BEHAVIOURAL RESPONSES OF *RHIPICEPHALUS APPENDICULATUS* NEUMANN 1901 AND *RHIPICEPHALUS EVERTSI* NEUMANN 1897 (ACARI: IXODIDAE) TO HOST AND NON-HOST SEMIOCHEMICALS

SIKA FRA KUTUA NOEL
B.Sc., M.Sc. (Zoology)

THESIS SUBMITTED IN PARTIAL FULFILMENT FOR
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY IN APPLIED ENTOMOLOGY
OF KENYATTA UNIVERSITY

DEPARTMENT OF ZOOLOGY
KENYATTA UNIVERSITY
P.O. BOX 43844
NAIROBI, KENYA

AUGUST 1996

DECLARATION

THIS THESIS IS MY OWN ORIGINAL WORK AND HAS NOT BEEN PRESENTED FOR A DEGREE IN ANY OTHER UNIVERSITY

Signed ..... Date 14th August, 1996

Sika Fra Kutua Noel

APPROVAL

THIS THESIS WORK HAS BEEN SUBMITTED FOR EXAMINATION WITH OUR APPROVAL AS KENYATTA UNIVERSITY AND ICIPE SUPERVISORS.

Signed ..... Date 16/08/96

Prof. Alloys Orago (PhD, CBiol, MBiol)
Associate Professor and Chairman,
Department of Zoology
Kenyatta University
P.O. Box 43844, Nairobi, Kenya.

Signed ..... Date 15th Aug 1996

Prof. Ahmed Hassanali (PhD)
Principal Research Scientist; Head, Chemical Ecology
Department and Deputy Director General,
ICIPE
P.O. Box 30772, Nairobi, Kenya.

DEDICATION

This thesis is dedicated:

to my late beloved wife

DEODATTE BUHENDWA CHIKURU

to my children

ERIC SIKA TOROMA

THIERRY SIKA MUHIRWA

DOUCELINE ISIMBI CIREZI

& LATE SANDRALINE SIKA

and to my father and mother

SIKA MALA'DE and ISIMBI MARIE-JEANNE

DEDICACE

A toi feue ma bien-aimée épouse, **Deodatte Buhendwa Chikuru**,

pour l'amour et le soutien durant ces années,

pour ta grandeur d'esprit et tes qualités rares,

nous quittant au moment où ton affectueuse

presence nous aurait été le plus nécessaire,

pour les sacrifices consentis à deux;

En témoignage de mon profond amour et de mon

éternelle reconnaissance;

A vous mes chers enfants,

Eric Sika Toroma

Thierry Sika Muhirwa

Douceline Isimbi Cirezi

& feue **Sandraline Sika**

En témoignage de mon amour paternel;

A vous mon père et ma mère,

Ernest Sika Mala'de et **Marie-Jeanne Isimbi**,

Pour avoir fait de moi ce que je suis;

Je dédie ce travail, fruit de dur labeur.

SIKA FRA KUTUA NOEL

ACKNOWLEDGEMENTS

A work of this magnitude could not have been carried out without the help of many people. My first and foremost gratitude and appreciation goes to Prof. A. Hassanali and Prof. A.S.S. Orago, my ICIPE and University supervisors respectively, for their continuous inspiration and guidance during the course of this study. I am also grateful to Dr. Samuel M. Waladde, former member of my supervisory committee who helped in shaping up some of the aspects of this thesis. Thanks are also due to Prof. J.M. Mueke and Dr. W. Lwande for their advice. Drs. K.S. Nokoe, A. Odulaja, J.O. Owino and Mr. D. Munyinyi have been very helpful in statistical analyses. I wish to thank too Drs. P.G.N. Njagi, S. Essuman, M.M. Rai, B. Torto and G. Melaku who made comments on various sections of this thesis. Thanks are also extended to Dr. R.K. Saini, and staff in the Behavioural Biology Department and the Rearing Unit of ICIPE who kindly attended to all my requests and needs. My appreciation goes to Messrs E. Nyandat, L.M. Moreka, N.K. Gikonyo, H. Odongo, D. Omondi, S.A. Ochieng and A. Chanzu for providing me with efficient technical assistance. I cannot forget the field assistance provided by Mr. Francis Achira.

During my stay at ICIPE, the families of Drs. N.N. Massamba and Mulenda Basimike have been a source of constant moral support. I am also thankful to Dr. Muhinda and the ARPPIS scholars of 1991 Class for their friendly cooperation and encouragement.

Last but not least, I thank ICIPE who provided me the UNDP scholarship through its African Regional Postgraduate Programme in Insect Science (ARPPIS).

Finally, the contribution of my family has been tremendous: especially the sacrifices my late wife and my children had to contend with and the moral support they gave throughout this study period, and yet accepting me as one of them.

ABSTRACT

On-host observations and laboratory behavioural assays were undertaken to investigate the role played by (a) bovine host-derived semiochemicals from the ear and anal region and (b) semiochemicals derived from conspecifics, in feeding site location by unfed adults of *Rhipicephalus appendiculatus* Neumann and *Rhipicephalus evertsi* Neumann. The potential of host-derived allomones from unpreferred feeding sites and that of a tick repellent plant, *Ocimum suave* Willd as disrupting factors to on-host orientation by the two tick species to their target sites were also assessed.

Orientative behaviour of *R. appendiculatus* on host was characterized by stereotyped sequences of behaviours which included stationary/scanning phase, random search, directional movement and arrestment closer to and at the site source.

Patterns of tick migratory paths to their respective feeding sites were characterized and quantified in both species. These were made up of runs with intermittent stops, all producing curvilinear tracks. The results of locomotory activities demonstrated a high proportion of completion of paths to the preferred feeding sites, along with high success rates of feeding site location (67.5-90.0% for *R. appendiculatus*; 69.8-85.6% for *R. evertsi*). The results also showed variations in mean walking speed between sexes, species and release points on the host. Mean velocity for males and females averaged 0.09 and 0.41cm/min in *R. appendiculatus* and 0.24 and 0.58cm/min in *R.*

evertsi.

Assays of tarsi-painting on *R. appendiculatus* and *R. evertsi* showed that these were capable of discriminating their respective feeding sites using olfactory cues. In both species, tarsi-coated individuals were less able to orient toward or locate efficiently the relevant anal or ear sites ($P<0.001$). *In situ* and laboratory assays have implicated primarily site-borne stimuli as mediating factors in this orientation. This kairomone-driven mediation appears to be enhanced by intraspecific signals emitted by feeding ticks present at the feeding site, since ear sites loaded with live ticks ($P<0.01$) or rinses of fed ticks ($P<0.01$) were more attractive, although not significantly, compared to control tick-free ears.

Specific behaviours leading to the location and finding of the feeding sites namely: arousal, activation, arrestment and aggregation in response to olfactory stimuli were examined. Arousal tests (in a Y-olfactometer) on *R. evertsi* and *R. appendiculatus* exposed to odour extracts from ear and anus resulted in scanning and residence response patterns that correlated with the stimulatory or inhibitory nature of each extract. Release of ticks *in situ* resulted in the activation of the tick by the host odours after a short latent period which decreased with decrease in distance from the feeding site. Likewise, walking speed of both species resulted in gradual decrease and eventual arrestment in the proximity to the feeding site. Tick velocity correlated with distance and arrestment was more evident at close range ($\leq$ 25 cm away from any site). On the other hand, artificial substrates impregnated with feeding site

materials (anal or ear extracts) caused walking arrestment of the relevant species ($P<0.01$) and evoked in the tick characteristic klinokinetic path patterns on the odourized arena.

Significant aggregation responses of male and female ticks of *R. appendiculatus* occurred on calves scrotum smeared with hexane rinses obtained from fed male and female ticks ($P<0.05$). The aggregation responses increased slightly with increasing concentrations of the rinses. The aggregating factor remains to be characterized.

Olfactometric assays showed that extracts in washings from various body parts, namely belly/axillae, neck/dewlap and leg were less or unattractive to the adults of *R. appendiculatus*. On the other hand, ear and anal extracts elicited strong attraction to the adults of *R. appendiculatus* ($P<0.001$) and *R. evertsi* ($P<0.001$), respectively, in a dose-dependent fashion. Nymphs and larvae of *R. appendiculatus*, in contrast, were significantly repelled ($P<0.01$). Trapped volatiles from the host were significantly attractive to the ticks than the washes ($P<0.001$), but the blend of the two was more attractive compared to the individual extracts. The additive effect of the two suggests a dual kairomonal set of components comprising short-range/contact signals and volatile components which mediate feeding selection in this tick.

Cross-assays involving the use of extracts from a site preferred by one tick species showed strong repulsive effect on the other species. Thus, a 'push-pull' mechanism has been proposed to explain highly successful tick orientation and feeding site finding on host and the difference in this regard in these two tick species. The

'pull' component is made of interspecific signals from the preferred sites augmented by intraspecific signals from successfully feeding ticks. The 'push' component comprised of repellents from unpreferred site which ensures that the tick does not mistakenly orient in that direction.

Repellent extracts from unpreferred feeding host sites and the essential oil of *Ocimum suave* (a tick repellent shrub) were used to study possible disorientation of *R. appendiculatus* and *R. evertsi* toward their respective feeding sites. Cattle repellent extracts and the essential oil of *O. suave* applied as smears to serve as barriers across tick migratory paths to the feeding sites, were disruptive at all concentrations tested. The host extract ($P < 0.05$) as well as the plant extract ($P < 0.001$) significantly reduced the success of *R. appendiculatus* and *R. evertsi* to reach their target site. Ear and anal extracts were found to have short-lived persistent effect on the target ticks, unlike the essential oil of *O. suave* which was effective for several days during the test period. This may be attributed to differences in concentration rather than intrinsic activity.

These results suggest possibilities of manipulating the host and feeding site location behaviour of ticks on- and off-host using appropriate semiochemicals as tactics in the management of tick populations.

TABLE OF CONTENTS

Page

Title page i

Declaration ii

Dedication iii

Acknowledgements ..... iv-v

Abstract ..... vi-ix

Table of Contents ..... x-xxiv

Chemical Ecology Glossary xxv

CHAPTER 1

1.0 **GENERAL INTRODUCTION AND LITERATURE REVIEW** 1

1.1 Introduction 1

1.2 Ticks and tick control methods 2

1.2.1 Biology, distribution and ecology of *Rhipicephalus appendiculatus* and *R. evertsi* 2

1.2.1.1 Taxonomic status of *R. appendiculatus* 2

1.2.1.2 Life cycle of *R. appendiculatus* 2

1.2.1.3 Distribution and ecology of *R. appendiculatus* 5

1.2.2 *Rhipicephalus evertsi* 7

1.2.3 Pest status and economic importance of the two tick species 9

1.2.4 Current tick control practices 12

1.2.4.1 Acaricides 12

Page

1.2.4.2 Non-acaricidal control alternatives 13

1.2.4.2.1 Ecological methods 13

1.2.4.2.2 Biocontrol 14

1.2.4.2.3 Naturally acquired host resistance to ticks 14

1.2.4.2.4 Immunological approaches/vaccine development 15

1.2.4.2.5 Anti-theilerial drug therapy 15

1.2.5 Semiochemicals in tick control 16

1.3 Responses of ticks to stimuli on- and off-host 16

1.3.1 Behavioural response patterns of ticks and factors that govern them 16

1.3.2 Orientation behaviour and factors that mediate them 17

1.3.3 Pheromone-mediated behaviours 18

1.3.3.1 Assembly (aggregation) off-host 18

1.3.3.2 The aggregation-attachment pheromone 19

1.3.3.3 Sex pheromones 19

1.3.3.4 The mounting and contact sex pheromones 20

1.3.3.5 Other tick pheromones 21

1.3.4 Responses of *R. appendiculatus* to host-derived kairomones 21

1.3.4.1 Responses of ticks to CO_2 and other bovine breath volatiles 22

1.3.4.2 Responses to other bovine-derived odours 22

1.3.4.3 Responses to feeding site stimuli 24

1.3.5 Tick movement and orientation in relation to semiochemicals 27

Page

1.3.5.1 Tick distribution and movement on host 27

1.3.5.2 On-host orientation mediated by kairomones/pheromones 28

1.4 Disruption of tick orientation on host with semiochemicals 29

1.4.1 Current semiochemicals-based monitoring and control methods 29

1.4.2 Allomonal effects of some plants, with particular reference to *Ocimum suave* 31

1.4.3 Disruptive effects of some host materials on ticks 33

CHAPTER 2

2.0 **OBJECTIVES OF THE STUDY** 34

2.1 Statement of the problem 34

2.2 Objectives of the study 34

2.2.1 General objectives 35

2.2.2 Specific objectives 35

CHAPTER 3

3.0 **GENERAL MATERIALS AND METHODS** 36

3.1 Ticks 36

3.2 Cattle 37

3.3 Experimental room 37

3.4 Sources of test semiochemicals 39

3.4.1 Host stimuli 39

3.4.1.1 Washes (gustatory and olfactory stimuli) 39

Page

3.4.1.2 Volatiles (olfactory stimuli) 41

3.4.1.3 Cow dung 43

3.4.2 Volatiles from the ticks 44

3.4.2.1 Solvent rinses 44

3.4.2.2 Volatiles from feeding ticks 45

3.4.3 Essential oils of plants 45

3.4.3.1 Plant material 45

3.4.3.2 Extraction of the essential oil 47

3.4.3.3 Estimation of the yield of essential oil and its eugenol content 47

3.4.3.4 Application of plant extracts 50

3.5 Solvents and chemical standards used 50

3.6 Gas chromatographic analyses of volatiles and essential oils 50

3.7 Olfactometers 53

3.7.1 T-tube olfactometer 53

3.7.2 Y-tube olfactometer 54

3.7.3 Paired vertical glass column 59

3.8 Experimental observations and procedures 63

3.8.1 Olfactometric bioassays 63

3.8.2 On-host behavioural studies 63

3.8.2.1 Crutch for observations 63

3.8.2.2 Preparation of cattle 64

3.8.2.3 Tick release on host 64

Page

3.8.3 On-host behaviour assays 65

3.8.3.1 Orientation behaviour to feeding sites 65

3.8.3.2 Disruption experiments 65

3.9 Behavioural studies layout 66

3.10 Statistical analyses 67

CHAPTER 4

4.0 **ON-HOST MOVEMENT PATTERNS OF *R. APPENDICULATUS* AND *R. EVERTSI* IN RESPONSE TO HOST-RELATED SEMIOCHEMICALS** .68

4.1 Introduction 68

4.2 Materials and methods 69

4.2.1 Feeding site selection 69

4.2.2 Orientative behaviour of *R. appendiculatus* on host 70

4.2.3 Pathways of *R. appendiculatus* and *R. evertsi* on host 72

4.2.4 On-host locomotory activity of *R. appendiculatus* and R. *evertsi* 73

4.3 Results 75

4.3.1 Selection of the feeding sites by *R. appendiculatus* and *R. evertsi* 75

4.3.2 Orientation behaviour of *R. appendiculatus* to the feeding site 82

4.3.3 Pathways of *R. appendiculatus* and *R. evertsi* to feeding sites 86

4.3.4 Locomotory activities of *R. appendiculatus* and *R. evertsi* 89

4.4 Discussion 93

Page

CHAPTER 5

5.0 **STUDY OF SOME SPECIFIC BEHAVIOURAL RESPONSES** 95

5.1 Introduction 95

5.2 Materials and methods 97

5.2.1 Arousal to host odours 97

5.2.2 Activation and arrestment behaviour of *R. appendiculatus* and *R. evertsi* to host odours 98

5.2.3 Aggregation responses of *R. appendiculatus* on bovine host 99

5.3 Results 100

5.3.1 Arousal of *R. appendiculatus* and *R. evertsi* to extracts from preferred and unpreferred feeding sites 100

5.3.2 Activation and arrestment responses of *R. appendiculatus* & *R. evertsi* to host odours 108

5.3.2.1 Activation and arrestment *in situ* 108

5.3.2.2 Walking arrestment of *R. appendiculatus* and *R. evertsi* on extract-impregnated artificial substratum (Whatman filter paper) 109

5.3.3 Aggregation response of *R. appendiculatus* on the natural bovine host 114

5.4 Discussion 119

5.4.1 Arousal to extracts from preferred and unpreferred feeding sites 119

5.4.2 Activation and arrestment in situ of *R. appendiculatus* and *R. evertsi*; walking arrestment on artificial substrate 118

5.4.3 Aggregation response 120

Page

CHAPTER 6

6.0 **ROLE OF HOST KAIROMONES: STIMULI RESPONSES UNDER LABORATORY CONDITIONS** 122

6.1 Introduction 122

6.2 Materials and methods 124

6.2.1 Preliminary studies of tick responses to host stimuli 124

6.2.1.1 Preliminary comparison of responses of *R. appendiculatus* instars to hexane extracts 124

6.2.1.2 Responses of *R. appendiculatus* adults to ear extracts using hexane, methanol and acetone solvents 124

6.2.2 Detailed responses of *R. appendiculatus* and *R. evertsi* adults to host odours stimuli 125

6.2.2.1 Responses of *R. appendiculatus* to odours from unpreferred feeding sites 125

6.2.2.2 Responses of *R. appendiculatus* and *R. evertsi* to stimuli from their respective and each other's feeding sites 126

6.2.2.3 Dose responses to feeding site extracts 126

6.2.2.4 Responses of *R. appendiculatus* to contact and air-borne volatile kairomones 127

6.3 Results 127

6.3.1 Responsiveness of adult and immature instars of *R. appendiculatus* to odour in ear wash extract 127

6.3.2 Relative attractiveness of 3 different solvent-extracted ear stimuli to *R. appendiculatus* 130

6.3.3 Responsiveness of *R. appendiculatus* adults to host odour extracts from selected body regions (non-feeding sites) 132

6.3.4 Responsiveness of *R. appendiculatus* and *R. evertsi* to odour extracts from their respective and each other's feeding sites 134

Page

6.3.5 Dose-responses of *R. appendiculatus* and *R. evertsi* to odour extracts from their respective and each other's feeding site 136

6.3.6 Responses of *R. appendiculatus* to contact and air-borne volatile kairomones 138

6.4 Discussion 140

CHAPTER 7

7.0 **DISRUPTION OF ORIENTATION BEHAVIOUR OF *R. APPENDICULATUS* AND *R. EVERTSI* ON HOST** 144

7.1 Introduction 144

7.2 Materials and methods 145

7.2.1 Responses of *R. appendiculatus* and *R. evertsi* to feeding sites modified by solvent washing 145

7.2.2 Responses of *R. appendiculatus* and *R. evertsi* to normal feeding sites treated with extracts from each other's feeding sites 146

7.2.2.1 *R. evertsi* orientation responses to anal area treated with the ear extract .146

7.2.2.2 *R. appendiculatus* orientation responses to the ear treated with the anal extract 147

7.2.3 Responses of *R. appendiculatus* and *R. evertsi* to signals from feeding sites modified by the essential oil of *O. suave* 149

7.2.4 Allomonal persistence of host materials and *O. suave* plant essential oil ... 152

7.3 Results 151

7.3.1 Responses of *R. appendiculatus* and *R. evertsi* to the respective feeding site modified by solvent washing 151

7.3.2 Orientation responses of *R. appendiculatus* and *R. evertsi* to their respective feeding sites modified by the presence of extracts from each other's feeding site 155

Page

7.3.3 Orientation responses of *R. appendiculatus* and *R. evertsi* to their feeding sites treated with *O. suave* essential oil 160

7.3.4 Allomonal persistence of host materials and *O. ocimum* essential oil 164

7.4 Discussion 169

CHAPTER 8 **GENERAL DISCUSSION** 171

CHAPTER 9 **CONCLUSIONS** 176

CHAPTER 10 **SUGGESTIONS FOR FUTURE WORK** 180

LIST OF FIGURES

Page

Figure 1 Map showing distribution of *R.appendiculatus* and other vectors of *Theileria parva* causing East Coast fever (ECF) in Africa 6

Figure 2 Distribution of theileriosis with *Theileria parva* (ECF) in Africa 10

Figure 3 Flow diagram showing the extraction procedure of odour materials collected from bovine host (body wash) 40

Figure 4 Components of a trapping system for air-borne volatiles for a host ... 42

Figure 5 Diagram showing a T-shaped glass tube olfactometer to test responses of adults to extracts 55

Figure 6 Diagram showing a Y-shaped glass tube olfactometer used to test responses of tick larvae and nymphs to host extracts 56

Figure 7 Diagram illustrating the delivery system in Y-tube olfactometer for larvae and nymphs 57

Figure 8 Diagram showing a Y-shaped tube olfactometer used for adult ticks in arousal experiments 58

Figure 9 Diagram showing a paired climbing column olfactometer for attractancy tests 61

Figure 10 Diagram showing a paired column climbing olfactometer for repellancy assays 62

Figure 11 Sampling areas of positive and negative orientation responses of *R. appendiculatus* to ear site on cattle 76

Figure 12 Sampling areas of positive and negative orientation responses of *R. evertsi* to anal site on cattle 77

Figure 13 Proportion of tarsi-painted *R. appendiculatus* orienting to the ear feeding site 81

Figure 14 Proportion of tarsi-painted *R. evertsi* orienting to the anal feeding site 81

Page

Figure 15 Feeding site orientation behaviour of *R. appendiculatus* and possible semiochemical signals implicated 84

Figure 16 General pathways followed by adult *R. appendiculatus* ticks to their feeding site (ear) from different release points 87

Figure 17 General pathways followed by *R. evertsi* ticks to their feeding site (anal area) from different release points 88

Figure 18 Proportion of *R. appendiculatus* locating successfully the ear site after release from 4 points on the host 90

Figure 19 Proportion of *R. evertsi* locating successfully the anal site after release from 2 points on the host 90

Figure 20 Frequency distribution of scanning activity and residence time of *R. appendiculatus* and *R. evertsi* adults exposed to ear extracts in a T-Tube olfactometer 106

Figure 21 Frequency distribution of scanning activity and residence time of *R. appendiculatus* and *R. evertsi* adults exposed to anal extracts in a T-Tube olfactometer 107

Figure 22 Walking arrestment of *R. appendiculatus* on ear extract-treated Whatman paper substrate: pathways and time responses 113

Figure 23 Walking arrestment of *R. evertsi* on anal extract-treated Whatman paper substrate: pathways and time responses 115

Figure 24 Responses of adult and immature instars of *R. appendiculatus* to ear odour extracts 131

Figure 25 Relative attractiveness of 3 solvent extracts of host ear to *R. appendiculatus* adults 133

Figure 26 Relative attractiveness of odour extracts of host body regions to *R. appendiculatus* adults 135

Figure 27 *R. appendiculatus* and *R. evertsi* adults responses to ear and anal odour extracts 137

Page

Figure 28 Dose response effects of ear and anal extracts on *R. appendiculatus* and *R. evertsi* adults 139

Figure 29 Effect of combining solvent extract and air-borne volatiles on the ear responses of *R. appendiculatus* 141

Figure 30 Types of barriers of repellent material around the ear base of the host 150

Figure 31 Effect of washing on orientation responses of *R. appendiculatus* to the ear site 154

Figure 32 Effect of washing on orientation responses of *R. evertsi* to the anal site 154

Figure 33 Gas Chromatograph profile of *O. suave* essential oil 165

Figure 34 Allomone persistence of the anal extract around the ear site as measured by *R. appendiculatus* responses 167

Figure 35 Allomone persistence of the ear extract around the anal site as measured by *R. evertsi* responses 167

Figure 36 Allomone persistence of *O. suave* essential oil around the ear as measured by *R. appendiculatus* responses 168

Figure 37 Allomone persistence of *O. suave* essential oil around the anal site as measured by *R. evertsi* responses 168

LIST OF PLATES

Page

Plate 1 Male of *R. appendiculatus* tick 4

Plate 2 Female of *R. appendiculatus* tick 4

Plate 3 Male of *R. evertsi* tick 8

Plate 4 Female of *R. evertsi* tick 8

Plate 5 Friesan exotic cattle breed (heifers) used in experiments and odours collection 38

Plate 6 Ayrshire exotic cattle breed (steers) used in experiments and odours collection 38

Plate 7 *Ocimum suave* plant 46

Plate 8 Photograph showing Clevenger apparatus used in this study for steam distillation of the essential oil from *O. suave* leaves 48

Plate 9 Photograph showing apparatus for the removal of solvent from hydrodistilled essential oil extract of *O. suave* 49

Plate 10 Hewlett Packard 5890 Gas Chromatograph (GC) equipment with a chromjet integrator used to analyse plant essential oils in this study 52

Plate 11 *R. appendiculatus* ticks scattered on calf scrotum treated with solvent (control) 117

Plate 12 Aggregation response pattern of *R. appendiculatus* ticks on scrotum treated with tick extracts 117

LIST OF TABLES

Page

Table 1 Dye colour group in relation to sites of the release of ticks on host 73

Table 2 Effects of tarsi-painting treatment on orientation responses of *R. appendiculatus* to the ear site 79

Table 3 Effects of tarsi-painting treatment on orientation responses of *R. evertsi* to the anal site 80

Table 4 Mean % *R. appendiculatus* orienting to ear signals augmented with various tick stimuli on the ear feeding site 85

Table 5 Locomotory activities of *R. appendiculatus* in relation to the location of the ear feeding site 91

Table 6 Locomotory activities of *R. evertsi* in relation to the location of the anal feeding site 92

Table 7 Scanning activity & residence time responses of *R. appendiculatus* and *R. evertsi* exposed to the ear odour extracts in treated olfactometer arm 104

Table 8 Scanning activity & residence time responses of *R. appendiculatus* and *R. evertsi* exposed to the anal odour extracts in treated olfactometer arm 105

Table 9 Activation and arrestment responses in situ of *R. appendiculatus* released in the vicinity of the ear odour source 109

Table 10 Activation and arrestment responses in situ of *R. evertsi* released in the vicinity of the anal odour source 110

Table 11 Time response for walking of *R. appendiculatus* arrested on Whatman filter paper substrate treated with ear extract 112

Table 12 Time response for walking of *R. evertsi* arrested on Whatman filter paper substrate treated with anal extract 114

Page

Table 13 Clustering response of *R. appendiculatus* on calf scrotum 118

Table 14 Effects of solvent washing on orientation responses of *R. appendiculatus* to the ear feeding site 155

Table 15 Effects of solvent washing on orientation responses of *R. evertsi* to the anal feeding site 156

Table 16 Effects of smears of various repellent extracts on orientation responses of *R. appendiculatus* to its preferred site 159

Table 17 Orientation responses of *R. appendiculatus* to the ear modified by the anal extract 160

Table 18 Orientation responses of *R. evertsi* to the anal region modified by the ear extract 161

Table 19 Orientation responses of *R. appendiculatus* to the ear modified by *O. suave* essential oil 163

Table 20 Orientation responses of *R. evertsi* to the anal region modified by *O. suave* essential oil 164

CHEMICAL ECOLOGY GLOSSARY*

Semiochemicals: chemical signals involved in interactions between organisms (=infochemicals, ecomones, ecochemicals).

Pheromones: chemical signals secreted by an organism to the outside and cause specific responses in receiving organisms of the 'same' species (= intraspecific semiochemicals).

Allelochemics: chemical signals that affect members of a species different from the emitter (= interspecific semiochemicals):

→ Kairomones: adaptatively favourable to the receiver (e.g. attractants, arrestants, stimulants);

→ Allomones: adaptatively favourable to the emitter (e.g. repellents, deterrents).

→ Synomones: adaptatively favourable to both.

* Lecture notes, Chemical Ecology Course, ICIPE, 1991.

CHAPTER ONE

1.0. GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Most ixodid ticks rely heavily on semiochemicals for their interspecific and intraspecific communication. Pheromones and other behaviour-modifying compounds (or semiochemicals) are known to regulate a number of tick functions such as host selection and location; mate location, recognition and copulation; selection of suitable habitat before dropping off host and assembly (a protective behaviour) during non-parasitic phases in the habitat. Likewise, host odours contain stimuli (bodily extracts, effluvia, carbon dioxide) which influence tick behaviour. However, the role played by specific chemical stimuli in attracting certain ticks to the preferred feeding sites on their host is not fully understood.

Two species of ticks, *Rhipicephalus appendiculatus* and *Rhipicephalus evertsi*, although differing in many of their biological aspects, show similar patterns of preference for predilection sites where they orient to in response to stimuli. This raises the possibility of manipulating this behaviour through appropriate intervention tactics. If feasible and compatible with other existing strategies, semiochemical-based tactics may be useful in Integrated Tick Management (ITM) and may significantly reduce the over-reliance on pesticides.

1.2 Ticks and tick control methods

1.2.1 Biology, distribution and ecology of *Rhipicephalus appendiculatus* and *R. evertsi* ticks

1.2.1.1. Taxonomic status of *Rhipicephalus appendiculatus*

Rhipicephalus appendiculatus belongs to the genus *Rhipicephalus* and the family Ixodidea. The genus whose most members are of African origin (Hoogstraal, 1956) comprises about 70 species worldwide and includes *Rhipicephalus zambeziensis* Norval, Walker & Colborne and *Rhipicephalus duttoni* Neumann, two species showing close morphological affinities with *R. appendiculatus*.

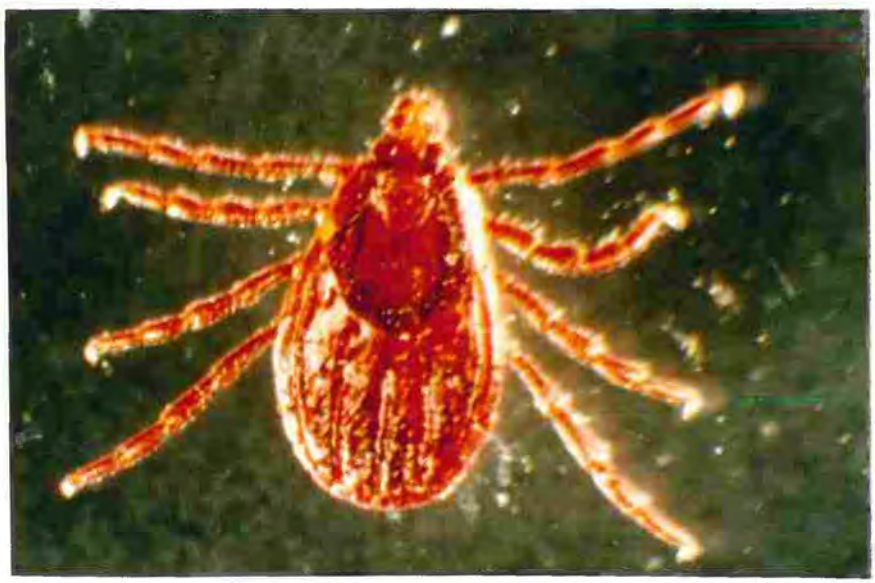
1.2.1.2 Life cycle of *R. appendiculatus*

The life history of *R. appendiculatus* (Plates 1 and 2) is relatively well documented (Elbl and Anastos, 1966; Yeoman and Walker, 1967; Newson, 1978). The species's life cycle is characterized by a three-host pattern as all the three stages must each locate and feed on a host animal. Larvae, nymphs and adults take a blood meal until they are fully engorged and then drop onto the ground where they pursue their non-parasitic phases. Larva and nymph can undergo moulting into the next stage while the adult males seek

for females to mate and can mate more than once (Punyua, 1978). After mating, the female undertakes the final and fast feeding phase which leads to full engorgement (Tukahirwa, 1976). The engorged female drops onto the ground and seeks a suitable oviposition site where she finally lays eggs. Generally, each stage feeds on a different host which may be the same (e.g. cattle), but the immature stages in the wild may in addition feed on unrelated hosts such as small mammals including rodents (Elbl and Anastos, 1966).

About 2-5% of a tick's life span is spent on a host during the cycle which may last up to 500 days (Hoogstraal, 1956; Branagan, 1973). The adults of *R. appendiculatus* mostly feed on the inner surface of host ear while feeding of larvae and nymphs takes place on many other parts of the host body (Elbl and Anastos, 1966; Yeoman and Walker, 1967).

Bovine cattle are the main host of *R. appendiculatus*, but goats and sheep also have been found infested. Infestations extend also to wild bovids (including buffalo, eland, water buck..) and many other ungulates.



Plates 1 and 2 Male (Upper) and female (Lower) of

Rhipicephalus appendiculatus (magnif.: 100-fold)

1.2.1.3 Distribution and ecology of *R. appendiculatus*

R. appendiculatus is a widespread tick species in Africa occurring in eastern, central and southern parts of the continent (Fig. 1). No populations of this species have been reported in West Africa (Hoogstraal, 1956). However, the latest information on distribution shows that it has spread now to 15 countries (Norval *et al.*, 1992a). The potential of the species spreading to new areas has been predicted based on their suitability (Sutherst and Maywald, 1985; Norval *et al.*, 1991b).

Relative humidity, associated with pluviometry and altitude are parameters of major significance in the ecology of this species. This restricts *R. appendiculatus* to cool and humid biotopes (of less than 30 °C daily maxima and at least 400 mm annual rainfall), preferably uplands savanna, with a vegetation cover made of woody or bushy grasslands (Yeoman and Walker, 1967). Climate as a macro factor plays a role in regulating populations as well as the seasonal dynamics and activity of different stages (Yeoman G.H., 1966; Short and Norval, 1981). Depending on rainfall pattern and host availability, one or two generations can be observed per year (Branagan, 1973; Newson, 1978).

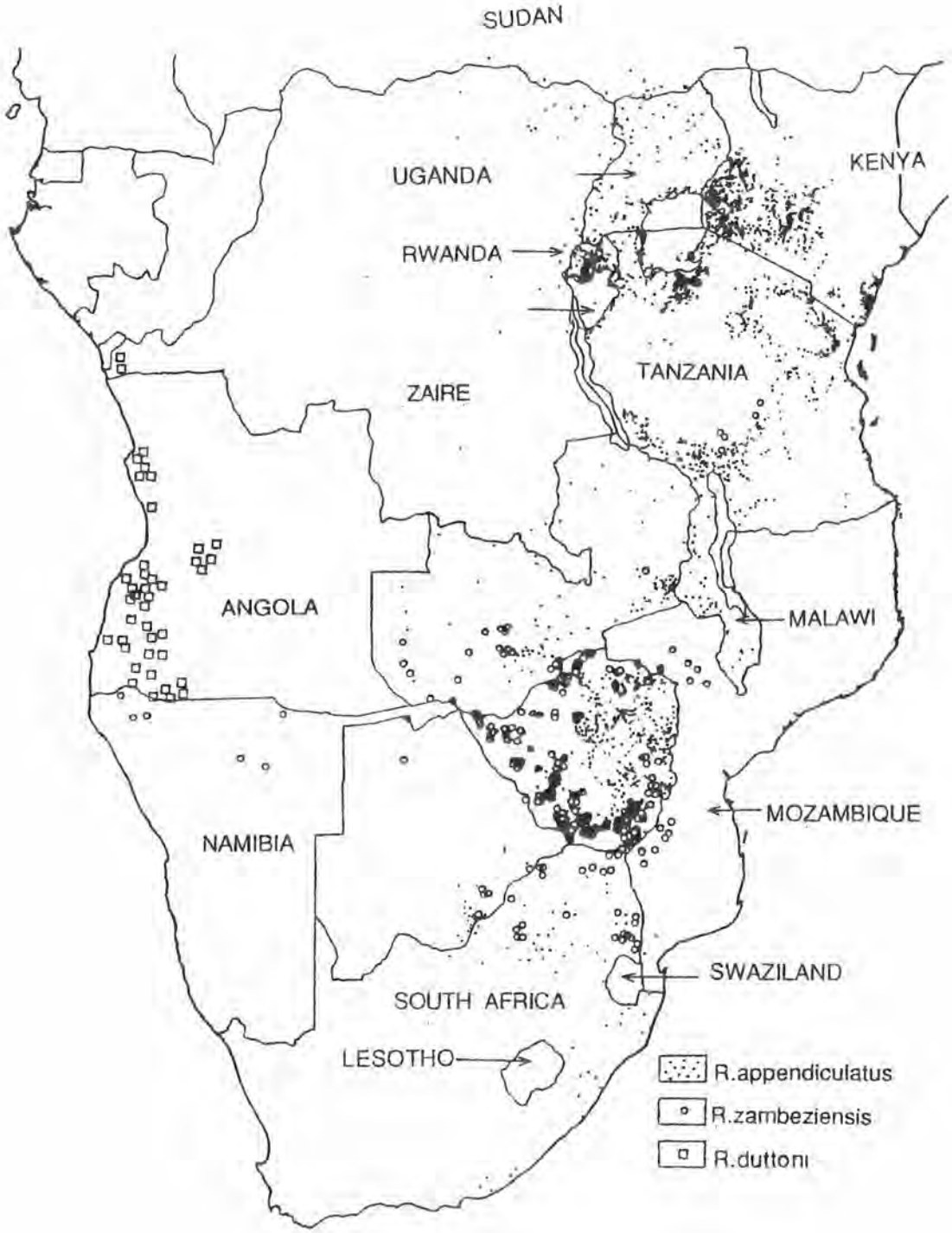
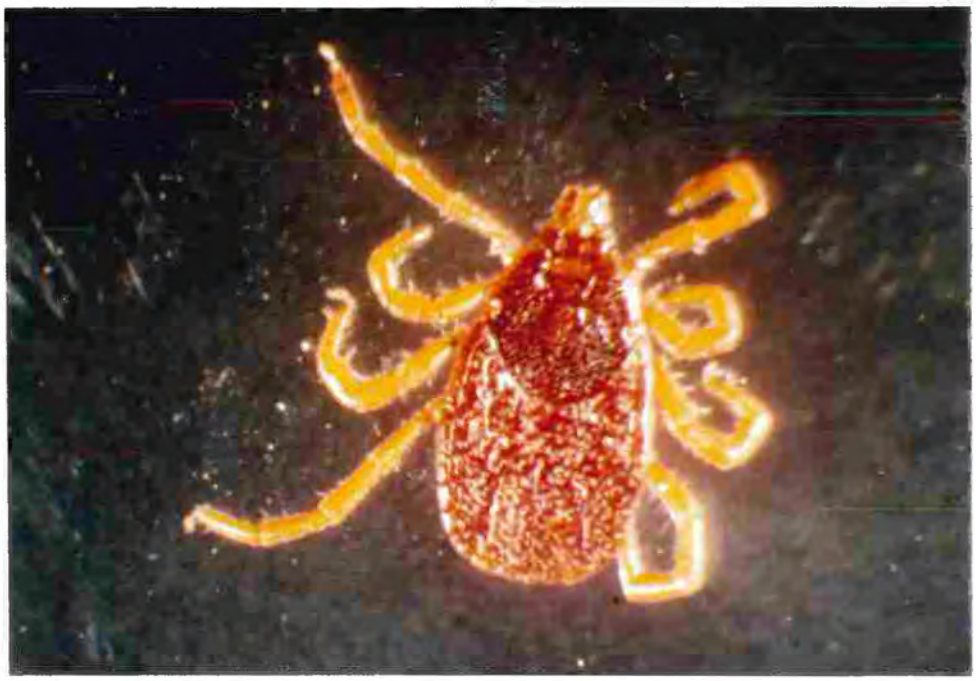


Fig. 1 Map showing the distribution of R. appendiculatus and other vectors of Theileria parva causing ECF in Africa (After Lessard et al; 1990)

1.2.2 *Rhipicephalus evertsi* species

The red-legged tick *Rhipicephalus evertsi* (Plates 3 and 4) is a two-host tick. Larvae and nymphs feed on one host whereas the engorged adult reattaches onto a second host. Two known subspecies of this species are *Rhipicephalus evertsi evertsi* Neumann and *Rhipicephalus evertsi mimeticus* Donitz. These together have a very wide geographic distribution in semi-arid areas of central, eastern and southern Africa. Few populations of this species have also been reported to extend towards the Arabian peninsula. The host range of the species includes both wild and domestic animals (FAO, 1984). *R. evertsi* is known to be highly specific with regard to the feeding site as adults attach mostly around the anal region of the host whereas larvae and nymphs are found deep in the ear. Based on this peculiar trait, *R. evertsi* was selected for comparison with *R. appendiculatus* in selected areas of this study.



Plates 3 and 4 Male (Upper) and female (Lower) of

Rhipicephalus evertsi (magnif.: 100 fold)

1.2.3 **Pest status and economic importance of the two tick species**

R. appendiculatus and *R. evertsi* are known vectors of agents that cause many economically important diseases in domestic livestock. Arguably, *R. appendiculatus* is seen as economically the most important vector among ticks and, concurrently with tsetse flies, poses a major threat to livestock industry development in Africa (Dipeolu, 1990).

The African brown ear tick *R. appendiculatus* is the major vector of the haemaprotozoan *Theileria parva parva*, the causative agent of East Coast fever (ECF)(Fig. 2) affecting livestock production in sub-saharan Africa. Another theilerial disease viz. the Corridor Disease, caused by *Theileria parva. lawrencei* affects buffalos and coexists with the ECF. The vectorial role of *R. evertsi* with respect to ECF is still uncertain, although the species is known to cause animal tick paralysis on the animal host and to transmit a number of animal diseases (FAO, 1984).

ECF-related mortality and morbidity are determined by several factors including the immune status of the host and virulence of *Theileria* strains (Norval *et al.*, 1991a). For example, exotic cattle, *Bos taurus* are highly susceptible with mortality of 90-100%. In contrast, indigenous cattle, *Bos indicus*, suffer much lower fatal cases of about 10-40% in calves due to acquired immunity (Sutherst *et al.*, 1978). Even in such cases, the young or adult cattle become ECF carriers after recovery (Young *et al.*, 1986). Cases of similar reservoirs are also found in wildlife (Young *et al.*, 1981).

Fig. 2 Distribution of Theileriosis with Theileria parva (ECF) in Africa (After Lessard et al., 1990)

Of the 200 million cattle in Africa, 38% live in ECF-affected areas which cover an estimated area of 156 m Ha (Mukhebi *et al.*, 1991). Eleven countries namely Burundi, Kenya, Malawi, Mozambique, Rwanda, Sudan, Tanzania, Uganda, Zaire, Zambia and Zimbabwe are under permanent risk. Apart from ECF, babesiosis, Nairobi Sheep Disease and Looping ill and a bite-borne toxaemia are also transmitted by *R. appendiculatus* (FAO, 1984).

Heavy infestations *per se* may cause numerous debilitating effects, resulting in cattle emaciation (due to exsanguination), irritation and wounds which serve as routes for infections (FAO, 1984). However, of real concern to farmers is loss of productivity in terms of milk and meat output. For example, in an ECF-infested area of Kenya, De Castro *et al.*(1985) evaluated decrease of liveweight of cattle of 12.8 kg for a 200 kg animal. Similarly, damages to hide are likely to downgrade their market value.

Besides cattle morbidity and mortality as well as severe economic reductions as a result of the direct and indirect actions of parasitism, stock management also requires tremendous financial inputs. For example, costs incurred for control operations have been estimated at US $ 7.02/head/year or US $ 1.08 per ha (ILRAD, 1990). Countries exposed to the threat of ECF also face immense financial burden due to importation of acaricides. Such drains of currency have been estimated to cost millions of US dollars annually in many African countries (Dipeolu, 1990).

1.2.4 **Current tick control practices**

1.2.4.1 **Acaricides**

Various approaches have been pursued worldwide to control cattle tick-borne diseases and their vectors. Primarily, these include the use of synthetic acaricides used in sprays, dips, dusts, etc. Acaricides that have been commonly used are of different chemical groups namely arsenates, organochlorines (e.g. DDT, dieldrin, toxaphene and lindane), organophosphates, carbamates, formadines, cyclic amidines and recently, ivermectins and pyrethroids.

There is a lot of awareness on problems arising from widespread use of pesticides (Wharton and Roulston, 1971; Matthewson, 1984; Nolan, 1990). The most noticeable are: the development of tick resistant strains, high cost of chemicals, toxicity to vertebrates and effects on both the environment and non-target species such as parasitoids and birds (Stutterheim and Brooke, 1981; Matthewson, 1984). As the development of new acaricides is becoming rather uneconomical (Norval *et al.*, 1992a), the current emphasis is on other improved formulations or release devices of existing acaricides such as ear-tags and pour-ons (Gladney, 1976; Young *et al.*, 1985).

1.2.4.2 **Non-acaricidal control alternatives**

Because of the above described shortcomings of chemical methods, promising non-pesticidal alternatives are being developed which could be incorporated into an integrated control strategy, among which the following are most important:

1.2.4.2.1 **Ecological methods**

Ecological methods for tick control include rotational grazing ("pasture spelling"), routinely applied against the one-host tick *Boophilus microplus* (Canestrini) in Australia, burning of pastures and habitat alterations (Sutherst *et al.*, 1979). Habitat modifications with anti-tick pastures such as anti-tick legumes of the genus *Stylosanthes* and molasses grass, *Melinis*, have been proposed (Thompson *et al.*, 1978; Sutherst *et al.*, 1982; Zimmerman *et al.*, 1984). Certain plants with repellent or acaricidal properties such as *Ocimum suave* (Willd), *Gynandra gynandropsis* (L.) Briggs, *Cleome monophylla* (L.), etc.. have also shown some promise (Malonza *et al.*, 1992; Mwangi *et al.*, 1995b; Ndungu *et al.*, 1995). Attractant plants currently explored may offer another way of ecologically managing certain tick species (Hassan *et al.*, 1994).

1.2.4.2.2 **Biocontrol**

Biological control of ticks includes the use of their various natural enemies such as predators, parasitoids and pathogens. Ticks are preyed on by oxpecker birds *Buphagus spp* or cattle egrets and by domestic chickens (Hassan *et al.*, 1991; Mwangi *et al.*, 1991). There are also records of parasitic wasps, especially *Ixodiphagus hookeri* attacking ticks (Mwangi *et al.*, 1991). Fungi such as *Beauveria bassiana*, *Metarhizium anisopliae* as well as a spectrum of bacteria are reported to be pathogenic to some tick species (Mwangi *et al.*, 1991). However, biocontrol agents have shown inherent limitations (Norval *et al.*, 1992b). Likewise, there are several genetic control packages such as sterilization techniques, hybrid sterility, cytoplasmic incompatibility (Galun *et al.*, 1972; Osburn and Knipling, 1982), but these have not achieved much practical success either (Galun *et al.*, 1972).

1.2.4.2.3 **Naturally acquired host resistance to ticks**

Naturally acquired host resistance is defined as the ability of certain cattle breeds to limit tick burden. This is brought about by exposure of the host to tick infestations and this phenomenon has shown some promise for tick control (Sutherst *et al.*, 1979). Zebu (*Bos indicus*) cattle or its crosses, unlike the exotic European breed (*Bos taurus*), are known to be genetically disposed to acquiring a higher resistance (Bonsma, 1981).

The ability to acquire resistance is a trait that can be inherited (Hewetson, 1972). Researchers in Australia and in Africa have demonstrated that this resistance can also, to some degree, be induced in susceptible hosts (Wikel and Allen, 1982; Jongejean *et al.*, 1986; Essuman *et al.*, 1991). In Africa, this resistance has been induced by numerous vector ticks including *R. appendiculatus*, *Amblyomma hebraeum* (Latif, 1984; Njau *et al.*, 1988; Dipeolu *et al.*, 1992). Attempts are being made to exploit this phenomenon for tick control.

1.2.4.2.4 **Immunological approaches/ vaccine development**

Immunological approaches to control ticks and the diseases they transmit have followed two directions. One approach is toward anti-theilerial vaccine targeting especially the surface-antigens of *Theileria parva* (Irvin and Morrison, 1989). The second one is based on immunization against the vector using tick immunogens (Johnston *et al.*, 1986; Jongejean *et al.*, 1986; Essuman *et al.*, 1991). Sources of immunogenic materials explored for anti-tick vaccines include whole tick extracts, salivary gland extracts, midgut antigens, etc.

1.2.4.2.5 **Anti-theilerial drug therapy**

Apart from the above described methods for controlling the vector, therapeutic drugs

against theilerial parasites are also in use of which naphtoquinones have been found to be the most effective (McHardy, 1989).

1.3 Semiochemicals in tick control

The control methods outlined above can hardly achieve success when applied singly, hence an integrated tick management (ITM) approach is necessary to deploy them in a cost-effective and complementary manner and to minimize the use of acaricides. A strategy that will incorporate semiochemicals is likely to be of special value as it has a considerable degree of species specificity (Shorey, 1976). Such an approach is also highly rated by environmentalists because of its other benefits such as maintaining a non-polluted environment, its safety to non-target organisms including humans and the fact that it does not give rise to resistance on target organisms (Newsom, 1967; Shorey, 1976; Perring and Mellanby, 1978).

1.3 Responses of tick to stimuli on- and off-host

1.3.1 Behavioural response patterns of ticks and factors that govern them

Semiochemical communication mediates a wide range of behavioural functions in arthropods such as host location, mate finding and habitat location. Bell and Tobin

(1982) suggested that sensory mechanisms involved in the orientation of arthropods to odours viz. are through an internal programme (idiothetic control) e.g. hunger and endogenous rhythms and by the detection of stimuli from the environment (allothetic control). Processing of the sensory information from the peripheral sensory neurones is in the Central Nervous System (CNS) (Baker, 1985) and the resultant motor patterns are generally manifested as non-oriented movements (kineses) or oriented movements (taxes) (Kennedy, 1977).

1.3.2 **Orientation behaviour and factors that mediate them**

Semiochemical factors mediating off-host and on-host events are of different classes and include intraspecific pheromones and host-derived interspecific allomones and kairomones. Pheromones are likely to elicit reactions at close distance or by contact as shown in numerous studies. At distance, host kairomones and some allomones are also known to play roles (Sonenshine, 1985; Sonenshine *et al.*, 1986). Details of how ticks utilize semiochemicals have been reviewed by Galun (1977); Sonenshine *et al.* (1982b, 1986); Sonenshine (1984, 1985) and Waladde and Rice (1982).

The reactions to chemicals that modulate arthropod behaviour often result in displacement towards or away from the odour source, aggregation, arousal and/or stimulation of ticks. More than one of these reactions can be integrated to form one resultant response (Bell and Tobin, 1982). In some instances, these reactions can consist

of a sequence of behaviour in order to achieve a specific function (e.g. courtship, copulation, etc...) (Sonenshine, 1985).

1.3.3 **Pheromone-mediated behaviours**

Different classes of pheromones are known to mediate tick behaviour on- and off-host:

1.3.3.1 **Assembly (aggregation) off-host**

This behaviour is mediated by environmentally persistent and non-specific pheromonal compounds whose function is arrestment of quiescent ticks at the pheromone-impregnated sites (Sonenshine *et al.*, 1982b). Unfed *R. appendiculatus* adults show assembly behaviour during dry periods (Sonenshine *et al.*, 1982b). Hassanali *et al.* (1989) confirmed that stressful conditions were conducive to assembly responses in that species. The assembly pheromone has been identified as guanine (Otieno *et al.*, 1985). Other purines generally elicit the same behaviour in other ticks (Dusbabek *et al.*, 1991).

1.3.3.2 **The aggregation-attachment pheromone**

These pheromones aggregate both sexes of adult ticks (and possibly nymphs) at the attachment sites on the host skin, at locations where other conspecifics are (Gladney *et al.*, 1974a; Rechav *et al.*, 1976, 1977). The male-produced pheromone present in most *Amblyomma spp* has been identified as a blend of O-nitrophenol (ONP), methyl salicylate and pelargonic acid (Schoni *et al.*, 1984). In *R. appendiculatus*, a similar aggregation pheromone is suspected to occur; in T-tube unfed ticks of both sexes were responsive to chemical stimuli from fed tick extracts and on rabbit, they prefer to attach nearby pre-attached conspecifics (Akinyi, 1991).

1.3.3.3 **Sex pheromones**

These are compounds that facilitate mate finding and recognition during mating. 2,6-Dichlorophenol (2,6-DCP) is the most widely reported sex attractant and was first identified in *Amblyomma americanum* (L.)(Berger, 1972). Its actual role in the mating behaviour of adult ticks is not fully understood. Wood *et al.*,(1975) showed in a T-tube assay that 2,6-DCP was attractive to *R. appendiculatus* male although they failed to detect it in tick extracts. The pheromones identified in the tick by these authors were phenol and p-cresol which were also present in *R. pulchellus* Gerstater extracts. Later, electrophysiological tests and chemical analyses demonstrated that 2,6-DCP is present in

both males and females of *R. appendiculatus* tick (McDowell and Waladde, 1985). According to this study, 2,6-DCP occurs also in the larvae but is not found in eggs. The presence of 2,6-DCP across stages led these workers to ask if the compound acts solely as a sex attractant in this tick. The amounts of 2,6-DCP released in the adult stage of *R. appendiculatus* were shown to be in higher concentrations in feeding females than in feeding males, suggesting a sex pheromone role (Akinyi, 1991). However, these differences may not be necessarily attributed solely to a sexual role. Whether the chlorinated phenol plays an aggregation role as suggested by its presence in both sexes and in larvae remains to be answered. Recently, 2,6-DCP was reported to be behaviourally inactive in *Boophilus microplus* in a host-simulated arena (De Bruyne and Guerin, 1994). Furthermore, work done at ICIPE highlighted the existence of a number of other halogenated phenols, in addition to 2,6-DCP and phenolic substances previously described by Wood *et al.* (1975). Among the new compounds are 2,6-Dibromophenol (2,6-DBP) and 2-bromo-6-chlorophenol (2-B-6-CP) (ICIPE, 1994). The role played by these halogenated phenols, individually and in blends in inter- and intra-specific interactions, requires further investigation.

1.3.3.4 **The mounting and contact sex pheromones**

These are specific classes of tick sex pheromones. The mounting sex pheromone (MSP) is known to elicit male copulation attempts (Hamilton and Sonenshine, 1988)

whereas the contact sex pheromone (CSP) facilitates courting male to guide its aedegus to the female gonopore (Sonenshine *et al.*, 1982a). MSP was further identified as cholesteryl oleate (Hamilton *et al.*, 1989). The nature of genital sex pheromone is not yet fully elucidated, although their fatty acid nature has been demonstrated (Allan *et al.*, 1988). *R. appendiculatus* is no exception as MSP was recently found to be released in this species too (Hamilton *et al.*, 1994).

1.3.3.5 **Other tick pheromones**

Possibility for the existence of a defensive pheromone in ticks (Yoder *et al.*, 1993) and a primer pheromone acting on fecundity (Khalil *et al.*, 1981) has been raised.

1.3.4 **Responses of *R. appendiculatus* to host-derived kairomones**

Apart from pheromone-mediated behaviours, host odours also influence extensively on-host orientation of the tick and their selection of feeding sites. Host-borne kairomonal stimuli fall into three classes based on their nature and the corresponding behaviour evoked: (i) CO_2 and other bovine breath volatiles; (ii) host body attractants and (iii) feeding site attractants.

1.3.4.1 **Responses of ticks to CO_2 and other bovine breath volatiles**

Carbon dioxide (5-10% of expired air of mammalian breath) is among host-originated odorants influencing the behaviour of several tick species, either as an attractant (Garcia, 1962; Neville, 1964) or a stimulant (Norval *et al.*, 1989b). Sauer *et al.* (1974) reported that CO_2 doses of 1-8% enhanced host seeking behaviour in the American lone star tick, *Amblyomma americanum*. Other compounds of interest found in trace amounts in ox breath are acetone, 1-octen-3-ol (octenol) and butanone. The blend of octenol and CO_2 was found to enhance catches of tsetse flies (Vale, 1980), but these components were ineffective and didn't evoke any attraction to *Amblyomma hebraeum* (Norval *et al.*, 1989b). Other detailed studies showed that CO_2's role in host location by ticks is more intricate, as elevated CO_2 in combination with other stimuli (e.g. heat, odour, temperature) elicit higher responses from ticks than when these stimuli were presented singly (Lees, 1948; Howell, 1975).

1.3.4.2 **Responses to other bovine-derived odours**

Ticks are haematophagous parasites and to secure bloodmeal, they have to locate their host. The role of host factors has been recognized by early workers who devised various *in vivo* and *in vitro* assays to study them (Lees, 1948; Howell, 1975; Doube and Kemp, 1979). Stimuli from hosts serve as a multi purpose indicator: for host proximity,

to guide to potential host and to elicit feeding responses once the contact with the appropriate site has been made (Howell, 1975). Waladde and Rice (1982) distinguished two types of inherent appetence behaviour used by tick vis-a-vis its host. In the questing type, passive ticks would await for a passing host to cling on. The hunting type in contrast would actively move to a suspected host and is likely to make use of host odours for orientation, alone or in conjunction with other cues. There are as many modalities in host location as there are species. In general, the presence of various stimuli has made possible a hierarchy with regard to whether these act at close, mid- or long-range distances during the host-finding process as observed for some insect groups (Shorey, 1976; Visser, 1986). According to some reports, attraction and attachment of tick to host skin are influenced by olfactory stimuli augmented by thermal stimuli (Lees, 1948; Howell, 1975). Rare cases of visual and/or acoustic stimuli have been mentioned (Webb, 1979; Waladde and Rice, 1982).

Host skin emanations as for CO_2, are assumed to function as kairomones. Olfactometric experiments indicated that many host parts do not play a kairomonal role as swabs of calves from legs, back, perineum and belly were unattractive to adults, nymphs and larvae of *R. appendiculatus* (Akinyi, 1991).

Host-derived stimuli eliciting attraction are not well understood nor identified. Carbon dioxide (CO_2), ammonia (NH_3) and butyric acid are among chemicals reported to be effective in host recognition, orientation to and on the host (Wilson *et al.*, 1972; Haggart and Davis, 1980; Waladde and Rice, 1982). Electrophysiological studies carried

out separately with the aforementioned gases and the acid showed that the ticks have olfactory receptors sensitive to these compounds, including 2,6-DCP (Haggart and Davis, 1980, 1981; Waladde, 1982; De Bruyne and Guerin, 1994).

1.3.4.3 **Responses to feeding site stimuli**

Two opposite behaviours exist among ticks in their selectivity for feeding sites on the host. The first involves unselective behaviour among species that feed on any part of the host body. Example of these is the sheep tick *Ixodes ricinus* (L.) which attaches to any body part of a wide variety of animals. The second category is made of ticks that exhibit a great deal of specialization in selecting their feeding site. Such specificity may serve to maximize survival and reproduction of the species (Chilton *et al.*, 1992). Adults of *R. appendiculatus* show a strong predilection for the inner part of bovine ear while the immatures show less selectivity - being found also on other parts of the host body. Likewise, adults of *R. evertsi* feed exclusively under the tail around the anal region while the larvae and nymphs attach deep inside the ear (Elbl and Anastos, 1966). *Amblyomma spp* demonstrate a similar attachment site preference on the belly and around the genitalia of bovine.

A wide range of factors ranging from physical to chemical ones have been related to the feeding site location by ticks. Observational data suggest that once on the host, ticks tend to avoid dessication and thus move to less exposed areas or parts of the host

(Roberts, 1971). Microenvironmental conditions specific to certain body areas of host e.g. the hygrometric index in the ear cavity, the skin temperature (which generates an environment which can dessicate tick), hence the irregular distribution of body temperature on the animal, have also been suggested to play a role in site preference of certain tick species (Roberts, 1971; Waladde *et al.*, 1991). The complex odorous environment crossed by individual ticks on their way to the preferred feeding site is also of importance. This offers an odour-permeated background (resulting from multiple secretions and volatile emissions on the skin surface of bovine host). Chemical compounds in sweats and other skin secretions, detectable by olfactory and/or tactile receptors, are believed to facilitate the selection of suitable feeding sites (Sonenshine *et al.*, 1986). Earlier, Balashov (1972) reported electrophysiological evidence on the detection of glucose and sucrose by the type A sensilla of the tick. More recent studies suggest ticks themselves also play a role in guiding other ticks of the same species to the suitable feeding site. This is the case of *Amblyomma spp* where an aggregation attachment pheromone component (ortho-nitrophenol) secreted by attached and feeding ticks has been confirmed as a stimulus that is utilized by the ticks to orient towards already settled conspecifics (Rechav *et al.*, 1976, 1977; Norval *et al.*, 1989a, 1989b). In the case of *R. appendiculatus*, olfactometric experiments using swabs impregnated with ear odour evoked attraction of adults but repelled nymphs and larvae of the same species (Akinyi, 1991). In the same study, ear swabs mixed with male tick extracts inhibited responses of these immature stages. No explanation was given to

account for these results. Likewise, for *R. evertsi*, although its feeding site propensity is known (Elbl and Anastos, 1966), adequate data are lacking to provide insights into its feeding site selection and the nature of the kairomonal signal(s) involved. Stimuli present in the host's anal region which attract *R. evertsi* are of interest, as in addition to body surface volatiles, it may include effluvium from the gut and volatiles from dung which is frequently passed out. In addition, do the many volatile pheromones known to be released in this 2-host tick (Goethe and Neitz, 1985) play a role?. Furthermore, how does this mediation system compare with that of *R. appendiculatus*?

The preferred sites may contain several behaviourally active compounds as demonstrated by relatively high rate of success in locating these by ticks. Do ticks as non-flying arthropods locate them by making progression through a stimulus gradient across an odour-permeated substratum, namely with host and tick odours? Or are specific blends of pheromones and/or kairomones components involved? To date, it is not known if the mechanism involved is one of the various ones used by insects to find their host plant or mate (Shorey, 1970; Visser, 1986). Which odour molecules are selected as cues, their mode of action and their specificity are intriguing questions that remain to be answered.

1.3.5 Tick movement and orientation in relation to semiochemicals

1.3.5.1 Tick distribution and movement on host

Little is known about the orientation patterns of most tick species on their mammalian hosts. Previous studies on *R. appendiculatus* were restricted mostly to determination of its ultimate distribution on different parts of the host body (Kaiser *et al.*, 1982, 1988). This 'static approach' using delineations of the host body regions have been useful in the recognition of attachment sites. It has also helped to clarify some of the vector-host relationships (Kaiser *et al.*, 1982). In *R. appendiculatus*, the relationship to the attachment sites on cattle have been documented by several workers. For example, Newson (1978) reported that 87% of adults of this species attached in the ear. This has been confirmed by other workers (Yeoman and Walker, 1967; Walker, 1974; Kaiser *et al.*, 1982; 1988). Further observations showed that as the infestation burden increased, infested areas extended first to the head and thereafter to other body zones. Likewise, available data for *R. evertsi* shows that almost 100% of individuals clustered around the anus (Kaiser *et al.*, 1982); but why and how remain unclear.

1.3.5.2 **On-host orientation mediated by kairomones/pheromones**

While on the host, different tick species exhibit various locomotory and exploratory behavioural patterns which relate to either mate finding or selection of feeding sites (Sonenshine *et al.*, 1986). For species like *R. appendiculatus* whose mating takes place on the host after attachment, females merely move to feeding sites and remain there until complete engorgement. The premating behaviour of the male is comparatively more intricate. Observations on *D. variabilis* (Say) and *A. americanum* showed that males first do not move very much from their attachment point (Gladney and Drummond, 1970). It is only after they become sexually active following a blood meal that they migrate, sometimes extensively over the host to seek the females (Gladney and Drummond, 1970; Sonenshine *et al.*, 1974; Kellum and Berger, 1977). This mate seeking behaviour may be mediated by pheromones which may also attract unfed conspecifics of both sexes to the feeding site. It has been assumed that stimuli from preferred feeding site may play a similar attraction role. Based on recent preliminary studies, it appears that these combined factors could account for tick attraction to these areas. But the site specific factor as well as the guiding cues are not yet known and identification of these would be facilitated if their roles and sources are elucidated.

1.4 Disruption of tick orientation on host with semiochemicals

1.4.1 Current semiochemicals-based monitoring and control methods

Unlike in insects where pheromones have been used extensively to control certain crop and orchard pests (Roelofs, 1979; Mitchell, 1981), semiochemical-assisted strategy has made little headway in the control of ticks and, where attempts have been made, it is only on trial bases. For example, CO_2, which is found in vertebrate breath acts as a tick kairomone, and has been tested for sampling and control under different synthetic preparations such as dry ice, compressed gas (Gray, 1985; Norval *et al.*, 1989b). Similarly, sex pheromones have been evaluated in a number of ways. Norval *et al.* (1989b) experimented a combination of CO_2 with O-nitro-phenol (ONP) in the field. According to these workers, increased catch of *A. hebraeum* (Koch) was obtained by the activating effect of CO_2 in synergy with the inherent attractant action of the pheromone. Other attempts have explored the use of pheromone/acaricide-treated areas of the host. This method proved to be very effective in killing lured ticks (Gladney *et al.*, 1974b; Rechav and Whitehead, 1978). A significant progress in the latter approach involves the use of impregnated objects or decoys with a slow release delivery system (Sonenshine *et al.*, 1992). Another technique which has achieved some degree of effectiveness is the confusant-killing method tested by Ziv *et al.* (1981). These authors incorporated the 2,6-DCP sex attractant pheromone in gelatin microcapsules (applied to host's fur) and

mixed with a pesticide (Propoxur) against the dog tick, *Dermacentor variabilis*. As a result, a majority of lured males were killed, while surviving females were left unmated by the induced pheromone-permeated background. This successful disruption of mating was achieved with 5.6 μg/ml of microencapsulated 2,6- DCP. Mixtures of an acaricide with an aggregation pheromone or a sex pheromone impregnated on plastic decoys were among other options showing promise. Hamilton and Sonenshine (1989) have patented a decoy coated with natural Mounting Sex Pheromone (MSP) and a pesticide. The decoys dispersed on the hair coat of the host were found highly lethal to males within 30 minutes and mating attempts by males with decoy resulted in 89% of death (Sonenshine *et al.*, 1992). A modified method of the above was tested by Norval *et al.* (1992b) who used plastic bands impregnated with components of the attractant-aggregation-attachment (AAAP) pheromone mixed with the pesticide flumethrin. The poisonous bands were attached to the tails of the cattle and as a result, there was a good killing of ticks as those attracted aggregated around the animal's rear and came into close contact with toxic chemicals.

Attracticides may not sound as ecologically fit for a pesticide-free environment, however since this method utilizes a behavioural trait which occurs regularly and predictably, its advantage lies on a reduced amount of acaricides being used and their tactical application on the host (Sonenshine, 1992).

1.4.2 Allomonal effects of some plants, with particular reference to the plant *Ocimum suave*

Most of the work on tick communication system have paid little attention to tick-plant interactions. Notwithstanding the fact that the non-parasitic stages of most tick species spend a sizeable time in the habitat (Hoogstraal, 1956; Branagan, 1973), where they find several resources including refuge from enemies, more congenial microhabitats, sites for oviposition, etc. On the other hand, in the course of the evolution, plants vis-a-vis insects (and other herbivores) have evolved defensive mechanisms in the form of repellents, phagodeterrents, toxicants and insecticides associated with secondary metabolites present in plants (Weaver *et al.*, 1991). In recent years, potency of essential oils and volatiles of plants have drawn considerable attention and have been documented for several arthropod species including ticks (Schoonhoven, 1968; Hassanali and Lwande, 1989; Schmutterer, 1990).

Work in progress at ICIPE has generated considerable base data suggestive of some plant components acting as allomones for some local tick species including *R. appendiculatus* (Malonza *et al.*, 1992; Mwangi *et al.*, 1995b; Ndungu *et al.*, 1995; Lwande *et al.*, 1996). It was found that *R. appendiculatus* preferred to ascend drier vegetation than fresh green ones, possibly because of an unknown allomonal factor (Mwangi *et al.*, 1995b). Some tropical legumes, especially those of the genus

Stylosanthes have been reported to exude sticky substances from the trichomes that trap and kill ascending larvae of *Boophilus microplus* tick (Sutherst *et al.*, 1982; Zimmerman *et al.*, 1984; Wilson *et al.*, 1989). In addition to trichomes themselves, glandular emanations of *Stylosanthes sp* were found to be also lethal to ticks (Sutherst *et al.*, 1982). Interestingly, in Kenya many farmers and cattlemen seem to link low infestation levels of ticks in certain pastures to the effects or proximity of some scented plants (pers. comm.). Many of these, identified as plants of the genus *Ocimum* including *O. suave* (Family Labiatiae) which was found to have insecticidal/acaricidal activity (Kokwaro, 1976; Mwangi *et al.*, 1995b) in addition to its medicinal usages, especially against cough, influenza, stomachaches (Kokwaro, 1976). In East Africa, *O. suave* leaves have been used as protectants against stored products pests (Chogo and Cranck, 1981; Hassanali *et al.*, 1990). The repellency responsible for this effect is often due to its essential oil made up of terpenoid constituents. Terpenoids are a class of naturally-occurring secondary metabolites normally built up from isoprene units (Ryan and Byrne, 1988). Hassanali *et al.* (1990) reported that the main repellent constituent of *O. suave* and *Eugenia caryophyllata* against the maize weevil *Sitophilus zeamais* was eugenol, a component of the essential oil of these plants. As reported by a number of workers, the level of repellency that terpenoids exert against insects and ticks may approach that of diethyltoluamide (DEET), widely recognized as a major synthetic repellent of many arthropod groups (Marinibettolo, 1983; Dobrotvorskii *et al.*, 1989; Mwangi *et al.*, 1995b). Although it has been conclusively shown that crude extracts of some repellent

plants are effective against *R. appendiculatus*, the active compounds have yet to be characterized.

Apart from botanical tick repellents, other synthetic compounds known to have anti-tick activity have been investigated (Hadani *et al.*, 1977). In addition, Skinner *et al.*(1983) reported that synthetic cyclic amides act as repellents to *R. sanguineus* (L.). Associated with these repellents include other effects such as knock down effect, toxicity, primer effect, etc (Matthewson *et al.*, 1981; Weaver *et al.*, 1991).

1.4.3 **Disruptive effects of some host materials on ticks**

Unlike the aforementioned effects obtained from exogenous sources (ie plant), very little attention has been paid to host stimuli as potential disrupting materials, nor to possible effects of transferring stimuli from preferred sites to unpreferred sites on the host. To our knowledge, cross-effects of stimuli extracts of feeding sites of different ticks have not been reported in the literature.

CHAPTER TWO

2.0 OBJECTIVES OF THE STUDY

2.1 Statement of the problem

Despite the accumulating knowledge about tick semiochemicals, very little is known about the functions of kairomonal and allomonal cues used by *R. appendiculatus* and *R. evertsi* to orient to their feeding sites on their natural bovine hosts. As a prerequisite to the establishment of the chemical basis of these behaviour components, detailed behavioural studies are needed to help examine the role of stimuli emanating from these preferred feeding sites and of others from non-feeding sites. No comparative data exist on the relative specificity of predilection sites and how this influences tick orientation and feeding behaviour. There is a need to establish the role of host odours on this peculiar behaviour which would provide clues on possible ways of disrupting tick orientation and feeding.

2.2 Objectives of the study

2.2.1 General objectives:

- to investigate arousal and orientation responses of *R. appendiculatus* to host odours.

- to examine possible ways of employing behaviour-modifying chemical stimuli to modify these elements of behaviour.

2.2.2 Specific objectives:

- to assess potential effects of host kairomones and/or tick pheromones on the orientation processes of *R. appendiculatus* and *R. evertsi* under laboratory conditions.

- to establish and compare movement patterns of both tick species towards their preferred feeding sites on their natural host.

- to assess effects of allomones from a plant (*Ocimum suave*) and odours derived from non-feeding sites on both species and their feeding site location behaviour.

CHAPTER THREE

3.0 GENERAL MATERIALS AND METHODS

3.1 Ticks

Rhipicephalus appendiculatus used in this study were obtained from ICIPE tick colony which was initially established with a strain originally obtained from Kenya Agricultural Research Institute (KARI), Muguga, Kenya, since 1952. *R. evertsi* were also kept in the same rearing facilities at ICIPE and were propagated from a strain provided by KARI/Muguga that originated from Mutara Ranch in Laikipia District, Kenya. The two species were reared as described by Bailey (1960) and Irvin and Brocklesby (1970). Tick-naive rabbits (New Zealand white breed, wt= ca 2 kg) served as feeding hosts for all tick instars and ear-sized cotton bags allowed confined ticks to feed on the ears. To help moulting of tick instars, these were held in a dark incubator maintained at a relative humidity (RH) of 85% and a temperature of 28°C. They were thereafter transferred in constant darkness at 18°C and 85% RH. Humidity was controlled by super saturated potassium chloride solution.

Unfed adult ticks (1-3 months old post-moult) were mainly used in this study. Where immature stages were used, these were 4-12 week post-moult. Preliminary trials had shown that ticks in these age groups were the most active.

Prior to behavioural assays, ticks were preconditioned at high humidity (> 85% HR) and were transferred into the test room 24 h before the experiments. They were used only once and then discarded, except where indicated otherwise.

3.2 **Cattle**

Two friesian steers, 325-345 kg with no prior exposure to ticks were used for tick feeding and on-host behaviour assays as well as for collecting odour samples. In addition, four Ayrshire calves weighing between 190 and 215 kg were used for experiments on disruption of feeding site location by the ticks. The two breeds (Plates 5 and 6) which are exotic grade cattle (*Bos taurus*) were fed *ad libitum* on hay and pellets and were kept under roofed sheds free of ticks.

3.3 **Experimental room**

The experimental room measuring 2,50m x 1,80m x 2,20m was maintained at 25.5 ± 0.5°C and 65 ± 5% RH with a dark-light photocycle of 12:12 h. During experiments, the light intensity from an overhead lamp and diffused with a white plain paper ranged between 600-1,500 lux which took into account the phototropic reactions of ticks. Generally, experiments were run between 0900 h and 1700 h.

Plates 5 and 6 Exotic cattle breeds used in experiments and for odours collection: Friesian heifers (Upper) and Ayrshire steers (Lower).

3.4 **Sources of test semiochemicals**

3.4.1 **Host stimuli**

Host odours and washes (kairomonal cues) and volatiles from ticks (pheromonal cues) were used in various sections of this study. Methods of collecting washings and volatiles by solvent extraction are described below.

3.4.1.1 **Washes (gustatory and olfactory stimuli)**

These were collected by solvent washing as outlined in the scheme in Fig. 3. Briefly, the ears, belly/axillae, dewlap, legs and anal areas of cattle were swabbed with cotton wool dipped in redistilled n-hexane. The swabs were washed using ca. 400 ml of n-hexane and the volume of the solvent wash was subsequently concentrated to 2 ml by vacuum evaporation (Buchi Rotary evaporator, Switzerland) at 30°C. The concentrates were then transferred into vials (8 ml) and the remaining hexane removed under nitrogen. The material was weighed and kept at 0-4°C and, later, dissolved in 2 ml of hexane for the assays. Methanol and acetone extracts were also prepared using the same procedure.

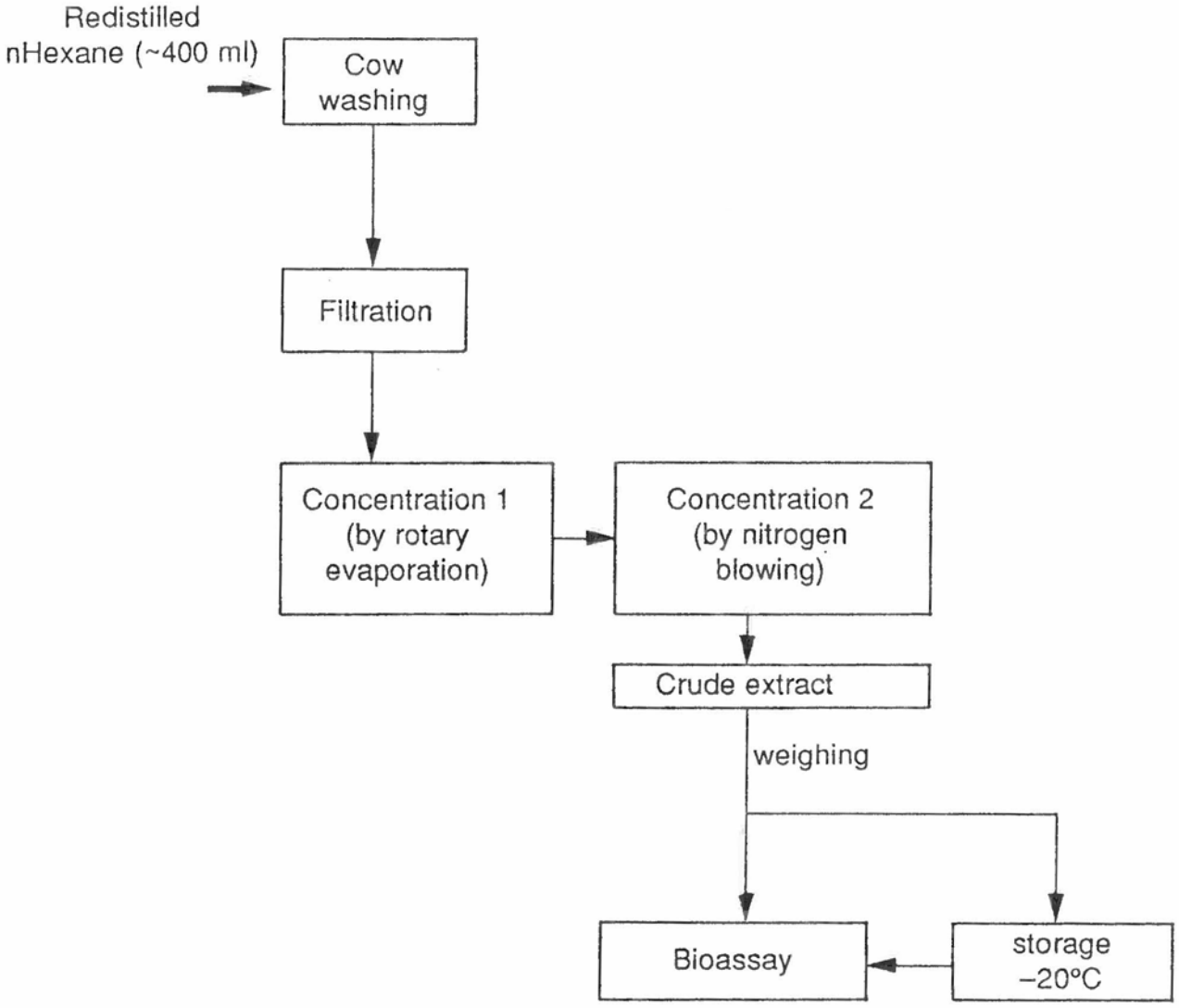


Fig.3 Flow diagram showing the extraction procedure of odour materials collected from bovine host (body wash)

3.4.1.2 **Volatiles (olfactory stimuli)**

Volatiles from ear parts were trapped using a modified method developed at ICIPE (Nyandat *et al.*, in prep.)(Fig. 4). In this method, odour traps were made of a fine wire gauze (5 cm length, 2.5 cm width) containing about 0.5 g of activated charcoal (mesh 0.2 μg;Chromopack) as the adsorbent. One side of the trap was covered with a sterile aluminium 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 into a 200 ml soxhlet extractor for 1 to 3 days and then dried and flushed of any contaminants with a stream of dry nitrogen at 60°C for 3 h. Used traps can be recycled in this way.

To collect volatiles, clean traps were attached onto the ear pinna or selected body region of cattle using adhesive plaster. After 24 h, the traps were removed and eluted with 2 ml of dichloromethane (Ch_2Cl_2) by immersion, washed with another 2 ml of the solvent and the eluates concentrated to 1000 μl. For olfactometric test, 100 μl aliquot were applied onto filter paper discs (Whatman no 1; 2 cm diam) to be plugged in the olfactometer arms. A fraction of the volatiles collected was kept at 0°C for chromatographic analyses.

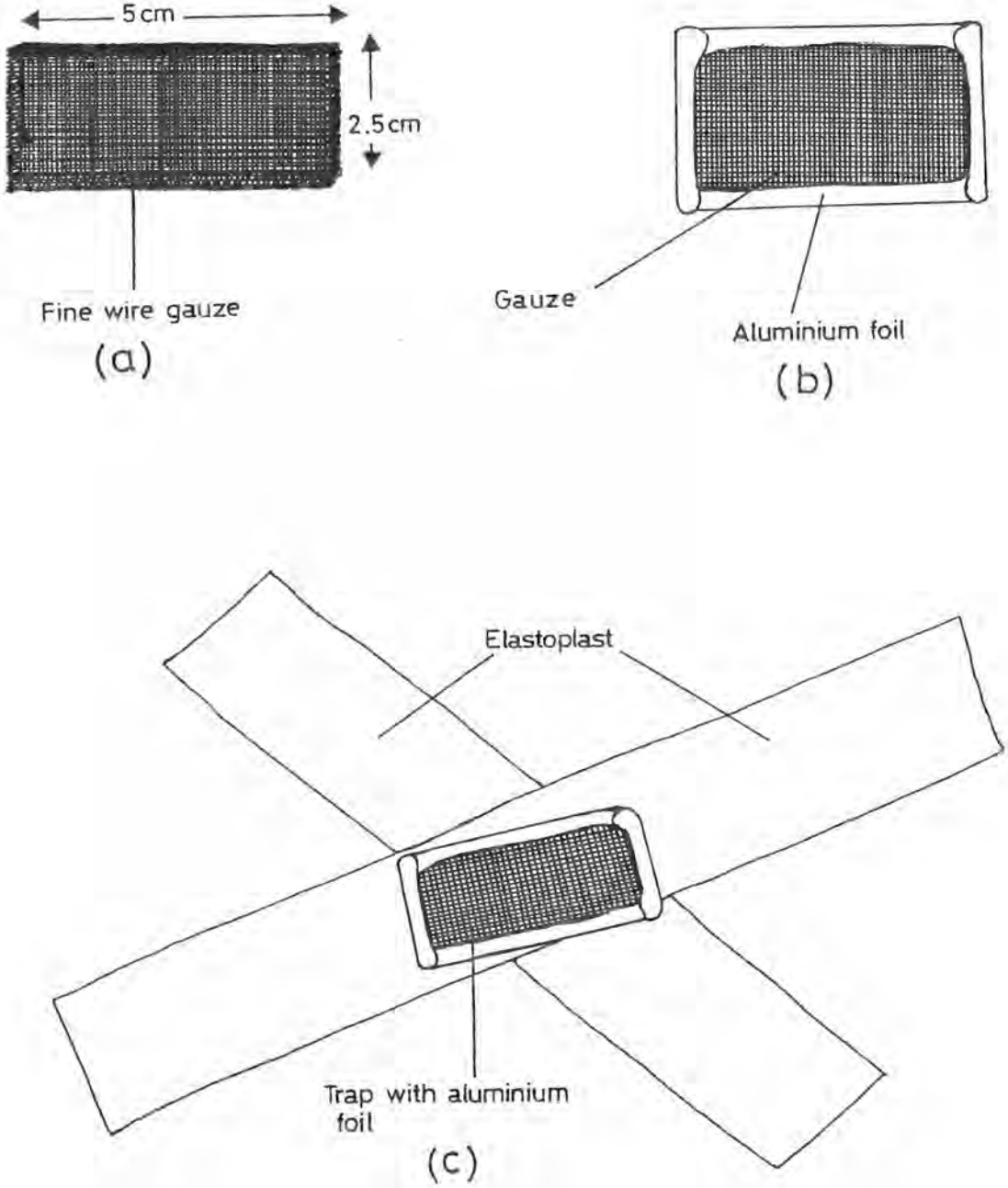


Fig. 4 Components of a trapping system for air-borne volatiles from a host

3.4.1.3 **Cow dung**

The dung was used either raw or in water suspensions. To test whether attractiveness of dung to *R.evertsi* was age-dependent (i.e. time after defecation), dung samples of three ages were used: (1) fresh wet dung of less than 2 h old after defecation (= level I); (2) fresh moist dung 24 h old or more (= level II), and (3) dry dung more than 3 days old (= level III). The fresh dung Level I elicited the best responses, and was used in subsequent assays. 2 g of the latter dung preparations was enclosed in fine wire gauze (mesh 0.5 mm) and plugged in either of the T-olfactometer arms. The control (blank) was a clean wire gauze of similar size attached to the other arm.

Water suspensions of the cow dung were prepared by mixing 1 g of freshly defecated dung in 10 ml of water and shaking it gently. The suspension was tested for the presence of kairomonal cue(s) with disruptive activity on *R. appendiculatus* as this tick migrates to the ear. For the assays, 1.0 ml of the water suspension extract was applied at the ear base of cattle the same day. The control was an untreated ear of cattle with the same volume of water applied on.

3.4.2 **Volatiles from the ticks**

These were either solvent rinses or volatile emissions from feeding ticks as detailed below.

3.4.2.1 **Solvent rinses**

Rinses of males of different feeding ages were used to investigate aggregation responses of *R. appendiculatus*. Male extracts were tested because it was assumed that like in other tick species such as *Amblyomma* sp. fed males release volatiles that mediate aggregation (Rechav *et al.*, 1976, 1977; Norval and Rechav, 1979). Fifty to hundred male ticks were allowed to attach and feed on rabbits and were forcibly removed at suitable feeding age (days). The ticks were then immersed in sufficient amount of n-hexane in a test tube (8ml) and the contents were frequently shaken and then stored at 0-4°C for 24 h. The rinses were filtered and the filtrate concentrated to 2 ml under gentle stream of nitrogen. Female rinses were prepared as described above for comparison. These hexane rinses were expressed as either male-equivalent (ME) or female-equivalent (FE). One tick-equivalent (TE) is the concentrate obtained from one tick during the collection period.

3.4.2.2 **Volatiles from feeding ticks**

Tick air-borne volatiles tested in attraction experiments to the feeding site were trapped and prepared in the same way as with air-borne volatiles from the feeding site (see section 3.4.1.2). Volatiles were collected from feeding ticks while attached on the ear of the host. The volatiles trapped were therefore a blend of volatile emissions from the feeding site of the host and volatiles from the feeding ticks.

3.4.3 **Essential oils of plants**

3.4.3.1 **Plant material**

The essential oil used in bioassays was extracted from *Ocimum suave* plants collected at Kabete, on the outskirts of Nairobi (Kenya), where they were found growing among other shrubs around human settlements (Plate 7). Taxonomic identity of samples were confirmed at the Herbarium in the Department of Botany, University of Nairobi.

Plate 7 *Ocimum suave* plant

3.4.3.2 **Extraction of the essential oil**

The fresh aerial parts of the plant collected from the field were transferred to the laboratory and stored at 4°C for not more than 24-48 h. The leaves of the plant were chopped and steam-distilled in a Clevenger type apparatus (Plate 8), according to the method of Guenther (1949). Further removal of hexane was done using Contes path (Plate 9). N-hexane extraction of the distillate yielded the essential oil in 0.60%. The steam-distillate was pretested for repellancy on test ticks using a Y-tube olfactometer and showed maximum repellency of ca. 100% at 1% solution.

Gas chromatography analyses were also performed on aliquots of the essential oil to determine its composition as detailed in section 3.6.

3.4.3.3 **Estimation of the yield of essential oil and its eugenol content**

The amount of essential oil obtained was deducted from the weight of fresh *O.suave*. In these extractions, 1 g of fresh plant material yielded 7 mg of the essential oil (0.7%). From gas chromatographic profiles, eugenol was about 60% of the essential oil.

Plate 8 Photograph showing Clevenger apparatus used for steam distillation of essential oils from *Ocimum suave* plant leaves

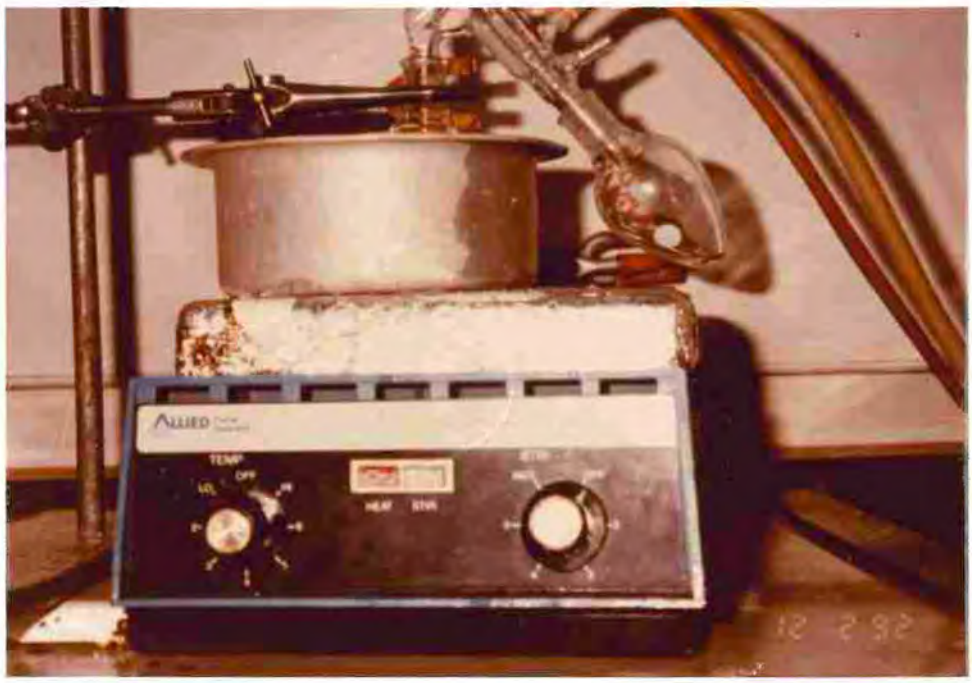


Plate 9 Photograph showing apparatus used for the removal of solvent from hydrodistilled essential oils of *Ocimum suave* extract

3.4.3.4 **Application of plant extracts**

Preparations of essential oils were applied on the ear and anal sites of the host to determine their disruptive activity against *R. appendiculatus* and *R. evertsi*. Indicated doses of test samples in hexane were applied onto either site. The controls were (essential) oil-free ear or anal area treated with the solvent alone.

3.5 **Solvents and chemical standards used.**

Spectrometric grade solvents and dichloromethane (purchased from Merck, Darmstadt, Germany) were used for extracting host materials. Further purification of solvents by redistillation was undertaken before their use for odour collection and for Gas Chromatography (GC). Standards of authentic samples used (eugenol) in GC runs were of GC grade and purchased from Aldrich Chemicals Co, Dorset, U.K..

3.6 **Gas chromatographic analyses of volatiles and essential oils.**

To obtain GC profiles of various extracts under study, 5 μl aliquots of the preparations in a solvent were injected into a Hewlett-Packard model 5890A Gas Chromatograph (GC)(Plate 10) equipped with a splitless injector and a flame-ionization detector (FID). Peaks were integrated on a Hewlett-Packard 3393A

integrator. Analyses of the essential oil of *Ocimum suave* were performed on a methylsilicone capillary column (50 m x 0.32 mm id; 0.2 μm film thickness). Nitrogen was used as the carrier gas and the oven temperature was programmed at an initial temperature of 60^{0}C held for 5 min, followed by a rate of 5^0/min to 175^{0}C held for 5 min and then 10^0/min rise to 220^{0}C at which it was held for 10 min. Co-injection of authentic eugenol was done to confirm the identity of this component.

Plate 10 Hewlett Packard 5890 Gas Chromatograph (GC) equipment (Left) with a chromjet integrator (Right) used to analyze odour and plant essential oils in this study.

3.7 **Olfactometers**

Three types of two-choice olfactometric set ups were used for quantifying tick responses to host odour extracts:

3.7.1 **T-tube olfactometer**

A T-tube olfactometer and bioassay method adapted from Wood *et al.* (1975) were used to test the attractiveness of odours to ticks. The olfactometer as illustrated in Fig. 5 had two parts made of a glass tubing, a vertical stem and two lateral arms joined to form a T-shaped continuous tube.

During tests, the vertical stem of T-tube was placed over a single unfed male or female tick and the tick was allowed to move up the tube and make a choice between either arm on reaching the T-junction. Test samples were dissolved in an appropriate solvent and known amounts applied onto filter paper discs (Whatman No.1; 2cm diam). After evaporation of the solvent, treated discs were plugged into either arm of olfactometer. The control arm was plugged with filter paper disc impregnated with the same amount of the solvent which was then allowed to evaporate. Orientation by the tick was recorded as positive if the arm with treated disc was chosen and negative if the tick chose the control arm.

3.7.2 **Y-tube olfactometer**

A Y-tube glass olfactometer adapted from Rechav *et al.* (1977) was used in experiments involving orientation studies of larvae and nymphs to host odours. The olfactometer, illustrated in Figs 6 and 7, was made of glass tubing and had a stem and two diverging arms set at an angle of 45°. At the junction of the two arms was a small outlet which was connected by a rubber tubing to a suction pump (Cole-Parmer, Air-cadet) which drew air from the olfactometer at a rate of 50-62 cm^3/min. The lower end of the vertical stem was extended into a bulb which served as chamber for immatures. On release of the ticks, this was stoppered. Batches of nymphs or larvae were introduced through the lower inlet of Y-tube and allowed to move up freely to the arms. The test procedures were as described for the T-tube olfactometer above.

In experiments to investigate arousal of ticks by host odours, a modified version of Y-tube with a shortened vertical stem (2 cm long) was used (Fig. 8). Individual ticks were introduced through the inlet and a polystyrene stopper was used to plug the bottom of the stem. Observations were made on the tick's responses in either arm (extract treated disc vs control solvent disc, Whatman No 1, 2 cm diam). The time spent (= residence period) in the olfactometer arm near or in contact with treated or control filter paper disc was recorded.

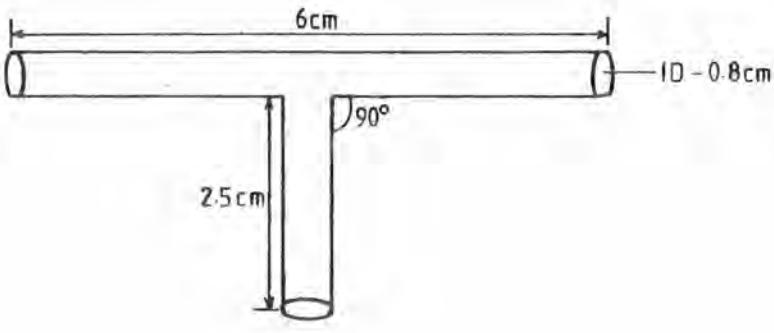


Fig. 5 Diagram showing a T - shaped glass tube olfactometer used to test responses of adults to extracts.

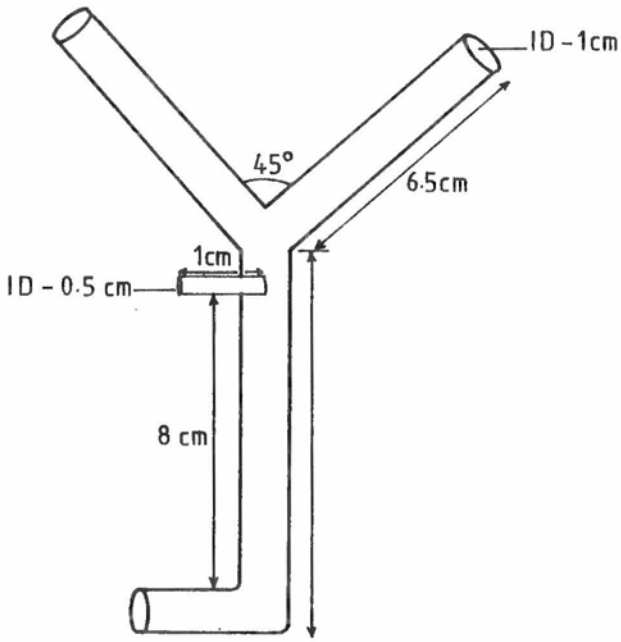


Fig. 6 Diagram of a Y- tube olfactometer used to test responses of tick larvae and nymphs to host extracts

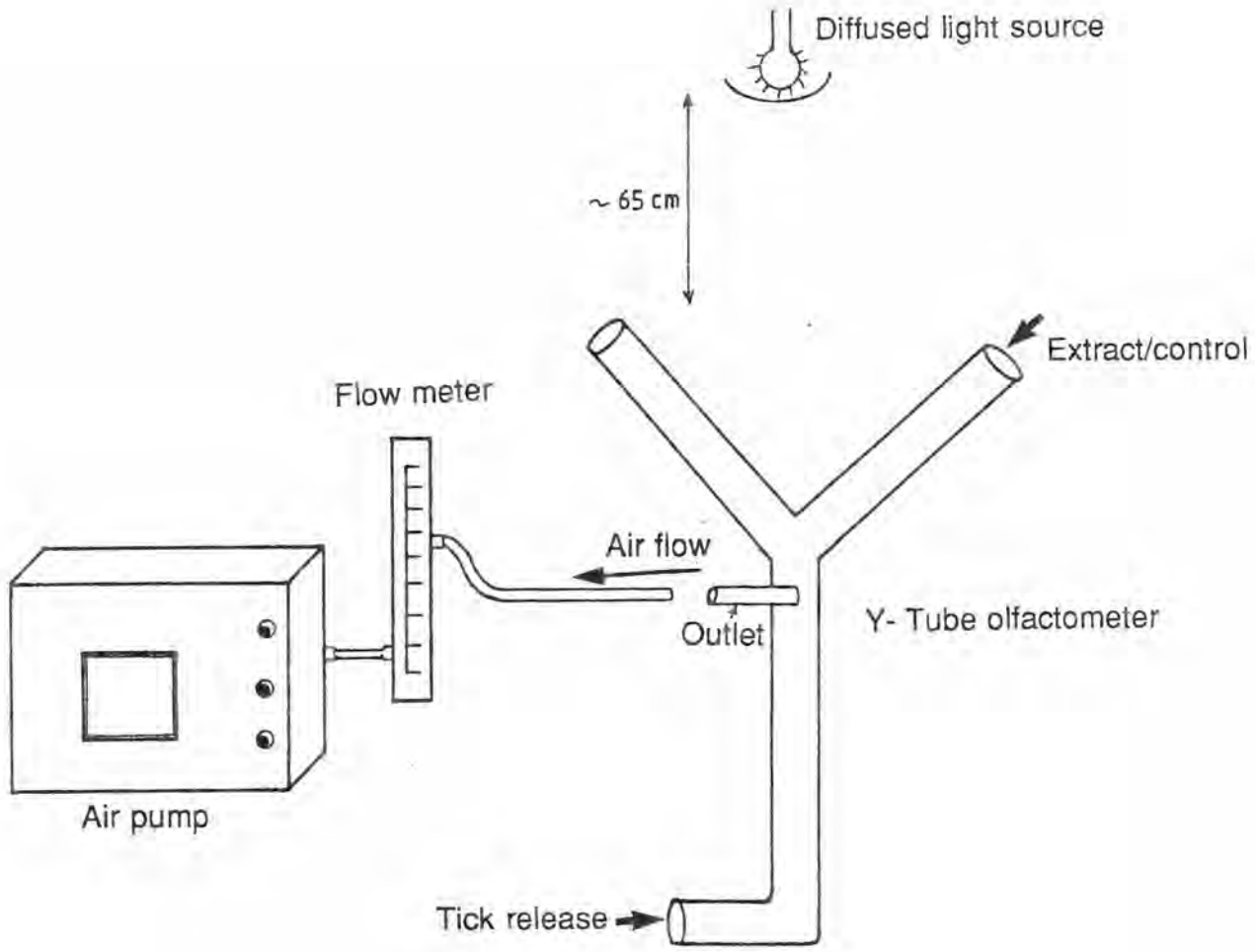


Fig. 7 Diagram illustrating odour delivery system in Y- tube olfactometer for larvae and nymphs ticks.

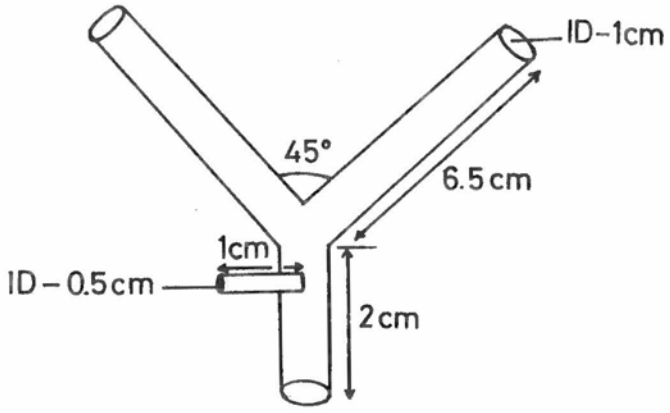


Fig. 8 Diagram showing a Y- shaped tube olfactometer used for adult ticks in arousal experiments

3.7.3 **Paired vertical glass column**

A modified form of climbing bioassay described by Browning (1976) was used for studies on dose-responses of ticks to extracts from the feeding sites. Two versions of the apparatus, one for attractancy and another for repellency tests are illustrated in Figs 9 and 10 respectively. The apparatus consisted of a square aluminium base lined up with wax-coated mosquito net. The wax lining was found to offer an appropriate texture for ticks wandering on the aluminium base and probably eliminated any cooling effect of water. On each base were two vertical aluminum rods of 0.8cm diameter. Two glass tubes (1 cm diam) were each slid fitted snugly over the aluminium rods and their tops were plugged with moist cotton wool. The glass tube was used since it is easier to clean. A second set of larger glass tubes (4.5cm diam) were each slid over the duo rod-glass tubing and held with clamps such that the lower ends were 4 cm above the aluminium base. The top ends were plugged with dry cotton wool to allow for free diffusion of extract odour in the glass columns which was necessary for creating a concentration gradient of stimulus. Strips of filter paper (Whatman No 1, 1cm x 2cm) impregnated with the extract or solvent (control) were rolled and inserted into the internal glass tubes 1 cm from the top end for attractancy tests or mid way for repellency tests. They were set 1 h prior to each trial to allow the stimulus concentration gradient to equilibrate. The apparatus base was immersed halfway in a tray of water, just above the water level to prevent ticks

from escaping. The assay was left to run for 1 h. For attraction assays, the number of ticks that reached the cotton wool at the top of the inner glass tubings on treated and control rods were counted. For repellency assays, the number of ticks that crossed the treated and control filter paper barriers were counted.

Any olfactometer in use was rotated 180° after each replicate or series of treatments to avoid any positional bias. After each test, the tubes or climbing columns of the olfactometer were washed thoroughly with hot detergent followed by distilled water and dried in oven or air to eliminate possibilities of contamination.

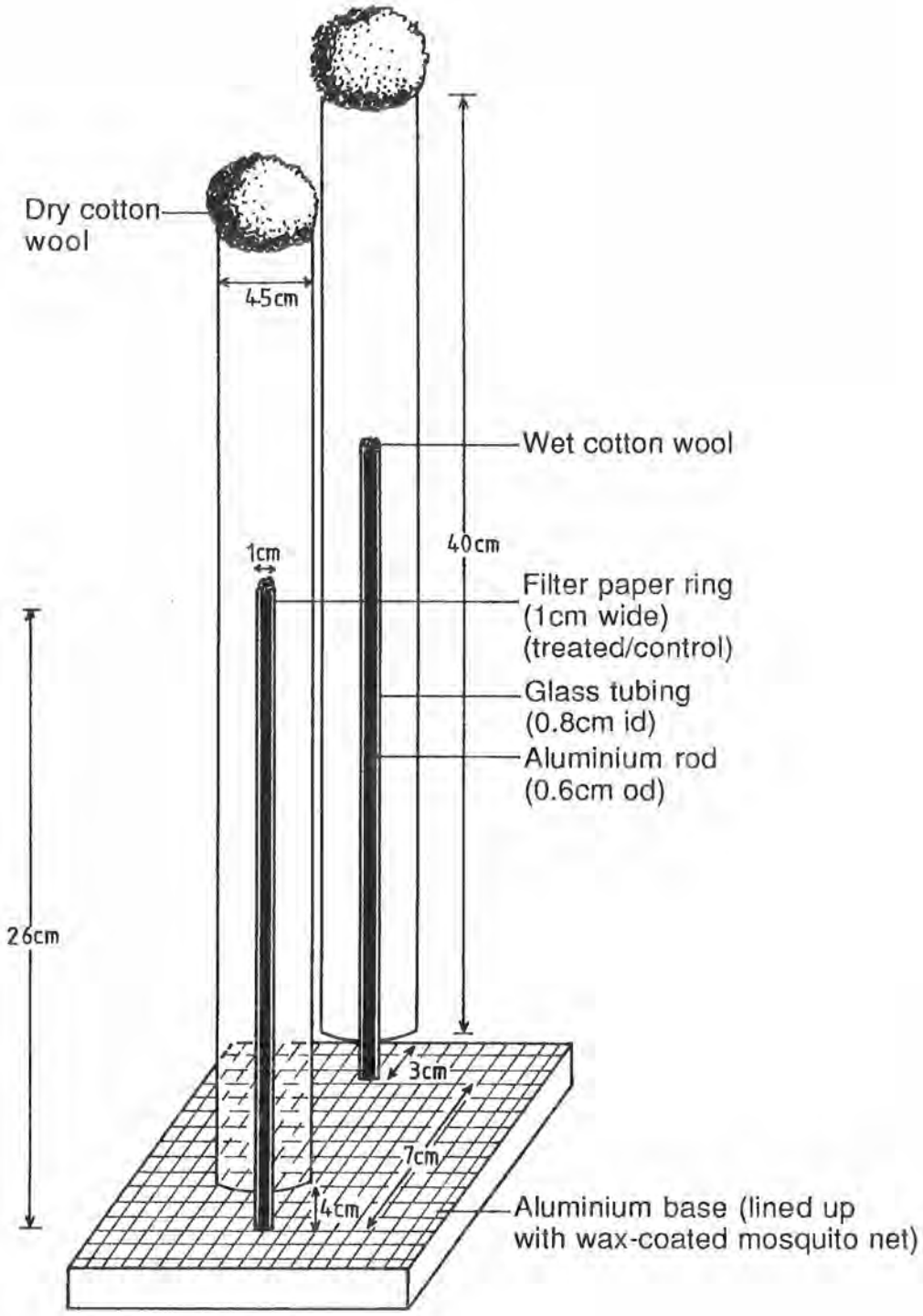


Fig. 9 Diagram showing a paired climbing column olfactometer for attractancy tests (Modified from Browning, 1976)

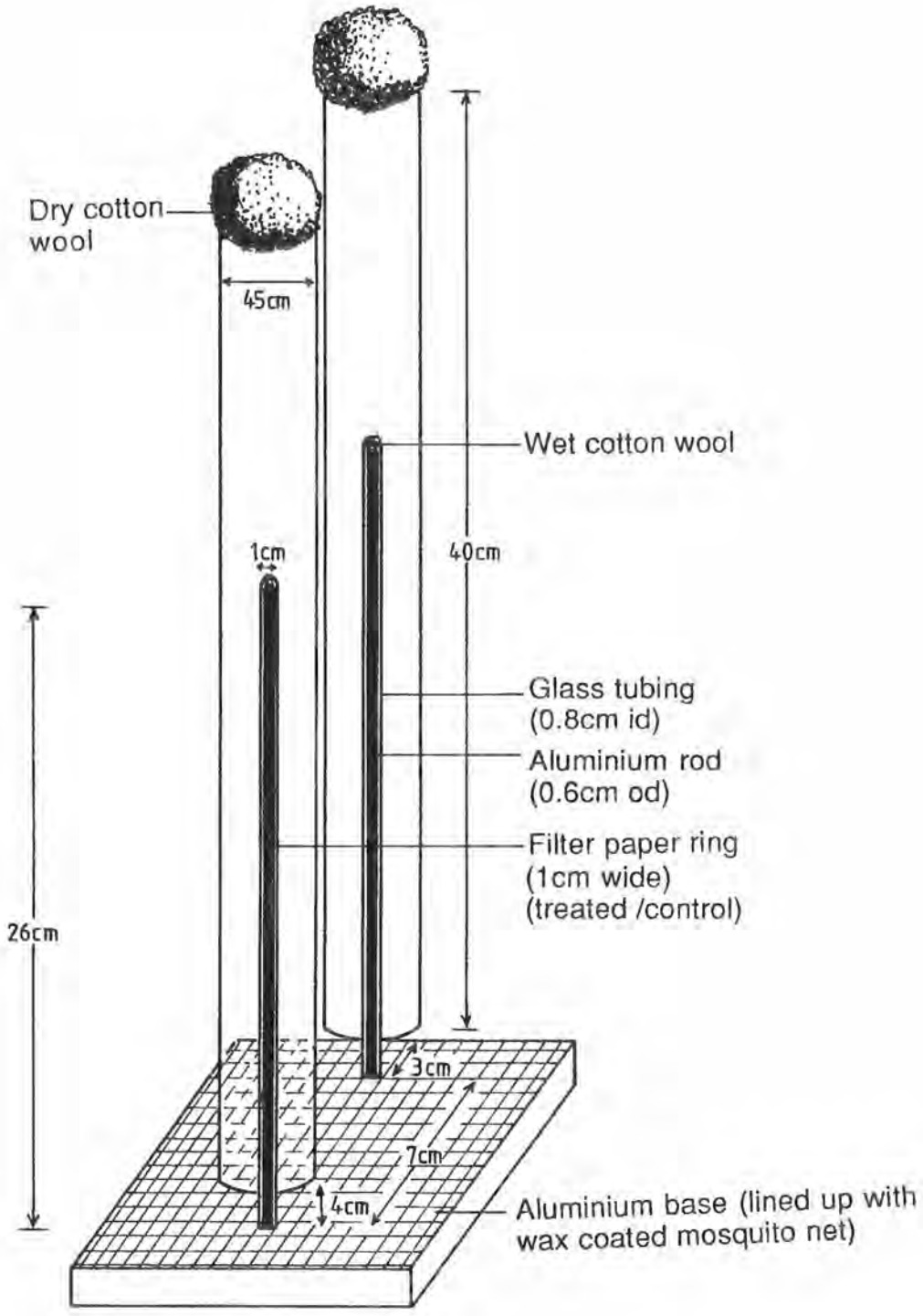


Fig. 10 Diagram showing a paired climbing column olfactometer repellency tests (Modified from Browning, 1976)

3.8. **Experimental observations and procedures**

3.8.1 **Olfactometric bioassays**

Responses of test ticks to extracts dispensed using calibrated Eppendorf pipettes, were based on the proportion of non-responding (NR) and responding individuals. Data from the latter ones only were analyzed. Unless expressed as mean percent of ticks moving to either arm, responses of ticks to various host odours and extracts were calculated as Percent Attractancy (PA) using the equation:

$PA = [(N_t - N_c)/(N_t + N_c) \times 100]$, where N_t and N_c are numbers of ticks recorded in treated and control arms of the olfactometer respectively. Values for the responses were rated as follows :

if $N_t > N_c$, attraction;

if $N_t < N_c$, repellency;

if $N_t = N_c$, no action.

3.8.2 **On-host behavioural studies**

3.8.2.1 **Crutch for observations**

The crutch for restraining cattle where behavioural assays on host were carried out

was that of ICIPE Headquarters, Nairobi, Kenya. The crutch frames restrained movements of the animal in all directions during experimentation. Observations were made between 0900 and 1600 h and under more or less stable atmospheric conditions (still air, ± same wind direction) but not during windy, overcast or rainy days. So, far, obervations were done in conditions close to natural ones.

3.8.2.2 **Preparation of cattle**

Unstressed animals fed beforehand were held in a standing position in crutch. Such experimental hosts kept tick-free for several weeks before assays were then loaded with ticks. Sometimes, a long period of restraint of the cattle was required to allow a sequence of observations on the effect of an extract to be made, in which case, the animal was transferred into an adjacent paddock to allow it the freedom of movement and access to food and water.

3.8.2.3 **Tick release on host**

This was done using a fine paint brush or hay straw. A stop watch was used to time behavioural responses of test ticks. These were monitored at 1h interval and behaviour(s) recorded after a 4hr period. At the end of the experiment, ticks were removed manually from the host and killed in methanol. While removing the attached

the ticks, care was taken not to damage the mouthparts.

3.8.3 **On-host behaviour assays**

3.8.3.1 **Orientation behaviour to feeding sites**

Ticks left to freely wander from any body part of bovine cattle were observed for their behaviour and responses. Location of ticks moving or non-moving on the host were recorded. Specific methods on aspects of feeding site location are detailed under the relevant sections.

3.8.3.2 **Disruption experiments**

Tests were conducted on animals under the same conditions as above but with test ingredients or compounds released on host. Emphasis here was on the site of application of these extracts which were to be at close proximity to the feeding site in order to elicit reaction from test ticks away or towards this site. Extracts in an appropriate medium/solution were dispensed using syringe and applied drop by drop on the selected area of the host.

3.9 **Behavioural studies layout**

The various behavioural experiments were specifically undertaken to investigate the following aspects:

1) Feeding site discrimination.
2) Migratory activity: locomotory speed, paths, feeding site finding success.
3) Observational studies on on-host orientative behaviour.
4) Aggregation responses in *R. appendiculatus*.
5) Arousal to host odours.
6) Arrestment responses
7) Stimulus responses to host kairomones under laboratory conditions.
8) Role of contact and air-borne volatile kairomones.
9) Disruption of tick orientation on host towards feeding sites.

Results of observations and experiments 1-3 and 4-6 are discussed in Chapter 4 and Chapter 5 respectively. Chapter 6 deals with results of 7-8 whereas results of 9 are emphasized in Chapter 7.

3.10 **Statistical analyses**

Treatment differences in bioassays and observation data were subjected to statistical analyses relevant to the type of experiments and the results obtained. Analyses of variances (ANOVA) and mean separation tests or other appropriate tests were performed using Statistical Analysis System (SAS, 1985). The statistical package used as reviewed by Sokal and Rohlf (1969) and essentially entailed means comparison of behaviour parameters of the data obtained.

CHAPTER FOUR

4.0 ON-HOST MOVEMENT PATTERNS OF *R. APPENDICULATUS AND R. EVERTSI* TICKS IN RESPONSE TO HOST-RELATED SEMIOCHEMICALS

4.1 **Introduction**

On the host, upon detecting the appropriate stimuli, ticks orient towards a target site which may be at varying distance from their positions. Often, the parasitic phases have to travel relatively large distances to reach the targeted site or mate (Gladney and Drummond, 1970; Sonenshine *et al.*, 1974; Kellum and Berger, 1977). Routine observations show that this phase is characterized by different movement patterns which include runs, stops, bouts, turns, etc. The settlement at 'preferred sites' is also related to host physical characteristics (texture, body temperature, skin humidity, etc..) as well as chemical attributes which are likely to provide optimum conditions for the attachment of ticks (Howell, 1975; Doube and Kemp, 1979). The migratory bouts which *R. appendiculatus* and *R. evertsi* perform during the search and location of their preferred feeding sites, imply a pattern of detection, location and finding which is displayed by the path's characteristics viz its linearity, turning rate, directionality, etc (Bell, 1983).

Mechanisms of site location by migrating ticks are not understood. To ascertain the mediation role of feeding site cues in the orientation process, movement patterns and spatial attachment patterns of both tick species need to be described. This could then provide a basis for studies on tick responses to host-related stimuli, especially those emanating from naturally preferred sites, and eventually, for behavioural manipulation of the ticks. Accordingly, observations were undertaken to investigate behavioural sequences associated with tick movement on the natural host, pathways of the trajectories as well as the locomotory activity patterns of the two ticks under study.

4.2 **Materials and methods**

4.2.1. **Feeding site selection**

To determine the ability of *R. appendiculatus* and *R. evertsi* ticks to discriminate for their respective feeding sites in relation to olfactory cues, ticks of both sexes with painted tarsi of the first leg pairs were released onto host at selected distances away from their feeding sites. Painting of tick's tarsi was performed using commercial lacquer (Radiant©, Grey color). Coating of tarsi which is known to obliterate olfactory receptors, especially the Haller's organ (Graf, 1975), was preferred to tarsectomy as it is a lesser traumatic treatment (Leahy *et al.*, 1975). Preliminary

observations showed that mortality or impairment of behaviour of lacquer-painted ticks were negligible several days post-treatment. Males and females treated in this way were kept 24 h before being tested. Batches of 20 *R. appendiculatus* of each sex were released about 11 cm away from each ear base, on the hypothetical line joining the two ears. Ticks reaching the feeding site were removed and counted at hourly intervals during a 4 h period. Those found in areas adjacent to the ear (head, neck, dewlap) were assumed to have been disorientated or to have missed the feeding site source.

Experiments on responses of tarsi-coated individuals of *R. evertsi* to olfactory stimuli from anal area were conducted as described above, except the release point was 10 cm away from the anus. Disorientated individuals scattered on hindleg, back, flank.. of the host were checked. For each species, the experiment was replicated 4 times. Percent of painted ticks and controls (unpainted) migrating to ears or anal region (including base of tail) was calculated and compared, after transformation (Square root) of data, using Least Square Difference (LSD) test.

4.2.2 **Orientative behaviour of *R. appendiculatus* on host**

Two sets of behaviour assays were undertaken and appropriate observations made to characterize different behavioural events relating to the whole migration process of the ticks on the host. To keep ticks behaving in conditions close to natural ones, no

attempt was made to alter natural cattle hairiness (mean height range = 0.5-3cm). The first experiment dealt with the description of different phases or behaviour components displayed from the point of contact with the host to the feeding site finding by *R. appendiculatus* as elicited by different semiochemical factors and observed under conditions of naturally-released body emanations from the host, including host/feeding site attractants. Up to 5 individual ticks were released and any behavioural trait initiated by the tick in terms of its occurrence, modification, phase transition, and termination, etc. was recorded.

As an extension to these observations, a further behavioural experiment was devised to study potential semiochemical signal(s) emitted by feeding ticks and this was done using three treatments. The first treatment was to test ticks which were attached and feeding on the cattle ears. More than 50 unfed ticks of mixed sexes were placed on the cow ear under cotton bag and allowed to feed. The cotton sleeve was opened 24 h after release to remove unattached or dead ticks and the number of attached ticks reduced to 50 per ear and the ears enveloped again. Ticks attached for a number of days were used since several reports indicated that males and females in *Amblyomma* species produce pheromones after 3 to 8 days post-attachment (Rechav *et al.*, 1977; Norval and Rechav, 1979). Some of the pheromone compounds being released are volatile and these may be attractants for conspecifics (Norval *et al.*, 1991c). On days 4 and 6 post-feeding, the ear bags were reopened and 20 unfed ticks of each sex released on the cattle head (mid-distance between the two

ears, ca. 10 cm) and their migration to either ear was recorded.

For the second treatment, feeding ticks were replaced by tick rinses obtained as described under section 3.4.2.1 (page 44). Evidence from previous studies on *Amblyomma* spp has shown that rinses of fed ticks possess attraction cues (Rechav *et al.*, 1976, 1977; Sonenshine *et al.*, 1982b). Tick rinses of different feeding ages amounting to 50 male-equivalents (ME) were applied onto each of tick-free ears of cattle. A similar amount of female-equivalents (FE) was used for comparison. In the third treatment, the ears were left untreated. Attraction to the ears was compared between treated ears and untreated ones and any additive attraction over the untreated ones was interpreted to mean combined attraction due to both interspecific and intraspecific signals. Differences in the distribution patterns of ticks for these 3 experiments were compared using ANOVA (SNK multiple range test). These observations together with the orientation of ticks to the ear were meant to provide insights on the way different host odours (including feeding site attractants) and tick odours (especially pheromone emissions from feeding ticks) are involved and integrated in the process of orientation to and location of the feeding site.

4.2.3 **Pathways of *R. appendiculatus* and *R. evertsi* on host**

Exploiting tick's common questing behaviour (Chiera, 1985) and assuming their vertical distribution pattern on vegetation (Punyua *et al.*, 1985), four points on the

cow were selected as release sites to monitor the pathways adopted by *R. appendiculatus* moving towards its feeding site. The selected sites, presumably among some of the points ticks are picked up by their passing hosts were: dewlap (DW), neck rim (NR), foreleg (FL)(external side, ~65 cm above the carpus) and hindleg (HL)(external side, ~70 cm above the carpus). For the study of *R. evertsi*'s routes to the anal region, these points were limited to the two latter. Free movement of individuals of each sex and species towards the relevant target, was observed during the migration. Observations involving individuals were preferred to avoid intraspecific effects (Sonenshine *et al.*, 1982b). Of the total number of ticks migrating, those reaching the feeding site were counted and the paths taken from different points sketched for analysis. At least 20 individuals were observed from each release point for each sex and species.

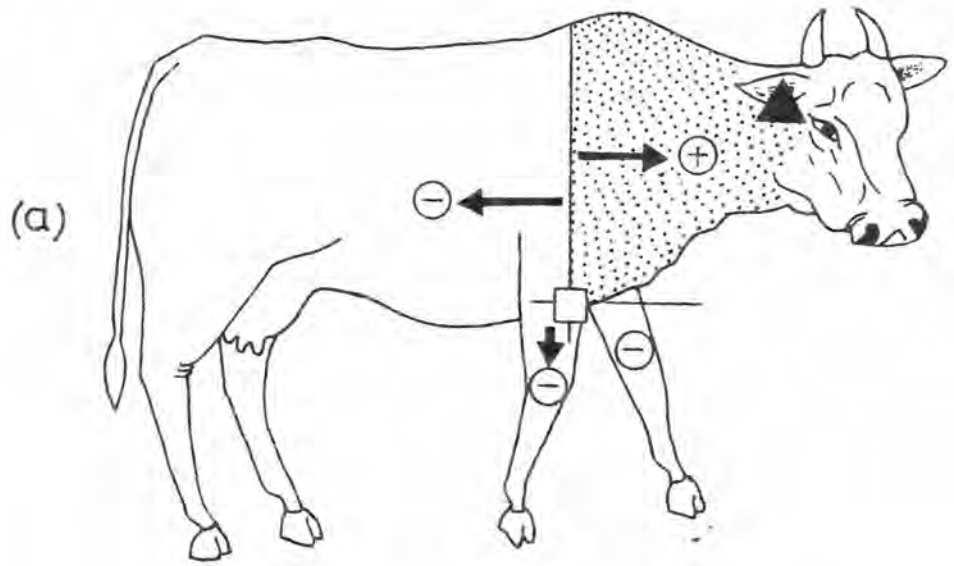
4.2.4 **On-host locomotory activity of *R. appendiculatus* and *R. evertsi*.**

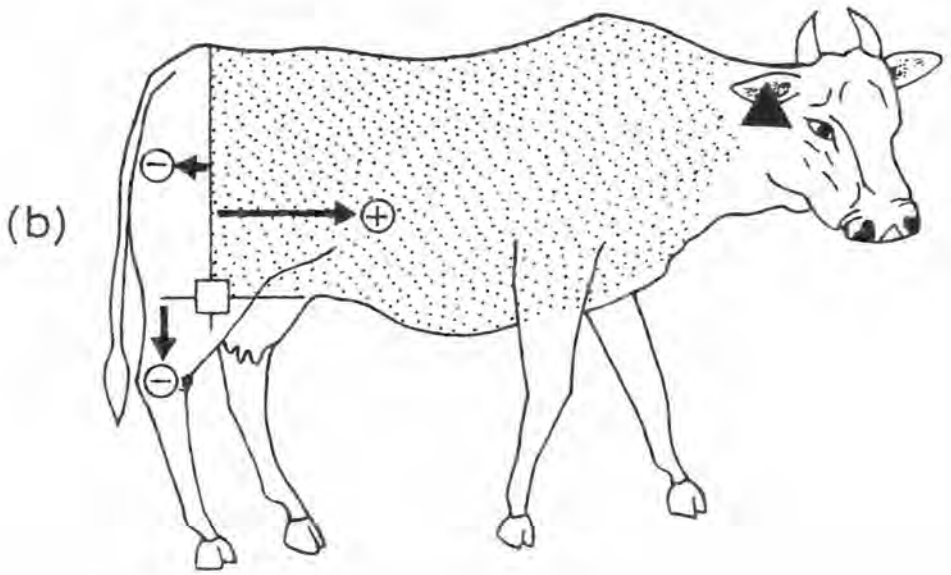
As a follow up of the above experiment, locomotory activities of adult *R. appendiculatus* and *R. evertsi* from the source to the target (feeding site) were determined. Parameters quantified were: the probability of moving to and away from the target feeding site (positive and negative migration respectively); the success rate of locating the feeding site. This was done using batches of 5 dye-marked ticks simultaneously released from selected body regions of the host and their behaviour

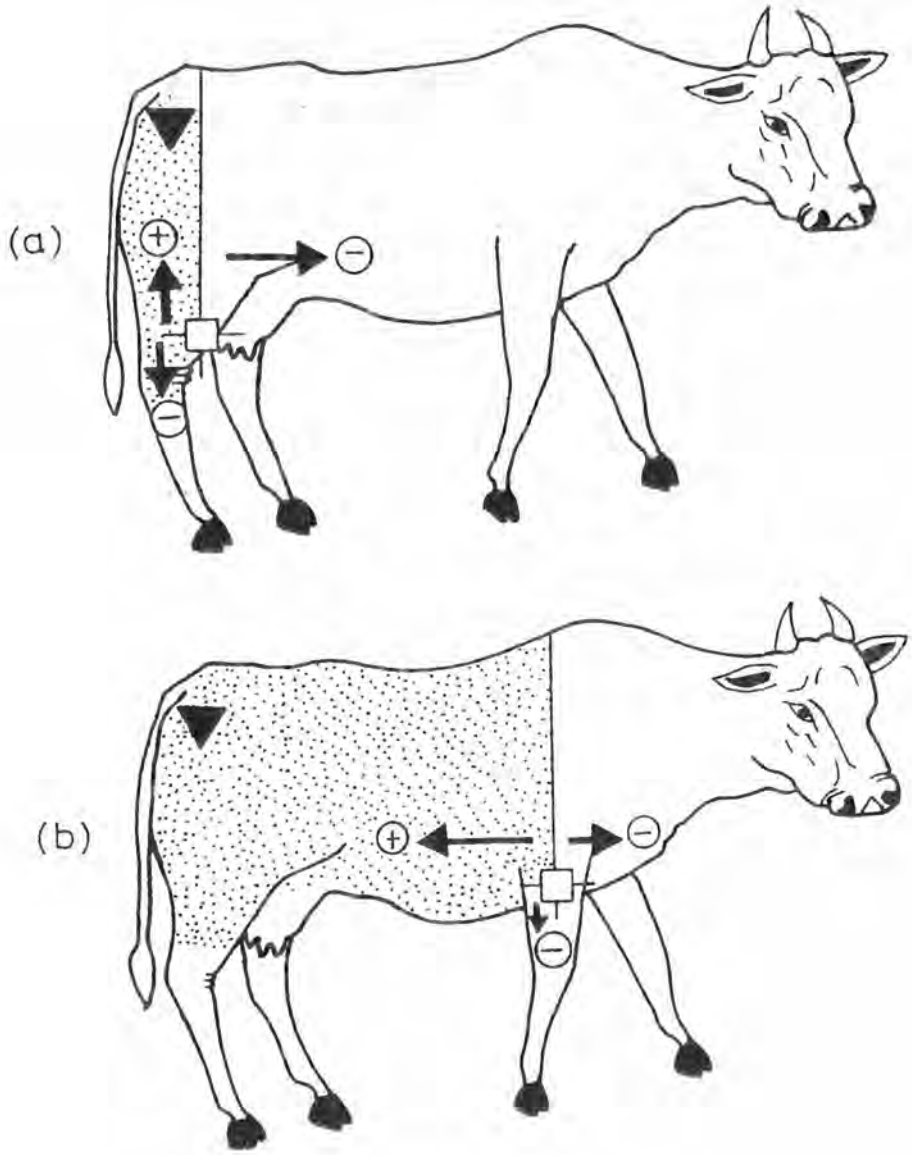
observed as they moved. A color code for dye (Humbrol Enamel, Hull, U.K.) was ascribed to each group with respect to the 4 sites of release (Tab. 1). Other parameters quantified were numbers of reorientations or directional adjustments from a release point (walking to and from across an arbitrary line towards the feeding site)(Fig. 11 and 12); the expected time to reach the target/feeding site and the drop out rate. For these experiments, the same release sites were selected for both species as mentioned in section 4.2.3. Individuals (at least 25 replicates) or batches of 5 individuals (5 replicates) were tested.

Tab. 1 Dye colour in relation to sites of release of ticks on host

Release point on the host	Dye colour group	
	R. appendiculatus	*R. evertsi*
Neck rim	Orange	-
Foreleg	Brown	Brown
Hindleg	Red	White
Back	White	-


(a)
⊕
⊖
⊖
⊖


(b)
⊖
⊕
⊖


(a)
(b)

4.3 RESULTS

4.3.1 Selection of the feeding site by *R. appendiculatus* and *R. evertsi*

Coating tick's tarsi (assuming by this treatment substantial occlusion of Haller's organ located on the 1st pair of legs) was shown to impact the discrimination efficiency of the target feeding site by both tick species (Figs 13 and 14). In *R. appendiculatus*, only 15.0% of tarsi-painted individuals reached the ear site during the 4h observation period as compared to 59.0% in unpainted ticks ($P<0.001$). The success rate for locating the ear dropped by 3.8 times between the treated and control groups (7.3 vs 1.9)(Table 2). Likewise, in *R. evertsi*, only 22.8% of coated ticks reached the anal area ($P<0.001$) compared to 43.4% of uncoated ones. A drop in the success rate for locating the anal site by 2.7 times due to the treatment was recorded (5.6 vs 1.4)(Table 3). In addition, significant number of coated ticks were found in areas adjacent to the respective feeding site ($P<0.001$), indicating a high rate of disorientation among treated ticks relative to controls (Tables 2 and 3).

Table 2 Effects of tarsi-painting treatment on orientation responses of *R. appendiculatus* to the ear site

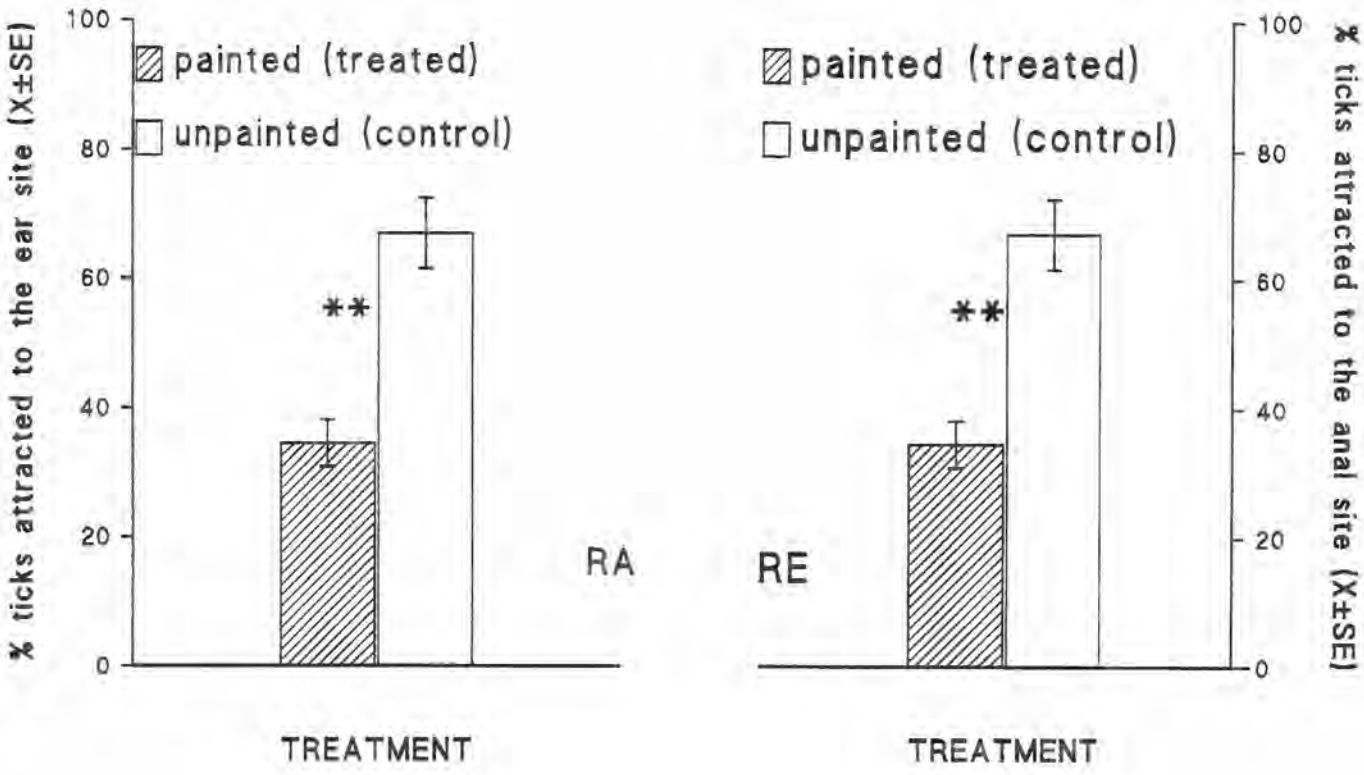
Behaviour parameter assessed	Response		LSD-Test
	Painted[1] (N=160)	Unpainted[1] (N=160)	
Total ticks moving (%)	45.3	55.9	ns
Reaching target FS (%)	15.0	59.0	**
Located off target FS[2] (%)	45.6	23.0	**
Stationary (%)	59.7	35.1	**
Drop-out (%)	36.9	19.8	**

FS = Feeding site (= ear);
[1] Pooled data for ♂ and ♀
[2] Areas adjacent to FS i.e. back, flank, hindleg, ..
*, significant; **, highly significant; ns= not significant

Table 3 Effects of tarsi-painting treatment on orientation responses of *R. evertsi* to the anal site

Behavior parameter assessed	Response		LSD-Test
	Painted[1] (N=160)	Unpainted[1] (N=240)	
Total ticks moving (%)	55.9	53.9	ns
Reaching target FS (%)	22.2	43.4	**
Located off target FS[2] (%)	40.7	10.5	**
Stationary (%)	44.1	46.1	ns
Drop-out (%)	63.1	36.7	**

FS = Feeding site (= anal area);
[1] Pooled data for ♂ and ♀
[2] Areas adjacent to FS i.e. back, flank, hindleg, ..
*, significant; **, highly significant; ns= not significant


% ticks attracted to the ear site (X±SE)
100
80
60
40
20
0
painted (treated)
unpainted (control)
**
RA
TREATMENT
painted (treated)
unpainted (control)
**
RE
TREATMENT
% ticks attracted to the anal site (X±SE)
100
80
60
40
20
0

4.3.2 **Orientation behaviour of *Rhipicephalus appendiculatus* to the feeding site**

Stereotyped sequence of behaviours during *R. appendiculatus* orientation from contact with host's fur to stimulus source are summarized in Fig. 1. Although variations occurred in the duration of each behavioural element at individual level, these stereotypes were consistently present irrespective of release sites of ticks and sexes. The orientation was made of runs and strides of varying intensities, alternating stops and bouts and, occasionally, walk-away and back-up movements. However, an initial short run, seemingly a reflexive movement observed sometimes following tick deposition on the host, was discarded from our observations.

Based on the migratory process features, four behavioural phases could be delineated. These were in order of their occurrence: (1) stationary/scanning phase, (2) initial movement/random search phase, (3) directional movement, (4) arrestment at feeding site and attachment. During the first phase, ticks made no translational movement. They were initially motionless but this was followed by outstretching of legs, and at times, they adopted a posture suggestive of scanning activity (questing-like). In the second phase, they initiated some random walking characterized by lateral displacements and frequent reversals in directions, although the majority displaced in the general direction of the feeding site. During the third phase, after about 1/3 to 2/3 of the course, tracks became less random and displayed some directional steering and faster displacement toward the feeding site. The final

approach phase took place relatively close to the odour site, when ticks became arrested as they approached the ear. Running speed slowed down considerably and eventually the ticks stopped. At the ear, they were completely arrested and after varying periods of time, insertion of their mouthpart pieces into host integument began as a result of the attachment process.

Observations carried out using 50 live ticks feeding on the ear or when extracts (50 TE) from fed ticks are applied on the ears, suggested the involvement of additional semiochemical signals in the orientation process. Indeed, comparative data showed more ticks orienting to the treated sites than to the natural (untreated) ones. The site attraction was enhanced by 1.4 fold with live ticks and 1.2 times fold with tick extracts as compared with natural tick-free ear, although the difference between the three treatments was not significant ($F=0.56$; $df= 1$; $P= 0.64$)(Table 4).

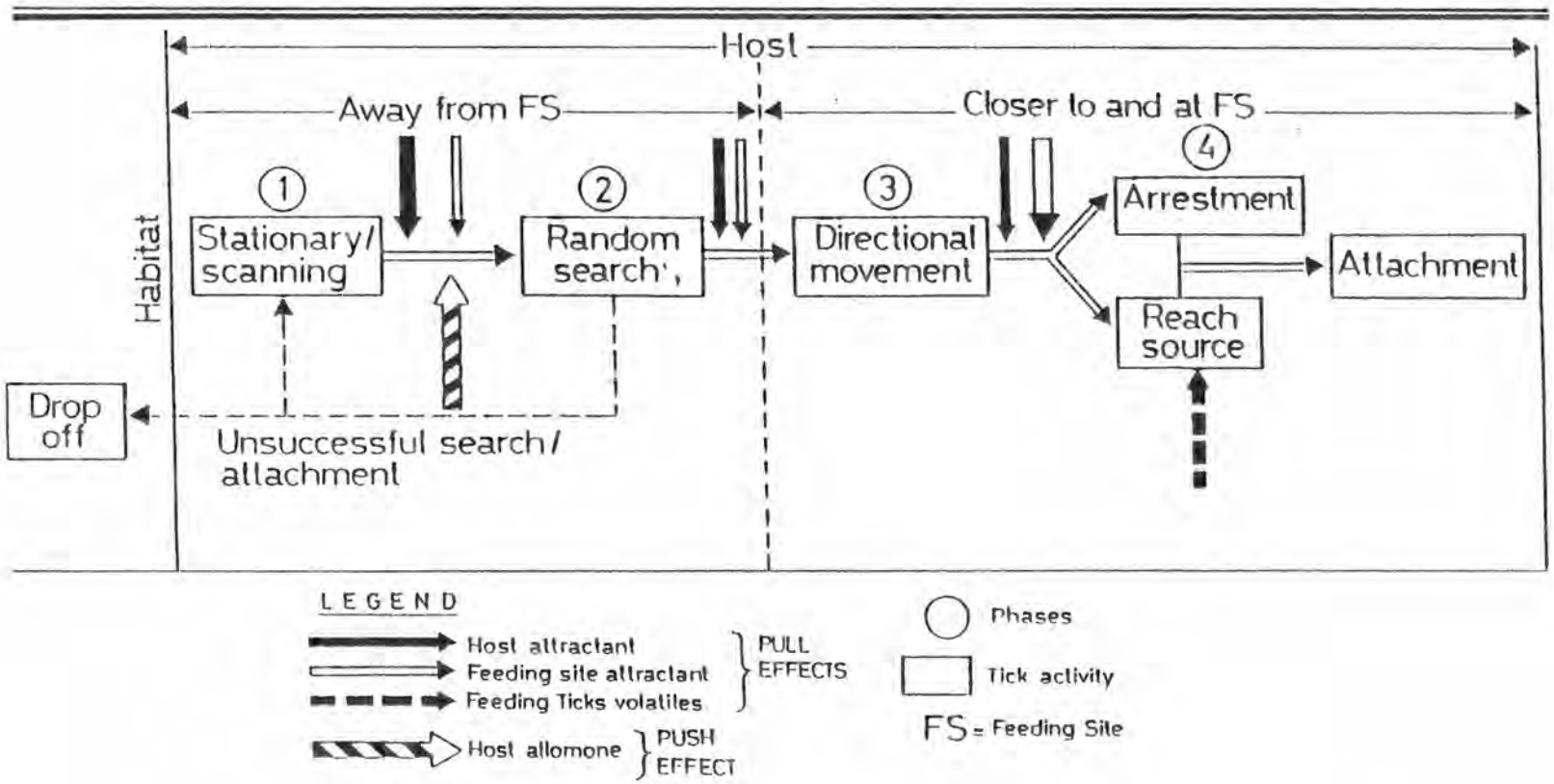


Fig. 15 Feeding site orientation behaviour of *R. appendiculatus* to the ear and possible semiochemical signals implicated

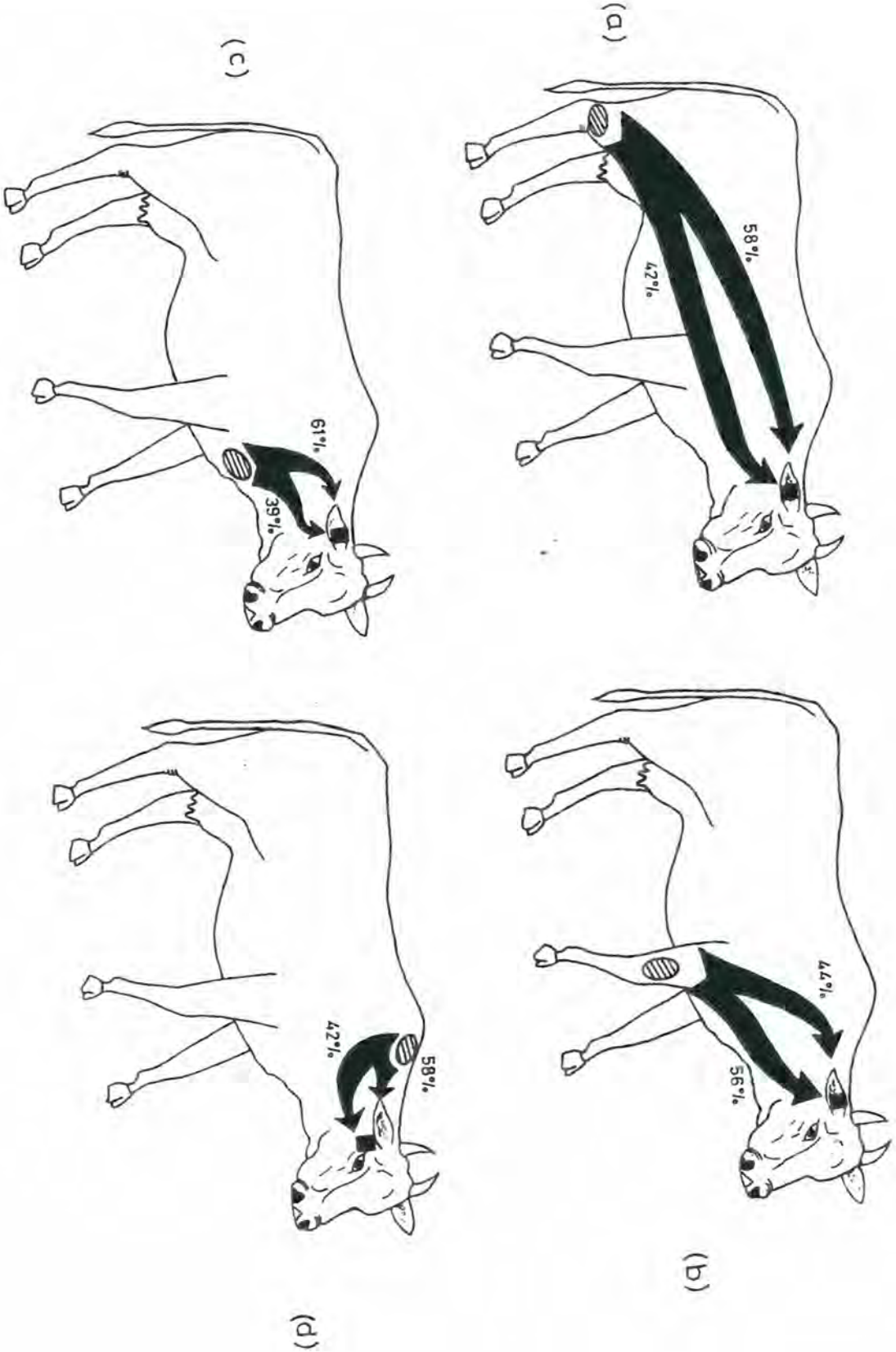
Table 4 Mean % *R. appendiculatus* orienting to ear site signals augmented with various tick stimuli on ear preferred feeding site

Behaviour component assessed	Ear site treatment		
	Untreated[1] (N=160)	+ 50 live ticks (N= 150)	+ 50 TE[2] (N= 150)
Total ticks moving (%)	52.7a	63.1a	57.8a
Reaching target FS (%)	67.0ab	78.0a	74.5a
Located off target FS[3] (%)	13.2a	16.1a	10.5a
Stationary (%)	47.3a	38.9a	42.2a
Drop-off (%)	30.5a	25.6a	28.4a

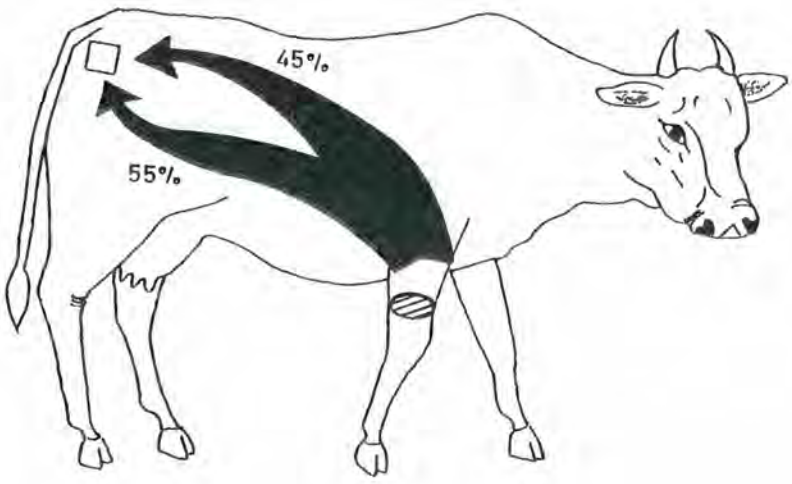
Pooled data (♂ and ♀);
FS = Feeding site (= ear area);
[1] Tick-free ear site;
[2] TE = tick-equivalent (pooled data for 50 male-equivalent [ME] and 50 female-equivalent [FE]);
[3] Areas adjacent to FS i.e. head, neck, dewlap;
Means within row with different letters are significantly different (ANOVA, SNK test, at $P<0.05$).

4.3.3 **Pathways of *R. appendiculatus* and *R. evertsi* to feeding sites**

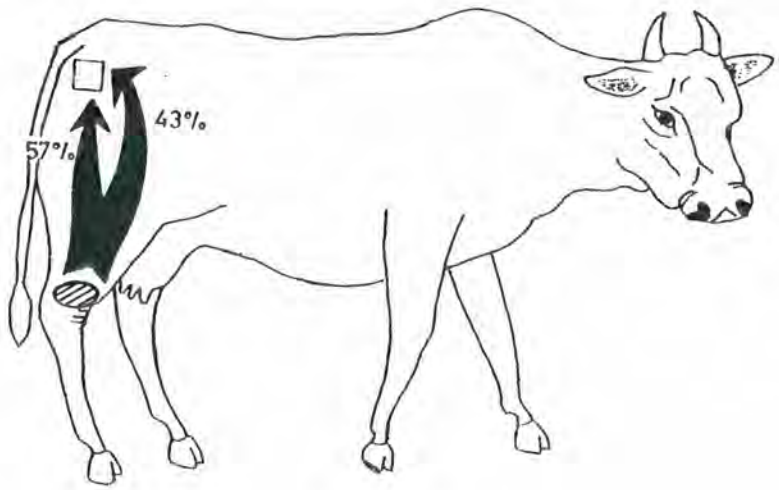
Individual ticks released on the host held in crutches under ambient conditions demonstrated highly orientated movement towards the appropriate feeding sites. This was true for both *R.evertsi* and *R. appendiculatus* which, irrespective of the release points, completed most paths to their target feeding sites (73.9% and 55.9% respectively). A few mobile ticks failed to complete their paths while others remained stationary at the release points. Some also dropped off the host at some point or another. Figures 16 and 17 depict the generalized pathways taken by individual ticks that were successful in locating their respective feeding sites. Most individual tracks were found to be curvilinear. Although not uniform between individuals, sex and species, these tracks were shown to display some directional steering as the tick approached the odour sources.


(a)
58%
42%
(b)
44%
56%
(c)
61%
39%
(d)
42%
58%

(a)



(b)



4.3.4 **Locomotory activities of *R. appendiculatus* and *R. evertsi*.**

Individual ticks of both species tested for their migratory activities, were found to move from all of the experimentally selected release points on the host. In *R. appendiculatus*, the number of ticks performing positive migration was relatively high with respect to the ear site, with the following order of successful target finding in relation to the release point: Neck Rim (NR)> Foreleg (FL)> Hindleg (HL)> Dewlap (DL) (viz 90.0%, 80.6%, 78.8% and 67.5% respectively)(Fig. 18) whereas relatively few (13.5%) performed negative migration. The number of reorientations accounted for 12.5 %, except in *R. appendiculatus* females (neck rim) where it averaged 31.7%. On average, 24.6% of ticks were found off-target site while mean percent drops-out was 7.4% (Table 5). Likewise, in *R. evertsi*, most individuals located the anal feeding site when released from HL (85.6%) and FL (69.8%)(Fig. 19). The number of reorientations was relatively high (overall avg 39%) for ticks departing from both HL and FL release sites. Number of ticks found off-target as well as drop out (11%) was low (Table 6). The timing during the course path at intervals showed that walking speed was consistently variable relative to sex, release point and species, ranging in *R. appendiculatus* between 0.09 cm/min for the lowest speed (NR) to 0.41 cm/min for the highest (DW) and, in *R. evertsi*, between 0.24 to 0.58 cm/min for FL and HL respectively (Tables 7 and 8).

Fig. 18 PROPORTION OF *R. APPENDICULATUS* LOCATING SUCCESSFULLY THE EAR FEEDING SITE AFTER RELEASE FROM 4 POINTS ON THE HOST

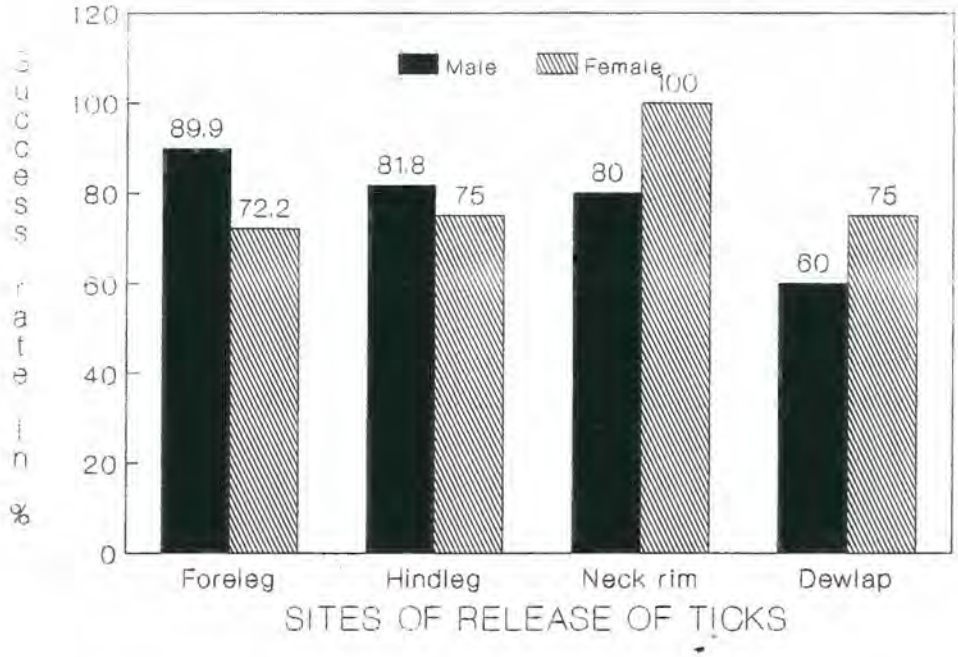


Fig. 19 PROPORTION OF *R. EVERTSI* LOCATING SUCCESSFULLY THE ANAL SITE AFTER RELEASE FROM 2 POINTS ON THE HOST

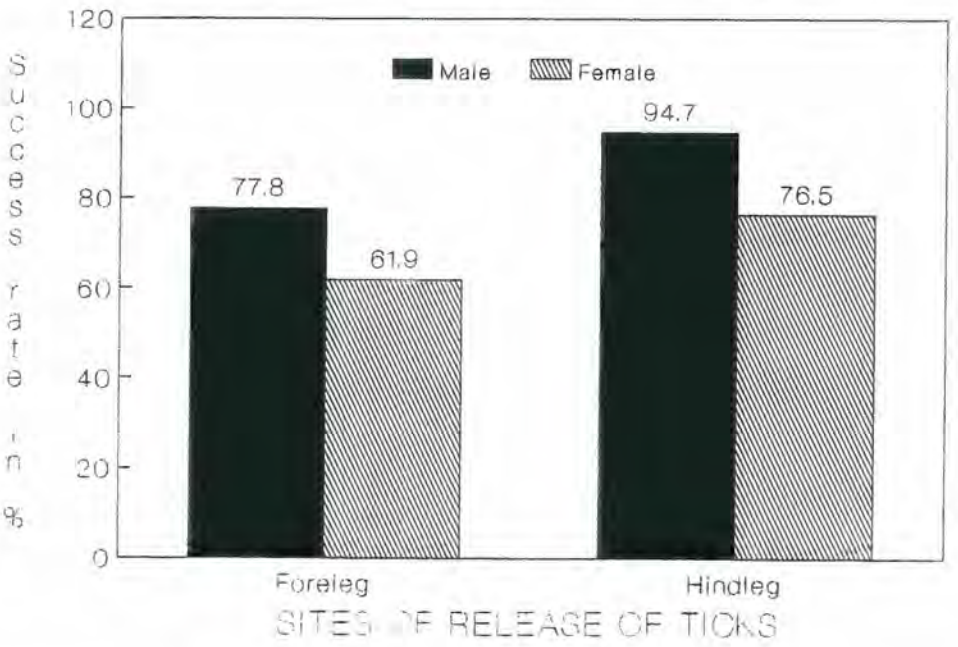


Table 5 Locomotory activities of *R. appendiculatus* in relation to the location of the ear feeding site

Response component	Release points on host			
	FL[1] (N=45)	HL[1] (N=45)	NR[1] (N=30)	DW[1] (N=60)
Total ticks moving	84.4	88.9	73.3	73.3
Moving towards target FS (%)	94.4	78.8	79.2	93.5
FS Location success (%)	80.6	78.8	90.0	67.5
Crossings (%)	18.4	14.7	27.3	17.0
Stationary (%)	15.6	11.1	26.7	26.7
Drop out (%)	6.0	6.7	10.0	26.7
Mean walking speed (cm/min ± SEM)	0.16±.04	0.09±.06	0.34±.10	0.41±.09
Mean time to reach FS[2] (min.)	10.4	14.4	1.6	2.9

FS = Feeding site (= ear);
FL=foreleg; HL= hindleg; NR= Neck rim; DW= Dewlap;
[1] pooled data for ♂ and ♀;
[2] time relative to mean distance to FS;

Table 6 Locomotory activities of *R. evertsi* in relation to the location of the anal feeding site

Response component	Release points	
	FL[1] (N=50)	HL[1] (N=50)
Total ticks moving	82.0	82.0
Moving towards target FS (%)	95.1	88.5
FS Location success (%)	69.8	85.6
Crossings (%)	24.4	53.6
Stationary (%)	18.0	18.0
Drop out (%)	6.0	16.0
Mean walking speed (cm/min ± SEM)	0.24±.05	0.58±.30
Mean time to reach FS[2] (min.)	30.2	2.9

FS = Feeding site (anal area);
FL=foreleg; HL= hindleg;
[1] pooled data for ♂ and ♀;
[2] time relative to mean distance to FS;

4.4 Discussion

In the present study, two tick species *R. appendiculatus* and *R. evertsi*, with different predilection feeding sites were observed for their on-host orientation behaviour under natural conditions. Aspects of tick orientation behaviour addressed included success rate in locating feeding sites, the pathways taken to reach the target site, effects of intraspecific and interspecific signals. Components of behaviour exhibited by ticks from the time of landing on the host to location of the feeding sites, such as locomotor activities, migration patterns, arrestment on arrival at the feeding site were determined.

Tarsi-coated ticks were less successful in locating the feeding site compared to untreated individuals. Tarsi-painting in ticks, like tarsectomy and palpectomy is known to suppress perception of cues including olfactory and contact stimuli (Graf, 1975; Leahy *et al.*, 1975). Coating the foreappendages of *R. appendiculatus* and *R. evertsi* probably resulted in partial loss of their sensory capability, probably as a result of blockage of the Haller's organ, which perceives primarily olfactory stimuli (Leonovitch, 1977; Waladde, 1987). Highly site-specific ticks, like the two species under study, must locate their site of predilection in the most efficient ways. Thermal and olfactory gradients are among the probable cues used as the guiding mechanisms (Shorey, 1976). Loss of ability to locate the feeding sites resulting from tarsi coating, strongly suggests that chemoreception is an important mechanism in feeding site

location in these ticks.

The stereotyped sequences which characterized the orientation behaviour of ticks on host culminated with the tick successfully finding its target. Four phases could be delineated. The first was characterized by lack of activity but reminiscent of an acclimatization and scanning behaviour. Noted, at times, was the waving of legs of the ticks, a trait associated with probing by many arthropods including ticks (Lees, 1948; Kramer, 1976; Bell *et al.*, 1983). This was followed by a search activity at first, with a lot of random movements, but with gradual progression toward the target site. Closer to the site, the movement assumed a clear directional character which brought the tick close to the feeding site. Lastly, there was arrestment of the tick at stimulus source, followed by attachment of mouthparts and engorgement. This orientation process implies the mediation of several interspecific chemical signals - a background host odour that retains the tick on the animal, a volatile signal emanating from the target feeding site which elicits an anemotactic response of the tick and a much less volatile short-range or contact signal that arrests the tick at the feeding site. Interestingly, the present study has shown that stimuli-depleted feeding site through solvent washing led to a significant depression in the oriented responses of the ticks, showing clearly the mediation of distance perceivable olfactory signals from such sites.

Of the above phases, the second which involves the perception of the signal from distance, probably distorted and interrupted by host and aerial movements is critical to

successful orientation by ticks. The progression is klinotactic in character, full of many apparently tentative strides, turns and stops. The many stops and turns could help the tick replenish its energy and readjust its directions (Shorey, 1970; Visser, 1986). According to Bell (1983), stop duration and stop frequency are important aspects of course control in an insect and ticks are no exception (Guerin *et al.*, 1992). In any case, this reflects investment of a lot of energy input by these ticks (Visser, 1988).

The present study has also implicated intraspecific olfactory signals from successfully feeding ticks in drawing unfed ticks to the feeding site. Thus, the presence of feeding ticks or rinses of fed ticks augmented the effect of interspecific signal present in the host ear. To date, identified pheromonal compounds in some *Amblyomma* spp. have been shown to attract other conspecifics to the host animal as well as to the feeding site (Schoni *et al.*, 1984). However, our results with *R. appendiculatus* suggests that emissions by feeding ticks may not be critical to this tick. On-host assays have shown that tick-free sites are still strongly attractive and inclusion of feeding ticks or extracts of fed ticks does not enhance success rate appreciably (Table 4). Therefore, in this species, pheromones emitted by feeding ticks may only augment a primarily kairomone mediated process of tick orientation to its feeding site.

Both *R. appendiculatus* and *R. evertsi* showed high success rates in orienting to their respective feeding sites irrespective of the release points. This implies that in

nature, ticks from different pick up points on host animal are able to detect the appropriate semiochemicals and orient toward the specific feeding sites. In summary, our results on on-host tick orientation behaviour suggest the mediation of potent semiochemicals and that a knowledge of these signals could open up novel tactics of manipulating tick behaviour on host or off-host.

CHAPTER FIVE

5.0 STUDY OF SOME SPECIFIC BEHAVIOURAL RESPONSES

5.1 Introduction

The success of ticks as ectoparasites is due in part to the plasticity and sophistication of their behaviours, of which host location, feeding site location and feeding represent an important set. Waladde and Rice (1982) gave a detailed scheme representing sequences of events culminating with successful feeding of ticks. In the present chapter, work undertaken on specific elements of behaviour involved in this chain of events will be described. These include some well known tick traits like aggregation behaviour, and other less documented ones like arousal, activation and arrestment responses.

Arousal as a primary reaction to any stimulus has formed the basis of a number of behavioural and electrophysiological studies on ticks. Various chemicals including host and tick odours are known to arouse ticks. Electrophysiological studies have focussed on the action potentials of ticks aroused by these compounds and stimuli (Haggart and Davis, 1980,1981; Waladde, 1987; De Bryune and Guerin, 1994).

Activation is seen as one of the ways the insects (and ticks) get access to environmental resources (Bell, 1988). Indeed, at this stage, information processing of

environmental parameters is very important before a decision is made by an arthropod to perform a certain function. Towards the end of any motion, arthropods which have been activated show a decline in their exploratory movements. Kennedy (1978) noted that following a motor output, an arrestment behaviour would manifest itself at the end of the attraction movement. To date, very little documented studies have been reported on these behavioural features of significance to ticks.

On the other hand, aggregation behaviour as a component of the feeding process has been highlighted in a number of tick species, particularly *Amblyomma spp.* An aggregation attachment pheromone (AAP) was shown to play an important role in the attraction and attachment of conspecifics near already attached ticks on the host (Gladney *et al.*, 1974a; Rechav *et al.*, 1976; 1977). The blend of chemical mediators involved has been identified for *Amblyomma variegatum* Fabricius (Schoni *et al.*, 1984). Routine observations showed that stages of *R. appendiculatus* display some gregarious behaviour. An example of this is clusters of adults confined in ear pinna of bovine hosts. Recently, evidence for an aggregation-like signal was presented from laboratory observations (Akinyi, 1991), but not confirmed on tick's natural bovine host. We report here the arousal responses of *R. appendiculatus* and *R. evertsi* to preferred site extracts under laboratory conditions. Activation *in situ* of the two species to host extracts together with the arrestment of their movements on an artificial substrate were also investigated. Lastly, confirmation of the production and mediation of an aggregation agent by *R. appendiculatus* on bovine host was also

sought.

5.2 Materials and methods

5.2.1 Arousal to host odours

In a Y-olfactometer, unfed *R. appendiculatus* were tested individually to determine their response times to ear and anal cues in terms of scanning activity (T_s) and residence period (T_r) in the olfactometer arm. Scanning activity comprises a display of activities such as raising, outstretching or waving of first pair of tarsi by the tick while probing or being aroused by a stimulus. Residence time is the period each individual tick spends in proximity of or in contact with the treated chamber (olfactometer arm). 1.0 mg of crude extract of the feeding site (see Fig. 8) on filter paper (Whatman no 1, 2cm diam) was plugged in either arm while the control arm was treated with the solvent alone. Each experiment lasted 3 minutes from the onset of tick release into the inlet. Ticks which did not respond within 5 minutes were discarded. The stimulus extract from the feeding site was first tested and then alternated with that of unpreferred feeding site. The test was performed using, for each extract, 8 replicates of 12 responsive ticks for each sex and species. Distribution frequency of each response (activation and residence time) during time intervals was obtained for comparison whereas General Linear Model (GLM),

followed by Student-Newman Keuls (SNK) test, was used on transformed data (square root) to rank scanning and residence means, along with correlation test for the two parameters.

5.2.2 **Activation and arrestment behaviour of *R. appendiculatus* and *R. evertsi* to host odours.**

The arrestment behaviour, defined as the cessation of ambulatory movement or activity toward source was investigated *in vivo* (on host) and *in vitro* bioassays. For the on-host assays, this behaviour parameter was studied by noting the time taken for ticks to (a) get into motion (activation) at varying distances from the source, and (b) to reach the feeding site while study of walking arrestment was on artificial substrate (filter paper, Whatman).

In the *in vivo* test, 5 *R. appendiculatus* of both sexes were released at gradually increasing distance away from the feeding site towards the neck region viz >35 cm, 35 cm, 25 cm, 15 cm, 5 cm and 0 cm from the base of the ear. In *R.evertsi*, release was made 5cm away from the anal region ($\pm$ 5-7 cm around anus) to positions towards the hindleg region 5 cm apart. Time elapsed for ticks to get activated after release and time taken to reach the respective feeding site were recorded. Activation time and progression towards site were compared. Arrestment parameter was defined by the relation between speed and distance to source. Each run of the experiment

lasted until the tick reached its feeding site. Five replicates from each release position were used for each tick species and sex.

In related experiments, arrestment effect on an artificial substrate for a walking tick was investigated in the laboratory. Whatman filter papers measuring 15 cm of diameter and consisting of 2 concentric areas were used as the arena. The inner circle (9 cm diam) was treated with odour sample or solvent (= hexane) and the outer circle (from 9-15 cm diam) was not treated. To prevent solvent and solute diffusion outward, only the inner substratum was impregnated with the extract/solvent and then, after evaporation of the solvent, glued over the large one. A tick was placed at the centre of the filter paper and allowed 3 opportunities of 3 minutes each to respond to odours, failing which it was discarded. Arrestment responses of *R. appendiculatus* and *R. evertsi* in the presence of anal and ear extracts were expressed as percentage time spent by wandering individuals on treated and untreated circles. Tracks of ticks through odourized and unodourized fields were also recorded. For each sex and species, the test was replicated 6 times, and data analysis was by Least Square Difference test on log transformed data means.

5.2.3 **Aggregation responses of *R. appendiculatus* on bovine host**

Evidence of aggregation responses to intraspecific signals on the natural host by adults of *R. appendiculatus* was sought using rinses of fed males of different feeding

ages. Aggregation cues have been found or suspected in rinses of some tick species (Sonenshine *et al.*, 1982b; Goethe and Neitz, 1985). Drops of 100 male-equivalents (ME) in 5 ml from rinses of 6 day-old fed males were applied on a 3cm discrete area of calves scrotum as described by Rechav *et al.* (1977). An identical amount of hexane alone was smeared in a similar way as above and served as control. These doses were extended to 25 and 50 ME to obtain dose-response relations. 100 ticks of mixed sexes were enclosed in a large bag glued to the scrotum 24 h before release. 24 h post-treatment, the bag was opened and the number and percent of ticks attached on extract-treated spot (3 cm diam) calculated and compared with the control spot. The dose effect was evaluated. The same amounts of female equivalent (FE) extracts of the same feeding age were also evaluated for comparison.

5.3 **RESULTS**

5.3.1 **Arousal of *Rhipicephalus appendiculatus* and *R. evertsi* to extracts from preferred and unpreferred feeding sites**

Results on arousal responses of *R. appendiculatus* and *R. evertsi* exposed to ear and anal odour extracts in a Y-tube olfactometer are shown in Tables 7 and 8 and Figs 20 and 21. Both tick species were aroused to host odours as well as to the

solvent, with varying sensitivity (Tables 7 and 8). Percent individuals aroused to ear and anal odours was 84.7% and 72.3% respectively for *R. appendiculatus* and 75.6% and 88.2% for *R. evertsi*. Scanning activity indices for both extracts suggested, although not clearly in the case of *R. evertsi*, the same level of responses, though values differed between the two species. On the other hand, indices for residence responses were longer for extracts from preferred feeding sites compared to that of unpreferred feeding sites (I_r 0.8 and 1.1 for males and females respectively for ear extracts vs 0.6 and 0.7 respectively for anal extracts for *R. appendiculatus*; for *R. evertsi*, these were I_r 0.9 and 0.8 respectively vs 0.4 and 0.5 respectively)(Tables 7 and 8). In the case of extracts derived from the favourable feeding site, the majority of ticks were still residing in the chamber beyond the set experimental time (3 min.), but very few did so when extracts from the unfavourable feeding site was tested; in the latter case, most of the ticks had left the chamber during the first 15 seconds apparently repelled by the odour (Figs 20 and 21). Our results indicate that with respect to most parameters, the responsiveness of *R. evertsi* was stronger than that of *R. appendiculatus*. No significant difference was noted with respect to both scanning and residence parameters between males and females of both species.

Table 7 Scanning activity & residence time of adult *R. appendiculatus* & *R. evertsi* exposed to ear odour extract in treated olfactometer arm

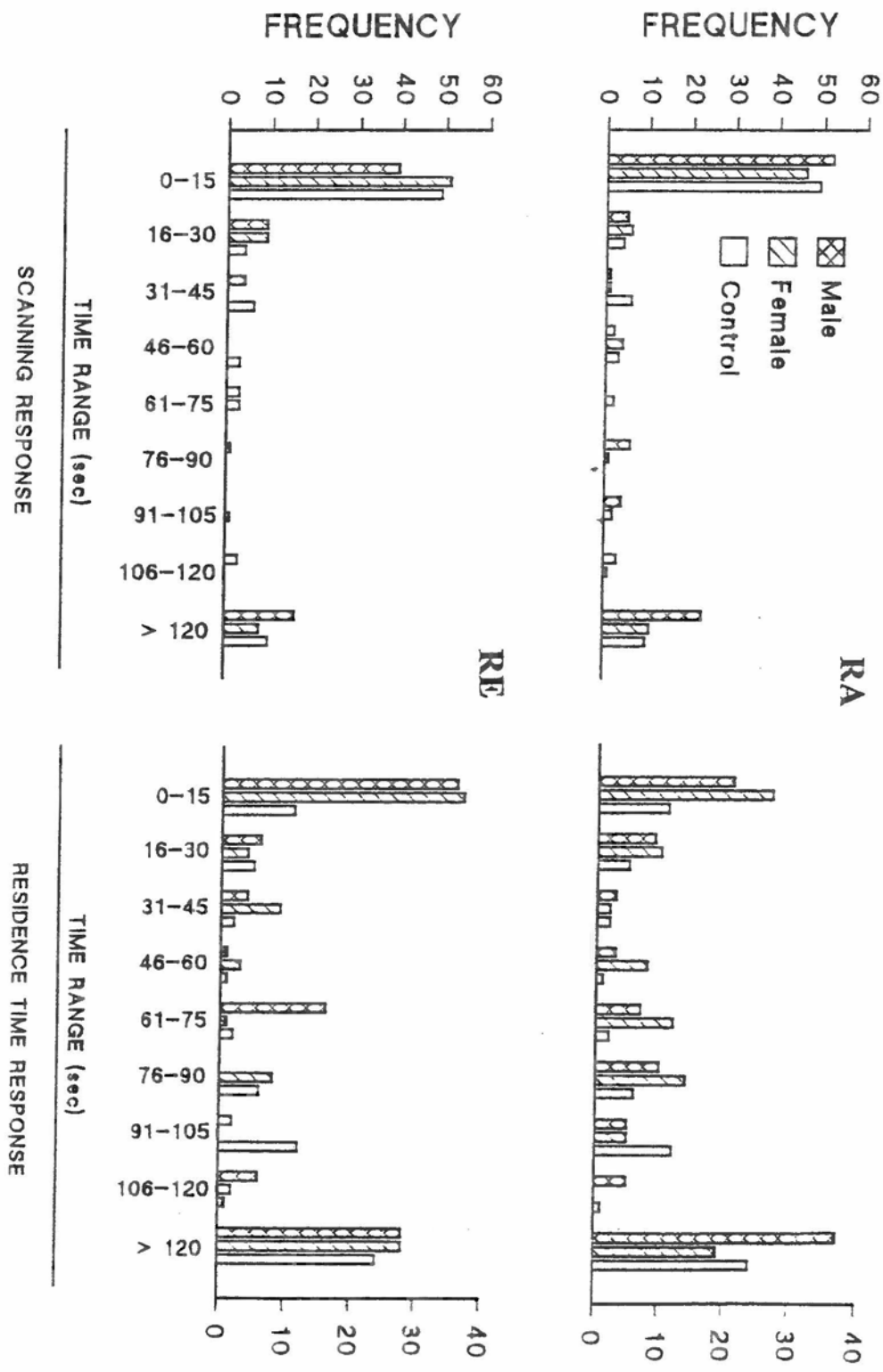
Responses time (sec)					
Parameter		*R.appendiculatus* (mean ±SE)		*R. evertsi* (mean ± SE)	
		Male	Female	Male	Female
Scanning	Trt	60.8 ± 7.2a	51.0 ± 6.9a	57.4 ± 8.4a	29.8 ± 6.4b
	C	47.6 ± 7.5b	37.2 ± 6.4b	35.9 ± 8.3b	52.5 ± 8.3a
	I_s	1.3	1.4	1.6	0.6
Residence	Trt	95.1 ± 7.2b	66.3 ± 6.0a	34.6 ± 6.1b	47.6 ± 6.8b
	C	115.4 ± 8.0a	63.1 ± 6.0a	89.0 ± 8.4a	99.7 ± 8.4a
	I_r	0.8	1.1	0.4	0.5
R coeff[1]		-0.31**	-0.44**	-0.26*	-0.21 ns

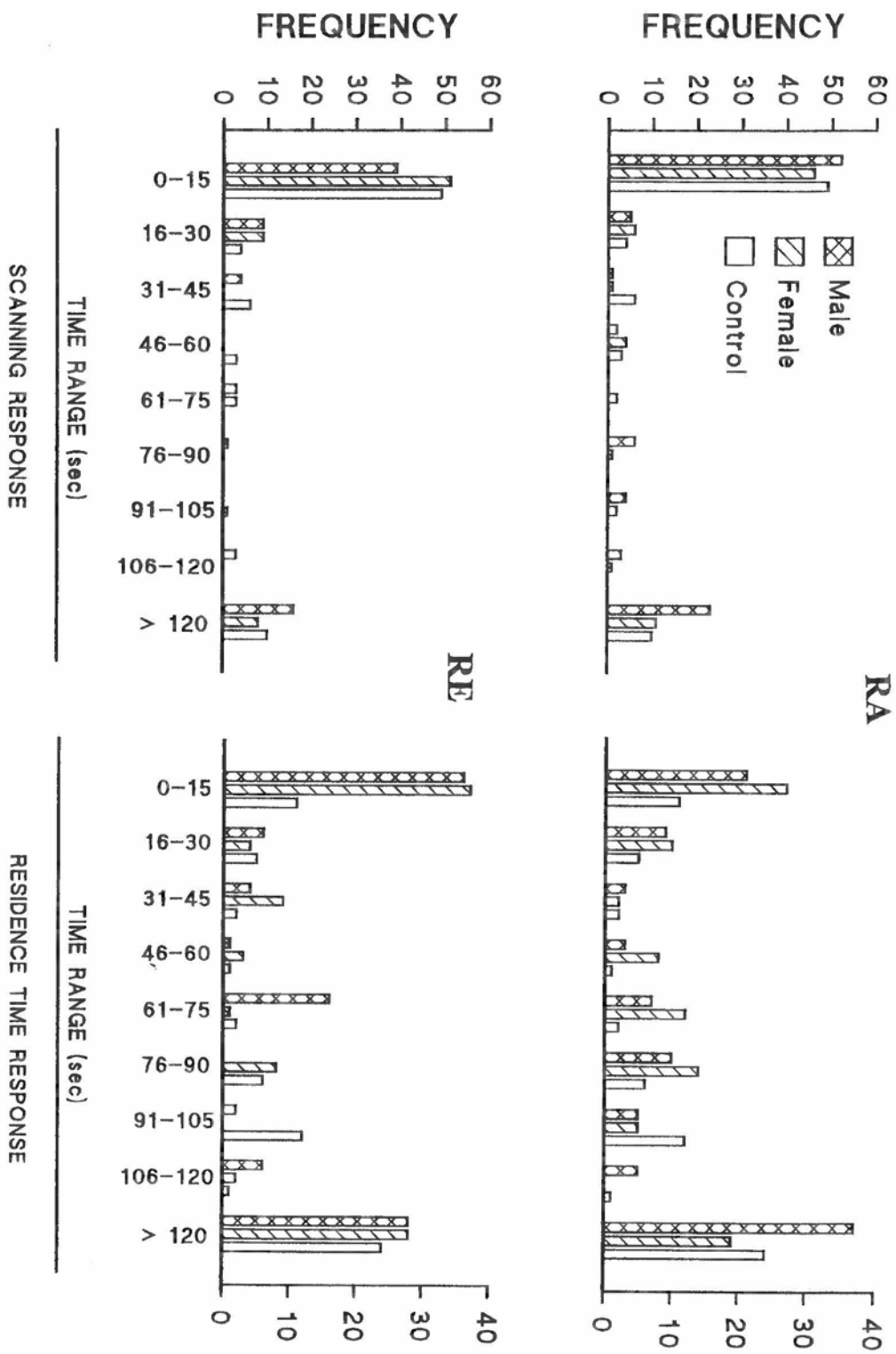
1n-hexane as trt vs blank control; trt= treatment (N=96); C= control (N=72);
I_s Scanning Index (= ratio trt/control);
I_r Residence Index (= ratio trt/control);
[1] R Correlation coefficient (Scanning/Residence);
Means in a row with the same letter in a species are not significantly different (Glm test/SNK).
*, P<0.05; **, P<0.01; ns = not significant

Table 8 Scanning activity & residence time of adult *R. appendiculatus* & *R. evertsi* exposed to anal odour extract in treated olfactometer arm

Responses time (sec)					
Parameter		*R.appendiculatus* (mean ± SE)		*R. evertsi* (mean ± SE)	
		Male	Female	Male	Female
Scanning	Trt	68.5 ± 7.6a	64.1 ± 7.6a	33.5 ± 8.4a	39.1 ± 6.3b
	C	47.6 ± 7.5b	37.2 ± 6.4b	35.9 ± 7.oa	52.5 ± 8.3a
	I_s	1.4	1.7	0.9	0.7
Residence	Trt	65.8 ± 6.8b	69.2 ± 7.3a	77.9 ± 6.0b	51.6 ± 6.8b
	C	115.4 ± 8.0a	63.1 ± 6.0b	89.0 ± 8.4a	99.7 ± 8.4a
	I_r	0.6	0.7	0.9	0.8
R coeff[1]		-0.31**	-0.44**	-0.26*	-0.21 ns

n-hexane as trt vs blank control; trt= treatment (N=72); C= control (N=72);
I_s Scanning Index (= ratio trt/control);
I_r Residence Index (= ratio trt/control);
[1] R Correlation coefficient (Scanning/Residence);
Means in a row with the same letter in a species are not significantly different (Glm test/SNK).
*, P<0.05; **, P<0.01; ns = not significant


FREQUENCY
0
10
20
30
40
50
60
Male
Female
Control
RA
RE
0-15
16-30
31-45
46-60
61-75
76-90
91-105
106-120
> 120
TIME RANGE (sec)
SCANNING RESPONSE
RESIDENCE TIME RESPONSE


FREQUENCY
FREQUENCY
Male
Female
Control
RA
RE
0-15
16-30
31-45
46-60
61-75
76-90
91-105
106-120
> 120
TIME RANGE (sec)
TIME RANGE (sec)
SCANNING RESPONSE
RESIDENCE TIME RESPONSE

5.3.2 Activation and arrestment responses of *R.appendiculatus* and *R. evertsi* to host odours

5.3.2.1 Activation and arrestment in *situ*

The latent times for *R. appendiculatus* and *R. evertsi* to get into motion in response to host odours near the feeding site are summarized in Tables 9 and 10. Also presented is the relation between tick's speed and its distance to the feeding site as a measure of the degree of arrestment. Irrespective of the distance away from the feeding site, *R. appendiculatus* activation occurred with varying periods of latency. This was found to decrease with decreased distance to the feeding site ($F = 4.26$; $df=6$; $P = 0.006$). Likewise, tick speed decreased in the vicinity of the ear feeding site and this arrestment correlated with distance only at close distance (< 15 cm) (Table 9). At distances > 15 cm, no correlation was found between the two parameters. In *R. evertsi*, the results showed similar trends as regards latency and velocity in relation to distance ($F = 3.58$; $df = 6$; $P=0.0025$). Except at distances > 15 cm, an arrestment pattern correlating with the feeding site closeness was also evident (Table 10). However, comparison between the two species showed that velocity but not latency varied between the two.

Table 9 Activation and arrestment responses in situ of *R. appendiculatus* released in the vicinity of ear odour source

Activation				
Distance from FS	Latency to activation (sec)	Velocity (cm/min)	R	Prob. (LSD Test)
0 cm	14.8 ± 1.7a	— [1]	—	—
5 cm	22.7 ± 2.9ab	48.4 ± 1.9c	0.69	P<0.05
15 cm	25.9 ± 2.8b	55.9 ± 1.3bc	0.65	P<0.05
25 cm	28.6 ± 7.1ab	60.7 ± 2.1b	0.34	ns
35 cm	28.5 ± 3.7ab	61.5 ± 2.3b	0.28	ns
> 35 cm	38.1 ± 5.1a	151.4 ± 10.a	0.20	ns

FS = Feeding site (= ear);
Detransformed mean ± SE;
[1] not applicable
R = Correlation coefficient latency/distance
N = 24 for each point (pooled data for ♂ and ♀);

Table 10 Activation and arrestment responses in situ of *R. evertsi* released in the vicinity of anal odour source

Activation				
Distance from FS	Latency to activation (sec)	Velocity (cm/min)	R	Prob. (LSD Test)
0 cm	19.3 ± 4.1a	— [1]	—	—
5 cm	20.7 ± 5.2a	41.9 ± 1.9c	0.71	P<0.05
15 cm	24.6 ± 5.4a	48.6 ± 0.9b	0.66	P<0.05
25 cm	37.8 ± 5.2a	65.5 ± 2.0b	0.59	ns
35 cm	35.5 ± 7.9a	43.3 ± 2.4c	0.43	ns
> 35 cm	45.2 ± 10.1 a	114.6 ± 5.5a	0.31	ns

FS = Feeding site (= anal area);
Detransformed mean ± SE;
[1] not applicable
R = Correlation coefficient latency/distance
N = 24 for each point (pooled data for ♂ and ♀);

5.3.2.2 Walking arrestment of *R. appendiculatus* and *R. evertsi* on an extract-impregnated artificial substratum (Whatman filter paper)

The ear-extract impregnated arena caused *R. appendiculatus* to walk more within it than the hexane-treated arena. This treatment also caused significant increase of the mean residence time of *R. appendiculatus* traversing the odourous area ($P < 0.01$). Indeed, test ticks resided longer in experimental arena moving therein compared to the control arena (28.1 ± 3.0 sec vs 14.7 ± 1.5 sec.), which represents a 191% increase of residence time in the treated over control arena (Table 11). Moreover, klinokinetic tracks (by both ♂ and ♀) were evident on ear-odourized Whatman filter paper substrate as opposed to more or less orthokinetic tracks away from the unodourized arena in the control experiment (Fig. 22). In some cases, repeated return to odour-impregnated areas was observed. In *R. evertsi*, walking was similarly arrested ($P < 0.01$) on the anal extract-impregnated arena compared to solvent-treated arena (avg 23.1 ± 2.7 sec vs 11.6 ± 1.9 sec), which represents a 199% increase of residence time in treated over the control arena. Pronounced klinokinetic tracks were also noted on extract-treated arena (Table 12; Fig. 23).

Table 11 Time response for walking of *R. appendiculatus* arrested on Whatman filter paper substrate treated with ear extract

Substrate (Whatman FP)	Residence time (sec)		LSD test
	Experimental	Control	
Inner circle (odourized)	28.1 ± 3.0	14.7 ± 1.5	**
Outer circle (Unodourized)	38.8 ± 4.0	22.8 ± 2.1	**

N= 12 (pooled data for ♂ and ♀)
*, P<0.05; **, P<0.01; ns = not significant
FP= Filter paper

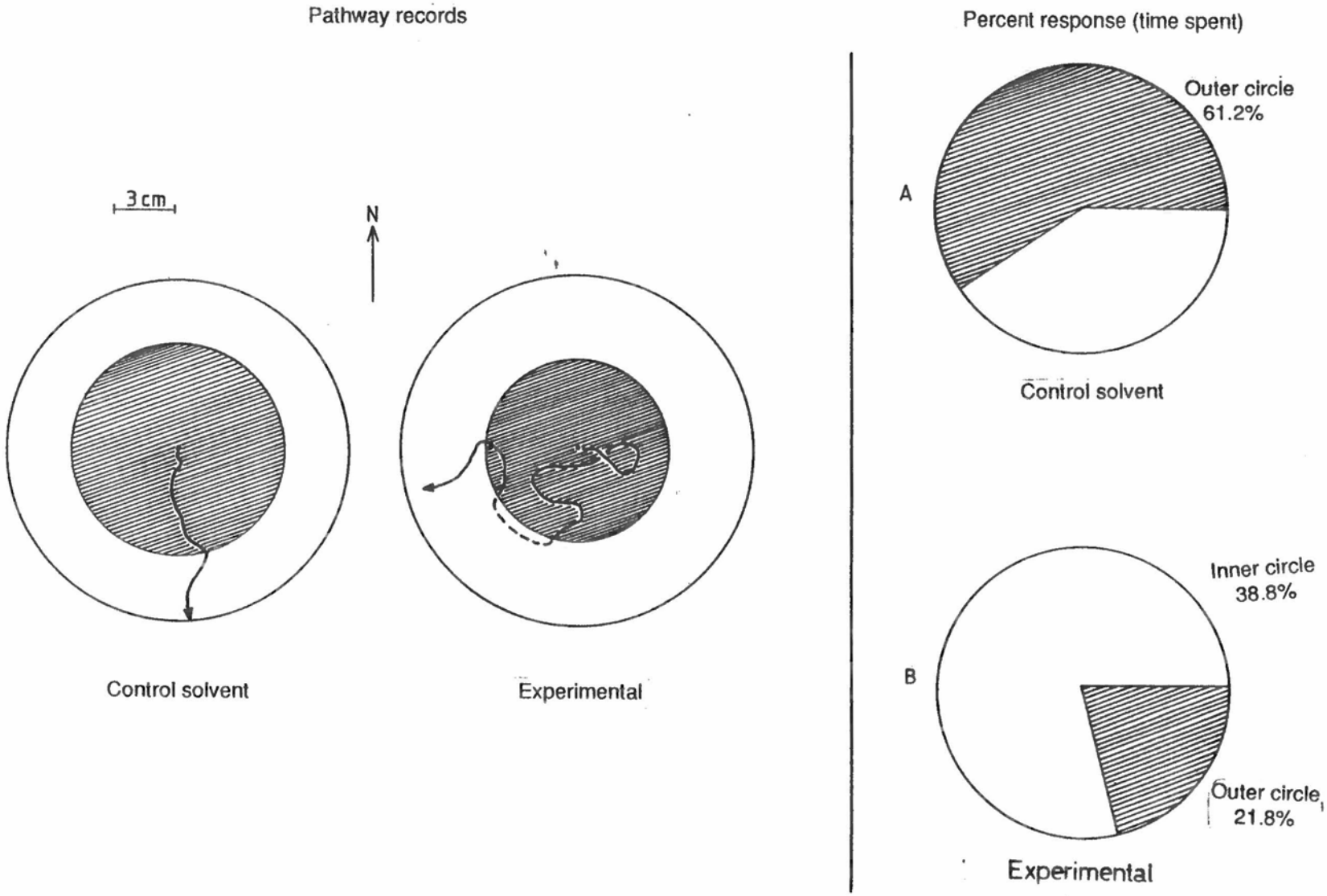


Fig. 22: Walking arrestment of R. appendiculatus on ear-extract odourized Whatman paper substrate: pathways and time responses

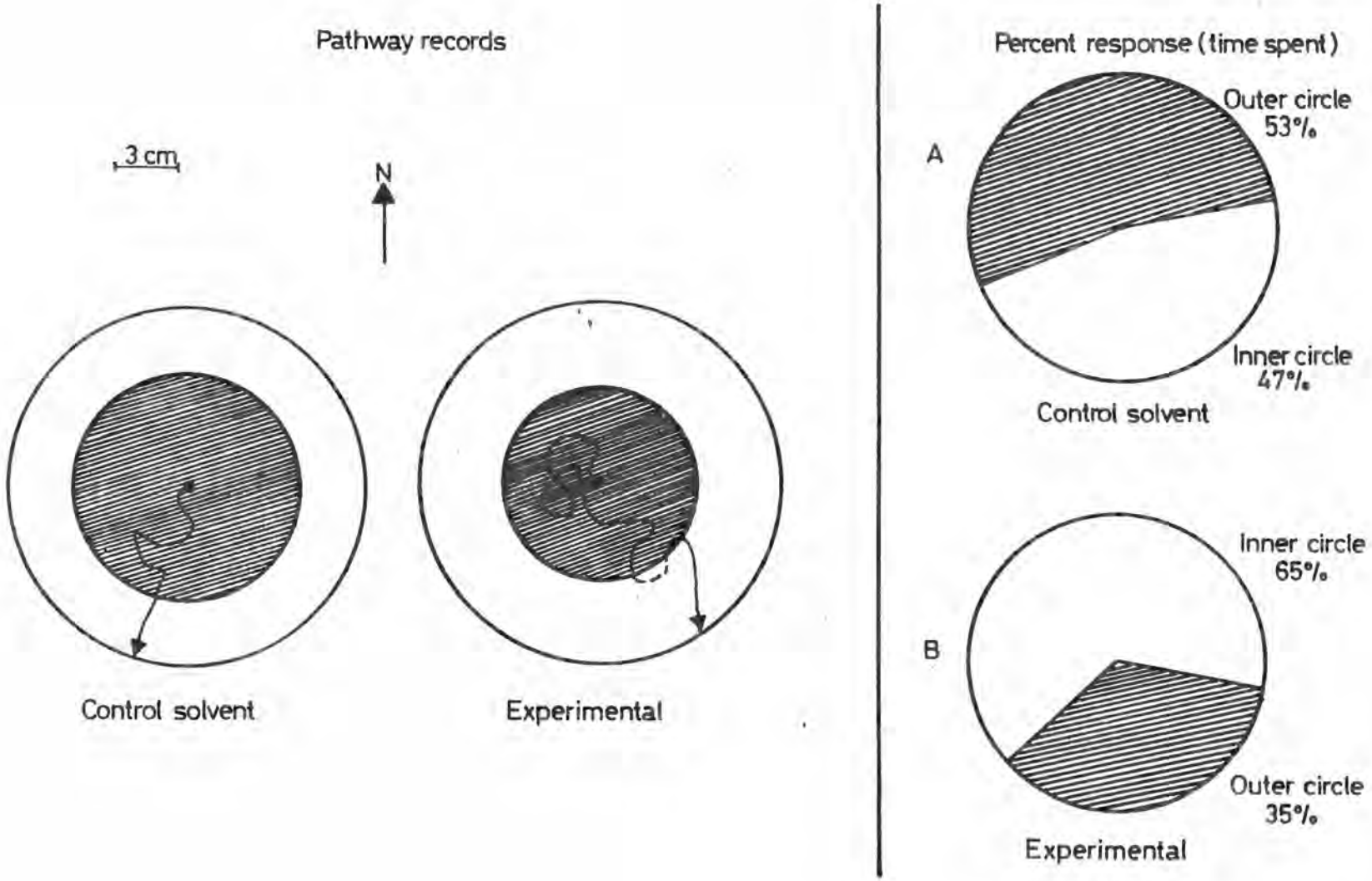


Fig. 23. Walking arrestment of R. evertsi on anal-extract odourized Whatman paper substrate: pathways and time responses

5.3.3 Aggregation response of *Rhipicephalus appendiculatus* on its natural bovine host

Rinses from fed males elicited significant aggregation responses of *R. appendiculatus* on the calf scrotum, with characteristic clusters of large numbers of ticks (Plate 11). On solvent-treated area of scrotum, ticks were found to display a scattered distribution pattern (Plate 12). Aggregation of male and female *R. appendiculatus* on the treated spot was significantly higher than on the untreated one ($P<0.05$). Results indicated that extracts of female ticks caused somewhat greater response than male extracts, but the difference was not significant (Table 13). No significant difference in tick attachment rate on the scrotum was observed between the experimental groups and their corresponding controls.

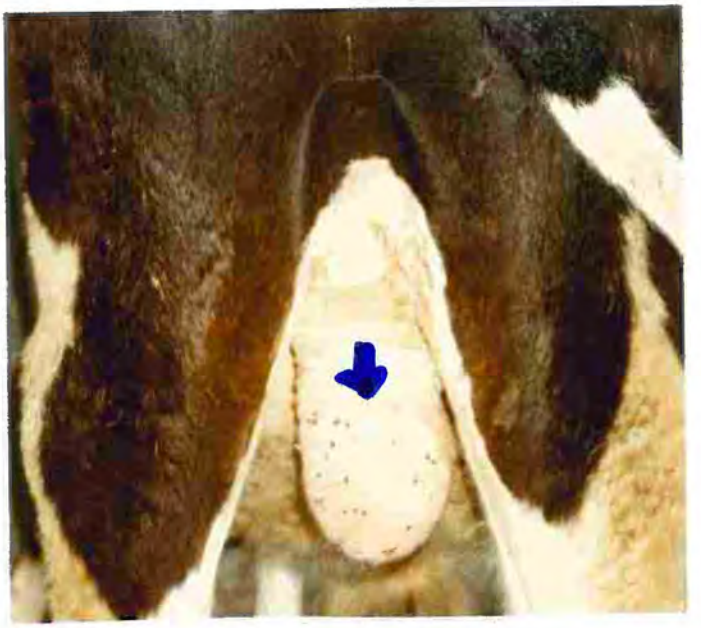


Plate 11 *R. appendiculatus* ticks scattered on calf scrotum treated with solvent (control)(Upper)

Plate 12 Aggregation response pattern of *R. appendiculatus* ticks on scrotum treated with tick extracts (Lower)

Table 13 Clustering response of *R. appendiculatus* on the calf scrotum smeared with tick extract

	responses		
	Attached (%)	On the spot	
		T	C
Male rinses			
25 ME	87.5	27a	13b
50 ME	91.0	34a	11b
Female rinses			
25 FE	94.5	29a	9b
50 FE	89.1	37a	12b

ME = Male-equivalent; FE = Female-equivalent;
N= 150 (3 replicates).
Means within a row with the same alphabet are not significantly different

5.4 Discussion

5.4.1 Arousal to extracts from preferred and unpreferred feeding sites

Studies were conducted to determine the arousal responses of *R. appendiculatus* and *R. evertsi* when exposed to odour extracts from preferred and unpreferred feeding sites. Following the tick's first encounter with the odour extract, a most perceptible reaction observed was the display of a set of behavioural features characteristic of scanning activity. Ticks in this phase could be seen waving the first pair of tarsi. This posture is used by ticks to probe and get the information about environmental parameters as insects do using their antennae (Lees, 1948; Bell *et al.*, 1983). In our study, the relatively short time range within which most of the scanning activity was shown to fall (from 0 to 30 sec.), indicates a well-defined pattern of response to the different extracts in both tick species. On the other hand, as regards to tick's residence time, a clear pattern emerged according to the stimulatory or inhibitory nature of the test extract. The permeation of the olfactometer arm by a favourable odour extract caused a longer residence time in the relevant tick species; in contrast, an unpreferred odour extract caused a shortened residence time as shown by the frequency distribution of these responses (Figs 20 and 21). In the latter case, ticks were reluctant to move to such unfavourable sites. This is consistent with the need for the tick to settle in its favourable environment and to move away from an

unfavourable one. This dual but opposite effect would facilitate settlement at a preferred site. This then would be followed by probing of the site and its eventually selection for feeding (Waladde, 1987). It remains to be shown whether the tick migrates to its preferred feeding site (hence avoiding unsuitable ones) through an evolutionary selection process because of the suitability of the site or only in response to prevailing local conditions of stimulation.

5.4.2 **Activation and arrestment *in situ* of *R. appendiculatus* and *R. evertsi*; walking arrestment on artificial substrate**

Activation is a common behaviour among ticks. Indeed, tick search activity prompts it to explore the surroundings in search of a resource e.g. a mate, an oviposition site, a shelter, etc. (Bell, 1983). Our results have shown that the host odours (either from the ear or anal area) induced swift activation responses from individuals released at relatively close distances from the feeding site. Various stimuli are known to activate ticks, including CO_2 which is part of host odours (Norval *et al.*, 1989b). Before ticks get activated, a pre-activation period was observed. This latent period may be associated with environmental scanning by the tick (Bell *et al.*, 1983). The similarity in latency response patterns between *R. evertsi* and *R. appendiculatus* is remarkable and suggests parallel adaptations of the two species to favourable semiochemicals from their respective feeding sites. Correlated

with this stimulation is the arrestment behaviour when the tick gets closer to its target site. Results of the on-host experiments showed that tick's walking speed decreased as the distance to the feeding site decreased, and indeed, close to the odour source, the velocity correlated with the distance, suggesting an arrestment response near the source. This is in agreement with reports on insects where the movement of the animal becomes inversely proportional to the pheromone concentration, resulting eventually in cessation of locomotion (Shorey, 1976). The cessation of the ambulatory movement of *R. appendiculatus* and *R. evertsi* at or close to the ear or anal site also may be a concentration effect of odour attractants, although the mediation of close range arrestant components cannot be ruled out.

In the studies on artificial substrates using extracts from feeding sites (ear and anal), both *R. appendiculatus* and *R. evertsi* spent more time in areas impregnated with the relevant extract. In fact, in some instances, ticks returned to the impregnated area. Waladde *et al.* (1991), using artificial feeding membrane treated with ingredients from the feeding site among others, noted a similar arrestment behaviour by *R. appendiculatus* on their feeding device. This arrestant role of feeding site stimuli is a way of allowing the tick to settle down once the site is found.

5.4.3 **Aggregation response**

The aggregation response elicited in *R. appendiculatus* on natural host using tick odour cues confirmed the existence of an intraspecific aggregating agent released by this tick. Clusters of individuals were found assembled in the area of calf scrotum treated with tick rinses compared to weak or no aggregation in controls. The aggregating cue attracted both males and females, fulfilling the criterion of an aggregating agent. Although relatively weak, the aggregation response pattern evoked in *R. appendiculatus* was similar to that reported in most *Amblyomma* species (Gladney *et al.*, 1974a; Rechav *et al.*, 1976,1977; Norval *et al.*, 1989a). While in *Amblyomma* spp, aggregation is mainly induced by male extracts, results of our study implicates both sexes as emitters of the aggregating cues, with females inducing a response greater or equal to that of the male. The difference in aggregation response pattern of *R. appendiculatus* from *Amblyomma* spp may be explained by the feeding site location behaviour of the species. Males of most tick species like *R. appendiculatus* do not attach initially to the feeding site whereas females move straight to the ear (Gladney and Drummond, 1970). As a result, settled females would then attract other conspecifics and the resulting stimulus package then will be complemented/amplified by male-released emissions.

In summary, these results implicate the mediation of two distinct sets of signals used by the two tick species under study to navigate to their

respective sites: an interspecific signal from the feeding site and an intraspecific signal from successfully feeding conspecifics.

CHAPTER SIX

6.0 ROLE OF HOST KAIROMONES: STIMULI RESPONSES UNDER LABORATORY CONDITIONS

6.1 Introduction

Previous bioassays and observational studies implicated but did not give adequate insight on the interplay of different components of host- and tick-derived semiochemicals on the process of host and feeding site location, as well as on mate finding by tick species under study. Potential semiochemical signals comprise host-derived odours (e.g. sweat, effluvia, breath chemicals including CO_2) and tick-derived odours (volatile emissions from feeding individuals) to which unfed ticks may be responsive. Analyses of emanations of bovine, a natural host to both *R. appendiculatus* and *R. evertsi* ticks, showed numerous organic substances among a range of phenolic compounds (Warnes, 1990), some of which could act as signals to the tick. Olfactometric experiments indicated that hexane washings from different parts of the host body elicited varying types of responses to *R. appendiculatus* (Akinyi, 1991). However, the extent to which host chemistry determines host selection and feeding site specificity is largely unknown (Sonenshine, 1985; Waladde, 1987). Indeed, to date no specific attractants have been reported from the feeding site

on host where a different batch of olfactory stimuli may operate to assist the tick to find its feeding site or mate (Sonenshine *et al.*, 1986). Is feeding at these localized sites due to unspecified olfactory attractants released from these areas? Adults of *R. evertsi* and *R. appendiculatus* ticks are known to be very selective as regard to their feeding sites. Surprisingly, from bioassay results, mixtures of ear extracts and 2,6-DCP, both individually attractive, were found to be repulsive to *R. appendiculatus* adults (Akinyi, 1991).

All the above olfactometric assays dealt with solvent washes of bovine parts probably made up largely of non-volatile or less volatile residues on the skin, which would provide contact or short-range signals. What is the part played in tick orientation by air-borne volatiles emitted by the feeding site and those from feeding individuals which may constitute important volatile semiochemical signals?

In the present study, behavioural assays were undertaken to identify these signals, particularly host kairomones. The stimulus response patterns of *R. appendiculatus* and *R. evertsi* to host odours from various host body regions, including those from their respective feeding sites and cross-responses to each other's feeding site odours were investigated. Responses to air-borne volatiles and contact stimuli as well as to their blends were also studied.

6.2 Materials and methods

6.2.1 Preliminary studies on tick responses to host stimuli

6.2.1.1 Preliminary comparison of responses of adult and immature instars of *R. appendiculatus* to hexane extracts

Ear wash extracts (2.5 mg/filter paper) were assayed in a Y-olfactometer against immature stages of *R. appendiculatus* for their attractiveness. A batch of approximately 50 nymphs each was introduced into the inlet tube. About 100 larvae were similarly tested using the same device and procedure. Assays using the nymphs were replicated 5 times and those for the larvae 3 times. The adult stage assayed in the T-olfactometer, consisted of 8 replicates using 12 individuals each. Responses of the two immature instars were meant to parallel those of adult ticks with the same dose of ear extract. Responses relative to their respective controls in both bioassays were compared using T-Student test.

6.2.1.2 Responses of *R. appendiculatus* adults to ear extracts using hexane, methanol and acetone solvents

Attractiveness of unfed *R. appendiculatus* adults to ear extracts of different

polarity using 3 different organic solvents was investigated. The objective was to see if the different blends extracted into the hexane, acetone and methanol solvents elicited different types or levels of responses. The extraction, as previously described in section 3.4.1.1 was carried out separately for each solvent. Extracts at doses ranging from 0.1 μg to 10 mg (with 10 fold incremental increase for intermediate doses) per Whatman filter paper No 1 measuring 2 cm in diameter, were tested in the T-olfactometer. Responses of 8 replicates using 12 ticks of each sex for each dose were analyzed using ANOVA and Student-Newman-Keuls (SNK) tests on arcsin transformed data.

6.2.2 Detailed responses of *R. appendiculatus* and *R. evertsi* adults to host odours stimuli

6.2.2.1 Responses of *R. appendiculatus* to odours from unpreferred feeding sites

Crude extracts of odours from selected host body regions other than that of the feeding site, namely belly/axillae, neck/dewlap and legs, were tested separately on unfed *R. appendiculatus* adults in T- olfactometer. 2.5 mg of extract was used in each test. Tests and replications used were as described under section 6.2.1.1.

6.2.2.2 **Responses of *R. appendiculatus* and *R. evertsi* to stimuli from their respective and each other's feeding sites**

In order to determine how differences in *R. evertsi* and *R. appendiculatus* orientation to their respective feeding sites might be related to differences on the effect of stimuli from these sources, 1.0 mg of wash extracts from the two areas were tested and cross-tested against each tick species in a T-olfactometer. Batches of 12 ticks replicated 8 times for each sex and species were used.

6.2.2.3 **Dose responses to feeding site extracts**

These assays were performed for each species as under section 6.2.2.2, but using a climbing pair column olfactometer, with 10-fold incremental increase in dose ranging from 0.1 μg to 10 mg. Four batches of 40 ticks of mixed sexes were released on the aluminum base of the olfactometer. The number of ticks climbing up the treated and untreated columns was noted and the response to stimulus compared and analyzed by ANOVA and SNK tests on square root transformed data.

6.2.2.4 **Responses of *R. appendiculatus* to contact and air-borne volatile kairomones**

N-hexane concentrates of extracts (1.0 mg) from ear and trapped air-borne volatiles from the ear and collected as specified in section 3.4.1.2, were tested separately (in T-olfactometer) and then, the blend of the two (ratio 1:1, v/v) extracts to see if there was any additive or synergistic effect. Attractancy of each test material was compared with their controls. Tests and replications for the individual extracts were as with in section 6.2.1.1, but attractancy between the three was analyzed using ANOVA and SNK tests.

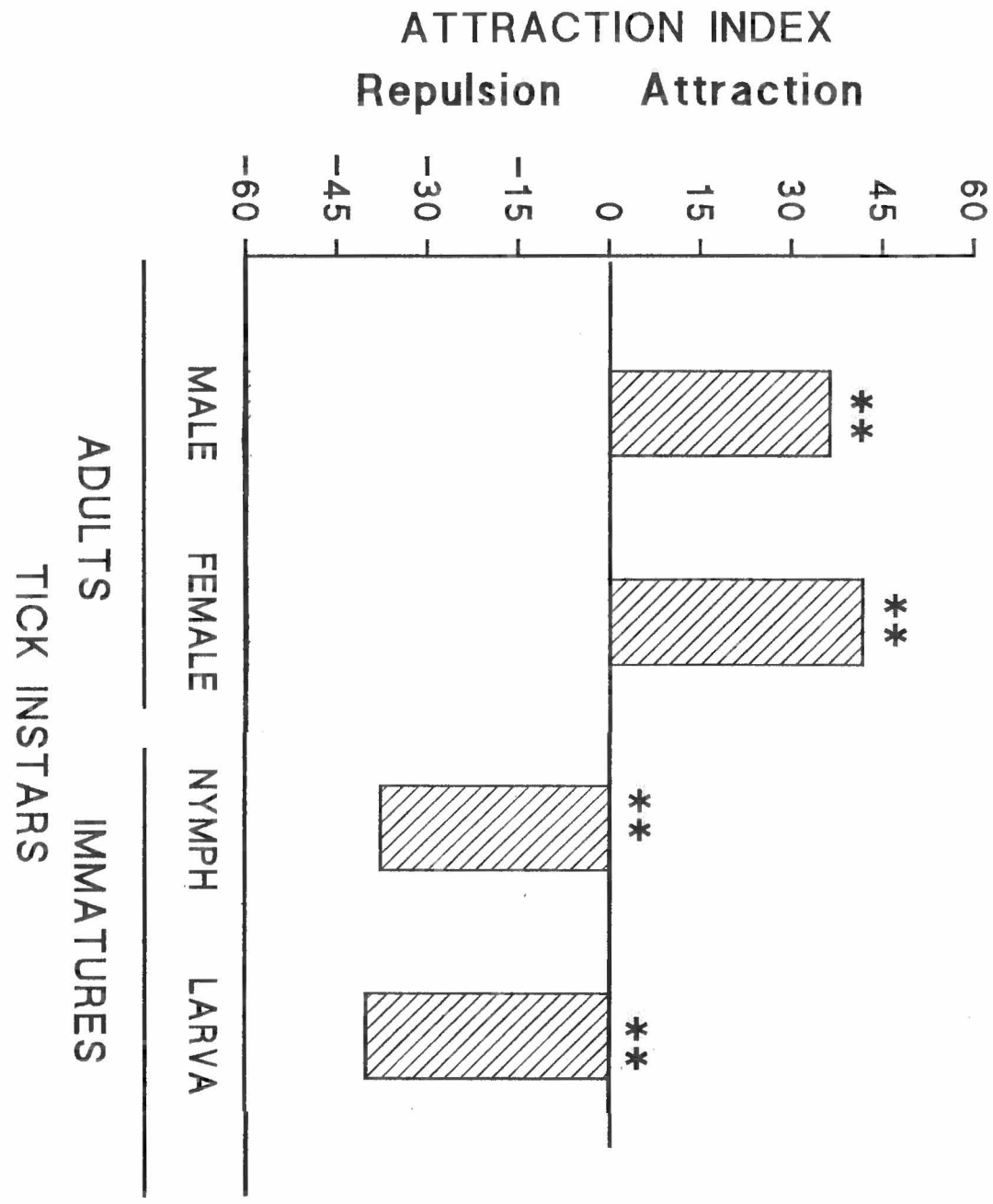
6.3 **RESULTS**

6.3.1 **Responsiveness of adult and immature instars of *R. appendiculatus* to the odour in ear wash extract**

Figure 24 summarizes the responses of unfed larvae and nymphs tested in Y-tube olfactometer to ear washes. These were opposed to those of adults in the T-tube. Crude ear extracts (2.5mg) evoked significant repellent effect to the immature stages ($P < 0.01$) with only 42.5% nymphs and 39.2% larvae moving up to the extract-

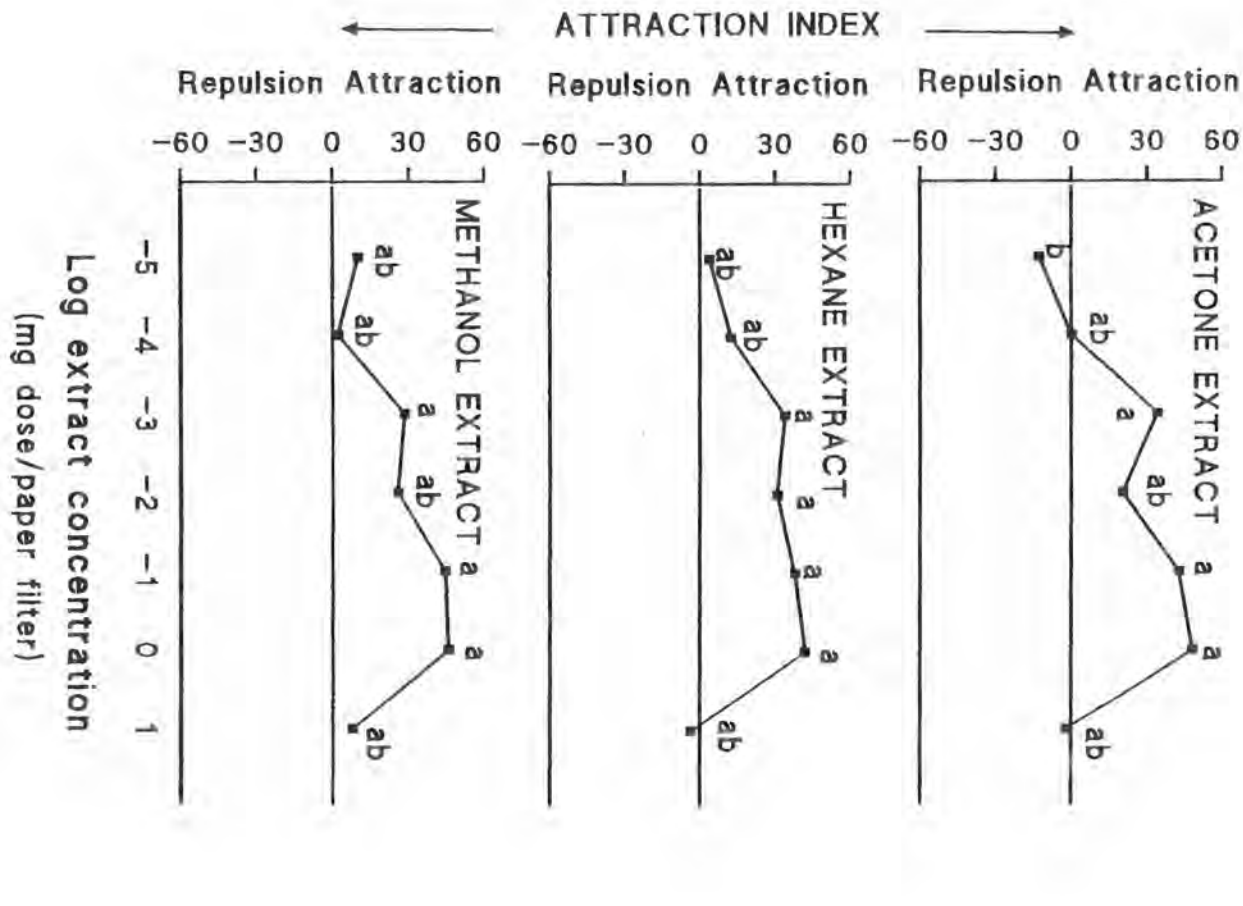
treated olfactometer arm compared to 57.5% and 60.8% to the respective controls (Attraction Index, I_A = -19.8 and -22.2 respectively). Conversely, ear extracts proved significantly attractive ($P < 0.001$) to the adult stage (70.9% for males and 69.5% for females)(I_A = 41.8 and 36.4 respectively).

In view of the above reactions of the instars to host odour cues, only adult ticks were used in all subsequent experiments. Henceforth, only unfed adult ticks are referred to in this work.


ATTRACTION INDEX
Repulsion
Attraction
-60
-45
-30
-15
0
15
30
45
60
MALE
FEMALE
NYMPH
LARVA
**
**
**
**
ADULTS
IMMATURES
TICK INSTARS

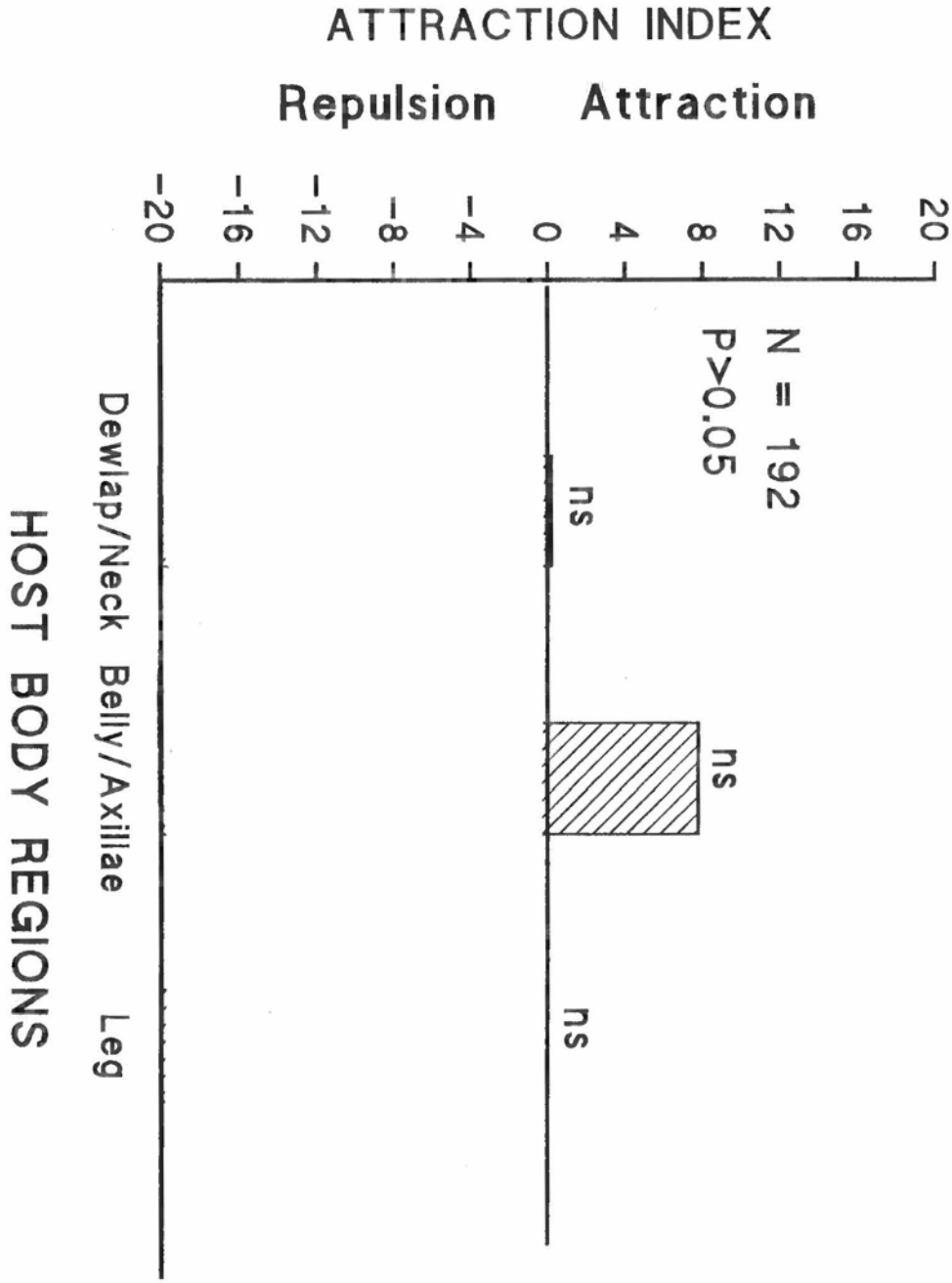
6.3.2 Relative attractiveness of 3 different solvent-extracted ear stimuli to *R. appendiculatus*

Figure 25 presents the results of bioassays performed with ear washes to determine which of the 3 organic solvents of different polarity was most effective in extracting active compounds. At most doses tested, acetone, hexane and methanolic extracts of ear evoked significant attraction to *R. appendiculatus* as compared to their respective controls (with the exception of 2 negative I_A for acetone extract, all $I_A > 0$). Dose-attractancy patterns varied little between the 3 types of washes within the concentration range tested (ANOVA, $F = 0.19$; $df = 2$; $P = 0.987$). Analysis of the data (ANOVA) suggested a parabolic relationship, that is at lower doses (0.1 μg and 1 μg) and at the highest dose (10 mg) used, weak or declining responses occurred for all the three extracts. As a result of these observations, hexane was used in all subsequent experiments because of its ease of evaporation.


ATTRACTION INDEX
Repulsion Attraction
−60 −30 0 30 60
ACETONE EXTRACT
HEXANE EXTRACT
METHANOL EXTRACT
Log extract concentration
(mg dose/paper filter)
−5 −4 −3 −2 −1 0 1

6.3.3 Responsiveness of *R. appendiculatus* adults to host odour extracts from selected body regions (non-feeding sites)

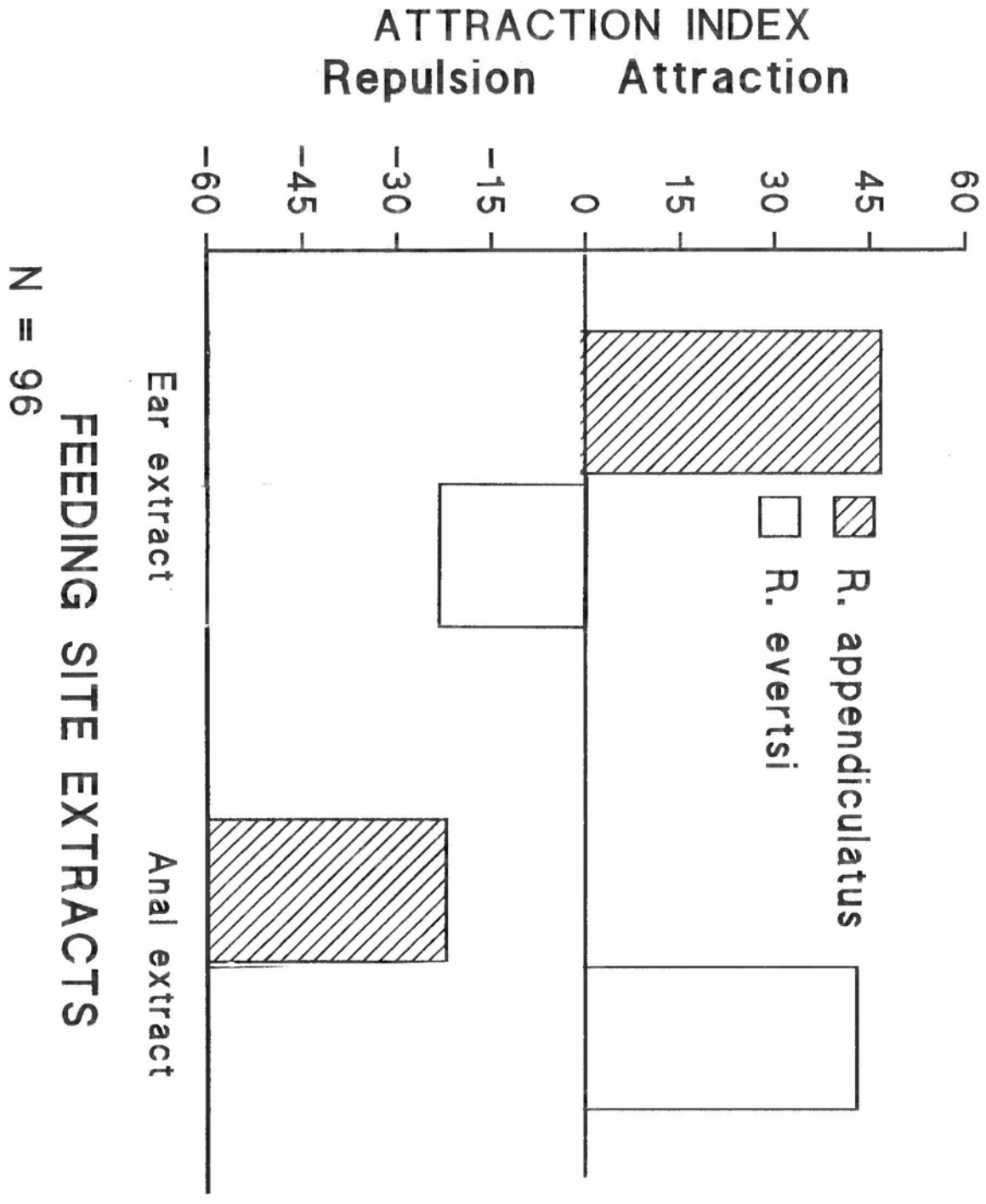
Odour extracts from body parts (ie belly/axillae, neck/dewlap and legs) of cattle other than those of its natural feeding site (ie ear) failed to evoke any significant attraction to *R. appendiculatus* in a T-tube olfactometer (Fig. 26). Attraction index of the washings from dewlap/neck region (I_A = 0.2) showed that this was unattractive (~ 50% moved up both treated and control arms; P= 0.51). Odour extracts from belly/axillae region and that of leg suggested that these might be marginally attractive (I_A = 7.8; 53,9%) and repulsive (I_A = -9.6; 45,2%) respectively.


ATTRACTION INDEX
Repulsion
Attraction
20
16
12
8
4
0
−4
−8
−12
−16
−20
N = 192
P>0.05
ns
ns
ns
Dewlap/Neck
Belly/Axillae
Leg
HOST BODY REGIONS

6.3.4 Responsiveness of *R. appendiculatus* and *R. evertsi* to odour extracts from their respective and each other's feeding sites

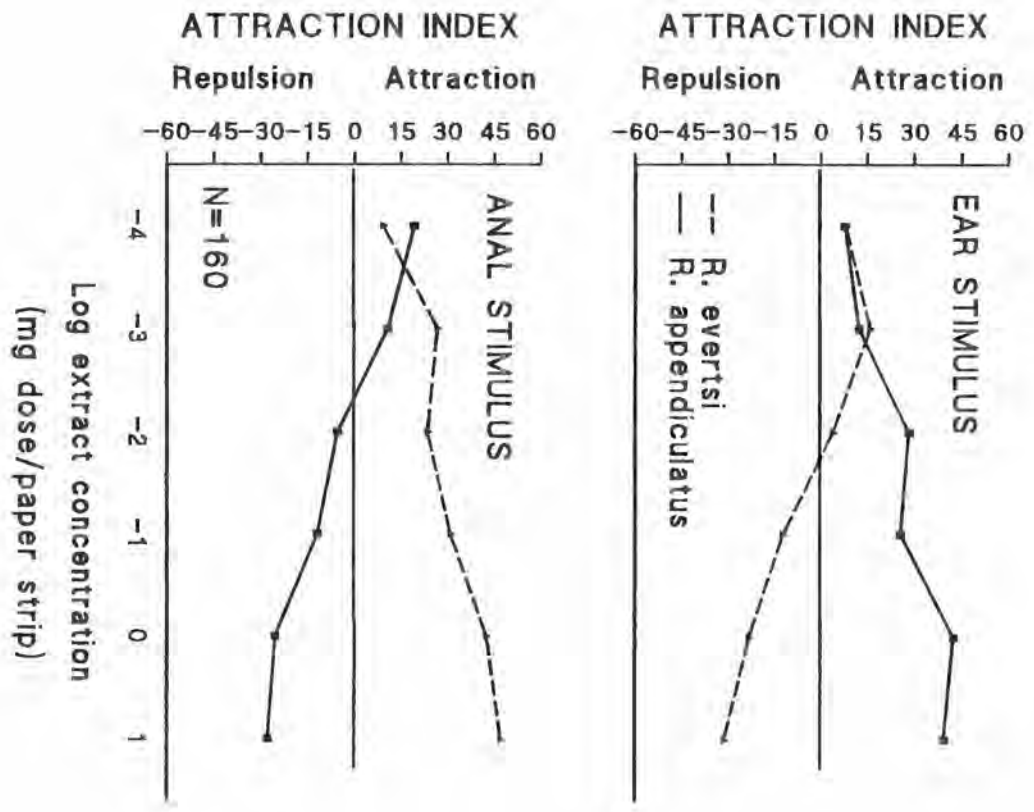
The responses of *R. appendiculatus* and *R. evertsi* adults to 2.5 mg of extracts from their natural feeding sites in a T-olfactometer showed positive attraction indices (Fig. 27). Also presented are cross-assays to 2.5 mg of extracts from the feeding site of the other tick. *R. appendiculatus* exhibited a highly significant preference ($P < 0.001$) for the ear odour extracts ($I_A = 46.8$), as ticks chose in greater numbers (73.4%) the ear extract-treated arm of the olfactometer than the hexane-treated arm. Similarly, a highly significant preference ($P < 0.001$) was also observed on *R. evertsi* with respect to the anal extract as 69.9% individuals moved to the treated arm of the olfactometer compared to the control ($I_A = 43.0$).

However, cross-assays with these extracts led to a different response pattern. Anal extract failed to evoke attraction to *R. appendiculatus* ($P < 0.001$), 61.1% individuals preferring to move to the untreated olfactometer arm ($I_A = -22.2$). Similarly, the ear extract had a repulsive effect on *R. evertsi* ($P < 0.001$), 63.6% of them preferring to move to the untreated arm ($I_A = -36.8$)(Fig. 27).


ATTRACTION INDEX
Repulsion Attraction
-60
-45
-30
-15
0
15
30
45
60
R. appendiculatus
R. evertsi
Ear extract
Anal extract
FEEDING SITE EXTRACTS
N = 96

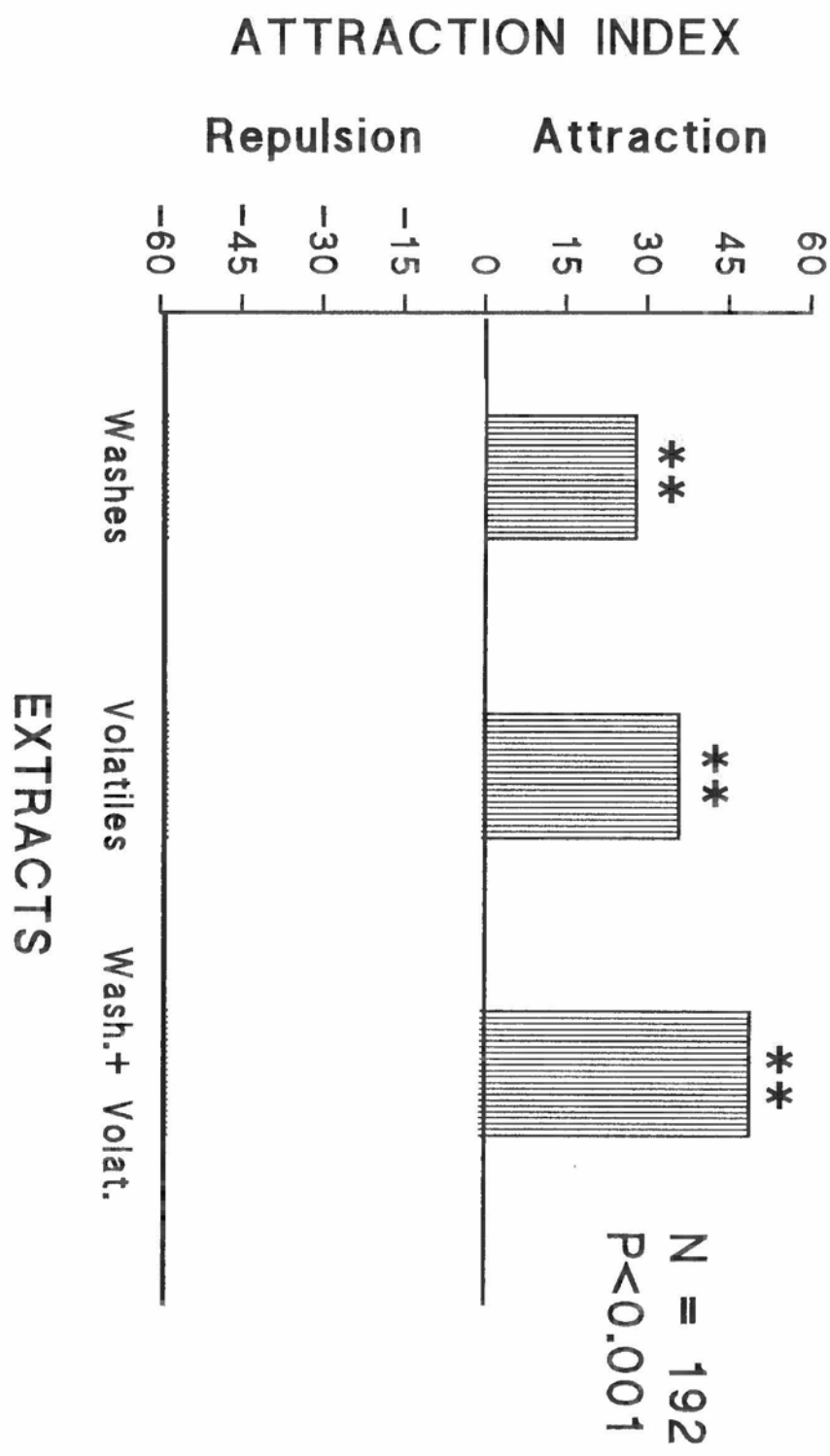
6.3.5 Dose responses of *Rhipicephalus appendiculatus* and *R. evertsi* to odour extracts from their respective and each other's feeding sites

When the two tick species were tested with extracts from their own and each other's feeding sites in a paired column olfactometer, all doses of ear extracts proved to be stimulatory for *R.appendiculatus* (I_A ranging from 7.8 to 42.6) as opposed to the inhibiting effect produced by most doses of anal extracts in the same species (positive I_A from 16.0 to 4.0, then negative I_A from -12 to -31). Increasing doses resulted in slight increase of the respective attractivity or repellancy of the extract (Fig. 28). For *R. evertsi*, it was similar but opposite, with the anal extract being attractive at all doses tested where no significant effect was observed (I_A from 9.2 to 47.2) and the ear extract repulsive, except at lower doses (positive I_A from 27.4 to 1.6, then negative I_A from -7.0 to -18.0). As with *R. appendiculatus*, *R. evertsi*'s responses to either extracts were dose-dependent (Fig. 28).


ATTRACTION INDEX
Repulsion
Attraction
-60 -45 -30 -15 0 15 30 45 60
ANAL STIMULUS
N=160
ATTRACTION INDEX
Repulsion
Attraction
-60 -45 -30 -15 0 15 30 45 60
EAR STIMULUS
-- R. evertsi
— R. appendiculatus
-4 -3 -2 -1 0 1
Log extract concentration
(mg dose/paper strip)

6.3.6 Responses of *Rhipicephalus appendiculatus* to contact and air-borne volatiles from the ear site

Figure 29 presents the responses of *R. appendiculatus* to contact and trapped air-borne volatiles from ear. Attraction indices of each extract and the blend (ratio 1:1, v/v) of the two (I_A = 28.0 and 36.0 for the extracts and volatiles respectively and 49.0 for the blend) showed that all were attractive relative to their controls ($P < 0.001$). The blend of the two evoked the stronger attraction over the individual compounds, although the difference was not statistically significant.


ATTRACTION INDEX
Repulsion
Attraction
-60
-45
-30
-15
0
15
30
45
60
**
**
**
Washes
Volatiles
Wash.+ Volat.
EXTRACTS
N = 192
P<0.001

6.4. **Discussion**

The mediation role of olfactory stimuli present in the bovine odour in the feeding site location behaviour of the two ticks was investigated. The study provides evidence that ear and anal odours are effective in attracting *R. appendiculatus* and *R. evertsi* respectively. The attractivity exhibited by the two extracts towards the two tick species corroborates their site preference behaviour under natural conditions (Elbl and Anastos, 1966; Yeoman and Walker, 1967; Kaiser *et al.*, 1982). The two species are known to infest the same host and, as implied in this study, the difference of reactions to site extracts suggests that their site preference is species specific. While the chemical basis of this specificity is still to be investigated, evidence provided by the bioassays reported here showed that ticks can narrow their preference to a particular site odour from a broad range of odour background such as those of different body regions of the natural host. Indeed, odours from other body parts such as belly/axillae, neck/dewlap and leg were not significantly attractive to *R. appendiculatus*. On the other hand, signals from predilection sites would appear to be strong, acting as potent kairomones for the relevant tick species.

The results presented here support those reported by Akinyi (1991) and show that the unattractiveness or weak attraction of these host signals to immatures indicate that the signal may be stage specific. The difference between stage responses may be a reflection of different degree of specialization and stage-host relationships, as

evidenced by the fact that *R. appendiculatus* immatures have many alternative hosts including small mammals (Elbl and Anastos, 1966). The implication of this is that the immature stages may rely on less specific host molecules. Alternatively, their olfactory sensitivity to the same site stimulus may be different.

The strong attraction evoked by hexane soluble stimuli and trapped air-borne volatiles suggest the mediation of a range of kairomonal components. Studies to elucidate the nature and structural features of kairomones from both the ear and anal sources are underway at ICIPE. Whether these include one or more of those previously reported to play a role in host recognition by ticks such as phenols, butyric acid, CO_2, ammonia (Wood *et al.*, 1975; Haggart and Davis, 1980, 1981; Waladde and Rice, 1982) remains to be seen. The additive interactions between solvent washings and air-borne volatile collection in the present study suggests that, at least in *R. appendiculatus* where it has been investigated, these kairomones may be made up of components of different volatilities with different functions. It may be hypothesized that the air-borne volatile emissions may steer mid or long-range attraction while contact and less volatile compounds would play the role of close range attractants and arrestants. Such a complex kairomone mediation system of different volatility and functionality is common in many insect groups, for example tsetse, mosquitoes, blackflies (Sutcliffe, 1985; Takken, 1991).

Site orientation displayed by the two tick species may also be influenced by other non-olfactory factors. Various cues have been implicated in site selection process in

ticks including humidity, body temperature, acoustic and visual cues (Howell, 1975; Doube and Kemp, 1979; Waladde and Rice, 1982). However, none of these has been studied in any detail; thus, their importance, if any, needs to be elucidated.

Ticks themselves also may play a role in guiding other ticks of the same species to the suitable feeding site. Ortho-nitrophenol, an aggregation attraction pheromone emitted by attached and feeding *Amblyomma* ticks, was reported to be a stimulus utilized by these ticks to orient towards already settled conspecifics (Schoni *et al.*, 1984; Diehl *et al.*, 1991; Norval *et al.*,1991c). Emissions by feeding ticks of *R. appendiculatus* attached on host ear, which were shown to enhance attraction of conspecifics to this area (Chapter 4 of this work), appear to play a similar role.

Although the results of the present study implicate site-specific kairomones from predilection sites of *R. appendiculatus* and *R. evertsi*, tick responses to the odours collected from each other's feeding sites suggest an additional mechanistic component in host location. The adults of the two ticks are repelled by odours of each other's feeding site. This "push" effect would further assist the tick in orientating toward the appropriate part of the host body and explains the relative success in the orientation of the ticks to their feeding sites even from long distances. Thus, our study implicates a "push-pull" effect in guiding the ticks to their respective feeding sites and provides an insight in the underlying semiochemical mechanism for the species isolation on the host. The phenomenon is also corroborated by the findings on the reluctance of a species to reside longer in any unfavourable odour site environment (arousal

experiments, Chapter 5).

The existence of allomones in host materials and their potency suggest that these two species can be manipulated through their own host-derived semiochemicals. This possibility is addressed in the next Chapter.

CHAPTER SEVEN

7.0 DISRUPTION OF ORIENTATION BEHAVIOUR OF *R. APPENDICULATUS* AND *R. EVERTSI* ON HOST

7.1 Introduction

Odour extracts from parts of bovine host were shown to produce different effects on the tick species under study (chapter 6 of this study). The ear extract was found most attractive for *R. appendiculatus* and the anal extract for *R. evertsi*. On the other hand, the former extract was repellent to *R. evertsi* and the latter repelled *R. appendiculatus*. The possibility of interfering with tick orientation to feeding sites using host extracts from such unpreferred feeding sites has not been previously recognized.

In addition, a number of plants are known to be repellent to insects and other arthropods. Such is the case with *Ocimum suave*, a locally available shrub used widely in herbal medicine and in protection against biting insects (Kokwaro, 1976). In the field where this shrub occurs, ticks are scarcely found, although they may occur in numbers in other patches without the plant (pers. comm., 1979). Could essential oils derived from such plants similarly be used to confuse ticks orienting to feeding sites?.

To explore these possibilities, limited trials were carried out to study the effectiveness of the two groups of disruptants (of host and non-host origins) on *R. evertsi* and *R. appendiculatus*. These included:

- responses of *R. appendiculatus* and *R. evertsi* to feeding sites modified by washing these with a solvent;
- effects of treating the feeding site with extracts from unpreferred sites;
- orientation of *R. appendiculatus* and *R. evertsi* to their respective feeding sites treated with the essential oils of *O. suave*;
- allomonal persistence of crude host extracts and plant essential oils when applied on cow.

7.2 Materials and methods

7.2.1 Responses of *R. appendiculatus* and *R. evertsi* to feeding sites modified by solvent washing.

The ability of *R. appendiculatus* and *R. evertsi* to discriminate their natural feeding sites from sites whose condition was modified through a solvent washing treatment was investigated. Hexane was used as the solvent. If, as believed, host odours are made of assortment of air-borne compounds of different volatility prone to extraction by organic solvents (Sonenshine *et al.*, 1982b), solvent washing of the site would be

expected to create a partial or complete absence of active compounds on a temporary basis.

Depending on the tick species, site surface of the ear or anal region was washed 3 times with 300 ml of n-hexane. The solvent was allowed to evaporate for 5 minutes and batches of 20 males and 20 females were released. Responses of migrating ticks to ear/anal site were recorded 4 h later. Controls were made up of animals with unwashed ear/anal area. The experiment (Day 0) was replicated 4 times in both treated animals and the controls for each sex and species. Further observations were made at different times (on day 1, 2, 4, 6 and 8) after treatment, until stimulation level of these sites returned to normal. In each case, fresh batches of test ticks were released and their migration noted.

7.2.2 **Responses of *R. appendiculatus* and *R. evertsi* to normal feeding sites treated with extracts from each other's feeding sites.**

7.2.2.1 ***R. evertsi* orientation responses to anal area treated with the ear extract**

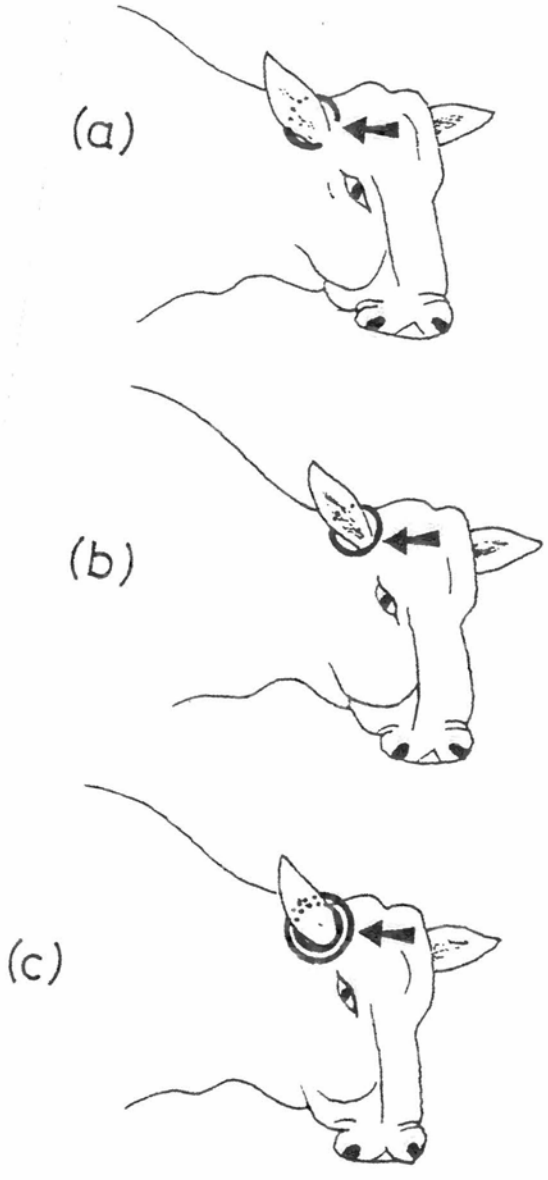
2 g of the ear extract in 1 ml of hexane was applied from a syringe around and 10 cm away from the anus to form a 'barrier' on the tick's orientation path. Untreated anal site (ie natural condition) served as the control. The number of ticks reaching the feeding site or located off-target site and dropping-off as a result of the repellent

effect of the extract was recorded and compared with that in the controls. The experiment was conducted for each sex using 3 replicates of 20 individuals each.

7.2.2.2 *R. appendiculatus* orientation responses to the ear treated with anal extract

This was conducted using 1 ml of solvent containing 2 g of the anal extract smeared around the ear base across *R. appendiculatus* path to the ear. This extract was subsequently replaced in all subsequent trials by 2 g of fresh faecal material (FM) which evoked equal disruption responses and which was easy to obtain. 3 batches of 20 ticks each for each sex were used. Parameters measured were as previously described (section 7.2.2.1, page 148).

So far, 2 ml of the suspensions of this FM was smeared near the ear base to set a repellent barrier across the orientation paths of *R. appendiculatus* and left to evaporate before the assays. Three types of barriers were used: single barrier (circle round the ear base), half barrier (semi-circular, leaving the upper half of the ear unprotected) and double barrier (two crown-circles around the ear base, 1 cm apart)(Fig. 30). The rest of the experiment was carried out as described for the anal extract. 3 replicates of 20 individuals were used in each test.


(a)
(b)
(c)

7.2.3 Responses of *R. appendiculatus* and *R. evertsi* to signals from feeding sites modified by the essential oil of *O. suave*

For both tick species, experiments similar to those in sections 7.2.2.1 and 7.2.2.2 were carried out to investigate the effects of *O. suave* essential oil on the ability of each of the two ticks to locate its feeding site. 5 μl of the essential oil of *O. suave* diluted in 1 ml of hexane and then mixed in 2 ml of liquid paraffin was dispensed with a syringe around the ear base (5cm away) or anus (10 cm away) across paths to these sites. The effect of the plant extract was monitored on the movement, orientation behaviour and spatial distribution of the ticks as described under the sections 7.2.1 and 7.2.2, pages 147-151. In both cases, feeding sites free of the essential oil smeared with a similar amount of paraffin served as controls.

7.2.4 **Allomonal persistence of host material and *O. suave* plant essential oil**

As an extension to the above tests (disruptive effects of both host and plant allomones), the persistence of fecal material (FM) for *R. appendiculatus*, that of ear extract for *R. evertsi* as well as that of *O. suave* essential oils for both tick species was investigated. The activity period of each host extract applied was evaluated on *R. appendiculatus* over a period of 3 days while the orientation responses of *R. appendiculatus* to the ear and that of *R. evertsi* to the anal area treated with the essential oil of *O. suave* was evaluated at 2 day intervals over a period of 14 days. Persistence of an extract was calculated by comparing its relative repellency on a specific day with a maximum theoretical repellency (MTR), set at 100%. Altogether four batches of 20 ticks of each sex were used in each assay. In addition, in order to check the presence of known repellent compounds in the plant essential oil, analyses of gas chromatograms of the oil were undertaken.

7.3 RESULTS

7.3.1 Responses of *R. appendiculatus* and *R. evertsi* to the respective feeding site modified by solvent washing

Results of solvent washing treatment of the feeding site on orientation responses of *R. appendiculatus* and *R. evertsi* towards their target sites showed that washed sites attracted less ticks than unwashed ones in both species (Figs 31 and 32). Attraction of *R. appendiculatus* to washed ears dropped significantly ($P<0.05$), from an average of 47.8% arrivals at untreated ear sites to an average of 35.2% reaching washed ears, which represents a drop of ~ 20% (Table 14). Washing treatment had a similar effect on *R. evertsi* ($P<0.05$), where an average 59.7% arrivals occurred at the unwashed anal site as compared to 44.3% reaching the washed site, which represents a drop of ~ 15% (Table 15). Comparison of treatment effectiveness indicated that solvent washing of ear caused greater reduction of test ticks reaching the area than did the corresponding treatment on anal area for *R. evertsi*. The lasting of effectiveness of this treatment on both sites was generally short and did not go beyond 24 hrs.

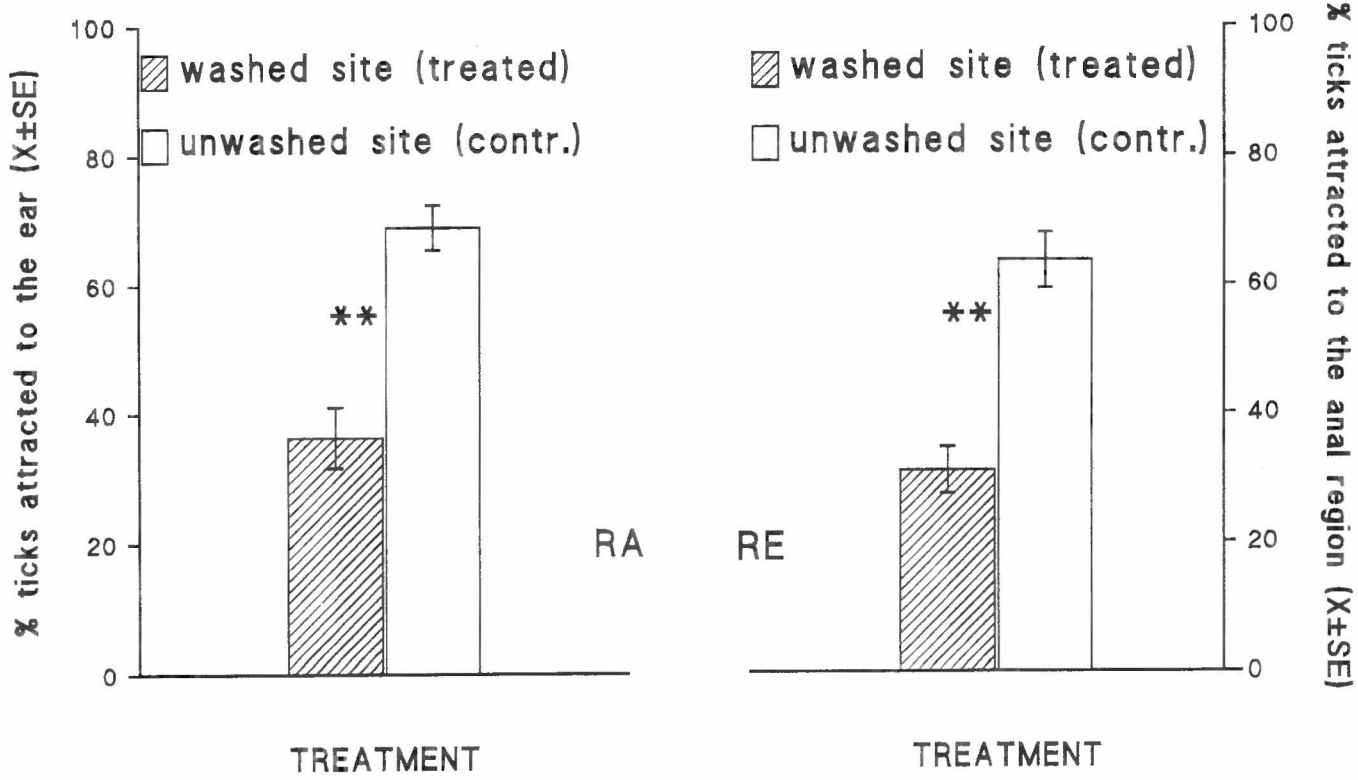

% ticks attracted to the ear (X±SE)
100
80
60
40
20
0
washed site (treated)
unwashed site (contr.)
**
RA
TREATMENT
RE
washed site (treated)
unwashed site (contr.)
**
100
80
60
40
20
0
% ticks attracted to the anal region (X±SE)
TREATMENT

Table 14 Effects of solvent washing on orientation responses of *R. appendiculatus* to the ear site

Parameters assessed	Site condition		LSD-Test
	Washed	Unwashed	
Total ticks moving	46.7	57.3	ns
Reaching target FS (%)	35.2	47.8	*
Located off target FS[1] (%)	39.5	28.3	ns
Unmoved (%)	53.3	42.7	ns
Drop-out (%)	37.0	44.6	ns

FS = Feeding site (= ear);
[1] Areas adjacent to FS i.e. head, neck, dewlap, ..
N= 160 (pooled data for ♂ and ♀);
*, P<0.05; **, P<0.01; ns = not significant

Table 15 Effects of solvent washing on orientation responses of *R. evertsi* to the anal site

Parameters assessed	Site condition		LSD-Test
	Washed	Unwashed	
Total ticks moving	61.2	69.3	ns
Reaching target FS (%)	44.3	59.7	*
Located off target FS[1] (%)	28.7	31.5	ns
Unmoved (%)	38.8	30.7	ns
Drop-out (%)	27.6	34.4	ns

FS = Feeding site (= anal area);
[1] Areas adjacent to FS i.e. back, flank, hindleg, ..
N= 160 (pooled data for ♂ and ♀);
*, P<0.05; **, P<0.01; ns = not significant

7.3.2 Orientation responses of *R. appendiculatus* and *R. evertsi* to transferred feeding site materials

Results of preliminary trials with *R. appendiculatus* showed that faecal material (FM) suspensions were equally disruptive as the anal extract. Suspensions of FM were placed across *R. appendiculatus* route to the ear to assess the orientation responses of this species. Smears of anal extracts applied dorsally on the ear and across *R. appendiculatus* route to the ear base did not show any significant difference. The results of disruption tests using 3 types of smear barriers across the ear base are presented in Table 16. It shows that the order of disruptive effectiveness by these smear types are: half circular barrier < single circular barrier < double circular barrier. Although the half-barrier repellent circle caused a reduction in the number of ticks arriving at the feeding site, the effect was not statistically significant compared to the control.

Tables 17 and 18 show results of further tests on orientation responses of *R. appendiculatus* and *R. evertsi* to their respective ear and anal feeding sites treated with extracts from the corresponding unpreferred feeding sites respectively. Orientation responses of either tick species to their normal feeding site was significantly disrupted. Thus, the number of *R. appendiculatus* reaching the ear treated with the anal extract ear dropped significantly compared to controls ($P < 0.01$), from an average of 56.4% at the untreated site to an average of 36.65%, which

represents a drop of ~ 20%. Similarly, limited trials carried out with ear extracts applied on anal area showed that these also disrupted *R. evertsi* orientation to its target site, the arrivals at the untreated anal site dropping from an average of 43.6% to 31.8% in control site, that is a drop of ~12.0% (Tables 17 and 18).

Moreover, observations of tick distribution showed that in treated hosts, more ticks were scattered in areas adjacent to the preferred site compared to the untreated hosts. In *R. appendiculatus*, 39.5% were found scattered off target, especially on the head, dewlap and neck. In *R. evertsi*, disoriented ticks (18.1%) were found scattered on the back, hindleg and flank (Tables 17 and 18).

Table 16 Effects of smears of various repellent extracts on the orientation responses of *R. appendiculatus* to its preferred feeding site.

Behaviour parameters assessed						
Type of treatment	Extracts applied	Size (N)	Reaching FS (ear)	Moved away /repelled	Dropping off/lost	Not moved
Circ. barrier	Oc. suave Anal extr. Control	80 80 120	8(10)b 4(5)b 44(36.5)a	8(10) 19(23.8) 9(7.4)	30(37.5) 26(32.4) 44(36.7)	34(42.5) 31(38.8) 23(19.2)
Half barrier	Oc. suave Anal extr. Control	80 80 120	20(25)ab 12(15)b 44(36.5)a	10(12.5) 12(15) 9(7.4)	16(20) 34(42.5) 44(36.7)	34(42.5) 22(27.5) 23(19.2)

FS = Feeding site; in bracket, percentage.
Means in a column with the same letter are not significantly different (Glm test/SNK).
N= (pooled data for ♂ and ♀).

Table 16 Effects of smears of various repellent extracts on the orientation responses of *R. appendiculatus* to its preferred feeding site.

Behaviour parameters assessed						
Type of treatment	Extracts applied	Size (N)	Reaching FS (ear)	Moved away /repelled	Dropping off/lost	Not moved
Circ. barrier	Oc. suave	80	8(10)b	8(10)	30(37.5)	34(42.5)
	Anal extr.	80	4(5)b	19(23.8)	26(32.4)	31(38.8)
	Control	120	44(36.5)a	9(7.4)	44(36.7)	23(19.2)
Half barrier	Oc. suave	80	20(25)ab	10(12.5)	16(20)	34(42.5)
	Anal extr.	80	12(15)b	12(15)	34(42.5)	22(27.5)
	Control	120	44(36.5)a	9(7.4)	44(36.7)	23(19.2)

FS = Feeding site; in bracket, percentage.
Means in a column with the same letter are not significantly different (Glm test/SNK).
N= (pooled data for ♂ and ♀).

Table 18 Orientation responses of *R. evertsi* to the anal site modified by the ear extract

Response component	Tick numbers (%)		LSD Test
	Treated site	Untreated site	
Reaching target FS	43.6	55.4	*
Off-target FS[1]	9.4	12.7	*
Drop-out	37.5	26.4	**
Unmoved	18.2	15.0	ns

FS = Feeding site (= anal area);
[1] Areas adjacent to FS i.e. back, flank, hindleg...
N = 120 (pooled data for ♂ and ♀).
*, $P<0.05$; **, $P<0.01$; ns = not significant

7.3.3 Orientation responses of *R. appendiculatus* and *R. evertsi* to their feeding site treated with *O.suave* essential oil

The results of orientation responses of *R. appendiculatus* and *R. evertsi* to their respective feeding sites treated with the essential oils of *O. suave* demonstrated a clear disruption effect on both species (Tables 19 and 20). The number of *R. evertsi* reaching the treated anal region dropped significantly (41.2% compared to 25.7%, ie a reduction of ~16%) on the treated animal ($P<0.05$). Likewise, in *R. appendiculatus*, it dropped from 56.4% (control) to 36.8% (treated), a reduction of ~20%. Also noted was significant increase of drop-outs and location off-target among ticks (Tables 19 and 20). GC profile of the essential oil of *O. suave* revealed the presence of eugenol and other terpenoid compounds (Fig. 33). The former compound is known as an effective repellent for a number of arthropods and its presence in the plant oil here indicate this component, alone, substantially or along the other terpenoids contribute to the repellent and disruption observed around treated sites.

Table 19 Orientation responses of *R. appendiculatus* to the ear modified by *O. suave* essential oil

Response component	Tick numbers (%)		
	Treated site	Untreated site	LSD Test
Reaching target FS	11.4	32.4	**
Off-target FS[1]	18.1	2.9	**
Drop-out	72.5	12.5	**
Unmoved	13.4	17.5	ns

FS = Feeding site (=ear);
[1] Areas adjacent to FS i.e. head, neck, dewlap...
N = 120 (pooled data for ♂ and ♀).
*, P<0.05; **, P<0.01; ns = not significant

Table 20 Orientation responses of *R. evertsi* to anal region modified by *O. suave* essential oil

Response component	Tick numbers (%)		
	Treated site	Untreated site	LSD Test
Reaching target FS	25.7	41.2	*
Off-target FS[1]	13.3	5.6	*
Drop-out	67.5	18.1	**
Unmoved	10.9	8.5	ns

FS = Feeding site (= ear);
[1] Areas adjacent to FS i.e. head, neck, dewlap...
N = 120 (pooled data for ♂ and ♀).
*, $P<0.05$; **, $P<0.01$; ns = not significant

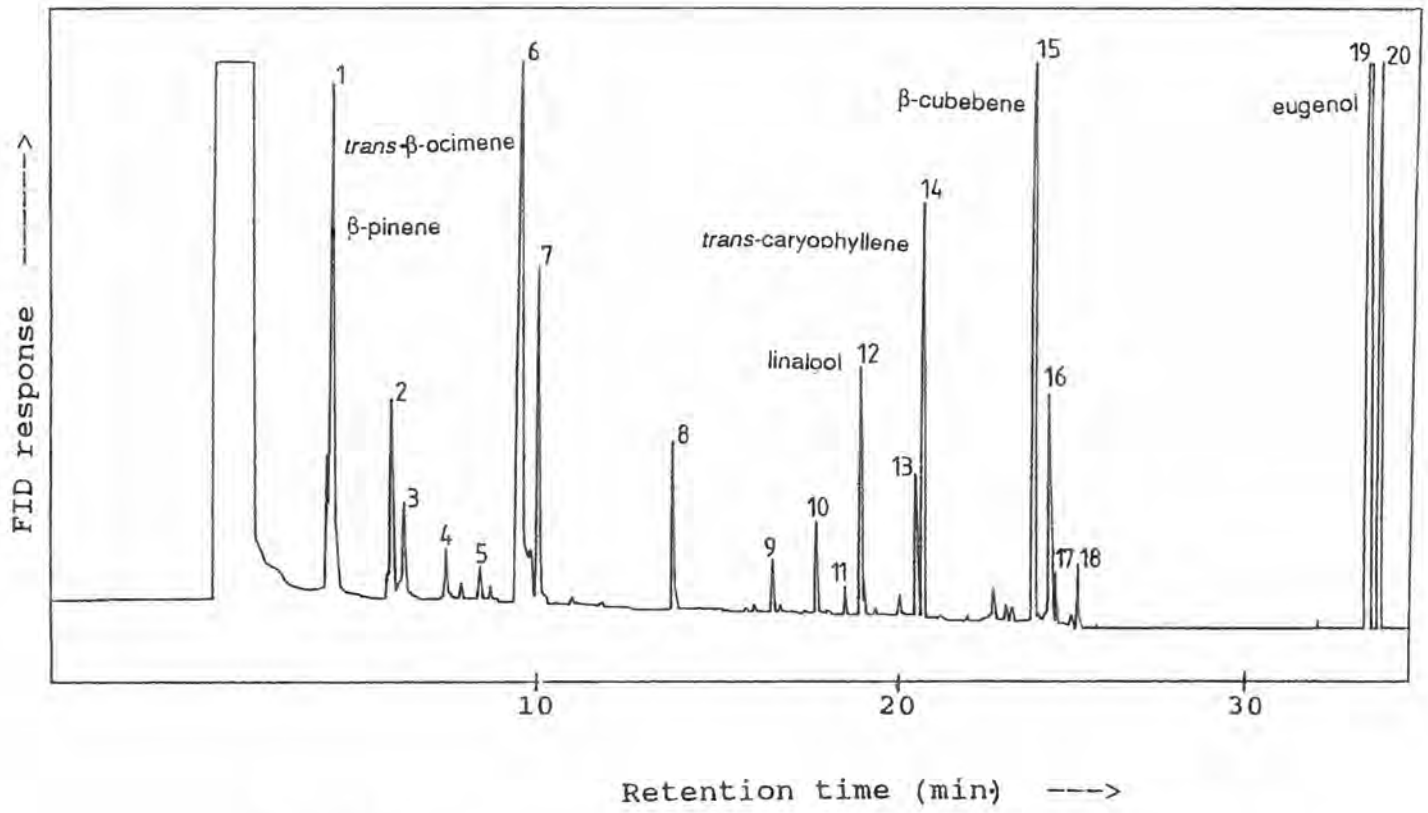


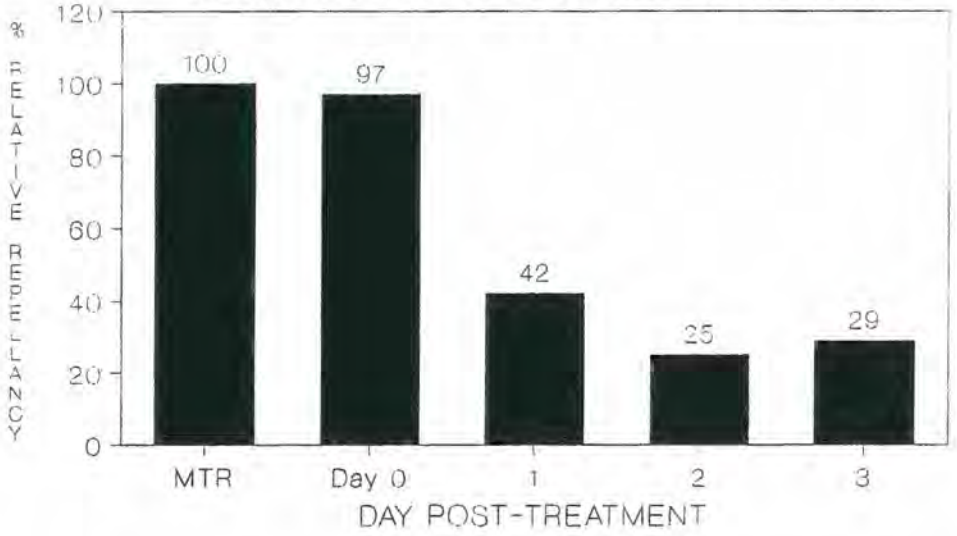
Fig. 33 Gas chromatograph profile of *Ocimum suave* essential oil

7.3.4 **Allomonal persistence of host materials and *O. ocimum* essential oil**

Results of persistence tests of the two categories of allomonal materials (host and plant derived) are presented in Figs 34-37. Anal and ear extracts were each found to cause significant ($P < 0.05$) disruption against the appropriate tick over a short period. The anal extract was effective on *R. appendiculatus* for less than 1 day (Figs 34 and 35). Similarly, the ear extract was effective against *R. evertsi* for the same period. In contrast, the effect of the essential oil lasted between 4-6 days (Figs 36 and 37).

Fig. 34

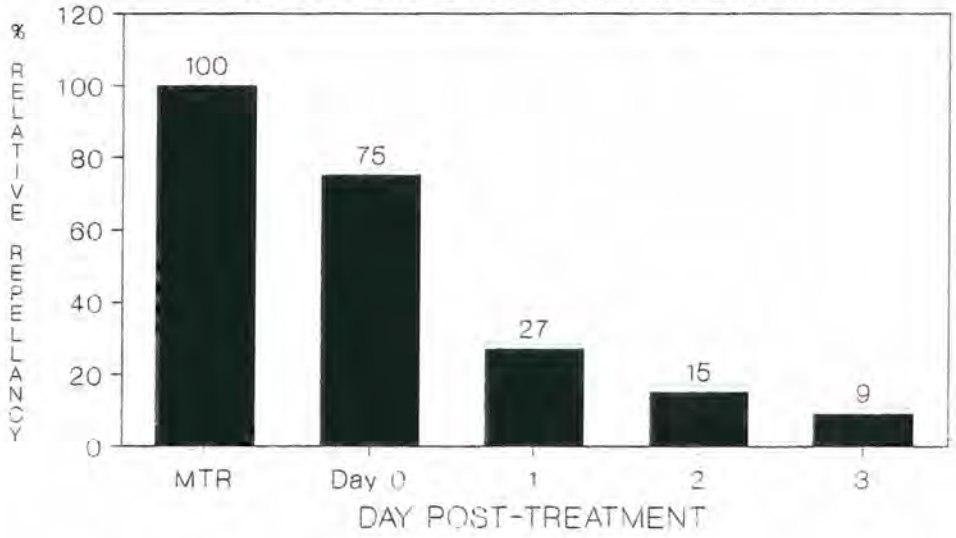
ALLOMONE PERSISTENCE OF THE ANAL EXTRACT AROUND THE EAR SITE AS MEASURED BY R. APPENDICULATUS RESPONSE



N = 120

Fig. 35

ALLOMONE PERSISTENCE OF THE EAR EXTRACT AROUND THE ANAL REGION AS MEASURED BY R. EVERTSI RESPONSE

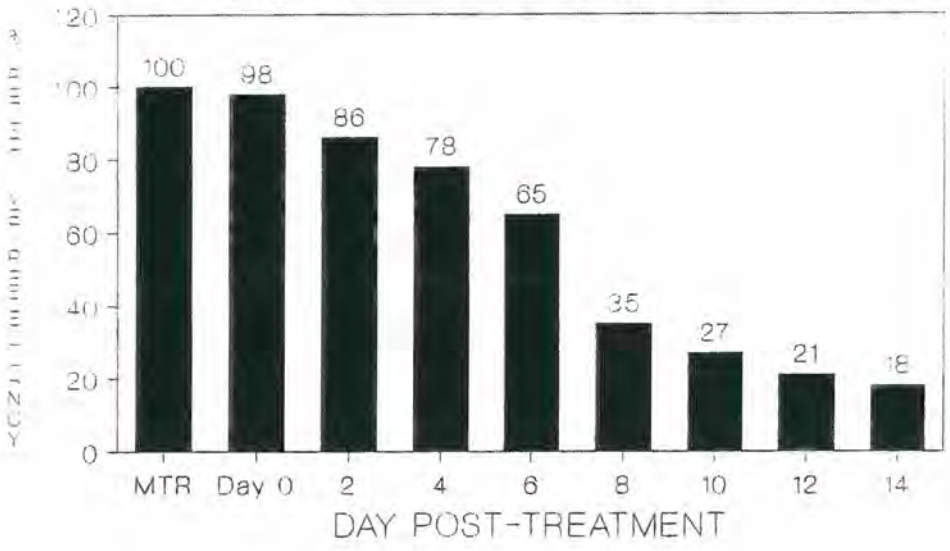


N = 120

MTR = Maximum Theoretical Repellancy

Fig. 36

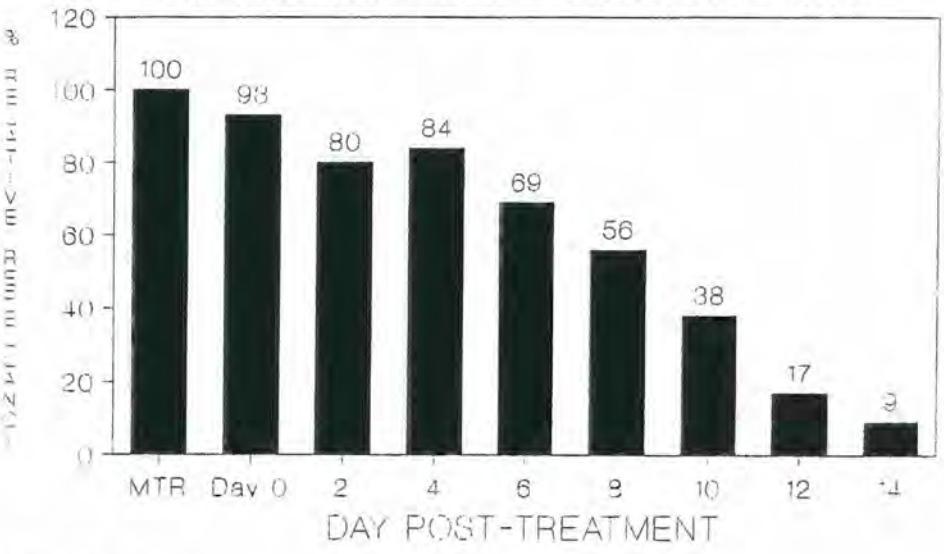
ALLOMONE PERSISTENCE OF O. SUAVE ESSENTIAL OIL AROUND THE EAR AS MEASURED BY R. APPENDICULATUS RESPONSE



N = 120

Fig. 37

ALLOMONE PERSISTENCE OF O. SUAVE ESSENTIAL OIL AROUND THE ANAL REGION AS MEASURED BY R. EVERTSI RESPONSE



N = 120

MTR = Maximum Theoretical Repellancy

7.4 **Discussion**

In the present study, the effects of various modifications of the feeding sites were evaluated. These included solvent washing, application of host allomones from unpreferred feeding sites and application of the oil of a tick repellent plant at the feeding site.

Natural feeding sites of ticks are believed to produce stimuli that attract the appropriate tick species (Gregson, 1973). Odours from such areas are likely to be made of organic compounds, some of which may be extractable by organic solvents. Our results show that washing of the ear and anal regions resulted in significant decrease of the number of *R. appendiculatus* and *R. evertsi* attracted to these sites. This reduced attractivity is very likely due to the partial depletion of attractive compounds from the washed sites. However, the site attractivity was restored after a short time suggesting that the depleted materials are quickly replenished.

Application of host derived allomonal materials, although crude and the active compounds probably present in small relative amounts, elicited significant disrupting effects. The effect of the materials, however, did not last long, in fact less than a day. This may be due to a high volatility of the active compounds or their low concentrations in the extracts, or both.

A similar modification of the olfactory properties of the feeding site was also induced by the essential oil of the plant *O. suave*. The reluctance of both *R.*

appendiculatus and *R. evertsi* to locate the otherwise favourable feeding site but which had been sealed with the essential oil treatment, is consistent with the repellent properties of this plant (Hassanali *et al.*, 1990; Mwangi *et al.*, 1995b). The disruptive effect may be caused largely by the presence of eugenol, the major component of *O. suave* oil, a known repellent of a number of arthropods including ticks (Chogo and Cranck, 1981; Hassanali *et al.*, 1990). This compound has been shown to have a repellency against a number of stored pests equal to or greater than that of DEET, a classical synthetic repellent (Hassanali *et al.*, 1990; Ndungu *et al.*, 1995). The GC profile of the essential oil of *O. suave* confirmed the presence of eugenol in addition to other terpenoid compounds which may also be involved in the repellent action of the oil.

Our results indicate that allomonal persistence of host extracts is lower compared to that of the plant extract. The difference in residual activity may be due to the nature of allomones involved. However, whereas the oil obtained from *O. suave* by hydrodistillation was relatively pure, solvent washings of the feeding sites, in all probability, gave a complex blend of compounds containing relatively low concentrations of the active compounds. So, a direct comparison of the two sets of allomones at this stage is not meaningful. This must await the identification of the actual active compounds from body extracts. In any case, from application point of view, formulations of the active compounds could substantially change the persistence and effectiveness of these allomones.

CHAPTER EIGHT

8.0 GENERAL DISCUSSION

In this section, the different findings described in the previous chapters will be discussed and attempt will be made to project an integrated view of the implication of the different results. In addition, unanswered questions and gaps identified during this study and areas that need to be further investigated will be highlighted. The prospects for the application of some of the knowledge gained on tick chemical ecology from the present study will be outlined.

The various *in vitro* and on-host assays that were undertaken have attempted to elucidate feeding site location behaviour of *R. appendiculatus* and *R. evertsi* which show distinct feeding site preferences on their bovine host. These studies have also attempted to explain the role of semiochemicals that may mediate this behaviour in the two tick species. The results of *in vivo* experiments indicate that on-host behaviour is mediated by chemical cues emanating from the feeding sites. Ticks whose olfactoreceptors (Haller's organ) were blocked, were shown to be endowed with the ability to perceive and discriminate between odours from preferred and unpreferred sites. Our observations suggest that chemo-orientation may be the primary orientation mechanism used by ticks to locate their feeding sites, although non-olfactory cues may also play a role. The mechanism involved in site location (anemotaxis or chemotaxis)

is not clear and its elucidation must await characterization of host odour semiochemistry using techniques such as gas chromatography-linked mass spectrometry (GS-MS) and electro-physiology.

The orientation behaviour of *R. appendiculatus* on the host revealed a set of sequential activities ranging from inactive/scanning phase to the onset of erratic movements that become increasingly directional, and, finally to arrestment at the odour source (feeding site). The well orchestrated phases show the complex sets of behavioural adjustment the tick exhibits as it progresses towards its feeding site. In part, this is designed to optimize the probability of success amid hazards such as grooming and other movements by hosts, changing aerial movements and associated distortion of the odour signal, wrong routing by the ticks, dessication etc..during transit (Roberts, 1971). Elucidating the role of host odour distribution and plume structure at each of these steps is a task ahead.

In the present study, some of the specific behaviours involved in the location of the feeding site such as arousal, activation, arrestment and aggregation have been examined. The results of arousal tests show that ticks get sensitized quickly to host odours particularly those from the feeding site. Scanning and residence patterns displayed by the two tick species in response to the stimulatory and inhibitory extracts to which ticks were exposed, revealed a complex set of reactions to these odour extracts. From the two patterns seen, the implication is that ticks use extensively externally-derived information (allothetic control) to guide themselves, in addition to

the internal information control (Bell, 1983). Activation and arrestment responses were studied *in situ* bioassays. Irrespective of the feeding site location and its distance, odour was shown to activate the ticks. Subsequently, as the tick approaches the feeding site, the odour elicited arrestment. Whether the same compounds elicit these two different responses at different concentrations or whether different groups of compounds with different volatilities are involved remain to be determined.

The aggregation responses of *R. appendiculatus* on bovine hosts to intraspecific male and female rinses were consistent with the behaviour of this tick species both in vegetation and on host. Individuals of this tick species are known to assemble on vegetation patches (Sonenshine *et al.*, 1982b). This assembly off host is believed to have a protective value against stressful environmental conditions (Sonenshine *et al.*, 1982b; Hassanali *et al.*, 1989). However, it is unlikely that the same role applies for aggregation propensity on host. In some *Amblyomma* spp, the aggregation-attachment pheromone produced by feeding ticks play an important role in guiding unfed conspecifics to the host animal and to the feeding sites (Rechav *et al.*, 1976, 1977; Norval and Rechav, 1979). The pheromone was characterized (Schoni *et al.*, 1984) and its potential in monitoring and control has been demonstrated (Norval *et al.*, 1991d, 1992; Sonenshine *et al.*, 1992). In the case of *R. appendiculatus*, the present results suggest that kairomones derived from the host constitute a primary set of attractants with the aggregating pheromone playing an augmentative role.

In addition, the results also indicate parallel response patterns of the two species

to stimuli derived from their own feeding sites and to that of each other's feeding sites. While the stimulus from one site acts as a strong kairomone (attractant) to the tick species that feeds at that site, it acts as an allomone (repellent) against the other tick. The result is a 'push-pull' effect that ensures that the tick is unlikely to wander off in the wrong areas of the host and enhances the probability of orienting toward its feeding site. The next step is to establish the nature of the chemicals involved. Attractants may be powerful agents for sampling and trapping and have been used together with acaricides to lure and toxicate ticks (Ziv *et al.*, 1981; Norval *et al.*, 1991d; Sonenshine *et al.*, 1992). On the other hand, the repellent effect of these host materials may be used to keep cattle infestation level low. The use of faecal material on the ear where large aggregates of *R. appendiculatus* ticks are found, may lower the number of this tick on cattle and small ruminants. Such materials may also reduce the number of ticks that successfully locate their feeding sites and their mates. The push-pull effect may also be exploited to attract ticks to devices treated with acaricides or pathogens.

The availability of plant repellents offers further possibilities in these directions. Whether plant or host animal-based allomones are more effective remains to be established. The essential oils of repellent plants such as *Ocimum suave* and *Gynandropsis gynandra* (Mwangi *et al.*, 1995a; Ndungu *et al.*,1995; Malonza *et al.*, 1992) are readily and widely available and offer possibilities of immediate exploitation.

The present study has therefore provided a basis for the development of tick control tactics using semiochemicals. As non-flying arthropods, ticks respond to odour attractants and repellents from relatively short distances. However, the results obtained by this research suggest that this may not be a disadvantage and behavioural manipulations on-host or off-host may represent effective and environmentally friendly methods of tick control.

CHAPTER NINE

9.0 CONCLUSIONS

The study has established that:

1. Orientation behaviour of unfed *R. appendiculatus* adults on host was made up of sets of stereotyped sequences of behaviours including stationary/scanning, random search, directional movement and arrestment close to and at the site source.

2. Pathways of *R. appendiculatus* and *R. evertsi* to their feeding sites were made of runs with intermittent stops, all producing curvilinear tracks of varying patterns with increasingly directional character as the tick progressed toward the feeding site. Individual ticks, irrespective of the release site on the host body, completed their paths to the feeding sites, along with high success rates of site location (69.8-85.6% for *R. evertsi*; 67.5-90.0% for *R. appendiculatus*). On average, *R. evertsi* moved faster than *R. appendiculatus* (0.24 and 0.58cm/min vs 0.09 and 0.41cm/min respectively for males and females).

3. Tarsi-coated individuals of both tick species were less effective in orienting or locating the relevant feeding sites, suggesting this location is primarily mediated by

odour, especially feeding site-borne kairomones.

4. Some specific behaviours relating to the location of the feeding sites on host such as arousal and activation and arrestment revealed that:

- Arousal tests on *R. appendiculatus* and *R. evertsi* exposed to odour extracts from ear and anal region resulted in scanning and residence (in Y-olfactometer arm) response patterns which correlate with the stimulatory or inhibitory nature of the extracts.

- *In situ* (host) assays, irrespective of the distance of the feeding site, the ticks became activated, with latent period to activation decreasing with decrease in distance from the feeding site. Likewise, walking speed of both spp resulted in gradual decrease and eventual arrestment in the proximity of the feeding site. On the other hand, *in vitro* assays, substrates impregnated with extracts from the preferred feeding site caused more walking arrestment of the relevant tick species. The nature of these arrestants needs to be characterized.

5. Olfactometric bioassays show that washing extracts from body parts (e.g. belly/axillae, neck/dewlap and leg) other than those of the feeding site were less or unattractive to the unfed adults of *R. appendiculatus*. Ear and anal extracts proved

strongly attractive to the adult stage of *R. appendiculatus* and *R. evertsi* respectively. Nymphs and larvae of *R. appendiculatus*, in contrast, were significantly not attracted by the ear extract, consistent with their lack of specific site preference to feed.

6. Both ear washes and trapped air-borne volatiles of the ear evoked significant attraction to *R. appendiculatus* adults. The blend of the two elicited a stronger attraction. The additive effect of the mixture suggests a dual kairomonal set of components comprising short-range/contact signals and volatile components which mediate feeding site selection in this tick.

7. Cross-assays involving the use of crude extracts from a site preferred by one tick evoked strong repulsion to the other tick species. Thus, for each species, a 'push-pull' mechanism is operational, with the repellent from unpreferred site pushing the tick away and the attractant from its feeding site pulling it toward itself.

8. Our results implicate the mediation of two sets of signals (intraspecific and interspecific) that ticks use to navigate to the feeding site: interspecific signals - volatiles emanating from the feeding site and contact or less volatile signals on one hand, augmented on the other by intraspecific signals from successfully feeding ticks at the feeding site.

9. The repellent host materials (ear extract for *R. evertsi*; anal extract for *R. appendiculatus*) were effective in disrupting orientation of each tick species to their feeding site. These disruptants need to be characterized.

10. The eugenol-rich essential oil of a tick repellent plant *O. suave*, was found to have similar disrupting effects on the orientation of both ticks to their target sites. Other effects of both host and plant-derived materials include increased disorientation and drop out among test ticks.

11. Although host extracts were less persistent than the plant repellent, a comparison between the two is inappropriate at this stage because of lack of knowledge on the concentration of active repellents in the host.

12. By manipulating and modifying on-host orientation and behaviour of the two tick species, these host and non-host semiochemicals have a potential for the development of tick control tactics as part of an Integrated Tick Management (ITM) system.

CHAPTER TEN

10.0 SUGGESTIONS FOR FUTURE WORK

Characterization of semiochemicals

- Detailed behavioural studies using fractionated extracts (acidic, basic, neutral and phenolic fractions) of ear and anal stimuli as well as tick-derived aggregating compounds for both *Rhipicephalus appendiculatus* and *R. evertsi* to locate active blends.

- Chemical characterization and identification of candidate active compounds implicated as host volatile kairomones and allomones and tick pheromones for each species by gas chromatography (GC)-linked electrophysiology and mass spectrometry (MS) techniques.

- High performance liquid chromatographic (HPLC) fractionation of less or non-volatile semiochemicals, followed by bioassay-guided location and purification of active compounds and their chemical characterization by the relevant spectrometric techniques.

- Detailed bioassays of all candidate compounds individually and in blends to identify active blends for each behavioural element.

Development of control tactics based on behavioural manipulation of the ticks on host

- Develop chemically stabilized, long-lasting, controlled-release dispensers for these attractants and repellents for field testing.

- Undertake modelling of tick on-host orientation process which can guide the development of intervention tactics.

- Develop, test and compare the use of (a) allomones (host and plant derived) as protectants of hosts and their feeding sites, (b) kairomones as baits to acaricide- or pathogen-treated targets on host, and (c) allomones and kairomones as protectants and baits in push-pull strategies.

- Undertake large-scale evaluation of promising tactics in the field.

Development of tactics for behavioural manipulation of the ticks off-host for monitoring and control

- Improve (a) existing olfactometric set-ups which exploit the climbing propensity of the ticks on the vegetation in the field and (b) incorporate attractant-impregnated baits to attract, trap and kill ticks.

- Test and optimize the above devices with various acaricidal and pathogenic formulations for control purpose.

Studies on the immature stages

- Detailed studies on responses of immature stages of both tick species to semiochemicals as for the adult stages.

REFERENCES

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

Allan S.A., Phillips J.S., Taylor D. & Sonenshine D.E.(1988). Genital sex pheromones of ixodid ticks: evidence for the role of fatty acids from the anterior reproductive tract in mating of *Dermacentor variabilis* and *D.andersoni*. **J.Ins.Physiol**. **34**(4):315-323.

Bailey K.P.(1960). Notes on the rearing of *Rhipicephalus appendiculatus* and their infection with *Theileria parva* for experimental transmission. **Bull.epiz.Afr. 8**:33-64.

Baker T.C.(1985). Chemical control of behavior.In: *Comprehensive Physiology, Biochemistry & Pharmacology*. Gilbert K. (Ed.).pp.621-672.

Balashov Y.S.(1972). Blood sucking ticks-vectors of disease of man and animals. **Misc.Publs.Ent.Soc.Am.**, 191-193.

Bell W.J. & Tobin R.T.(1982). Chemo-orientation. **Biol.Rev. 57**:219-260.

Bell W.J. (1983). Chemo-orientation in walking insects. In: *Chemical Ecology of Insects* (Bell & Cardé, Eds). Chapman & Hall. London.

Bell W.J., Tobin R.T., Vogel G. & Surber F. (1983). Visual course control of escape responses in the cockroach *Blaberus cranifer*: role of internal and external orientation information. **Physiol.Entom. 8**:121-132

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

Bonsma J.C.(1981). Breeding tick-repellent cattle. In: *Tick biology and control*: Proc.Intern.Conf. Whitehead G.B. & Gibson J.D.(Eds), Rhodes Univ., Grahamstown. pp.67-77.

Branagan D.(1973). The developmental periods of the ixodid *R. appendiculatus* under laboratory conditions. **Bull.ent.Res. 63**:155-168.

Browning T.S.(1976). The aggregation of questing ticks *Rhipicephalus pulchellus* on grass stems with observation on *R. appendiculatus*. **Physiol.Entom. 1**:107-114.

Chiera J.W.(1985). The distribution and climbing behaviour of *Rhipicephalus appendiculatus* on grass stems. **Insect Sc.Applic. 6**:213-215.

Chilton N.B., Andrews R.H. & Bull C.M.(1992). Interspecific differences in the movements of female ticks on reptiles. **Intern.J.Parasitol. 22**(2):239-242.

Chogo J.B. & Crank G.(1981). Chemical composition and biological activity of the Tanzanian plant *Ocimum suave*.**J.Nat.Prod. 44**:308-311.

De Castro J.J., Cunningham M.P., Dolan T.T., Dransfield R.D., Newson R.M., Young A.S.(1985). Effects on cattle of artificial infestations with the tick *Rhipicephalus appendiculatus*. **Parasitol. 90**:21-33.

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**(2):143-154.

Diehl P.A., Guerin P., Vlimant M. & Steullet P.(1991). Biosynthesis, production site and emission rates of aggregation-attachment pheromone in males of two *Amblyomma* ticks. **J.chem.Ecol. 17**(5):833-847.

Dipeolu O.O.(1990). Research on ticks of livestock in Africa: review of the trends, advances and milestones in tick biology and ecology in the decade 1980-1989. **Insect Sci.Appl. 10**:723-740.

Dipeolu O.O., Mongi A.O., Essuman S., Amoo A.O. & Ndungu N.J.(1992). Studies on naturally acquired immunity to African ticks. II: Observations on cattle exposed to *Rhipicephalus appendiculatus* under varying periods of repeated infestations. **Veter.Parasitol. 41**:293-320.

Dobrotvorskii A.K., Tkachov A.V., Demenkova L.I.(1989). [Studies of the repellent activity of some plants extracts against *Ixodes persulcatus*]. **Izvestiya**.Akademi Nauk SSSR [russian] **6**:62-66.

Doube B.M. & Kemp D.H.(1979). The influence of temperature, relative humidity and host factors on the attachment and survival of *Boophilus microplus* larva to skin slices. **Intern.J.Parasitol. 9**(5):449-455).

Dusbabek F., Simek P., Jegorov A., Triska J.(1991). Identification of xanthine and hypoxanthine as components of assembly pheromone in excreta of argasid ticks. **Exper.Appl.Acarol. 11**(4):307-316.

Elbl A. & Anastos G.(1966). The Ixodid ticks of Central Africa, Vol.III. Genus *Rhipicephalus*. **Ann.Mus.Roy.Afr.Centr.** ser.8, Sci.zool.147, pp.53-57.

Essuman S., Dipeolu O.O. & Odhiambo T.R.O.(1991). Immunization of cattle with a semi-purified fraction of solubilized membrane-bound antigens extracted from the midgut of the tick *R. appendiculatus*. **Exp.Appl.Acarol. 13**:65-73.

FAO (1984). Tick and Tick-borne disease control: A practical field manual. Vol.I. Food and Agriculture Organization, Roma, 621pp.

Galun R.(1977). Responses of blood-sucking arthropods to vertebrate hosts. In: *Chemical control of insect behavior: Theory & Application*, Shorey H.H.& McKelvey J.J.Jr (Eds) Wiley, Interscience, pp.103-15.

Galun R., Stenberg S. & Mango C.(1972). The use of sterile females for the control of the tick *Argas persicus*. **Israel J.Entomol.** VII, 109-115.

Garcia R. (1962). Carbon dioxide as an attractant for certain ticks. **Ann.Ent.Soc.Amer.**, Vol.55(5)p.605Gladney W.J.(1971). Mate seeking of female *Amblyomma maculatum* on a bovine. **Nature (London) 232**:401-402.

Gladney W.J. (1976). Insecticide-impregnated ear-tags and pours-on, new devices for tick control. **J.med.Entom.,13**:213-215

Gladney W.J. & Drummond R.O.(1970). Migration of male lone star ticks on the host in relation to mating. **J.econ.Entom. 63**:1214-16.

Gladney W.J., Ernst S.E. & Grabbe R.R.(1974a). The aggregation response of the Gulf coast tick on cattle. **Entomol.Soc.Am. 67**:750-752.

Gladney W.J., Ernst S.E., Oehler D.D.(1974b). The Gulf Coast tick: evidence of a pheromone produced by males. **J.med.Entom. 11**:303-306.

Goethe R. and Neitz W.H. (1985) Investigations into the participation of male pheromones of *Rhipicephalus evertsi evertsi* during infestation. **Onderst.J.Vet.Res., 52**:67-70

Graf J.F.(1975). Ecologie et éthologie d'*Ixodes ricinus* L en Suisse (Ixodoidea:Ixodidae). Cinquième note: mise en évidence d'une phéromone sexuelle chez *I. ricinus*. **Acarologia 17**:436-441.

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

Gregson J.D.(1973). Tick paralysis - An appraisal of natural and experimental data. Can.Dept.Agr., Monogr.No 9, 109pp.

Guenther E.(1949). The essential oils.Vol.1. D.Van Nostrand, New York.

Guerin P.M., Steullet P., Kröber T., Diehl P.A., Vlimant M., De Bruyne M., Cordas T., Falk-Vairant J., Kuhnert F.and Lösel P.M.(1992). In: Proc.1st Int.Conf.tick-borne pathogens at the host-vector interface (Munderloh U.G. & Kurtii T.J., Eds). Minnesota Univ., MN. pp.314-323.

Hadani A., Ziv M. & Rechav Y.(1977). A laboratory study of tick repellents. **Entomol.Exp.Appl. 22**(1):53-59.

Haggart D.A. & Davis E.E.(1980). Ammonia-sensitive neurones on the first tarsi of tick *Rhipicephalus sanguineus*. **J.Ins.Physiol. 26**:517-523.

Haggart D.A. & Davis E.E.(1981). Neurons sensitive to 2,6-Dichlorophenol on the tarsi of the tick *Amblyomma americanum* (Acari:Ixodidae). **J.med.Entom. 18**(3):187-193.

Hamilton J.G.C. & Sonenshine D.E.(1988). Evidence for occurrence of mounting sex pheromone on body surface of female *Dermacentor variabilis* and *D. andersoni*. **J.chem.Ecol. 14**(1):401-410.

Hamilton J.C.G. and Sonenshine D.E. (1989). United States Patent no 8,884,361.

Hamilton J.G.C., Sonenshine D.E. & Lusby W.R.(1989). Cholesteryl oleate: mounting sex pheromone of the tick *Dermacentor variabilis*. **J.Ins.Physiol. 35**(11):873-879.

Hamilton J.G.C., Papadopoulos E., Harrison S.J., Llyod C.M. and Walker A.R. (1994). Evidence for a mounting sex pheromone in the brown ear tick *Rhipicephalus appendiculatus*. **Exper.Appl.Acarol., 18**(6):331-338.

Hassan S., Dipeolu O.O., Amoo A.O. & Odhiambo T.R.(1991). The predation of livestock ticks by chickens. **Vet.Parasitol.** 38:199-204.

Hassan S.M., Dipeolu O.O. & Malonza M.M.(1994). Natural attraction of livestock ticks by the leaves of a shrub. **Trop.Anim.Hlth.Prod. 26**:87-91.

Hassanali A. & Lwande W.(1989). Antipest secondary metabolites from African plants. 3rd Chem.Congr.N.Amer. Arnason J.T., Philogène B.J.R. & Morand P.(Eds).pp.78-94.

Hassanali A., Nyandat E., Obenchain F.A., Otieno D.A. & Galun R.(1989). Humidity effects on response of *Argas persicus* to guanine, an assembly pheromone of ticks. **J.chem.Ecol. 15**:791-797.

Hassanali A., Lwande W., Ole-Sitayo N., Moreka L., Nokoe S., & Chapya A.(1990). Weevil repellent constituents of *Ocimum suave* leaves and *Eugenia caryophyllata* cloves used as grain protectants in parts of Eastern Africa. **Discov.Innov. 2**(20):91-95.

Hewetson R.W.(1972). The inheritance of resistance by cattle to cattle tick. **Aust.Vet.J. 48**:299-303

Hoogstraal H.(1956). Ticks of the Sudan. African Ixodoidea. Vol.1., U.S.Navy, Washington. 615pp.

Howell F.G.(1975). The roles of host-related stimuli in the behavior of *Argas cooleyi.* **J.med.Entom. 11**(6):715-725.

ICIPE, 1979 Annual Report, pp.47, 48

ICIPE, 1994 Scientific Annual Report, pp.121, 122

ILRAD, 1990 Annual Scientific Report 1990, p.79.

Irvin A.D. & Brocklesby D.W.(1970). Rearing and maintaining *Rhipicephalus appendiculatus* in the laboratory. **J.Inst.Anim.Technol. 21**:106-112.

Irvin A.D. & Morrison W.J. (1989). Vaccines against *Theileria parva.* In: Wright I.G.(Ed.). Veterinary protozoan and haemoparasitic vaccines. CRC Press Inc., Boca Raton, FL. pp.115-130.

Johnston L.A.Y., Kemp D.H. & Pearson R.D.(1986). Immunization of cattle against *Boophilus microplus* using extracts deriving from adult female ticks : effects of induced immunity on tick populations Int.J.Parasitol. **16**:27-34.

Jongejan F., Pegram R.G., Zivkovic D., Hensen E.J., Mwase E.T., Thielemans M.J.C., Cosse A., Niewold T.A., El Said A. & Uilenberg G.(1989). Monitoring of naturally acquired and artificially induced immunity to *Amblyomma variegatum* and *Rhipicephalus appendiculatus* ticks under field and laboratory conditions. **Exper.Appl.Acarol. 7**:181-199.

Kaiser M.N., Sutherst R.W., Bourne A.S.(1982). Relationship between ticks and Zebu cattle in Southern Uganda. **Trop.Anim.Hlth Prod. 14**(2):63-74.

Kaiser M.N., Sutherst R.W., Bourne A.S., Gorissen L., Floyd R.B.(1988). Population dynamics of ticks on Ankole cattle in five ecological zones in Burundi and strategies for their control. **Prevent.vet.med. 6**:199-222.

Kennedy J.S.(1977). Olfactory responses to distant plants and other odor sources. In: *Chemical control of insect behavior, Theory & Application.* pp.67-91. Shorey & Mckelvey (Eds). John Wiley, New York.414pp.

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

Kokwaro JO(1976). Medicinal plants of East Africa.Nairobi. East Afri. Literature Bureau.

Kramer E.(1976) The orientation of walking bees in odour field with small concentration gradients. **Physiol. Entomol.**, **1**:27-37.

Latif A.A.(1984). Resistance to natural tick infections in different breeds of cattle in Sudan. **Insect Sci.Applic. 5**:95-97.

Leahy M.G., Karuhize G., Mango C., Galun R.(1975). An assembly pheromone and its perception in the tick *Ornithodoros moubata*. **J.med.Entom. 12**:284-87

Lees A.D.(1948). The sensory physiology of the sheep tick, *Ixodes ricinus*. **J.exper.Biol., 23**:379-410.

Leonovitch S.A. (1977) Electron microscopy studies of Haller's organ of the tick *Ixodes persulcatus* Ixodidae. **Parazitologiia 11**:340-346.

Lessard P., L'Eplattenier R., Norval R.A.I., Kundert K., Dolan T.T., Croze H., Walker J.B., Irvin A.D. & Perry B.D.(1990). Geographical information systems for studying the epidemiology of cattle diseases caused by *Theileria parva*. **Veter.Record. 126**:255-262

Lwande W., Ndakala A.J., Hassanali A., Moreka L., Nyandat E., Ndungu M., Amiani H., Gitu, Malonza M.M.& Punyua D.K (1996). *Gynandropsis gynandra* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) repellents.

Malonza M.M., Dipeolu O.O., Amoo A.O. & Hassan S.M.(1992). Laboratory and field observations on anti-tick properties of the plant *Gynandropsis gynandra*. **Veter.Parasit. 42**:123-136.

Marinibettolo G.B.(1983). The role of natural products in plant insect and plant-fungi interaction. In: *Natural products of innovative pest management*. Whitehead & Bowers (Eds). Pergamon Press, pp.187-222

Mathewson M.D.(1984). The future of tick control: A review of the chemical and non-chemical options. In: *Impact of disease on livestock production in the tropics*. Riemann HP & Burridge MP (Eds), Elsevier, Amsterdam, pp.559- 568.

Mathewson M.D., Hughes G., MacPherson I.S. & Bernard C.P. (1981). Screening techniques for the evaluation of chemicals with activity as tick repellents. **Pesticide Science 12**(4):455-462.

McDowell P.G. & Waladde S.M.(1985). 2,6-dichlorophenol in the tick *Rhipicephalus appendiculatus* Neumann, a reappraisal. **J.chem.Ecol. 12**:69-81.

McHardy N.(1989). Buparvaquone - the new antitheilerial: A review of its efficacy and safety. Proc.Workshop on East Coast fever immunization, Lilongwe, Malawi. Dolan T.T.(Ed.) ILRAD, Nairobi, pp.158-165.

Mitchell E.R.(1981). Management of insect pests with semiochemicals: concepts and practice. Plenum Press. N.Y.

Mukhebi A.W., Perry B.D. & Kruska R.(1991). Estimated economics of theileriosis control in Africa. **Prevent.Veter.Med., 12**:73-85

Mwangi E.N., Dipeolu O.O., Newson R.M., Kaaya G.P. & Hassan S.M.(1991). Predators, parasitoids and pathogens of ticks: a review. **Biocontr.Sc.Technol. 1**:147-156.

Mwangi E.N., Essuman S., Kaaya G.P., Nyandat E., Munyinyi D. & Kimondo G.M.(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. & Kimondo G.M.(1995b). Repellent and acaricidal properties of *Ocimum suave* plant against *Rhipicephalus appendiculatus* ticks. **Exper.Appl.Acarol. 19**(1):11-18.

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

Neville E.M.(1964). The role of carbon dioxide as a stimulant and attractant to the sand tampan *Ornithodoros savigyni*. **Onderst.J.Vet.Res. 31**:59-68.

Newsom L.D.(1967). Consequence of insecticide use on non-target organisms. **Ann.Rev.Ent. 12**:257-286.

Newson R.M.(1978). The life cycle of *Rhipicephalus appendiculatus* on the Kenyan coast. In: *Tick-borne diseases & their vectors*. Wilde J.K.H.(ed.). CTVM

Njau B.C., Nyindo M. & Mutani A.(1988). Acquired resistance in rabbits to immature stages of *Rhipicephalus evertsi evertsi*. **Vet.Res.Com. 12**:367-373.

Nolan J.(1990). Acaricide resistance in single and multi-host ticks and strategies for its control. **Parasitologia** 32:145-153.

Norval R.A.I., Andrew H.R., Yunker C.E.(1989a). Pheromone mediation of host-selection in bont ticks (*Amblyomma hebraeum*). **Science 243**:364-5.

Norval R.A.I., Butler J.F. & Yunker C.E.(1989b). 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. **Exper.Appl.Acarol. 7**:171-180.

Norval R.A.I., Lawrence J.A., Young A.S., Perry B.D., Dolan T.T. & Scott J.(1991a). *Theileria parva*: Influence of vector, parasite and host relationships on the epidemiology of theileriosis in southern Africa. **Parasitol. 102**:347-356.

Norval R.A.I., Perry B.D., Gebreab F.& Lessard P.(1991b). East Coast fever: A problem of the future for the horn of Africa? **Prevent.Veter.Med. 10**:163-172.

Norval R.A.I., Peter T., Yunker C.E., Sonenshine D.E. & Burridge M.J.(1991c). Responses of ticks *Amblyomma hebraeum* and *A.variegatum* to known or potential components of the aggregation attachment pheromone. I.Long range attraction. **Exper.Appl.Acarol. 13**:11-18.

Norval R.A.I., Yunker C.E., Duncan I.M. & Peter T.(1991d). Pheromone/acaricide mixtures in the control of tick *Amblyomma hebraeum*: Effects of acaricides on attraction and attachment. **Exper.Appl.Acarol. 11**:233-240.

Norval R.A.I., Sonenshine D.E., Meltzer M., Burridge M.J. and C.E. Yunker (1992b) An attractant decoy for controlling bont ticks. Patent pending.

Norval R.A.I., Perry B.D. & Young A.S.(1992a). The epidemiology of theileriosis in Africa. Acad.Press, London, 481pp.

Nyandat E., Hassanali A., Moreka L. and Lwande W., The study of efficiency trapping volatiles on porapack Q, reverse phase C_{18}-bonded silica, activated charcoal and glasswool from *Rhipicephalus appendiculatus* [in prep.]

Osburn R.L. and Knipling E.F. (1982). The potential use of sterile male hybrid *Boophilus microplus* as a supplemental eradication technique. **J.Med.Entom., 19**:637-644).

Otieno D.A., Hassanali A., Obenchain F.D., Sternberg A. & Galun R.(1985). Identification of guanine as an assembly pheromone of ticks. **Insect Sci.Appl. 6**(6):667- 670.

Perring P.H. & Mellanby K.(1978). Ecological effects of pesticides. Acad.Press, London. 194pp.

Punyua D.K., Newson R.M. & Mutinga M.J.(1985). Diurnal and seasonal activity of unfed adult *R. appendiculatus* in relation to some intrinsic and extrinsic factors. I. Factors regulating activity. **Insect Sci.Applic. 6**:63-70.

Purnell L.P. & Joyner R.(1972). Sterilization of *R. appendiculatus* ticks. Intern. J. Parasiltol. Vol. 58:345-347.

Rechav Y. & Whitehead G.B.(1978). Field trials with pheromone-acaricide mixtures for control of *Amblyomma hebraeum*. **J.econ.Entomol. 71**(1):149-151.

Rechav Y., Whitehead G.B. & Knight M.M.(1976). Aggregation response of nymphs to pheromone(s) produced by males of tick *Amblyomma hebraeum*. **Nature 259**:563-4.

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

Roberts J.A.(1971). Behaviour of larvae of the cattle tick *Boophilus microplus* on cattle of differing degrees of resistance. **J.Parasitol. 57**:651-656

Roelofs W.L. (1979). Establishing efficacy of sex attractants and disruptants for insect control. Ent.Soc.Amer, Maryland, 97pp.

Ryan M.F. and Byrne O. (1988). Plant-insect coevolution and inhibition of acetylcholinesterase. **J.chem.Ecol. 14**(10):1965-1975.

Sauer J.R., Hair J.A. & Houts M.S.(1974). Chemo-attraction in the lone star tick. 2. Responses to various concentrations of CO_2. **Ann.Ent.Soc.Am. 67**:150-152.

Schoni R., Hess E., Blum W. & Ramstein K.(1984). The aggregation-attachment pheromone of the tropical bont tick *Amblyomma variegatum*. Isolation, Identification and action of its components. **J.Ins.Physiol. 30**:27-30

Schoohoven L.M.(1982). Chemosensory basis of host plant selection. **Ann.Rev.Entomol. 13**:115-136.

Schumutterer H. (1990). Properties and potential of natural pesticides from the neem trees. **Ann.Rev.Entomol.**, **35**:271-297.

Shorey H.H.(1970). Sex pheromones of Lepidoptera. In: *Control of insect behavior by natural products*. Wood, Silverstein & Nakajima (Eds).Acad.Press, New York.

Shorey H.H.(1976). Animal communication by pheromones. Acad.Press, New York. 167pp.

Short, N.J. & Norval R.A.I. (1981). Regulation of seasonal occurrence in the tick *Rhipicephalus appendiculatus*. **Trop.Anim.Hlth.Prod. 13**:19-26.

Skinner W.A., Kosentreter U. & Eluard T.(1983). Tick repellents II. N-substituted azacyclopentanes and azacyclopentenones. **J.Pharm.Sci. 72**(11):1355-1356.

Sokal R.R. & Rohlf F.J.(1969). Biometry. The principles and practices of statistics in biological research. Freeman W.H. and Co, San Francisco, CA, 776pp.

Sonenshine D.E.(1984). Tick pheromones. In: *Current topics in pathogen-Vector-Host research* (Harris K.F.,Ed.) Praeger Press, New York, Vol.2, pp.225-263.

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

Sonenshine D.E.(1992). Biology of ticks. Plenum Press. New York. 354pp.

Sonenshine D.E., Silverstein R.M., Layton E.C. & Homsher P.J.(1974). Evidence for the existence of a sex pheromone in two species in ixodid ticks. **J.med.Entom. 11**:307-315.

Sonenshine D.E., Rechav Y., and Silverstein R.M.(1982a). Evidence of a sex pheromone in male of *Dermacentor variabilis*. J.Med.Ent. Vol.19:206-211.

Sonenshine D.E., Silverstein R.M., Rechav Y.(1982b). Tick pheromone mechanisms. In: *Physiology of ticks*. Obenchain F.D. & Galun R.(Eds).pp 439-468. Pergamon Press, Oxford.

Sonenshine D.E., Taylor D, & Carson (1986). Chemically mediated behavior in Acari: Adaptations for finding hosts and mates. **J.chem.Ecol. 12**(5):1091-1108.

Sonenshine D.E., Hamilton J.G.C. James S., Phillips K.P.E. & Norval R.A.I.(1992). Innovative techniques for control of tick disease vectors using pheromones. Proc.1st Intern.Conf.on tick-borne pathogens at the host vector host interface, Minnesota.

Sonenshine D.E., Khalil G.M., Homsher P.J. & Mason S.N.(1982a). *Dermacentor variabilis* and *D.andersoni*: Genital sex pheromones. **Exper.Paras. 54**:317-330.

Stutterheim C.J. & Brooke R.K.(1981). Past and present distribution of the yellow-billed oxpecker in south Africa. **South Afr.J.Zool. 16**:44-49.

Sutcliffe J.F.(1985). Black fly host location: a review. **Can.J.Zool. 64**:1041-1053.

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

Sutherst R.W. & Maywald G.F.(1985). A computerized system for matching climates in ecology. **Agric.Ecosyst.Environ. 13**:281-299

Sutherst R.W., Norton G.A., Barlow N.D., Conway G.R., Birley M. & Cummins H.N.(1979). An analysis of management strategies for cattle tick (*Boophilus microplus*) Control in Australia. **J.Appl.Ecol. 16**:359-382.

Sutherst R.W., Wharton R.H. & Utech K.B.W.(1978). Guide to studies on tick ecology CSIRO, Div.Entomol., CSIRO Techn. Pap. No 14, Australia, 59pp.

Takken W.(1991). The role of olfaction in host-seeking of mosquitoes: a review. **Insect Sci.Appl.** Vol. **12**, No 1/2/3/pp.287-295.

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

Tukahirwa E.M.(1976). The feeding behaviour of larvae, nymphs and adults of *R. appendiculatus*. **Parasitol. 72**:65-74.

Vale G.A.(1980). Field studies of the responses of tsetse flies and other Diptera to carbon dioxide, acetone and other chemicals. **Bull.Ent.Res. 70**:563-570.

Visser J.H.(1986). Host odor perception in phytophagous insect. **Ann.Rev.Entomol. 31**:121-44.

Waladde S.M.(1982). Tip-recording from ixodid tick olfactory sensilla: responses to tick related odours **J.Comp.Physiol.** A. **148**:411-418.

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

Waladde S.M. & Rice M.(1982). The sensory basis of tick feeding behavior. In: *Physiology of ticks*. Obenchain F.D. & Galun R.(eds.). Pergamon Press. Oxford. pp.71-118.

Waladde S.M., Ochieng S.A. & Gichuhi P.M.(1991). Artificial-membrane feeding of the ixodid tick *R. appendiculatus*, to repletion. **Exper.Appl.Acarol. 11**:297-306.

Walker J.B.(1974). The Ixodid tick of Kenya. Commonwealth Institute of Entomology, London.

Walker J.B., Norval R.A.I. & Corwin N.B.(1981). *Rhipicephalus zambeziensis* Sp.nov., a new tick from Eastern and Southern Africa, together with redescription of *R. appendiculatus*. **Onderst.J.Vet.Res. 48**:87-104.

Warnes M.L.(1990). Responses of *Glossina m. morsitans* and *G. pallidipes* to the skin secretions of oxen. **Bull.Ent.Res. 80**:91-97.

Weaver D.K., Dunkel F.V., Nteruzubanza L.,Jackson L.L. & Stock D.T.(1991). The efficacy of linalool, a major component of freshly-milled *Ocimum canum*, for protection against post-harvest damage by certain stored product coleoptera. **J.Stored Prod.Res. 27**(4):213-220

Webb J.P.(1979). Host locating behavior of nymphal *Ornithodoros concanensis*. **J.med.Entom., 16**(5):437-447

Wharton R.H. & Roulston W.J.(1970). Resistance of ticks to chemicals. CSIRO Div.Entomol., Long Pocket Lab.,

Wikel S.K.& Allen J.R.(1982). Immunological basis of host resistance to ticks In: *Physiology of ticks*. Obenchain F.D & Galun R.(Eds). Pergamon Press, Oxford, pp.169-195.

Wilson J.G., Kinzer D.R., Sauer J.R. & Hair J.A.(1972). Chemo-attraction in the lone star tick. 1. Response of different developmental stages to carbon dioxide administered via traps. **J.med.Entomol. 9**:254-252.

Wood W.F., Leahy M.G., Galun R., Prestwich G.D., Meinwald J., Purnell R.E. & Payne R.C.(1975). Phenols as pheromones of ixodid ticks: a general phenomenon? **J.chem.Ecol. 1**(4):501-509.

Yeoman G.H.(1966). Field vector study of epizootic East Coast fever. II Seasonal studies of *Rhipicephalus appendiculatus* on bovine and non-bovine hosts in East Coast fever enzootic, epizootic and free zones. **Bull.epiz.Dis.Afr. 14**:113-140.

Yeoman G.H. & Walker J.B.(1967). The Ixodid ticks of Tanzania. Commonwealth Institute of Entomology, London.

Yoder J.A., Pollack R.J. and Spielman (1993). An anti-diversionary secretion of ticks: first demonstration of an acarine allomone. **Insect Physiol., 39**(5):429-436.

Young A.S., Leitch B.L. & Newson R.M.(1981). The occurrence of a *Theileria parva* carrier state in cattle from an East Coast fever endemic area of Kenya. Proc.Intern.Conf. ILRAD, Nairobi. Irvin A.D., Cunningham M.P. & Young A.S.(Eds), Martinus Nijhoff Publrs, The Hague, pp.60-62.

Young A.S., De Castro J.J. & Kiza-Aura P.P.(1985). Control of African ticks by application of acaricide impregnated ear tags to cattle. **Bull.Ent.Res. 75**:609-619.

Young A.S., Leitch B.L., Newson R.M. & Cunningham M.P.(1986). Maintenance of *Theileria parva* infection in an endemic area of Kenya. **Parasitol. 93**:9-16.

Zimmerman R.H., Garris G.I. & Beaver J.S.(1984). Potential of *Stylosanthes* plants as a component in an integrated pest management approach to tick control. **Prevent.Veter.Med.** 2:579-588.

Ziv M., Sonenshine D.E., Silverstein R.M., West Jr & Gingher K.H.(1981). Use of sex pheromone, 2,6-dichlorophenol, to disrupt mating by American dog tick, *Dermacentor variabilis*. **J.chem.Ecol.** 7(5):829-840.