AN ASSESSMENT OF THE USE OF BOTANICAL EXTRACTS AND PHEROMONES FOR THE OFF-HOST AND ON-HOST CONTROL OF *AMBLYOMMA VARIEGATUM TICK*

ALIOUNE TOURE

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN VETERINARY ENTOMOLOGY IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY, KENYA.

APRIL, 2005

DECLARATION

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

Alioune Toure — 08/04/2005 Date

We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

Dr. Sammy Shimenga Kubasu

Department of Biological Sciences, Kenyatta University

Signature — 13/4/2005 Date

Dr. Ellie O. Osir

Molecular Biology & Biotechnology Unit, ICIPE

Signature — 8/4/05 Date

Prof. Ahmed Hassanali

Behavioural & Chemical Ecology Department, ICIPE

Signature — 12/4/05 Date

DEDICATION

To

My wife Fatou SECK, my mother, my late father, my son, my country, Senegal and my host country, Kenya.

ACKNOWLEDGEMENTS

I thank the ALMIGHTY ALLAH.

I express my deepest gratitude to my supervisors Dr. Sammy Kubasu, Dr. Ellie Osir and Prof. Ahmed Hassanali for their academic supervision, guidance and encouragements, without which this thesis would not have been completed. I am also very thankful to Dr. Rosebella Maranga for her advice and technical support.

I am thankful to the Director General of ICIPE and the Capacity Building staff: Prof. Ole MoiYoi, Dr. Ochieng-Odero, Lucy Theuri, Lisa Omondi and Margaret Ochanda for all the support accorded to me during my study period. I also thank Dr. Vitalis Musewe, the former Chief of the Capacity Building Unit and Lizzie Chongo'ti. I thank the staff of the Molecular Biology and Biotechnology Unit, especially Dr. Daniel Masiga, Dr. Remy Pasquet, Rosekellen Njiru, Daniel Amin, Mark Gacau Kimondo, James Kabii, Harrison Kibogo, Benjamin Omondi and the late David Ochaka for their technical assistance. I am also thankful to the Behavioural and Chemistry Department staff, in particular Dr. Wilbert Lwande, Edward Nyandat, Onesmus Wanyama and John Angira for their assistance and advice. I am also thankful to Prof. Lev and Anthony Wanjoya of the Biostatistics Unit for their support and advice. The support of Dr. Nguya Maniania, John Mwangi and Francis Onyango is greatly acknowledged.

I express my gratitude to the Director of the National Veterinary Research Centre (Muguga, KARI) and all his collaborators for allowing me to use their facilities for my field work. My special thanks to Drs. Muraguri Gitonga, Priscilla Ngumi, Samuel G. Ndungu and David Wesonga and the technicians Bernard Chege, Peter Ominde and Ester Njuguna for their technical advice and assistance. My sincere thanks go to all the casual workers both at ICIPE and Muguga who helped me carry out the field experiments.

I express my gratitude to Professor Bocar Sally Galledou of the University of Dakar for the extraction of the oil of the plants collected from Senegal. I am also very grateful to Dr. Abdoulaye Niassy for his advice and support. The support of Serigne Thierno Ngom, Dr. Paly Cissé, Arfang Mané, Baba Sarr, Dr. Ibrahima Sarr, Dr. Serigne Tacko Kandji, Dr. Imam Cherif Mohamed Habib Kane and all my colleagues at the Directorate of Veterinary Services, the Directorate of Plant Protection and the Ceres/Locustox Foundation is greatly acknowledged.

I thank all my fellow students at ICIPE, the Senegalese community in Nairobi, all my friends in Kenya and all the staff of ICIPE, for all the assistance to me during the study.

I am very thankful to my sponsor, the Ministry for Foreign Affairs of the Netherlands / DSO Programme, for the financial support through the African Regional Postgraduate Programme in Insect Science (ARPPIS) of ICIPE.

Last but not least, I am very grateful to my wife Fatou Seck for successfully managing the family during my long absence from Senegal.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Page

LIST OF ACRONYMS AND ABBREVIATIONS

AAAP	Attraction-Aggregation-Attachment Pheromone
ANOVA	Analysis of Variance
ARQU	Animal Rearing and Quarantine Unit
CO_2	Carbon Dioxide
DF	Degree of Freedom
F	F value
ICIPE	International Centre of Insect Physiology and Ecology
ITM	Integrated Tick Management
KARI	Kenya Agricultural Research Institute
NVRC	National Veterinary Research Centre
NCE	Neem Cake Extracts
SAS	Statistical Analysis System
SE	Standard Error
SNK	Student-Newman-Keuls

ABSTRACT

The livestock tropical bont tick, *Amblyomma variegatum* Fabricius, is a pest of major economic importance in Africa where it is the vector of *Cowdria ruminantium* that causes Heartwater, disease in cattle. Its control has relied mainly on commercial acaricides, which have many problems associated with, environmental pollution, development of resistant strains of ticks against acaricides and escalating costs. It has, therefore, become necessary to look for alternative methods of tick control, which are environmentally friendly, relatively cheap and which involve farmers directly. In this regard, the purpose of this study investigated the potential of pheromones in attracting ticks to a trap off-host and toxicating them with Neem products and the repulsion of ticks on-host using repellent plant. The use of pheromone components comprising Attraction-Aggregation-Attachment Pheromone (AAAP), 1-octen-3-ol and 2,6-dichlorophenol to attract *A. variegatum* to traps containing botanical extracts on the vegetation was explored. The possibility of enhancing attraction of ticks to AAAP with 1-octen-3-ol and 2,6-dichlorophenol was first investigated in a T-tube olfactometer in the laboratory. Whereas males were attracted to increasing proportions of 1-octen-3-ol, females were repelled. A combination of 8 ng of 1-octen-3-ol with 1.1 mg of AAAP was adopted as an optimum for the attraction of both sexes, enhancing the attraction of ticks by 20% ($p<0.0001$). Unlike 1-octen-3-ol, 2,6-dichlorophenol did not improve the attraction of *A. variegatum* in the laboratory ($p=0.0667$). In the field, the attraction of *A. variegatum* to different doses of AAAP+1-octen-3-ol combination from various distances was investigated. The longest distance from which ticks were significantly attracted was 7 m. The effects of continuous and intermittent release of carbon dioxide (CO_2) on the performance of AAAP and 1-octen-3-ol in attracting ticks in the field were also investigated. The results showed that CO_2 increased the range of attraction to 8 m and the continuous and the intermittent release of CO_2 were not significantly different. The efficacy of traps treated with Neem Cake Extracts (NCE) and baited with AAAP, 1-octen-3-ol and CO_2 was evaluated. The attraction to the traps as well as the mortality of *A. variegatum* were significant ($p<0.0001$). The mortality of attracted ticks was dependent on the concentration of NCE and the time of exposure. 98% mortality was recorded for a concentration of 30% of NCE. The volatiles of two of the predilection feeding sites of *A. variegatum* (scrotum and lower dewlap) were tested against the odour of the ear. The scrotum and the lower dewlap were found very attractive to the ticks. The possibility of using plant extracts with repellent properties in reducing the number of ticks arriving and attaching at predilection sites was also investigated. *Boscia senegalensis*, *Ocimum suave* and *Ocimum kilimandscharicum* were tested as repellents. *Ocimum kilimandscharicum* was found to repel adult *A. variegatum* by 33%. This technology of using botanical traps baited with pheromones and CO_2 as well as repellent plants could be improved and can be transferred to farmers where it can be incorporated in an Integrated Tick Management Strategy.

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Ticks constitute the most important livestock pest in Africa. They are found in the entire African continent, causing enormous production losses (Punyua, 1992). Ticks affect around 800 million cattle and a similar number of sheep throughout the world (Sutherst *et al.*, 1982). The major tick-borne diseases of livestock are theileriosis (transmitted by *Rhipicephalus spp.*, *Hyalomma spp.* and *Amblyomma spp.*), babesiosis (transmitted by *Boophilus spp.),* anaplasmosis (transmitted by *Boophilus spp, Dermacentor spp.*) and cowdriosis or heartwater (transmitted by *Amblyomma spp).* Moreover, ticks may cause toxicosis that is pathological or pathophysiological by inoculating non-infectious substances during feeding on man and animals (Gothe, 1984). Dermatophilosis, associated with *Amblyomma* infestations, is also an important livestock disease (Barré, 1997).

Economic losses due to East Coast fever alone, which is transmitted by *Rhipicephalus appendiculatus*, have been estimated at US$ 200 million annually (Lewis, 2001). In Senegal, cowdriosis causes major losses on the exotic and indigenous animals. The cattle imported for milk, sheep and goats are mostly affected by this disease (Guèye *et al.,* 1993a). Apart from mortality, tick infestation causes serious economic losses in cattle, e.g. reductions in growth rates, reduction in milk production, damaged skins and others (Norval *et al.*, 1992a).

Pastoralists depend heavily on their herds for food supply, social standing and commercial purposes while peasant farmers rely on their livestock for milk, ploughing and as a source of cash income (Norval *et al.*, 1992a). It is therefore important to control ticks.

The control of ticks in Africa, as in other continents, has relied mainly on commercial acaricides since their introduction in South Africa in 1890 (Kaaya, 1994). The use of synthetic chemical acaricides is associated with many problems including environmental pollution, development of resistant strains of ticks, escalating costs (Dipeolu, 1991) and contamination of food with toxic residues (Norval et al., 1992a). Several acaricides have been withdrawn from the market due to toxicity and tick resistance (Kaaya, 1994). It is estimated that Africa spends about US$ 720 million annually to import acaricides (Kaaya, 1994). Even when purchased, other logistical, economic and social problems make their use in communal dips very inefficient. For instance, in some countries, it has been estimated that only 3% of cattle dips are functional at any time (Kaaya, 1994). It has, therefore, become necessary to look for alternative methods of tick control which are environmentally friendly, relatively cheap and which directly involve farmers in tick management (Mwangi *et al.*, 1995a).

Acaricides have therefore to a large extent failed to control ticks and for this reason alternative tick control strategies have been sought (Maranga, 1998). These alternative tick control strategies may be incorporated into an Integrated Tick Management (ITM) system, which is now thought to be the most effective way of controlling livestock ticks (Maranga, 1998).

1.2 LITERATURE REVIEW

1.2.1 Ticks

Ticks belong to the Phylum Arthropoda, Class Arachnida, Order Acarina and Suborder Ixodoidea (Nyindo, 1992). There are two major families of ticks, Argasidae (soft ticks) and Ixodidae or hard ticks (Nyindo, 1992; Maranga, 1998). The Ixodidae family has 13 genera (Okello-Onen *et al.*, 1999): *Amblyomma*, *Rhipicephalus*, *Haemaphysalis*, *Dermacentor*, *Hyalomma*, *Boophilus*, *Ixodes*, *Aponomma*, *Anomalohimalaya*, *Cosmiomma*, *Margaropus*, *Rhipicentor* and *Nosoma*.

The life cycle of ticks consists of eggs, larvae which have 3 pairs of legs, nymphs with 4 pairs of legs and adults with 4 pairs of legs (Nyindo, 1992). According to their life cycle, Ixodid ticks may be divided into three groups; one-host ticks if they complete their life cycle on one host. Only the engorged and mated female drops off to lay eggs on the ground. Two-host ticks require two hosts: the intermediate stages (larvae and nymph) are found on the same host. The larva molts to nymph on the host and stays on it. The nymphs feed on the same host as the larva and engorged nymph drops off and molts to adult on the ground, which seeks a new host (Nyindo, 1992). Thirdly, three-host ticks; each instar leaves its host after engorging and molts on the ground to the next instar which seeks another host. The intermediate stages of some of 3-host ticks feed on rodents, reptiles or birds in the wild while the adult stage seeks large mammals such as cattle or sheep (Nyindo, 1992).

Ticks in the genera *Amblyomma* are three-host ticks with 102 species described (Okello-Onen *et al.*, 1999). The *Amblyomma* species differ in life cycle, hosts, distribution, predilection sites and transmitted diseases (FAO, 1998). The two most important species are *A. variegatum* and *A. hebraeum*. Other species of significance are *A. americanum*, *A. cajennense* and *A. maculatum* (FAO, 1998).

An *A. variegatum* female produces up to 20,000 eggs that hatch between 4-13 weeks and then the larvae engorge within 4-20 days before molting in 2-7 week time while the nymphs engorge within 5-20 days and molt in 14-60 days. Adult females engorge within 10-20 days. The adult feeds on many species of mammals while larvae and nymphs feed mainly on birds, reptiles and small mammals (FAO, 1998).

Amblyomma variegatum is widely distributed in southwestern Africa, Ethiopia, southern Africa (Figure 1.1) and Caribbean where the tropical bont tick has been introduced to from Africa during the last centuries to the islands of Indian Ocean and also to numerous islands of the Lesser Antilles and Puerto Rico (Uilenberg *et al.*, 1984).

In Africa, many studies showed the predominance of *A. variegatum*. Walker and Koney (1999), studying the tick population of Ghana found *A. variegatum* in all samples from sheep, goats and cattle. Tick fauna of Ghana is similar to those of other West African countries (Walker and Koney, 1999).

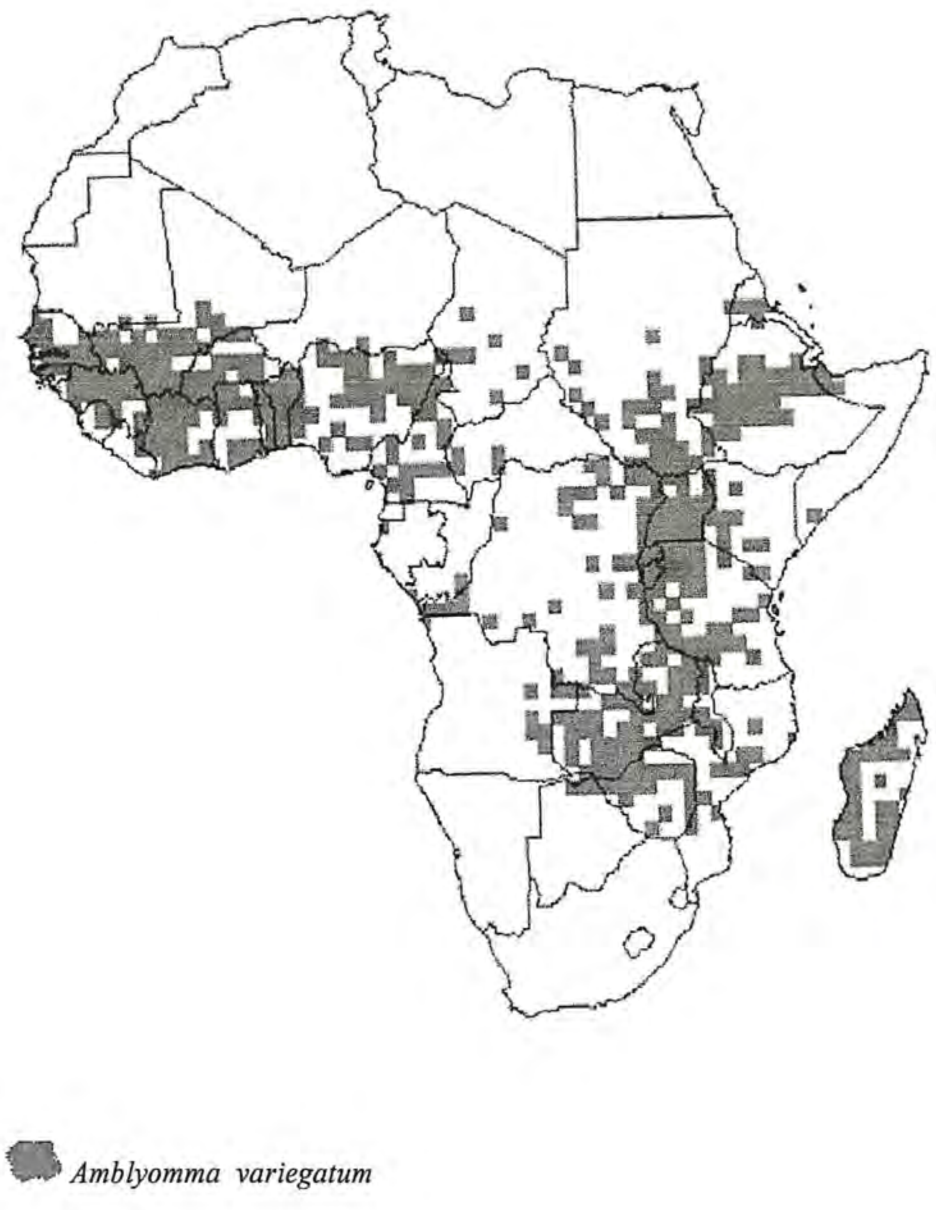

Figure 1.1: Distribution of *Amblyomma variegatum* in Africa (Walker *et al.*, 2003)

In Senegal, *A. variegatum* is abundant and well distributed, mainly in the zone of Niayes, Guinean zone and South-Soudanian zone (Guèye *et al.*, 1993b, 1993c). It was found in the Sahelian area of Senegal before the drought period (Guèye *et al.*, 1987). *Amblyomma variegatum* is the predominant tick species in Cameroon followed by *Rhipicephalus lunulatus*, *Hyalomma sp*. and *Boophilus sp*. (Tawah, 1992). *Amblyomma variegatum* is, hence, one of the major parasitic ticks of cattle in Africa and cattle are the most important domestic hosts for all stages of its life cycle (Macleod *et al.,* 1977).

In general, most ticks found on the domestic hosts attach to the front parts, below the body midline, whereas, on the natural hosts, 60% of the ticks attach to the hindquarters (Spickett and Heyne, 1988). In the case of *A. variegatum*, adults and nymphs attach mainly in the ventral surface of the host including the lower dewlap, brisket, abdomen, axillae and genitalia (Macleod *et al.*, 1977; Dioli *et al.*, 2001). Abiotic factors such as moist and warmer conditions could explain these predilection sites (Kiara *et al.*, 1994).

The importance of *A. variegatum* ticks in Africa has stimulated research into methods of controlling them. This present study aimed at contributing in building a new environmentally friendly technology for controlling *A. variegatum* ticks.

1.2.2 Tick control

1.2.2.1 Conventional methods

There are many types of conventional tick control methods (Nyindo, 1992). Arsenic dips were the first effective method for controlling ticks and tick-borne diseases and were used in many parts in the world (George, 2000). The dips method is fast and effective but the dip tanks used are costly to build (Maranga, 1998) and requires proper supervision to keep the concentration of the dip optimum (Nyindo, 1992). Hand spraying is also another way of applying acaricides, which is suitable for small scale farmers and effective in keeping animals free from ticks (Nyindo, 1992).

Systemic acaricides, mainly the macrocyclic lactone endectocides such as moxidectin, ivermectin, and eprinomectin in many formulations are also tried in the control of ticks and their efficacy is well documented. Guglielmone *et al.* (2000) demonstrated that 0.5% moxidectin pour-on provided over 95% control of *Boophilus microplus* for 25 naturally infected heifers between days 7 to 21 post infection, as compared to a similar group of untreated heifers. The oral or pour-on administration is more convenient for the farmers. There are also alternative ways of administrating acaricides such as injection and collars, which are devices impregnated with acaricides. Impregnated collars with flumethrin and propoxur were found efficient against adult *R. sanguineus* on dogs (Fourie *et al.*, 2003).

Since their introduction into South Africa, acaricides have been the commonest method of tick control in Africa, leading to many problems such as development of resistant tick strains and escalating costs (Dipeolu and Ndungu, 1991; Kaaya, 1992). It has been demonstrated *in vitro*, using a known permethrin resistant strain of Mexican *B. microplus*, an increase in acaricide resistance in five generations (Davey and George, 1998). The results were then evaluated by treating heifers infested with the resistant strain in permethrin dips and no difference was found in tick numbers, reproductive value index and biological parameters between the treated and untreated groups. In South Africa, the resistance of *B. decoloratus* against some acaricides such as chlorfenvinphos and cypermethrin has been also reported (Mekonnen *et al.*, 2002). In addition, acaricides are an enormous financial burden, mainly for African countries. It is estimated that Africa spends US$ 720 million annually for importing acaricides (Odhiambo, 1992).

It is, therefore, necessary to look for alternative methods of control of ticks, which are environmentally friendly and economically affordable.

1.2.2.2 Alternative control methods

1.2.2.2.1 Traditional practices

Barré (1988) reported that some pasture and herd exploitation methods such as soil purification could be applied successfully to decrease cattle infestation with *A. variegatum*. This method can be obtained by temporary over-grazing followed by a "trap" animals treatment. This purification can be maintained by limiting grazing to a 6 pm-6 am period, when very few engorged ticks drop from hosts and infest pastures and when very few flat ticks are active in locating hosts.

Bush burning is also reported to be used by farmers for controlling ticks (Fasanmi and Onyima, 1991). Farmers in Nigeria believe that burning surrounding bush would reduce the menace of tick infestation by burning of the eggs of the tick, as well as eliminating possible intermediate host for pests and diseases (Adekunle *et al.*, 2002). Hand-picking has been also reported as a traditional method for controlling ticks (Fasanmi and Onyima, 1991; Mekonnen *et al.,* 1992).

Pasture spelling is another traditional means of controlling ticks (Wilkinson, 1957; Kaaya, 1992). It consists of denying hosts to free living larvae. Nevertheless, pasture spelling or rotational grazing is recommended for established and organized farms with the necessary capital and management skill (Fasanmi and Onyima, 1991; Kaaya, 1992). Pasture spelling may also be ineffective in ticks with longer life cycles (Kaaya, 1992) like *R. appendiculatus*, whose adults can survive in the pastures as long as 18 months (Mwangi, 1990). These traditional practices could be associated to pheromones and plants-based methods, which attract and kill the ticks on the ground and are more practical for small scale farmers. These pheromones and plants-based methods are also appropriate to the African 3-host ticks, which spend 95-97% of their life time in the vegetation (Punyua, 1992),

1.2.2.2.2 Host resistance

Many workers have reported several cases of host resistance in response to tick infestation. Solomon and Kaaya (1998) concluded that *A. variegatum* and *B. decoloratus* fed on Boran x Friesan crossbred cattle showed higher survival than ticks fed on the two indigenous cattle breeds. Norval (1992), studying *Amblyomma hebraeum* on cattle, found that *Bos indicus* and *B. indicus* x *B. taurus* breeds of cattle became less heavily infested than *B. taurus* breeds. The indigenous cattle are more resistant to ticks than the crossbred animals. Stachurski (1993) reported a great variability of the individual infestations by *A. variegatum* adults on naturally infested Gudali zebus. Some of the animals had a tick burden 10 to 16 times higher than that of the least parasitized cattle of the herd. The selection of resistant breeds could be used as a component of tick control strategy. Tick control is greatly facilitated by the high degree of natural resistance possessed by indigenous cattle (Chhabra, 1992).

Vaccination is the most promising and well developed non-chemical control of ticks (Jonsson, 1997). Workers in Australia have developed a commercial vaccine, which contains a recombinant Bm86 antigen preparation, derived from a glycoprotein of the tick's gut (Willadsen *et al.*, 1995). The results of the trials carried out with the product showed 20% to 30% reduction in the number of females engorging, 30% reduction in the weight of engorging ticks and 60% to 80% reduction in the weight of eggs laid per gram of female ticks. The effect of vaccination with TICKGARD (Bm86) against *B. microplus* has been also reported by Jonsson *et al.* (2000) who found a 56%

reduction in tick numbers in the field over one generation. These results were confirmed later by Patarroyo *et al.* (2002) who reported that synthetic peptides derived from the Bm86 glycoprotein had a high efficacy of 81.05% in immunizing cattle against *B. microplus*. The efficacy of the Bm86 has also been assessed in *B. microplus* and other tick species by De Vos *et al.* (2001) who conducted vaccine trials in cattle using TICKGARD and found a reduction of reproductive capacity of 74% for *B. microplus* and 70% for *B. decoloratus* and a reduction of 95% in the number of nymphs engorging for *H. dromedarii*.

The Bm86 vaccine failed to protect cattle against *R. appendiculatus* and *A. variegatum* ticks (De Vos *et al.*, 2001), demonstrating the need in developing multivalent anti-tick vaccines and new innovative technology such as pheromones and plants against ticks.

1.2.2.2.3 Botanicals

The use of anti-tick botanicals is another way of improving control methods of ticks in an Integrated Tick Management Programme. Many plants have shown acaricidal and repellence properties. Mwangi *et al.* (1995a), reported a study on the climbing behaviour of *R. appendiculatus* on *Melinis minutiflora* where all instars of the tick avoided climbing on the green *M. minutiflora* whereas most larvae, nymphs and adults climbed on the control plant (*Pennisetum clandestinum*), due to the long trichomes on the stems and the leaves and a strong volatile, which is repulsive to the ticks (Mwangi *et al.*, 1995a). A 5% concentration of *M. minutiflora* extracts in petroleum jelly applied on rabbit ears prolonged feeding periods of all instars of *R. appendiculatus* (Mwangi *et al.*, 1995a).

Thompson *et al.* (1978) reported in field studies on *B. microplus* that *M. minutiflora* and *Andropogon gayanus* reduced the tick populations in 14 days. Many other grasses of the genus *Stylosanthes* are reported to have anti-tick properties. Zimmerman *et al.* (1984) observed that *S. viscosa* and *S. scabra* reduced the survival of *B. microplus* larvae and *A. variegatum* larvae and nymphs. Anti-tick grasses such as *Stylosanthes spp*. and *M. minutiflora* could be planted in pastures to immobilize and kill ticks in vegetation, thus reducing the numbers of ticks attaching on cattle (Kaaya, 2000).

Other botanical extracts are also reported to have anti-tick properties. Mwangi *et al.* (1995b) observed that an oil extract from the leaves of a tropical shrub *Ocimum suave* repelled as well as killed all stages of *R. appendiculatus*. A 10 % solution of this oil protected rabbits for 5 days against attaching larvae and killed all immature and more than 70 % of adults. The repellence of the essential oil of the shrubs *Cleome monophylla* and *C. hirta* when evaluated against *R. appendiculatus* showed a high level of repellence on *R. appendiculatus* (Ndungu *et al.*, 1995a, 1995b; Lwande *et al.*, 1999). The shrub *Gynandropsis gynandra* was evaluated against *R. appendiculatus* and the repellency observed was higher than that of the commercial arthropod repellent *N,N*-diethyltoluamide (Lwande *et al.*, 1999).

Plants of the family Meliaceae have also been evaluated against many tick species. Seed oil of *Azadirachta indica* has been reported to kill *A. variegatum* larvae (Ndumu *et al.*, 1999), larvae of *R. pulchellus* (Ismail *et al.*,

2002) and larvae and adult of *Hyalomma dromedarii* ticks (DiefAlla *et al.*, 2003). The effect of *Melia azedarach* has been also assessed on *B. microplus* larvae and females (Borges *et al.*, 2003). The larvae were killed while *Melia azedarach* extracts inhibited partially or totally egg production and embryogenesis in the adult females.

A tick control strategy using these plants would be effective and affordable to many resource poor farmers in Africa and elsewhere since the seeds of grasses and other anti-tick plants are inexpensive and the technology is simple and easy to apply (Kaaya, 2000). Farmers would also easily adopt the technology since most of them are already using one or more of these traditional methods of tick control (Kaaya, 2000).

1.2.2.2.4 Biological control

Biological control is one of the non-chemical methods, which consists of the use of parasitoids, pathogens or predators. Parasitoids such as *Ixodiphagus hookeri* have been shown to be effective against *A. variegatum* (Mwangi *et al.*, 1997) in a field trial. The engorged nymphs declined by 95% within 4 months after the release of 150,000 *I. hookeri.* The adult tick population started to decrease 6 to 7 months after the parasitoid releases began and this could be attributed to the long life of the adult ticks that had been in the environment (Knipling and Steelman, 2000). Hu *et al.* (1993) reported also the effectiveness of this parasitoid in *Ixodes dammini*. Whereas much is unknown about tick parasitoids, the evidence is strong that released parasitoids could achieve effective control of tick populations (Knipling and Steelman, 2000). Much research needs to be done in this field since it has been reported that the nymphs of *R. appendiculatus* were not infected by the parasitoid (Mwangi, 1990) and because of its life cycle, *B. microplus* is probably unsuited to control by this method (Jonsson, 1997).

Various predators of ticks have been reported with birds being the most common (Kaaya, 1992). Holm and Wallace (1989) reported predation of *Anystis baccarum* which fed upon the larvae of the cattle tick *B. microplus*. Six groups of animals are confirmed to be predators of free-living engorged female of *R. appendiculatus*: ants (*Pheidole megacephala*), rodents (*Mastomys natalensis, Lemniscomys striatus*), birds (*Motacilia aguimp, Spreo superbus*), lizards (*Mabuya striatus*), shrew (*crocidura nigrofusca*) and spiders have been reported a s predators (Mwangi *et al.*, 1991). Barré *et al.* (1991) reported in Guadeloupe (French West Indies) other animal species that are predators of the tick *A. variegatum*, among them the most effective predator was the tropical fire ant, *Solenopsis geminata.* Birds (cattle egret), mice (*Mus musculus*) and insects (*Megaselia scalaris*) were also found to feed on ticks (Barré *et al.*, 1991). Predation of ticks by domestic chicken has also been reported (Hassan and Dipeolu, 1993).

Fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* and a spectrum of bacteria are reported to be pathogenic to some tick species. Kaaya and Hassan (2000) found in the field that fungal oil formulations (10^9 conidia per ml) induced 100% mortality in larvae of *R. appendiculatus* and *A. variegatum* when ticks were sealed in nylon tetrapacks and infected with the entomogenous fungi, *Beauveria bassiana* and *Metarizium anisopliae* and maintained in potted grass. Mortalities in nymphs varied between 80-100% and in adults 80-90%. Maranga (1998) investigated *B. bassiana* and *M. anisopliae* in fungi/pheromones traps on *A. variegatum* in the field and recorded 79% tick mortality for ticks exposed to fungi in the field using the trap and then incubated in the laboratory. The mortality was 66.2% for ticks which were exposed to the fungi in the field and then left in the vegetation for three weeks.

Fungi could be a suitable biocontrol agent but their safety to non-target organisms needs to be investigated (Maranga, 1998). The self propagation of fungi isolates could make difficult their safe handling by farmers who are already using plants in the control of ticks.

1.2.2.2.5 Pheromones

Pheromones are compounds released by individuals of a species and regulate the behaviour of other animals of the same species (Kalson and Luscher, 1959; Sonenshine, 1984). Three types of pheromones are identified, which include sex pheromones, which are produced by fed females of hard ticks and are attractive to males. Berger (1972) first identified from the female Lone Star tick, *Amblyomma americanum*, 2,6-dichlorophenol, which is believed to be a sex pheromone of this tick. Later workers (Chow *et al.*, 1975), in a study on *Rhipicephalus sanguineus*, confirmed 2,6-dichlorophenol as a sex pheromone. A female *R. sanguineus*, produces 2-3 ng of 2,6-dichlorophenol (Chow *et al.*, 1975). A study on *Dermacentor variabilis* revealed that 2,6-dichlorophenol also acts as an attachment stimulant for this tick (Yoder and Stevens, 2000).

The second category of pheromones is the assembly pheromones, which induce clustering of ticks in natural habitats. They are the most common of all chemical cues utilized by ticks (Sonenshine, 1991). They are interspecific, affect all life stages (Sonenshine, 1985; 1991) and provoke ticks to cease movement and cluster in groups, hence their name. The aggregation of ticks occur in natural holds in their environment, such as cracks, crevices, ledges, caves and nests of rodents or burrowing animals (Sonenshine, 1985; 1991). These assemblies are most likely in response to ensure survival of the population, from preventing desiccation. Large numbers would also ensure the recruitment of sexual partners for mating (Petney and Bull, 1981). Moreover, clustering of ticks in animal nests would promote host contact and allow for blood meals to be taken (Sonenshine, 1991). The Attraction-Aggregation-Attachment Pheromone (AAAP) attracts ticks to the site of feeding individuals and induces them to aggregate and attach around the site (Rechav *et al.*, 1976).

The AAAP is produced by feeding males of *Amblyomma spp*. It induces unfed conspecific female and male ticks to migrate, aggregate and attach around the emitting source. After initial attachment, males must feed for about four days before becoming sexually mature at which time they start to emit AAAP which is necessary for the attachment of unfed females (Norval and Rechav, 1979; Rechav *et al.*, 1976). Adult males of the bont tick, *A. hebraeum*, produce AAAP after feeding on a host for approximatively seven days (Rechav *et al.*, 1977).

Male *Anocentor nitens* (Acari: Ixodidae) were found to be attracted to females that reached sexual maturity after six days of feeding (Borges and Ribeiro, 1999). Rechav *et al.* (1976, 1977) showed significant attraction of *A. hebraeum* nymphs by fed males or hexane extract of fed males.

Another volatile, 1-octen-3-ol, contributes to the aggregation response of *Amblyomma spp* (McMahon *et al.*, 2000). These workers found 1-octen-3-ol in volatiles of various life-stages of both *A. variegatum* and *A. hebraeum*. 1-octen-3-ol was also found in unfed *A. variegatum* adults on a servosphere (McMahon *et al.*, 2000) and is common in cattle breath (Hall *et al.*, 1984) where it acts as an important kairomone for tsetse flies and other bloodfeeding insects, including mosquitoes, ceratopogonids, tabanids (French and Kline, 1989).

Host searching behaviour by unfed bont ticks is stimulated by carbon dioxide (Norval *et al.*, 1987, 1989a; Norval, 1992; Yunger *et al.*, 1992; Maranga, 1998). Other arthropods such as *Argas cooleyi* (Howell, 1975), *Ornithodoros turicata* (Olusola and Jerry, 1991), mosquitos (Kline *et al.*, 1991) are also stimulated by carbon dioxide. Kline *et al.* (1991) reported that the effect of CO_2 is increased by 1-octen-3-ol and that the effect of CO_2 and octenol was additive for *Culex spp.* and *Diachlorus ferrugatus* and synergistic for *Aedes taeniorhynchus*.

The AAAP produced by males of *A. variegatum* after a six-day feeding, is composed of ortho-nitrophenol, methyl salicylate and nonanoic acid in the ratio of 2: 1: 8, one tick producing 11 micrograms (Schöni *et al., 1984*). 2,6-dichlorophenol is produced by *R. sanguineus* at 2-3 ng per female

(Chow *et al.*, 1975). 1-octen-3-ol is detected over freshly moulted ticks at levels of 1 ng per tick (McMahon *et al.*, 2000).

Pheromone(s)-acaricides mixtures have been studied for use in the control of tick (Norval *et al.*, 1991). These authors combined a pheromone (AAAP) and different acaricides: amitraz (amidine), flumethrin (synthetic pyrethroid) and chlorfenvinphos (organophosphate). The results showed flumethrin as the compound of choice for use in pheromone/acaricide mixtures. Gladney *et al.* (1974) applied a mixture of aggregation-attachment pheromone of *A. maculatum* and the insecticide Isobenzan to a specific site on a bovine. Female *A. maculatum* were attracted to the pheromone-baited site, attached and were killed by the insecticide. Rechav and Whitehead (1978) reported that body sites on cattle treated with a mixture of fed male *A. hebraeum* extract and the insecticide toxaphene attracted and killed adults and nymphs of this species. The mixture was effective for 4 days in both laboratory and field trials.

The use of fungi, instead of chemical acaricides, has been investigated (Maranga, 1998). Using AAAP with CO_2 and studying a suitable control method of *A. variegatum*, this author found that it is possible to attract up to 79% of released *A. variegatum* from a radius of 5 m to a point where they were exposed to fungal infections of *Metarhizium anisopliae and Beauveria bassiana* in a special device. These fungi/pheromones traps on *A. variegatum* in the field recorded 79% tick mortality for ticks exposed to fungi in the field using the trap and then incubated in the laboratory. The mortality was 66.2% for ticks which were exposed to the fungi in the field and then left in the vegetation for three weeks.

These previous investigations combined either commercial acaricides or fungi to AAAP, while this present study aimed in testing the efficacy of anti-tick plants in traps baited with AAAP, 1-octen-3-ol and CO_2 and then in increasing the performance of AAAP of attracting and killing ticks.

1.3 JUSTIFICATION FOR THE STUDY

Amblyomma variegatum is one of the most important ticks in Africa and is well distributed in cattle rearing regions. It is a widely distributed species in Africa, occurring across the continent from Senegal through West Africa into the Central African Republic, southern Sudan and Ethiopia. It is prevalent in most of eastern Africa (Kenya, Uganda, Tanzania, Rwanda and Burundi) as well as Malawi, Zambia, Democratic Republic of Congo, eastern Angola, Namibia, north eastern Botswana, Zimbabwe and Mozambique (Norval *et al.*, 1992a, Walker *et al.*, 2003).

Amblyomma variegatum transmits heartwater, a killer of livestock in Africa. The escalating acaricides cost, the problem of tick resistance to the chemical acaricides, their environmental pollution as well as their residual contamination of milk and meat, call for new alternative methods of tick control. A number of these methods are used in Integrated Tick Management (ITM) programs: farm management practices, host resistance and immunization, genetic control, biological control (predation, parasitoids, and pathogens), pheromones and the potential use of botanical extracts. Nonetheless the effectiveness of this ITM programme has to be improved and the aim of this proposed study was to contribute to this by developing and testing a specific and effective technology which will enable farmers to control ticks.

Most of the tick control effort has been directed on the parasitic stages on their mammalian hosts. However, the African 3-host ticks spend 95-97% of their time in the environment/vegetation (Punyua, 1992). It is, therefore, important to develop effective and environmentally friendly methods of controlling both the free tick stages in their environment and the on-host ticks. The purpose of this study was to investigate the potential use of pheromones (the attraction-aggregation-attachment pheromone, 1-octen-3-ol and 2,6-dichlorophenol) and botanical extracts for the control of *A. variegatum*.

1.4 HYPOTHESES

(a) 1-octen-3-ol and 2,6-dichlorophenol cannot enhance the response of *Amblyomma variegatum* to the Attraction-Aggregation-Attachment Pheromone (AAAP).

(b) Host volatiles do not play a key role on the orientation process of *Amblyomma variegatum*.

1.5 OBJECTIVES OF THE STUDY

1.5.1 Main objective

The main objective of this study was to develop and optimize methods for tick control by integrating host / site-searching behaviours of *Amblyomma variegatum* and phytochemicals with acaricidal or repellent properties.

1.5.2 Specific objectives

(a) To investigate the response of *A. variegatum* to combinations of 1-octen-3-ol and 2,6-dichlorophenol with AAAP.

(b) To investigate the effect of intermittent release of carbon dioxide on the attraction of *A. variegatum to* AAAP with/without 1-octen-3-ol or 2,6-dichlorophenol.

(c) To develop a suitable formulation of plants extracts that may be used in the control of *A. variegatum* in traps baited with AAAP, 1-octen-3-ol and CO_2.

(d) To study pathways used by *A. variegatum* to locate the preferred feeding sites on-host and the signals involved.

(e) To investigate the possibility of using botanical repellents to prevent *A. variegatum* from locating their predilection sites.

CHAPTER 2

MATERIALS AND METHODS

2.1. TICKS

The unfed adult *Amblyomma variegatum* ticks (Plate 2.1) used were obtained from the Animal Rearing and Quarantine Unit (ARQU) of the International Centre of Insect Physiology and Ecology (ICIPE) and were between 2 to 3 months of age. The ticks were reared on adult naïve New Zealand white rabbits.

Plate 2.1: *Amblyomma variegatum* adult male (A) and female (B)

2.2. RABBITS

Naive adult New Zealand white rabbits were obtained from ARQU. They were kept in rabbit cages and fed on commercial pellets and clean piped water. The rearing procedure was as described by Kubasu (1992; 1997), Kubasu *et al.* (1992) and Kiara *et al.* (1994).

2.3. PHEROMONES

2.3.1 The Attraction-Aggregation-Attachment pheromone (AAAP)

The synthetic AAAP was obtained from SIGMA-ALDRICH Chemical Company Limited of the Old Brickyard-New Road Gillingham-Dorset SP8 4XT (England). The AAAP consists of three chemical products: ortho-nitrophenol, methyl salicylate and nonanoic acid. The mixing of these components was carried out as described by Schöni *et al.* (1984) and Maranga (1998). The AAAP components were then prepared by mixing them at a ratio: 2:1:8, for ortho-nitrophenol, methyl salicylate and nonanoic acid, respectively. First, 200 mg of ortho-nitrophenol was weighed using a balance, after which it was placed in clean dry universal bottle. Then, 85 μl an equivalent of 100 mg of methyl salicylate and 883 μl of nonanoic acid (an equivalent of 800 mg of nonanoic acid) were added to the ortho-nitrophenol. The volume of methyl salicylate and nonanoic acid was calculated from the relation: Volume = Mass/Density. This mixture was then dissolved in 1 ml of dichloromethane that was used as the solvent and this formed the AAAP stock solution. One micro liter of this solution contains 0.2 mg of ortho-nitrophenol, 0.1 mg of methyl salicylate and 0.8 mg of nonanoic acid. The AAAP was used at 1.1 mg. The use of this dose is based on responses observed by Maranga (1998).

2.3.2 1-octen-3-ol

1-octen-3-ol was dissolved in dichloromethane (McMahon *et al.*, 2000). Varying octenol doses were added to AAAP (Table 3.1)

Table 3.1: Doses of AAAP and 1-octen-3-ol used in the laboratory

Doses	AAAP	1-octen-3-ol (ng)
D0	1.1 mg	0
D1	1.1 mg	5
D2	1.1 mg	6
D3	1.1 mg	7
D4	1.1 mg	8
D5	1.1 mg	9
D6	1.1 mg	10
D7	1.1 mg	11
D8	1.1 mg	12
D9	1.1 mg	13
D10	1.1 mg	14
D11	1.1 mg	15

2.3.3 2,6-dichlorophenol

This chemical product was dissolved in dichloromethane at ratio: 1:10. The use of 10 % solution of 2,6-dichlorophenol is based on responses observed in an olfactometer (Yunger *et al.*, 1992; Norval *et al.*, 1991).

Varying doses of 2,6-dichlorophenol were added to AAAP (Table 3.2)

Table 3.2: Doses of AAAP and 2,6-dichlorophenol used in the laboratory

Doses	AAAP	2,6-dichlorophenol (mg)
D0	1.1 mg	0
D1	1.1 mg	7.5
D2	1.1 mg	10
D3	1.1 mg	12.5
D4	1.1 mg	15
D5	1.1 mg	17.5
D6	1.1 mg	20
D7	1.1 mg	22.5
D8	1.1 mg	25
D9	1.1 mg	27.5
D10	1.1 mg	30
D11	1.1 mg	32.5
D12	1.1 mg	1
D13	1.1 mg	0.1
D14	1.1 mg	0.01
D15	1.1 mg	2
D16	1.1 mg	4

CHAPTER 3

RESPONSES OF *AMBLYOMMA VARIEGATUM* TO VARYING COMBINED DOSES OF AAAP, 1-OCTEN-3-OL AND 2,6-DICHLOROPHENOL IN THE LABORATORY

3.1. INTRODUCTION

The Attraction-Aggregation-Attachment Pheromone (AAAP) attracts ticks to the site of feeding individuals and induces them to aggregate and attach around the site. The potential of pheromones in controlling ticks was first proposed by Gladney *et al.* (1974). Gladney's technique was later used to attract and kill *A. hebraeum* on cattle treated with an extract of a male and an acaricide (Rechav and Whitehead, 1978). Some of the components of the AAAP of *A. variegatum* were identified and their attractiveness tested by Schöni *et al.* (1984). Hess and de Castro (1986), Norval *et al.* (1989a) and Yunker *et al.* (1992) also tested and found attractive some AAAP components on *A. hebreum* or *A. variegatum*.

The AAAP is produced by feeding males of *Amblyomma spp*. They induce unfed conspecific female and male ticks to migrate, aggregate and attach around the emitting source. After initial attachment, males must feed for about four days before becoming sexually mature (Norval *et al.*, 1989b) at which time they start to emit an attraction-aggregation-attachment pheromone (AAAP) which is necessary for the attachment of unfed females (Rechav *et al.*, 1976; Norval and Rechav, 1979).

The AAAP of six-day fed male of *A. variegatum* is composed of ortho-nitrophenol, methyl salicylate and nonanoic acid in the ratio of 2: 1: 8, one tick producing 11 µg (Schöni *et al.,* 1984). Studies carried out have demonstrated strong responses of *A. variegatum* to AAAP (Maranga, 1998). Furthermore it has been reported that 1-octen-3-ol contributes to the aggregation response of *Amblyomma spp* (McMahon *et al.*, 2000). Another volatile, 2,6-dichlorophenol is also reported to be a sex stimulant (Chow *et al.*, 1975; Yoder and Stevens, 2000).

Most of the studies carried out off host investigated only the response of the tick to a single pheromone. The aim of the experiments reported in this chapter was to assess the response of *A. variegatum* to varying combined doses of AAAP, 1-octen-3-ol and 2,6-dichlorophenol.

3.2. MATERIALS AND METHODS

3.2.1 T-tube olfactometer method

The T-tube olfactometer method as described by Maranga (1998) was used. It presents alternative choices to ticks. Tubes used for adult tick bioassays measured 4 x 4 cm with an inner diameter of 1.5 cm. A small filter paper carrying the test material was placed in one arm of the T-tube and a blank having dichloromethane in the other arm. A tick was introduced into the base of the tube and was allowed to climb up the tube to select one of the two arms. The number of ticks (15 unfed adult males and 15 unfed adult females), choosing the test site as well as the blank was recorded. Four replications were done for each dose of pheromone. Various amounts of 1-octen-3-ol and 2,6-dichlorophenol (Table and Table) were mixed with the standard blend of 1.1 mg of AAAP previously shown to be optimum for attraction of *Amblyomma variegatum* in a T-tube olfactometer in the laboratory.

The tick response to each pheromone dose was tested by introducing ticks at the base of the T-tube. The ticks were then allowed to choose between the pheromone(s) and the control (dichloromethane). One tick was investigated at a time. Responses of the male and female ticks were recorded separately. The filter paper carrying the test material and the control was discarded and the T-tubes were washed in detergent and rinsed in dichloromethane after every treatment. The T-tubes were then dried in the oven at 100° C for 30 minutes. All the experiments were carried out in a hood for sucking out air (to avoid accumulation of pheromones in the working area).

3.2.2 Data analysis

Tick responses to various blends of AAAP+1-octen-3-ol (Table) and AAAP+2,6-dichlorophenol (Table) were recorded and entered on Microsoft Excel. Percentage Attraction (PA) values were calculated using the formula: PA = [(Nt - Nc)/ (Nt + Nc)] x 100, where N_t and N_c are number of ticks recorded in treated and control arms of the olfactometer respectively as described by Ndungu *et al.* (1995a). Then all the percentages were subjected to an arc-sine transformation before an Analysis of Variance (ANOVA) was carried out to test for differences in attraction due to dose, sex or their interaction, using the Statistical Analysis System Software (SAS, 1988). When there were significant differences between treatments, Student-Newman-Keuls multiple range tests was applied for separation of means at 0.05 significance level.

3.3 RESULTS

1-octen-3-ol enhanced the attraction of *A. variegatum* to the blend of 1.1 mg of AAAP in the T-tube olfactometer (Table 3.3). 1.1 mg of AAAP attracted up to 53.27% of the ticks while the number attracted to the test material significantly increased to 72.86% when 8 ng of 1-octen-3-ol was added (Table 3.3). The other treatments did not significantly differ from the standard blend of 1.1 mg of AAAP. 8 ng of 1-octen-3-ol gave the highest attraction (72.86±2.7) of *A. variegatum* in the olfactometer (Figure 3.1). Significant differences were noted between some doses in attracting ticks in the T-tube olfactometer (Figure 3.1) and between the sexes of the ticks (3.3). The results showed that whereas the males were attracted to the increasing proportions of 1-octen-3-ol, females were repelled (Figure 3.2; Table 3.3).

The adjunction of 2,6-dichlorophenol to the standard 1.1 mg of AAAP did not improve the attraction of the ticks ($p = 0.0667$), the doses seemed to be repelling the ticks in the olfactometer (Figure 3.4).

Table 3.3: Mean percentage number (±SE) of ticks attracted to the doses of AAAP and 1-octen-3-ol in the olfactometer

Dose	Female	Male	Average
D0	49.7±2.47abA	56.83±2.74bcA	53.26±2.29b
D1	54.86±3.79abA	47.66±4.58cA	51.26±3.11b
D2	57.77±2.47abA	66.77±11.93abcA	62.27±6.04ab
D3	53.33±4.44abA	64.68±6.17abcA	59.014.12ab
D4	68.88±4.44aA	76.83±1.03abA	72.86±2.7a
D5	48.88±4.44abB	69.04±4.76abcA	58.96±5.36ab
D6	49.22±2.40abB	58.88±1.41abcA	54.05±2.49b
D7	55.55±5.87abB	80.03±2.33abA	67.79±6.16ab
D8	55.55±5.87abA	64.34±3.67abcA	59.95±3.67ab
D9	46.66±3.84abB	86.5±3.71aA	66.58±9.22ab
D10	51.11±9.68abA	73.16±6.66abcA	62.13±7.20ab
D11	31.5±7.32bB	75.05±8.6abA	53.27±10.9b

Means (±SE) between doses bearing the same small letters are not significantly different at 0.05 level based on the SNK test.

Means (±SE) within doses bearing the same capital letters are not significantly different at 0.05 level based on the SNK test.

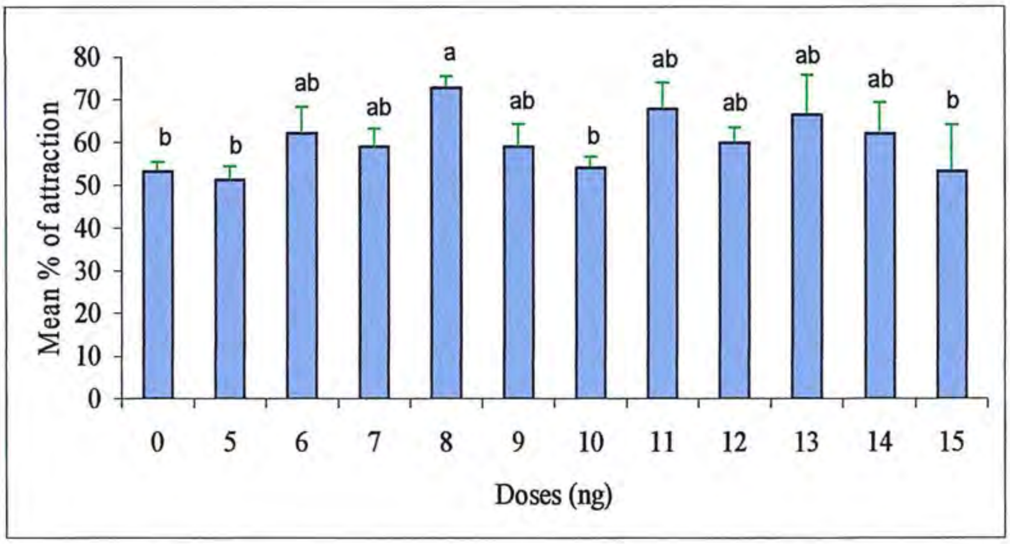

Figure 3.1: Attraction of both sexes of *A. variegatum* to standard AAAP blended with different doses of 1-octen-3-ol. Means (±SE) bearing the same letters are not significantly different at the SNK test ($p \leq 0.05$).

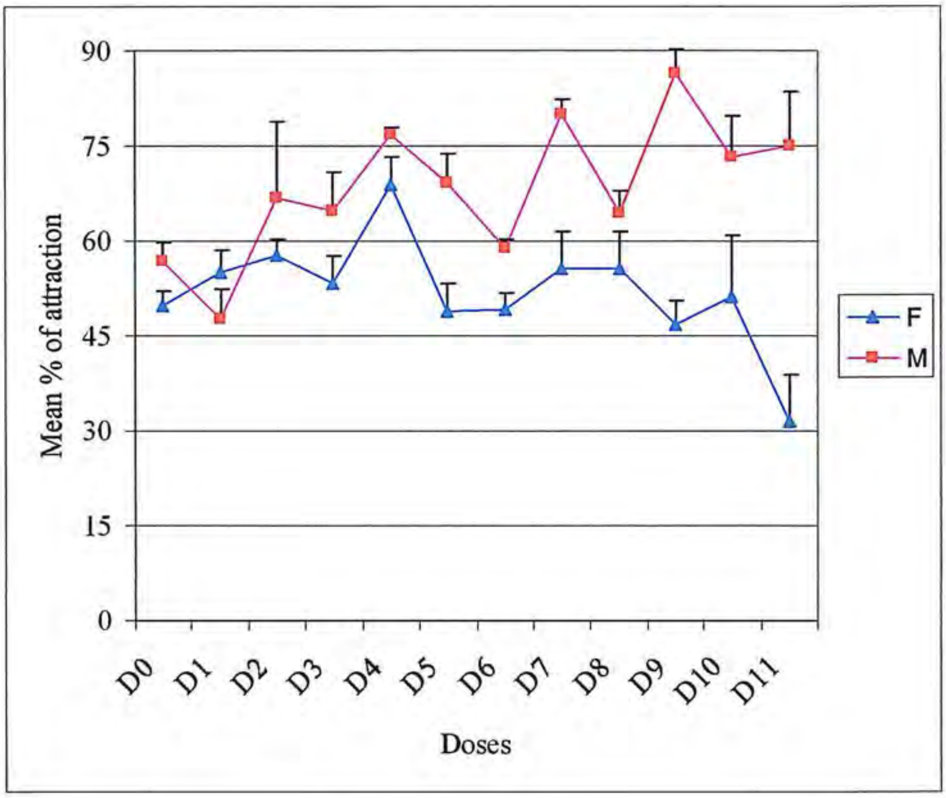

Figure 3.2: Attraction of different sexes of *A. variegatum* to standard AAAP blended with different doses of 1-octen-3-ol.

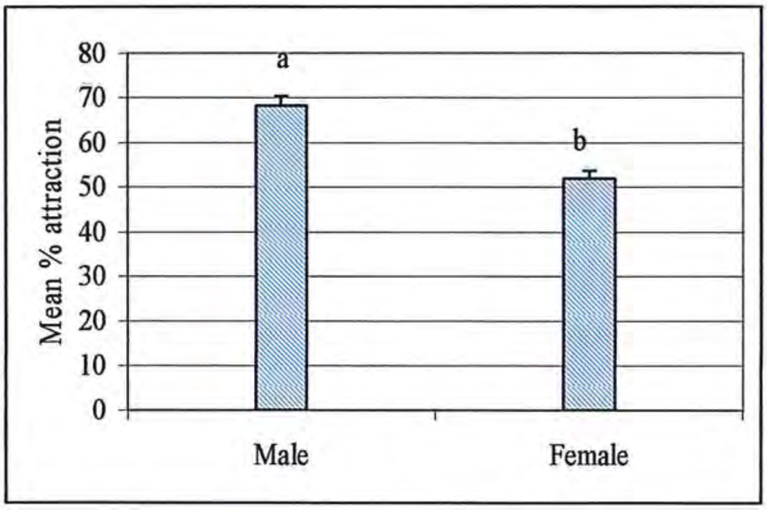

Figure 3.3: Attraction of female and male *A. variegatum* to AAAP blended with 1-octen-3-ol in the laboratory.

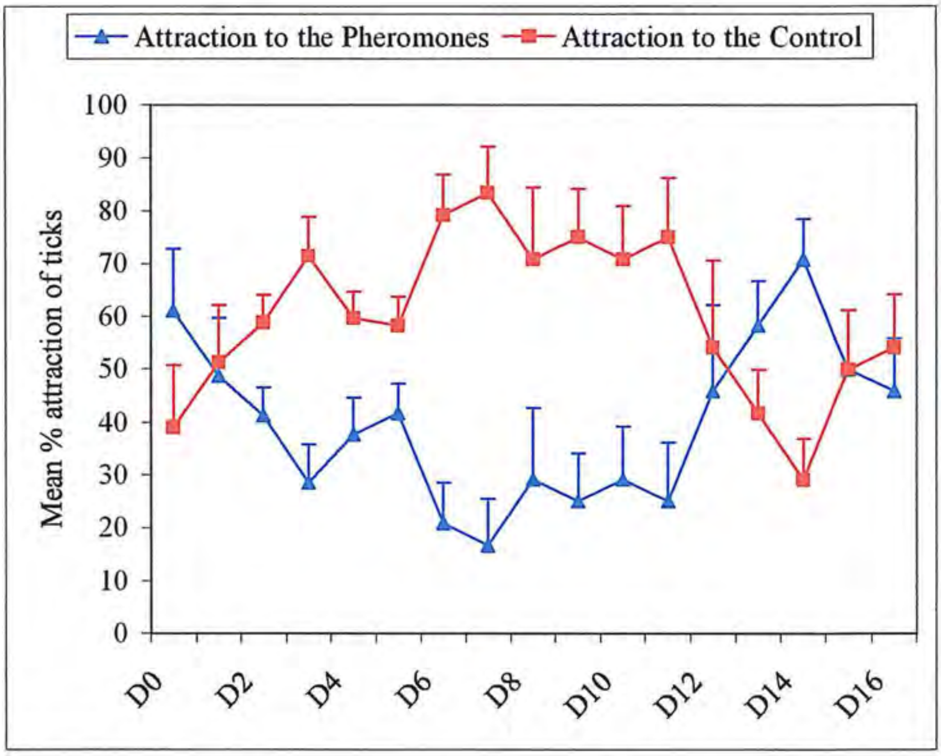

Figure 3.4: Mean (±SE) of the responses of *Amblyomma variegatum* to AAAP and 2,6-dichlorophenol in the olfactometer.

3.4 DISCUSSION

The study involved two compounds, AAAP and 1-octen-3-ol, which is present in volatiles over various life-stages of both *A. variegatum* and *A. hebraeum* (McMahon *et al.*, 2000). Emitted by the host (Hall *et al.*, 1984; Steullet and Guerin, 1994), 1-octen-3-ol is found attractive to unfed *A. variegatum* adults on a servosphere (McMahon *et al.*, 2000).

The results showed that 1-octen-3-ol enhanced the attraction of A. variegatum to the standard blend of 1.1 mg of AAAP. When used separately, AAAP attracted 53.27% of the ticks in the T-tube olfactometer while the number attracted to the test material increased up to 72.86% when 8 ng of 1-octen-3-ol was combined to AAAP. These results are in agreement with the findings of Osterkamp *et al.* (1999) who found that 1-octen-3-ol was additive to a bovine odour blend while studying the questing behaviour of *B. microplus* and *I. ricinus*. While the odour blend induced a questing response of 80.3%, this performance decreased to 50.9% when 1-octen-3-ol was removed from the mixture. Osterkamp *et al.* (1999) also found that *B. microplus* responded with high sensitivity to 1-octen-3-ol than *I. ricinus* and suggested that 1-octen-3-ol may contribute to the cattle –specificity of the questing response of *B. microplus*. In mosquito, Kline *et al.* (1990) demonstrated the potential of 1-octen-3-ol as a mosquito attractant while evidence of the additive effect of 1-octen-3-ol to CO_2 was later highlighted on *Ae. taeniorhynchus* (Kline *et al.*, 1991).

A combination of 8 ng of 1-octen-3-ol with 1.1 mg of AAAP was observed to be attracting the maximum of ticks in the olfactometer; higher blends did not attract more ticks. This is in agreement with the findings of McMahon and Guerin (2000) on *A. variegatum* adults in a servosphere, whereby a 3:1 ratio of o-nitrophenol and methyl salicylate was found less attractive than a 1:1 binary mix of both compounds at the same dose. High amounts of 1-octen-3-ol have been also found to be unattractive to *A. variegatum* adults, which may be making parsimonious use of this compound (McMahon *et al.*, 2000), demonstrating that ratios of attractant components are critical in eliciting responses of this tick (McMahon and Guerin, 2000). Whereas males were attracted to the increasing proportions of 1-octen-3-ol females were repelled (Figure). Similar results were obtained on *A. variegatum* by Maranga (1998) using AAAP only while Norval *et al.* (1991) reported higher attachment rates of males than females on rabbit ears treated with o-nitrophenol and methyl salicylate.

Apart from few cases, the adjunction of 2,6-dichlorophenol to the standard blend of 1.1 mg of AAAP had a repellent effect on ticks (Figure). The results of this study are at variance with earlier reports, which described 2,6-dichlorophenol as a sex pheromone (Berger *et al.*, 1972; Chow *et al.*, 1975) and an attachment stimulant (Yoder and Stevens, 2000). The findings of this study are in agreement with Akinyi (1991) who found that mixtures of ear extracts and 2,6-dichlorophenol, both individually active, were repulsive to *R. appendiculatus* adults.

2,6-dichlorophenol appears to be also inactive to ticks when not combined in a blend with host volatiles. Khalil *et al.* (1981), working on *Hyalomma dromedarii*, observed that the ticks responded to 2,6-

dichlorophenol near the host but no response when it was offered in an air stream as a single substance.

De Bruyne and Guerin (1994) reported that 2,6-dichlorophenol had no effect on adult *B. microplus* males when the compound was presented alone. These findings are in agreement with McMahon and Guerin (2000) who did not find any effect of 2,6-dichlorophenol on the behaviour of *A. variegatum* on the servosphere.

CHAPTER 4

AMBLYOMMA VARIEGATUM ATTRACTION USING VARIOUS DOSES OF PHEROMONES IN THE FIELD

4.1 INTRODUCTION

The attraction of ticks in the field by pheromones has been reported. *Amblyomma variegatum* females were attracted up to 1 m to the AAAP (Schöni *et al.*, 1984). They concluded that ortho-nitrophenol induces orientation and dynamic aggregation and that methyl salicylate and nonanoic acid, together with ortho-nitrophenol, induce mounting and clasping behaviour. Hess and de Castro (1986) and Norval *et al.* (1989a) found, in field experiments, that ortho-nitrophenol is a long-range attractant used by the ticks to locate hosts and attached feeding males. More recently, adult *A. variegatum* ticks were attracted to the AAAP at a distance of 5 meters in the field (Maranga, 1998).

The purpose of this study was to investigate the optimal dose of the combined pheromones which attracts the ticks from the longest distance possible in the field.

4.2 MATERIALS AND METHODS

4.2.1. Ticks

Two to three months old unfed adult males and females of *A. variegatum* from ARQU were used. The ticks were counted in batches of 10 (5 males and 5 females) and placed in separate vials. The ticks were marked using the artist's paint (Rowney Georgian oil colour, made in England, London HA 35 RH). Males and females were marked on the lower quarter of the dorsal side (Maranga, 1998).

4.2.2. Experimental location

The experiments were carried out at the National Veterinary Research Centre, Muguga-KARI: Latitude 1° 13' S, Longitude 36° 18' E, Altitude 2070 m (Obiri *et al.*, 1994). The Muguga climate is a modified equatorial type with a mean monthly temperature of 18°C. Mean annual rainfall is 1005 mm, distributed bimodally with peaks in April and October (Obiri *et al.*, 1994).

The experiments were carried out in 18 separate plots. Each plot was prepared by measuring a circular plot of 7 meters of radius. Then the plot was marked from the centre using wooden pegs at intervals of 1 m in a straight line. A small circle of 10 cm radius was made at the centre of the plot and all the grass cut. Each plot had a barrier of 5 m uncleared grass surrounding it (Maranga *et al.*, 2003) as shown in plate 4.1.

Plate 4.1: An experimental plot with distances separated with wooden pegs (**A**) and a Petri dish carrying a dose of pheromones on filter paper (**B**)

4.2.3 Source of pheromones and doses used

The blend of 1.1 mg of AAAP and 8 ng of 1-octen-3-ol observed to attracting most *A.variegatum* ticks in the laboratory experiment was used and five doses were prepared by increasing the initial dose by a factor of 2 (Table 4.1). The controls were made using dichloromethane alone. The blends were placed on Whatman's filter paper fixed to the bottom side of Petri dishes using laboratory parafilm (Maranga *et al.*, 2003).

Table 4.1: Doses used in the experiments

Doses	AAAP	1-octen-3ol (ng)
D1	1.1 mg	8
D2	2.2 mg	16
D3	4.4 mg	32
D4	6.6 mg	48
D5	8.8 mg	64
D6	Dichloromethane	

4.2.4. Tick release

The ticks marked with different colours were released from premarked distances. Ticks were released from three different angles downwind from the centre of the plots where the source of pheromone(s) was placed.

The direction of the wind was monitored using a thin thread attached to a wooden stick of 1m long at the centre of the plot. Ticks coming to the target (pheromones source) were collected and recorded, noting down their colour and sex in each of the plots. The experiment was repeated three times. Each replicate was observed for three days using a fresh source of pheromones.

4.2.5 Data analysis

Tick responses to the standard blend of 1.1 mg of AAAP to five doses of 1-octen-3-ol were recorded and entered on Microsoft Excel. Analysis of Variance (ANOVA) was then performed on the data after square root transformation to test for differences in attraction due to dose, sex, distance of release or their interactions. Student-Newman-Keuls test at 0.05 significance level was performed where there were significant differences for separation of means.

4.3. RESULTS

The attraction of the ticks to the blends of AAAP and 1-octen-3-ol is highly significant, compared to the control (Table 4.2). All doses are significant different from the control and the combination of 2.2 mg of AAAP and 16 ng of 1-octen-3-ol was the most attractive dose on average (Table 4.2). The results showed a gradient of reactivity depending on the distance from the source of pheromones with the least number of ticks being attracted from the furthest distance from the source. Ticks exposed to the combination of 2.2 mg of AAAP and 16 ng of 1-octen-3-ol were significantly attracted at 6 m, compared to the control (Table 4.2). The tick attraction at 7 m was not significant, compared to the control. Unlike the laboratory tests (Table 4.2), female ticks were significantly more attracted in the field (Figure 4.1).

Table 4.2: Mean percentage number (± SE) of ticks attracted to AAAP blended with 1-octen-3-ol in the field.

Doses	Chemicals		Distance (m)							Dose
	AAAP	1-octen-3-ol	1	2	3	4	5	6	7	Average
D1	1.1 mg	8 ng	25.55±8.50abA	5.55±2.67bcAB	3.33±2.27abB	4.44±1.40abAB	6.66±4.21bAB	3.33±1.49abB	0.00±0.00aB	6.98±1.83b
D2	2.2 mg	16 ng	36.66±10.14aA	23.33±5.89aAB	13.33±2.98aABC	16.66±4.79aABC	13.33±2.98aABC	8.89±1.40aBC	4.44±2.22aC	16.66±2.36
D3	4.4 mg	32 ng	26.66±7.50abA	10.00±2.27abAB	13.33±7.09abAB	10.00±3.75abAB	2.22±1.40bB	3.33±2.27abB	2.22±1.40aB	9.68±1.98b
D4	6.6 mg	48 ng	38.88±8.67aA	18.89±5.28abB	10.00±4.47abBC	5.55±2.04abBC	4.44±2.81bBC	4.44±2.22abBC	0.00±0.00aC	11.74±2.48
D5	8.8 mg	64 ng	27.77±5.28abA	10.00±4.47abB	5.55±3.18abB	5.55±3.18abB	2.22±1.40bB	8.88±5.87abB	8.89±4.76aB	9.84±1.91b
D6	Dichloromethane		5.55±2.67bA	0.00±0.00cB	0.00±0.00bB	0.00±0.00bB	0.00±0.00bB	0.00±0.00bB	0.00±0.00aB	0.79±0.46c

Means (±SE) between doses bearing the same small letters are not significantly different at 0.05 level based on the SNK test.
Means (±SE) within doses bearing the same capital letters are not significantly different at 0.05 level based on the SNK test.

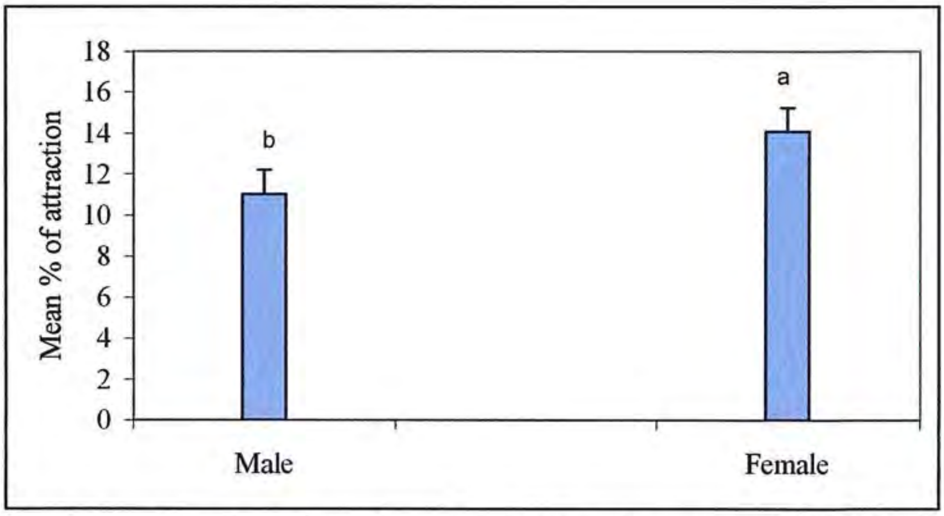

Figure 4.1: Attraction of female and male *A. variegatum* to AAAP blended with 1-octen-3-ol in the field

4.4 DISCUSSION

It was observed that the longest distance from which ticks were significantly attracted by the combined doses of AAAP and 1-octen-3-ol was 6 m. Hess and Castro (1986), Barré *et al.* (1997) and Maranga *et al.* (2003) previously reported an attraction of *A. variegatum* at 1, 8 and 5 meters respectively. The second record involved AAAP and CO_2 with a Caribbean strain. The findings of this study are in agreement with them.

The results showed a gradient of reactivity depending on the distance from the source of pheromones with the least number of ticks being attracted from the furthest distance from the source. This is in agreement with Maranga *et al.* (2003). These authors used only AAAP whereas our experiment involved AAAP and 1-octen-3-ol. The combination of 1-octen-3-ol and AAAP was also effective in attracting *A. variegatum* in the field. The same observations were made by Osterkamp *et al.* (1999) who demonstrated that 1-octen-3-ol enhanced the questing behaviour of *Boophilus microplus*. As suggested by McMahon *et al.* (2000), 1-octen-3-ol may be, for ticks, a cue for aggregation with conspecifics and for host finding when in search for blood meal. The combination of 2.2 mg of AAAP and 16 ng of 1-octen-3-ol significantly attracted the maximum of ticks on average in the field. This amount of AAAP is less than the 6.6 mg found by Maranga *et al.* (2003) as attracting the highest number of ticks in the field.

To my knowledge, this is the first time that the attraction of *A. variegatum* ticks to 1-octen-3-ol has been demonstrated in the field. McMahon *et al.* (2000) first reported the attractiveness of 1-octen-3-ol to ticks in a servosphere.

CHAPTER 5

OPTIMISATION BY CARBON DIOXIDE OF A SEMIOCHEMICAL BLEND FOR THE ATTRACTION OF *AMBLYOMMA VARIEGATUM* TICKS.

5.1 INTRODUCTION

Wilson *et al.* (1972), Eads *et al.* (1982) and Gray (1985) demonstrated the attraction effect of CO_2 on *Amblyomma americanum*, *Dermacentor andersoni* and *Ixodes ricinus* where the CO_2 effect was used for tick sampling. The combination of CO_2 and pheromones was later reported by Norval *et al.* (1989a) as attractant of *Amblyomma hebraeum*. They demonstrated that males and females were activated by carbon dioxide and attracted to the aggregation-attachment pheromone in the field. Maranga *et al.* (2003) reported the attraction up to 5 m of *A. variegatum* to AAAP combined with carbon dioxide.

The purpose of this study was to investigate the effect of CO_2 on the performance of traps baited with AAAP and 1-octen-3-ol in attracting *A. variegatum*. Also investigated was the most efficient way of delivering the carbon dioxide in the field.

5.2 MATERIALS AND METHODS

5.2.1 Ticks

The ticks used in this experiment are as described in section 4.2.1.

5.2.2 Experimental location

The experiments were carried out at the National Veterinary Research Centre, Muguga-KARI as described in section 4.2.2 except that 15 plots measuring 8 m of radius were used.

5.2.3 Source of pheromones and carbon dioxide

The blend of 2.2 mg of AAAP and 16 ng of 1-octen-3-ol observed in a previous field study as attracting the most important number of ticks was used with 300 g of CO_2. The CO_2 was provided using dry ice bought from CARBACID-Nairobi. The pheromones were placed on Whatman's filter paper fixed to the side of a Petri-dish, while the carbon dioxide was contained in another Petri dish and either released continuously or intermittently. The continuous release meant a presence of CO_2 during the daily experimental period (from 8 to 12:30) while the dry ice for the other plots was delivered at regular intervals of 1.5 hours. The controls were made using only 300 g of carbon dioxide (continuous or intermittent release).

Table 5.1: Doses used in the experiments

Doses	AAAP	1-octen-3-ol	Carbon dioxide
D1	2.2 mg	16 ng	300 g *
D2	2.2 mg	16 ng	300 g **
D3	2.2 mg	16 ng	0
D4			300 g *
D5			300 g **

* Continuous Release

** Intermittent Release

5.2.4 Tick release

The release of the ticks was carried out as described in section 4.2.4.

5.2.5 Data analysis

Tick responses to various combined doses of AAAP, 1-octen-3-ol and CO_2 were recorded and entered on Microsoft Excel. Analysis of Variance (ANOVA) was then performed on the data after square root transformation to test for differences in attraction due to dose, sex, distance of release or their interactions. Mean values were compared using the Student-Newman-Keuls test at a significance level of 0.05.

5.3 RESULTS

The attraction of ticks to the combined dose of 2.2 mg of AAAP, 1-octen-3-ol and 300 g of CO_2 delivered intermittently was significant (Table 5.2), compared to the results of pheromones without CO_2 and when the carbon dioxide was only applied. The method of applying the carbon dioxide did not have any significant effect either when it was combined to the pheromones or delivered separately (Table 5.2). The only case where the intermittent release of CO_2 had a significant effect on the attraction of ticks to the pheromones was at 7 m, compared to the pheromones with continuous application of CO_2. The release point of the ticks had a significant role in the attraction, the ticks attracted from 5 to 8 for all doses was less than those coming from 1 m. The doses made up of CO_2 only attracted few proportions of ticks mainly from the two first meters (Table 5.2). Differences have been found between sexes in the attraction of ticks to the blends and males were observed responding more than females (Table5.1).

Table 5.2: Mean percentage number (± SE) of ticks attracted from various distances in the field.

Treatment	Distance (m)								Dose
	1	2	3	4	5	6	7	8	Average
AAAP+1-octen-3-ol+$CO_{2\ (continuous\ release)}$	71.11±10.97aA	41.11±5.55bA	53.33±12.29abA	24.44±5.06bcA	15.55±6.59cAB	10.00±3.33cA	8.88±3.71cA	8.88±4.09cA	29.16±3.97A
AAAP+1-octen-3-ol+$CO_{2\ (intermittent\ release)}$	61.11±12.81aA	27.77±8.50bA	32.22±8.84bAB	17.77±7.02bcA	21.11±4.36bA	8.88±2.81bcA	2.22±1.40cB	8.88±3.29bcA	22.50±3.43AB
AAAP+1-octen-3-ol	40.00±6.44aB	36.66±5.64aA	18.88±2.67abBC	27.77±6.75abA	14.44±6.30bcAB	5.55±4.36cdAB	0.00±0.00dB	3.33±2.27cdA	18.33±2.62B
$CO_{2\ (continuous\ release)}$	18.88±6.75aC	6.66±2.98bcB	6.66±1.72bC	2.22±1.40bcB	0.00±0.00cC	2.22±1.40bcAB	0.00±0.00cB	1.11±1.11cA	4.72±1.25C
$CO_{2\ (intermittent\ release)}$	15.55±6.59aC	1.11±1.11bB	5.55±3.18bC	3.33±2.27bB	2.22±1.40bBC	0.00±0.00bB	1.11±1.11bB	0.00±0.00bA	3.61±1.15C

Means (±SE) between doses bearing the same Capital letters are not significantly different at 0.05 level based on the SNK test.
Means (±SE) within doses bearing the same small letters are not significantly different at 0.05 level based on the SNK test.

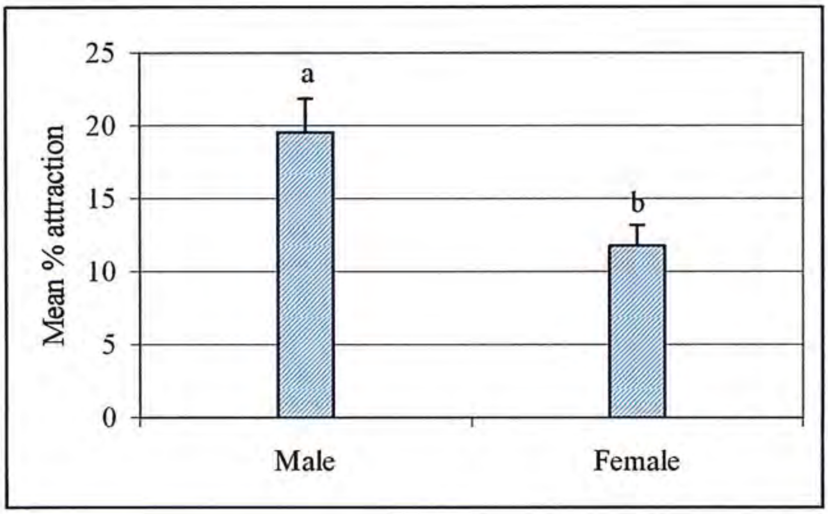

Figure: 5.1: Attraction of Male and Female *A. variegatum* in the field

5.4 DISCUSSION

Ticks were attracted up to 8 meters in the field. The pheromones with intermittent application of carbon dioxide were significantly more attractive to ticks than those without CO_2 (Table 5.2). The findings are in agreement with Yunker *et al.* (1990) who reported that 2% of adult *A. variegatum* released at 4 m from a source of CO_2 were recovered compared to a recovery of 54% when a mixture of pheromone extracts and CO_2 were used. Similar observations were made by Maranga *et al.* (2003) on *A. variegatum* whereby 34.17% of the ticks released 1 m from a source of AAAP were recovered. The recovery increased to 98.33% when 500 g of CO_2 were added to the pheromone. The presence of the carbon dioxide enhances substantially the responses of the ticks to the pheromones (Maranga *et al.*, 2003).

Norval *et al.* (1987) on *A. hebraeum* and Maranga *et al.* (2003) on *A. variegatum* suggested that host location in these ticks involves two sequential processes: activation and a non-directional searching activity stimulated by CO_2; and a directional movement to the pheromones. The findings of this study are in agreement with these suggestions. Whereas 6% of adult *A. variegatum* moved 3 m upwind to a CO_2 source (300 g), over 53% of the ticks were attracted when AAAP was also present. CO_2 largely functions to mark the presence of a potential host, the pheromone that is emitted by successfully feeding ticks helps to signal the presence of an appropriate host (Maranga *et al.*, 2003). It also helps to orient the ticks to their feeding sites where male-female contact can also take place.

The results of this study are at variance with those of Barré *et al.* (1997) who discovered that carbon dioxide is an effective attractant of a Caribbean strain of *A. variegatum*. In three experiments, CO_2 alone attracted ticks released at 0.5-6 m of the source, sometimes in large numbers (66% of adult ticks in one trial with 500 g of dry ice and 43% in another with 250 g of dry ice). CO_2 was also found attractive to *A. hebraeum* and *A. variegatum* nymphs (Norval *et al.*, 1992) which were respectively attracted to CO_2 sources at distances of 15 and 10 m while AAAP enhanced the elicited responses. These findings are in agreement with the observations of Stachurski (2000) in Burkina Faso where cattle stimuli were found powerful enough to attract *A. variegatum* ticks, males as well as females, and induce their attachment. It was observed that ticks were picked up on the pastures by the hosts, whether the cattle were infested or not. The cattle stimuli suggested by Stachurski (2000) may include other volatiles than CO_2. Such volatile components of mammalian hosts might be particularly effective in stimulating the ticks (Maranga *et al.*, 2003). This behaviour of the Caribbean strain mostly appears different from that of most *A. hebraeum* and *A. variegatum* in Africa for which attractants other than CO_2 are required to catch the ticks in significant number (Norval *et al.*, 1987, 1989, 1992; Maranga *et al.*, 2003).The presence of CO_2 is crucial for the attraction of *A. variegatum* adults as well as it enhances that of the nymphs (Norval *et al.*, 1992). These workers suggested that nymphs and adults use the AAAP to discriminate between suitable hosts on which AAAP-emitting males are already attached and potentially unsuitable ones on which no males are attached.

The basis of use of CO_2 was investigated and it was found no significant difference between the intermittent and the continuous release of CO_2. This intermittent use being more economical, this finding could be more useful for the farmers who are going to use such a technology.

CHAPTER 6

MORTALITY OF *AMBLYOMMA VARIEGATUM* TICKS EXPOSED TO NEEM CAKE OIL IN TRAPS BAITED WITH PHEROMONES AND CARBON DIOXIDE IN THE FIELD.

6.1 INTRODUCTION

The trapping of ticks has been reported for many years. Wilson *et al.* (1972), Eads *et al.* (1982) and Gray (1985) demonstrated the attraction effect of CO_2 respectively on *Amblyomma americanum*, *Dermacentor andersoni* and *Ixodes ricinus*. The CO_2 effect was used for tick sampling.

Later on, studies have been carried out on the attraction of ticks in the field by pheromones. *A. variegatum* female was attracted up to 1m to the Attraction-Aggregation-Attachment Pheromone (Schöni *et al.*, 1984). They concluded that ortho-nitrophenol induces orientation and dynamic aggregation and that methyl salicylate and nonanoic acid, together with ortho-nitrophenol, induce mounting and clasping behaviour. Hess and de Castro (1986) and Norval *et al.* (1989a) found, in field experiments, that ortho-nitrophenol is a long-range attractant used by the ticks to locate hosts and attached feeding males.

The combination of CO_2 and pheromones was later reported by Norval *et al.* (1989a) as attractant of *Amblyomma hebraeum*. They demonstrated that males and females were activated by carbon dioxide and attracted to the aggregation-attachment pheromone in field experiments. Maranga (1998) reported the attraction up to 5 m of *Amblyomma variegatum* to carbon dioxide combined to AAAP.

These findings in the attraction of ticks by CO_2 and pheromones allowed the use of pheromone traps for the control of ticks. The use of pheromone/acaricide mixtures to control *Amblyomma hebraeum* has been reported (Rechav and Whitehead, 1978; Norval *et al.*, 1991). Later, Maranga (1998) investigated the efficacy of fungi traps baited with AAAP for the control of *A. variegatum*.

For the best of my knowledge, no work has been carried out using pheromone/ botanical extracts. The aim of this experiment was to test the efficacy of attracting and killing ticks of Neem Cake Extracts traps baited with AAAP, 1-octen-3-ol and CO_2.

6.2 MATERIALS AND METHODS

6.2.1 Ticks

The ticks used in this experiment were as described in section 4.2.1.

6.2.2 Experimental location

The experiments were carried out at the National Veterinary Research Centre, Muguga-KARI as described in section 4.2.2 except that 12 plots measuring 8 m of radius were used. The traps were built up from the design of Maranga (1998) and modified to three levels for placement of the pheromones, the CO_2 and the neem cake extracts, respectively (Plate 6.1).

6.2.3 Preparation of the neem cake extracts (NCE)

The Neem cake was obtained from the ICIPE Neem factory. A quantity of 500 g of cake (0.6% of azadirachtin) was mixed with hexane during three days. The mixture was then filtered and the solvent evaporated. The oil was then formulated in 10, 20 and 30% using paraffin oil (Mwangi *et al.*, 1995b). The doses were then made as it follows:

- D1: AAAP (2.2 mg) +1-octen-3-ol (16 ng) +CO_2 (500 g) + NCE 10% (8 ml)
- D2: AAAP (2.2 mg) +1-octen-3-ol (16 ng) +CO_2 (500 g) + NCE 20% (8 ml)
- D3: AAAP (2.2 mg) +1-octen-3-ol (16 ng) +CO_2 (500 g) + NCE 30% (8 ml)
- D4: AAAP (2.2 mg) +1-octen-3-ol (16 ng) +CO_2 (500 g) + liquid paraffin (8 ml)

Plate 6.1: Trap set in the field, carrying AAAP and 1-octen-ol (**1**), CO_2 (**2**) and Neem Cake Extracts (**3**).

6.2.4 Tick release

The release of the ticks was carried out as described in section 4.2.4. The ticks which came in contact with the NCE as well as those in the control plots were taken to the laboratory and their mortality assessed for 21 days.

6.2.5 Data analysis

Tick responses to various combined doses of AAAP, 1-octen-3-ol and CO_2 were recorded and entered on Microsoft Excel. Analysis of Variance (ANOVA) was carried out to test for differences in attraction due to doses, sex or their interaction on the data after square root transformation, using the Statistical Analysis System Software (SAS, 1988). A Survival Analysis was also performed and differences in the survival curves were analysed by the Lifetest procedure (SAS) and the significant level was based on Wilcoxon Rank tests of equality over strata. Mean values were compared using the Student-Newman-Keuls test at a significance level of 0.05.

6.3 RESULTS

The effectiveness of the traps in attracting ticks was tested and the results showed strong attraction of *A. variegatum* to the blends of AAAP, 1-octen-3-ol and CO_2 (Table 6.1). The different doses on average did not show any significant differences in attracting ticks (Table 6.1).

The Neem Cake Extracts had significant lethal effect on the ticks compared to the control on average (Table 6.2) and there was no difference between 10%, 20% and 30% concentrations of NCE. The mortality of the ticks in the control group was minimal through out the 21 day-exposure and no significant difference was noted from the first to the last day (Table 6.2).

The survival curves between the doses were compared and the rank tests for homogeneity indicated a significant difference between the doses with NCE compared to the control ($P<0.0001$) for the Log-rank test and the Wilcoxon test, as earlier indicated in the SNK test. The log-rank test, which places more weight on larger survival times, is equally significant as the Wilcoxon test, which places more weight on early survival times. The mean survival time in days (Figure 6.1) showed that ticks exposed to the control and the lower concentrations of 10% and 20% live significantly longer than those exposed to 30% of Neem Cake Extracts. The three first days of exposure caused more mortality compared to the following time of exposure and it was noted with NCE 30%, NCE 20% and NCE 10% 83%, 79% and 66% mortality respectively (Figure 6.2).

Table 6.1: Mean percentage number (± SE) of ticks attracted to the traps from various distances

Doses	Distance (m)								Dose average
	1	2	3	4	5	6	7	8	
AAAP+1-octen-3-ol+CO_2+NCE 10%	57.77±6.51aB	37.77±5.16bA	37.77±2.58bA	17.77±3.32cC	14.44±2.54cA	14.44±2.17cA	6.66±1.32dB	18.88±6.15cA	27.89±1.07A
AAAP+1-octen-3-ol+CO_2+NCE 20%	53.33±8.29aB	21.11±2.17bC	25.55±3.28bB	27.77±4.51bB	7.77±1.45dB	11.11±4.03dA	6.66±1.32dB	11.11±1.78cA	24.05±0.99A
AAAP+1-octen-3-ol+CO_2+NCE 30%	68.88±4.54aA	27.77±3.15cB	37.77±4.44bA	36.66±5.81bA	20.00±3.84dA	10.00±2.03eA	7.77±2.86efB	4.44±1.20fB	27.80±1.19A
AAAP+1-octen-3-ol+CO_2+liq. Paraffin	53.33±5.28aB	35.55±4.34bA	21.11±3.54cC	28.88±4.13bB	16.66±1.54cdA	18.88±6.14cdA	11.11±1.52dA	3.33±1.23eB	25.82±0.99A

Means (±SE) between doses bearing the same Capital letters are not significantly different at 0.05 level based on the SNK test.
Means (±SE) within doses bearing the same small letters are not significantly different at 0.05 level based on the SNK test.

Table 6.2: Mean percentage number (±SE) of ticks killed after being exposed to the Neem Cake Extracts.

Days after exposure	Treatments			
	AAAP+1-octen-3-ol+CO2+NCO 10%	AAAP+1-octen-3-ol+CO2+NCO 20%	AAAP+1-octen-3-ol+CO2+NCO 30%	AAAP+1-octen-3-ol+CO2+liq. paraffin
1	41.29±9.67aA	35.59±6.51aA	51.48±5.10aA	0.42±0.26bA
2	16.79±4.43aB	32.64±4.01aA	22.09±5.95aB	0.87±0.55bA
3	8.15±2.93aC	11.18±1.35aB	9.75±0.44aC	0.00±0.00bA
4	7.06±3.29aCD	6.50±3.58aC	4.85±1.80aD	0.00±0.00aA
5	2.81±0.75aCD	0.00±0.00bD	1.75±1.10abE	0.42±0.26bA
6	1.59±0.63aCD	2.22±0.81aCD	0.00±0.00bE	0.00±0.00bA
7	0.44±0.27abD	1.48±0.46aCD	0.52±0.32abE	0.00±0.00bA
8	1.31±0.83aD	0.74±0.46aCD	1.10±0.35aE	0.42±0.26aA
9	2.16±0.98aCD	0.00±0.00bD	0.00±0.00bE	0.84±0.53abA
10	1.59±0.63aCD	1.48±0.93aCD	0.00±0.00aE	0.00±0.00aA
11	0.57±0.36abD	2.22±0.81aCD	0.52±0.32abE	0.00±0.00bA
12	0.44±0.27aD	0.74±0.46aCD	1.10±0.35aE	0.00±0.00aA
13	0.00±0.00aD	0.00±0.00aD	0.00±0.00aE	0.61±0.39aA
14	1.75±1.10aCD	0.74±0.46aCD	0.93±0.30aE	1.04±0.34aA
15	1.59±0.63aCD	0.00±0.00bD	0.00±0.00bE	0.42±0.26bA
16	0.00±0.00aD	0.00±0.00aD	0.52±0.32aE	0.42±0.26aA
17	0.44±0.27abD	1.48±0.93abCD	1.51±0.09aDE	0.00±0.00bA
18	0.57±0.36aD	0.00±0.00aD	1.69±0.64aDE	0.61±039aA
19	0.00±0.00aD	0.00±0.00aD	0.00±0.00aE	0.00±0.00aA
20	0.00±0.00aD	0.00±0.00aD	0.00±0.00aE	0.00±0.00aA
21	1.45±0.48aCD	0.00±0.00bD	0.00±0.00bE	0.00±0.00bA
Treatment average	4.28±0.96a	4.62±0.96a	4.65±1.09a	0.29±0.05b

Means (±SE) between doses bearing the same small letters are not significantly different at 0.05 level based on the SNK test.
Means (±SE) within doses bearing the same capital letters are not significantly different at 0.05 level based on the SNK test

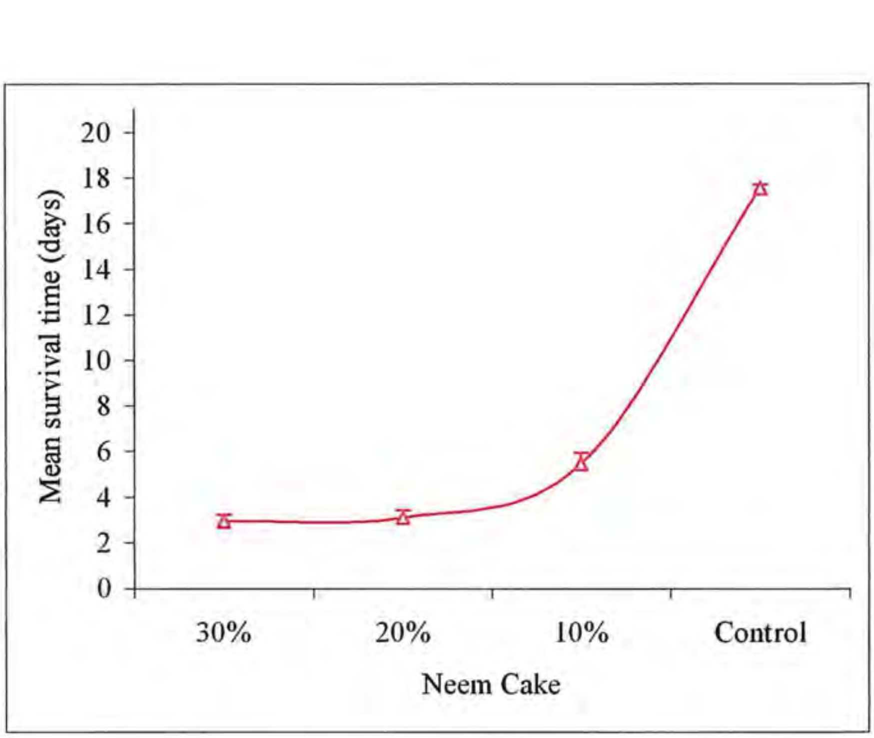

Figure 6.1: Mean survival time of *A. variegatum* ticks exposed to Neem Cake Extracts.

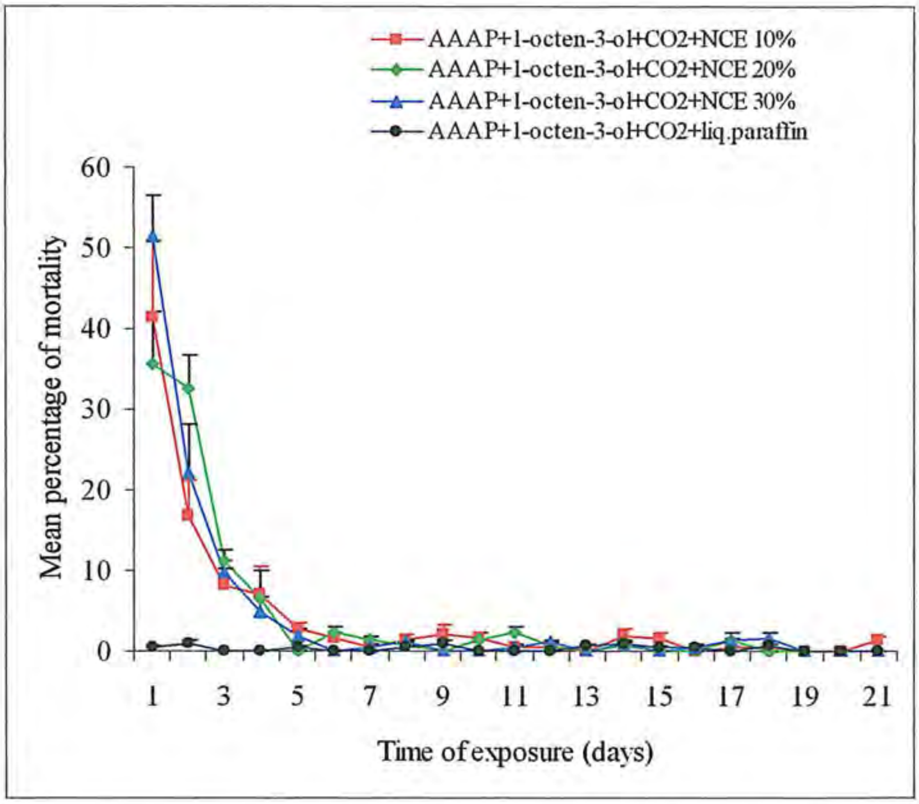

Figure 6.2: Mean percentages (± SE) of mortality of *A. variegatum* ticks exposed to Neem Cake Extracts after 3 weeks

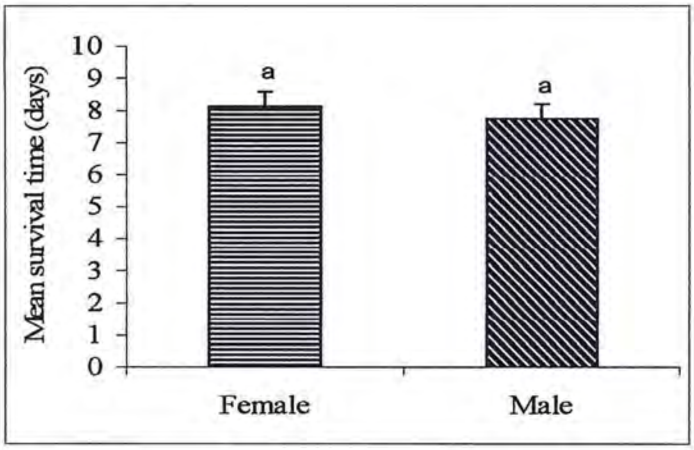

Figure 6.3: Mean survival time (±SE) of male and female *A. variegatum* ticks exposed to the NCE

6.4. DISCUSSION

The Neem Cake Extract (NCE) used in this study had 0.6% of Azadirachtin. Azadirachtin is the predominant insecticidal active ingredient in the seeds, leaves, and other parts of the Neem tree (Mulla, 1999) and has been reported to be acaricidal against *A. variegatum* and other tick species (Ndumu *et al.*, 1999; Ismail *et al.*, 2002; DiefAlla *et al.*, 2003).

Early as well as high mortalities were noted (Figure 6.1; Figure 6.2). This study also found that the acaricidal toxicity of the NCE varied with the concentration when tested against adult *A. variegatum*. Higher concentration of NCE produced higher mortalities than the lower concentration (Figure 6.2; Table), significant differences being only noted in the NCE groups and the control. The mortality of the ticks was also time-dependent (Figure 6.2; Table 6.2) and in all doses with NCE, the most important number of ticks died during the three first days of exposure. 83%, 79% and 66% of the ticks died in the groups where 30%, 20% and 10% concentrations were applied, respectively.

In this regard, the results of this study are similar to those of Ndumu *et al.*, (1999) who found, in a study on larvae of *A. variegatum* in Nigeria that a concentration of 100% of undiluted Neem seed oil killed all the larvae after 48 hours. Similar conclusions were reported by Ismail *et al.*, (2002). Working on the 3-host tick *Rhipicephalus pulchellus* in Ethiopia, they found that the 40% Neem oil was the most effective and showed the highest toxicity compared to 10%. Investigating the effect of Neem on *Hyalomma anatolicum*, Abdel-Shafy and Zayed (2002) used a commercial formulation of *A. indica*, Neem Azal F, and obtained similar results. Most of the studies investigated only tick larvae which are "supposed" to be more susceptible than adults.

The efficacy of Neem on *Boophilus microplus* has also been reported (Mansingh and Williams, 1998). The authors investigated the crude ethanol extracts of the Neem leaves on engorged *B. microplus* and found an acaricidal index of 68% and concluded that Neem was very effective against this tick. The results of this study showed more efficiency in controlling ticks. The use of the NCE in this study caused 98% of mortality to the ticks exposed to a concentration of 30%. Derivatives of *Azadirachta indica*, a native of Burma and the arid regions of the Indian sub-continent, have been traditionally used by farmers in Asia and Africa to control insect pests of household, agricultural and medical importance (Kaaya, 2003). These derivatives are less toxic to natural enemies of insect pests compared to the pests themselves (Schmutterer, 1990) and could be useful in controlling ticks and therefore be incorporated in an Integrated Tick Management (ITM), which would involve the use of all known and effective means of tick control depending to their compatibility with each other, climate conditions and livestock management system (Kaaya and Hassan, 2000).

CHAPTER 7

STUDIES ON LOCATION PATHWAYS OF *AMBLYOMMA VARIEGATUM* ON CATTLE.

7.1 INTRODUCTION

The selection of host by *A. variegatum* involves some chemicals. After identifying the suitable host, ticks attach to the feet (Stachurski, 2000). The attachment to the feet is temporary and not very strong. Ticks need the host to lie down before moving and attaching to the predilection sites (Stachurski, 2000).

The lower dewlap, the ventral surface and the genitalia area are known to be preferred attachment sites of *A. variegatum* adults. Sika (1996) provided evidence of the attraction of *R. appendiculatus* to the ear odours while *R. evertsi* ticks prefer the anal region. Later on, Demas *et al.* (2000) suspected involvement of kairomones for the attachment of *A. variegatum* nymphs on the heels of cattle but little is known about the signals involved for locating and attaching to the preferred feeding sites by *A. variegatum* adults.

The purpose of this study was to identify the pathways and signals used by *A. variegatum* to locate the preferred feeding sites on-host.

7.2 MATERIALS AND METHODS

7.2.1 Collecting volatiles

Volatiles were trapped using adsorbent sachets (4 x 2.5 cm) made up of stainless wire gauze and filter paper containing about 0.5 g of reverse-phase C_{18}-bonded silica, as described by Gikonyo *et al.* (2002). One side of the trap was covered with a sterile Aluminum foil paper while the other face was left open to allow for diffusion of odours from the host into the trap. Prior to use, traps were first cleaned by putting them in a 200 ml Soxhlet extractor for 2 days and then dried and flushed of any contaminants with a stream of dry nitrogen at 60° C for 3 hours (Gikonyo *et al.*, 2002)

To collect volatiles, 3 clean traps were attached overnight onto the lower dewlap, the scrotum and the ear using adhesive plaster to each of the two zebu cattle. The following day, the traps were removed and eluted with 2 ml of dichloromethane by immersion, washed with another 2 ml of the solvent. The eluates were then kept at 0° C for gas chromatographic analyses and bioassays.

7.2.2 Bioassays

The T-tube olfactometer method as described by Maranga (1998) was used. It presents alternative choices to ticks. Tubes used for adult tick bioassays measure (4 x 4) cm with an inner diameter of 1.5 cm. A small filter paper carrying the respective test materials was placed in the arms of the T-tube. Ticks were introduced into the base of the tube and were allowed to climb up the tube to select one of the two arms. The number of ticks (unfed adult male and female), choosing one arm or another was recorded. Four replications were done for each dose of volatile. The tick response to each dose of volatile was tested by introducing ticks at the base of the T-tube. One tick was investigated at a time and responses of the male and female ticks were recorded separately.

The filter paper carrying the test materials was discarded and the T-tubes were washed in detergent and then rinsed in dichloromethane after every treatment. The T-tubes were then dried in the oven at 100° C for 30 minutes. All the experiments were carried out in a hood for sucking out air (to avoid accumulation of the odours in the working area). The percentage attractiveness (PA) values were calculated using the formula: $PA = [(N_D - N_E)/ (N_D + N_E)] \times 100$ (Ndungu *et al.*, 1995a), where N_D and N_E are numbers of ticks recorded in the arms of the olfactometer respectively treated with the dewlap odour and the ear odour. Similar formula was used to calculate the percentage attractancy when scrotum odour was tested versus ear odour and scrotum volatiles versus dewlap odour. No control was set.

7.2.3 Data analysis

Tick response to the volatiles collected from different parts of the cattle were recorded and entered on Microsoft Excel. A t-test was carried out using the Statistical Analysis System Software (SAS, 1988) to compare the effect of the volatiles collected from the dewlap, the scrotum and the ear.

7.3 RESULTS

The volatiles from the scrotum significantly attracted more ticks when tested against the ear odour ($P<0.0001$) and the dewlap odour ($P = 0.0004$). The volatiles from the lower dewlap were more attractive than the odour from the ear ($P = 0.0005$). The scrotum and the dewlap volatiles are all attractive to ticks (Figure 7.1) than the ear volatiles but the scrotum kairomones attracted more ticks than the volatiles from the lower dewlap.

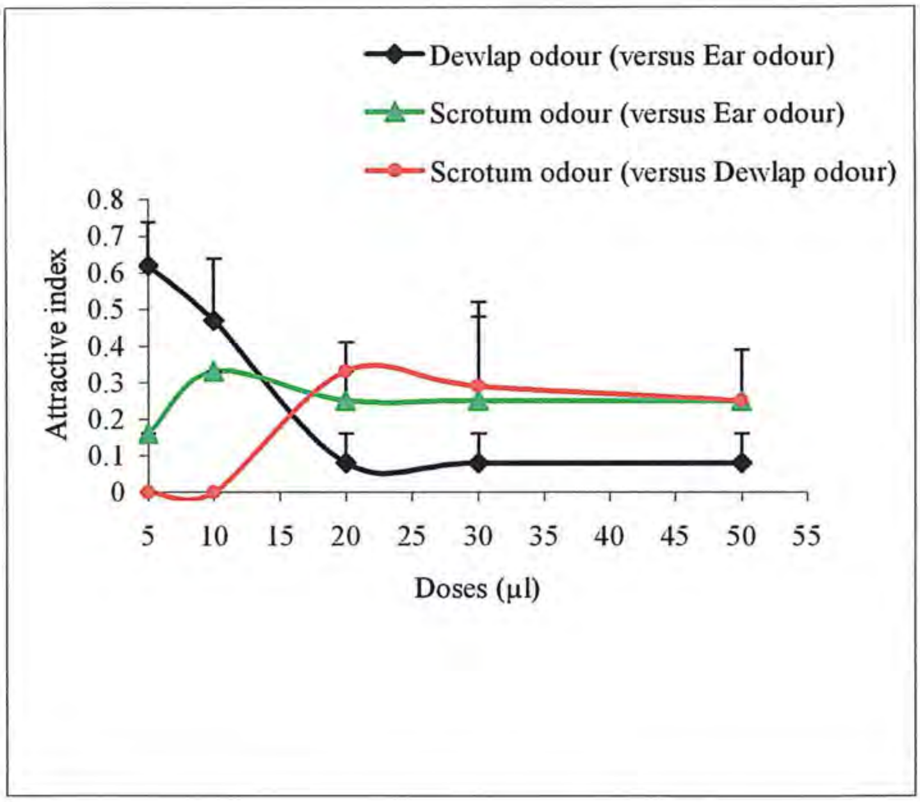

Figure 7.1: Attractiveness of the odour of some feeding sites to *A. variegatum* in a T-tube olfactometer.

7.4 DISCUSSION

The questing behaviour of Ixodid ticks serves for identification and localisation of approaching hosts and is evoked by carbon dioxide, vibrations, visual and odour stimuli (Osterkamp *et al.*, 1999). Once the tick is on the host, host specificity and site of feeding are enhanced by odour, temperature and taste stimuli (Waladde, 1987). The stimuli involved may be non-specific, acting on all tick species or may be highly specific for a given species. The olfactory capability of ticks reside in some wall-pore sensilla borne on the first leg tarsi, which serve as functional antennae to detect volatiles from hosts and conspecifics (McMahon *et al.*, 2003).

Host odours are potentially the most specific distance stimuli encountered by arthropod parasites (Osterkamp *et al.*, 1999). In this regard, the volatiles of two of the predilection feeding sites of *A. variegatum* (scrotum and lower dewlap) were tested against the odour of the ear. The odours of the scrotum and the lower dewlap were found to be attractive to the ticks. The attraction appeared to be dependent to the doses up to a certain level of volatile from which the response of ticks decreases and becomes stable (Figure 7.1). This demonstrates that the volatiles of the feeding sites are critical in attracting ticks.

The attachment of *A. variegatum* to the predilection sites occurs in two steps. Stachurski (2000) demonstrated that *A. variegatum* ticks do not invade the hosts by the head when grazing as suggested earlier for *A. hebraeum*. Instead, Stachurski (2000) reported a two-stage process for *A. variegatum* ticks, which start by attaching on the feet near the hooves of the hosts

(Appendix 2). Carroll *et al.* (1995) found that kairomones from external glands on the legs of white-tailed deer, including the interdigital glands located between the hooves, arrested host-seeking *I. scapularis* ticks. In a similar manner, kairomones could be responsible for the attachment of host-seeking *A. variegatum* nymphs on the heels of cattle (Demas *et al.*, 2000). As long as the cattle do not lie down, nearly all the ticks picked up in the pasture are temporarily attached to the feet, the attachment to the predilection sites (scrotum, lower dewlap, ventral surface) occurring later.

The attraction of other tick species is also documented. Sika (1996) reported that *R. evertsi* ticks have strong predilection for the anal region, whereas *R. appendiculatus* prefer attaching to the inner side of the ears. Later on, Wanzala *et al.* (2004) confirmed these results in an orientation process test. Evidence was then provided that *R. appendiculatus* was attracted to the ear volatiles and repelled by the anal volatiles. On the other hand, *R. evertsi* was repelled by the ear volatiles and attracted to the anal odours. The results of this study on *A. variegatum* are in agreement with these findings on *R. appendiculatus* and *R. evertsi.*

Ticks parasitoids are also reported to be attracted to the volatiles of the feeding sites of their hosts (Demas *et al.*, 2000). These authors investigated *I. hookeri* and found that its attraction to cattle is mediated by cattle waste odours as well as odours from the feeding sites of *A. variegatum*, its host.

The attraction to the host odours can be very specific. Osterkamp *et al.* (1999) reported that *I. ricinus* and *B. microplus* showed specific response to different host odours, chemical factions of host emanations and artificial odour blends. *Ixodes ricinus* was equally stimulated by all mammalian odours tested, whereas *B. microplus* always preferred bovine odours. By fractionating bovine odour, these authors showed that phenolic compounds only stimulated *B. microplus* and not *I. ricinus*, indicating that phenols might signal some cattle specificity.

A. variegatum may be using other signals than the host odours in selecting the predilection feeding sites since the most important number of ticks attracted to any of the volatiles tested was 62% (Figure 7.1). The findings are in agreement with the observations of Wanzala *et al.* (2004) on the feeding sites selection process of *R. appendiculatus* and *R. evertsi* whereby other signals may be involved. AAAP may play a role and Demas *et al.* (2000) suspected it in the finding of the host habitat and hosts by *I. hookeri* while McMahon and Guerin (2000) found that the ratios of the components of the AAAP are important in attracting *A. variegatum* adults, and that responses to one of the main pheromone component, o-nitro phenol are both enhanced and modified in the presence of host odour.

The attraction of *A. variegatum* appeared to be driven by both the AAAP and a blend of host odours comprising 1-octen-3-ol, CO_2 and other components. In the case of *A. variegatum*, it would be interesting to fully test the orientation process of this tick on cattle.

CHAPTER 8

USE OF BOTANICAL EXTRACTS TO PREVENT *AMBLYOMMA VARIEGATUM* TICKS FROM ATTACHING ON CATTLE IN THE FIELD.

8.1 INTRODUCTION

The livestock tropical bont tick, *Amblyomma variegatum* Fabricius (Acari: Ixodidae) is a pest of major economic importance in Africa where it is the vector of *Cowdria ruminantium*, which causes Heartwater in animals. The control of ticks is currently accomplished almost entirely by short-interval use of synthetic acaricides (Lwande *et al.*, 1999). So, acaricides have been the commonest method of tick control in Africa, leading to many problems such as environmental pollution, development of resistant tick strains and escalating costs (Kaaya, 1992).

To save African countries from the high costs of imported acaricides and to replace those rendered unusable by tick resistance, it is important to consider possibilities of using indigenous African plants as sources of acaricides (Kaaya, 2000).

In this regard, the use of anti-tick plants has been investigated. Some plants or plant extracts have been reported to be repellent to ticks. Mwangi *et al.* (1995a) observed in an experiment that all instars of *R. appendiculatus* were repelled by *Melinis minutiflora*. Later, Kaaya and Saxena (1998) reported that a concentration of 25% of Neem oil sprayed on deticked zebu cattle grazing on natural pastures infested with ticks reduced infestation by adult ticks by 44-62% for 5 days.

The aim of this study was to test the repellency of *Boscia senegalensis*, *Ocimum suave* and *O. kilimandscharicum* against adult *A. variegatum* in the field.

8.2 MATERIALS AND METHODS

8.2.1 Experimental site

The experiment was carried out in Muguga, at the National Veterinary Research Centre as described in section 4.2.2.

8.2.2 Animals

Eight Boran cattle were used. Before starting the experiment, the animals were subjected to two acaricidal treatments. This was to obtain animals free of ticks.

8.2.3 Pastures

The animals were grazing in a paddock of 200 x 100 m from 8:00 to 17:00. From 18:00, the animals were kept in a night paddock. The pastures were seeded with 8,000 male and female *A. variegatum*. The original number of ticks seeded in the grass served through all the experiments.

8.2.4 Tick counts

Adult *A. variegatum* attached to the animals were counted once a day and removed when animals were transferred to the night paddock. The number, sex and attachment sites of the ticks were noted.

8.2.5 Plants extracts

The repellency experiments involved three plants: *Boscia senegalensis*

(PERS.) LAM ex POIR., *Ocimum suave* Willd and *Ocimum kilimandscharicum* Guerke. The leaves of *B. senegalensis* are traditionally used in West Africa in cereal protection against pathogens, pharmacologic applications and food processing (Dicko *et al.*, 2001). The leaves have some polysaccharide hydrolases (Dicko et al., 2001) as well as methyl-isothiocyanate, methyl-cyanide and sulphur, compounds responsible for the biocide activity of the plant (Lognay *et al.*, 1993; 1994). The extracts of the plant were obtained by distillation of the leaves and the stems. The extraction was performed in the Laboratory of Organic Chemistry and Informatics, at the University of Dakar, Senegal. The oils of *O. suave* and *O. kilimandscharicum* were obtained from the leaves of these plants by hydrodistillation at the Bioprospecting Unit of ICIPE. Several concentrations of the oil (10%, 20% and 30%) in Vaseline were made according to Mwangi *et al.*, (1995b) and applied to the hooves of the three groups composed of 2 animals. The control animals were only smeared with Vaseline.

8.2.6 Data analysis

In the cases of *Ocimum suave* and *O. kilimandscharicum*, the percentage of repellency (PR) was calculated using the formula:

$PR = [(N_C - N_T)/ (N_C + N_T)] \times 100$ (Ndungu et al., 1995a), where N_C and N_T are numbers of ticks recorded in control and treated groups every day. The percentages were then subjected to an arc-sine transformation before a GLM test was carried out to test for differences in repellency due to doses, sex or their interaction, using the Statistical Analysis System Software (SAS, 1988).

The data on the attraction of ticks in the study of *Boscia senegalensis* were subjected to a square root transformation before the GLM test. A SNK test at 5% level was performed where applicable (Table) to compare means of transformed data of the different groups.

8.3 RESULTS

The oil of *O. kilimandscharicum* obtained by hydrodistillation showed repellency against *A. variegatum* in the field. The groups did not significantly differ at the SNK test. Consequently, the repellency of 10%, 20% and 30% did not significantly differ (Table 8.1). In the case of *B. senegalensis*, the treated animals (group 5%) significantly attracted more ticks while the same number of ticks was collected by the 10% treatment group and the control while *O. suave* did not show any repellency against *A. variegatum* (Table 8.1).

Table 8.1: Repellent plants study: statistical tests and results

Plants	Doses tested	Mean % attraction	Statistical tests and results
Ocimum suave	10%	23.75±5.72	Mixed procedure GLM test
	20%	39.25±12.65	
	30%	30.00±10.65	
Ocimum kilimandsharicum	10%	12.29±9.16A	GLM test SNK
	20%	23.77±10.45A	
	30%	33.67±6.72A	
Boscia senegalensis	5%	18.9±1.54AB	GLM test SNK
	10%	23.95±2.22A	
	Control	15.76±1.68B	

Mean (±SE) between doses, within the same and bearing the same capital letters are not significantly different at 0.05 level based on the SNK test

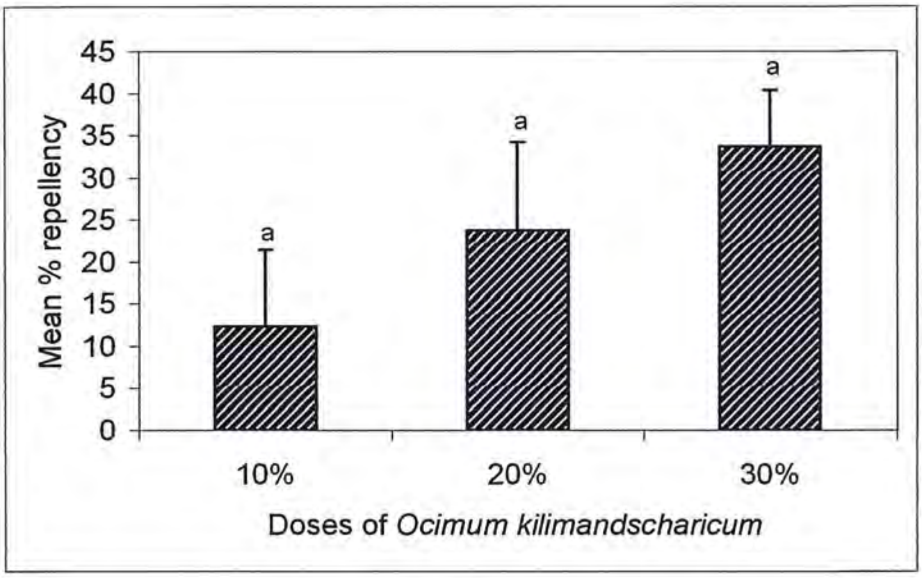

Figure 8.1: Mean percentage repellency (±SE) of *O. kilimandscharicum* to *A. variegatum* ticks in the field.

8.4 DISCUSSION

Economic and environmental considerations demand an integrated tick management strategy in which environmentally friendly acaricides replace the synthetic ones (Mansingh and Williams, 1998). In this regard, this study investigated the level of protection of cattle conferred by extracts of some plants. The results showed that *B. senegalensis* and *O. suave* are not effective in repelling *A. variegatum* in the field. Dicko *et al.* (2001) reported a traditional use of the leaves in cereal protection against pathogens. Seck *et al.* (1993) provided evidence of the efficacy of *B. senegalensis* on many grain storage pests. An acetone extract of *B. senegalensis* in a glass desiccator caused 100 % mortality in adult *Sitotroga cerealella* within 1.5 hours while for *Callosobruchus maculatus* and *Prostephanus truncates*, the LT_{50} values were 2.3 hours and 3.8 hours respectively. Further investigations of the effect of *B. senegalensis* in ticks in field should take into account the stability of the extracts. Relative humidity and temperature are some parameters, which could affect the efficiency of the extracts. The seeds of *B. senegalensis* are known to lose viability after moisture contents drop below 22% (Danthu *et al.*, 2000).

This study did not demonstrate also any repellency of *O. suave* against *A. variegatum* in the field. The results are at variance with those of Mwangi *et al.* (1995b). These authors tested the repellency of the essential oil of *O. suave* against *R. appendiculatus* and found that a concentration of 20% in Vaseline, applied three times in a week, reduced by 68.8% the attachment of ticks in the ears of cattle. This difference in the results with *A. variegatum* and *R. appendiculatus* could be due to the treated animal body area. The study of

Mwangi *et al.* (1995b), because of the natural attachment of *R. appendiculatus* in the inner side of the ears, which were smeared, allowed the oil to be protected. The oil was much more exposed to sunlight and grasses when the feet of the cattle were treated in this investigation. The sensitivity of the different ticks species could also affect the results, *A. variegatum* adult seems be less sensitive than *R. appendiculatus*. The repellency of *O. suave* to other species of pests is well documented. Seyoum (2003) reported that the seeds and leaves of *O. suave* repel 53% of mosquitoes by thermal expulsion while the oil showed repellent properties against *Sitophilus zeamais* when assessed 1h after application in an olfactometer (Hassanali *et al.*, 1990).

The use of *O. kilimandscharicum* as a storage grain protectant is also well documented. Jembere *et al.* (1995) reported the efficacy of the leaves and the essential oil of *O. kilimandscharicum* against storage grain pests. Seyoum (2003) provided evidence of the efficacy of the plants in repelling mosquitoes. The little repellency in our study (33%) may be due to the way the oil was used, smearing the feet of the cattle with the oil exposed it to sunlight and grasses. Further investigation would address the stability of such oils for better results.

CHAPTER 9

GENERAL DISCUSSION, CONCLUSION AND SUGGESTIONS

9.1 GENERAL DISCUSSION

The control of ticks in Africa relies mainly on synthetic chemical acaricides. The problems associated with the use of these pesticides, namely environmental pollution, development of resistant strains of ticks and escalating costs have stimulated research into alternative tick control methods. This study was carried out in contributing to environmentally friendly, relatively cheap ways of alleviating tick burden in the livestock industry.

This study evaluated the possibility of enhancing attraction of ticks to the Attraction-Aggregation-Attachment Pheromone (AAAP), produced by feeding males of *Amblyomma spp* with 1-octen-3-ol, which is present in volatiles of various life-stages of both *A. variegatum* and *A. hebraeum* (McMahon *et al.*, 2000). AAAP attracts unfed males and females to the site of feeding individuals and induces them to aggregate and attach around the site. 1-octen-3-ol has been shown also to elicit aggregation of the two sexes off-host (McMahon *et al.*, 2000). The test was carried out in a T-tube olfactometer in the laboratory. Males were more attracted to increasing proportions of 1-octen-3-ol than females. Similar trend was obtained by previous studies (Maranga, 1998) where AAAP only was used. A combination of 8 ng of 1-octen-3-ol with 1.1 mg of AAAP was observed as an optimum for the attraction of both sexes. Unlike 1-octen-3-ol, 2,6-dichlorophenol did not improve the attraction of *A. variegatum* in the laboratory by AAAP. This is in agreement with Akinyi (1991) who reported that mixtures of ear extracts and 2,6-dichlorophenol, both individually active, were found to be repulsive to *R. appendiculatus* adults. 2,6-dichlorophenol is a sex pheromone (Berger *et al.*, 1972; Chow *et al.*, 1975) and an attachment stimulant (Yoder and Stevens, 2000), but also seems to be inactive to ticks when it is not integrated in a blend with host volatiles (Khalil *et al.*, 1981). The results of the study are similar to the findings of previous experiments on 2,6-dichlorophenol, which might act on-host. It would be useful to investigate 2,6-dichlorophenol blended with host volatiles.

In the field, the attraction of *A. variegatum* to different doses of AAAP+1-octen-3-ol combination from various distances was investigated. The longest distance from which ticks were attracted was 7 m. The findings of our study are in agreement with Hess and Castro (1986) and Maranga *et al.* (2003) who previously reported attraction of *A. variegatum* respectively from 1 and 5 meters. The ticks reacted to the pheromones depending on the distance from the source with the least number of ticks being attracted from the furthest distance. 1-octen-3-ol improves the attraction of ticks by AAAP in the field. As suggested by McMahon *et al.* (2000), 1-octen-3-ol may be, for *A. variegatum*, a cue for aggregation with conspecifics and for host finding when in search for blood meal. Like *A. variegatum*, 1-octen-3-ol also enhances the questing behaviour of *Boophilus microplus* (Osterkamp *et al.*, 1999).

The effects of continuous and intermittent release of CO_2 on the performance of AAAP and 1-octen-3-ol in attracting ticks in the field were also investigated. CO_2 is important in host-seeking in ticks and the results showed that CO_2 increased the range of attraction to 8 m. Norval *et al.* (1987) on *A. hebraeum* and Maranga *et al.* (2003) on *A. variegatum* suggested two sequential processes of the host location in these ticks, from activation and a non-directional searching stimulated by CO_2 to a directional movement to the pheromones. The findings of this study are in agreement with these suggestions. CO_2 largely functions to mark the presence of a potential host, the pheromone that is emitted by successfully feeding ticks helps to signal the presence of an appropriate host (Maranga *et al.*, 2003). The findings did not support a significant attraction power to CO_2 alone, unlike Barré *et al.* (1997) who reported, using a Caribbean strain of *A. variegatum,* that CO_2 alone attracted ticks. The basis of use of CO_2 was investigated. The findings showed no significant difference between the continuous and the intermittent deliveries of CO_2.

The study evaluated the efficacy of traps treated with Neem Cake Extracts (NCE) and baited with AAAP, 1-octen-3-ol and CO_2. The NCE used in this study has an amount of 0.6% of Azadirachtin, which is the predominant insecticidal active ingredient in the seeds, leaves, and other parts of the Neem tree (Mulla, 1999). The mortality of attracted ticks, assessed for 21 days, was dependent on the concentration of NCE compared to the control and the time of exposure. Most of the previous studies on the efficacy of Neem investigated only tick larvae which are "supposed" to be more sensitive than adults. The findings are comparable to those of Ndumu *et al.*, (1999) in a study on larvae of *A. variegatum* in Nigeria, where undiluted Neem seed oil killed all the larvae after 48 h.

As host odours are potentially the most specific distance stimuli encountered by arthropod parasites (Osterkamp *et al.*, 1999), the behaviour of *A. variegatum* ticks was evaluated in this with regard to the volatiles of two of its predilection feeding sites (scrotum and lower dewlap) and the odour of the ear. The odours of the scrotum and the lower dewlap were very attractive to the ticks. The attraction seems to be dependent to the doses up to a certain level of volatile from which the response of ticks decreases and becomes stable. This demonstrates that the volatiles of the feeding sites are critical in attracting ticks. Similar behaviour is found in other species. It has been reported that *Rhipicephalus evertsi* ticks have strong predilection for the anal region, whereas *R. appendiculatus* prefer attaching to the inner side of the ears (Sika, 1996; Wanzala *et al.*, 2004). Stachurski (2000) demonstrated that the attachment of *A. variegatum* to the predilection sites occurs in two steps. *A. variegatum* ticks attach first on the feet near the hooves before moving to the predilection sites when the hosts lay down. As suggested by Demas *et al.* (2000), kairomones could be responsible for the attachment of host-seeking *A. variegatum* nymphs on the heels of cattle. The orientation process of *A. variegatum* could be investigated to fully understand the signals involved.

Considering the possibilities of using plants as sources of acaricides, the study investigated the efficacy of plant extracts in preventing ticks to attach to their predilection sites. *Boscia senegalensis*, *Ocimum suave* and *O. kilimandscharicum* were tested as repellents. The results showed that *B. senegalensis* and *O. suave* are not effective in repelling *A. variegatum* in the field. In the best of my knowledge, *B. senegalensis* has never been tested on ticks even though its effects on storage grain pests are documented (Seck *et al.*, 1993; Dicko *et al.*, 2001). Further investigation on this plant should take into account its stability in the field since it has been reported that the seeds lose viability below 22% of relative humidity (Danthu *et al.*, 2000). *O. suave* was not found repellent against *A. variegatum* in the field, while its repellent properties are confirmed in *R. appendiculatus* ticks (Mwangi *et al.*, 1995b), mosquitoes (Seyoum, 2003) and storage grain insects (Hassanali *et al.*, 1990). *O. kilimandscharicum* was found to repel adult *A. variegatum*. The plant extracts need to be stabilized for a better use in the field.

This technology of using botanical traps baited with pheromones and CO_2 as well as repellent plants could be further refined and incorporated into ITM Technology.

9.2 CONCLUSIONS

The study has established that:

1. The attraction of *A. variegatum* ticks by the Attraction-Aggregation-Attachment Pheromone (AAAP) is improved by 1-octen-3-ol in the laboratory. The blend of the two elicited a stronger attraction of the ticks.

2. Carbon dioxide increased the range of perception of AAAP blended with 1-octen-3-ol to 8 m by *A. variegatum* ticks in the field and the continuous and the intermittent deliveries of CO_2 are not significantly different.

3. The efficacy of traps treated with Neem Cake Extracts (NCE) and baited with AAAP, 1-octen-3-ol and CO_2 was demonstrated. A concentration of 30% of NCE in liquid paraffin killed 98% of ticks after 21 days of exposure.

4. The host odours play a key role in attracting *A. variegatum* ticks to the predilection feeding sites. The scrotum and the lower dewlap were significantly more attractive to the ticks than the ear.

5. Some repellent properties of the essential oil of *Ocimum kilimandscharicum* are promising in preventing *A. variegatum* ticks to locate and attach to their predilection feeding sites.

9.3 SUGGESTIONS FOR FURTHER INVESTIGATIONS

Behavioural tests could be carried out on *A. variegatum* nymphs and larvae with AAAP and 2,6-dichlorophenol respectively blended with 1-octen-3-ol. Further investigations could also look at the orientation process on host and the responses of *A. variegatum* adults to 2,6-dichlorophenol and AAAP blended with other host odours components. Therefore the process of host finding in this tick could be better understood.

The chemical characterisation of the odours of the feeding sites of *A. variegatum* by Gas chromatography and Gas chromatography-mass spectrometry techniques is necessary to identify the compounds of the different odours.

The technology of exposing *A. variegatum* ticks to Neem Cake Extracts in traps should be compared to those using fungi or synthetic acaricides alongside a comprehensive socio-economic evaluation.

Further investigations on the essential oils of plants candidate for repellency studies should address their stability in the field. In this regard, a chemical characterisation of these oils would be of great importance.

REFERENCES

Abdel-Shafy, S. and Zayed, A.A. (2002). In vitro acaricidal effect of plant extract of Neem seed oil (*Azadirachta indica*) on egg, immature, and adult stages of *Hyalomma anatolicum excavatum* (Ixodoidea: Ixodidae). *Veterinary Parasitology, 106, 89-96.*

Adekunle, O. A., Oladele, O. I. and Olukaiyeja, T. D. (2002). Indigenous control methods for pests and diseases of cattle in Northern Nigeria. *Livestock Res. Ru. Devel., 14 (2).* ***http: / / www . cipav .org .co / lrrd / lrrd 14 / 2 / adek142 .htm.***

Akinyi, S. (1991). Studies on pheromones and other semiochemicals of the African brown ear tick *Rhipicephalus appendiculatus*. Msc thesis. Kenyatta University. Kenya.

Barré, N. (1988). Mesures agronomiques permettant une diminution des populations de la tique *Amblyomma variegatum. Revue Elev. Med. Vet. Pays Trop., 41 (4): 387-393.*

Barré, N., Mauleon, H., Garris, G.I. and Kermarrec, A. (1991). Predators of the tick *Amblyomma variegatum* (acari: Ixodidae) in Guadeloupe, French West Indies. *Exp. & App. Acarol., (1991) 12: 163-170.*

Barré, N. (1997). Les tiques des ruminants dans les Petites Antilles: biologie, importance économique, principes de lutte. *INRA Prod. Anim., 10 (1): 111-119.*

Barré, N., Garris, G.I. and Lorvelec, O. (1997). Field sampling of the tick *Amblyomma variegatum* (Acari: Ixodidae) on pastures in Guadeloupe;

attraction of CO_2 and/or tick pheromones and conditions of use. *Exp. & App. Acarol., (1997) 21 (2): 95-108*

Berger, R.S. (1972). 2,6-dichlorophenol, as a sex pheromone of the lone star tick. *Science, 177: 704-705.*

Borges, L.M.F. and Ribeiro, M.F.B (1999). Presence of sex pheromones in *Anocentor nitens* (Acari: Ixodidae). *J. Med. Entomol., 36: 50-54.*

Borges, L.M.F., Ferri, P.H., Silva, W.J., Silva, W.C. and Silva, J.G. (2003). In vitro efficacy of extracts of Melia azedarach against the tick *Boophilus microplus*. *Med. Vet. Entomol., 17: 228–231*

Carroll, J. F., Klun, J. A. and Schmidtmann, E. T. (1995). Evidence for kairomonal influence on selection of host-ambush sites by adult *Ixodes scapularis* (Acari: Ixodidae). *J. Med. Entomol. 32: 119-125.*

Chhabra, M.B. (1992). Tick infestations of livestock in India and their control. *Insect Sci. Applic. Vol.13, No. 4: 649-655.*

Chow, Y.S., Wang, C.B. and Lin, L.C. (1975). Identification of a sex pheromone of the female brown dog tick, *Rhipicephalus sanguineus*. *Ann. Entomol. Soc. Amer. Vol. 68. N°3.*

Danthu, P., Guèye, A., Boye, A., Bauwens, D. and Sarr, A. (2000). Seed storage behaviour of four Sahelian and Sudanian tree species (*Boscia senegalensis*, *Butyrospermum parkii*, *Cordyla pinnata* and *Saba senegalensis*). *Seed Sci. Research, Vol. 10, No. 2, 183-187(6).*

Davey, R.B. and George, J.E. (1998). In vitro and in vivo evaluations of a strain of *Boophilus microplus* (Acari: Ixodidae) selected for resistance to permethrin. *J. Med. Entomol., 35: 1013-1019.*

De Bruyne M. and Guerin, P.M. (1994). Isolation of 2,6-dichlorophenol from the cattle tick *Boophilus microplus*: receptor cell responses but no evidence for a behavioural response. *J. Insect Physiol. 40: 143-154.*

Demas, F. A., Hassanali, A., Mwangi, E., Kunjeku, E. C. and Mabveni, A. R. (2000). Cattle and *Amblyomma variegatum* odors used in host habitat and host finding by the tick parasitoid, *Ixodiphagus hookeri. J. Chem. Ecol. Vol. 26, No. 4: 1079-1093.*

De Vos, S., Zeinstra, L., Taoufik, A., Willadsen, P. and Jongejan, F. (2001). Evidence for the utility of the Bm86 antigen from *Boophilus miroplus* in vaccination against other tick species. *Exp. Appl. Acarol., 25(3): 245-261.*

Dicko, M. H., Searle-Van Leeuwen, M. J. F., Traore, A. S., Hilhorst, R. and Beldman, G. (2001). Polysaccharide hydrolases from leaves of Boscia senegalensis: properties of Endo-(1→3)-β-D-Glucanase. *Appl. Biochem. And Biotechn., Vol. 94, No. 3: 225-241 (17).*

DiefAlla, H. A., Azzam, M. A., Hamdy, I. H., Salah, M. K. (2003). Acaricidal effects cardiac glycosides, Azadirachta indica and Neem oil against the camel tick, *Hyalomma dromedarii* (Acari: Ixodidae). *Pest Manage. Sci., Vol. 59 (11): 1250-1254.*

Dioli, M., Jean-Baptiste, S. and Fox, M. (2001). Ticks (Acari: Ixodidae) of the one-humped camel (*Camelus dromedarius*) in Kenya and Southern Ethiopia: species, composition, attachment sites, sex ratio and seasonal incidence. *Revue Elev. Med. Vet. Pays Trop., 54 (2): 115-122.*

Dipeolu, O.O. (1991). Current approach to control of African ticks on livestock, ICIPE, Kenya: In: Modern Acarology. (Ed. F. Dusbabeck and V. Bukwal, Volume I, Academia, Prague, 219-228.

Dipeolu, O. O. and Ndungu, J. N. (1991). Acaricidal activity of "Kupetaba", a ground mixture of natural products against *Rhipicephalus appendiculatus. Vet. Parasit. 38: 327-338.*

Eads, R.B., Smith, G.C. and Maupin, G.O (1982). A CO_2 platform trap for taking adult *Dermacentor andersoni* (Acari: Ixodidae). *Proc. Entomol. Soc. Wash., 84: 342-348.*

FAO, (1998). Ticks and Tick-borne Disease Control: a practical field manual, vol.1.

Fasanmi, F. and Onyima, V.C. (1991). Current concepts in the control of ticks and tick-borne diseases in Nigeria - A review. *Insect Sci. Appli. Vol. 13, No. 4: 615-619.*

Fourie, L. J., Stanneck, D. and Horak, I. G. (2003). The efficacy of collars impregnated with flumethrin and propoxur against experimental infestations of adult *Rhipicephalus sanguineus* on dogs. *Jou. S. Afr. Vet. Ass. Vol. 74, No.4: 123-126.*

French, F.E. and Kline, D.L. (1989). 1-octene-3-ol, an effective attractant for Tabanidae (Diptera). *J. Entomol. 26: 459-461.*

George, J.E. (2000). Present and future technologies for tick control. *Ann. N.Y. Acad. Sci., 916: 583-588.*

Gikonyo, N.K., Hassanali, A., Njagi, P.G. N., Gitu, P. and Midiwo, J. O. (2002). Odor composition of preferred (Buffalo and ox) and nonpreferred (waterbuck) hosts of some savanna tsetse flies. *J. Chem. Eco., Vol. 28, No. 5.*

Gladney, W.J., Ernst, S.E., and Oehler, D.D. (1974). The Gulf Coast tick: Evidence of a pheromone produced by males. *J. Med. Entomol., 11: 303-306.*

Golob, P., Moss, C., Dales, M., Fidgen, A., Evans, J. and Gudrups, I. (1999). The use of spices and medicinals as bioactive protectants for grains. FAO, Agriculture Services Bulletin No 137, Food and Agriculture Organisation of the United Nations, Rome.

Gothe, R. (1984). Ticks Paralyses: Reasons for Appearing during Ixodid and Argasid Feeding. In Current Topics in Vector Research, vol. II, 199-224 (Ed. By Kerry F. Harris).

Gray, J.S. (1985). A carbon dioxide trap for prolonged sampling of *Ixodes ricinus* L. populations. *Exp. Appl. Acarol., 1: 35-44*

Guèye, A., Camicas, J.L., Diouf, A. and Mbengue, M. (1987). Tiques et hémoparasitoses du bétail au Sénégal. 2. La Zone sahélienne. *Revue Elev. Med. Vet. Pays Trop., 40 (2): 119-125.*

Guèye, A., Mbengue, M. & Diouf, A. (1993a). Epidémiologie de la cowdriose au Sénégal. I. Etude de la transmission et du taux d'infection d'*Amblyomma variegatum* (fabricius, 1794) dans la région des Niayes. *Revue Elev. Med. Vet. Pays Trop., 46 (3): 441-447.*

Guèye, A., Mbengue, M., Dièye, T., Diouf, A., Seye, M. and Seye, M.H. (1993b). Cowdriosis in Senegal: some epidemiological aspects. *Revue Elev. Med. Vet. Pays Trop., 46 (1-2): 217-221.*

Guèye, A., Martinez, D., Mbengue, M., Dieye, T. and Diouf, A. (1993c). Epidémiologie de la cowdriose au Sénégal. II. Résultats de suivis sérologiques. *Revue Elev. Med. Vet. Pays Trop., 46 (3): 449-454.*

Guglielmone, A.A., Mangold, A.J., Munoz Cobenas, M.E., Scherlin, N., Garcia Posse, F., Anziani, O.S. and Lappolo, M. (2000). Moxidectin pour-on for control of natural populations of the cattle tick *Boophilus microplus* (Acarina: Ixodidae). *Vet. Parasit., 87: 237-241.*

Hall, D.R., Beevor, P.S., Cork, A., Nesbitt, B.F. and Vale, G.A. (1984). A potent olfactory stimulant and attractant for tsetse isolated from cattle odours. *Insect Sci. Appl. 5: 335-339.*

Hassan, S.M. and Dipeolu, O.O. (1993). Livestock tick predation by chickens: the rate of digestion of ticks in the alimentary tract of chickens. *Bio. Sc. and Technol., (1993), 3: 79-83.*

Hassanali, A., Lwande, W., Ole-Sitayo, N., Moreka, L., Nokoe, S. and Chapya, A. (1990). Weevil repellent constituents of *Ocimum suave* leaves and *Eugenia caryophyllata* cloves used as grain protectants in parts of Eastern Africa. *Discovery and Innovation,* 2(2): 91-95.

Hess, E. and de Castro, J.J. (1986). Field tests of the response of *Amblyomma variegatum* (Acari: Ixodidae) to the synthetic aggregation-attachment pheromone and its components. *Exp. Appl. Acarol.*, 2: 249-255.

Holm, E. and Wallace (1989). Distribution of some Anystid mites (Acari: ixodidae) in Australia and Indonesia and their role as possible predators of the cattle tick, *Boophilus microplus* (Acari: Ixodidae). *Exp. & App. Acarol., 6: 77-83.*

Howell, F.G. (1975). The roles of host-related stimuli in the behavior of *Argas cooleyi* (Acarina: Argasidae). *J. Med. Entomol., 6: 715-723.*

Hu, R., Hyland, K. E. and Mather, T. N. (1993). Occurrence and distribution in Rhode Island of Hunterellus hookeri (Hymenoptera: Enccyrtidae), a wasp parasitoid of *Ixodes dammini. J. Med. Entomol, 30: 277-280.*

Ismail M.H., Chitapa, K. and Solomon, G. (2002). Toxic effect of Ethiopian Neem Oil on larvae of cattle tick, *Rhipicephalus pulchellus* Gerstaeker. *Kasetsart J. (Nat. Sci.) 36: 18-22.*

Jembere, B, Obeng-Ofori, D. a nd H assanali, A. (1995) Products derived from the leaves of *Ocimum kilimandscharicum* (Labiatae) as post-harvest grain protectants against the infestation of three major stored product insects pests. *Bulletin of Entomological Research,* 85: 361-367.

Jonsson, N. N. (1997). Control of cattle ticks (*Boophilus microplus*) on Queensland dairy farms. *Aust. Vet J. Vol. 75, No. 11: 802-807.*

Jonsson, N.N., Matschoss, A.L., Pepper, P., Green, P.E., Albrecht, M.S., Hungerford, J. and Ansell, J. (2000). Evaluation of tickGARD (PLUS), a novel vaccine against *Boophilus microplus*, in lactating Holstein-Friesian cows. *Vet. Parasitol. 88 (3-4): 275-285.*

Kaaya, G.P. (1992). Non-chemical agents and factors capable of regulating tick populations in nature: a mini review. *Insect Sci. Appli. Vol. 13, No.*

4: 587-594.

Kaaya, G.P. (1994). Development of efficient and environmentally acceptable Tsetse and Tick control strategies for the Tropical developing World: Ticks Research. In Annual Scientific Report/ICIPE, 113-114.

Kaaya, G. P. and Saxena, R. C. (1998). Evaluation of neem products for the control of the African ticks *Rhipicephalus appendiculatus*, *Amblyomma variegatum* and *Boophilus decoloratus*. In: ICIPE Annual Scientific Report 1995-1997: 165-167.

Kaaya, G.P. (2000). The potential of anti-tick plants as components of integrated tick control strategy. *Ann. N. Y. Acad. Sci., 916: 576-582.*

Kaaya, G.P. and Hassan, S. (2000). Entomogenous fungi as promising biopesticides for tick control. *Exp. App. Acarol. 24: 913–926.*

Kaaya, G.P. (2003). Prospects for innovative tick control methods in Africa. *Insect Sci. Applic., Vol. 23, No. 1, 59-67.*

Karlson, P. and Luscher, M. (1959). "Pheromone": A new term for a class of biologically active substance. *Nature, 183: 55-56.*

Khalil, G.M., Nada, S.A. and Sonenshine, D.E. (1981). Sex pheromone regulation of the mating behaviour in the camel tick *Hyalomma dromedarii* (Ixodoidea: Ixodidae). *J. Parasitol. 67: 70-76.*

Kiara, H.K., Essuman, S., Osir, E.O., and Okwakpam (1994). Rearing *Amblyomma variegatum* ticks in the laboratory: a simple technique to enhance attachment. *Med. & Veter. Entomol., 8: 395-397.*

Kline, D. L., Takken, W., Wood, J. R. and Carlson, D. A. (1990). Field studies on the potential of butanone, carbon dioxide, honey extract, 1-

octen-3-ol, lactic acid, and phenols as attractants for mosquitoes. *Med. Vet. Entomol. 4: 383-391.*

Kline, D.L., Wood, J.R. & Cornell, J.A. (1991). Interactive effects of 1-octen-3-ol and carbon dioxide on mosquito (Diptera: Culicidae): surveillance and control. *J. Med. Entomol., 28(2): 254-258.*

Knipling, E. F. and Steelman, C. D. (2000). Feasibility of controlling *Ixodes scapularis* ticks (Acari: Ixodidae), the vector of Lyme disease, by Parasitoid Augmentation. *J. Med. Entomol. 37(5): 654-652.*

Kubasu, S.S. (1992). The ability of *Rhipicephalus appendiculatus* (Acarina: Ixodidae) ticks in Kenya to become infected with *Theileria parva* (Apicomplexa: Theileridae). *Bull. of Entomol. Res., 82: 349-353.*

Kubasu, S.S., Young, A.S. and Karuhize, G.R. (1992). Isoenzyme caracterisation of the tick *Rhipicephalus appendiculatus* Neumann (Acarina: Ixodidae) in Kenya. *Insec. Sc. & its Appli., 13 (4): 475-480.*

Kubasu, S.S. (1997). Some biological differences of *Rhipicephalus appendiculatus* (Acarina: Ixodidae) populations in Kenya. Tropical Biodiversity and Systematics. Proceedings of the International Symposium on Biodiversity and Systematics in Tropical Ecosystems, Bonn, 1994. Zoologisches Forschungsinstitut and Museum Alexander Kœnig, Bonn, 1997. Pp 213-222.

Lewis, R. (2001). The Institute for Genomic Research (TIGR) tackles East Coast Fever. Sequencing *Theileria parva* may aid vaccination efforts. *The Scientist. 15 (1)* http://www.thescientist.com.

Lognay, G., Seck, D., Marlier, M., Haubruge, E., Gaspar, C. and Severin, M. (1993). Identification of elemental sulphur (S8) in *Boscia senegalensis* (PERS.) LAM ex POIR. leaves. *Bull. Rech. Agron. Gembloux, 28 (4) : 521-531.*

Lognay, G., Marlier, M., Seck, D., Haubruge, E., Wathelet, J. P., Coulibaly, A. D., Gaspar, C. and Severin, M. (1994). Détection des molécules responsables de l'activité biocide des feuilles de *Boscia senegalensis* (PERS.) LAM ex POIR. Par échantillonnage d'espace de tête et spectrométrie de masse couplée à la chromatographie en phase gazeuse. *Bull. Rech. Agron. Gembloux, 29 (1) : 125-136.*

Lwande, W., Ndakala, A.J., Hassanali, A., Moreka, L., Nyandat, E., Ndungu, M., Amiani, H., Gitu, P.M., Malonza, M.M. and Punya, D.K. (1999). *Gynandropis gynandra* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) repellents. *Phytochemistry 50: 401-405.*

Macleod, J., Colbo, M. H., Madbouly, M.H. and Mwanaumo, B. (1977). Ecological studies of Ixodid ticks (Acari: Ixodidae) in Zambia. 111. Seasonal activity and attachment sites on cattle, with notes on other hosts. *Bull. of Entomol. Res., 67: 161-173.*

Mansingh, A. and Williams, A.D.L. (1998). Pesticidal potential of tropical plants-II. Acaricidal activity of crude extracts of several Jamaican plants. *Insect Sci. Applic. Vol. 18, N°. 2: 149-155.*

Maranga, R.O. (1998). Innovative control methods for *Amblyomma variegatum* (Fabricius, 1794), using entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* in traps baited with the Attraction-Aggregation-Attachment pheromone. Ph.D. thesis, Jomo Kenyatta University of Agriculture and Technology, Kenya.

Maranga, R.O., Hassanali, A., Kaaya, G.P. and Mueke, J.M. (2003). Attraction of *Amblyomma variegatum* (ticks) to the attraction-aggregation-attachment pheromone with or without carbon dioxide. *Exp. & App. Acarol., 29: 121-130.*

McMahon, C. and Guerin, P.M. (2000). Responses of the tropical bont tick, *Amblyomma variegatum* (Fabricius), to its aggregation-attachment pheromone presented in an air stream on a servosphere. *J. Comp. Physiol. A., 186: 95-*

McMahon, C., Guerin, P.M. and Syed, Z. (2000). 1-octen-3-ol isolated from bont ticks attracts *Amblyomma variegatum*. Institute of Zoology, University of Neuchâtel, Rue Emile-Argand 11, 2007-Neuchâtel, Switzerland.

McMahon, C., Krober, T. and Guerin, P.M. (2003). In vitro assays for repellents and deterrents for ticks: differing effects of products when tested with attractant or arrestment stimuli. *Med. Vet. Entomol., 17: 370-378.*

Mekonnen, S., De Castro, J., Gebre, S., Hussein, I. and Regassa, A. (1992). Ticks, tick-borne diseases and their control in western Ethiopia. *Insect Sci. Applic. Vol. 13, No. 4: 661-664.*

Mulla, M.S. (1999). Activity and biological effects of Neem products against arthropods of medical and veterinary importance. *J. Am. Mosq.*

Control Assoc. 15 (2): 133-52.

Mwangi, E. N. (1990). Mortality in the non-parasitic stages of Rhipicephalus appendiculatus and other ticks of livestock, with reference to predators, parasites, pathogens and climatic factors and their role in tick life cycles. Ph.D thesis, Kenyatta University, Nairobi, Kenya.

Mwangi, E.N., Newson, R.M. and Kaaya, G.P. (1991). Predation of free-living engorged female *Rhipicephalus appendiculatus*. *Exp. & App. Acarol., 12: 153-162.*

Mwangi, E.N., Essuman, S., Kaaya, G.P., Nyandat, E., Munyinyi, D. and Kimondo, M.G. (1995a). Repellence of the tick *Rhipicephalus appendiculatus* by the grass *Melinis minutiflora*. *Trop. Anim. Hlth. Prod., 27: 211-216.*

Mwangi, E.N., Hassanali, A., Essuman, S., Nyandat, E., Moreka, L. and Kimondo, M. (1995b). Repellent and acaricidal properties of *Ocimum suave* against *Rhipicephalus appendiculatus* ticks. *Exp. & App. Acarol., 19, 11-18.*

Mwangi, E. N., Hassan, S. M., Kaaya, G. P.and Essuman, S. 1997. The impact of *Ixodiphagus hookeri,* a tick parasitoid, on *Amblyomma variegatum* (Acari: Ixodidae) in a field trial in Kenya. *Exp. Appl. Acarol. 21: 117-126.*

Ndumu, P.A., George, J.B. and Choudhury, M.K. (1999). Toxicity of neem seed oil (Azadirachta indica) against the larvae of *Amblyomma variegatum* a three-host tick in cattle. *Phytother. Res. 13 (6): 532-4.*

Ndungu, M., Lwande, W., Hassanali, A., Moreka, L. and Chhabra, S.C. (1995a). *Cleome monophylla* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) and maize weevil (*Sitophilus zeamais*) as repellents. *Entomolo. Exp. & App., 76: 217-222.*

Ndungu, M. W., Chhabra, S.C. and Lwande, W. (1995b). *Cleome hirta* essential oil as livestock tick (*Rhipicephalus appendiculatus*) and maize weevil (*Sitophilus zeamais*) repellent. *Fitotherapia 70: 514-516.*

Norval, R.A.I. and Rechav, Y. (1979). An assembly pheromone and its perception in the tick *Amblyomma variegatum* (Acarina: Ixodidae) *J. Med. Entomol., 16: 507-511.*

Norval, R.A.I., Yunker, C.E. and Butler, J.F. (1987). Field sampling of unfed adults of *Amblyomma hebraeum* Koch. *Exp. App. Acarol., 3: 213-217*.

Norval, R.A.I., Yunker, C.E., Gibson, J.D. and Deem, S.L.D. (1988). Field sampling of unfed nymphs of *Amblyomma hebraeum. Exp. Appl. Acarol., 4: 173-177.*

Norval, R.A.I., Butler, J.F. and Yunker, C.E. (1989a). Use of carbon dioxide and natural or synthetic aggregation-attachment pheromone of the bont tick, *Amblyomma hebraeum*, to attract and trap unfed adults in the field. *Exp. App. Acarol., 7: 171-180.*

Norval, R.A.I., Andrew, H.R. and Yunker, C.E. (1989b). Pheromone-mediation of host selection in bont ticks (*Amblyomma hebraeum* Koch). *Science, 243: 364-365.*

Norval, R.A.I., Yunker, C.E., Duncan, I.M. and Peter, T. (1991). Pheromone/acaricide mixtures in the control of the tick *Amblyomma hebraeum*: Effects of acaricides on attraction and attachment. *Exp. & App. Acarol., 11: 233-24019.*

Norval, R.A.I. (1992). Host susceptibility to infestation with *Amblyomma hebraeum. Insect Sci. Applic. Vol. 13, No. 4: 489*-494.

Noval, R.A.I., Perry, B.D. and Young, A.S. (1992a). The epidemiology of theileriosis in Africa. 481 pp. ISBN 0-12-521740-4. Academic press, London.

Norval, R.A.I., Peter, T. and Meltzer, M.I. (1992b). A comparison of the attraction of nymphs and adults of the ticks *Amblyomma hebraeum* and *A. variegatum* to carbon dioxide and the male-produced aggregation-attachment pheromone. *Exp. & App. Acarol., 13: 179-186.*

Nyindo, M. (1992). Animal diseases due to Protozoa and Rickettsia, ISBN 9966-9860-0-6, English Press, Nairobi, Kenya.

Obiri, J.A.F., Giathi, G. and Massawe, A. (1994). The effect of Cypress Aphid on *Cupressus lusitanica* Orchards in Kenya and Tanzania. *E. Afri. Agric. For. J., 227-234.*

Odhiambo, T.R. (1992). Foreword to "Symposium on status and recent advances in tick management in Africa". *Insect Sci. Applic. Vol. 13, No. 4, p. 467.*

Okello-Onen, J., Hassan, S.M. and Essuman, S. (1999). Taxonomy of African Ticks: An Identification Manual. 124 pp. ICIPE Sciences Press, Nairobi.

Olusola, A.A. and Jerry, F.B. (1991). Field e valuation o f C arbon Dioxide baits for sampling Ornithodoros turicata (Acari: Argasidae) in Gopher Tortoise Burrows. *J. Med. Entomol. 28 (1): 45-48.*

Osterkamp, J., Wahl, U., Schmalfuss, G. and Haas, W. (1999). Host-odour recognition in two tick species is coded in a blend of volatiles. *J. Comp. Physio, 185: 59-67.*

Patarroyo, J.H., Portela, R.W., De Castro, R.O, Pimentel, J.C, Guzman, F., Patarroyo, M.E., Vargas, M.I., Prates, A.A. and Mendes, M.A. (2002). I mmunization of cattle with synthetic peptides derived from the *Boophilus microplus* gut protein (Bm86). *Vet. Immunol. Immunopathol. 88(3-4): 163-172.*

Petney, T.N. and Bull, C.M. (1981). A non-specific aggregation pheromone in two australian reptile tcks. *Anim. Behav. 29: 181-185.*

Punyua, D.K. (1992). A review of the development and survival of ticks in the tropical Africa. *Insect Sci. Appl. 13 (4): 537-544.*

Rechav, Y., Whitehead, G.B. and Knight, M.M. (1976). Aggregation response of nymphs t o p heromone(s) p roduced b y males of the t ick *Amblyomma hebraeum (Koch). Nature, 259: 563-564.*

Rechav, Y., Parolis, H., Whitehead, G.B. and Knight, M.M. (1977). Evidence for an assembly pheromone(s) produced by males of the bont tick, *Amblyomma hebraeum* (Acarina: Ixodidae). *J. Med. Entomol., 14: 71-78.*

Rechav, Y. and Whitehead, G.B. (1978). Field trials with pheromone-acaricide mixtures for control of *Amblyomma hebraeum. J. Econ.*

Entomol., 71: 149-151.

Schmutterer, H. (1990). Properties and potentials of natural pesticides from Neem tree, *Azadirachta indica. Annu. Rev. Entomol. 35: 271-297.*

Schöni, R., Hess, E., Blum, W. and Ramstein, K. (1984). The aggregation-attachment pheromone of tropical bont tick *Amblyomma variegatum* Fabricius (Acari, Ixodidae): isolation, identification and action of its components. *J. Insect. Physiol., 30: 613-618.*

Seck, D., Lognay, G., Haubruge, E., Wathelet, J. P., Marlier, M., Gaspar, C. and Severin, M. (1993). Biological activity of the shrub *Boscia senegalensis* (PERS.) Lam. ex poir. (Capparidaceae) on stored grain insects. *J. Chem. Ecolo*, 19(2): 377-389

Seyoum, A. A. (2003). Studies of the use of repellent plants and plant products against the main malaria vectors in Eastern Africa. Ph.D thesis, Kenyatta University, Nairobi, Kenya

Sika, F. K. N. (1996). Behavioural responses of *Rhipicephalus appendiculatus* Neumann 1901 and Rhipicephalus evertsi Neumann 1897 (Acari: Ixodidae) to host and non-host semiochemicals. Ph.D. thesis, Kenyatta University, Kenya.

Solomon, G. and Kaaya, G.P. (1998). Development, reproductive capacity and survival of *Amblyomma variegatum* and *Boophilus decoloratus* in relation to host resistance and climatic factors under field conditions. *Veter. Parasit., 75, 241-253.*

Sonenshine, D.E. (1984). Ticks pheromones. In "Current Topics in Vector Research", Vol. 2, 225-263. Ed. By Kerry F. Harris.

Sonenshine, D.E. (1985). Pheromones and other Semiochemicals of the Acari. Ann. *Rev. Entomol. 30: 1-28.*

Sonenshine, D.E. (1991). Biology of ticks. Vol 1 pp. 331-369. Oxford University Press, New York.

Spickett, A. M. and Heyne, H. (1988). A survey of Karoo tick paralysis in South Africa. Onderstepoort *J. Vet. Res., 55: 89-92.*

Stachurski, F. (1993). Variability of cattle infestation by *Amblyomma variegatum* and its possible utilisation for tick control. *Revue Elev. Med. Vet. Pays Tropicaux, 46 (1-2): 341-348.*

Stachurski, F. (2000). Invasion of West African cattle by the tick *Amblyomma variegatum. Med. & Veter. Entomol., 14: 391-399.*

Steullet, P. and Guerin, P.M. (1994). Identification of vertebrate volatiles stimulating olfactory receptors on tarsus I of the tick *Amblyomma variegatum* Fabricius (Ixodidae) I. Receptors within the Haller's organ capsule. *J. Comp. Physiol. 174: 27-38.*

Sutherst, R.W., Raymond, J.J. and Schnitzerling, H.J. (1982). Tropical legumes of the genus *Stylosantes* immobilize and kill cattle ticks. *Nature, 295: 320-321.*

Tawah, C.L. (1992). Comparative study of tick burdens in Gudali and Wakwa cattle under natural infestation in the subhumid highlands of Wakwa, Cameroon. Preliminary observations. *Revue Elev. Med. Veter. Pays Trop., 45 (3-4): 310-313.*

Thompson, K.C., Rao, J. and Romero, T. (1978). Anti-tick grasses as the basis for developing practical tropical tick control packages. *Trop. Anim. Hlth. Prod. 10: 179-182.*

Uilenberg, G., Barre, N., Camus, E., Burridge, M.J. and Garris, G.I. (1984). Heartwater in the Caribbean. *Prev. Vet. Med., 2: 255-267.*

Walker, A.R. and Koney, E.B.M. (1999). Distribution of ticks (Acari: Ixodida) infesting domestic ruminants in Ghana. *Bull. Entomol. Res., 89: 473-479.*

Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Pena, A., Horak, I.G., Latif, A.A., Pegram, R.G. and Preston, P.M. (2003). Ticks of Domestic Animals in Africa: A Guide to Identification of Species. Biosciences Reports, 42 Comiston Drive, Edinburgh EH10 5QR, Scotland, U.K.

Waladde, S. M. (1987). Receptors involved in host location and feeding in ticks. *Insect Sci. Applic. Vol. 8, Nos 4/5/6, 643-647.*

Wanzala, W., Sika, N. F. K., Gule, S. and Hassanali, A. (2004). Attractive and repellent host odours guide ticks to their respective feedings sites. *Chemoecology, Vol. 14, Nos. 3-4: 229-232.*

Wilkinson, P.R. (1957). The spelling of the pasture in cattle tick control. *Aust. J. Agric. Res. 8, 414-423.*

Willadsen, P., Bird, P., Cobon, G.S. and Hungerford, J. (1995). Commercialisation of a recombinant vaccine against *Boophilus microplus*. *Parasitol., 110:843-850.*

Wilson, J.G, Kinzer, D.R., Sauer, J.R. and Hair, J.A. (1972). Chemo-attraction in the Lone Star tick (Acarina: Ixodidae). 1. Response of different developmental stages to carbon dioxide. *J. Med. Entomol., 9: 245-252.*

Yoder, J.A. and Stevens, B.W. (2000). Attraction of immature stages of the American dog tick (*Dermacentor variabilis*) to 2,6-dichlorophenol. *Exp. Appl. Acarol., 24: 159-164.*

Yunker, C.E., Andrew, H.R., Norval, R.A.I. and Keirans, J.E. (1990). Interspecific attraction to male-produced pheromones of two species of *Amblyomma* ticks (Acari: Ixodidae). *J. Insect Behav. 3: 557-565.*

Yunker, C.E., Peter, T., Norval, R.A.I., Sonenshine, D.E., Burridge, M.J. and Butler, J.F. (1992). Olfactory responses of adult Amblyomma hebraeum and A. variegatum (Acari: Ixodidae) to attractant chemicals in laboratory tests. *Exp. Appl. Acarol., 13: 295-301.*

Zimmerman, R.H., Carris, C.I. and Beaver, J.S. (1984). Potential of *Stylosanthes* plants as a component in an integrated pest management approach to tick control. *Prev. Vet. Med. 2: 579-588.*

APPENDIX 1: Some of the plants used in the study:

Ocimum kilimandscharicum

Azadirachta indica (Golob *et al.*, 1999)

APPENDIX 2: Preliminary attachment of *A. variegatum* in the field: female attaching in the hind heel (A) and the front heel (B) of a cattle.

A