	0	0	0	0	0
	C3	0	0	0	**0**	**0**	**0**	0	0	6	16	2	6	3	0	0	0	0	5
	BSS1	0	8	0	**8**	**1**	**7**	0	0	0	12	2	8	0	0	0	0	0	0
	BSS2	0	14	3	**17**	**4**	**13**	3	0	3	9	6	5	0	0	0	0	1	1
	BSS3	0	3	0	**3**	**1**	**2**	0	0	0	6	5	7	0	0	0	0	0	0

Date	Treatment	Female		Male	Total mosquitoes	Genera		Ara	Bla	Col	Dip	Hem	Hym	Lep	Neu	Odo	Ort	Pha	Thy
		Blood meal	No blood meal			*Aedes*	*Culex*												
29-Mar-13	**FS1**	0	3	0	**3**	**0**	**3**	1	0	6	14	8	7	0	0	0	0	0	0
	FS2	0	17	1	**18**	**9**	**9**	0	0	2	29	3	16	0	0	1	0	0	0
	FS3	0	23	4	**27**	**5**	**22**	0	0	0	16	6	6	0	0	1	0	0	0
	C1	0	30	1	**31**	**11**	**20**	0	0	1	9	1	57	0	0	0	0	0	1
	C2	1	29	2	**32**	**8**	**24**	1	0	14	38	12	11	6	0	2	0	0	0
	C3	0	16	2	**18**	**9**	**9**	0	0	3	13	3	3	0	0	1	0	0	0
	BSS1	0	34	1	**35**	**16**	**19**	0	0	14	16	4	13	2	0	2	0	0	0
	BSS2	1	34	0	**35**	**6**	**29**	2	0	2	20	0	8	5	0	0	0	0	0
	BSS3	0	23	3	**26**	**9**	**17**	0	0	7	20	6	14	0	0	0	0	0	0
31-Mar-13	**FS1**	0	23	3	**26**	**7**	**19**	0	0	2	23	2	7	3	0	0	0	0	0
	FS2	1	39	2	**42**	**8**	**34**	1	0	3	17	2	11	2	0	0	0	0	0
	FS3	0	25	3	**28**	**5**	**23**	2	0	1	19	9	8	1	0	0	0	0	0
	C1	0	1	0	**1**	**0**	**1**	0	0	1	9	3	4	0	0	0	0	0	0
	C2	0	4	0	**4**	**1**	**3**	0	0	3	14	2	9	0	0	0	0	0	0
	C3	0	1	0	**1**	**0**	**1**	0	0	0	14	3	2	0	0	0	0	0	0
	BSS1	0	7	1	**8**	**1**	**7**	0	0	2	7	9	3	0	0	0	0	0	0
	BSS2	0	4	0	**4**	**2**	**2**	0	0	6	13	4	2	0	0	0	0	0	4
	BSS3	0	1	0	**1**	**0**	**1**	2	0	0	3	7	3	0	0	0	0	0	0
4-Apr-13	**FS1**	0	2	0	**2**	**0**	**2**	0	0	0	2	5	7	0	0	0	0	0	0
	FS2	0	3	1	**4**	**1**	**3**	1	0	2	6	1	1	0	0	0	0	0	0
	FS3	0	2	0	**2**	**0**	**2**	0	0	1	7	6	1	0	0	0	0	0	0
	C1	0	0	0	**0**	**0**	**0**	0	0	0	1	0	0	0	0	0	0	0	0

Date	Treatment	Female		Male	Total mosquitoes	Genera		Ara	Bla	Col	Dip	Hem	Hym	Lep	Neu	Odo	Ort	Pha	Thy
		Blood meal	No blood meal			*Aedes*	*Culex*												
	C2	0	5	0	**5**	**2**	**3**	1	0	1	23	2	2	1	0	0	0	0	0
	C3	0	12	1	**13**	**4**	**9**	1	0	1	3	1	2	1	0	0	0	0	0
	BSS1	0	5	1	**6**	**1**	**5**	0	0	2	15	6	13	2	0	0	0	0	0
	BSS2	0	23	4	**27**	**8**	**19**	0	0	5	21	6	15	0	0	0	0	0	0
	BSS3	0	13	2	**15**	**6**	**9**	0	0	1	9	2	16	2	0	0	0	0	0
	FS1	0	20	2	**22**	**6**	**16**	0	1	2	35	14	12	6	0	0	0	0	0
	FS2	0	5	0	**5**	**2**	**3**	1	0	1	23	2	2	1	0	0	0	0	0
	FS3	1	27	5	**33**	**18**	**15**	1	1	0	39	10	8	1	0	0	0	0	0
6-Apr-13	**C1**	0	15	2	**17**	**8**	**9**	0	0	0	8	1	4	0	0	0	0	0	0
	C2	0	22	2	**24**	**5**	**19**	0	0	2	9	4	9	0	0	0	0	0	0
	C3	0	4	1	**5**	**3**	**2**	2	0	4	11	1	3	0	0	0	0	0	0
	BSS1	0	19	0	**19**	**13**	**6**	0	0	0	22	20	0	0	0	0	0	0	0
	BSS2	0	31	4	**35**	**8**	**27**	0	0	1	12	2	3	1	0	0	0	0	0
	BSS3	0	1	0	**1**	**0**	**1**	0	0	0	4	1	2	1	0	0	0	0	0
	FS1	0	13	1	**14**	**4**	**10**	0	0	0	0	1	0	0	0	0	0	0	0
	FS2	0	43	4	**47**	**15**	**32**	0	0	2	10	2	2	0	0	0	0	0	0
	FS3	0	10	0	**10**	**8**	**2**	0	0	1	6	0	4	1	0	0	0	0	0
8-Apr-13	**C1**	0	14	1	**15**	**7**	**8**	0	0	9	13	11	13	3	0	1	0	0	0
	C2	0	8	1	**9**	**4**	**5**	0	0	3	19	3	9	0	0	3	0	0	0
	C3	0	0	0	**0**	**0**	**0**	0	0	1	0	0	0	0	0	0	0	0	0
	BSS1	1	24	3	**28**	**9**	**19**	0	0	6	27	6	23	13	0	0	0	0	2
	BSS2	0	26	1	**27**	**5**	**22**	2	0	12	32	5	18	8	0	0	0	0	0
	BSS3	0	12	1	**13**	**6**	**7**	0	0	0	13	1	10	0	0	0	0	0	0
	FS1	1	29	2	**32**	**11**	**21**	0	0	0	9	4	12	2	0	0	0	0	0
	FS2	0	19	4	**23**	**16**	**7**	0	0	6	16	6	7	0	0	0	0	0	0

Date	Treatment	Female		Male	Total mosquitoes	Genera		Ara	Bla	Col	Dip	Hem	Hym	Lep	Neu	Odo	Ort	Pha	Thy
		Blood meal	No blood meal			*Aedes*	*Culex*												
	FS3	0	6	0	**6**	**2**	**4**	0	0	2	8	0	3	3	0	1	0	0	0
10-Apr-13	**C1**	0	1	0	**1**	**0**	**1**	0	0	0	4	1	2	1	0	0	0	0	0
	C2	0	8	0	**8**	**7**	**1**	0	0	0	3	0	2	0	0	0	0	0	0
	C3	0	3	0	**3**	**2**	**1**	1	0	0	2	0	2	0	0	0	0	0	0
	BSS1	0	7	2	**9**	**6**	**3**	0	0	0	8	1	2	0	0	0	0	0	0
	BSS2	0	4	0	**4**	**1**	**3**	0	0	1	3	1	1	1	0	0	0	0	0
	BSS3	0	4	0	**4**	**2**	**2**	0	0	0	4	2	2	0	0	0	0	0	0
	FS1	0	7	0	**7**	**6**	**1**	0	1	1	10	1	8	4	0	0	0	0	0
	FS2	0	6	0	**6**	**3**	**3**	0	0	2	8	1	1	1	0	0	0	0	0
	FS3	0	7	1	**8**	**5**	**3**	0	0	0	7	1	7	4	0	2	0	0	0
12-Apr-13	**C1**	0	4	3	**7**	**3**	**4**	2	0	0	4	2	2	0	0	0	0	0	0
	C2	0	1	0	**1**	**0**	**1**	0	0	0	5	3	1	0	0	0	0	0	0
	C3	0	7	0	**7**	**3**	**4**	0	0	0	2	0	0	0	0	0	0	0	0
	BSS1	0	8	1	**9**	**5**	**4**	0	0	2	6	2	5	0	0	0	0	0	1
	BSS2	0	5	0	**5**	**1**	**4**	0	0	1	6	6	2	6	0	0	0	0	0
	BSS3	0	3	0	**3**	**0**	**3**	0	0	2	11	1	6	0	0	0	0	0	0
	FS1	0	7	1	**8**	**8**	**0**	1	0	0	23	6	8	7	0	0	0	0	0
	FS2	0	8	1	**9**	**5**	**4**	0	0	1	35	4	6	0	0	0	0	0	0
	FS3	0	13	0	**13**	**4**	**9**	0	0	0	16	2	1	0	0	0	0	0	0
19-Aug-14	**C1**	0	8	4	**12**	**5**	**7**	0	2	2	13	0	10	8	0	0	0	0	0
	C2	0	0	0	**0**	**0**	**0**	0	0	0	0	0	0	0	0	0	0	0	0

Date	Treatment	Female		Male	Total mosquitoes	Genera		Ara	Bla	Col	Dip	Hem	Hym	Lep	Neu	Odo	Ort	Pha	Thy
		Blood meal	No blood meal			*Aedes*	*Culex*												
	C3	1	5	2	**8**	**6**	**2**	2	0	0	5	0	2	0	0	0	0	0	0
	BSS1	0	23	0	**23**	**5**	**18**	0	0	0	27	3	13	28	0	0	0	0	0
	BSS2	0	0	0	**0**	**0**	**0**	0	0	0	0	0	0	0	0	0	0	0	0
	BSS3	0	6	0	**6**	**4**	**2**	0	0	1	1	1	3	2	0	0	0	0	0
	FS1	0	1	0	**1**	**1**	**0**	0	1	3	14	2	9	1	0	0	0	0	0
	FS2	0	13	1	**14**	**8**	**6**	0	4	1	13	4	9	2	0	0	0	0	0
	FS3	0	2	3	**5**	**2**	**3**	1	0	0	4	0	4	6	0	0	0	0	0
22-Aug-14	**C1**	0	4	3	**7**	**6**	**1**	0	0	0	5	0	5	2	0	0	0	0	0
	C2	0	0	0	**0**	**0**	**0**	0	0	0	0	0	0	0	0	0	0	0	0
	C3	0	2	1	**3**	**1**	**2**	0	0	1	1	0	0	1	0	0	0	0	0
	BSS1	0	7	6	**13**	**9**	**4**	0	0	2	14	2	2	2	0	0	0	0	0
	BSS2	0	2	9	**11**	**2**	**9**	0	0	2	6	2	0	1	0	0	0	0	0
	BSS3	0	4	1	**5**	**3**	**2**	1	0	0	0	0	0	0	0	0	0	0	0
	FS1	0	4	4	**8**	**4**	**4**	0	0	0	13	1	1	1	0	0	0	0	0
	FS2	0	5	0	**5**	**3**	**2**	0	0	3	7	1	3	1	0	0	0	0	0
	FS3	0	6	4	**10**	**9**	**1**	0	0	0	11	1	0	3	0	0	0	0	0
25-Aug-14	**C1**	0	6	2	**8**	**3**	**5**	0	0	1	13	1	3	1	0	0	0	0	0
	C2	0	4	1	**5**	**1**	**4**	0	0	0	7	0	2	11	0	0	0	0	0
	C3	0	4	3	**7**	**0**	**7**	4	0	0	2	0	2	4	0	0	0	0	0
	BSS1	1	6	5	**12**	**5**	**7**	0	0	0	18	1	1	1	0	0	0	0	0
	BSS2	0	0	0	**0**	**0**	**0**	0	0	0	5	0	0	0	0	0	0	0	1
	BSS3	0	5	0	**5**	**3**	**2**	0	0	0	2	0	2	0	0	0	0	0	0
	FS1	0	15	14	**29**	**5**	**24**	1	0	2	19	2	7	1	0	0	0	0	0

Date	**Treatment**	**Female**		**Male**	**Total mosquitoes**	**Genera**		**Ara**	**Bla**	**Col**	**Dip**	**Hem**	**Hym**	**Lep**	**Neu**	**Odo**	**Ort**	**Pha**	**Thy**
		Blood meal	**No blood meal**			***Aedes***	***Culex***												
	FS2	0	8	3	**11**	**3**	**8**	0	0	0	5	2	3	6	1	0	0	0	1
	FS3	0	1	1	**2**	**0**	**2**	0	0	0	6	0	2	1	0	0	0	0	0
28-Aug-14	**C1**	0	5	0	**5**	**0**	**5**	0	0	0	5	1	2	1	1	0	0	0	0
	C2	0	9	2	**11**	**2**	**9**	1	0	1	5	8	4	2	0	0	0	0	0
	C3	0	6	2	**8**	**4**	**4**	0	0	0	12	0	0	1	0	0	0	0	0
	BSS1	0	11	0	**11**	**3**	**8**	1	0	1	29	2	7	4	0	0	0	0	0
	BSS2	0	4	3	**7**	**2**	**5**	1	0	0	23	5	7	4	0	0	0	0	0
	BSS3	0	2	2	**4**	**0**	**4**	0	0	0	19	0	1	3	0	0	0	0	0
	FS1	0	7	5	**12**	**3**	**9**	0	0	0	16	0	2	3	0	0	0	0	0
	FS2	0	4	5	**9**	**2**	**7**	0	0	0	14	2	6	3	1	0	0	0	0
	FS3	0	7	2	**9**	**3**	**6**	1	0	0	18	0	1	3	0	0	0	0	0
9-Sep-14	**C1**	0	4	3	**7**	**2**	**5**	1	0	0	10	7	6	6	1	0	0	0	0
	C2	0	5	0	**5**	**3**	**2**	0	0	1	6	5	3	2	0	0	0	0	0
	C3	0	1	1	**2**	**2**	**0**	3	0	3	7	1	4	2	0	0	0	0	0
	BSS1	0	22	12	**34**	**7**	**27**	1	0	3	22	0	12	7	0	0	0	0	0
	BSS2	0	5	1	**6**	**2**	**4**	0	0	0	17	5	4	1	1	0	0	0	0
	BSS3	0	0	0	**0**	**0**	**0**	0	0	1	4	0	1	0	0	0	0	0	0
	FS1	0	12	3	**15**	**2**	**13**	0	0	1	9	13	4	4	1	0	0	0	0
	FS2	0	5	1	**6**	**3**	**3**	0	0	2	0	2	4	2	0	0	0	0	0
	FS3	0	3	7	**10**	**1**	**9**	1	0	0	7	0	1	2	0	0	0	0	0
18-Sep-14	**C1**	0	0	0	**0**	**0**	**0**	0	0	0	0	0	0	0	0	0	0	0	0
	C2	0	14	15	**29**	**3**	**26**	0	0	0	8	1	5	4	0	0	0	0	0
	C3	0	17	0	**17**	**1**	**16**	0	0	0	3	0	3	3	1	0	0	0	0

Date	Treatment	Female		Male	Total mosquitoes	Genera		Ara	Bla	Col	Dip	Hem	Hym	Lep	Neu	Odo	Ort	Pha	Thy
		Blood meal	No blood meal			*Aedes*	*Culex*												
	BSS1	0	17	15	**32**	**5**	**27**	0	0	0	18	5	11	3	0	0	0	0	0
	BSS2	0	13	2	**15**	**3**	**12**	1	0	0	2	9	2	2	0	0	0	0	0
	BSS3	0	11	2	**13**	**2**	**11**	2	0	0	10	0	1	5	0	0	0	0	0
	FS1	0	12	10	**22**	**4**	**18**	3	0	0	13	3	4	7	0	0	0	0	0
	FS2	0	9	2	**11**	**5**	**6**	0	0	0	23	4	10	0	0	0	0	0	0
	FS3	0	8	1	**9**	**3**	**6**	0	0	2	15	0	2	1	0	0	0	0	0
24-Sep-14	**C1**	0	1	1	**2**	**2**	**0**	0	0	2	6	4	2	0	0	0	0	0	0
	C2	0	9	8	**17**	**3**	**14**	0	0	0	8	0	0	2	0	0	0	0	0
	C3	0	5	0	**5**	**2**	**3**	0	0	0	7	0	2	1	0	0	0	0	0
	BSS1	0	0	0	**0**	**0**	**0**	0	0	1	0	2	3	0	0	0	0	0	0
	BSS2	0	24	14	**38**	**9**	**29**	0	1	0	17	5	11	5	0	0	0	0	0
	BSS3	0	6	0	**6**	**6**	**0**	1	0	0	8	1	6	2	0	0	0	0	0
	FS1	0	10	9	**19**	**4**	**15**	1	0	0	13	4	4	2	0	0	0	0	0
	FS2	0	12	5	**17**	**6**	**11**	0	0	3	10	1	3	6	0	0	0	0	0
	FS3	0	8	1	**9**	**2**	**7**	0	0	1	9	1	7	1	0	0	0	0	0
30-Sep-14	**C1**	0	0	0	**0**	**0**	**0**	0	0	0	3	1	2	1	0	0	0	0	0
	C2	0	3	5	**8**	**8**	**0**	0	0	10	9	0	1	9	0	0	0	0	0
	C3	0	12	2	**14**	**2**	**12**	0	0	0	14	0	3	3	0	0	0	0	0
	BSS1	0	0	0	**0**	**0**	**0**	0	0	0	3	0	51	0	0	0	0	0	0
	BSS2	0	4	10	**14**	**5**	**9**	1	0	1	7	1	3	4	0	0	0	0	0
	BSS3	0	10	1	**11**	**5**	**6**	0	0	0	6	2	21	2	0	0	0	0	0
	FS1	0	16	16	**32**	**6**	**26**	0	0	1	27	4	7	3	0	0	0	0	0
	FS2	0	14	18	**32**	**4**	**28**	0	0	1	18	1	2	7	0	0	0	0	0
	FS3	0	8	1	**9**	**2**	**7**	0	0	1	12	0	3	2	0	0	0	0	0

Date	Treatment	Female		Male	Total mosquitoes	Genera		Ara	Bla	Col	Dip	Hem	Hym	Lep	Neu	Odo	Ort	Pha	Thy
		Blood meal	No blood meal			*Aedes*	*Culex*												
10-Jul-14	**C1**	0	0	0	**0**	**0**	**0**	0	0	1	2	0	8	0	0	0	0	0	0
	C2	0	8	4	**12**	**9**	**3**	1	0	0	7	1	10	2	0	0	0	0	0
	C3	0	0	1	**1**	**0**	**1**	0	0	0	17	0	0	0	0	0	0	0	0
	BSS1	0	9	16	**25**	**9**	**16**	0	0	0	20	1	3	3	0	0	0	0	0
	BSS2	0	28	15	**43**	**19**	**24**	0	0	1	4	0	1	5	2	0	0	0	0
	BSS3	0	17	4	**21**	**3**	**18**	1	0	0	17	0	5	3	0	1	0	0	0
	FS1	0	21	20	**41**	**13**	**28**	0	0	0	16	0	4	2	1	0	0	0	0
	FS2	0	10	11	**21**	**6**	**15**	3	0	2	21	3	3	5	0	0	0	0	0
	FS3	0	5	5	**10**	**4**	**6**	0	0	0	19	0	9	0	0	0	0	0	0
10-Oct-14	**C1**	0	7	2	**9**	**2**	**7**	0	0	0	18	0	4	1	0	0	0	0	0
	C2	0	7	5	**12**	**5**	**7**	0	0	1	21	1	3	1	0	0	0	0	0
	C3	0	4	1	**5**	**0**	**5**	0	0	1	3	0	2	0	1	0	0	0	0
	BSS1	0	2	0	**2**	**0**	**2**	0	0	0	7	1	20	0	1	0	0	0	0
	BSS2	0	13	9	**22**	**3**	**19**	0	0	0	18	1	0	3	0	0	0	0	0
	BSS3	0	4	0	**4**	**1**	**3**	1	0	0	50	0	3	0	0	0	0	0	0
	FS1	0	19	5	**24**	**6**	**18**	1	0	1	22	0	2	3	0	0	0	0	0
	FS2	0	7	9	**16**	**9**	**7**	0	0	1	37	0	3	0	0	0	0	0	0
	FS3	0	8	3	**11**	**2**	**9**	0	0	0	47	0	3	0	0	0	0	0	0
14-Oct-14	**C1**	0	1	0	**1**	**0**	**1**	0	0	0	4	0	18	0	0	0	0	0	0
	C2	0	6	3	**9**	**3**	**6**	0	0	1	12	8	3	1	0	0	0	0	0
	C3	0	6	1	**7**	**0**	**7**	0	0	0	8	0	2	0	0	0	0	0	0
	BSS1	0	6	7	**13**	**4**	**9**	0	0	0	53	1	3	2	1	0	0	0	0
	BSS2	0	5	4	**9**	**2**	**7**	0	0	1	9	1	1	0	0	0	0	0	0
	BSS3	0	7	8	**15**	**8**	**7**	0	0	0	66	0	5	0	0	0	0	0	0

Date	**Treatment**	**Female**		**Male**	**Total mosquitoes**	**Genera**		**Ara**	**Bla**	**Col**	**Dip**	**Hem**	**Hym**	**Lep**	**Neu**	**Odo**	**Ort**	**Pha**	**Thy**
		Blood meal	**No blood meal**			***Aedes***	***Culex***												
	FS1	0	10	4	**14**	**3**	**11**	0	0	0	26	2	2	0	0	0	0	0	0
	FS2	0	10	7	**17**	**9**	**8**	2	0	0	5	0	1	1	0	0	0	0	0
	FS3	0	18	7	**25**	**9**	**16**	0	0	1	29	0	6	1	0	1	1	0	0
28-Oct-14	**C1**	0	11	8	**19**	**6**	**13**	0	0	0	76	12	4	2	0	0	0	0	0
	C2	0	17	6	**23**	**5**	**18**	9	0	2	53	4	3	3	0	0	0	0	0
	C3	0	6	3	**9**	**8**	**1**	0	0	0	10	0	7	2	0	1	0	0	0
	BSS1	0	0	0	**0**	**0**	**0**	1	0	0	0	28	13	0	0	0	0	0	0
	BSS2	0	10	2	**12**	**3**	**9**	0	0	1	42	11	9	4	0	0	0	0	0
	BSS3	0	4	1	**5**	**1**	**4**	0	0	0	47	0	5	0	0	0	0	0	0
	FS1	0	17	14	**31**	**11**	**20**	3	0	2	17	2	4	1	0	0	0	0	0
	FS2	0	13	1	**14**	**3**	**11**	2	1	7	87	17	31	2	0	0	0	0	0
	FS3	0	19	3	**22**	**4**	**18**	1	0	0	32	0	13	2	0	5	0	0	0
11-Nov-14	**C1**	0	9	2	**11**	**6**	**5**	0	0	0	6	5	2	1	0	0	0	0	0
	C2	0	0	0	**0**	**0**	**0**	7	0	2	4	15	23	0	0	0	0	0	0
	C3	0	9	1	**10**	**5**	**5**	1	0	2	57	0	7	2	0	0	0	0	0
	BSS1	0	29	11	**40**	**16**	**24**	1	0	1	29	7	4	6	0	0	1	0	0
	BSS2	0	28	2	**30**	**14**	**16**	4	0	2	53	10	7	4	0	0	0	0	0
	BSS3	0	12	2	**14**	**5**	**9**	0	0	0	35	0	18	2	0	1	0	0	0
	FS1	0	24	5	**29**	**8**	**21**	2	0	4	21	28	11	1	0	0	0	0	0
	FS2	0	31	4	**35**	**6**	**29**	3	0	0	112	6	17	1	0	0	0	0	0
	FS3	0	24	5	**29**	**4**	**25**	0	0	0	32	5	10	2	1	6	0	0	0
12-Feb-15	**C1**	0	0	0	**0**	**0**	**0**	0	0	0	10	0	5	3	0	0	0	0	0

Date	Treatment	Female		Male	Total mosquitoes	Genera		Ara	Bla	Col	Dip	Hem	Hym	Lep	Neu	Odo	Ort	Pha	Thy
		Blood meal	No blood meal			*Aedes*	*Culex*												
	C2	0	6	0	**6**	**2**	**4**	1	0	1	64	0	3	2	0	0	0	0	0
	C3	0	9	2	**11**	**5**	**6**	1	0	0	41	0	6	1	0	0	0	0	0
	BSS1	0	0	0	**0**	**0**	**0**	0	0	1	1	2	25	3	0	0	0	0	0
	BSS2	0	5	1	**6**	**0**	**6**	0	0	0	42	0	10	2	0	0	0	0	0
	BSS3	0	18	0	**18**	**6**	**12**	0	0	1	63	1	2	1	0	0	0	0	0
	FS1	1	8	1	**10**	**2**	**8**	0	0	0	31	0	8	2	0	2	0	0	0
	FS2	0	0	0	**0**	**0**	**0**	3	0	1	1	6	4	1	0	0	0	0	0
	FS3	0	14	1	**15**	**8**	**7**	0	0	2	27	2	8	2	0	7	0	0	0
26-Feb-15	**C1**	0	0	0	**0**	**0**	**0**	1	0	0	1	0	0	0	0	0	0	0	0
	C2	0	6	0	**6**	**2**	**4**	1	0	1	24	1	26	2	0	0	0	0	0
	C3	1	7	0	**8**	**5**	**3**	0	0	1	12	0	6	1	0	1	0	0	0
	BSS1	0	0	0	**0**	**0**	**0**	4	0	1	7	11	19	1	0	0	0	0	0
	BSS2	0	0	0	**0**	**0**	**0**	4	0	0	8	8	15	1	0	0	0	0	0
	BSS3	0	26	0	**26**	**9**	**17**	0	0	0	11	0	4	4	0	4	0	0	0
	FS1	0	0	0	**0**	**0**	**0**	1	0	0	0	5	51	0	0	0	0	0	0
	FS2	0	0	0	**0**	**0**	**0**	0	0	1	11	0	9	1	0	0	0	0	0
	FS3	0	24	1	**25**	**8**	**17**	0	0	0	7	2	3	0	0	0	0	0	0