MECHANISMS OF LOCATION OF *Amblyomma variegatum* (FABRICIUS) AND OTHER LIVESTOCK TICKS BY THE PARASITOID *Ixodiphagus hookeri* HOWARD.

by

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A thesis submitted in partial fulfilment of the

requirements for the degree of

Doctor of Philosophy

Department of Biological Sciences

Faculty of Science

University of Zimbabwe

June 1997

DECLARATION

I hereby declare that the material contained in this thesis is my own original work and has not been submitted for a degree in any other University.

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APPROVAL

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To my late father, mother, wife and sisters
whose love, interest and support were fountains of inspiration to me

ABSTRACT

The control of *Amblyomma variegatum* and other economically important ticks, like *Rhipicephalus appendiculatus,* has relied mainly on the use of acaricides worldwide. However, due to the drawbacks of chemical control, future tick control will have to move more in the direction of integrated approaches emphasizing more regular use of environmentally-friendly non-chemical methods, such as tick parasitoids.

Ixodiphagus hookeri, one of the seven species of tick parasitoids known, occurs naturally in Kenya and is specific to *A. variegatum* ticks. This study investigated the host location cues used by the parasitoids, visual evaluation and recognition of hosts, acceptance of *A. variegatum* and *R. appendiculatus* nymphs by the parasitoids for oviposition as well as the suitability of the two tick species for the development of parasitoid immatures. Observations were also made on the host seeking and dispersal behaviour of the parasitoids both in the laboratory and the field.

Experiments carried out in a Y-tube olfactometer showed that *I. hookeri* females were not attracted to grass odours (*Cynodon dactylon*, *Eragrostis superba* & *Digitaria seriata*). Cattle waste odours (urine and dung) and odours from tick-free dewlaps and heels were attractive to the parasitoids. Odours from tick-free cattle ears and scrota were not attractive. Off-host *A. variegatum* nymphs did not attract the parasitoids, while feeding nymphs were attractive.

Experiments done in a T-tube olfactometer showed that hexane washes and faeces of *A. variegatum* nymphs were attractive to the parasitoids. Gas chromatographic analyses of *A. variegatum* and *R. appendiculatus* washes and faecal extracts showed that there were differences in the chemical composition of volatiles emitted by the two tick species.

Petri-dish/vial bioassays revealed that the parasitoids recognized and evaluated the sizes, feeding status and species of hosts visually before contact. Movement and colour were not important in visual host recognition.

Glass vial experiments showed that the parasitoids oviposited more in host nymphs than in non-host nymphs. The conditioning of *R. appendiculatus* nymphs with *A. variegatum* nymphal odours and integument prolonged the time these nymphs were attacked by the parasitoids, however, oviposition was rare. Scanning electron microscopy revealed differences in the appearance of the integument surfaces of the two tick species. All *R. appendiculatus* and unfed *A. variegatum* nymphs attacked were not suitable for the development of *I. hookeri* immatures while fed *A. variegatum* nymphs attacked yielded progeny. *I. hookeri* females spent less time ovipositing in parasitized than in unparasitized hosts. Dermal gland secretions from mechanically disturbed fed *A. variegatum* nymphs had a short-lived repellent effect on the parasitoids.

In the field, parasitoids searched longer on cattle heels for hosts compared to other parts of the legs. *A. variegatum* nymphs collected from cattle heels were more parasitized than nymphs collected from the other body regions. In the field and wind tunnel, the parasitoids dispersed through crawling, jumping and flight. Jumping was more common while flight was rare. Release distances did not significantly influence the numbers of parasitoids reaching the odour sources.

CHAPTER 1: GENERAL INTRODUCTION

1.1. The tick problem

Ticks have been recognized as ectoparasites of domestic animals since ancient times. The earliest reference to ticks is from an Egyptian papyrus scroll dated 1550 BC (Obenchain & Galun, 1982). Globally, ticks surpass all other arthropods in the number and variety of diseases which they transmit to domestic animals and wildlife while they also act as vectors of human diseases (Obenchain & Galun, 1982). Estimates of economic losses due to ticks and tick-borne diseases are in the billions of dollars worldwide, although precise figures for most of the countries are lacking. Ticks affect approximately 800 million cattle and a similar number of sheep throughout the world (Sutherst *et al.*, 1982).

In Africa about 90% of the currently estimated 200 million cattle are infested with ticks, a significant proportion (70%) with multiple tick species (Dipeolu *et al.*, 1992). Annual global loss resulting from livestock death or diminished productivity was estimated in 1979 to be US$ 7,000 million (McCosker, 1979). Dipeolu (1991) estimated the overall loss in Africa due to ticks at US$ 720 million per year, while McCosker (1979) believes that Africa's annual global loss in livestock production attributed to ticks is greater than those of the rest of the world put together. Debilitating diseases transmitted by ticks include theileriosis, heartwater, cutaneous streptothricosis, babesiosis, anaplasmosis, Nairobi sheep disease and Q fever. Besides the diseases they transmit, ticks may cause direct economic damage to livestock. Tick infestations can cause milk and weight loss, damage hides, and predispose animals to bacterial and fungal infections, as well as screw-worm attack (Bram, 1983; Norval *et al.*, 1992a). It has been estimated that, on average, one female tick can cause weight loss of four grams in cattle (McCosker, 1979).

Africa is a continent renowned for its wild and domestic animal diversity. The major domestic livestock are cattle, sheep, goats, camels, pigs, water buffalo, horses, mules, donkeys and poultry (Norval *et al.*, 1992b). The livestock are a primary investment resource, which generate food (meat, dairy products), cash

income, fuel, manure, draught power for crop production, clothing, employment and capital stock. These livestock are also a store of wealth which provides a sense of security, prestige, social status and cultural value (Norval *et al.*, 1992b). Livestock will continue to be a major component of African farming for the foreseeable future due to the traditional values of the farmers, the climate and the terrain (Young *et al.*, 1988). As ticks and tick borne diseases occur in areas of highly productive land in Africa, it is on this continent that they present the greatest problem (Young *et al.*, 1988).

The majority of people living in Africa are subsistence farmers and a greater development of the livestock industry is required in Africa than any other continent (Young *et al.*, 1988). Of the developing world, the African continent has 17% of the cattle and 26% of the small ruminant populations; these percentages change to 11 and 17% respectively when compared to world populations (FAO, 1986a,b). The human population of Africa represents 15.3% of the developing countries' and 11.5% of the world's population (FAO, 1986a,b). In tropical Africa, the self-sufficiency ratio for meat and milk has declined during the last twenty years (Tacher, 1986). The ratio for meat was 1.0 for the period 1961-1977 and it is expected to decline to 0.6 or 0.4 by the year 2000. The ratio for milk was 0.9 in 1961-1965 and it is projected to decline constantly to 0.4 or 0.3 by the year 2000 (ILCA, 1980). Hence, it is imperative for livestock production to be improved in the whole of Africa. The tick problem is aggravated by the occurrence of large populations of wild ungulates such as buffalo (*Syncerus caffer*), giraffe (*Giraffa camelopardalis*), eland (*Taurotragus oryx*), greater kudu (*Tragelaphus strepsiceros*) etc., (Dinnik *et al.*, 1963; Yeoman & Walker, 1967; Ferrar & Kerr, 1971) maintaining both tick vectors and tick borne diseases (Grootenhuis & Young, 1981).

1.2. Economic importance of *Amblyomma variegatum* and other livestock ticks

1.2.1. *Amblyomma variegatum*

In sub-Saharan Africa, as many as 175 million cattle may be exposed to cowdriosis (Latif, 1992). Of the twelve species of *Amblyomma* known to be capable of

ACKNOWLEDGEMENTS

My first vote of thanks go to the Heavenly Father who gave me the strength and courage to successfully produce this thesis. I am also very grateful to my University of Zimbabwe (UZ) supervisors, Dr. Edna Kunjeku and Mrs. Audrey Mabveni, and my International Centre of Insect Physiology and Ecology (ICIPE) supervisors, Dr. Esther Mwangi and Prof. Ahmed Hassanali for their sustained interest, guidance and encouragement.

Special thanks go to the African Regional Postgraduate Programme in Insect Science (ARPPIS) coordinated by Dr. Vitalis Musewe, ICIPE, UZ and the German Academic Exchange Service (DAAD) for their superb collaboration which gave me a chance to do this project.

Thanks also go to Messrs. Joseph Mugane, Edwin Njamura, Byrum Wabwoba and Owen Olukohe (Technicians, Tick Biology); and Messrs. Edward Nyandat, Lamberts Moreka, James Mbai, Dalmas Omondi and Willis Bundi (Technicians, Chemical Ecology) for the technical assistance, interest and advice while Mr. Onesmus Wanyama is thanked for assisting in the GC-MS analyses. Mr. David Munyinyi and Dr. Adedapo Odulaja are sincerely thanked for statistical consultations. Thanks also go to Dr. Mary Owaga for helping with the identification of the grasses, and to Mr. John Mwangi (National Museums of Kenya) for the identification of the ants. Further thanks go to Dr. Shawgi Hassan for fruitful discussions in tick ecology.

Appreciation also go to Messrs. Fred Thuo and Patrick Muchisu for supplying the ticks for this study while Dr. Morris Odindo and the staff of the large animal unit are thanked for the supply of the rabbits. Drs. William Overholt and Adele Ngi-song are thanked for the loan of the Y-tube olfactometer set-up and the interest they have shown in this study. Thanks Drs. Hassane Mahamat and Wilber Lwande for the permission to use the wind tunnel.

Thanks also go to Mrs. Joyce Mureithi and Mr. Peter Lisamula for scanning the tick integument and printing the micrographs, respectively. Thank you all ARPPIS colleagues and friends for being so supportive during my study since we were all going through the same problems.

A special vote of thanks go to my dearest wife, Sylvia, for her patience, understanding as well as the constant moral support and love. Finally, I wish to express my sincere gratitude to my late father, mother and sisters for their support, prayers and encouragement.

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transmitting *Cowdria ruminantium*, the tropical bont tick, *A. variegatum* is undoubtedly the most important (Walker & Olwage, 1987). Besides heartwater, this three-host tick can also transmit Nairobi sheep disease and can harbour Q fever of man and animals. In addition, its bites are severe and may result in the formation of septic wounds and abscesses, inflammation of the teats of cows and considerable damage to hides and skins (Walker, 1970). The bite wounds have been shown to form a route of infection for bovine lymphangitis. Bites of this tick also cause severe irritation and inflammation in humans (Walker, 1970).

A. variegatum is not only an extremely efficient vector but it is also far more widely distributed than the other important *Amblyomma* species (Walker & Olwage, 1987; Fig. 1.1). In Africa, *A. variegatum* occurs south of the Sahel, right across the continent from Senegal through west Africa, the Central African Republic, southern Sudan and Ethiopia to the extreme north-western tip of Somalia. It is absent from the desert areas of the Horn of Africa but is prevalent in most of eastern Africa (Kenya, Uganda, Tanzania, Rwanda and Burundi), as well as Malawi, much of Zambia, Zaire and eastern Angola. South of Zambia its range extends into the Caprivi strip of Namibia, north-eastern Botswana, north-western Zimbabwe and southern and central Mozambique. *However, A. variegatum* is excluded from arid zones (Walker & Olwage, 1987; Norval *et al.*, 1992b). *A. variegatum* has also been introduced into Madagascar with cattle imported from mainland Africa and now occurs virtually throughout the island (Uilenberg *et al.*, 1979 in Walker & Olwage, 1987).

Cattle are the most important domestic hosts of all stages of the life cycle of *A. variegatum* (MacLeod & Colbo, 1976). On cattle, *A. variegatum* adults and nymphs feed mainly on the lower dewlap, brisket, abdomen, axillae and genitalia (Hoogstraal, 1956; Yeoman & Walker, 1967; Walker, 1974; Norval *et al.*, 1992b). Nymphs also attach on the legs, especially around the hooves (Yeoman & Walker, 1967; Walker, 1974). Larvae are widely distributed over the body (Hoogstraal, 1956; MacLeod *et al.*, 1977; Kaiser *et al.*, 1982). Sheep, goats and other domestic animals also become parasitized but to a lesser degree (Hoogstraal, 1956).

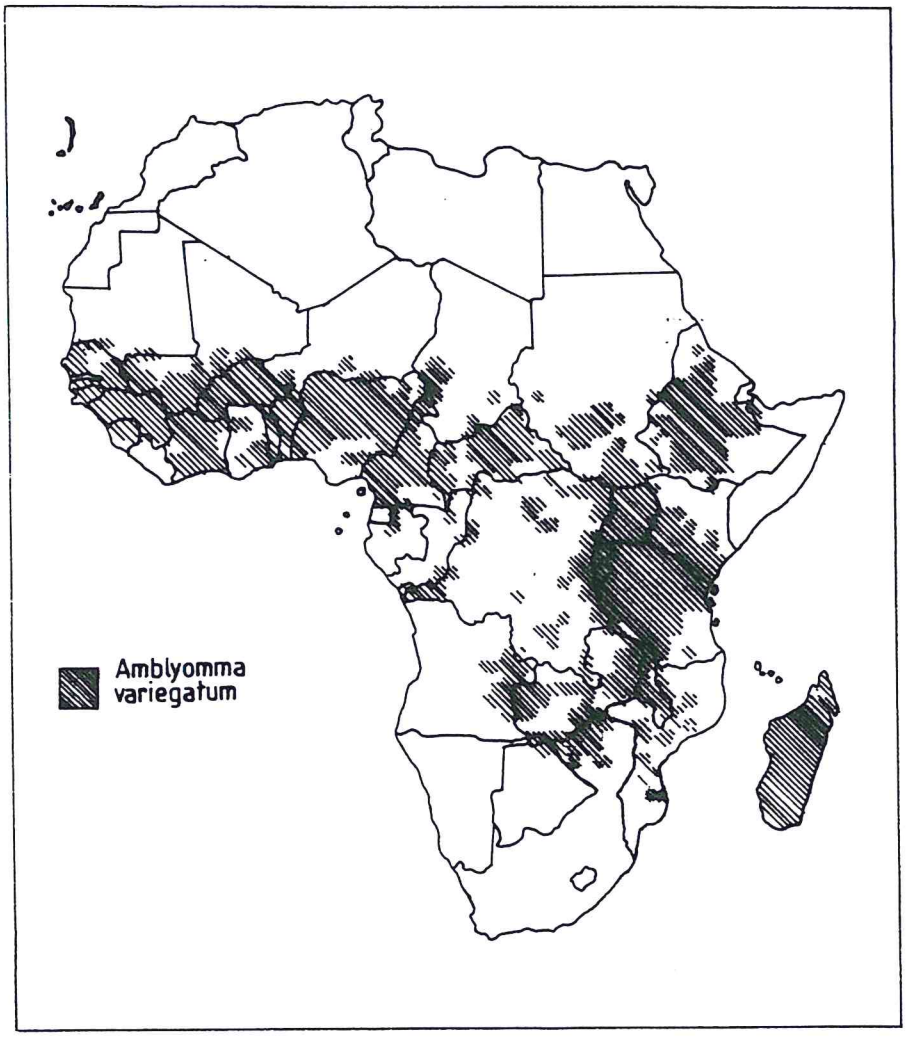

Fig. 1.1. Distribution of *A. variegatum* in Africa (from Norval *et al.*, 1992b)

The wild hosts parasitized by adult *A. variegatum* are mainly medium and large herbivores, the most important being buffalo (*Syncerus caffer*). The immature stages have a wider host range and, in addition to herbivores, parasitize a wide variety of small mammals, birds and lizards (Hoogstraal, 1956).

1.2.2. *Rhipicephalus appendiculatus*

R. appendiculatus is the major vector of *Theileria parva*, the causative organism of East Coast Fever (ECF) in cattle. In Africa, 38% of the total cattle population is in the ECF-affected areas (eleven countries of eastern, central and southern Africa)(ILRAD Annual Scientific Report, 1990; Norval *et al.*, 1992b). Theileriosis of cattle in Africa, particularly ECF, has undoubtedly had more impact on the development of the beef and dairy cattle industries in Africa than any other livestock disease complex (McCosker, 1991).

R. appendiculatus can also carry *T. mutans* and *T. lawrencei* (Walker, 1970), and is also associated with babesiosis, toxicosis, Nairobi sheep disease and tick-bite fever of man (Barnett, 1961; Walker, 1970). Moreover, anaemia and wounds through which secondary infections may occur account for debilitating effects on cattle, while damage caused on hides result in their depreciation in value (Barnett,1961). Furthermore, heavy infestations can cause mortality among several antelope species and predispose them to infestation with screw-worm fly (*Chrysomya bezziana*) larvae (Ferrar & Kerr, 1971; Lewis, 1981; Lightfoot & Norval, 1981; Norval & Lightfoot, 1982).

The distribution of *R. appendiculatus* in Africa has been assembled by Lessard *et al.* (1990)(Fig. 1.2). This tick has been collected from 15 countries, including, from north to south, the Central African Republic, Sudan, Zaire, Uganda, Kenya, Rwanda, Burundi, Tanzania, Zambia, Malawi, Mozambique, Zimbabwe, Botswana, Swaziland and South Africa. However, the distribution of *R. appendiculatus* is not continuous, even in those countries in which it occurs, mainly because of climate, vegetation and host availability.

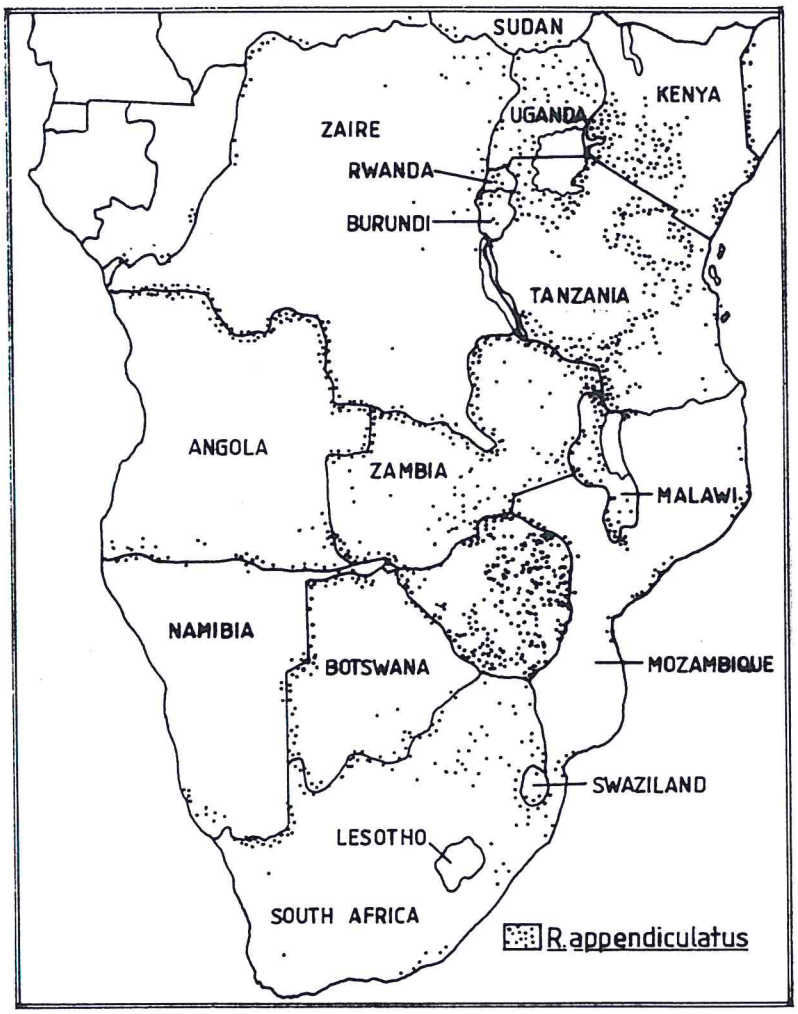

Fig. 1.2. The distribution of *R. appendiculatus* in Africa (from Norval *et al.*, 1992b)

R. appendiculatus has a wide host range. Adults parasitize medium to large size ungulates while the immature stages feed on most ungulate species and a wide variety of other mammals, including carnivores and lagomorphs (Norval *et al.*, 1992b). Among domestic animals, cattle are the major hosts and become heavily infested with larvae, nymphs and adults. Sheep, goats, horses, donkeys and mules are also parasitized but not usually to the same extent. Several wild ungulate species such as buffalo (*Syncerus caffer*), giraffe (*Giraffa camelopardalis*), eland (*Taurotragus oryx*), greater kudu (*Tragelaphus strepsiceros*), bushbuck (*Tragelaphus scriptus*), waterbuck (*Kobus ellipsiprymnus ellipsiprymnus* and *K. ellipsiprymnus defassa*), sable antelope (*Hippotragus equinus*), impala (*Aepyceros melampus*), reedbuck (*Redunca arundinum* and *R. redunca*), klipspringer (*Oreotragus oreotragus*) and duiker (*Sylvicapra grimmia*) can also become heavily infested (Dinnik *et al.*, 1963; Yeoman & Walker, 1967; Ferrar & Kerr, 1971; Walker, 1974; Lewis, 1981; Lightfoot & Norval, 1981; Matthysse & Combo, 1987; Colborne, 1988). Adults attach in large numbers on the ears of their hosts but are also found on other parts of the body (Norval *et al.*, 1992b). Larvae and nymphs show much less preference for the ears and frequently attach on other parts of the body such as the head, legs, neck and dewlap (Baker & Ducasse, 1967; Kaiser *et al.*, 1982; Colborne, 1988).

1.3. Tick control

1.3.1. Acaricides

The introduction of exotic cattle breeds to the African continent during the nineteenth century demanded intensive tick control. This consisted of acaricide application aimed at keeping animals totally free of ticks to prevent transmission of pathogens causing tick-borne diseases; this usually involved frequent applications of acaricides throughout the year. This practice expanded rapidly to include indigenous cattle, and in many countries was enforced through legislation (Pegram *et al.*, 1995).The practice was continued on a wide scale until the past decade, when financial constraints, coupled with increasing cost of the acaricides made it prohibitively expensive (Pegram *et al.*, 1995).

Chemicals to control ticks are applied topically to cattle by spraying or dipping, pour-on (acaricide applied topically along the mid-line (backbone) of the animal), or by impregnated devices such as ear tags, ear bands, horn bands, neck bands and tail decoys. Some compounds can be administered systemically as low-level feed additives, or in the form of oral sustained-release boluses, or may lend themselves to formulation as controlled-release injections (Drummond *et al.*, 1981; Schröder, 1987; Radostits *et al.*, 1994). However, because populations of the most economically important ticks are maintained by wildlife, the topical, ingestible and injectable acaricide formulations currently available for pets and domestic livestock are of limited value (Schmidtmann, 1994).

Self-medicating methods use certain aspects of host behaviour to deliver acaricides in a highly selective manner, thereby minimizing the amounts of acaricide dispersed into the environment and to the host (Sonenshine, 1993). For example, Damminix (promising method for controlling *Ixodes dammini* in the United States), which consists of simple biodegradable cardboard tubes containing permethrin-impregnated cotton, can be placed in the host habitat. White-footed mice use cotton to line their nests, thereby treating themselves and their nest mates with acaricides (Sonenshine, 1993). The development of delivery systems for applying acaricide to wildlife is promising and needed in developing host-targeted tick control measures (Schmidtmann, 1994). A self-medicating device developed in Zimbabwe for treating livestock, wild antelope and other wild ungulates delivers an oily liquid from an overhead reservoir due to the shaking of the pole supporting it as the animal inserts its head to seize food from a container at its base (Duncan & Monks, 1992).

Despite the obvious need, tick control is often a formidable and costly task. Some of the greatest obstacles had been pointed out by Sonenshine (1993) as:

(1) the extremely wide dispersal of most ticks in the vegetation, making it difficult for acaricides to reach them without also contaminating the natural environment with unacceptably large quantities of toxicants;

(2) the attached, fixed position of the ticks while feeding on a host, requiring whole body treatment to reach even the most sheltered locations;

(3) the secretive habits of many tick species, especially argasid ticks, hiding in caves, burrows, cracks, crevices and other shelters where acaricides cannot reach them;
(4) the immense reproductive capacity of ticks, which requires frequent treatment of animals or habitats to overcome the continuing threat of reinfestation; and
(5) the remarkable longevity of most ticks, enabling them to persist unseen for months or even years following attempts to control them.

Additional factors, such as the plasticity of their host selection process, the ability of certain species to suppress the host's homeostatic mechanisms and/or evade host immune responses, the appearance of acaricide resistance and the ability of many species to survive abnormally harsh environmental conditions present further obstacles to tick control (Sonenshine, 1993). Early reports documented examples of tick resistance to cyclodiene acaricides, lindane and, occasionally, organophosphorous acaricides (Nolan, 1990). Recently the first evidence of resistance to pyrethroids appeared in cattle ticks in Australia (Nolan *et al.*, 1989). Resistance is one of the factors stimulating interest in alternative methods of tick control, or at least, in methods for minimizing the amounts of acaricide required to control ticks (Sonenshine, 1993). Furthermore, the contamination of the environment with toxins, contamination of milk and meat, and yet unassessed threat to biodiversity resulting from the interaction of ticks and host animals with non-target organisms, such as oxpeckers, are some of the factors counting against excessive acaricide usage (Wharton & Norris, 1980; Matthewson, 1984; Hassanali, 1994).

Annual importation costs of acaricides have been estimated at US$ 6-10 million for Kenya (Chema, 1984), US$ 10 million for Zambia (Pegram *et al.*, 1988) and US$ 150 000 for Burundi (Niyonzema and Klitz, 1986). Attempts to control ticks in the vegetation by spraying acaricides from aeroplanes or machine-powered sprayers followed soon after the success achieved in controlling crop pests by these methods. Early examples include attempts to control cattle ticks, *Boophilus annulatus*, in the southern United States; and American dog ticks in Long Island,

New York (Glasgow & Collins, 1946) and Nova Scotia, Canada (Mckiel *et al.*, 1967). However, whereas the highly focussed distribution of crop pests in precisely defined open fields made those pests vulnerable to aerial or mechanical sprayers, tick distribution is very different (Sonenshine, 1993). Most ticks, especially ixodid ticks, are dispersed throughout the vegetation of their habitats and, even while questing, are sheltered in microhabitats under a protective cover of trees, bushes or other wild plants, often in complex, mixed vegetative communities. Although certain acaricide formulations can penetrate dense ground cover to kill ticks, large quantities need to be applied periodically leading to increasing environmental contamination and, eventually, growing public resistance to these methods (Sonenshine, 1993). Most farmers in Africa are resource-poor and it is therefore important that pest control strategies are aimed at making pest control affordable and readily available to these farmers.

1.3.2. Habitat modification and other cultural practices

Habitat modification alters the natural habitat where ticks survive and quest for hosts. This strategy includes mechanical clearing of ground cover and understorey vegetation, removal of leaf litter and in some cases, even partial removal of the forest canopy to expose the ground level to intense sunlight, herbicidal treatment or controlled burning to kill vegetation and all stages of ticks on grazing land. Habitat modification does not require broad scale release of dangerous toxicants or slaughter of wildlife. However, it is labour intensive and must be repeated to prevent regrowth of the vegetation in which ticks thrive (Sonenshine, 1993). Although burning also makes way for new grasses to sprout and replace the dried pasture after rains, pasture agronomists frown at this since it destroys the remaining pasture available to livestock during the dry season and also exposes these soils to erosion and desertification.

Hand picking of ticks is favoured by the pastoralists since it is time saving and can be carried out at any time and in conjunction with other husbandry practices such as milking (Maina, 1986).

Hand picking is practised with some measure of success where tick infestation is light, such as in zero grazed animals. However, it is directed at engorging female ticks thereby leaving the larvae and nymphs which are usually smaller in size but are capable of transmitting a good number of tick-borne diseases (Fasanmi & Onyima, 1992).

1.3.3. Host eradication and pasture spelling

Host eradication is one of the earliest attempts to control tick-borne diseases by destroying the tick host and was carried out in Montana in an effort to control Rocky Mountain spotted fever (Sonenshine, 1993). Beginning in 1911, thousands of Columbian ground squirrels, pine squirrels, chipmunks and other small rodent hosts of *Dermacentor andersoni* were killed by trapping, shooting and dissemination of poisoned baits. Eventually, the practice was discontinued as ineffective and extremely costly (Cooley, 1932). However, host eradication has been successful where the target environment was isolated and reinvasion by the same host species was precluded.

Deprivation of hosts by excluding them from selected areas (pasture spelling) has been suggested as an alternative to dipping for effective tick control (Sutherst *et al.*, 1978). This strategy is relatively easy to use with livestock , where herds of domestic stock may be kept from a specific, tick-infested pasture for several years (Sonenshine, 1993; Schmidtmann, 1994). Since mating of most ixodid ticks occurs on the host, mating interference due to host deprivation provides additional opportunities for tick control. Furthermore, with less numbers of ticks on livestock and with infrequent dipping (Whitnall & Bradford, 1947; Harrison, 1950), pasture spelling is believed to gradually replace resistant ticks by less resistant strains (Wilkinson, 1957). However, pasture spelling requires the farmer to divide the ranch into paddocks, thus investing in fencing, high level managerial input and water supply (Fasanmi & Onyima, 1992).

At the level of the pastoralist, pasture spelling is compounded by the fact that pastoralists have no grazing land of their own and available grazing land is used for

livestock grazing throughout the year, regardless of tick-infestations. On the other hand, small scale farmers in Africa own small portions of land which are not large enough for pasture spelling while very few of these farmers can afford to purchase fencing material. Pasture spelling is therefore recommended for established and organized farms with the necessary capital and management skills (Fasanmi & Onyima, 1992).

1.3.4. Acquired resistance of livestock to ticks, anti-tick vaccines and immunity

The repeated feeding of ticks on a host commonly leads to a progressive expression of resistance. This acquired resistance is characterized by a longer duration of tick attachment, reduced engorgement weight, lessened fecundity, impaired viability of eggs and tick death (Wikel, 1988). Research efforts have capitalized on the above-mentioned effects of acquired resistance on ticks through developing an anti-tick vaccine that will trigger the host's immune system to interfere with or suppress tick feeding. Although prospects for the production of anti-tick vaccines through the use of molecular techniques and tick antigens other than salivary gland secretions are promising, only one anti-tick vaccine is known thus far. Australian scientists have developed a vaccine against *Boophilus microplus*. It has been found that vaccination leads to the death of ticks both on the host and after engorgement (Kemp *et al.*, 1986). Immunization of livestock against ticks using concealed antigens (antigens not normally encountered by the animal during tick feeding) is another form of tick control. Most of these antigens are obtained from tick internal organs and haemolymph (Mbogo *et al.*, 1992).

To be able to immunologically attack these concealed antigens which are in the tick haemolymph, the animal antibodies must cross the tick midgut. Since the vaccination of cattle with extracts derived from whole *Boophilus microplus* ticks caused extensive gut damage in these ticks when fed on vaccinated cattle (Kemp *et al.*, 1986; Agbede & Kemp, 1986), it is postulated that the passage of animal antibodies across the tick midgut into the haemolymph may be increased if ticks

feed on animals immunized with concealed antigens (Mbogo *et al.*, 1992).

1.3.5. Reproductive interference

Radiation-induced sterility in males (SIT, i.e. sterile insect technique) has been used with great success in eradicating the screwworm, *Cochliomyia hominivorax*, from the United States (Knipling, 1979). SIT has also been used successfully to eradicate tsetse flies from Burkina Faso (Clair *et al.*, 1990) and Nigeria (Olandunmade *et al.*, 1990). Limitations of SIT for suppressing tick populations are: the need for mass rearing of ticks for inundative releases and concern that released ticks may cause further economic losses or transmit diseases (Sonenshine & Matter, 1994).

Hybrid sterility is the result of mating among similar species of ticks giving rise to sterile offspring. Furthermore, subsequent mating between hybrid males and wild-type females results in the production of non-viable eggs (Sonenshine & Mather, 1994). Interspecific matings resulting in sterile hybrids have been reported for several tick species, including *B. microplus* and *B. annulatus* (Graham *et al.*, 1972), *B. microplus* and *B. decoloratus* (Spickett & Malan, 1978), *A. variegatum* and *A. hebraeum* (Rechav *et al.*, 1982), and at least under laboratory conditions between *R. appendiculatus* and *R. zambeziensis* (Wouters, 1990).

Tick pheromones, especially the attraction, aggregation and attachment pheromone (AAAP) lead to finding of suitable hosts, pairing of sexes, copulation and spermatophore transfer (Schmidtmann, 1994). Hosts on which sexually mature (fed) males are present attract unfed adults and nymphs from the surrounding habitat, whereas potential hosts on which no fed males are present do not attract these ticks (Norval *et al.*, 1994). In agricultural situations where regularly dipped cattle and untreated alternative wildlife hosts share the same pastures, unfed adults and nymphs attach in much larger numbers on the alternative hosts than on the cattle as AAAP-producing males will be feeding on wildlife hosts (Norval *et al.*, 1994). AAAP have been used in pheromone-baited acaricides to attract and kill female *A. maculatum* ticks (Gladney *et al.*, 1974).

Cytoplasmic incompatibility and distorted sex ratios are other methods available for reproductive interference (Knipling *et al.*, 1968; Spickett, 1987; Sonenshine, 1993; Schmidtmann, 1994). Cytoplasmic incompatibility occurs when individuals from diferent populations are crossed resulting in reduced reproductive potentials. The reduction is due to incompatibility factors in the cytoplasm causing sterility in individual eggs (Pedigo, 1996). The sperm enters an egg and stimulate meiosis, but does not fuse with the egg pronucleus to form a zygote (Pedigo, 1996). The selective killing of a particular sex (e.g females killed through an AAAP-baited acaricide) will lead to less members of that sex in the population (distorted sex ratio) and the subsequent failure of the opposite sex to locate mates for sucessful reproduction.

1.3.6. Biological control

Biological control is the direct or indirect human manipulation of living natural organisms on pest organisms in deliberate attempts to reduce populations of the pest to such levels that economic damage is eliminated or significantly reduced (Beirne, 1962). In tick biological control, anti-tick plants, predators, pathogens, natural host resistance to tick infestations and parasitoids are used. Biological control of ticks has almost always been presented as the antithesis of chemical control (Spickett, 1987). The role of biological agents in regulating tick populations is poorly appreciated, and for the most part poorly investigated (Schmidtmann, 1994). Scientists have been encouraged to research the use of biological methods of control, the main drive coming from socio-economic and environmental pressures resulting from the alternative, namely, the use of toxic and increasingly expensive chemicals (Spickett, 1987). The drawbacks of acaricide application have made the development of cheap, effective and environmentally acceptable tick control methods a continuing priority in Africa (Amoo *et al.*, 1993).

For several decades it has been known that the molasses grass, *Melinis minutiflora* possesses tick-repellent properties. Columbian farmers reported that cattle grazing molasses grass carried fewer ticks than those on other improved or

native pastures (Thompson *et al.*, 1978). In an experiment conducted with six grass species (i.e. *Cynodon dactylon* (star grass), *Brachiaria decumbens* (signal grass), *Melinis minutiflora* (molasses grass), *Pennisetum clandestinum* (Kikuyu grass), *Hypparrhenia rufa* (Jarugua grass) and *Andropogon gayanus* (Gamba grass)), *M. minutiflora* and *A. gayanus* were found to have the highest anti-tick repellent properties for larvae of *Boophilus microplus* (Thompson *et al.*, 1978).

Other plants possessing anti-tick properties belong to the genus *Stylosanthes*. These are highly nutritious varieties of tropical legumes covered with glandular hairs (trichomes) that secrete a viscous fluid capable of entrapping ticks (Sutherst *et al.*, 1982). Plants with acaricidal constituents include tobacco, camphor, derris and turpentine (Cremlyn, 1978). Rotenoids from *Derris elliptica*, *Lonchcarpus laxiflorus* and *Tephrosia vogelii* and pyrethrins from *Chrysanthenum cinerariaefolium* have also been studied for acaricidal properties (Cremlyn, 1978; Matthewson, 1984). Microencapsulation of pyrethrin protects it from sunlight and increases its persistence as a toxicant (Simpkin & Galun, 1983). An oil extracted from the leaves of a tropical shrub, *Ocimum sauve,* has been shown to repel as well as kill all stages of *R. appendiculatus* (Mwangi *et al.*, 1995), while the shrub, *Gynandropsis gynandra* repels and kills ticks (Malonza *et al.*, 1992).

Birds like the red-billed oxpecker (*Buphagus erythrorhynchus*), yellow-billed oxpecker (*Buphagus africanus*), cattle egret (*Ardeola ibis*), pee-wee (*Grallina cyanoleuca*), starling (*Sturnus vulgaris*), magpie (*Pica pica*), Indian myna (*Acridotheres tristis*), American robin (*Turdus migratorius*), African pied wagtail (*Motacilia agwimp*) and the superb starling (*Spreo superbus*) are known to prey on ticks either on or off the host (reviewed by Mwangi *et al.*, 1991). Domestic chickens have also been reported to predate effectively on ticks in cattle sheds (Mwangi, 1990) and those attached to cattle ears (Hassan *et al.*, 1989) with indigenous African chickens being the most effective.

Tortoises, ants, spiders, lizards, rodents and shrews are also known to prey on ticks. Pathogens like *Proteus mirabilis*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus thuringiensis*,

Aspergillus fumigatus, *Beauveria bassiana*, *Penicillium insectivora*, *Aspergillus parasiticus*, *Beauveria tenella*, *Cephalosporium coccorum*, *Paecilomyces fumosoroseus*, *Metarhizium anisopliae*, *Rickettsia prowazeki*, *Wolbachia persicus* and nematodes belonging to the families Steinernematidae and Heterorhabditidae have been found to be capable of killing ticks (reviewed by Mwangi *et al.*, 1991).

It has long been recognized that various breeds of cattle differ in their response to tick infestations. Some breeds have the ability to reduce the number of ticks they carry and are considered naturally resistant, while the sensitive breeds cannot control the number of ticks on them (Rechav, 1992). Zebu, *Bos indicus*, cattle are regarded as most resistant to tick infestations when compared to European breeds, *Bos taurus* (Elder *et al.*, 1980).

Hymenopteran wasps (parasitoids) are also known to control ticks (Spickett, 1987; Mwangi *et al.*, 1991; Schmidtmann, 1994). These free-living wasps oviposit only in larval and nymphal ticks. The eggs of tick parasitoids lie dormant through periods of tick inactivity and moulting, hatch with blood feeding, and the developing larvae then consume the internal contents and pupate in the corpse (Schmidtmann, 1994). Female parasitoids lay multiple eggs per tick and oviposit in more than one tick (Cole, 1965; Schmidtmann, 1994).

1.3.6.1. Tick parasitoids

So far, all tick parasitoids described are hymenopteran wasps belonging to the family Encyrtidae in the superfamily Chalcidoidea. The first tick parasitoid to be identified was *Ixodiphagus texanus* Howard. This parasitoid was collected from the nymphal stages of the ticks *Haemaphysalis leporispalustris*, *Dermacentor variabilis* and *Ixodes dentatus* and was described by Howard in 1907 (Howard, 1907; Smith & Cole, 1943). Since that report, *Ixodiphagus mysorensis* Mani was collected from an unidentified *Ornithodoros* in India; *Ixodiphagus hirtus* Nikol'skaya was collected from *Ixodes persulcatus* and *Ixodiphagus biroi* Erdos was collected from an unknown host (Cole, 1965; Mwangi *et al.*, 1991).

Hunterellus (=Ixodiphagus) hookeri Howard was collected from *Rhipicephalus sanguineus* (Howard, 1908). Since then this parasitoid has been found parasitizing fifteen species of ticks. Other parasitoids, first named *Ixodiphagus caucurtei* and *Habrolepis caniphia*, were later transferred to the species *H. hookeri* (Cole, 1965). In Namibia (then called Southwest Africa), *Hunterellus (=Ixodiphagus) theilerae* Fiedler was collected from a nymph of *Hyalomma transiens* which was feeding on a wild hare (Fiedler, 1953). This parasitoid was recorded again from *Rhipicephalus oculatus* in Transvaal, South Africa (Fiedler, 1953). *Hunterellus (=Ixodiphagus) sagarensis* Geevargheese was collected from *Haemaphysalis bispinosa* in India (Geevargheese, 1977), and was later reported in Japan from *Haemaphysalis longicornis* (Tachikawa, 1980). The identification of the two genera, *Ixodiphagus* and *Hunterellus*, has so far been based on morphology alone (Mwangi *et al.*, 1994). *Hunterellus* has been formally synonymized to *Ixodiphagus* (Trjapitzin, 1985).

1.3.6.1.1. *Ixodiphagus hookeri*

I. hookeri has the most cosmopolitan distribution of all the tick parasitoids and has been reported from England, France, USSR, India, China, Brazil, USA, Mexico and Cuba. In Africa, it was reported from Nigeria (Philip, 1931), South Africa (Cooley & Kohls, 1934), Ivory Coast (Graf, 1979), Mozambique (Santos Dias, 1948), Uganda and in Kenya it was reported from the Mombasa area (Philip, 1954) and the Trans-Mara area (Mwangi *et al.*, 1994). Recently, this parasitoid was found in the Kuja River Basin and Busia area of Kenya (E. Mwangi, pers. comm.). In nature, *I. hookeri* has been collected from *Rhipicephalus sanguineus*, *Dermacentor parumapertus*, *Ixodes ricinus*, *Hyalomma aegyptium*, *R. oculatus*, *R. evertsi*, *R. appendiculatus*, *Hyalomma asiaticum*, *Haemaphysalis concinna*, *Haemaphysalis japonica*, *I. persulcatus* and *I. orenulatus* (Cole, 1965), *Amblyomma variegatum* (Mwangi *et al.*, 1994), *A. nutalli* (Graf, 1979) and *A. tholloni* (Santos Dias, 1948).

Although *I. hookeri* was collected from *R. appendiculatus* in other locations, there is no proof of natural parasitism of this species in Kenya. In the Trans-Mara area of Kenya, *I. hookeri* naturally infects 50% of *A. variegatum* nymphs on cattle

(Mwangi *et al.*, 1994). Graf (1979) found a parasitoid from *A. nutalli*, which he reported to be closely related to *I. hookeri*. It was, however, absent from *A. variegatum* collected from the same neighbourhood. Santos Dias (1948) has also recorded it from *A. tholloni* from Maputo, Mozambique. Cooley (1930) mentioned that a parasitoid was noticed emerging from *A. hebraeum* in South Africa, but it was never identified, and has not been mentioned again since that time. In a study conducted by Bowman *et al.* (1986) with *I. texanus* using *R. sanguineus*, *A. americanum* and *A. maculatus* as hosts, it was concluded that the *Amblyomma* ticks were not suitable hosts.

The life cycle of *I. hookeri* begins when the female wasp lays her eggs in the body of the tick host by piercing the integument with her ovipositor. Oviposition takes about 2-20 seconds (Wood, 1911; Cooley, 1928). All the ixodiphagines studied are able to oviposit in recently detached fully fed ticks, while most identified species oviposit and develop only in the nymphal stage of the host (Davis, 1986). *I. hookeri* will oviposit in unattached unfed hosts (Cooley & Kohls, 1928) and has also been found to oviposit in larvae (Cooley & Kohls, 1934). The parasitoid's eggs seem to be killed in adults (S. Bengaly, pers. comm.). Cooley and Kohls (1928) found that during the harsh winters in the United States of America, parasitoid eggs or very young larvae diapause in larval ticks during metamorphosis of the fed larvae and remain inactive in the resulting hibernating unfed nymphs until the nymphs take a blood meal.

The first indication of parasitism is swelling in the host, soon followed by an irregular striped appearance, caused by the larvae of the parasitoid as seen through the skin of the host. The larvae continue to feed, gradually reducing the body contents of the host, until only the empty shell remains (Cooley, 1928). The adult parasitoid emerges through a hole which it gnaws in the body wall of the tick, mating takes place immediately and lasts for only a few seconds. The female begins immediately to search for a tick host in which to oviposit (Wood, 1911; Cooley, 1928).

Accumulated information on the biology of *I. hookeri* by Wood (1911), Cooley (1928) and Cooley and Kohls (1928) helped make this parasitoid the focal point of attempts to release tick parasitoids as agents for tick control in the late 1920's and early 1930's. Larrousse *et al.* (1928) released *I. hookeri* collected in France on an island off the coast of Massachusetts for controlling the American dog tick, *Dermacentor variabilis*. This release apparently had no measurable effect on the abundance of American dog ticks, but it presumably led to the establishment of *I. hookeri* in North America in association with *Ixodes dammini* (Schmidtmann, 1994). In a subsequent study conducted in the same area, Smith and Cole (1943) recovered one male and four female *I. hookeri* from an *I. dammini* nymph attached to a meadow mouse, but 284 other *I. dammini* nymphs and 2143 nymphal *D. variabilis* were not parasitized.

More recent reports indicate that 27% of host-seeking nymphal *I. dammini* on Naushon Island, near the release site of *I. hookeri* by Larrousse in 1926, were infected with *I. hookeri* (Mather *et al.*, 1987b). This parasitoid population is of further interest in that only nymphs devoid of *Borrelia burgdorferi*, the aetiologic agent of Lyme disease, were parasitized. This relationship was judged to reflect an antagonism, conceivably mediated by the tick 'immune' system, between *I. hookeri* and *B. burgdorferi* (Schmidtmann, 1994). Indeed, it was subsequently observed that nymphs derived from larvae that fed previously on white-footed mice were not parasitized by *I. hookeri*, whereas some nymphs from larvae that fed on white-tailed deer, which are not a source of *B. burgdorferi*, were parasitized (Mather *et al.*, 1987b). This relationship provides useful insight into the host-searching behaviour of *I. hookeri*, as well as clarifying the potential for using *I. hookeri* to suppress *I. dammini* populations.

Since larval *I. dammini* in eastern USA feed largely on small rodents, particularly white-footed mice, the potential for suppressing *I. dammini* with *I. hookeri* appears to be limited, even where wasp density is high (Schmidtmann, 1994). Although *I. dammini* is as abundant on Naushon Island as in certain other sites, risk of infection by either *B. burgdorferi* or *Babesia microti* is less.

Introduction of *I. hookeri* onto Naushon Island seems to have benefited the residents, but not in the manner originally intended (Mather *et al.*, 1987b).

In another trial on Naushon Island, an estimated 44 000 adult *I. hookeri* were released in a cranberry bog, along with 55 meadow voles each carrying about 20 parasitized nymphs. A second release consisted of 47 000 wasps released in a beach grass habitat, along with 6 000 female wasps and 32 white-footed mice each carrying from 20 to 25 parasitized ticks (Schmidtmann, 1994). Although immature *D. variabilis* density was low in the cranberry bog following release of *I. hookeri*, none of the 24 nymphs collected from rodents were infected. A year later only 27 *D. variabilis* nymphs were collected from mice, none of which were infected with *I. hookeri* (Schmidtmann, 1994).

Theiler (1969) summarized the Naushon Island control efforts, stating 'the results of work done on *I. hookeri* were so disappointing that no one has had the courage to try it again'. However, many basic questions about the biology of *I. hookeri* and other tick parasitoids remain to be answered. In the Smith & Cole (1943) study, *I. hookeri* was mass reared and released for control of *D. variabilis* irrespective of the host location cues utilized by these parasitoids. As parasitoids of *Ixodes* ticks, the host searching behaviour of *I. hookeri* females could favour the location of immature *Ixodes* ticks on white-footed mice, *Peromyscus leucopus*, that frequent woodland habitats, rather than immature *D. variabilis* on meadow voles in grassland vegetation (Schmidtmann, 1994).

White-footed mice and/or woodland odours could have attracted the parasitoids more than the meadow voles and/or grassland odours. Questions about the timing of experimental release relative to the phenology of both parasitoid and host, a key factor requisite to successful use of parasitoids for control of insects (Stinner, 1977), also remained unresolved. Efforts could have failed because of inadequate numbers of parasitoids released as compared to the geographical area covered by the releases (Morton, 1928; Smith & Cole, 1943; Cole, 1965). On the other hand, the behaviour of Ixodiphagini has been little studied; least known is how the female parasitoids find their hosts (Davis, 1986).

Females have been seen in the fur of animals (Philip, 1931). They may therefore first search for the host animal of the tick, the ticks would then be found and parasitized whilst they are still attached (Davis, 1986). It is quite possible that the parasitoids may prefer to find the ticks on the ground as unfed larvae, fed larvae, unfed nymphs or fed nymphs, or may choose the ticks on the host animals or search for both off-host and on-host ticks (Cooley & Kohls, 1934).

Even if adequate numbers of *I. hookeri* parasitoids are released, the success of the biological control programme still depends on whether these parasitoids can locate the host ticks. It seems likely now that the failure of *I. hookeri* to control the American dog ticks could have been grossly due to the fact that some fundamental issues concerning the host searching behaviour of female parasitoids were overlooked. Studies are thus needed to investigate host-parasitoid interactions carefully, especially the host location aspect, so that *I. hookeri* can be fully incorporated into integrated tick management as a safe and more specific component. Effective and economic use of natural enemies requires that we understand how they interact with their hosts and other factors in the environment. In foraging for food and hosts, parasitoids use an intricate system of cues and tactics. Without such information, we will be attempting to use parasitoids in a 'black box', with the odds stacked against us (Lewis *et al.*, 1994). With proper knowledge, we can utilize the parasitoids' foraging to our advantage. It seems as if *I. hookeri* performs better in Africa judged from the 80% parasitization rate for the nymphs collected near Hartebeestpoort Dam and at a location on Pienaar's River in South Africa (Cooley, 1934), the more than 90% parasitization rate for *R. sanguineus* in Nigeria (Philip, 1931) and the 50% parasitization rate for *A. variegatum* nymphs in the Trans-Mara area of Kenya (Mwangi *et al.*, 1994).

The improved use of parasitoids as biological control agents is at the forefront of our quest for more effective, lasting, and environmentally safe technology for pest management (Knipling, 1992). Various avenues are available for utilizing parasitoids, including the importation of new species, mass-propagation and release of parasitoids, and habitat management techniques to increase the abundance and

performance of feral and released individuals. Our limited understanding of parasitoid-host interactions and other factors affecting their efficiency is a central barrier to consistently effective use of parasitoids with all of these approaches (Knipling, 1992).

In selecting potential biocontrol agents, Stinner (1977) outlined needs for the following baseline information: (1) an appreciation for the general adaptive features of the agent;

(2) an analysis of the agent's searching capacity; and

(3) knowledge of the rate of predator increase relative to prey increase. Other components of successful biocontrol agent establishment and release programmes include pre-release conditioning, determination of optimum release times, distribution and number. Thus, in the absence of even rudimentary information about tick parasitoids, it is indeed premature to judge the potential of tick parasitoids in regulating tick populations (Schmidtmann, 1994).

1.3.7. Integrated Tick Management (ITM)

In the early 1980s research was designed by scientists and donors to evaluate the tick control problem. This resulted in revision of control methods in several countries and the introduction of the concepts of ITM, and integrated tick and tick-borne disease control (Young *et al.*, 1988; FAO, 1990; Tatchell, 1992). This concept can be summarized as the combination of one or more methods of tick control with methods of control of the pathogens that cause tick-borne diseases in such a way that maximum benefit is gained for minimum cost (Pegram *et al.*, 1995). However, the current methods of tick control in many countries still rely almost entirely on the use of synthetic acaricides (Schröder, 1987; Latif, 1992; Hassanali, 1994; Radostits *et al.*, 1994).

The exploration and realization of the power of alternatives to chemical control and the integration of these methods into ITM is long overdue and need to be re-evaluated seriously if animal production is to be rescued from the 'claws' of ticks and tick-borne diseases.

Implicit in the ITM concept is the maximum use of natural enemies supplemented with selective acaricides when necessary (Croft & Brown, 1975).

1.4. Objectives of this study

The main objective of this study is to investigate the chemical and physical cues utilized by *I. hookeri* in the selection of *A. variegatum* nymphs since *I. hookeri* seems to be host as well as host stage specific. Special emphasis is on host habitat location, host location, host suitability and acceptance.

Specific objectives are to:

(1) Investigate chemical cues which *I. hookeri* uses to locate *A. variegatum* nymphs;

(2) Investigate physical cues which *I. hookeri* uses to recognize *A. variegatum* nymphs;

(3) Compare chromatograms of some of the semiochemicals found useful in the host location process and those which are not useful;

(4) Examine and compare host acceptance and suitability of *A. variegatum* and *R. appendiculatus* nymphs to *I. hookeri*;

(5) Attempt the induction of parasitization in *R. appendiculatus* nymphs through using *A. variegatum* semiochemicals;

(6) Carry out field experiments to investigate the behaviour of *I. hookeri* between release and finding of the tick hosts.

(7) Determine the dispersal behaviour of *I. hookeri*.

CHAPTER 2: LITERATURE REVIEW

2.1. Host selection in insect parasitoids

Considering the small size of both parasitoids and their hosts, and also the structural complexity of the environments inhabited by most parasitoids, finding a suitable host appears a formidable task (Godfray, 1994). Several strategies are employed for host selection by different parasitoid species. The strategies that have evolved appear to be related in part to the specificity of the parasitoid-host association (Vinson, 1977). These strategies depend on the biology of the host, the environment in which the host lives, the host strategy for exploiting its environment, the relationship the host has with other organisms in its environment, and also on the types or modalities of stimuli available to the parasitoid and the distances over which these stimuli operate (Vinson, 1984a).

While the host evolves to avoid and defend itself against parasitoids, the parasitoid evolves to exploit all means available to locate and successfully parasitize its host. Often this involves the parasitoid's response to organisms other than its host (Vinson, 1984a). While the host attempts to conceal itself it must continue to communicate, feed, reproduce and defend itself in order to survive. The parasitoid exploits these activities, particularly the host's need to communicate, as communication often involves the release of chemicals that can reveal the host's whereabouts (Vinson, 1984a).

Parasitoid host selection has received some attention, due to its importance in the control of insect pests. Through an understanding of host selection it may be possible to manipulate the behaviour of parasitoids to the benefit of biological control. Some progress in this direction has been made (Gross *et al.*, 1975; Lewis *et al.*, 1975a,b), and the future can be viewed with optimism (Greenblatt & Lewis, 1983). The problem of finding hosts is complicated by the fact that the host is usually killed and the immature parasitoid must undergo a period of change to the free-living adult stage before the process of host selection is initiated (Vinson, 1984a).

The female parasitoid may emerge in a location where hosts are present, but in the improper stage for attack. While some of these difficulties are overcome by the synchronization of the biology of the parasitoid and the host, in most cases the proper host stage is not present in the immediate environment where the female emerges (Vinson, 1984a, 1985).

The female may emerge in a location where the host population is no longer present, having moved during the period required by the parasitoid to develop into a reproductive adult. The female may also be forced to seek new host communities either because all the hosts were already used or because it failed in the competition for hosts, it needed to seek food or a mate or it was deterred by predators or by environment perturbations such as habitat destruction, high winds or floods (Vinson, 1984a, 1985).

In nature, female parasitoids seek hosts on a variety of substrates, moving in and between them in a complex non-random manner (van Alphen & Vet, 1986). They respond to a hierarchy of physical and/or chemical stimuli which lead them to their potential hosts. Unfortunately, there is no information on host searching cues of parasitoids of ticks or other blood sucking livestock pests in literature. Forty species of pupal parasitoids of tsetse flies, another blood sucking pest of livestock, are known (Laird, 1977). Experimentally, the tsetse parasitoids (*Syntomosphyrum* spp.) will oviposit in the puparia of a wide variety of the higher Diptera, including species of *Musca*, *Dacus*, *Lucilia*, *Chrysomyia*, *Sarcophaga*, *Calliphora*, *Phormia*, *Stomoxys* (Laird, 1977) and *Drosophila* (Saunders, 1961). Unfortunately, there is also no literature on how these parasitoids locate the host pupae. Therefore, the host selection process for other parasitoids, notably, the parasitoids of herbivorous pests will be used to give a general overview of the host selection process although the odour sources and the nature of odours may differ.

A basic premise is that a species that is totally dependent on a given host for reproduction is a highly specialized organism having exceptional host-finding capabilities (Knipling, 1992). While several detection mechanisms may be used, the most effective appear to involve chemical cues produced by the host or by the

plant/animal harbouring the host (Knipling, 1992). Lewis *et al.* (1975a,b), and Loke and Ashley (1984) determined that kairomone (Table 2) signals intensify host searching and increase the host finding capability of parasitoids.

Semiochemicals are chemicals that mediate inter- and intraspecific interactions (Nordlund, 1981). Semiochemicals are divided into pheromones and allelochemicals (Nordlund & Lewis, 1976). These chemicals have been categorized, based on the nature of the interaction (producer/acquirer) and cost benefit analysis (the energy required to produce the semiochemical weighted against the benefit gained from the emission of the semiochemical)(Nordlund & Lewis, 1976; Dicke & Sabelis, 1988). Dicke and Sabelis (1988) suggested terminology for chemicals utilized in the producer-acquirer relationship as outlined in Table 2.

The distribution of hosts is often clumped in time and/or space (Southwood, 1966). The distribution of these host clumps changes spatially and seasonally, further complicating host selection. There are two basic host selection strategies that parasitoids may employ (Vinson, 1984b). One is ambush and the other searching (Vinson, 1984a, 1985). The ambush strategy generally requires the attacker to hide either physically or cryptically. Some parasitoids have evolved an ambush strategy. For example, some potential host species lay egg masses which are either produced infrequently, widely dispersed or concealed (e.g. preying mantid eggs are covered with a frothy liquid which hardens to form a protective case around the eggs while grasshopper and locust eggs are concealed in egg pods)(Godfray, 1994; Vinson, 1984a). These eggs are exploited by some phoretic parasitoids (Clausen, 1976), whose females often wait for a transporting host, to which they attach to be carried to where the host female lays eggs. The egg parasitoid, *Mantibaria* (=*Rielia*) *mantis,* attaches itself to an adult mantid (*Mantis religiosa*) and wait until the female oviposits. The parasitoid jumps off and parasitizes the egg before the frothy liquid hardens as the parasitoid is unable to attack host egg masses after the frothy liquid has hardened (Godfray, 1994).

Table 2. Infochemical terminology (Dicke & Sabelis, 1988)

Infochemical: A chemical that, in the natural context, conveys information in an interaction between two individuals, evoking in the receiver a behavioural or a physiological response that is adaptive to either of the interactants or both.

Pheromone: An infochemical that mediates an interaction between organisms of the same species whereby the benefit is to the origin-related organism, to the receiver, or both.

Allelochemicals: An infochemical that mediated an interaction between two individuals that belong to different species.

Allomone: An allelochemical that is emitted by an organism and which evokes a behavioural and physiological response in the receiver. However, the response of the receiver is adaptively favourable to the emitter, not the receiver.

Kairomone: An allelochemical that is emitted by an organism and which evokes a behavioural and physiological response in the receiver. The response of the receiver is adaptively favourable to itself, not the emitter.

Synomone: An allelochemical that is emitted by an organism and which evokes a behavioural and physiological response in the receiver. The response of the receiver is adaptively favourable to both itself and to the emitter.

The parasitoid thus gains early access to many potential hosts (egg masses). Another example of ambush is provided by species which lay many eggs in a potential host community, relying on chance host encounter for contact, as in certain parasitoid Diptera (Tripp, 1961). The searching strategy falls conveniently into five types; (1) host-to-host, (2) random search, (3) direct orientation, (4) hierarchy of cues and (5) a series of discrete cues (Vinson, 1977).

The process that results in successful parasitism can generally be divided into five steps: (a) host habitat location, (b) host location, (c) host acceptance, (d) host suitability, and (e) host regulation (Salt, 1935; Laing, 1937; Flanders, 1953; Doutt, 1964; Vinson, 1975). Some authors have inserted further divisions, for example dividing host acceptance into examination, probing, drilling and oviposition (Vinson 1985). However, it must be remembered that these divisions are arbitrary, in some cases one step may be absent or divided into more steps, or several steps may overlap (Arthur, 1981; Weseloh, 1981). Furthermore, the host searching strategies utilized by parasitoids in the location and acceptance of their hosts depend on the types of cues provided by the host or its environment (Vinson, 1984a).

2.1.1. Host habitat location

A particular habitat may be selected because it harbours a preferred host species. On the other hand, hosts may be attacked, not because they are preferred but because they are accessible in a particular environment (Orphanides & Gonzalez, 1970). There are also several reports of parasitoids being attracted to a potential host habitat regardless of the presence or absence of hosts (Nishida, 1956; Vinson, 1975), and others where a parasitoid will search and attack hosts in some habitats but not in others (Taylor, 1932; Arthur, 1962). Vinson (1991) suggested that physical factors such as temperature, light, wind and humidity play a major role in causing parasitoids to aggregate in a particular macrohabitat, thus influencing host selection. Other factors involved in habitat location include biological factors, such as food sources for the adults (Wolcott, 1942; Simmonds *et al.*, 1975), refuge sites, and the presence or absence of competitors and predators (Vinson, 1991). In addition, the flying and crawling habits of the parasitoid play a role in habitat preference (Weseloh, 1972).

There are species which use a wide range of habitats and hosts, such as *Colastes braconius*, which attacks a broad range of leafmining insect larvae, belonging to Diptera, Coleoptera and Lepidoptera, in both herb and tree zones (Shaw, 1983).

For some parasitoids, habitat choice is unimportant, like for *Pachycrepoideus vindemiae* which attacks *Drosophila* spp. pupae in urban environments, woods and open landscape, or the aphelinids *Encarsia tricolor*, *E. partenopea* and *E. formosa* which attack whiteflies on many different species of host plants (van Alphen & Vet, 1986). However, it is apparent that host plants play a major role in most of the parasitoid-host relationship, especially in parasitoid-herbivorous pest relationships.

It has been estimated that there are at least 100 000 chemical compounds produced during the growth and development of the more than 200 000 species of flowering plants (Metcalf & Metcalf, 1992). Plant extracts were found to stimulate parasitism in the field (Altieri *et al.*, 1981). Altieri *et al.* (1981) found that parasitization by *Trichogramma* spp. was significantly higher in weedy soybeans or in soybeans interplanted with corn than in weed-free soybean monocultures. A detailed understanding of the role of host plants in host finding by larval parasitoids was provided by Turlings *et al.* (1990; 1991a). They found that the host generalist parasitoid, *Cotesia marginiventris*, is stimulated to more oriented flights by feeding damage than by either frass or larvae. Caterpillar feeding resulted in the production, by the plant, of unique volatiles that are used by the parasitoid to locate hosts (Lewis *et al.*, 1994). *Microplitis croceipes* females have been found to respond to extracts of frass from larvae reared on cotton and soybean, but not on corn (Sauls *et al.*, 1979; Nordlund & Sauls, 1981).

The complexity of volatile stimuli released may depend on the physiological state of the plant (Hedin, 1976), including age or health (Ahmad, 1983) and also the cultivar (Elzen *et al.*, 1985). Flower scents attract and/or arrest the movement of potential natural enemies. Parasitoids are carnivorous only as larvae, while as adults they require nutrition from flowers (Wolcott, 1942; Leuis, 1967). The nutritional requirements of the adult are often associated with the type of reproductive strategy of the female. Females of some species emerge with a full complement of eggs which are often deposited during a relatively short period of time after which the female produces no more eggs. Such pro-ovigenic females (Flanders, 1950) generally do not require food, thus, the need for food would

probably not play a role in habitat preference (Vinson, 1985).

On the other hand, most species are synovigenic (Flanders, 1950), producing eggs throughout their lifetime. Such females need food in the form of nectar, honeydew and host fluids (Vinson, 1985). The success of the large wasp, *Larra americana*, in controlling the mole cricket, *Scapteriscus vicinus*, in Puerto Rico apparently depended on the presence of the two weeds, *Borreria verticillata* and *Hyptis atrorubens* from which the adult wasps obtain nectar (Wolcott, 1942).

In a few parasitoid-host relationships, organisms in association with a host are apparently responsible for providing cues to the host's habitat. Fungi associated with the galleries of siricid woodwasps have been found to attract and initiate probing behaviour in their parasitoids (Madden, 1968; Spradberry, 1970). There are also reports of visual orientation to a microhabitat, for example, the response of *Diaparsis truncatis* to wooden models of asparagus beetles (van Alphen & Vet, 1986), and also the involvement of sound, for example, the tachinid, *Euphasiopterix ochracea* is attracted to the calls of male crickets (Cade, 1975). However, olfaction seems to be the most common sensory mode of habitat location (van Alphen & Vet, 1986). Stimuli used in habitat location (i.e long range) are generally plant volatiles or host pheromones (Vinson, 1981).

2.1.2. Host location

Once the female parasitoid has found a particular habitat, the area of search has already been effectively reduced. The female now has to search for a potential host community. For this purpose, the female must be in the 'proper physiological condition' and the proper conditions of light, humidity, wind, temperature and time of day must be present (Vinson, 1985). The parasitoid should respond to the spatial distribution of the hosts and to the presence of other searching parasitoids in a way that leads to maximum encounter rate (Hassell & Godfray, 1992).

Research into host location by parasitoids falls into two main schools. One apparent has concentrated on trying to understand the behavioural mechanisms used by the parasitoid to locate their hosts (Godfray, 1994).

This research programme, started in the 1930s, has been spectacularly successful in revealing the complex assemblages of cues used by the parasitoids in host location (Godfray, 1994).

The origins of the second apparent are more recent and lie in the explosion of interest in behavioural ecology in the 1970s. A cornerstone of the new field is optimal foraging theory, which seeks to predict the feeding behaviour of animals on the assumption that behaviour is optimized by natural selection (Godfray, 1994).

Host location and attack is traditionally discussed using a conceptual model first developed by Salt (1935) and Laing (1937). Salt (1935) divided host location and attack into 'ecological' and 'physiological' components, the former incorporating habitat and to a certain extent host location, while the latter referred chiefly to host acceptance. The division of successful parasitism into the hierarchical processes of host habitat location, host location, host acceptance, host suitability and host manipulation is primarily 'for our convenience in thought and communication' (Vinson, 1981). This conceptual model, however, has tended to emphasize a static hierarchical view of parasitoid behaviour (Godfray, 1994).

A much more dynamic model has been proposed recently by Lewis *et al.* (1990) and Vet *et al.* (1990). They first point out that stimuli will vary in their information content and that the parasitoid should respond to the stimulus most closely associated with the host. Thus host habitat location is redundant if the parasitoid is able to locate the host directly. They envisage a naive parasitoid (not experienced host odours before) being born with an innate set of 'response potentials' to different stimuli; a parasitoid presented with a number of stimuli will react to the one with the highest response potential. The ranking of different stimuli will be fine-tuned by natural selection to maximize the parasitoid's chance of successful host location, on the other hand the ranking of different stimuli will change over the life of the parasitoid (Lewis *et al.*, 1990; Vet *et al.*, 1990).

Parasitoid biologists have made enormous advances in recent years in understanding the cues and stimuli used by parasitoids to locate hosts (Vinson 1976, 1981, 1984a,b, 1985; Waage 1978; Jones 1981; Weseloh 1981; Nordlund

et al. 1988; Vet & Dicke 1992). The intense selection pressure that parasitoids experience in locating hosts is well illustrated by the variety of subtle cues used in host searching.

Natural enemies are faced with a great variety of stimuli they may use to locate their victim. Both plants and hosts produce odours and thus potential information (Vet & Dicke, 1992). The appropriateness and usability of information ultimately depends on two factors: (a) its reliability in indicating herbivore presence, accessibility, and suitability and (b) the degree to which stimuli can be detected. It is assumed that the use of information that is both reliable and easy to detect enhances searching efficiency and consequently Darwinian fitness (Vet & Dicke, 1992). Stimuli derived from the host itself are generally the most reliable sources of information. Ideally, the infochemicals should tell natural enemies whether a herbivore is present, which species it belongs to, how many there are, whether the herbivore should be parasitized or eaten, and whether it is readily accessible or hidden (Vet & Dicke, 1992). However, due to their production in minute quantities and the continuous selection for inconspicuousness, host-derived stimuli are hard to detect over long distances (Dicke *et al.*, 1990; Turlings *et al.*, 1991b).

Stimuli from plants, on the other hand, are usually more readily available because of the plant's relatively larger biomass but are less reliable predictors of host presence. So natural enemies are challenged to combine the advantageous characteristics of information from both trophic levels (Vet & Dicke, 1992).

Natural enemies may deal with the reliability-detectibility problem in three ways: (a) by resorting to the use of more conspicuous infochemicals from host stages different from the one under attack (i.e. infochemical detour), (b) by focussing their responses on stimuli created by specific interactions of the host and its food, and (c) by learning to link easy-to-detect stimuli to reliable but hard-to-detect stimuli (Vet & Dicke, 1992). Hosts have to feed and defecate, which unavoidably results in emission of volatiles that may reveal their presence. The more successful the host is in avoiding information conveyance, the more natural enemies have to turn to information from plants (Vet & Dicke, 1992).

Natural enemies attacking non-feeding stages of the host, such as eggs and pupae may solve the reliability-detectibility problem by resorting to information from other stages (infochemical detour), provided that these stages supply reliable information on the presence of the host stage of interest (Vet & Dicke, 1992). The juvenile stage most closely associated with the adult insect is the egg, and some egg parasitoids use the adult sex pheromone in host location (Lewis *et al.*, 1982). For instance, the egg parasitoids, *Trichogramma* sp., respond to the sex pheromone of their host, the moth *Helicoverpa (=Heliothis) virescens* (Lewis *et al.*, 1982). The sex pheromone is adsorbed and retained on the surface of the leaf and thus provides information about the past presence of a sexually active adult (Noldus & Potting, 1991).

Other important sources of arrestant or short range attractant chemicals include frass and honeydew. The mandibular gland secretions of the larvae of *Plodia interpunctella* attracted and stimulated oviposition in *Nemeritis canescens*, leading to increased parasitization (Mossadegh, 1980). Substances associated with silk produced by the labial gland of the gypsy moth, *Lymantria dispar*, act as short-range cues for the parasitoid, *Cotesia* (=*Apanteles*) *melanoscelus* (Weseloh, 1976, 1977, 1981). Mattiacci & Dicke (1995a) found that *Cotesia glomerata* discriminates between feeding damage done by young and old *Pieris brassicae* caterpillars on a cabbage leaf after landing on the leaf. This discrimination is mediated by cues from frass, silk and herbivore-damaged leaf tissue (Mattiacci & Dicke, 1995b) and increases the probability of the survival of *C. glomerata* through the avoidance of encounters with the aggressive fifth instar (older) larva. Furthermore, the avoidance of old *P. brassicae* caterpillars reduces the possibility of encapsulation of *C. glomerata* eggs as encapsulation of eggs is more frequent in older larvae than in younger larvae (Mattiacci & Dicke, 1995b).

Infochemicals can be classified as attractant chemicals which insects use to locate hosts, and arrestant chemicals which, while not providing directional information, reveal the possible presence of the host in the near vicinity (Dethier *et al.*, 1960; Kennedy, 1978; Waage, 1978).

Arrestant chemicals tend to have higher molecular weight and lower volatility and elicit area-restricted search in the parasitoid, while attractant chemicals have a higher volatility and urge the parasitoid to move towards them (Dethier *et al.*, 1960; Shorey, 1976; Waage, 1978; Hassell & Godfray, 1992).

Waage (1979) suggested that the response of a parasitoid to the arrestant chemical wanes during search within a patch until a threshold is reached at which point the insect leaves the patch. The discovery of a host acts to increase the responsiveness to the arrestant and thus lead to the insect staying longer in patches containing greater numbers of the host. Such behaviour is also known as success-motivated searching and often involves both a decrease in the speed of searching (orthokinesis) and an increase in the rate of turning (klinokinesis)(Godfray, 1994). Similar behaviour is observed when a parasitoid enters an area contaminated by a host-associated chemical (Waage, 1978; Loke & Ashley, 1984; Gardner & van Lenteren, 1986). Nelson and Roitberg (1995) found that the leafminer parasitoid, *Opius dimidiatus*, resets its 'giving-up time' (GUT), or increases search intensity, by adding an amount of search time that increased with each successive oviposition. Conversely, encounters with parasitized hosts decreased search intensity, by subtracting an amount of search time with each successive encounter.

Short range chemicals have also been shown to be important in host location. Hendry *et al.* (1973) have shown that *Orgilus lepidus* is attracted to an infested site by heptanoic acid, and a second unidentified compound elicits ovipositor probing upon contact. The influence of contact chemicals on the behaviour of a parasitoid is difficult to separate from that of touch and texture since these chemicals elicit response only on contact (Vinson, 1976). It has been shown that insects normally not recognized as hosts can be attacked if they are contaminated by odours from the parasitoid's habitual host (Thorpe & Jones, 1937; Tawfik, 1957; Vinson, 1975). Although many of these treated hosts are attacked, they are not all accepted as ovipositional sites (Vinson, 1975). Host larvae that were solvent extracted to remove the host-seeking stimulants were not recognized as hosts by *Cardiochiles nigriceps*, however if the stimulants were re-applied, the larvae were attacked but

no eggs laid (Hays & Vinson, 1971). Other factors seem to be responsible for host acceptance and egg release.

Although the detection of chemical cues seems to be the most frequent method of host location, some parasitoids make use of other senses. A few parasitoid flies are known to be attracted by the sound of their hosts, for example, the attraction of the sarcophagid, *Colcondamyia auditrix* to cicadas (Soper *et al.* 1976). Richerson & Borden (1972) suggested that the braconid, *Coeloides brunneri*, used infra-red radiation to detect its host, a bark beetle. However, it is possible that convection or conduction rather than radiation were responsible for heat perception (Weseloh, 1981). Substrate vibration (Vet & Bakker, 1985; Vet & van Alphen, 1985) is often used by the parasitoid, especially those attacking concealed hosts. Movement of the host detected visually, frequently guide parasitoids in the final stage of host location (Monteith, 1956, 1963).

The aphid parasitoid, *Aphidius funebris*, only seemed to detect the presence of an aphid by antennal tapping or when the aphid kicked while the parasitoid was passing it. After initial contact with the host colony, however, *A. funebris* 'remembers' the position of the aphids as indicated by the differences between the search speeds away and towards the colony (Weisser, 1995). This is called associative learning (i.e. response to a new stimulus is added to a parasitoid's behavioural repertoire after it has been encountered together with a stimulus toward which the parasitoid has already an established response). A relatively small number of parasitoids with large eyes attack swiftly moving adult insects which they intercept in flight. Detection of hosts is visual (Godfray, 1994). Conopids, which are large robust flies mimic the flight of their hosts (i.e. bees and wasps) before pouncing on the host and laying an egg in mid-flight (Raw, 1968).

Over long periods of evolutionary time, hosts may have evolved to avoid parasitism by changing their feeding pattern or phenology. Phytophagous hosts living inside plant tissue may conceal themselves so that they provide no external visual cues for the searching parasitoid. Leaf-mining insects often only eat part of the green leaf mesophyll, perhaps so that an obvious white mine is not produced

(Vet *et al.*, 1991; Vet & Dicke, 1992). 'Snacking'(i.e. eating small amounts of food and then moving to a new feeding site) reduces the usefulness of the cues produced while the host is feeding (Mauricio & Bowers, 1990). Some lepidopteran caterpillars cut off partially eaten leaves which might otherwise have acted as cues for a variety of predators and parasitoids (Heinrich, 1979). Host larvae often carefully ensure that frass falls off the host plant and does not contaminate the feeding area (Price, 1981).

Some hesperid butterflies can explosively eject frass over 1 meter (Frohawk, 1913). Some hosts remain absolutely motionless in the presence of the parasitoids so that movement does not reveal their position (Richerson & De Loach, 1972). Some tropical nymphalid butterflies hide in rolled leaves and plug the entrance with their head capsule since the head capsule appears too hard to be pierced by the ovipositor of a parasitoid wasp and also affords some protection against the larvae of tachinid flies which lay their eggs on the exposed part of the caterpillar (De Vries, 1987).

2.2. Host acceptance and suitability

After a parasitoid locates a host, it is faced with a sequence of decisions concerned with oviposition. The hosts encountered will often vary in their quality as food for the developing young, in the time needed for their attack and parasitism, and possibly in the risks of mortality to the female during oviposition (Godfray, 1994). Parasitoids should obviously attack the best hosts and ignore any hosts in which the probability of successful larval development is limited. There are two main types of optimal host acceptance models. The most straightforward type of model assumes that the parasitoid's decision is unaffected by its internal physiological state, for example, by the number of eggs in its oviduct. These models are termed 'static optimization models' in contrast to 'dynamic models', which allow the parasitoid's decision to vary with internal state (Godfray, 1994). The acceptance of hosts have been attributed to a number of factors. Shape, size and age, movement, sound, surface texture, vision and thickness of the cuticle have all been shown to be

important in host acceptance, although chemicals again play a role.

Parasitoids use a variety of cues to assess the quality of their hosts. Many species, especially those attacking immobile hosts, spend a great deal of time examining the host externally and internally. For more active hosts, such as leaf-feeding Lepidoptera, host examination is relatively fast (Vinson, 1984a). This information will help the parasitoid in decisions concerning the suitability of the encountered host, and thus will lead to the acceptance or rejection of the host. External examination in *Trichogramma* spp. starts with the 'drumming' of their antennae over its surface (Salt, 1935). Klomp and Teerink (1962) suggested drumming set up vibrations in the host which the female parasitoid monitored in order to determine host size and in turn regulate the number of eggs deposited.

In some cases a parasitoid may walk over the host with ovipositor tapping and acceptance is prolonged (Strand & Vinson, 1983). If the host is accepted at this stage, the parasitoid will attempt to probe the host with the ovipositor (Salt, 1935). Salt (1935) also found that size was the most important attribute leading to host acceptance. Larger *Trichogramma evanescens* females tended to accept larger host eggs. Many parasitoid wasps especially ichneumonids have very noticeable white tips or bands on their antennae. It is possible that these markings assist the wasp in assessing the size of the host (Godfray, 1994).

Host movement is also frequently a prerequisite for host acceptance (Arthur, 1981). However, in egg parasitoids movement may inhibit oviposition since egg parasitoids are frequently unable to develop in mature eggs and the movement of the developing embryo is used as a signal that the eggs are too old to support a larva (Salt, 1938; Jackson, 1958). van Alphen and Janssen (1982), and van Alphen and Vet (1986) found that *Asobara tabida*, a parasitoid of drosophilids, rejects small species and those with a thick cuticle prior to piercing.

Odour is of primary importance in initiating oviposition in *Campoletis sonorensis*, and a cylindrical shape more readily accepted than other shapes (Schmidt, 1974), while hairiness and odour were important to *Apanteles melanoscelus* (Weseloh, 1974).

Parasitoids can quickly evaluate the visual cues such as host shape, size, colour and movement without the risks associated with host handling (Gerling *et al.*, 1990). The three aphid parasitoids *Ephedrus californicus*, *Monoctonus paulensis* and *Praon pequodorum* use visual cues in host recognition and evaluation (Michaud & Mackauer, 1995). The role of sound in host acceptance is not clear yet.

Chemical cues perceived through receptors in the antennae and tarsi are undoubtedly of great importance in host acceptance. Non-volatile chemicals present on the surface of the host (contact semiochemicals) may be the final stimuli for oviposition (Godfray, 1994). Gravid European corn borer, *Ostrinia nubilalis*, females used chemical constituents in the host plants to elicit oviposition response (Udayagiri & Mason, 1995).

Arthur (1981) reviews a number of studies which have found that solvent extracts of host cuticle are recognized by parasitoids as oviposition stimuli. After the solvent treatment, the actual host may be markedly less attractive to the parasitoid. Chemical cues may act in conjunction with shape, size and texture. After external examination, many parasitoids insert their ovipositor into the host to obtain additional information about its suitability (Godfray, 1994). Parasitoids frequently insert their ovipositor into a host but do not go on to lay an egg. The ovipositor is normally covered with sensillae and it seems likely that the insect rejects the host after perceiving that it is unsuitable for oviposition (Vinson, 1976; Godfray, 1994). The parasitoid may assess the suitability of the host using chemical cues, or possibly by detecting the heartbeat of a healthy host (Fisher, 1971). Parasitoids can oviposit in false hosts, eg. *Itoplectis conquisitor* would oviposit into false hosts made of plastic film containing host haemolymph and hidden in leaf rolls similar to those made by the real hosts (Arthur *et al.*, 1969, 1972; Hegdekar & Arthur, 1973).

The successful development of a parasitoid to the imago depends on several factors. These factors include evasion of or defense against the host's internal defense system, competition with other parasitoids that occur within the host, the presence of toxins detrimental to the parasitoid egg or larva due to the host's food choice, the host's nutritional suitability and inadequacy (Vinson & Iwantsch, 1980),

insecticide treatment, exposure to juvenile hormone mimics or other insect growth regulators, pathogenic infections, location of a host in an environment unsuitable for the parasitoid and hormonal balance (Vinson, 1984a). Nicotine, a toxin, was shown to reduce the emergence of *Apanteles congregatus* when its host, the tobacco hornworm, fed on tobacco (Gilmore, 1938).

The nutritional suitability of a host is believed to be influenced by the nutrient level, quality, availability and presence of accessory growth factors, genetic make-up of the host, and host age (Vinson & Iwantsch, 1980). Host nutritional insufficiency can lead to pre-emergence mortality or unsuccessful emergence (House & Barlow, 1961; Wylie, 1963).

Host size influences the size of the emerging parasitoids as well as the sex ratio. In gregarious parasitoids, smaller individuals result from a large number of eggs present in a single host (Wylie, 1965; Vinson & Iwantsch, 1980; Godfray, 1994). Large hosts have been reported to yield female parasitoids, while smaller hosts yielded males (Clausen, 1939). Recently, host density has also been shown to influence the factors affecting sex ratios (King *et al.*, 1995). Decreases in the duration of the parasitoid's development with increasing host age at the time of initial parasitization have also been noted (Vinson & Baras, 1970; Beckage & Riddiford, 1978). This effect may be due to nutrient supply, as older hosts can presumably supply more abundant nutrients to developing parasitoids than younger hosts (Smilowitz & Iwantsch, 1973). However, it has been shown that *Trichogramma* spp. lay more eggs in younger host eggs than in older eggs. It is believed that older host eggs provide fewer resources for the developing parasitoid larvae than young eggs (Marston & Ertle, 1969; Pak, 1986; Godfray, 1994).

The failure of parasitoids to survive in unnatural hosts can be due to nutritional unsuitability, nutritional inadequacy or the parasitoid's inability to obtain certain nutrients present in the host's tissues at the proper time (Vinson & Iwantsch, 1980). Raimo (1975) found that *Apanteles melanoscelus* could vector nuclear polyhedrosis virus from one host to another during oviposition through mechanical transmission via the ovipositor.

Death of *Campoletis sonorensis* in *Helicoverpa (=Heliothis) virescens* was found to be due to premature death of the pathogen-infected host (Irabagon & Brooks, 1974).

Juvenile hormone and ecdysone analogues (JHA and EA) applied to hosts can be beneficial, have no effect or be lethal to the contained parasitoids (Vinson & Iwantsch, 1980). JHA greatly reduced the number of *Encyrtus infelix* emerging from *Saissetia coffeae* (Hamlen, 1975). Parasitoids in hosts treated with JHA concentrations higher than 0.05% died either as larvae or pupae, and many parasitoids treated just prior to their pupation died as larval-pupal intermediates (Vinson & Iwantsch, 1980). Synthetic JHA and ecdysone added to an artificial rearing medium greatly enhanced the survival of embryos and first instars of *Ageniaspis fusciollis* (Vinson & Iwantsch, 1980).

Pediobius foveolatus failed to develop in *Epilachna varivestis* larvae treated with diflubenzuron (Dimilin), a chitin synthesis inhibitor for insect control, at a concentration below the LC_{50}. *Lymantria dispar* is not able to survive a 0.2g/liter concentration of applied Dimilin, but some tachinids parasitizing this host can survive this dose (Demolin, 1978 in Vinson & Iwantsch, 1980). The host's hormonal balance may play an important role in determining the suitability of a particular host or host stage for a parasitoid (Vinson, 1984a). The synchronization of the parasitoid's development with that of the host has been demonstrated many times (Fisher, 1971). Synchronization allows the emergence of parasitoids to coincide with the presence of susceptible host stages. To achieve synchronization, parasitoids often diapause or enter resting stages when hosts are unavailable. Overwintering of tick parasitoids in ticks has been reported. (Larrousse *et al.*, 1928; Cooley & Kohls, 1934). *I. hookeri* parasitizes feeding larval *Dermacentor andersoni* ticks in August. The eggs of the parasitoids overwinter (diapause) in the moulting larvae and start to develop only after the nymphs, resulting from larval moult, had engorged in the following spring (Cooley & Kohls, 1934). After these parasitoids have successfully passed the winter in the egg stage, the adults would again become active in August, thus completing their life cycle in time to attack the larval

ticks of the current season (Cooley & Kohls, 1934). The onset and breaking of diapause depend on the effect of the host's endocrine activity on the parasitoid as well as the effect of the parasitoid on the host's endocrine system (Vinson, 1984a). The parasitoid rests until activated by specific (hormonal) changes that occur in the host (Schneider, 1951 in Vinson, 1985). On the other hand, *Aphidius plantensis*, had a juvenilizing effect on its host (Johnson, 1959).

2.2.1. Host defense mechanisms

The host can mount passive and active defenses against the parasitoid besides the avoidance behaviour which was discussed under host location (section 2.1.2). One of the most common responses is violent wriggling (Askew, 1971; Matthews, 1974). The movement may be sufficient to throw the parasitoid off, or to prevent it from laying an egg. The very smooth surface of some butterfly pupae may be an adaptation to prevent parasitoids from gaining a firm footing on the wriggling host (Cole, 1959; Godfray, 1994). The cocoons of a number of genera will jump if disturbed (Godfray, 1994), aphids kick violently when attacked (Kouamé & Mackauer, 1991), gregarious caterpillars like *Pieris brassicae* simultaneously jerk their bodies to frighten generalist parasitoids (Godfray, 1994), many lepidopterans (e.g. larval *Plathypena scabra*) deliberately jump off the plant but remain attached to a silk thread (Yeargan & Braman, 1986).

Hosts also exude drops of sticky liquid from the mouth. This liquid may contain toxins or it may coagulate and surround the parasitoid with gum. Aphids secrete a waxy substance which may deter parasitoids (Godfray, 1994). Some hosts bleed copiously when wounded, and it is possible that the coagulating haemolymph deters the parasitoid from laying an egg (Vinson, 1990). Some host species turn on their attackers and kill them sometimes (Bridwell, 1919, 1920). Some parasitoids deliberately allow themselves to be attacked by predacious hosts, for example, *Lasiochalcidia igiliensis* incites antlions (*Myrmeleon* sp.) to emerge from their burrows and seize the wasp's heavily armoured legs. From this position, the wasp is able to lay an egg through the membrane between the host's head and

thorax (Steffan, 1961 in Godfray, 1994). Other hosts are guarded from parasitoids, for example, ants in the genus *Formica* drive away the braconid parasitoid, *Xenostigmus bifasciatus*, from aphids (*Cinara* spp.) and may even form a protective screen between the aphids and the parasitoids (Stary, 1970).

Hosts can also escape a particular parasitoid by attacking a plant lacking those stimuli used by the parasitoid to locate the potential host community. This idea is supported by the observation that some parasitoids attack a range of unrelated hosts on a particular plant instead of selecting phylogenetically related hosts feeding on various plants (Cross & Chesnut, 1971; Askew & Shaw, 1974).

After oviposition into a host, the last obstacle is the internal defense or immune mechanism of the host. Many endo-parasitoids evade the host's internal defense mechanism by careful placement of their progeny within certain tissues (ganglion, fat body) or stage (eggs) of the host that afford the parasitoid better protection (Salt, 1963). However, this concept should not be accepted at face value, since even particular host tissues or stages have a defense against invasion by foreign materials (Vinson & Iwantsch, 1980; Vinson, 1984a). The host defense most often described is encapsulation (Salt, 1970), a cellular defense reaction in which many haemocytes surround and isolate any foreign material that invades the haemocoel.

Brodeur & Vet (1995) found that there was a relationship between encapsulation and the host range of *Cotesia glomerata* and *C. rubecula*. In some cases a non-cellular capsule is formed, believed to consist of melanin (Götz, 1986). A third means of parasitoid elimination is cuticular encystment (Arthur & Ewen, 1975). After parasitization, the host forms a small cyst from the posterior dorsal cuticle which consists of a separation of the cuticle from the epidermis with a space containing haemolymph. The parasitoid egg or larva migrates into the cyst which is shed at the next moult. There is little doubt that chemicals play an important role in the internal defense and counter-defenses that have evolved during the evolution of the various parasitoid-host relationships (Vinson, 1984a,b).

If a host is attacked by a parasitoid and is unable to mount a physiological or behavioural defense, it is doomed and will not pass genes to future generations (the only exceptions are hosts that are attacked as adults or mature nymphs and which may mate or reproduce before death)(Godfray, 1994). Selection may act on the host to commit suicide because by killing the parasitoid eggs or larvae inside it, it protects conspecifics from attack (Shapiro, 1976; Smith Trail, 1980).

Shapiro (1976) suggested that some nymphalid butterfly caterpillars commit suicide. A case of adaptive suicide was recorded for the pea aphid, *Acyrthosiphon pisum* (Mc Allister & Roitberg, 1987). When disturbed, parasitized aphids from dry areas were more likely to drop from the plant than healthy aphids. The risk of death after dropping is high in dry areas.

2.2.2. Host discrimination

Most parasitoids require the entire host for their development. Since many parasitoids are solitary species and only one progeny normally survives from a parasitized host, re-parasitizing such hosts would be a waste of the parasitoid's time and resources (Knipling, 1992). For this reason, some parasitoids have evolved capabilities to minimize intraspecific and interspecific competition. Some species leave a marking pheromone that is recognized by conspecifics and perhaps by other species. Some species of parasitoids will avoid previously parasitized hosts upon encountering them, but others apparently have not evolved discrimination behaviour and will re-parasitize the hosts (Vinson & Iwantsch, 1980; Knipling, 1992; Godfray, 1994).

Marking takes time and there are likely to be metabolic costs to the production of the marking chemical; so what are the advantages of marking? A review by Roitberg & Mangel (1988) explains the evolution of marking as follows: (1) marking may improve the efficiency of the parasitoid population by increasing the evenness of host attack, however, this suggestion relies on population-level selection and is hard to justify; (2) marking may allow the female to avoid a host she has just attacked; and (3) the mark may be an altruistic act to help related

females forage efficiently, this explanation requires limited dispersal by adult parasitoids so that females are likely to encounter hosts attacked by relatives; and (4) marking might alert other females that the host has already been attacked.

The function of marking lies in the benefit to the ovipositing female. However, other parasitoids encountering the same host will recognize the mark and use the information to their own benefit. Thus, a mark should not last any longer than it is of benefit to the marker. A mark helps the marker to differentiate between hosts that she has previously parasitized and unparasitized hosts while she is searching in a patch, and also to deter a second female from laying in a host she has already oviposited into (van Alphen & Visser, 1990). By the time the mark wears off, the risk of successful competition from the offspring of a second parasitoid will be small, and the internal changes to the host, caused by the developing parasitoid are likely to be obvious to potential superparasitoids, so that long-acting oviposition deterrents may be unnecessary (Godfray, 1994). The mark is thus important during the period when the second clutch (eggs from the second parasitoid) could still win in competition for the host. This may explain why marks are water-soluble and do not last long (van Alphen & Visser, 1990).

Many parasitoids should be more willing to attack recently parasitized hosts where their larvae have the greatest probability of survival (van Lenteren, 1981; Strand, 1986; Mackauer, 1990). It is believed that some parasitoids use their antennae to discover marks left on the host's exterior by females in earlier attacks (Wilson, 1961); to detect heart beat in parasitized and unparasitized hosts (Ullyett, 1936; Simmonds, 1954); to detect eggs already on the host (Smith, 1935); or in conjunction with the prothoracic feet to detect chemical traces left on the host in an earlier attack (Salt, 1937).

Many others probably recognize chemical or physical conditions in parasitized hosts that differ from those in unparasitized hosts with the ovipositor (Salt, 1937; Lloyd, 1939). Wylie (1965) reported that *Nasonia vitripennis* laid fewer eggs on parasitized than on unparasitized pupae of the house fly, *Musca domestica*, but that the discrimination was through the detection of chemical and/or physical conditions

detected through the insertion of the ovipositor and not because of physical or chemical traces on the surface of parasitized hosts. The female parasitoid is believed to detect the venom injected by the former parasitoid before she laid her eggs and the internal injury to the host resulting from the insertion of the parasitoid's ovipositor.

Previous oviposition experience has a strong influence on the parasitoid's response to the external mark (van Lenteren, 1976), however, even frequently ovipositing females sometimes cannot detect the external mark after certain periods of time. For example, the marks of *Trichogramma embryophagum*, *T. pretiosum* and *Telenomus heliothidis* persist for 12-18 hours (Klomp *et al.*, 1980), and that of *Telenomus fariai* for 3 days (Bosque & Rabinovich, 1979). van Lenteren and Bakker (1975) and Klomp *et al.*, (1980) concluded that selective avoidance of parasitized hosts must be acquired through experience with unparasitized hosts. In contrast, van Alphen *et al.* (1987) maintained that the ability to discriminate between parasitized and unparasitized hosts need not be learned.

Visser *et al.* (1992) found that parasitoids having pre-patch experience with parasitized hosts parasitized a significantly greater proportion of the already parasitized host larvae placed in a test patch than did wasps having pre-patch experience with unparasitized hosts. Henneman *et al.* (1995) found that the general experience of egg laying profoundly influenced the tendency to accept hosts, especially parasitized ones, in the eucoilid parasitoid, *Leptopilina heterotoma*. The findings of Bjorksten and Hoffmann (1995) that host preference in two strains of *Trichogramma* sp. nr *ivelae* was more strongly affected by oviposition experience than pre-adult experience also support the belief that oviposition and associative learning can streamline host discrimination. Naive *Ooencyrtus nezarae* females and females that had not oviposited for some time, preferred recently parasitized eggs of the platispid bug, *Megacopta punctatissimum* to unparasitized eggs (Takasu & Hirose, 1991). When attacking parasitized hosts, the wasps make use of the hole drilled in the chorion by the first female. This reduces their handling time from about twenty to nine minutes (Takasu & Hirose, 1991).

Long considered to be the result of mistakes made by imperfect animals, superparasitism is now recognized as adaptive in a number of situations (van Alphen & Visser, 1990; Godfray, 1994). A spectacular example of superparasitism was recorded by Tothill *et al.* (1930) who found 72 eggs of the solitary tachinid, *Ptychomyia remota* on a larva of the zygaenid moth, *Levuana iridescens*. During conspecific-superparasitism, eggs deposited in a host parasitized by another female are potential competitors of the other female's offspring, whereas in self-superparasitism, eggs deposited in a host parasitized by the same female will increase competition among siblings.

Self-superparasitism in solitary parasitoids therefore means a waste of time and the egg, whereas conspecific superparasitism can be advantageous under a wider range of conditions because of the probability of elimination of the nonsib competitor from the parasitized host (van Alphen & Visser, 1990). If parasitoids are deprived of hosts, they are more willing to superparasitize. Host deprivation may be used by the parasitoid as an indication that hosts are rare and thus superparasitism is a more favourable strategy. On the other hand, a female which has not attacked a host for a long period can be reasonably sure that a parasitized host encountered was found to be suitable by the former female, and that it can lay an egg without risking self-superparasitism (Godfray, 1994).

Both solitary and gregarious ecto-parasitoids (lay eggs on the host) have been observed to destroy eggs on previously parasitized hosts. A superparasitizing female solitary pteromalid parasitoid, *Pachycrepoides vindemiae*, will destroy the egg of the first female laid on *Drosophila* pupae in the space between the pupa and the puparium (Nell & van Lenteren, 1982 in Godfray, 1994). Some, but not all strains of the braconid, *Bracon hebetor* are ovicidal, using their ovipositor to destroy eggs already present on the host (Godfray, 1994).

2.3. Dispersal

It is perhaps not surprising that we know relatively little about parasitoid dispersal because measuring the movement of small insects presents technical problems

(Godfray, 1994). Some indirect evidence suggests that parasitoids regularly move quite large distances. Askew (1968) and Copland and Askew (1977) noted that even quite small parasitoids may be collected at some distance from the nearest habitats where their hosts are found.

Parasitoids disperse in various ways. In some species, flight of the adults may commonly be observed under certain conditions in nature. For example, *Praon abjectum* (aphid parasitoid) adults may often be found to fly and disperse over the plant, either searching for mates or hosts under favourable weather conditions, but otherwise prefer to run under less favourable conditions (Stary, 1970). The flight range of a parasitoid also depends on the dispersal of its host. For example, aphid parasitoids which parasitize earlier aphid instars will not need to disperse as much as the other parasitoids which attack late alate instars which are capable of long distance dispersal (Stary, 1970).

In order to recover released *I. hookeri* parasitoids from the field, Cooley and Kohls (1934) trapped or shot rodents and held the nymphs recovered from them under observation. Soon they realized that very little is known about the manner and rate of dispersal of this parasitoid and that failure to recover the parasitoids in a given area is not positive proof that the parasitoids have not become established in that locality.

Mwangi *et al.* (1994) reported that the size of the containers used for the mass rearing of *I. hookeri* was crucial in terms of providing flying space. Parasitoids placed in glass vials (7.6cm x 2.5cm) and glass jars (15cm x 10cm) moved sluggishly and rested most of the time as compared to those in perspex boxes (36cm x 30cm x 20cm). *I. hookeri* parasitoids are also believed to be capable of free flight in the field since they parasitize ticks feeding on cattle (Mwangi *et al.*, 1994).

2.4. Infochemicals and pest management

Ever since the first discovery of infochemical use by natural enemies in the early 1970s, investigators have speculated on possibilities for applying these infochemicals in pest management.

Similarly, the discovery of learning in parasitoids has provoked thoughts on improvement of natural enemy efficiency through behavioural manipulation (Vet & Dicke, 1992). Chemicals which stimulate host-searching behaviour in parasitoids have been identified for a number of Hymenoptera species: *Cardiochiles nigriceps*, *Trichogramma evanescens*, *T. pretiosum*, *Trissolcus* spp., *Telenomus* spp., *Microplitis croceipes* and *Aphidius nigriceps* (Nordlund *et al.*, 1981). Over time, some other parasitoids have been added to this list.

Taking advantage of the fact that many parasitoids seek out particular habitats and are guided by volatiles emanating from plants, some researchers have applied certain plant extracts on crop plants to reinforce the host location behaviour of parasitoids and have improved parasitization rates (Altieri *et al.*, 1981). Spraying of plant produced synomones attracted ovipositing female parasitoids, enhancing the parasitization of *Helicoverpa zea* and *Anagasta kuehniella* by *Trichogramma* wasps under soyabean field and greenhouse conditions respectively (Altieri *et al.*, 1981; Altieri & Letourneau, 1982; Altieri *et al.*, 1993). There is also potential for using stimulatory plant extracts to disrupt oviposition behaviour or to attract the European corn borer females away from valuable crops (Udayagiri & Mason, 1995).

Although pheromones have been used to control insect pests, few attempts have been made to apply this strategy against ticks. Early demonstrations of pheromone-enhanced kill of *Amblyomma maculatum* and *A. hebraeum* excited much interest (reviewed by Sonenshine, 1985). However, no commercial use of these pheromone-acaricidal mixtures occurred despite subsequent identification of the male attractant-aggregation-attachment pheromone (AAAP) of *A. variegatum* (Schoni *et al.*, 1984), a closely related species. Sonenshine *et al.* (1985) applied sex pheromone-impregnated microcapsules along with a pesticide to the fur of dogs infested with *Dermacentor variabilis*. The pheromone background confused and ultimately killed most mate-seeking males before they could inseminate female ticks. Norval *et al.* (1996) incorporated components of the AAAP (o-nitrophenol and methyl salicylate), 2,6-dichlorophenol (sex attractant), phenylacetaldehyde and one of three different acaricides (cyfluthrin, flumethrin or alphacypermethrin) into plastic

bands which were attached to the tails of cattle. When released into tick-infested pastures, the combination of carbon dioxide from the animal's breath and AAAP attracted ticks sheltering in the soil or vegetation to the treated animals. Most ticks (79.2-99.3%) that infested these animals were killed. The decoys remained active for many weeks. The sex attractant, 2,6-dichlorephenol has been used to disrupt mating of *Dermacentor variabilis* (Ziv *et al.*, 1981).

These pheromone-assisted tick control strategies provide important advantages not found with conventional methods of pesticide delivery, especially (1) reduced and localized pesticide use and where sex pheromones are used (2) total disruption of reproduction (Sonenshine *et al.*, 1992). Pavis *et al.* (1994) showed that dermal gland secretions of *A. variegatum* have an antibiotic activity against the bacteria species *Bacillus thuringiensis* and *Serratia marcescens*, and a repellent effect on a potential predator, the fire-ant, *Solenopsis geminata*. The activation and sampling of ticks using carbon dioxide has also been well exploited (Garcia, 1965).

Although ticks use both attractant and arrestant infochemicals in both host seeking, mate finding and copulation (Obenchain & Galun, 1982; Sonenshine *et al.*, 1986; Gothe, 1987; Sonenshine, 1993; Norval *et al.*, 1994; Schmidtmann, 1994), so far, none of these chemicals have been evaluated for their action on tick parasitoids. The application of chemical ecology to tick management is clearly in its embryonic stage unlike the case of insects where a group of infochemicals (sex pheromones, aggregation pheromones and kairomones) have found widespread use in insect pest management (Hassanali, 1994). Clearly, identification and elucidation of the roles of different groups of infochemicals that have been implicated in different stages of the life of the tick as well as their effects on other trophic levels could provide means of developing more effective monitoring tools and highly specific behavioural approaches to controlling ticks. It could also provide a basis of knowing and predicting the fate of some of the organisms that interact with and share the environment with the tick (Hassanali, 1994), such as tick parasitoids.

CHAPTER 3: MATERIALS AND METHODS

3.1. Parasitoids

Ixodiphagus hookeri (Plate 3.1) was obtained from the colony maintained at the ICIPE for seven years. The parasitoids were originally obtained from *Amblyomma variegatum* ticks collected from cattle in the Trans-Mara area of Kenya and was maintained on *A. variegatum* nymphs fed on New Zealand White rabbits. The nymphs were parasitized while still feeding on the rabbit ears and the replete parasitized nymphs were kept in aluminium cans (Plate 3.2) at 28°C, 80% relative humidity (r.h.) and a 12:12 (light:dark) photocycle until the emergence of the parasitoids. A single hole (diameter = 2cm) was bored in the centre of the can's lid to allow air and light to pass through. Freshly emerged mated, naive (i.e. no previous experience with the odours tested) female parasitoids were used within four hours after emergence in all experiments. The length of the antennae and courtship behaviour were used to separate the females from the males. The males have longer antennae than the females and were always courting (touch the antennae and head of the females with their antennae while rapidly moving left and right in front of the females) and attempting to mount the females.

3.2. Ticks

3.2.1. *Amblyomma variegatum*

A. variegatum ticks (Plate 3.3) were obtained from the ICIPE colony. The colony was occasionally boosted with ticks collected from the Trans-Mara area of Kenya. Adults were fed on the scrota of Friesian calves. Fed, mated adult females were kept at 96% r.h. and 28°C for egg laying and hatching of the eggs. Larvae and nymphs were fed on New Zealand White rabbit ears inside cotton sleeves. A hard leather collar was placed around the neck of the rabbit to avoid the scratching of the feeding ticks (Plate 3.4). Unfed adults, nymphs and larvae were kept at 96% r.h. and 18°C. Moulting nymphs and larvae were kept at 18°C and 85% r.h.

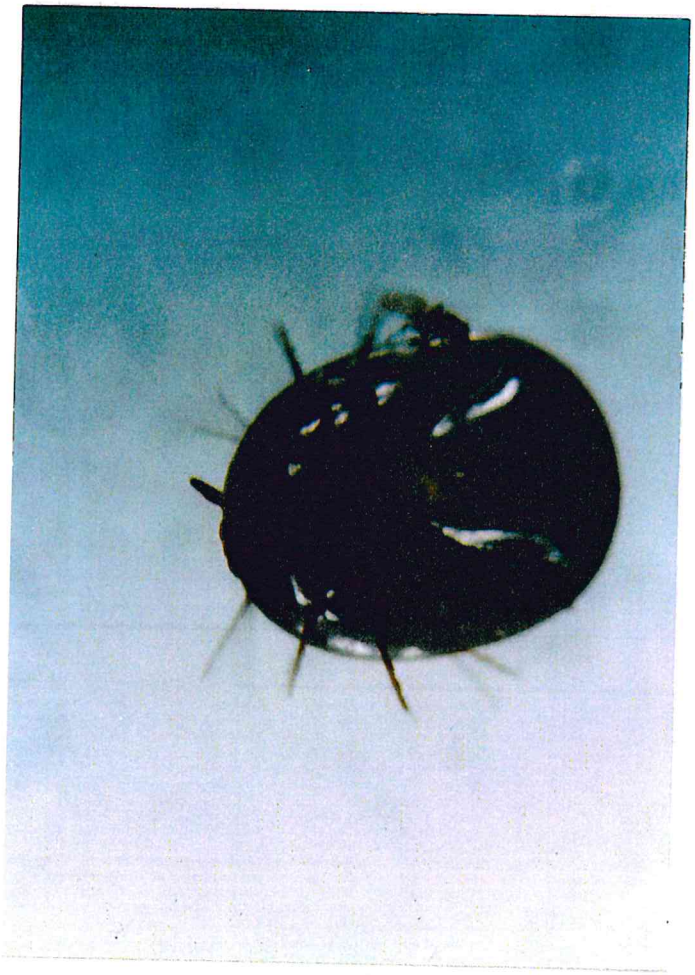

Plate 3.1. *I. hookeri* female parasitizing an *A. variegatum* nymph (magnification: x15)

Plate 3.2. Aluminium cans in which parasitized replete *A. variegatum* nymphs were kept till the emergence of the parasitoids (height = 20cm, diameter = 20cm)

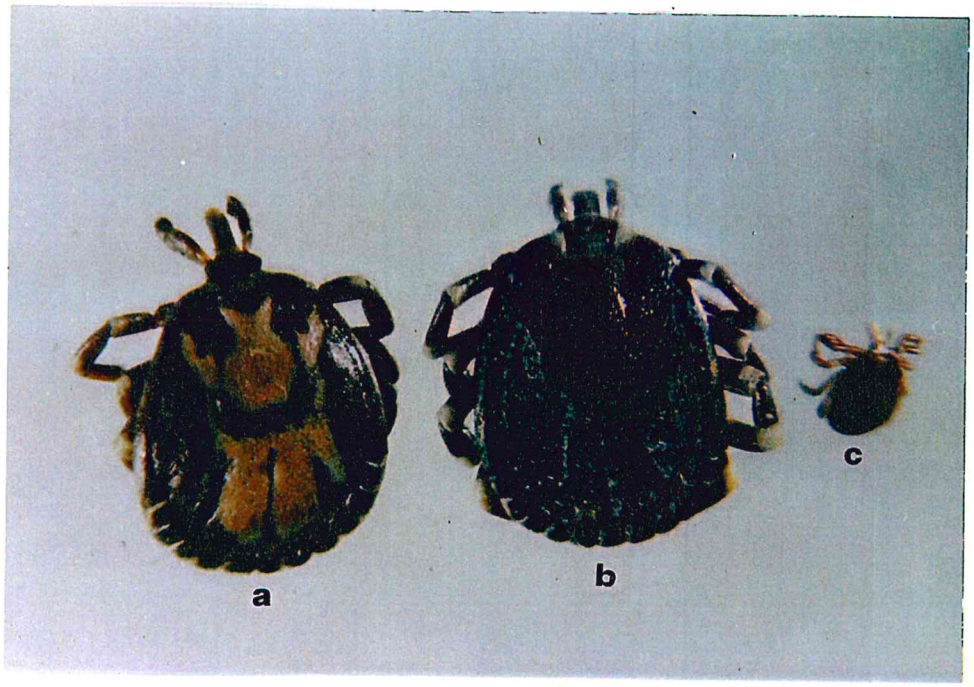

Plate 3.3. *A. variegatum* ticks: (a) unfed male (b) unfed female (c) unfed nymph (magnification: x15)

3.2.2. *Rhipicephalus appendiculatus*

R. appendiculatus ticks (Plate 3.5) were also obtained from the ICIPE tick colony. The colony was established from a strain originally kept at the East African Veterinary Research Organization, Muguga, Kenya and was maintained in the laboratory using the methods described by Bailey (1960) and Branagan (1974). Moulting and egg-laying ticks were kept at 85% r.h. and 28°C. Larvae and nymphs were fed on New Zealand White rabbit ears inside cotton sleeves as described above. After moulting, unfed ticks were kept at 96% r.h. and 18°C.

3.3. Vertebrate hosts of the tick

3.3.1. Rabbits

New Zealand White rabbits were used to feed larval and nymphal ticks. The rabbits were obtained from the ICIPE colony which was initiated from parent stock from Hylyne, Germany. The colony was occasionally boosted with New Zealand White rabbits from a pure breed from Sasumua Farm, Njoro, Kenya and were maintained on commercial pellets.

3.3.2. Calves

Three tick-free bull calves (one Friesian, one Ayrshire and one Ayrshire x Charolais, all less than 15 months old) were maintained on commercial concentrates and hay. The calves were obtained from the Ngong Veterinary Farm near Nairobi, Kenya. Body surface and waste product odours were collected from these calves while the scrota were also used for tick feeding. Two white zebu calves infested with *A. variegatum* (mainly adults) and *R. appendiculatus* ticks (larvae, nymphs and adults) were borrowed from farmers on Rusinga Island for the studies on the field behaviour of *I. hookeri*. These calves grazed freely.

3.4. Grasses

Fresh green *Cynodon dactylon*, *Eragrostis superba* and *Digitaria seriata* were uprooted from a paddock near the ICIPE, Nairobi, Kenya.

The culms of the grasses were cut just above the roots and used immediately for bioassays. The grasses were identified using the key outlined by Müller (1984) and confirmed by Dr. Mary Owaga, a former ICIPE ecologist.

3.5. Bioassay set-ups

3.5.1. Exploring olfactometers

Three different airflow olfactometers were investigated to find the set-up which yielded the best response of the parasitoids to the test odour sources. The olfactometers tested were: (i) a Y-tube olfactometer with a suction outlet at the base of the stem and (ii) a Y-tube olfactometer with a suction outlet at the junction of the two arms.

3.5.1.1. Y-tube olfactometer with suction at the base of the stem

The Y-tube olfactometer (Fig. 3.1) described by Sabelis and van de Baan (1983) and Steinberg *et al.* (1992) was used. The olfactometer was made out of a 4cm inner diameter glass tube and had the following specifications: stem = 15cm, each arm = 14cm, PVC joints = 6.4cm, glass segments = 10cm. Two flat-bottom flasks (500ml), one for each arm, were connected to flowmeters and then to the arms of the olfactometer with Tygon tubing. The airflow through the olfactometer was visualized by passing ammonium chloride fumes through it. Ammonium chloride fumes were produced in one of the flasks by passing 25% ammonium hydroxide (NH_4OH) vapour over concentrated hydrochloric acid (HCl) and drawn through the olfactometer with the aid of a pump (Cole-Parmer Pressure/Vacuum Air Cadet, Model No. 400-3902, Barnant Co.) at an airflow rate of 1.5l/min in either arm. The separation of the fumes and the air could be seen in the stem of the olfactometer and thus, the olfactometer was confirmed to be symmetrical. Turbulence at the junction of the arms was reduced to levels not detectable with the human eye when the airflow was equal in both arms. The response of parasitoids to cattle urine (found to be attractant) was also tested and the orientation of the parasitoids to the odour plume could be seen in the stem of the olfactometer before the arms were

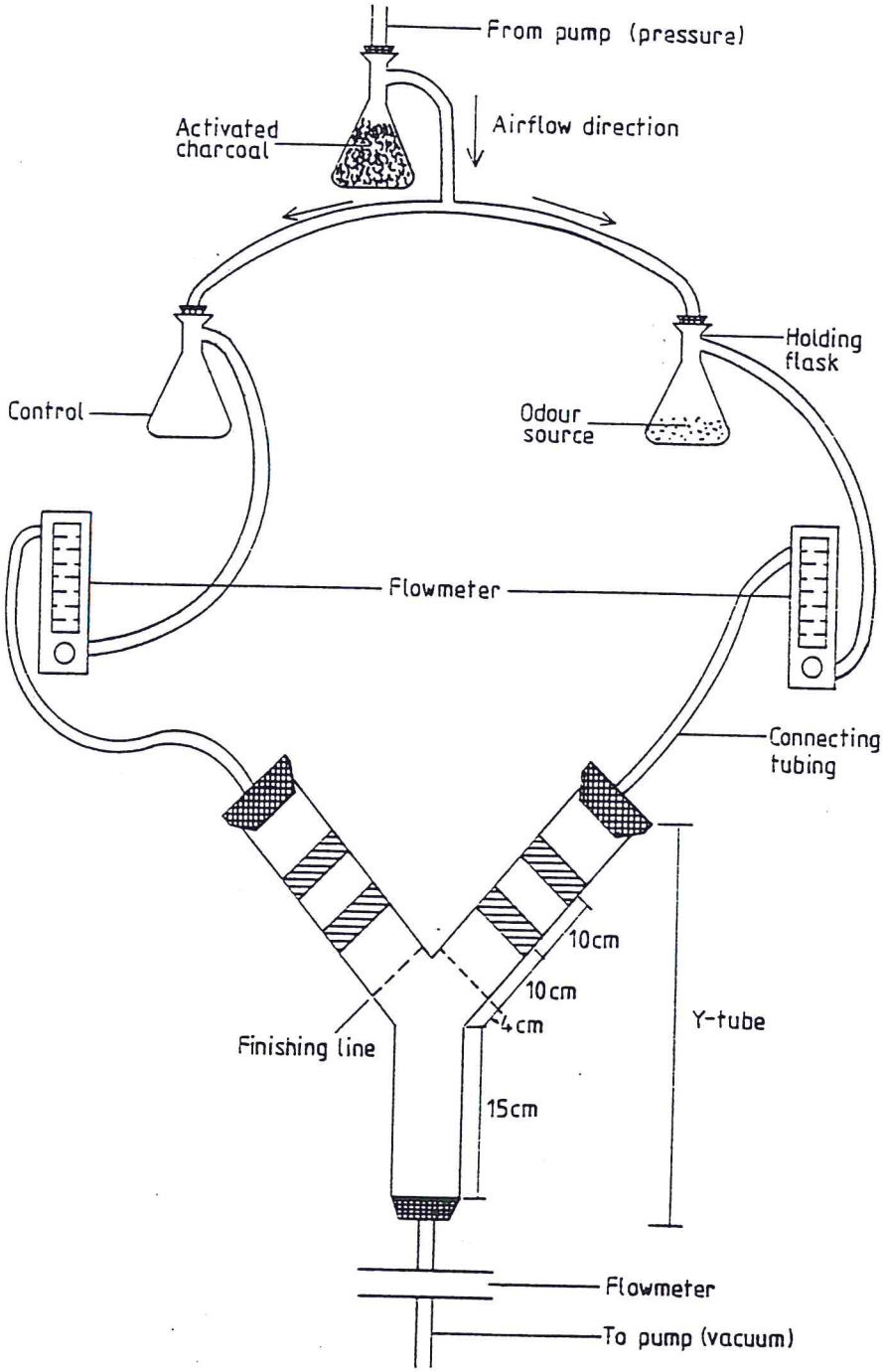

Fig. 3.1. Y-tube olfactometer set-up

entered. The parasitoids were introduced at
odours were sucked right down to the point
evaluate the odours right away and respond ac
best response of the parasitoids to the odour
this study for bioassays.

3.5.1.2. Y-tube olfactometer with suction at

The Y-tube described in section 3.5.1.2
(length = 5.5cm) at the junction of the two arm
revealed that turbulence at the junction o
olfactometer than the one described above (s
the air had to be sucked out through the same
could not be controlled by adjusting the airflow
remained in the stem of this olfactometer with
to the response in the olfactometer described
not reach the base of the stem where the
olfactometer seemed to confuse the parasitoi
for bioassays in this study.

3.5.2. Experimental procedures and bioassay

All bioassays were conducted at room tempe
light intensity of 900-950lux (photometer, LI-

3.5.2.1. Y-tube olfactometer

The Y-tube olfactometer (Fig. 3.1) describe
investigate the distant orientation of *I. hooke*
odours (distance between the odour source a
parasitoids was > 1m). The major modifica
described by Sabelis and van de Baan (1983) a
odour and control source containers which we

The Y-tube was held in a vertical position with the arms facing upward with the aid of a clamp. A white uniform background was created through a box (60cm x 60cm x 40cm) with an open top and front to facilitate easy detection of the parasitoids in the olfactometer and to cut out the influence of the surroundings. A pump (Cole-Parmer Pressure/Vacuum Air Cadet) was used to push air through an activated charcoal filter and then into the arms of the olfactometer at a flowrate of 1.5l/min in each arm while another pump of the same make removed the air from the olfactometer at 3.0l/min at the base of the stem. Parasitoids were released individually (to avoid interference or facilitation among parasitoids) in the stem of the Y-tube and left for five minutes to choose one of the arms. To take care of any confusion at the junction of the olfactometer arms and to give the parasitoids a chance to modify their choice, a finishing line (4cm past the junction) was delineated with thin strips of sticky tape on each arm. Once a female crossed this line that arm was recorded as the choice. Besides the choice, response time (time between the introduction of the parasitoid and the crossing of the finishing line) and retention time (time the parasitoid spends in the choice arm) were also recorded. After five minutes, the insect was removed and the olfactometer rinsed with acetone, washed in distilled water and dried at 50°C to eliminate possible odours left by the test parasitoid before the next parasitoid was introduced. The connections of the test and control flasks to the arms of the olfactometer were reversed after every five insects tested to rule out the effect of asymetrical bias in the olfactometer or its surroundings and also to take care of any handedness (more tendency to turn to the left or right) in the parasitoids.

3.5.2.2. T-tube olfactometer

The close-range orientation of *I. hookeri* females to host-derived infochemicals was tested in a glass T-tube olfactometer (Fig 3.2). The arms and stem of the olfactometer were 2cm long each. Test materials were placed in fine wire meshes and inserted directly into the arms. Parasitoids were released individually in the stem and once a parasitoid reached the end of an arm that arm was recorded as the

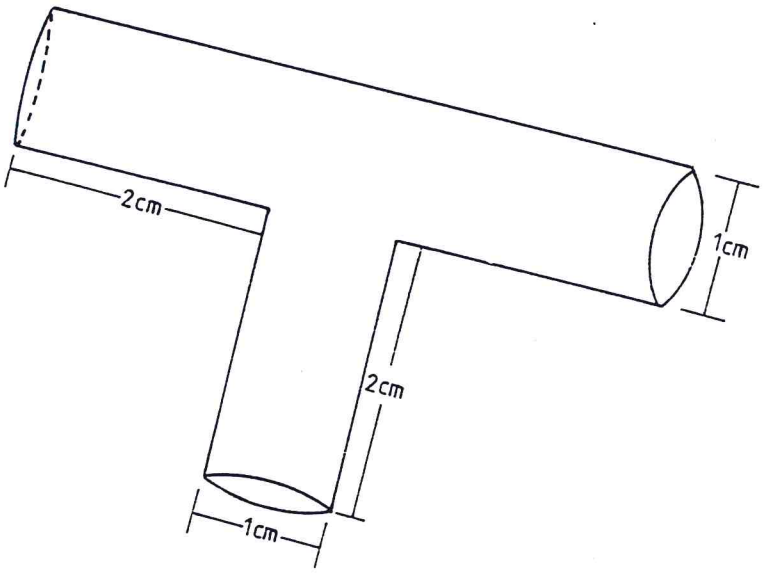

Fig. 3.2. T-tube olfactometer set-up

choice. The cleaning of this olfactometer was the same as that described for the Y-tube olfactometer.

3.5.2.3. Petri dish/vial set-up

Visual host evaluation and recognition by *I. hookeri* females were tested in the Petri dish/vial set-up. Glass petri-dishes (Pyrex, inner diameter = 9cm, height = 1.8cm) and 50cm x 12cm glass vials were used. The test materials were placed inside the vials and the vials tightly closed with polythene tops to eliminate the interference of any host-derived semiochemical. After closure, the test and empty control vials were rinsed with acetone, rinsed with distilled water, wiped with cotton wool (cleaned in hexane and dried in oven at 100°C), air-dried and sealed with parafilm. Whenever live mobile nymphs were tested, cotton wool was placed in the hollow insides of the tops to prevent ticks from entering the tops and thus becoming invisible to the parasitoid. The test and control vials were then placed horizontally opposite each other in the petri-dish. A single female parasitoid was released in the centre of the petri-dish (between the two vials) and the dish closed. Each female was observed for ten minutes before removal. Examination time, examination frequency and the direct detection of the test materials were recorded. Examination time is the total time during which the parasitoid actively ran on and/or antennated the vial. Stops (no active running or antennating of the vial) on the vial lasting more than ten seconds were regarded as resting and not recorded. Examination frequency is the number of times the parasitoids contacted the vials for examination. The straight running of the parasitoids to the vial and contacting the vial at the spot where the test material was visible was recorded as direct detection. The set-up was cleaned after every insect tested.

3.5.2.4. Vial set-up

Acceptance behaviour of the parasitoid was tested in glass vials (5.0cm x 1.3cm). An inverted vial was placed over a single female parasitoid which in turn

climbed to the top of it. The vial was then placed over a single nymphal tick. Pre-oviposition, oviposition and pre-contact times were recorded in the case of *A. variegatum* nymphs, while attack time (i.e. the time a parasitoid spent with its ovipositor inserted in a nymph) was recorded for *R. appendiculatus* nymphs. An observation was terminated after five minutes or at the first contact between the parasitoids and the nymphs.

3.6. Collection and bioassay of odours

3.6.1. Grasses

To investigate the possible role pasture grasses play in the location of off-host *A. variegatum* nymphs and nymphs feeding on cattle grazing in pastures, three grasses, *Cynodon dactylon*, *Eragrostis superba* and *Digitaria seriata* were tested for their attraction to *I. hookeri* females using the Y-tube olfactometer (section 3.5.2.1). Fifteen grams each of fresh *C. dactylon*, *E. superba* and *D. seriata* grasses were tested separately against control air (charcoal-filtered air). Grasses were replaced with fresh ones after every 50 minutes. This experiment was replicated three times with 20 parasitoids per replicate.

3.6.2. Cattle

3.6.2.1. Waste products

To test the possible role of cattle waste products in the location of the host habitat and hosts by mated naive *I. hookeri* females, fresh urine, seven-day-old urine and fresh dung were tested for attraction in the Y-tube olfactometer (section 3.5.2.1). All fresh products tested were bioassayed within two hours after collection. Fresh urine was collected directly from urinating calves and ten millilitres poured into a holding flask and tested against control air.

To test the response of the parasitoids to odours from old urine, portions of fresh urine were left in stoppered vials (7.5cm x 2.4cm) for seven days for fermentation before being bioassayed. Fresh dung was also collected from calves, 15 grams transferred to a holding flask and tested against control air.

This experiment was replicated three times with 20 parasitoids per replicate.

3.6.2.2. Cattle body surface odours

To investigate the possible role cattle body surface odours play in the location of the host habitat and hosts by mated naive *I. hookeri* females, swabs were taken on dry cotton wool and bioassayed against control cotton wool in the Y-tube olfactometer. The swabs were taken from four known feeding sites of *A. variegatum* ticks on cattle (i.e. dewlap, scrotum, hind heels and front heels, Fig. 3.3) and the main feeding site of *R. appendiculatus* nymphs on cattle, the ear. All swabs were tested immediately after being taken and were replaced with fresh ones after every 30 minutes. This experiment was replicated three times with 20 parasitoids per replicate.

3.6.3. *A. variegatum* nymphs

3.6.3.1. Off-host nymphs

3.6.3.1.1. Live nymphs

Since off-host ticks are known to produce assembly pheromones which attract different life stages of the same species as well as other species (Sonenshine *et al.*, 1982), volatiles from ten, thirty or fifty nymphs (fed or unfed) were bioassayed against control air in the Y-tube olfactometer. This was done to test whether the assembly of nymphs in an area could amplify any chemical signal(s) used by the parasitoids to locate these nymphs. This experiment was replicated three times with 20 parasitoids per replicate.

3.6.3.1.2. Washes

To investigate the role of less volatile and non-volatile infochemicals on the body surfaces of *A. variegatum* and *R. appendiculatus* nymphs in the location of these nymphs by mated naive *I. hookeri* females, three hundred unfed nymphs of each species were submerged in 10ml of redistilled hexane for ten hours and the

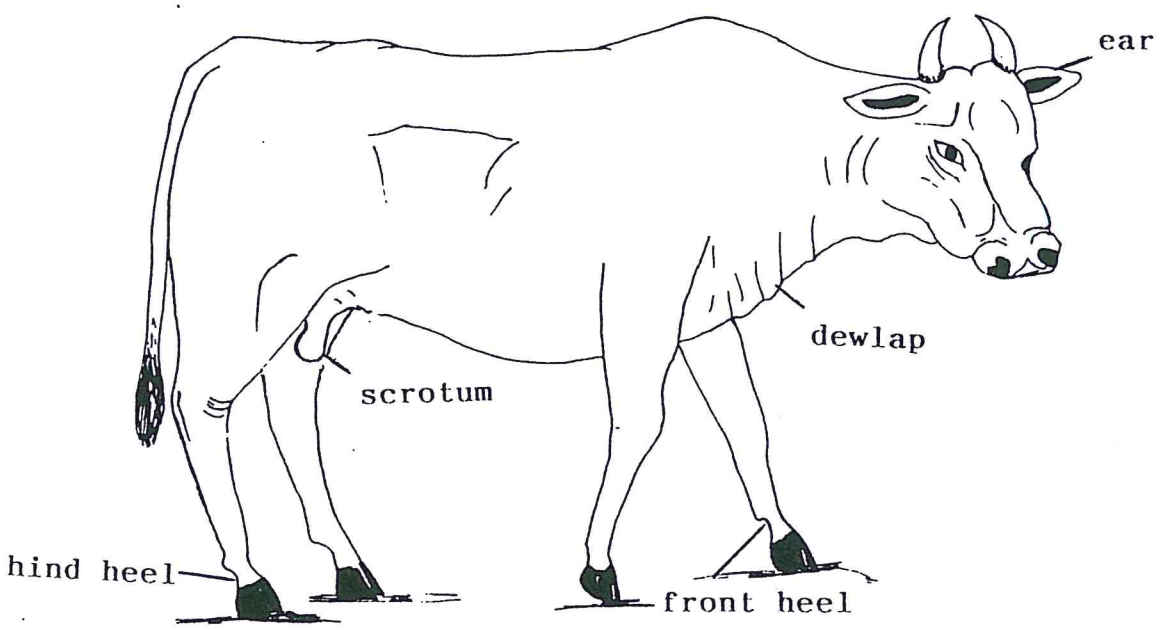

Fig 3.3. Areas on calf body surface from which volatiles were collected

samples bioassayed in the T-tube olfactometer. Ten μl of the concentrated (section 3.7.2) samples were placed on filter paper discs (Whatman, no. 1) and tested against the same amount of hexane on discs. The discs were placed directly in the arms of the T-tube and were replaced after every ten insects tested. This experiment was replicated three times with 30 parasitoids per replicate. The hexane washes were analyzed using the gas chromatograph (GC, section 3.7.2.1) and the chromatograms inspected visually.

3.6.3.2. On-host nymphs

3.6.3.2.1. Volatiles from tick-infested and tick free calf scrota

To investigate the possible attraction of a blend of volatiles from *A. variegatum* nymph-infested calf scrota, fifty nymphs were fed on calf scrota inside cotton scrotal bags (Plate 3.6). The scrotal bags were held in place at the base of the scrota with stic-tite glue and the nymphs allowed to feed for four days before volatiles were trapped. Volatiles were trapped overnight for 18 hours on activated charcoal (Chrompack) placed in fine wire-mesh pockets (3cm x 2cm) and placed in turn inside the scrotal bags. As controls, volatiles were trapped from tick-free scrota as described above. After trapping, the charcoal was eluted with redistilled dichloromethane (DCM). Ten μl of the concentrated (section 3.7.2) tick-infested and tick-free samples were placed on filter paper discs and tested separately against the same amount of concentrated DCM on discs in the Y-tube olfactometer for their attraction to naive mated *I. hookeri* females. The discs were replaced after every ten insects tested. The samples were analyzed using the gas chromatograph (section 3.7.2.1) and the chromatograms inspected visually. This experiment was replicated three times with 20 parasitoids per replicate.

3.6.3.2.2. Faeces from feeding nymphs

To investigate the role infochemicals from host-derived products play in the location of hosts by mated naive *I. hookeri* females, the faeces of feeding

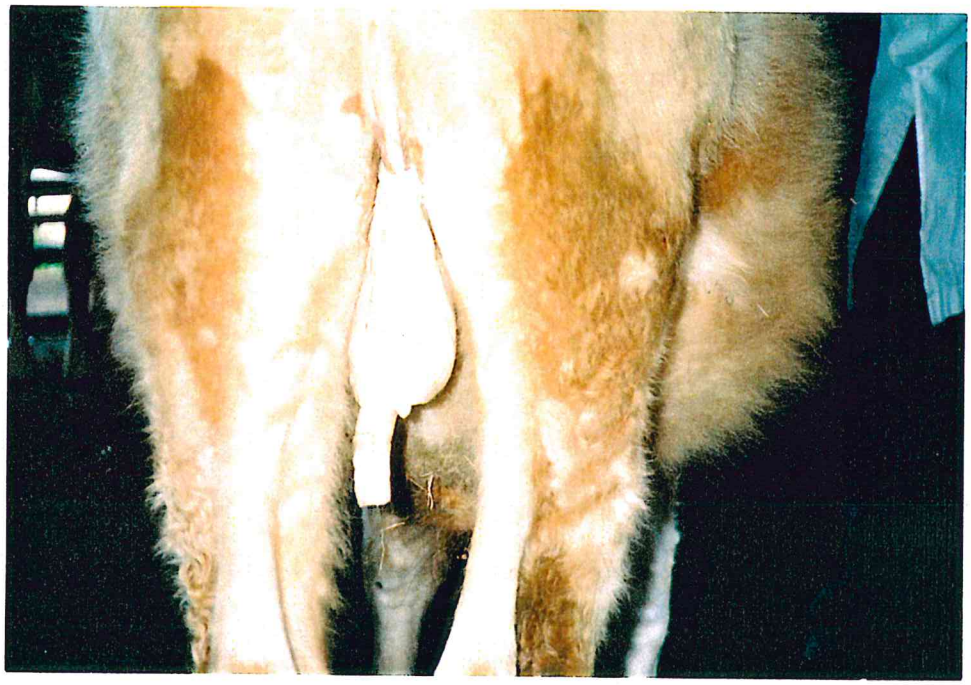

Plate 3.6. Ticks feeding on a calf's scrotum inside a scrotal bag

A. variegatum and *R. appendiculatus* nymphs were collected. The faeces (0.3g) were extracted with redistilled hexane for ten hours and the extracts bioassayed in the T-tube olfactometer (section 3.5.2.2). Ten μl of the concentrated samples were placed on filter paper discs and tested separately against the same amount of hexane on discs for their attraction to the parasitoid. This experiment was replicated three times with 30 parasitoids per replicate. The discs were replaced after every ten insects tested. The extracts were also analyzed using the gas chromatograph and the chromatograms inspected visually.

3.7. Storage and analysis of volatiles

3.7.1. Storage

All volatiles collected were stored in small media bottles with teflon-lined tops (4ml, Sigma, UK) in a freezer at -20°C until the time for analyses. Outside the freezer, the volatiles were always kept in ice to avoid the possible evaporation of compounds.

3.7.2. Analysis

Just before analysis, the volatiles were concentrated under a gentle stream of nitrogen to 100μl. Aliquots of volatile collections were analyzed by gas chromatography.

3.7.2.1. Gas chromatography (GC)

GC analyses were performed on a Hewlett-Packard (HP) 5890 Series II gas chromatograph (Plate 3.7) equipped with a flame ionization detector (FID) (both detector & injector temperatures =250°C) and fitted with a HP Ultra1 crosslinked capillary column (Methyl Silicon, 50m (length) x 0.22mm (inner diameter) x 0.33μm (film thickness)). Nitrogen was used as the carrier gas at a flowrate of 0.35ml/min. The GC oven temperature was initially set at 40°C (5 minute hold), increased at a rate of 7°C/min to 180°C (0 minute hold), increased at a rate of 10°C/min to a final temperature of 280°C (30 minute hold) for all the

other samples. For nymphal washes, the oven temperature was initially set at 150°C (2 minute hold), increased at a rate of 25°C/min to 270°C (2 minute hold), increased at a rate of 7°C/min to a final temperature of 280°C (30 minute hold). Chromatographic peaks were integrated using a HP Series II 3396 integrator. Two μl of each sample were injected for analyses.

3.8. Visual host evaluation and recognition

Visual evaluation (assessment of information about the host, such as size, colour, species, movement etc. from a distance without handling the host) of *A. variegatum* nymphs by *I. hookeri* parasitoids and the recognition of *A. variegatum* and *R. appendiculatus* nymphs were tested in the petri-dish/vial set-up described in section 3.5.2.3. Test and control nymphs were placed singly in glass vials (50cm x 12cm), sealed and the two vials presented to naive (i.e. no experience with the test material before) mated *I. hookeri* females in a glass petri-dish (Pyrex, inner diameter = 9cm, height = 1.8cm) for evaluation and recognition testing. The vials were laid flat in the petri-dish and the dish closed to keep the parasitoids in. All fed nymphs were tested on the day they dropped from the rabbit ears since nymphs changed colour and the integument hardened in preparation for moulting beyond two days post drop. Examination time, examination frequency and the direct and indirect detection of the nymphs were recorded.

3.8.1. Size

To test the effect of host size on the evaluation and recognition of hosts by *I. hookeri* females, the parasitoids were presented with a single live large fed nymph (6.5mm-7mm from the tip of the abdomen to the base of the mouthparts) and a single live small fed nymph (4.5mm-5mm)(Plate 3.8). This experiment was replicated three times with ten parasitoids per replicate.

Plate 3.8. Sizes of fed *A. variegatum* nymphs tested: large(left) small(right) (magnification: x15)

3.8.2. Movement

To test the effect of host movement on the evaluation and recognition of hosts by *I. hookeri* females, the parasitoids were presented with a single live fed mobile nymph and a single dead (killed by freezing) fed nymph. Killing with chemicals (e.g chloroform) was avoided because of possible traces of chemicals which could influence the experiment. This experiment was replicated three times with ten parasitoids per replicate.

3.8.3. Feeding state

To test the effect of nymphal engorgement with blood on the evaluation and recognition of these nymphs by *I. hookeri* females, the parasitoids were presented with a single live fed nymph (Plate 3.1) and a single live unfed nymph (Plate 3.3). This experiment was replicated three times with ten parasitoids per replicate.

3.8.4. Colour

To test the colour preference of *I. hookeri* females, the parasitoids were presented with a single dead (killed by freezing) fed nymph (grey) and a single mummified yellow-brown or dark brown nymph (Plate 3.9). The parasitoids were also presented with a single mummified yellow-brown or a single dark brown mummy. This experiment was replicated three times with ten parasitoids per replicate.

3.8.5. Species

To test whether *I. hookeri* females could recognize *A. variegatum* nymphs, the parasitoids were presented with a single live fed *A. variegatum* nymph and a single live fed *R. appendiculatus* nymph (Plate 3.10). This experiment was replicated three times with ten parasitoids per replicate.

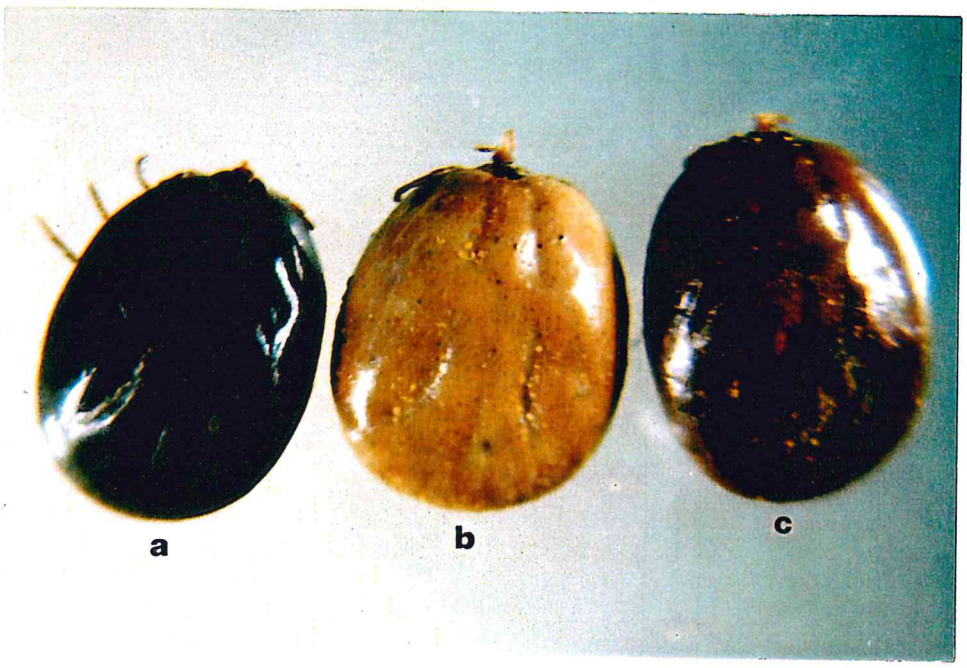

Plate 3.9. Colours of *A. variegatum* nymphs (live and mummified) tested: (a) grey (b) light brown (c) dark-brown (magnification: x15)

Plate 3.10. Replete *A. variegatum* (left) and *R. appendiculatus* (right) nymphs (magnification: x15)

3.9. Host acceptance and suitability

3.9.1. Acceptance

The acceptance of hosts and non-hosts for oviposition by mated, naive *I. hookeri* females was tested using the glass vial set-up described in section 3.5.2.4. All replete nymphs were tested on the day they dropped from the rabbit ears since fed nymphs changed colour and the integument hardened in preparation for moulting beyond two days post drop. The hardened integument made oviposition in these nymphs difficult.

3.9.1.1. Species

To investigate the acceptance of host and non-host nymphs for oviposition, fed *A. variegatum* (host) and *R. appendiculatus* (non-host) nymphs (Plate 3.10) were presented singly to *I. hookeri* females. The percentage of nymphs attacked and the attack time (i.e. the time a parasitoid spent with its ovipositor inserted in a nymph) were recorded. Each nymph was dissected within 30 minutes after the attack, stained with Giemsa stain, and the presence or absence of eggs recorded. Since encapsulation of *I. hookeri* eggs in *R. appendiculatus* nymphs is suspected (Bengaly, pers. comm.), the study assumed that the dissection of *R. appendiculatus* nymphs within 30 minutes would still reveal the parasitoid eggs. This experiment was replicated three times with ten parasitoids per replicate.

3.9.1.2. Parasitization

To investigate the effect of encounters with parasitized hosts on foraging naive, mated *I. hookeri* females, a single fed, unparasitized *A. variegatum* nymph was presented to four parasitoids, one at a time, at five minute intervals. Pre-oviposition and oviposition times of each of the parasitoids in this nymph were recorded. This experiment was replicated twice with ten nymphs and 40 parasitoids per replicate.

3.9.1.3. Repellence of *I. hookeri* by mechanically disturbed *A. variegatum* nymphs

To test the effect of dermal gland secretions from disturbed fed *A. variegatum* nymphs on the behaviour of *I. hookeri* females, the legs of nymphs (two days after drop) were twisted with a pair of forceps (mechanical disturbance) until droplets were visible on the dorsal, lateral and ventral cuticle. These droplets were not secreted unless the nymphs were disturbed. Pavis *et al.* (1994) believe that these droplets are secreted by the dermal glands and therefore this study will refer to these secretions as dermal gland secretions. Pre-contact time (time between the introduction of the tick into the vial and the first contact between the parasitoid and the tick) was recorded at introduction (0 minutes), 10 minutes, 30 minutes and 50 minutes. An observation was stopped at the first contact between the tick and the parasitoid. This experiment was replicated three times with ten parasitoids per replicate.

3.9.1.4. Induction of parasitism in *R. appendiculatus*

3.9.1.4.1. Rubs

To enhance the acceptance of fed *R. appendiculatus* nymphs by *I. hookeri* females for oviposition, *A. variegatum* and *R. appendiculatus* nymphs were gently held with forceps and rubbed against each other. This was done to transfer infochemicals on the body surfaces of *A. variegatum* nymphs to *R. appendiculatus* nymphs since hexane made the nymphs sluggish or killed them when hexane washes were applied topically.This experiment was replicated three times with ten parasitoids per replicate.

3.9.1.4.2. Washes

To create the effect of the presence of many *A. variegatum* nymphs in the area, three hundred unfed *A. variegatum* nymphs were washed in redistilled hexane and ten μl of the concentrated washes applied on filter paper discs (Whatman no. 1) in a marked area.

The solvent was allowed to evaporate and fed *R. appendiculatus* nymphs (rubbed against fed *A. variegatum* nymphs, section 3.9.1.4.1) presented singly to *I. hookeri* females in inverted glass vials placed in the marked area. This was done to enhance the acceptance of fed *R. appendiculatus* nymphs by the parasitoids for oviposition. As controls, *R. appendiculatus* nymphs were also presented to the parasitoids in the absence of the odours. The percentage of nymphs attacked and the attack time (i.e. the time a parasitoid spent with its ovipositor inserted in a nymph) were recorded. After the attack, the nymphs were dissected, stained with Giemsa stain, and the presence or absence of parasitoid eggs in the nymphs recorded. This experiment was replicated three times with ten parasitoids per replicate.

3.9.1.4.3. *A. variegatum* integument

To enhance the acceptance of fed *R. appendiculatus* nymphs by *I. hookeri* females for oviposition, pieces of the integument of fed *A. variegatum* nymphs were pasted on the backs of fed *R. appendiculatus* nymphs. To obtain the integument, *A. variegatum* nymphs were placed in a petri-dish containing distilled water and punctured once at the posterior end. All contents were squeezed out of the nymphs through the punctured holes by gently pressing the nymphs. The empty nymph shells were transferred to another petri-dish and gently pressed again in distilled water to remove the remaining contents. Small pieces (3mm x 3mm) were cut from the dorsal sides (backs) of the nymphs, briefly blotted dry on filter paper, 10 μl of the concentrated unfed *A. variegatum* nymphal washes applied, air-dried for 30 seconds and placed on the backs of fed *R. appendiculatus* nymphs (Plate 3.11). These nymphs were presented singly to *I. hookeri* females. The percentage of nymphs attacked and the attack time were recorded. After the attack, the nymphs were dissected, stained with Giemsa stain, and the presence or absence of parasitoid eggs in the nymphs recorded. This experiment was replicated three times with ten parasitoids per replicate.

Plate 3.11. Replete *R. appendiculatus* nymph with *A. variegatum* nymphal integument on its dorsum (magnification: x15)

3.9.1.4.3.1. Scanning electron microscopy (SEM)

To reveal possible differences in the surface appearances of the integuments *A. variegatum* and *R. appendiculatus* nymphs, the integuments of unfed nymphs were studied with the aid of a SEM (Jeol JSM-T330A Scanning Electron Microscope, Plate 3.12). Five ticks of each species were fixed in 2.5% gluteraldehyde for six days. After fixation, the ticks were washed three times with sodium cacodylate buffer (NaCac, 0.05M); dehydrated in 30%, 70%, 90% and absolute alcohol for ten minutes at each concentration while being shaken gently on a Titertek shaker (Flow laboratories). Dehydration in absolute alcohol was done 4 times and the specimens were left overnight in absolute alcohol. After 24 hours, the specimens were mounted on stubs with double-sided sticky tape and dried for five days in the desiccator whereafter they were coated with gold under vacuum for four minutes at 10mA ion current in a JFC-1100E ion sputter (Jeol, Fine coat). The integument textures of the specimens were then scanned and detected as backscattered beams.

3.9.2. Suitability of *A. variegatum* and *R. appendiculatus* nymphs for the development of *I. hookeri* immatures

To investigated the suitability of *A. variegatum* and *R. appendiculatus* nymphs for the development of *I. hookeri* immatures, fed and unfed *A. variegatum* nymphs as well as fed *R. appendiculatus* nymphs were exposed singly to mated, naive *I. hookeri* females in glass vials (section 3.5.2.4). *R. appendiculatus* nymphs were conditioned with *A. variegatum* nymphal washes and integument pieces (section 3.9.1.4.3). *R. appendiculatus* nymphs not attacked by the parasitoids within five minutes were re-introduced after being re-conditioned with *A. variegatum* nymphal washes and integument pieces cut from the dorsa of *A. variegatum* nymphs and pasted onto the dorsa of *R. appendiculatus* nymphs (section 3.9.1.4.3). Attacked nymphs were kept in aluminium cans (Plate 3.2) at 28°C, 80% r.h. and a 12:12 (light:dark) photocycle until the

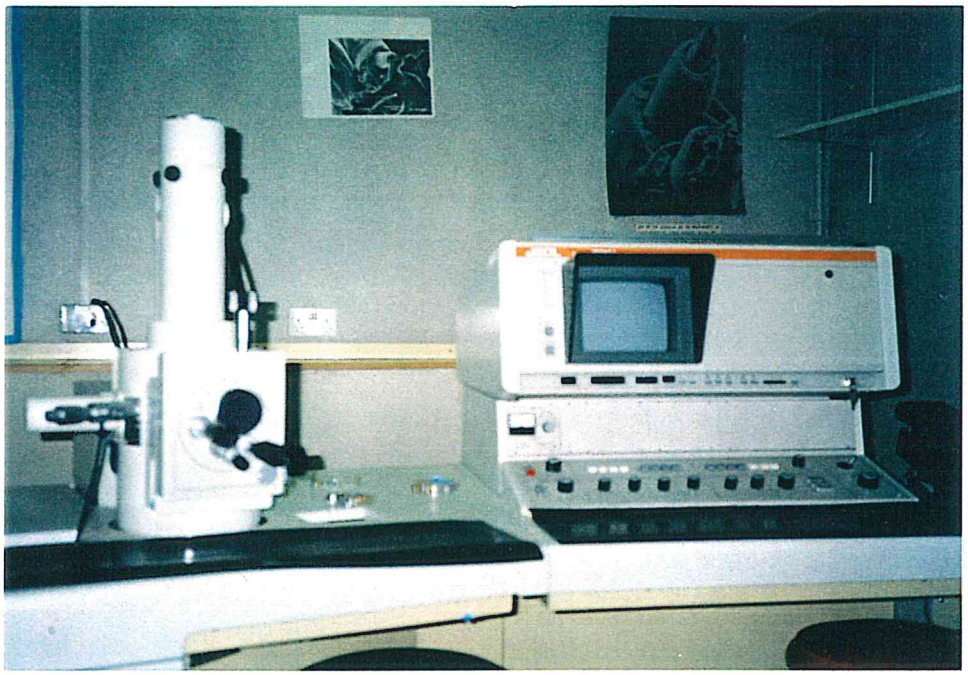

Plate 3.12. Scanning electron microscope (Jeol JSM-T330A)

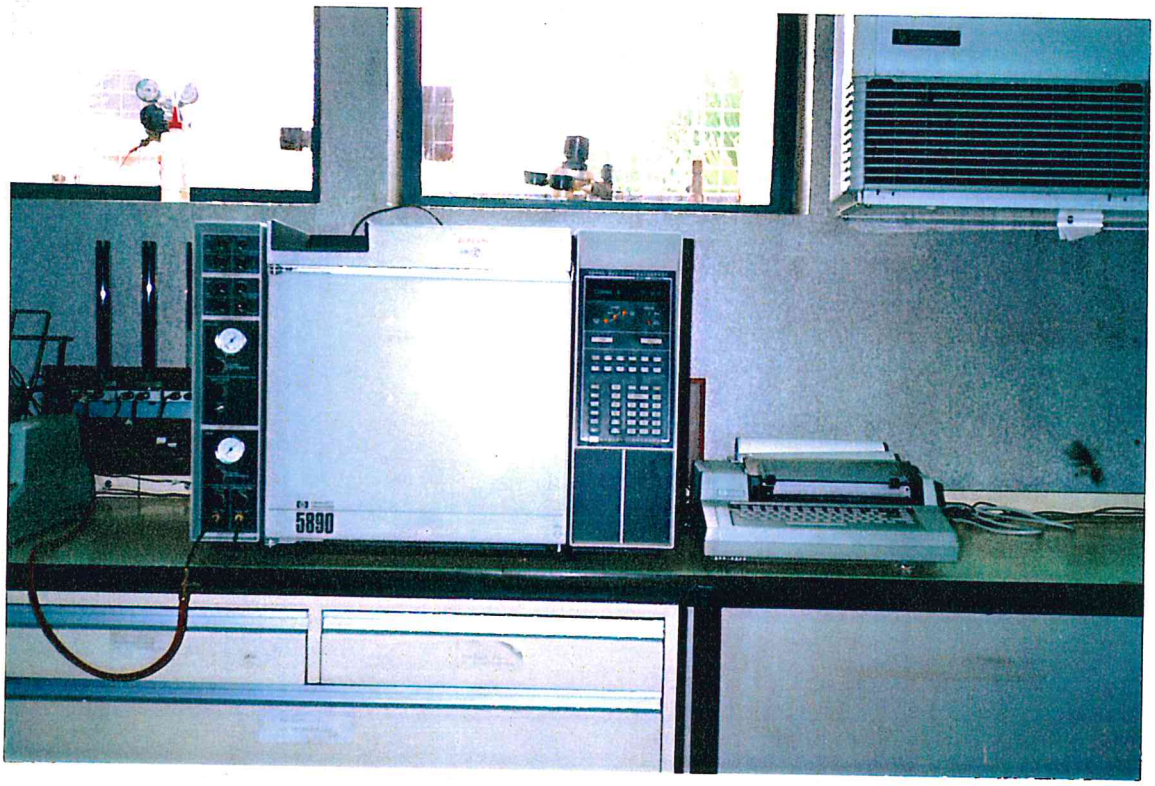

Plate 3.7. A Hewlett-Packard (HP) 5890 Series II gas chromatograph (GC)(left) coupled to a HP Series II 3396 integrator(right)

emergence of the parasitoids, moulting or death of the nymphs. The numbers of nymphs attacked, yielding progeny, moulting, killed through parasitization within 30 days and those alive after 30 days as well as the number of progeny per female parasitoid and the feeding success of *A. variegatum* nymphs fed on rabbit ears 24 hours after exposure to the parasitoids were recorded. This experiment was replicated three times with 20 parasitoids per replicate.

3.10. Field and laboratory observations on the host seeking and dispersal behaviour of *I. hookeri*

3.10.1. Field observations

3.10.1.1. Host seeking behaviour

All experiments were carried out at 65-90% r.h. and 24-32°C. Tick-free and tick-infested bull calves were used for observations. Parasitoids were released downwind from the calves. The observer was positioned close to the animals due to the small size of the parasitoids.

3.10.1.1.1. Parasitoid releases on cattle

To investigate the searching behaviour of mated naive *I. hookeri* females on tick-free cattle (zero grazed) the parasitoids were released singly high up on the shoulders of three tethered tick-free bull calves (one Friesian, one Ayrshire and one Ayrshire x Charolais). Searching time was recorded for the various parts of the front legs searched (i.e. shoulder, upper leg, middle leg and lower leg; Fig. 3.4) since almost all the parasitoids released searched on the front legs alone for the duration of the experiment. Each insect was observed for 20 minutes or until it left the animal (if this occurred earlier). This experiment was done between 08h00 and 12h00 hours and was replicated three times with ten parasitoids per replicate.

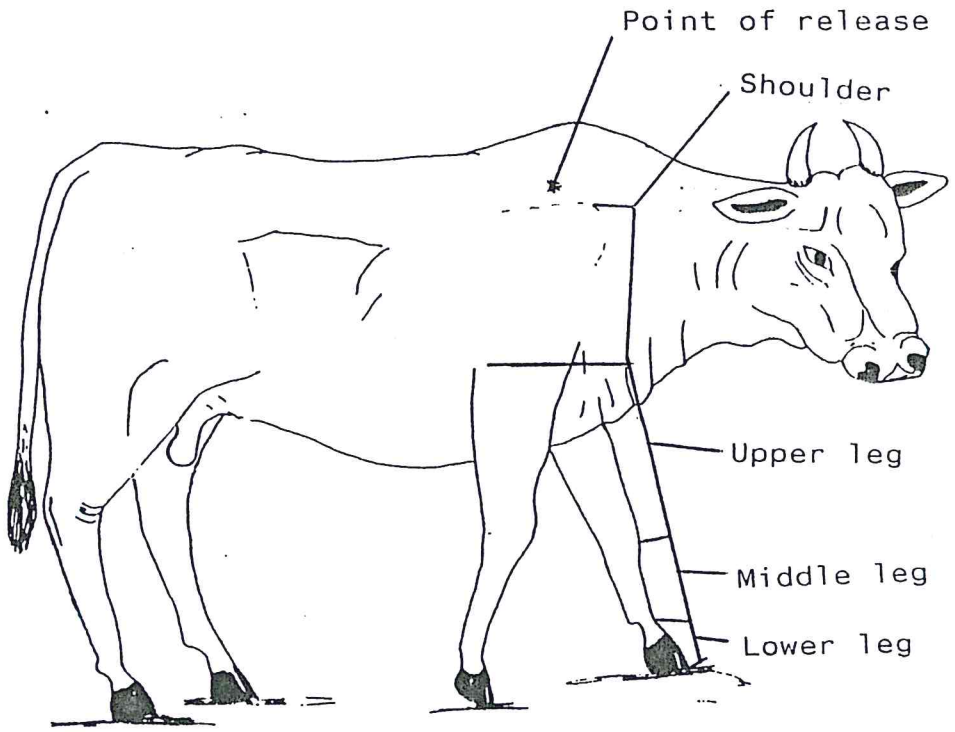

Fig. 3.4. Areas on the front legs of calves which were searched by *I. hookeri* parasitoids released on the shoulders of calves

3.10.1.1.2. Parasitization of *A. variegatum* nymphs per feeding site

Unfed, partially fed and close-to-repletion *A. variegatum* nymphs were collected from free ranging cattle in Trans-Mara and Kuja River to investigate the field parasitization rates of on-host *A. variegatum* nymphs encountered on various body regions of cattle by the parasitoids. Frontlegs, hindlegs, bellies, scrota, udders, dewlaps and ears of free ranging cattle in Kuja River and Trans-Mara areas of Kenya were inspected for nymphs and the nymphs removed with a pair of forceps. Collections were done between 08h00 and 12h00 hours before farmers released the cattle for grazing. Close-to-repletion nymphs collected from different body parts were kept separately in aluminium cans (Plate 3.2) at 28°C, 80% r.h. and a 12:12 (light:dark) photocycle until signs of parasitism appeared or the nymphs moulted. Unfed and partially fed nymphs collected were dissected and the presence or absence of parasitoid eggs or larvae scored. One collection was made in Trans-Mara (September 1995), while two collections were made in Kuja River (September 1995 & January 1997).

3.10.1.1.3. Field dispersal behaviour

To study the movement of mated naive *I. hookeri* females from the point of release to tick-infested cattle, two white zebu calves (Plate 3.13) infested with *A. variegatum* (mainly adults feeding on the dewlaps, legs, bellies and scrota) and *R. appendiculatus* (larvae, nymphs and adults feeding on the ears and heads) ticks were borrowed from farmers on Rusinga Island. The parasitoids were released 50cm, 100cm, 200cm and 300cm from tethered white calves. Since the distant orientation and movement of the parasitoids towards tick-infested calves was investigated, all the selected release distances fell outside the 20cm range over which short-range volatiles have been reported to act (Hendry *et al.*, 1973). The distances were measured from half the body length of the calves (shoulder to base of tail) in a down wind direction to give the parasitoids a chance to approach the calves from any angle. The cotton stopper was removed from a vial containing ten freshly emerged female parasitoids and the vial placed

Plate 3.13. Tethered calves used in field observations of the dispersal and host seeking behaviour of *I. hookeri* females

upright among the grasses. After leaving the vials, most parasitoids climbed onto the grasses, therefore, the grass blades were taken as the release platforms. Movement of the parasitoids on the grasses was not recorded as dispersal since these parasitoids were still at the release site, however, movement away from the grasses in the direction of the calves was recorded. Dispersal in the opposite direction was not recorded. The behaviour of the parasitoids at the release site, the number of parasitoids dispersing within ten minutes, numbers reaching the calves and those still at the release site after one hour were recorded. The released parasitoids were observed for one hour whereafter all the visible parasitoids in the test section (section between the release site and the calves) were removed with the aid of an aspirator before the next release. This experiment was replicated six times with ten parasitoids per replicate and was carried out between 07h00 and 18h00 hours.

3.10.2. Laboratory observations

3.10.2.1. Wind tunnel dispersal behaviour

The distant orientation and movement of mated naive *I. hookeri* females in response to cattle urine and body surface odours was investigated in a flat-bed wind tunnel (180cm x 45cm x 45cm) described by Inayatullah *et al.* (1994)(Fig. 3.5). The parasitoids were released at 50cm and 100cm from the odour source (fresh urine and swabs from front heels on cotton wool). The distances selected fell outside the activity range of close-range volatiles as explained above (section 3.10.1.1.3). Charcoal-filtered air was moved through the tunnel at a speed of 2cm/s and sucked out of the tunnel at the same speed at the downward end which was covered with a wire screen. Preliminary experiments showed that higher wind speeds arrested parasitoid movement. A double-layered muslin buffer was placed 30cm away from the fan on the upwind side to ensure laminar air flow in the tunnel. Three doors (15cm x 15cm), located at the top of the tunnel, allowed entry for the manipulation of the parasitoids. Air was supplied to the laboratory from a 16m high chimney equipped with a fan.

An exhaust fan ran continuously to vent odours from the room. Illumination was provided by two 60W incandescent bulbs placed 1.5m from the top of the wind tunnel. The odour source was placed 5cm above the tunnel floor (same height as the vials the parasitoids were released from) against the double-layered muslin buffer and held in place with a wire-mesh. Cotton wool stoppers of vials containing parasitoids were replaced with cotton wool used for swabs as mentioned above and the vials held in the windtunnel room for at least ten minutes pre-test acclimation. A single parasitoids was allowed to crawl on top of an inverted vial (5.0cm x 1.3cm) and the vial together with the parasitoid introduced into the wind tunnel 50cm or 100cm away from the muslin buffer. The vial was taken as the release platform, therefore, the movement of the parasitoid on the vial was not recorded as dispersal since the parasitoid was still at the release site, however, movement away from the vial in the direction of the odour source was recorded. Dispersal in the opposite direction was not recorded. Each female was observed for 15 minutes before being removed from the tunnel. Females not responding within this time were given a second chance through re-introduction. The amount of time the parasitoids spent on the platform (vial); amount of time spent on running, jumping, flying as well as the distances reached through each of these activities; total time taken to reach the longest distance; sitting time on tunnel floor; as well as the numbers of parasitoids reaching the muslin buffer were recorded. All the experiments were conducted at 27-30°C, and 50-60% r.h., between 08h00 and 17h00 hours. This experiment was replicated three times with 20 parasitoids per replicate.

3.11. Statistical analysis

Log transformation was used, where needed, to normalize the data distribution. Mean times and distances measured were separated by ANOVA/SNK (Analysis of Variance/ Student-Newman Keuls means separation procedure, Statistical Analysis System (SAS) Institute, 1988), while percentage responses were analyzed using the log likelihood ratio test (G-test) for goodness of fit (Sokal &

Rohlf, 1981).

CHAPTER 4: RESPONSE OF *Ixodiphagus hookeri* TO ODOURS FROM GRASSES, CATTLE AND *Amblyomma variegatum* NYMPHS

4.1. Introduction

In parasitoids, two phases of host finding behaviour can be recognized: (1) location of the host's habitat and (2) location of the host within its habitat. The majority of parasitoids respond to volatile kairomones or synomones in the long-distance location of their hosts (van Alphen & Jervis, 1996). These chemicals may originate from: (1) the host itself (e.g. faeces or frass) and may be part of intraspecific communication signals such as sex and aggregation pheromones, or may be produced during moulting or feeding; (2) from the organism the host feeds on; or (3) from some interaction between the host and the organism it feeds on.

Less volatile and non-volatile (i.e. short range) host products may also be used in host location (Weseloh, 1981). Lewis (1970) reported that *Microplitis croceipes* responds by antennation to 13-methylphentariacontane in the frass of *Helicoverpa zea*. *Microterys nietneri* (=*flavus*) responds to fructose and sucrose as well as to some unidentified compounds in the honeydew secreted by its host *Coccus hesperidum* (Vinson *et al.*, 1978). Host-derived cues become more important the closer foraging natural enemies come to their hosts (Vet & Dicke, 1992).

Although *I. hookeri* has been reported to be capable of parasitizing *R. appendiculatus* ticks in other locations (Cole, 1965), the Kenyan *I. hookeri* strain is specific to *A. variegatum* ticks. *A. variegatum* ticks, feed on a variety of vertebrates of which cattle are the most important domestic hosts (MacLeod & Colbo, 1976). When not on the host these ticks are dispersed throughout the vegetation.

Since the cues leading *I. hookeri* parasitoids to *A. variegatum* nymphs are not known, the first objective of this study was to investigate the chemical (olfactory) cues used by *I. hookeri* in the location of the habitat of *A. variegatum* nymphs and the subsequent location of these nymphs.

Grass, cattle and nymphal odours were investigated as possible sources of infochemicals in the Y and T-tube olfactometers.

Three pasture grasses, *C. dactylon*, *E. superba* and *D. seriata* were bioassayed for their possible role in host habitat and host location behaviour of mated naive *I. hookeri* females since off-host *A. variegatum* ticks are found on the grasses. Furthermore, pasture grasses are important as food for cattle and odours from these grasses could be used for possible location of cattle feeding on these grasses and the subsequent location of nymphs feeding on these cattle.

Cattle waste (dung and urine) and body surface odours were bioassayed for their possible role in host habitat (cattle) location which could lead to the subsequent location of *A. variegatum* nymphs feeding on these cattle by the parasitoids. Portions of the urine were left for seven days in stoppered vials and bioassayed since old fermented cattle urine has been found to enhance the attraction of tsetse flies to cattle (Owaga *et al.*, 1988; Madubunyi *et al.*, 1996). Swabs were also taken from known feeding sites of *A. variegatum* and *R. appendiculatus* ticks on cattle and bioassayed for their possible role in the host habitat and host location behaviour of *I. hookeri* females.

Furthermore, the role of odours from fed and unfed off-host nymphs in host location was investigated. Since off-host ticks are known to produce assembly pheromones which attract different life stages of the same species as well as other species (Sonenshine *et al.*, 1982), odours from ten, thirty and fifty nymphs were bioassayed for attraction to the parasitoids. This was done to test whether the assembly of nymphs in an area could amplify any chemical signal(s) used by the parasitoids to locate these nymphs. *A. variegatum* nymphs were also fed on calf scrota and volatiles from these nymphs trapped and bioassayed against volatiles from tick-free scrota to test whether the presence of feeding nymphs on cattle enhances the location of these nymphs. The two volatile samples were analyzed using the gas chromatograph (Plate 3.7) and the chromatograms compared.

To investigate the role of less volatile and non-volatile infochemicals on the body surfaces of *A. variegatum* and *R. appendiculatus* nymphs, unfed nymphs were washed in hexane and the samples bioassayed. The two washes were analyzed using the gas chromatograph and the chromatograms compared.

Faeces were also collected from feeding *A. variegatum* and *R. appendiculatus* nymphs and extracted with hexane to investigate the role that infochemicals from this host-derived product play in the location of these nymphs by mated naive *I. hookeri* females. The two extracts were also subjected to gas chromatography and the chromatograms compared.

4.2. Results

4.2.1. Response to grass odours

The possible role of pasture grass odours in the location of questing *A. variegatum* nymphs (on grasses) and nymphs feeding on cattle grazing in pastures by mated naive *I. hookeri* females was investigated. Three pasture grasses, *C. dactylon*, *E. superba* and *D. seriata* were bioassayed separately in the Y-tube olfactometer against control air (section 3.6.1). The arm of the olfactometer chosen as well as the response time (time between the introduction of the parasitoid and the crossing of the finishing line) and retention time (time the parasitoid spends in the choice arm) were recorded.

I. hookeri females were not significantly attracted by any of the grass odours when compared to control air (G-test, $P<0.05$, Fig. 4.1).

The response times of the parasitoids to *C. dactylon* and *D. seriata* odours were not significantly different from the response to control air (Table 4.1). Response time to *E. superba* odours was significantly slower than the response to control air (Table 4.1).

The retention times of the parasitoids in *C. dactylon* and *D. seriata* odours were not significantly different from the retention in control air (Table 4.1). *E. superba* odours retained the parasitoids significantly longer than control air (Table 4.1).

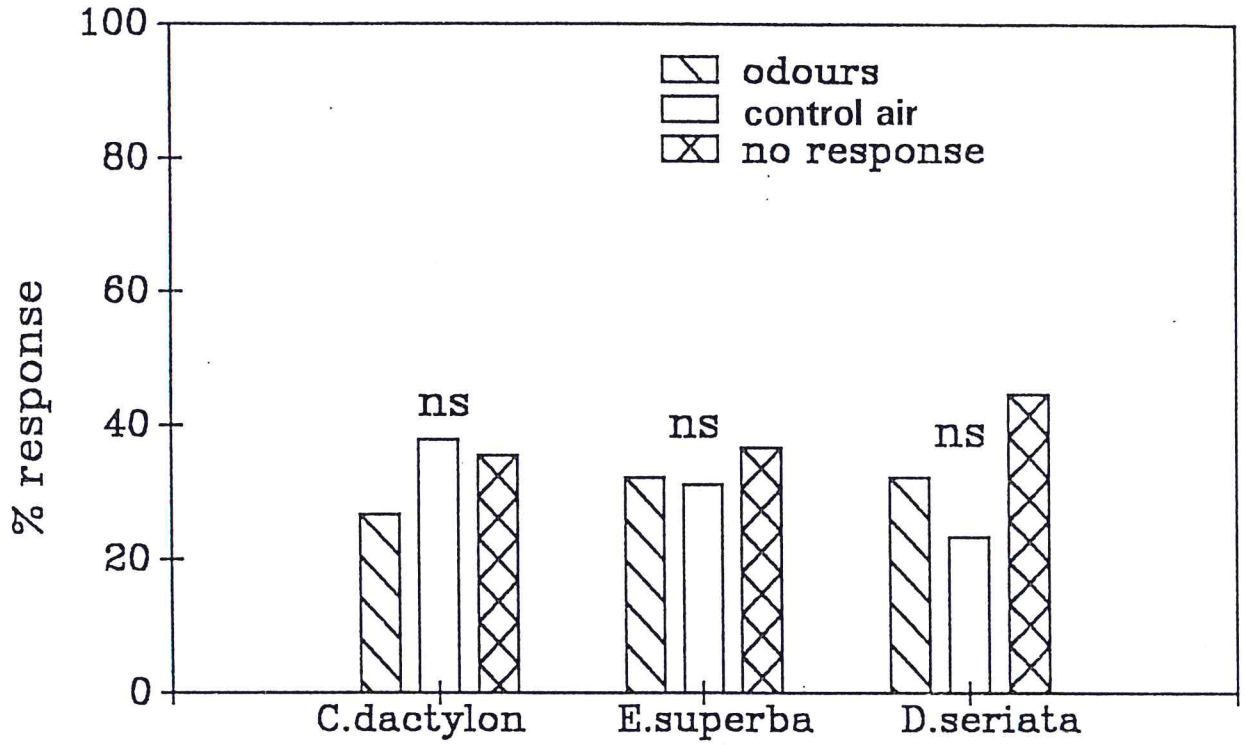

Fig. 4.1. Response of mated naive *I. hookeri* females to grass odours. Significance: ns = not significant (G-test, $P < 0.05$)

Table 4.1: Response and retention times of mated naive *I. hookeri* females exposed to grass odours

Test material	Response time(sec) [1]Mean ± SE	Retention time(sec) [1]Mean ± SE
C. dactylon	3.2 ± 0.09^{c}	4.5 ± 0.10^{b}
Control air	3.2 ± 0.07^{c}	4.7 ± 0.04^{ab}
E. superba	3.8 ± 0.14^{b}	5.0 ± 0.07^{a}
Control air	3.3 ± 0.09^{c}	4.6 ± 0.14^{b}
D. seriata	4.2 ± 0.14^{a}	4.8 ± 0.11^{ab}
Control air	3.9 ± 0.11^{ab}	4.7 ± 0.08^{ab}

Values followed by the same superscript letters in the same column are not significantly different (SNK, $P<0.05$). [1]Means are log-transformed

4.2.2. Response to cattle odours

4.2.2.1. Waste products

The possible role of cattle waste products in the location of the host habitat and hosts by mated naive *I. hookeri* females was tested in the Y-tube olfactometer (section 3.6.2.1). Fresh urine, seven-day-old urine and fresh dung were tested separately against control air for attraction. The arm of the olfactometer chosen as well as the response and retention times were recorded.

All calf waste products tested were significantly attractive to the parasitoids compared to control air (G-test, $P<0.001$, Fig. 4.2). When tested against control air, fresh urine attracted the parasitoids significantly more ($X^2=22.50$, $df=1$, $P<0.001$, Fig. 4.2). Seven-day-old urine also attracted significantly more parasitoids than the control air ($X^2=29.26$, $df=1$, $P<0.001$, Fig. 4.2). The attraction of the parasitoids to fresh dung was also significantly higher than the attraction to control air ($X^2=15.42$, $df=1$, $P<0.001$, Fig. 4.2).

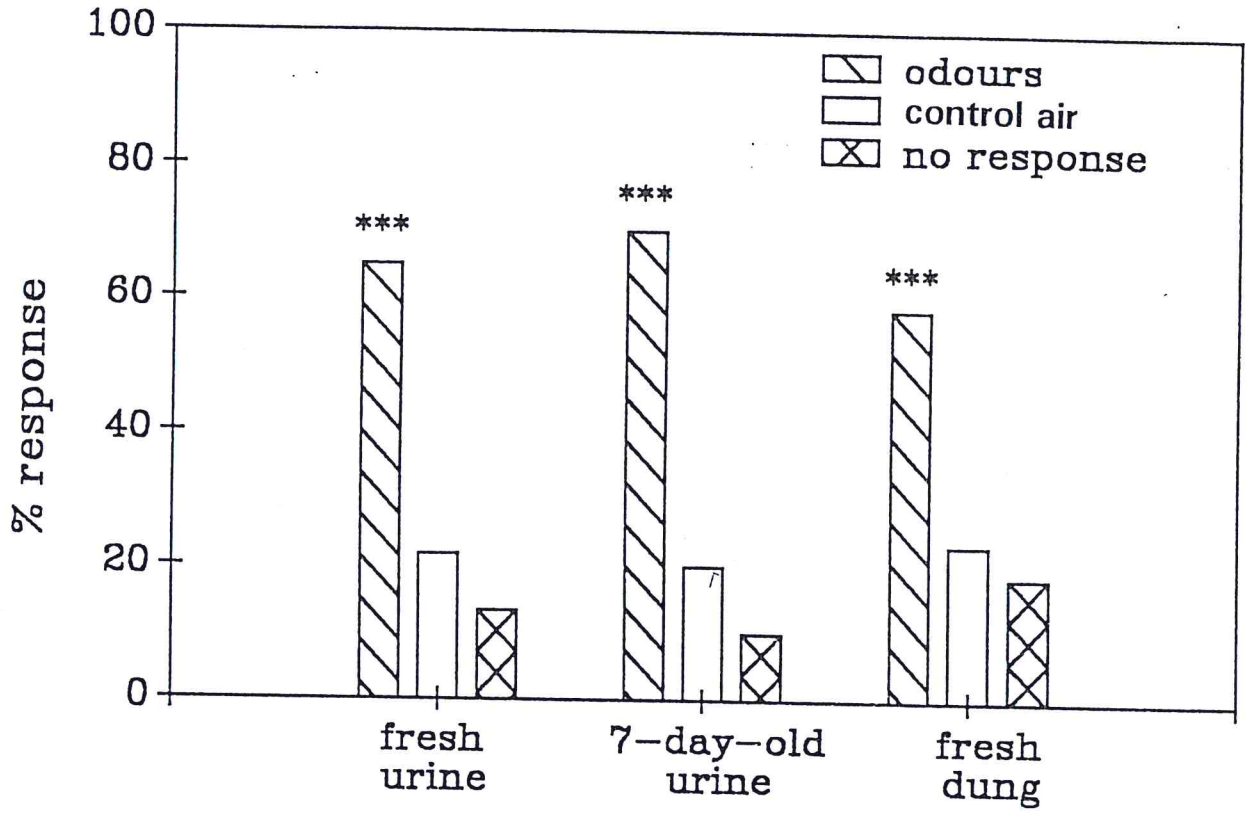

Fig. 4.2. Response of mated naive *I. hookeri* females to cattle waste odours. Significance: *** = significant at $P<0.001$ (G-test)

The response times of the parasitoids to dung, fresh urine and seven-day-old urine odours were not significantly different from the response to control air (Table 4.2).

Moreover, the retention times of the parasitoids in dung, fresh urine and seven-day-old urine odours were not significantly different from the retention in control air (Table 4.2).

4.2.2.2. Tick-free cattle body surface odours

The possible role of cattle body surface odours in the location of tick-free cattle by mated naive *I. hookeri* females was investigated. Swabs were taken on clean dry cotton wool from the dewlap, scrotum, hind heels and front heels (feeding sites of *A. variegatum* ticks, Fig. 3.3) and the ear (feeding site of *R. appendiculatus* nymphs) and tested separately against control clean dry cotton wool in the Y-tube olfactometer (section 3.6.2.2) for their attraction to the parasitoids. The arm of the olfactometer chosen as well as the response and retention times were recorded.

Three out of five body surface odours tested were significantly attractive to *I. hookeri* females compared to control cotton wool (G-test, Fig. 4.3). The ear odours did not attract the parasitoids significantly more compared to control cotton wool (G-test, $P<0.05$, Fig 4.3). The dewlap attracted significantly more parasitoids compared to control cotton wool ($X^2=4.11$, $df=1$, $P<0.05$, Fig. 4.3). The attraction of the parasitoids to front heel odours was also significantly more than the attraction to control cotton wool ($X^2=39.87$, $df=1$, $P<0.001$, Fig. 4.3). Scrotal odours did not attract the parasitoids more compared to control cotton wool (G-test, $P<0.05$, Fig. 4.3). Hind heels attracted significantly more parasitoids compared to control cotton wool ($X^2=33.68$, $df=1$, $P<0.001$, Fig. 4.3).

The response times of the parasitoids to ear, dewlap, front heel, scrotal and hind heel odours were not significantly different from the response to cotton wool (control) (Table 4.3).

Table 4.2. Response and retention times of mated naive *I. hookeri* females exposed to cattle waste products

Test material	Response time(sec) [1]Mean ± SE	Retention time(sec) [1]Mean ± SE
Dung	2.9 ± 0.10[b]	4.8 ± 0.07[ab]
Control air	3.0 ± 0.17[b]	4.7 ± 0.17[ab]
Fresh urine	3.5 ± 0.06[a]	4.9 ± 0.08[ab]
Control air	3.4 ± 0.13[a]	4.6 ± 0.13[b]
Seven-day-old urine	3.5 ± 0.07[a]	4.9 ± 0.04[ab]
Control air	3.4 ± 0.13[a]	5.0 ± 0.12[a]

Values followed by different superscript letters in the same column are significantly different ($P<0.05$).
[1]Means are log-transformed

The retention times of the parasitoids in ear, dewlap, front heel and scrotal odours were not significantly different from the retention in control air (Table 4.3). Retention time in hind heel odours was significantly shorter than the retention in control air (Table 4.3).

4.2.3. Response to *A. variegatum* nymphal odours

4.2.3.1. Off-host nymphs

The attraction of mated naive mated *I. hookeri* females to volatiles from ten, thirty or fifty nymphs (fed or unfed) was investigated in the Y-tube olfactometer (section 3.6.3.1.1). This was done to test whether the assembly of nymphs in an area could amplify any chemical signal(s) used by the parasitoids to locate these nymphs. The arm of the olfactometer chosen as well as the response and retention times were recorded.

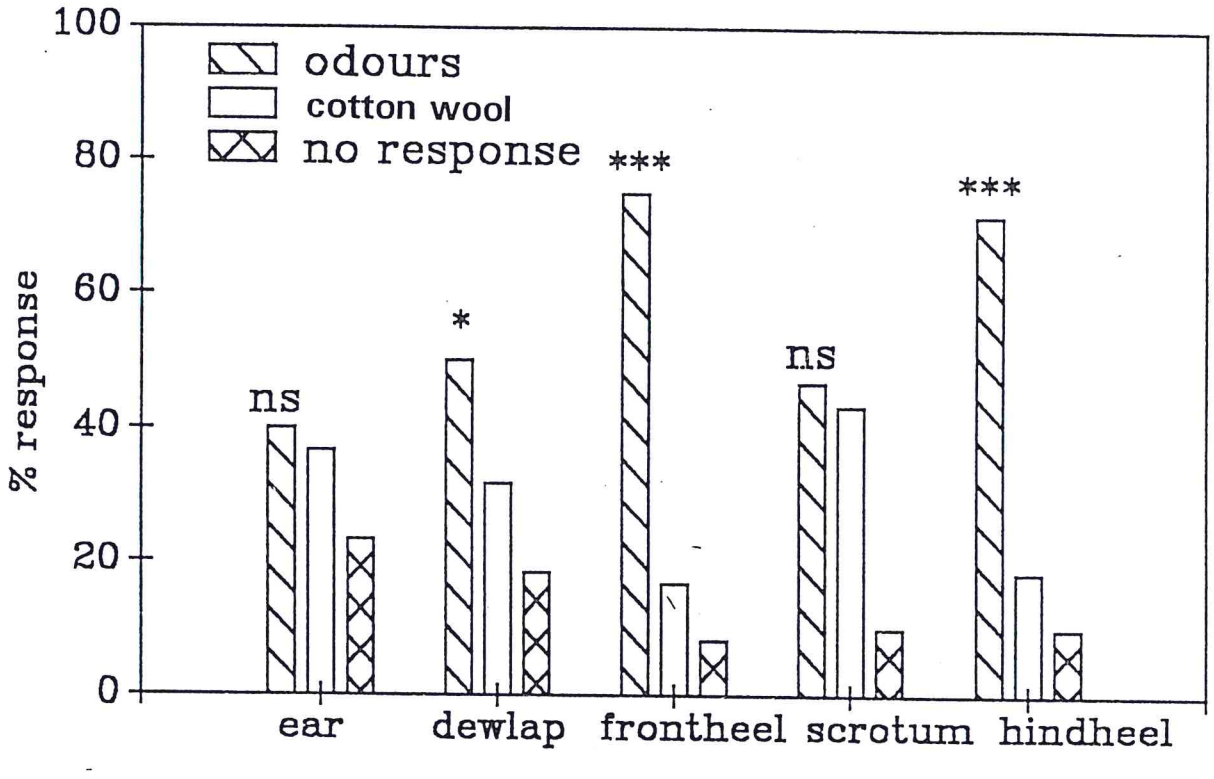

Fig. 4.3. Response of mated naive *I. hookeri* females to tick-free calf body surface odours. Significance: ns = not significant at $P<0.05$, * = significant at $P<0.05$, *** = significant at $P<0.001$ (G-test)

Table 4.3. Response and retention times of mated naive *I. hookeri* females exposed to tick-free cattle body surface odours

Test material	Response time(sec) [1]Mean ± SE	Retention time(sec) [1]Mean ± SE
Ear	3.2 ± 0.11^{b}	4.6 ± 0.09^{b}
Cotton wool	3.3 ± 0.11^{abc}	4.6 ± 0.12^{b}
Dewlap	3.7 ± 0.15^{abc}	4.8 ± 0.08^{b}
Cotton wool	3.6 ± 0.15^{abc}	4.5 ± 0.10^{b}
Front heel	3.1 ± 0.07^{c}	4.8 ± 0.09^{b}
Cotton wool	3.3 ± 0.12^{abc}	4.9 ± 0.13^{ab}
Scrotum	3.8 ± 0.15^{a}	4.9 ± 0.09^{ab}
Cotton wool	3.7 ± 0.13^{ab}	4.9 ± 0.07^{ab}
Hind heel	3.4 ± 0.11^{abc}	4.6 ± 0.09^{b}
Cotton wool	3.7 ± 0.18^{ab}	5.2 ± 0.04^{a}

Values followed by different superscript letters in the same column are significantly different ($P<0.05$).
[1]Means are log-transformed

4.2.3.1.1. Unfed nymphs

The attraction of *I. hookeri* females to odours from 10, 30 and 50 unfed nymphs was not significantly different from the attraction to control air (G-test, $P<0.05$, Fig. 4.4). The response times of the parasitoids to 10, 30 and 50 unfed nymphs were not significantly different from the response to control air (Table 4.4).

The retention times in the odours of 10 and 50 unfed nymphs were not significantly different from the retention in control air (Table 4.4). The retention time in the odours of 30 unfed nymphs was significantly longer than the retention in control air.

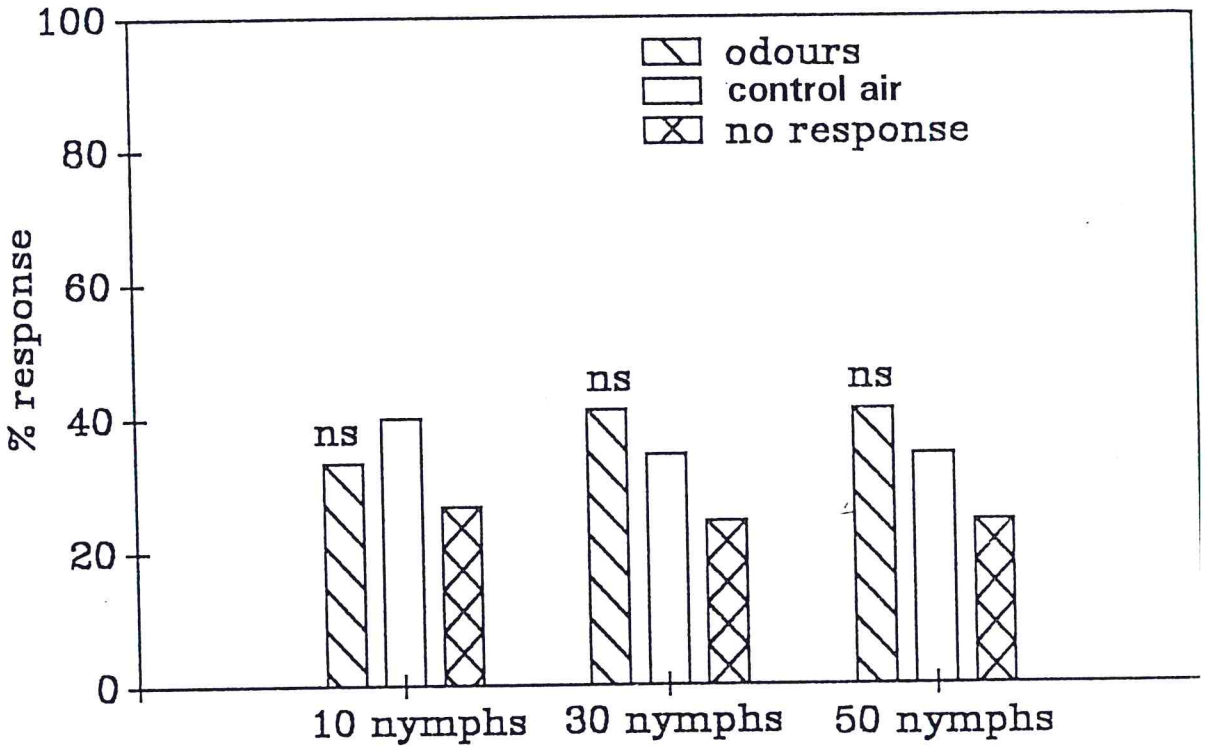

Fig. 4.4. Response of mated naive *I. hookeri* females to odours from various numbers of unfed *A. variegatum* nymphs. Significance: ns = not significant at $P < 0.05$,(G-test)

Table 4.4. Response and retention times of mated naive *I. hookeri* females responding to odours from various numbers of unfed *A. variegatum* nymphs.

Number of nymphs	Response time(sec) [1]Mean ± SE	Retention time(sec) [1]Mean ± SE
10	3.1 ± 0.05^{c}	4.8 ± 0.08^{a}
Control air	3.4 ± 0.10^{bc}	4.7 ± 0.07^{ab}
30	3.1 ± 0.11^{c}	4.9 ± 0.07^{a}
Control air	3.3 ± 0.14^{bc}	4.4 ± 0.15^{b}
50	3.9 ± 0.11^{a}	4.8 ± 0.09^{a}
Control air	3.6 ± 0.13^{ab}	5.0 ± 0.09^{a}

Values followed by the same superscript letters in the same column are not significantly different ($P<0.05$). [1]Means are log-transformed

4.2.3.1.2. Fed nymphs

The attraction of mated naive *I. hookeri* females to odours from 10, 30 and 50 fed off-host nymphs was not significantly different from the attraction to control air (G-test, $P<0.05$, Fig. 4.5).

The response times of the parasitoids to 10, 30 and 50 fed nymphs were not significantly different from the response to control air (Table 4.5).

The retention times of the parasitoids in the odours of 10, 30 and 50 fed nymphs were not significantly different from the retention in control air (Table 4.5).

4.2.3.2. On-host nymphs

The attraction of mated naive *I. hookeri* females to volatiles from tick-free and *A. variegatum* nymph-infested calf scrota was investigated in the Y-tube olfactometer (section 3.6.3.2.1). Volatiles trapped from tick-free and tick-infested scrota was eluted with redistilled dichloromethane (DCM) and tested against the same amount of DCM. This was done to test whether tick feeding on cattle would make the location of these ticks by *I. hookeri* easier.

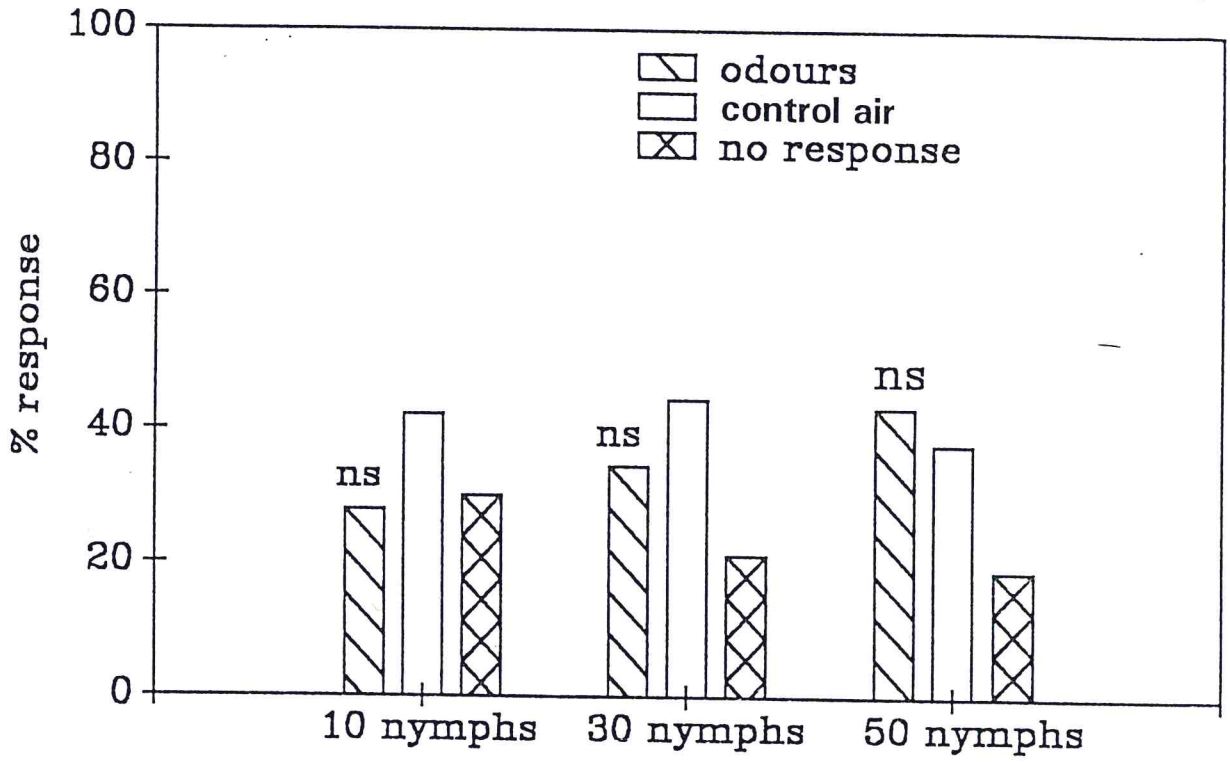

Fig. 4.5. Response of mated naive *I. hookeri* females to odours from various numbers of fed *A. variegatum* nymphs. Significance: ns = not significant at $P < 0.05$ (G-test)

Table 4.5. Response and retention times of mated naive *I. hookeri* females responding to odours from various numbers of fed *A. variegatum* nymphs.

Number of nymphs	Response time(sec) [1]Mean ± SE	Retention time(sec) [1]Mean ± SE
10	3.7 ± 0.14[a]	4.8 ± 0.07[abc]
Control air	3.4 ± 0.10[a]	4.7 ± 0.07[bcd]
30	3.6 ± 0.12[a]	4.5 ± 0.11[d]
Control air	3.3 ± 0.09[a]	4.6 ± 0.11[cd]
50	3.4 ± 0.09[a]	4.9 ± 0.05[ab]
Control air	3.6 ± 0.10[a]	5.0 ± 0.05[a]

Values followed by the same superscript letters in the same column are not significantly different ($P<0.05$). [1]Means are log-transformed

The arm of the olfactometer chosen as well as the response and retention times were recorded. The tick-free and tick-infested scrotal samples were also subjected to gas chromatography (section 3.7.2.1.) and the chromatograms compared.

Volatiles from tick-free calf scrota were not significantly attractive to the parasitoids compared to control air (G-test, $P<0.05$, Fig. 4.6). However, scrota on which *A. variegatum* nymphs were feeding for 4 days were significantly attractive to the parasitoids compared to control air ($X^2=14.14$, $df=1$, $P<0.001$, Fig. 4.6).

The response times of the parasitoids to odours from tick-free and infested scrota did not differ significantly from the response to DCM (control)(Table 4.6).

Furthermore, the retention times of the parasitoids in the odours from tick-free and infested scrota were not significantly different from the retention in control air (Table 4.6).

The chemical profile of the odours emitted by tick-free and tick-infested calf scrota differed (Fig. 4.7). The tick-free scrotal sample had more peaks than the tick-infested scrotal sample. Peaks 1 to 5 were present in the tick-free sample only.

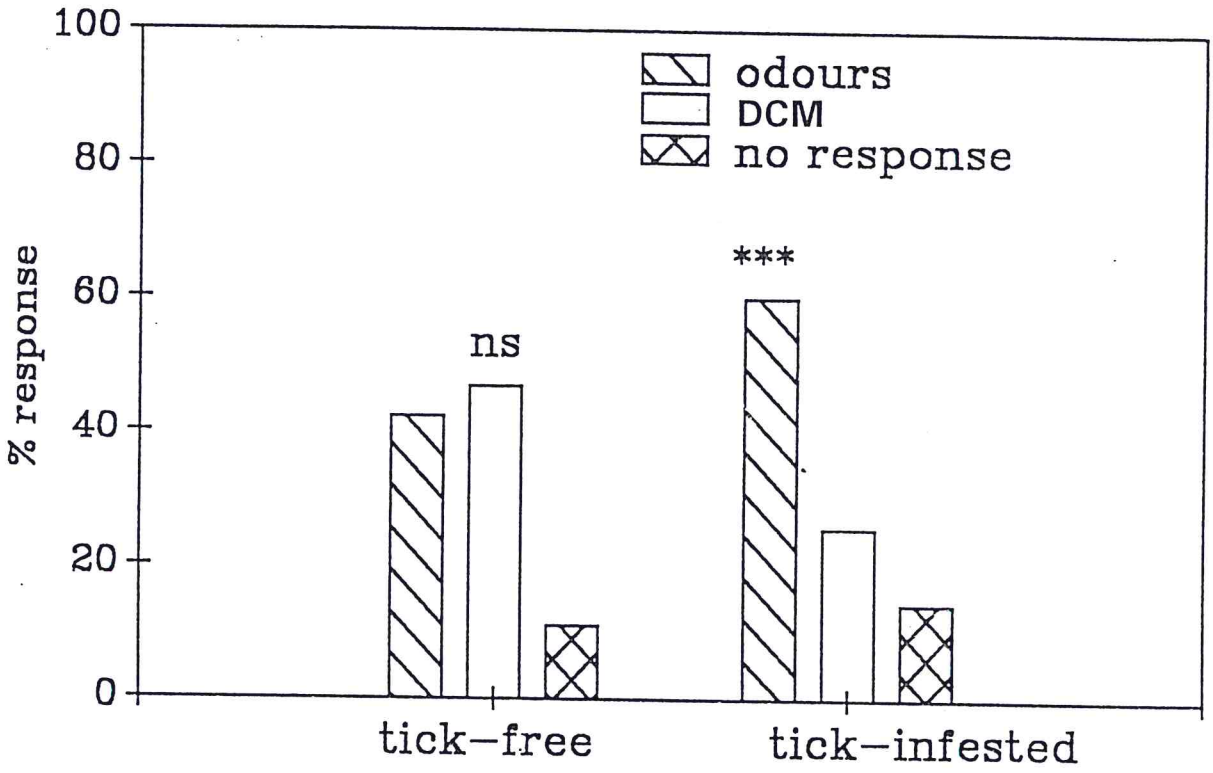

Fig. 4.6. Response of mated naive *I. hookeri* females to odours from tick-free and infested scrota. Significance: ns = not significant at $P < 0.05$, * * * = significant at $P < 0.001$ (G-test)

Table 4.6. Response and retention times of mated naive *I. hookeri* females exposed to volatiles from tick-free and *A. variegatum* nymph infested calf scrota

Test	Response time(sec) [1]Mean ± SE	Retention time(sec) [1]Mean ± SE
Tick-free	3.4 ± 0.08[a]	4.9 ± 0.07[a]
DCM	3.4 ± 0.07[a]	4.9 ± 0.06[a]
Infested	3.2 ± 0.05[a]	4.9 ± 0.06[a]
DCM	3.3 ± 0.10[a]	5.0 ± 0.10[a]

Values followed by the same superscript letters in the same column are not significantly different ($P<0.05$). [1]Means are log-transformed

All the other peaks, notably peaks 6 to 8, were present in both samples. However, the compounds represented by these peaks were more abundant in the tick-infested scrotal sample (Fig. 4.7).

4.2.3.3. Nymphal washes

The role of less volatile and non-volatile infochemicals on the body surfaces of *A. variegatum* and *R. appendiculatus* nymphs in the location of these nymphs by mated naive *I. hookeri* females was investigated in the T-tube olfactometer (section 3.6.3.1.2). Unfed *A. variegatum* and *R. appendiculatus* nymphs were washed in hexane and the samples bioassayed separately against hexane. The arm of the olfactometer chosen by the parasitoids was recorded. Washes of the two tick species were also analyzed using the gas chromatograph and the chromatograms compared.

I. hookeri females were attracted significantly to hexane washes of *A. variegatum* nymphs compared to hexane alone ($X^2=40.09$, $df=1$, $P<0.001$, Fig. 4.8). On the other hand, washes of *R. appendiculatus* nymphs did not attract the parasitoids significantly more than hexane (G-test, $P<0.05$, Fig 4.8).

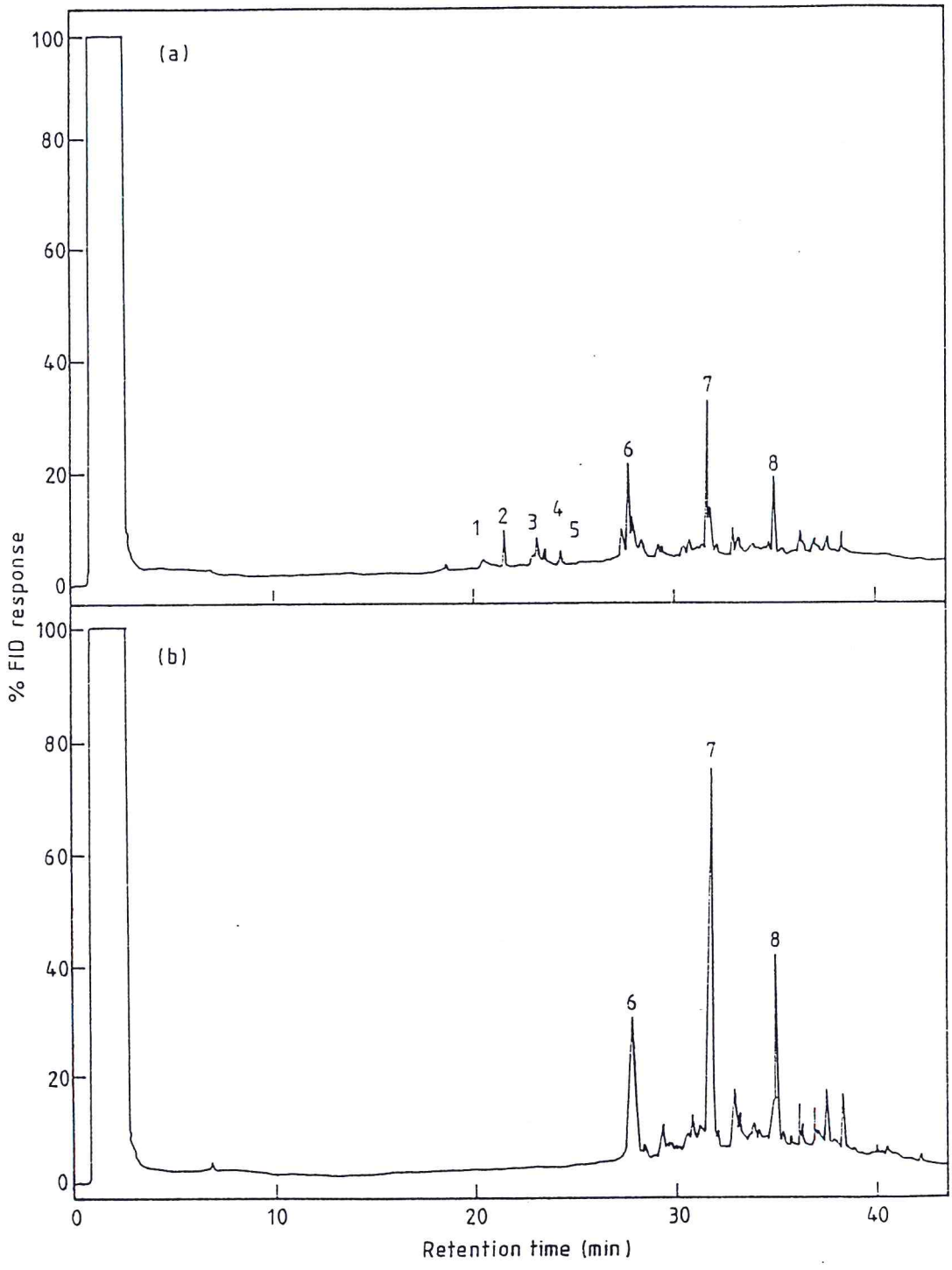

Fig. 4.7. Gas chromatograms of volatiles released by (a) tick-free and (b) infested calf scrota (FID = flame ionization detector)

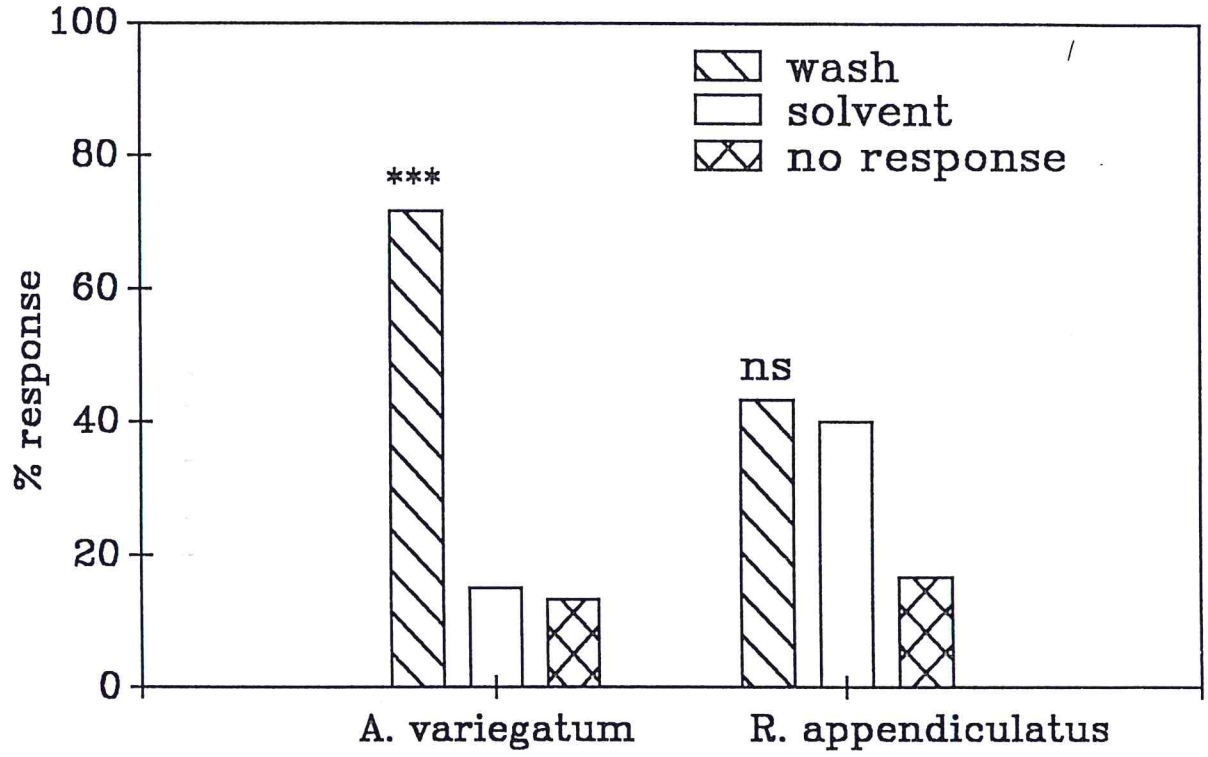

Fig. 4.8. Response of mated naive *I. hookeri* females to hexane washes of unfed *A. variegatum* and *R. appendiculatus* nymphs. Significance: ns = not significant at $P<0.05$, *** = significant at $P<0.001$ (G-test)

The chemical profiles of unfed *A. variegatum* and *R. appendiculatus* nymphs differed (Fig. 4.9). More peaks appeared in the *A. variegatum* wash compared to the *R. appendiculatus* wash. Peaks 1,2,5,6,8,10,12,13 and 15 were specific to *A. variegatum* washes while peak 7 was specific to the *R. appendiculatus* wash. Peaks 3,4,9,11 and 14 appeared in both samples but were more abundant in *A. variegatum* washes (Fig. 4.9).

4.2.3.4. Faeces from feeding nymphs

To investigate the role of infochemicals from host-derived waste products in the host location behaviour of mated naive *I. hookeri* females, the faeces of feeding *A. variegatum* and *R. appendiculatus* nymphs were extracted with hexane and bioassayed separately against hexane in the T-tube olfactometer (section 3.6.3.2.2). The arm of the olfactometer chosen by the parasitoids was recorded. The two extracts were also subjected to gas chromatography and the chromatograms compared.

The parasitoids were attracted significantly more to hexane extracts of the faeces from feeding *A. variegatum* nymphs (host) compared to the attraction to hexane alone ($X^2 = 25.89$, $df = 1$, $P < 0.001$, Fig. 4.10). The attraction of the parasitoids to the extracts of *R. appendiculatus* (non-host) faeces was not significantly different compared to the attraction to hexane alone (G-test, $P < 0.05$, Fig. 4.10).

The chemical profiles of the faecal extracts of feeding *A. variegatum* and *R. appendiculatus* nymphs differed (Fig. 4.11). Peak 5 was specific to *A. variegatum* extracts while peaks 3,9 and 15 were specific to *R. appendiculatus* extracts. Peaks 1,2,4,6,7,8, 10,11,12,13,14,16 and 17 appeared in both samples but were more abundant in *R. appendiculatus* extracts (Fig. 4.11).

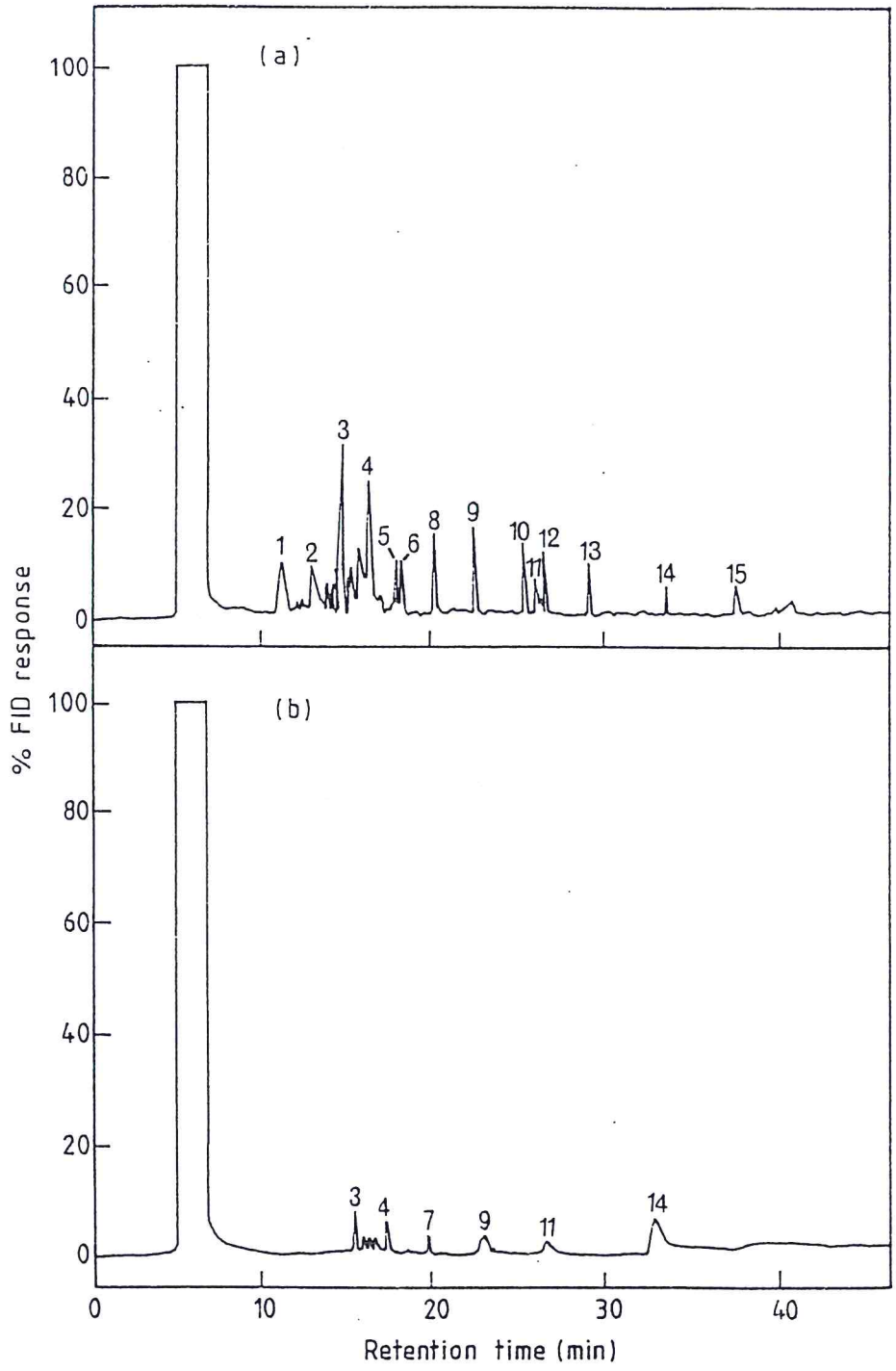

Fig. 4.9. Gas chromatograms of nymphal washes: (a) *A. variegatum* and (b) *R. appendiculatus* (FID = flame ionization detector)

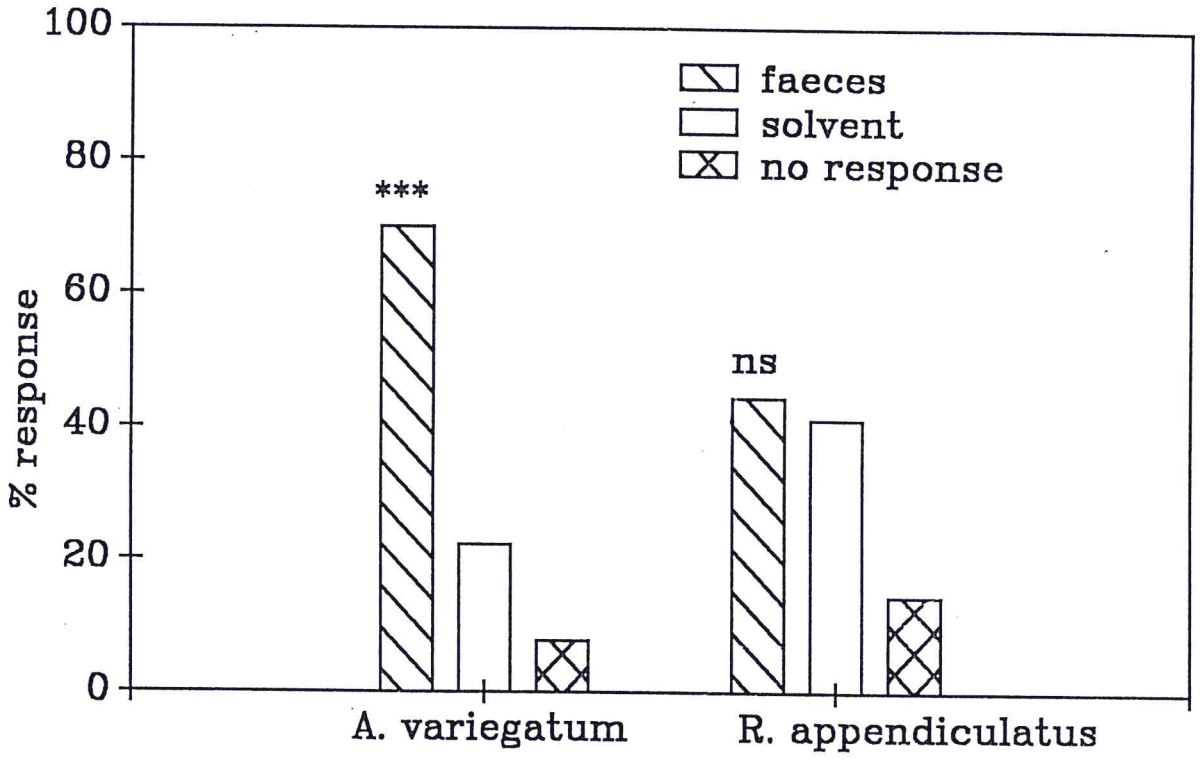

Fig. 4.10. Response of mated naive *I. hookeri* females to hexane extracts of faeces from feeding *A. variegatum* and *R. appendiculatus* nymphs

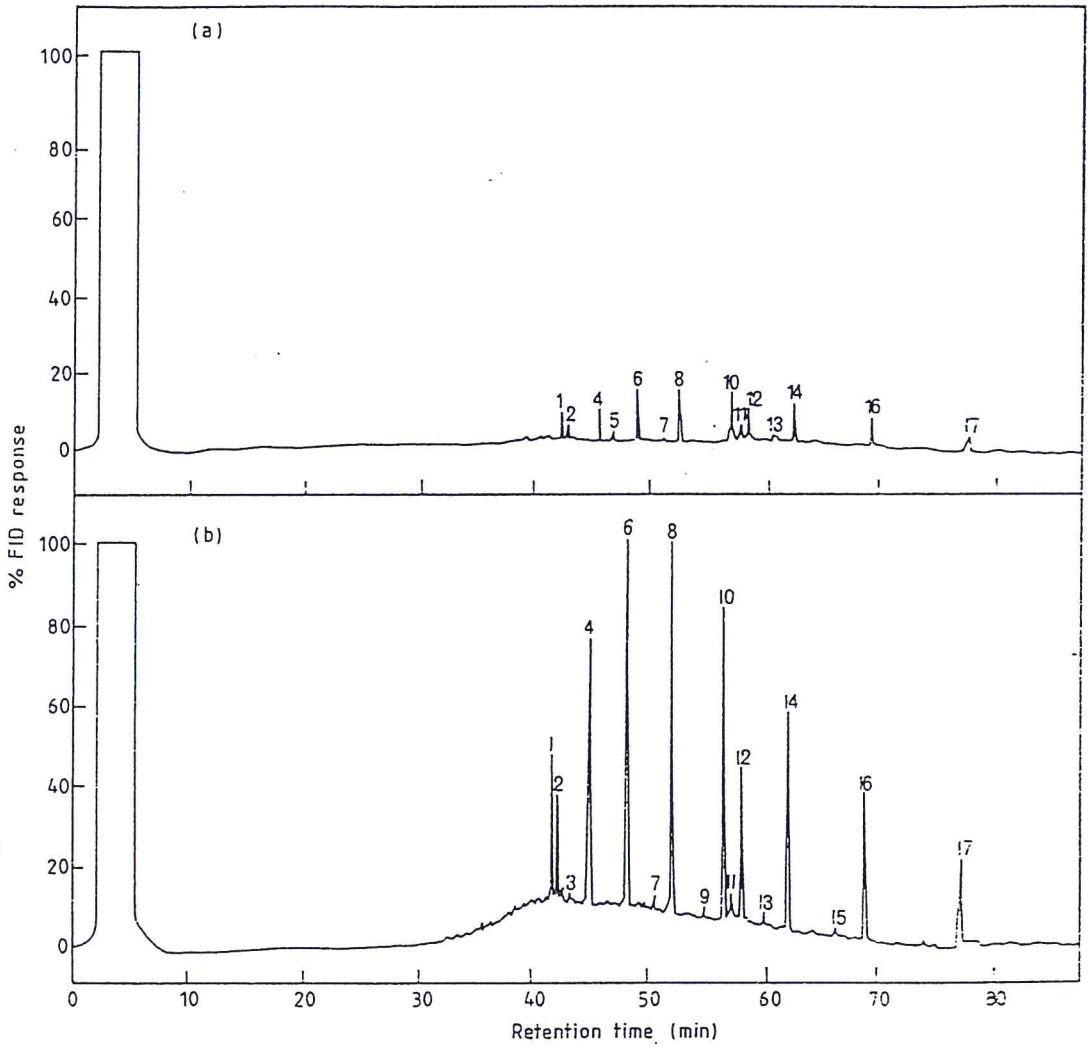

Fig. 4.11. Gas chromatograms of hexane extracts of faeces from feeding nymphs: (a) *A. variegatum* and (b) *R. appendiculatus* (FID = flame ionization detector)

4.3. Discussion

Plant odours have been found to play an important role as long-range attractants for parasitoids of herbivorous pests (Vinson, 1985; Nordlund *et al.*, 1988; Whitman, 1988; Williams *et al.*, 1988; Lewis *et al.*, 1990). In contrast, host location cues for parasitoids of haematophagous livestock ectoparasites (i.e. ticks and tsetse flies) are not known. In this study, mated naive *I. hookeri* females were not attracted to odours from three grasses (*C. dactylon*, *E. superba* and *D. seriata*) in Y-tube olfactometer bioassays (Fig. 4.1).

Although the parasitoids responded significantly slower to *E. superba* odours compared to control air, they were retained significantly longer in the odours of this grass (Table 4.1). The immediate significance of this behaviour is not clear since *E. superba* did not attract the parasitoids significantly more compared to control air (Fig. 4.1) despite its significantly long retention of the parasitoids compared to control air. On the other hand, the lack of significance in response and retention times between *C. dactylon* and *D. seriata* odours and their controls (Table 4.1) suggests that the parasitoids searched both arms of the olfactometer for similar amounts of time since there were no attractive odours present in either. Since the host of *I. hookeri*, *A. variegatum*, feeds on cattle the observed poor response to grasses might have been due to the unreliability of grass odours as predictors of the presence of host ticks which are likely to secure a blood meal and sustain the development of the parasitoid immatures. On the other hand, the role of volatiles released by grasses in response to cattle feeding in the finding of cattle by *I. hookeri* females may give further insight into the role, if any, of grass odours.

However, the observed lack of attraction of the parasitoids to grass odours could apply only to the Kenyan strain of *I. hookeri* and the degree to which this strain has co-evolved with its host, *A. variegatum*, since host-seeking *Ixodes scapularis* nymphs collected from the leaf litter and vegetation in Connecticut, USA, were parasitized by *I. hookeri* (Stafford *et al.*, 1996). It can be speculated that the Connecticut strain of *I. hookeri* could be more attracted to grass odours than the Kenyan strain since the parasitoids in Connecticut locate and parasitize off-host

nymphs.

Cattle odours are more reliable predictors of the presence of ticks which are likely to be successful in feeding and hence will provide enough food for the developing parasitoid larvae. The significant response of the parasitoids to cattle waste odours (Fig. 4.2) is consistent with the well-documented utilization of cattle waste product cues by many tsetse fly species to locate cattle (Owaga, 1984,1985; Owaga *et al.*, 1988; Vale *et al.*, 1988; Dransfield *et al.*, 1990). Some of the compounds identified from urine are now used extensively as odour baits for sampling and control of the *morsitans* group of tsetse (Vale, 1993; Mihok *et al.*, 1996).

The parasitoids are attracted significantly to both fresh and aged (seven-day-old) urine (Fig. 4.2) unlike tsetse flies which are significantly attracted to aged urine only (Madubunyi *et al.*, 1996). The attraction of certain tsetse species, e.g. *Glossina pallidipes* Austen and *G. morsitans* Westwood to buffalo and cattle urine is attributable to their phenolic constituents (Owaga *et al.*, 1988). These phenols are abundant in aged urine (kept in stoppered vials for more than two days) while probably absent in fresh cattle urine (Madubunyi *et al.*, 1996). The microbial invasion and subsequent fermentation of urine cause the gradual accumulation of the phenols in aged urine (Okech & Hassanali, 1990). It is thus likely that *I. hookeri* females, unlike tsetse flies, utilize other non-phenolic constituents in urine for the location of cattle and the subsequent location of the nymphs feeding on these cattle.

In addition to the cattle waste odours, *I. hookeri* females also utilize odours from the feeding sites of *A. variegatum* ticks on cattle for host habitat and host location (Fig. 4.3). Skin-based constituents of animal body odours are chiefly secretions and exudations of glands that are part of mammalian dermatography and are more likely than urine to possess compounds that are species or strain-specific (Madubunyi *et al.*, 1996).

The waste products could help in the initial orientation of the parasitoids to the cattle while the odours from the feeding sites could help in 'mapping out' the

areas to be searched to avoid searching less rewarding areas. The dung and urine odours might also help in the 'mapping out' of feeding sites since these sites (e.g. belly, dewlap, scrotum, udder and heels) become contaminated with urine and dung as the animals lie down in cattle sheds contaminated with dung and urine. Protection from the sun and animal grooming, thinness of the skin, sparseness of the hair coat and the importance of vascularization are believed to govern feeding site selection in ticks (Fourie & van Zyl, 1991; L'Hostis *et al.*, 1994).

The observation that the parasitoids were attracted to tick-free cattle odours underline the importance of cattle odours as possible long and medium-range attractants. Attraction to uninfested host habitats have also been reported for parasitoids of herbivorous pests. For example, *Campoletis sonorensis* is attracted to uninfested food plants of its host (Elzen *et al.*, 1983); *Macrocentrus grandii* is attracted to several plant species including its host's food, uninfested maize (Ding *et al.*, 1989); *Cotesia flavipes* and *C. sesamiae* are strongly attracted to odours from several graminaceous plants (Ngi-song *et al.*, 1996).

The ear (predilection site of *R. appendiculatus* ticks) odours were not attractive to the *I. hookeri* (Fig. 4.3), supporting the argument that animal odours narrow down the area to be searched by the parasitoids. Although tick-free scrota were not attractive to the parasitoids, the scrota became attractive when infested with *A. variegatum* nymphs. The increased attraction could be due to odours released by the: (a) feeding nymphs, (b) scrota in response to tick feeding, or (c) blend of nymphal and scrotal odours. Unfortunately, this study did not investigate the odours from feeding *A. variegatum* nymphs and those from the scrotum in response to tick feeding separately because of problems encountered in separating these odours from each other. Similar findings have been reported in several tritrophic systems (reviewed by Vet & Dicke, 1992). Dicke *et al.* (1990) reported that Lima bean and cucumber plants infested with two-spotted spider mites, *Tetranychus urticae*, emitted a blend of volatiles that attracted the predatory mites, *Phytoseiulus persimilis*. Furthermore, *Cotesia flavipes* and *C. sesamiae* preferred odours from host-infested plants over uninfested plants (Ngi-song *et al.*, 1996).

The significant attraction of the parasitoids to heel odours is of further interest since most of the nymphal *A. variegatum* ticks were found on the heels of free ranging cattle during field collections (Chapter 6). Yeoman and Walker (1967) and Walker (1974) also found that *A. variegatum* nymphs feeding on cattle legs attach mainly around the hooves. Caroll *et al.* (1995) found that kairomones from external glands on the legs of white-tailed deer, including the interdigital glands located between the hooves, arrested host-seeking *Ixodes scapularis* ticks. Since interdigital glands are found in most species of deer (Cervidae), antelope (Bovidae) and pronghorns (Antilocapridae) (Wood *et al.*, 1995), a similar arrestment mechanism from cattle on host-seeking *A. variegatum* nymphs might be at work in conjunction with waste product contamination around the hooves. The attraction of the parasitoids to heel odours suggests that the heels might be the converging zones of both the ticks and the parasitoids.

Although the cattle waste odours and all the tick-free feeding site odours of nymphal *A. variegatum* ticks on cattle (excluding the scrotal odours) were significantly attractive to the parasitoids, surprisingly, the parasitoids did not respond significantly faster, nor were they retained significantly longer in these odours compared to control air. Furthermore, the parasitoids were retained significantly longer in control air compared to hind heel odours (Tables 4.2 & 4.3) although the hind heel odours were significantly attractive to the parasitoids (Fig. 4.3). This finding gives an insight into the host searching behaviour of *I. hookeri* females. Firstly, the similar response times to odours and controls suggest that mated naive *I. hookeri* females take time to respond to cattle odours. A newly emerged adult parasitoid faces the problem of which host or microhabitat to search for (Godfray, 1994). A faster response to cattle odours in experienced females may be expected, since associative learning during host searching has been reported for various parasitoids. For example, *Leptopilina heterotoma* females showed a dramatic increase in initially weak responses to host substrate odours after having experienced them in association with oviposition in host larvae (Vet, 1988).

Response to attractive test odours may become faster and more refined as the females become experienced during searching (Turlings *et al.*, 1993).

Finally, the lack of significant retention of the parasitoids in almost all of the cattle odours might suggest that the parasitoids gave up when no host-derived (*A. variegatum* nymphal) signals were encountered, prompting the parasitoids to continue their search in the control arm or the stem of the Y-tube olfactometer. It is crucial for a 'time-tight' parasitoid like *I. hookeri* which lives for 1-2 days to adjust its giving-up time (GUT) in order to maximize its searching efficiency. Van Steenis *et al.* (1996) found that the GUT of the aphid parasitoid, *Aphidius colemani*, was strongly influenced by the number of aphids encountered.

Off-host ticks are known to produce assembly pheromones which result in the assembly of these ticks during the dry season, facilitating survival in suitable micro-environments (Sonenshine *et al.*, 1982). This study anticipated that the increase in the number of unfed and fed nymphs would amplify any kairomonal signal(s) used by the parasitoids and hence lead to increased attractiveness of these nymphs to the parasitoids. However, in Y-tube bioassays, odours from unfed and fed nymphs were not significantly attractive to the parasitoids when compared to control air despite the increase in the number of nymphs (Figs. 4.4 & 4.5). This finding shows that an increase in the number of off-host nymphs do not enhance the detection of these nymphs by the parasitoids. Furthermore, the observed decline in the retention of the parasitoids when the number of unfed nymphs were increased from 30 to 50 (Table 4.4) also suggests that an increase in the numbers of off-host nymphs does not necessarily bring about a longer retention of the parasitoids in the odours of these nymphs. Together with the earlier finding that grass odours are not attractive to the parasitoids, the above-mentioned findings stress that off-host nymphs are not targetted for parasitization by *I. hookeri* females.

Mwangi *et al.* (1994) found that none of the 196 unfed questing *A. variegatum* nymphs collected from grasses and subsequently fed on rabbit ears were parasitized by the Kenyan *I. hookeri* strain.

The lack of parasitization in host-seeking unfed *A. variegatum* nymphs could be due to the possibility that these unfed nymphs might fail outright to find a host to feed on, and therefore the eggs laid in such nymphs would be wasted since a bloodmeal is required for the development of the parasitoids inside the nymph (Chapter 5). Graf (1979) found that *A. nuttalli* unfed nymphs parasitized by an undescribed tick parasitoid had a great deal of difficulty in feeding normally and died rapidly. Findings on host suitability for *I. hookeri* development are reported in Chapter 5.

Moreover, fed ticks hide in top soil litter after detachment awaiting the complete digestion of the bloodmeal and the subsequent moulting while avoiding dehydration (Knülle & Rudolph, 1982). Subsequently, these ticks become inaccessible and unattractive to host-seeking female parasitoids since they have left the areas 'mapped out' by the feeding site odours on the animal. On the other hand, if *I. hookeri* had to parasitize fed, off-host nymphs, it would have to locate these nymphs soon after detachment in order to synchronize the development of its eggs with the internal physiology of the nymph before complete digestion of the blood by the nymph takes place. Furthermore, the integuments of fed nymphs harden in preparation for moulting beyond two days post drop. In the laboratory, parasitoids had problems in piercing these hardened nymphs with their ovipositors.

Furthermore, the lack of significant response and retention at the odours of the off-host unfed and fed nymphs relative to the controls in almost all of the experiments in the Y-tube olfactometer (excluding the significantly longer retention in odours from 30 unfed nymphs, Table 4.4) could have been due to the tendency to give up when no additional host-derived signals were encountered. It is known that parasitoids remain longer in areas containing hosts and increase search intensity if they encounter and oviposit in the hosts (Nelson & Roitberg, 1995).

The significant attraction of *I. hookeri* females by feeding on-host nymphs (Fig. 4.6) supports the suggestions by Davis (1986) and Mwangi *et al.* (1994) that parasitization of *A. variegatum* nymphs may take place while the ticks are on the vertebrate host. Parasitization of feeding *A. variegatum* nymphs assures female *I. hookeri* parasitoids that there will be a blood meal available in the nymph for the

successful development of their eggs. *A. variegatum* nymphs are known to feed for an average of 6 days before detachment (Hoogstraal, 1956). For ixodid ticks, the basic pattern of feeding is one of slow weight increase for some days followed by a rapid increase in weight on the final day of engorgement (Balashov, 1972). Graf (1979) found that the highest percentage parasitization was obtained when parasitization of *A. nuttalli* nymphs by an undescribed parasitoid took place towards the end of feeding or immediately after detachment of the nymphs. The lack of significant response and retention to feeding nymphal odours and their controls (Table 4.6) could also have been due to the tendency of the parasitoids to give up when no additional host-derived signals were encountered.

The chemical profiles of tick-free and infested scrota show that the response of the parasitoids to these odour sources have a chemical basis. The absence of peaks 1 to 5 (probable repellent compounds) in the tick-infested scrotal sample could have been responsible for the significant attraction of the parasitoids to tick-infested scrota.

Furthermore, tick feeding on calf scrota increased the relative abundance of volatiles emitted by the scrota, notably the volatiles represented by peaks 6 to 8. Since more of these volatiles are present when scrota are fed on, the parasitoids can utilize these odours better. The attraction of various parasitoids to host-infested habitats have been reported. For example, *Cotesia flavipes* is attracted to stemborer-infested plants (Potting, 1996). Studies into the chemical nature of volatiles and the identification of behaviourally active components from tick-infested scrota may shed more light on the tritrophic interaction between the parasitoids, feeding *A. variegatum* nymphs and cattle. Unfortunately, this study did not go into that aspect.

The significant attraction of the parasitoids to hexane washes of its host, *A. variegatum*, in T-tube bioassays (Fig. 4.8) underline the importance of host-derived kairomones the closer the parasitoids come to their hosts. The close-range discrimination between *A. variegatum* and *R. appendiculatus* nymphs by *I. hookeri* females may lie in the difference in less and non-volatile kairomones present on the

body surfaces of the two tick species. Small glands (Type I) and large wax glands (Type II) on the integuments of the two tick species are considered to be potential sources of infochemicals (Pavis *et al*, 1994; Schulze, 1942 in Walker *et al.*, 1996). The large wax glands (Type II) in *Amblyomma* ticks have been implicated in the production of a defensive allomone (Pavis *et al.*, 1994), the production of aggregation-attachment pheromones (Diehl *et al.*, 1991) and wax waterproofing (Lees, 1947; Balashov, 1960). In *R. appendiculatus* ticks, the small glands (Type I) are speculated to be producing the mounting sex pheromone, although there is no direct evidence (Walker *et al.*, 1996). It is also believed that these small glands produce a secretion that adds to the wax waterproofing, while the large wax glands are speculated to be producing a defensive allomone (Walker *et al.*, 1996). The infochemicals responsible for the discrimination of the parasitoids between the washes of the two tick species could have been secreted by these glands.

Visual inspection of the chromatograms (Fig. 4.9) show that the chemical composition of body surface semiochemicals of the two tick species differ. Nine peaks were specific to *A. variegatum* washes while one peak was specific to *R. appendiculatus* washes. Furthermore, volatiles represented by peaks appearing in both samples were more abundant in *A. variegatum* washes. Cuticular components (obtained through washes) have been shown to be species-specific. For example, the analysis of cuticular hydrocarbons has been used to tackle a variety of taxonomic problems in insects (Phillips *et al.*, 1988). In ticks, cuticular hydrocarbon analysis has revealed variation of several taxa (Hunt, 1986; Estrada-Peña *et al.*, 1992; 1994a,b). Moreover, Tucker & Leonard (1977) found that host acceptance in *Brachymeria intermedia*, a solitary parasitoid of lepidopterous pupae, is mediated by a cuticular kairomone which elicits antennal drumming and drilling of the host. The identification of the compounds in *A. variegatum* and *R. appendiculatus* nymphal washes might reveal the compound(s) responsible for the parasitoid's host specificity. Schulze (1942 in Walker *et al.*, 1996) suggests that the dermal glands could produce chemical means of recognition between the opposite sexes and individuals of the same species.

I. hookeri females were also significantly attracted by volatiles from the faeces of feeding *A. variegatum* nymphs, another set of host-derived kairomones (Fig. 4.10). Host faeces are one of the chemical cues available at the emergence site of a parasitoid and might be learned by the parasitoid before it starts searching for hosts (Godfray, 1994). Frass of many insects have been shown to contain host-location kairomones. Agelopoulos *et al.* (1995) found that infochemicals from larval *Pieris rapae* and *P. brassicae* faeces were utilized by their parasitoid, *Cotesia rubecula* in host location, while Alborne *et al.* (1995) reported a host-specific recognition kairomone for the parasitoid, *Microplitis croceipes* from the frass of its host, *Helicoverpa zea*.

Defaecation by attached ticks is an indicator of active feeding (Waladde *et al.*, 1991), a very important pre-requisite for the development of immature *I. hookeri* parasitoids inside the tick. It is, therefore, crucial that the parasitoids respond to these odours and parasitize promising hosts. Biochemical analysis of excreta from many adult tick species have revealed the presence of guanine, '2nd purine', hematin, protein, various amino acids (Hamdy, 1977), xanthine and hypoxanthine (Dusbabek *et al.*, 1991). Guanine, xanthine and hypoxanthine have been found as components of assembly pheromones (Dusbabek *et al.*, 1991) which induce clustering of ticks in the natural habitat, off the host, thereby fostering mating and host-finding success (Sonenshine, 1986).

Visual inspection of the chromatograms of hexane extracts of the faeces show that the chemical composition of the volatile fractions of the faeces of feeding *A. variegatum* and *R. appendiculatus* ticks differ (Fig. 4.11). One peak was specific to *A. variegatum* faeces while 3 peaks were specific to *R. appendiculatus* faeces. Volatiles represented by peaks present in both samples were more abundant in *R. appendiculatus* faeces (Fig. 4.11). We may conclude that there is a chemical basis for the significant response of *I. hookeri* females to hexane extracts of the *A. variegatum* faeces compared to the solvent alone. The lack of significant response to the faecal extracts of *R. appendiculatus* nymphs could also have been due to the observed differences in the chemical profiles of the faeces of the two tick

species. The identification of the compounds in these extracts might further reveal the compound(s) responsible for the parasitoid's host specificity.

In conclusion, the findings of this study show that *A. variegatum* nymphs are parasitized while feeding on the host. *I. hookeri* females utilize tick host odours (e.g. waste product odours) in the initial (distant) orientation towards the host, while *A. variegatum* feeding site odours on the host help in identifying the areas to be searched. The difference in attachment/feeding site selection between *A. variegatum* and *R. appendiculatus* ticks makes the searching on cattle easier for the parasitoids. Since this study highlighted the dependence of *I. hookeri* females on cattle odours to locate nymphs feeding on them, it is clear that cattle numbers in an area will affect the field performance of the parasitoids. Hu *et al.* (1993) suggested that *I. hookeri* may require a critical density of a vertebrate host of the tick to successfully locate the ticks on them. It is therefore necessary to investigate how phenomena like drought, cattle diseases and the resultant death of cattle affect the field performance of the parasitoids.

Host-derived cues like faeces and short-range (2-20cm; Hendry *et al.*, 1973) volatiles help the parasitoids in the final steps of the host location process. *I. hookeri* females are more attracted to cattle infested with *A. variegatum* nymphs but will also search tick-free cattle.

Odours from cattle do not retain the parasitoids although they are attractive, suggesting that *I. hookeri* females combine physical cues with volatile cues in host location (e.g. chemotactile cues), and that the parasitoids will give up searching if they do not encounter additional host signals. The varied responses of *I. hookeri* to odours from cattle (long and or medium-range cues), on and off-host ticks (short-range and or contact cues), and tick-derived odours (short-range and or contact cues) may be based on differences in the chemical nature of the odours.

CHAPTER 5: VISUAL HOST EVALUATION, RECOGNITION, ACCEPTANCE AND SUITABILITY

5.1. Introduction

Many foragers use visual cues to locate and evaluate resources (Bell, 1991). For parasitoids of hosts that can defend themselves such as aphids (Gardner *et al.*, 1984; Gerling *et al.*, 1990), visual information has the added advantage in that the information can be assessed from a distance without handling the host. The ease with which an insect can visually detect an item is a function of the item's dimensions, pattern, contrast against the background, distance between the insect and the item and the intensity of illumination (Prokopy & Owens, 1983). Parasitoids may enhance search efficiency by learning both olfactory and visual cues (Wäckers & Lewis, 1994)

Generally, specific host-associated stimuli need to be present for oviposition behaviour in parasitoids following location of prospective hosts (van Alphen & Jervis, 1996). Host size, shape, movement, sound, surface texture, vision, colour and thickness of the cuticle are all known to be important in host recognition and subsequent acceptance (Salt, 1958; Michaud & Mackauer, 1995; van Alphen & Jervis, 1996).

The hosts encountered by a parasitoid will often vary in size, age, species, quality as food for the developing young, time needed for their attack and parasitism, possible risks of mortality to the female during oviposition and internal defenses (Godfray, 1994). There is no certainty that a parasitoid egg laid in or on a host will develop successfully to the adult stage.

Unparasitized hosts which provide the highest chances of offspring survival are often preferred for oviposition over parasitized hosts (van Alphen & Visser, 1990). However, self and conspecific superparasitism occur and are believed to be adaptive in a number of situations (van Alphen & Visser, 1990; Godfray, 1994).

Besides the internal defenses, hosts can also launch external passive and active defenses. Among active defenses, the secretion of repellent substances has been reported.

In ticks, evidence of an allomone which regulates tick behaviour in certain mixed species populations has been reported (Khalil *et al.*, 1983). Pavis *et al.* (1994) reported an allomonal secretion of *A. variegatum* ticks.

This study investigated the possible use of physical cues by *I. hookeri* females in the evaluation and recognition of hosts. The ability of the parasitoids to evaluate and discriminate between host sizes, movement, feeding status and colour as well as the ability to recognize the host tick (*A. variegatum*) and discriminate between the host and non-host (*R. appendiculatus*) was investigated.

Furthermore, this study examined the acceptance of *A. variegatum* and *R. appendiculatus* nymphs for oviposition by *I. hookeri* females. The effect of encounters with parasitized hosts and an allomone (repellent) secreted by *A. variegatum* nymphs on the host acceptance behaviour of naive, mated *I. hookeri* females was investigated. *A. variegatum* nymphal washes, rubs and integument pieces were used to enhance the acceptance of *R. appendiculatus* nymphs by *I. hookeri* females for oviposition and hence induce parasitism in this vector of East Coast Fever for which no parasitoid has so far been found in Kenya. *R. appendiculatus* nymphs attacked (ovipositor inserted in nymph) by the parasitoids were dissected and the presence or absence of parasitoid eggs recorded.

A. variegatum and *R. appendiculatus* integuments were scanned with the aid of a scanning electron microscope (SEM) to reveal possible differences in the surface appearances of the integuments of the two tick species.

This study also investigated the suitability of *A. variegatum* and *R. appendiculatus* nymphs for the development of *I. hookeri* immatures. Fed and unfed *A. variegatum* nymphs and fed *R. appendiculatus* nymphs were exposed to the parasitoids and the percentages of nymphs attacked, yielding progeny,

moulting, killed and alive as well as the progeny produced per female recorded.

5.2. Results

5.2.1. Visual host evaluation and recognition

The visual evaluation of the size, movement, feeding status (fed or unfed) and colour of *A. variegatum* nymphs as well as the recognition of *A. variegatum* and *R. appendiculatus* nymphs by naive, mated *I. hookeri* females were investigated in the petri dish/vial set-up (section 3.5.2.3). Test and control nymphs were placed singly in glass vials (50cm x 12cm), sealed to prevent volatile odours from escaping from the vials and the two vials presented to naive (i.e. no experience with the test material before) mated *I. hookeri* females in a glass petri-dish for evaluation and recognition testing. The vials were laid flat in the petri-dish and the dish closed to keep the parasitoids in. The time the parasitoids spent on the vials examining their contents/nymphs (examination time), the frequency at which the vials were visited by the parasitoids for examination (examination frequency) and the percentages of parasitoids detecting the nymphs directly (running straight to vial and contacting it at the spot where the nymphs were visible) and those which detected them indirectly (not contacting the vial at the spot where the nymphs were visible) were recorded.

I. hookeri females examined larger *A. variegatum* nymphs significantly longer than the smaller nymphs of the same species. There was no significant difference between the examination times of live and dead *A. variegatum* nymphs (Table 5.1). Furthermore, fed *A. variegatum* nymphs were examined significantly longer than unfed nymphs. There was no significant difference between the examination times of grey replete *A. variegatum* nymphs and yellow-brown mummies tested against each other (Table 5.1). The difference in examination times between grey nymphs and dark brown mummies was not significant. The examination times of yellow-brown and dark brown mummies tested against each other were also not significantly different.

Table 5.1. Examination times and frequencies of mated naive *I. hookeri* females presented with *A. variegatum* and *R. appendiculatus* nymphs and mummies in sealed glass vials

Test material	Examination time(s) [1]Mean ± SE	Examination frequency [1]Mean ± SE
A. variegatum		
(a) Size		
Large	3.5 ± 0.13^{a}	1.7 ± 0.12^{ab}
Small	2.6 ± 0.18^{bcd}	1.5 ± 0.13^{abcd}
(b) Movement		
Live	2.4 ± 0.21^{cd}	1.1 ± 0.09^{e}
Dead	2.4 ± 0.20^{cd}	1.1 ± 0.10^{de}
(c) Feeding status		
Fed	2.9 ± 0.16^{abc}	1.7 ± 0.07^{abc}
Unfed	2.1 ± 0.18^{d}	1.4 ± 0.11^{bcde}
(d) Colour		
Grey	3.7 ± 0.14^{a}	1.9 ± 0.10^{a}
Yellow-brown	3.7 ± 0.12^{a}	1.6 ± 0.10^{abc}
Grey	3.4 ± 0.15^{a}	1.5 ± 0.07^{abcd}
Dark brown	3.2 ± 0.26^{ab}	1.2 ± 0.12^{cde}
Yellow-brown	3.3 ± 0.21^{ab}	1.4 ± 0.09^{bcde}
Dark brown	3.1 ± 0.25^{abc}	1.1 ± 0.09^{de}
(e) Species		
A. variegatum	3.2 ± 0.18^{ab}	1.8 ± 0.08^{ab}
R. appendiculatus	2.4 ± 0.22^{cd}	1.3 ± 0.11^{cde}

Values followed by different superscript letters in the same column are significantly different (SNK, $P<0.05$). [1]Means are log-transformed

A. variegatum nymphs were examined significantly longer than *R. appendiculatus* nymphs (Table 5.1).

The frequencies at which large and small *A. variegatum* nymphs were examined were not significantly different (Table 5.1). The examination frequencies of live and dead *A. variegatum* nymphs were also not significantly different.

Furthermore, the frequencies at which fed and unfed *A. variegatum* nymphs were examined were not significantly different (Table 5.1). Replete grey *A. variegatum* nymphs and yellow-brown mummies were examined at similar frequencies.

The examination frequencies of grey nymphs and dark brown mummies were also not significantly different (Table 5.1). The examination frequencies of yellow-brown and dark brown mummies were not significantly different. Finally, the frequency at which *A. variegatum* nymphs were examined was significantly more than that of *R. appendiculatus* nymphs (Table 5.1).

In all tests, there were more females which detected the test materials directly (ran straight to vial and contacted the vial at the spot where the test material was visible) than females which did not display this behaviour (i.e. indirect detection) (G-test, Fig. 5.1). Direct detection was more pronounced than indirect detection when large *A. variegatum* nymphs were tested against small nymphs ($X^2 = 22.47$, $df = 1$; $P < 0.001$, Fig. 5.1). Parasitoids detecting fed and unfed nymphs directly were significantly more than those which did not display this behaviour ($X^2 = 16.37$, $df = 1$; $P < 0.001$, Fig. 5.1).

Furthermore, direct detection of *A. variegatum* and *R. appendiculatus* nymphs was significant compared to indirect detection ($X^2 = 11.32$, $df = 1$; $P < 0.001$, Fig. 5.1). The percentage of *I. hookeri* females which detected dead and live *A. variegatum* nymphs directly was significanty more than the females which detected these nymphs indirectly ($X^2 = 4.01$, $df = 1$; $P < 0.05$, Fig.5.1). Direct detection of replete grey *A. variegatum* nymphs and yellow-brown mummies tested against each other was significantly more than the indirect detection of these test materials ($X^2 = 7.13$, $df = 1$; $P < 0.01$, Fig. 5.1). Significantly more parasitoids detected grey nymphs and dark brown mummies tested against each other directly compared to those which did so indirectly ($X^2 = 4.01$, $df = 1$; $P < 0.05$, Fig. 5.1). Direct detection of yellow-brown and dark brown mummies tested against each other was significantly different from the indirect detection of these test materials ($X^2 = 7.13$, $df = 1$;

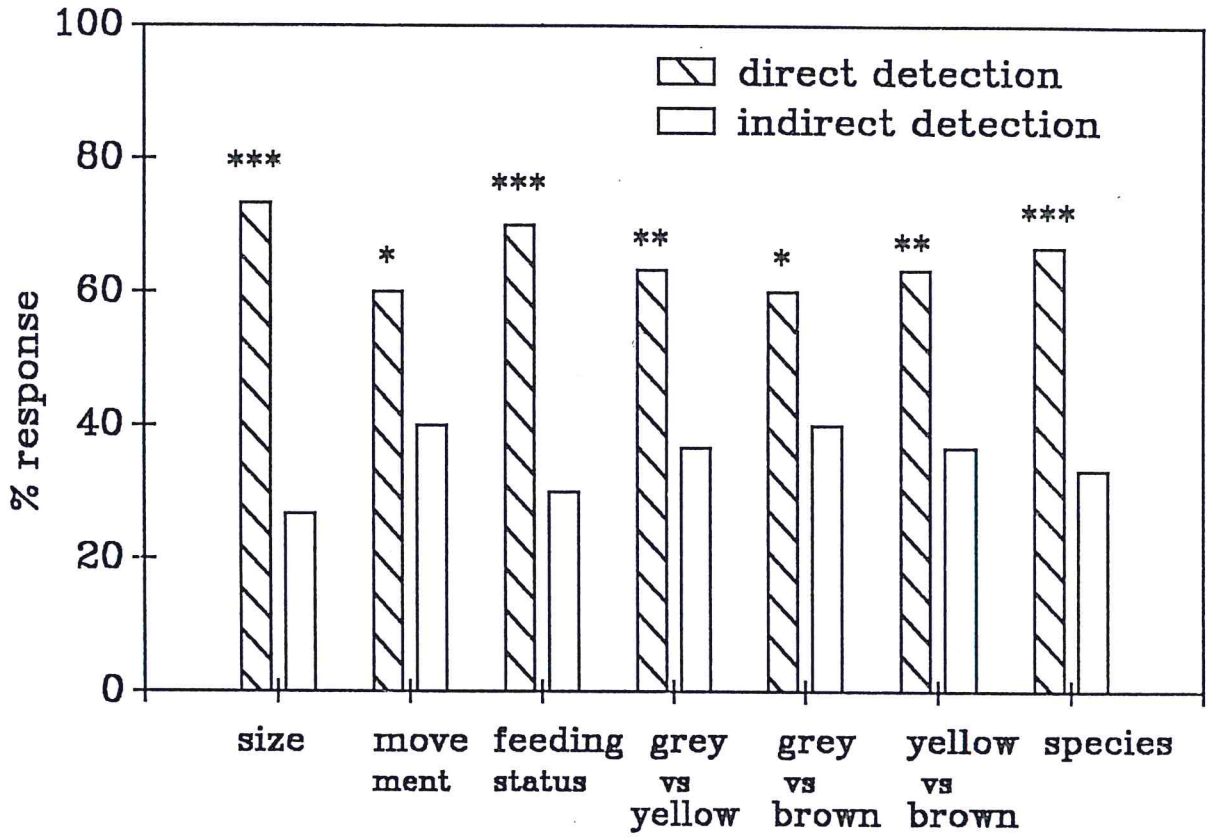

Fig. 5.1. Percentage of mated naive *I. hookeri* females which detected the test material directly and those which did so indirectly

$P<0.01$, Fig. 5.1).

5.2.2. Host acceptance and suitability

5.2.2.1. Acceptance

5.2.2.1.1. Species

To investigate the acceptance of host and non-host nymphs for oviposition, fed *A. variegatum* and *R. appendiculatus* nymphs were presented singly to naive, mated *I. hookeri* females in glass vials (sections 3.5.2.4. & 3.9.1.1.).

The percentage of nymphs attacked and the attack time (i.e. the time a parasitoid spent with its ovipositor inserted in a nymph) were recorded. After the attack, the nymphs were dissected, stained and the presence or absence of parasitoid eggs in these nymphs recorded.

The percentage of *A. variegatum* nymphs attacked by *I. hookeri* females (96.7%) was significantly more than *R. appendiculatus* nymphs attacked (16.7%)(Table 5.2).

The mean attack time of the parasitoids was significantly longer in *A. variegatum* nymphs than in *R. appendiculatus* nymphs (Table 5.2). A single attack lasted between 10-241 seconds in *A. variegatum* nymphs while it lasted for 2-6 seconds in *R. appendiculatus* nymphs (Table 5.2).

The percentage of *A. variegatum* nymphs parasitized (96.7%) was significantly more than *R. appendiculatus* nymphs parasitized (6.7%) ($X^2 = 108.92$, $df = 1$; $P<0.001$, Table 5.2).

5.2.2.1.2. Parasitization

To investigate the effect of encounters with parasitized hosts on foraging naive, mated *I. hookeri* females, a single fed unparasitized *A. variegatum* nymph was presented to four naive, mated female parasitoids, one at a time, at five minute intervals in glass vials (sections 3.5.2.4. & 3.9.1.2.). Pre-oviposition and oviposition times of each of the parasitoids in this nymph

Table 5.2. Acceptance of replete *A. variegatum* and *R. appendiculatus* nymphs by mated naive *I. hookeri* females for oviposition

Species	N	% Attacked	Mean attack time(sec) [1]Mean ± SE	Range of attack time(secs)	% Oviposited in
A. variegatum	30	96.7[a]	4.3 ± 0.12[a]	10 - 241	96.7[a]
R. appendiculatus	30	16.7[b]	1.2 ± 0.07[b]	2 - 6	6.7[b]

Values followed by different superscript letters in the same column are significantly different ($P<0.05$). [1]Means are log-transformed

were recorded.

Parasitization of *A. variegatum* nymphs by *I. hookeri* females increased the pre-oviposition time between the first (26.0 seconds) and second (47.9 seconds) females significantly (Fig. 5.2). Pre-oviposition times of the second, third and fourth females were similar. On the other hand, parasitization of the nymphs decreased the oviposition times of superparasitizing females. The oviposition time of the first female (141.2 seconds) was significantly longer than that of the second female (82.9 seconds)(Fig. 5.2). Oviposition times of the second, third and fourth females were similar (Fig. 5.2).

5.2.2.1.3. Repellence of *I. hookeri* by mechanically disturbed *A. variegatum* nymphs

To test the effect of dermal gland secretions from disturbed fed *A. variegatum* nymphs on the behaviour of naive, mated *I. hookeri* females, the legs of the nymphs were twisted with a pair of forceps until droplets were visible on the body surface (section 3.9.1.3). Twisted (disturbed) and undisturbed nymphs were presented singly to *I. hookeri* females in glass vials (section 3.5.2.4.) and the time the parasitoids spent before contacting these nymphs (pre-contact time) recorded at introduction (0 minutes), 10 minutes, 30 minutes and 50 minutes.

I. hookeri females stayed significantly longer (72.7 seconds) before contacting disturbed *A. variegatum* nymphs at introduction (0 minutes) compared to the pre-contact time for undisturbed nymphs (21.0 seconds)($X^2 = 30.03$, $df = 1$; $P < 0.001$, Fig. 5.3). However, the repellent effect waned within ten minutes and the pre-contact times of disturbed and undisturbed nymphs were no longer significantly different after 10 minutes (G-test, $P < 0.05$, Fig. 5.3). On the other hand, pre-contact time for undisturbed nymphs remained almost constant over the entire test period (17.3-21.0 seconds)(Fig. 5.3).On the other hand, pre-contact time for undisturbed nymphs remained almost constant over the entire test period

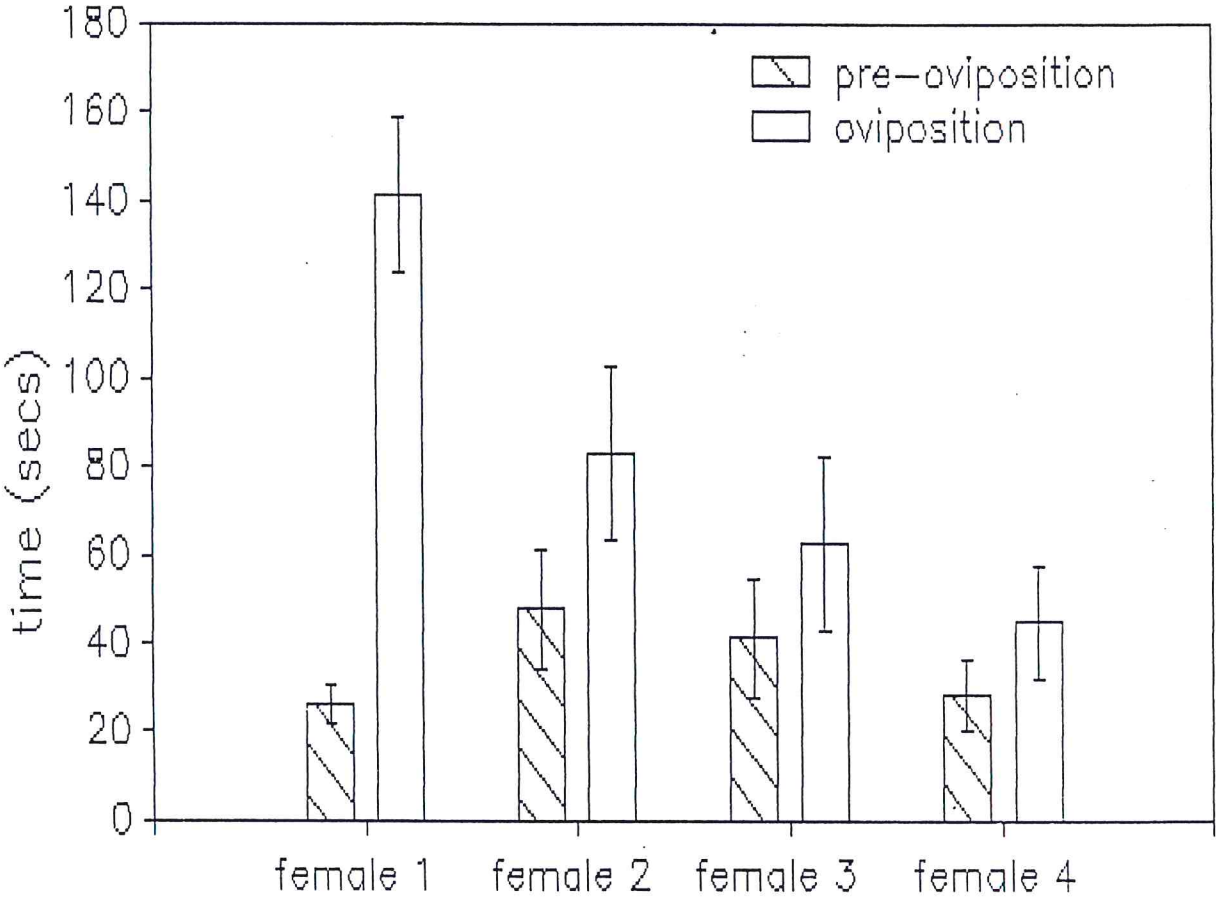

Fig. 5.2. Superparasitization of a single replete *A. variegatum* nymph by three mated naive *I. hookeri* females (first female parasitized an unparasitized nymph while females 2,3 & 4 were superparasitizing)

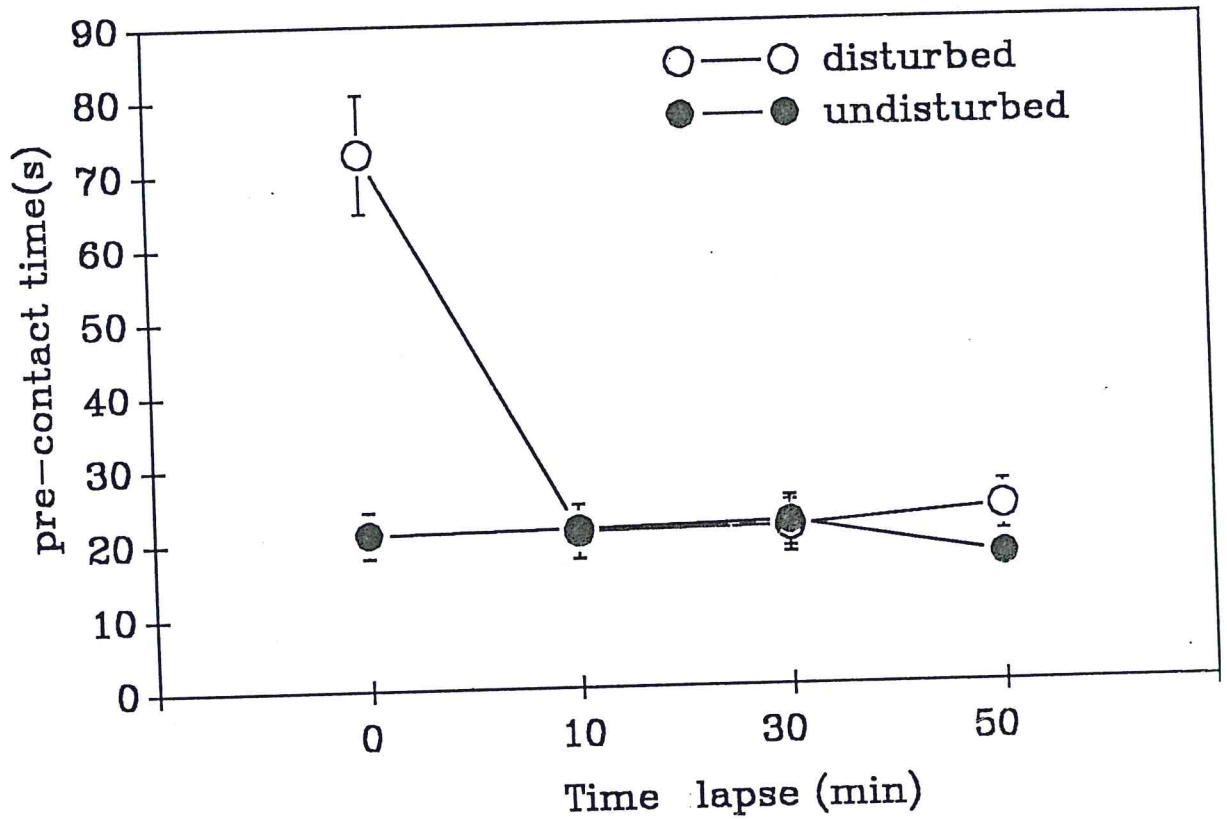

Fig. 5.3. Repellence of mated naive *I. hookeri* females by disturbed *A. variegatum* nymphs

(17.3-21.0 seconds)(Fig. 5.3).

5.2.2.1.4. Induction of parasitism in *R. appendiculatus*

To enhance the acceptance of fed *R. appendiculatus* nymphs by naive, mated *I. hookeri* females for oviposition, *A. variegatum* nymphal washes in hexane, integument pieces (3x3cm) and rubbing of *R. appendiculatus* and *A. variegatum* nymphs against each other were employed (section 3.9.1.4.). *R. appendiculatus* nymphs were presented singly in glass vials (section 3.5.2.4.) to the parasitoids in the absence and presence of *A. variegatum* nymphal washes in hexane, rubs and integument pieces. The percentage of nymphs attacked and the attack time were recorded. After the attack, the nymphs were dissected, stained and the presence or absence of parasitoid eggs in the nymphs recorded. *A. variegatum* and *R. appendiculatus* nymphal integuments were also scanned with the aid of a SEM to reveal possible differences in the surface appearances of the integuments of the two tick species.

When *R. appendiculatus* nymphs were presented in the presence of *A. variegatum* nymphal washes, the percentage of parasitoids attacking these nymphs increased significantly from 16.7% to 36.7% (Table 5.3). A combination of *A. variegatum* nymphal washes and integument pieces further increased the percentage of parasitoids attacking *R. appendiculatus* nymphs significantly from 36.7% in the presence of washes alone to 50% (Table 5.3).

The presence of *A. variegatum* nymphal washes increased the mean attack time of the parasitoids on *R. appendiculatus* nymphs significantly compared to the mean oviposition time in the absence of the washes (Table 5.3). The presence of a combination of *A. variegatum* washes and integument further increased the mean oviposition time of the parasitoids in *R. appendiculatus* nymphs significantly compared to the mean oviposition times in *R. appendiculatus* nymphs in the absence or presence of *A. variegatum* washes

Table 5.3. Acceptance of replete *R. appendiculatus* nymphs by mated naive *I. hookeri* females in the presence of *A. variegatum* odours and integument pieces

Test	N	% Attacked	Mean attack time(sec) [1]Mean ± SE	Range of attack time(sec)	% Oviposited in
Absence of *A. variegatum* odours	30	16.7[a]	1.2 ± 0.07[a]	2 - 6	6.7[a]
A. variegatum hexane washes	30	36.7[b]	2.6 ± 0.13[b]	3 - 48	10.0[a]
A. variegatum hexane washes & integument	30	50.0[c]	3.0 ± 0.14[c]	4 - 90	13.3[a]

Values followed by different superscript letters in the same column are significantly different ($P<0.05$). [1]Means and standard errors are log-transformed

(Table 5.3).

The longest attack time (90 seconds) was recorded when *R. appendiculatus* nymphs were presented to the parasitoids in the presence of a combination of *A. variegatum* nymphal washes and integument pieces, while the shortest attack time (2 seconds) was recorded in the absence of the washes and integument pieces (Table 5.3).

The presence of *A. variegatum* nymphal washes and integument pieces did not significantly increase the percentage of *R. appendiculatus* nymphs oviposited in by the parasitoids (Table 5.3).

Visual inspection of the scanning electron micrographs of the integument surfaces (dorsal aspect) of unfed *A. variegatum* (Plate 5.1) and *R. appendiculatus* (Plate 5.2) nymphs revealed differences between the integuments of the two tick species. Punctations (pits on the surface of the scutum) of *A. variegatum* nymphs are deeper and more pronounced, while the scuta of *R. appendiculatus* nymphs are smoother. Furthermore, the scuta of *A. variegatum* nymphs have rounded v-shaped posterior margins (Plate 5.1) while, those of *R. appendiculatus* nymphs have bell-shaped posterior margins (Plate 5.2).

The grooves separating the festoons (uniform, more or less rectangular areas separated by grooves along the posterior submarginal area of the dorsum) are longer in *R. appendiculatus* nymphs (Plate 5.2) compared to *A. variegatum* nymphs (Plate 5.1). *A. variegatum* nymphs have more festoons (eleven) than *R. appendiculatus* nymphs (two).

Bases capituli of *R. appendiculatus* nymphs are hexagonal with sharp lateral angles. The palps are short and blunt-ended (Plate 5.2; Walker, 1970). The bases capituli of *A. variegatum* nymphs are subtriangular with rounded lateral angles. The palps of *A. variegatum* nymphs are longer than those of *R. appendiculatus* nymphs.

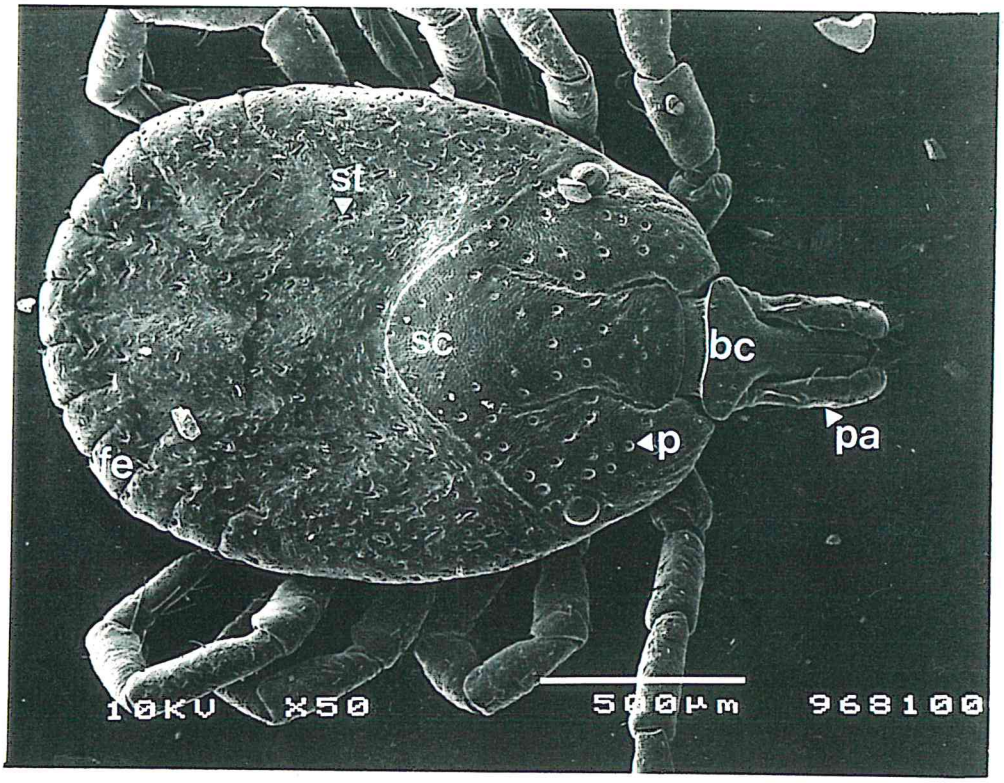

Plate 5.1. Scanning electron micrograph of an unfed *A. variegatum* nymph. st = seta, fe = festoon, sc = scutum, p = punctation, bc = basis capituli, pa = palp

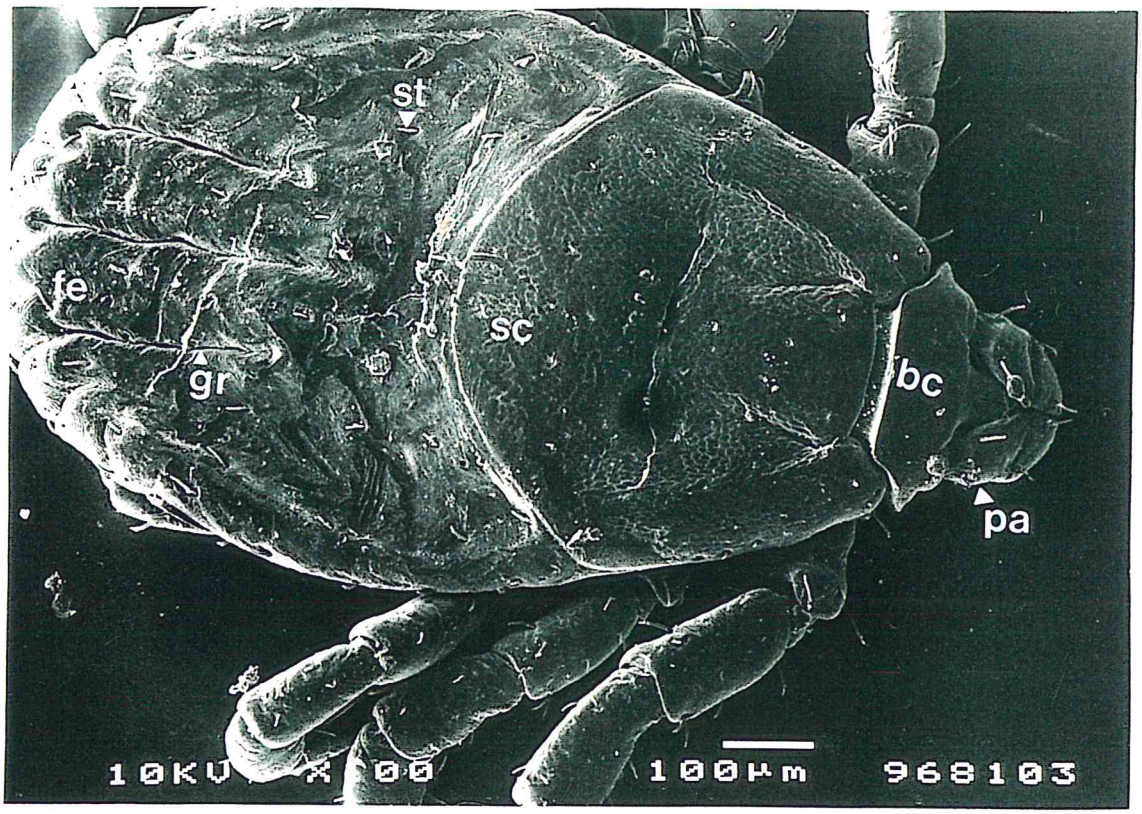

Plate 5.2. Scanning electron micrograph of an unfed *R. appendiculatus* nymph. gr = grooves separating festoons

Finally, the dorsal setae on the integument of *A. variegatum* nymphs are more numerous than those of *R. appendiculatus* and are concentrated along the margins of the dorsum, while those of *R. appendiculatus* nymphs are spread out more regularly along the entire dorsum (Plates 5.1 & 5.2).

5.2.2.2. Suitability of *A. variegatum* and *R. appendiculatus* nymphs for the development of *I. hookeri* immatures

To investigate the suitability of *A. variegatum* and *R. appendiculatus* nymphs for the development of *I. hookeri* immatures, fed and unfed nymphs were exposed singly to mated, naive *I. hookeri* females in glass vials (section 3.5.2.4). *R. appendiculatus* nymphs were conditioned with *A. variegatum* nymphal washes and integument pieces. Attacked nymphs were kept in aluminium cans until the emergence of the parasitoids, moulting or death of the nymphs. The percentages of nymphs attacked, yielding progeny, moulting, killed through parasitoid attack within 30 days and those alive after 30 days as well as the number of progeny per female parasitoid and the feeding success of *A. variegatum* nymphs fed on rabbit ears 24 hours after exposure to the parasitoids were recorded.

Significantly more *A. variegatum* nymphs (100%) were attacked by the parasitoids compared to *R. appendiculatus* nymphs attacked (83.3%)(Table 5.4). The percentage of replete *A. variegatum* nymphs yielding parasitoids (83.3%) was significantly more than that of nymphs fed after being attacked by the parasitoids (20.0%)(Table 5.4). Unfed *A. variegatum* and *R. appendiculatus* nymphs attacked did not yield progeny. All the *R. appendiculatus* nymphs moulted into adults within 30 days despite 83.3% being attacked by the parasitoids, while significantly less *A. variegatum* nymphs moulted into adults after being attacked (16.7% of replete nymphs and 36.7% of nymphs fed after parasitoid attack, Table 5.4). Furthermore, significantly more of the nymphs fed after being attacked by the parasitoids moulted than nymphs parasitized after

Table 5.4. Suitability of *A. variegatum* and *R. appendiculatus* nymphs for the development of *I. hookeri*

Test	% Attacked	% Yielding progeny	% Moulting	% Killed through attacks within 30 days	% Alive after 30 days	Progeny/ female [1]Mean ± SE
A. variegatum						
Fed	100.0[a]	83.3[a]	16.7[c]	83.3[b]	16.7[c]	72.3 ± 3.33[a]
Unfed	100.0[a]	0.0[c]	0.0[d]	90.0[a]	10.0[d]	0.0 ± 0.00[b]
Fed after parasitoid attack	100.0[a]	20.0[b]	36.7[b]	63.3[c]	36.7[b]	5.8 ± 0.92[c]
R. appendiculatus						
Fed	83.3[b]	0.0[c]	100.0[a]	0.0[d]	100.0[a]	0.0 ± 0.00[b]

Values followed by different superscript letters in the same column are significantly different (SNK, $P<0.05$). 43.3% of A. variegatum nymphs fed after parasitoid attack died before engorgement (Table 5.5). [1]Means and standard errors are untransformed

feeding to repletion on rabbit ears (Table 5.4).

Significantly high percentages of *A. variegatum* nymphs were killed through parasitoid attack within 30 days (83.3% of the replete nymphs, 90.0% of the unfed nymphs and 63.3% of the nymphs fed after parasitoid attack) while none of the *R. appendiculatus* nymphs were killed (Table 5.4). Mortality was the highest among unfed nymphs (never fed before or after attack), followed by the replete nymphs and finally by the nymphs fed after attack.

Significantly more *R. appendiculatus* nymphs (100%) were alive after 30 days compared to *A. variegatum* nymphs (16.7% of replete nymphs, 10.0% of unfed nymphs and 36.7% of nymphs fed after parasitoid attack, Table. 5.4). Survival of *A. variegatum* nymphs exposed to the parasitoids was the highest in nymphs fed after parasitoid attack, followed by replete nymphs, and finally unfed nymphs.

A. variegatum nymphs parasitized after repletion yielded significantly more progeny (mean = 72.3) than those parasitized before feeding (mean = 5.8)(Table 5.4). Nymphs which were never fed and replete *R. appendiculatus* nymphs did not yield any progeny (Table 5.4). From the *A. variegatum* nymphs parasitized 24 hours before being fed on rabbit ears, 56.7% engorged fully while 43.3% died before engorgement (Table 5.5).

Table 5.5. Feeding success of parasitized unfed *A. variegatum* nymphs fed on rabbit ears 24 hours after parasitoid attack

Test	N	% Engorged	% Dead before engorgement
fed after parasitoid attack	60	56.7	43.3

5.3. Discussion

This study provides evidence that *I. hookeri* females evaluate their hosts visually in the final host location and recognition stage before any physical contact takes place (Table 5.1). Visual host evaluation before contact has also been shown to be employed by other parasitoids, e.g. *Aphidius ervi*, a parasitoid of aphids (Battaglia *et al.*, 1995). In all tests, the percentage of parasitoids which showed signs of visual detection of the test material (direct detection) were significantly more than those which did not do so. Among other functions, the visual cues help the parasitoids to differentiate between sizes, feeding status (fed or unfed) and species of the host.

Host size has a major influence on parasitoid fitness as it determines the maximum amount of food available for the developing parasitoid (Godfray, 1994). The longer examination time at larger nymphs suggests that *I. hookeri* females prefer larger hosts when presented with a choice. The discrimination between fed and unfed *A. variegatum* nymphs could have been due to the preference for larger hosts since engorged nymphs are bigger than unfed ones. Furthermore, the longer examination of replete *A. variegatum* nymphs compared to *R. appendiculatus* nymphs could also have been due to size since replete *A. variegatum* nymphs are larger than their *R. appendiculatus* counterparts. However, this discrimination could also be governed by other factors since nymphs of other *Amblyomma* ticks are also discriminated

against (E. Mwangi, pers. comm.).

Movement of the nymphs did not play a significant role in host recognition as the duration of examination time and frequencies at which both dead and live nymphs were visited were similar (Table 5.1). This could be due to the sessile habit of feeding on-host ticks (Chapter 4 discussed the location and parasitization of on-host ticks). Since feeding *A. variegatum* nymphs attach firmly with their long mouthparts to the vertebrate host (Kemp *et al.*, 1982) and show almost no movement, movement seems not to be important in host evaluation which might lead to subsequent host acceptance. In the laboratory, the female parasitoids frequently probe dead mummies they emerge from with their ovipositors, further demonstrating the insignificant role movement plays in host evaluation and acceptance.

It is evident that the parasitoids did not prefer any of the colours tested in this study over the other (Table 5.1). *A. variegatum* nymphs change colour from dark red-brown (unfed, Plate 3.3(c)) to grey (fed, Plate 3.10(a)) during feeding, while parasitized fed nymphs change colour from grey to yellow-brown or dark brown when becoming mummified (Plate 3.10(b) & (c)). Since the parasitoids emerge from mummified nymphs, they could have learned at the emergence site to associate these colours with potential hosts while the recognition of grey nymphs as potential hosts could be innate as the parasitiods were not exposed to grey nymphs before the experiment. Parasitoids have to look for engorging on-host nymphs which become grey as they fill with blood.

The apparent inability of the parasitoids to discriminate between the colours of mummies and fed *A. variegatum* nymphs is contrary to the preference of certain colours reported for some aphid parasitoids. *Aphidius rhopalosiphi* prefers green over brown colour morphs of the aphid, *Sitobion avenae* (Ankersmit *et al.*, 1986). However, it is consistent with the inability of some host ticks to discriminate between colours.

For example, adult *Hyalomma truncatum* ticks do not discriminate between blue, green, red and yellow colours (Kopp & Gothe, 1995). Furthermore, it is possible that the parasitoids were attracted by the shape of the nymphs and not the colour since mummified nymphs still have the puffed-up shape (appearance) of fed nymphs. Host selection in the aphid parasitoid, *Monoctonus crepidis* is influenced by aphid size and shape (Griffiths, 1960).

The visual discrimination of *I. hookeri* females between *A. variegatum* and *R. appendiculatus* nymphs (Table 5.1) together with the chemical discrimination discussed in Chapter 4 leaves little room for accidental oviposition of the parasitoids in these non-hosts.

The frequencies at which the test materials were visited do not reflect the importance of the these materials as visual cues. For example, the parasitoids visited large and small nymphs at similar frequencies (Table 5.1), yet they spent significantly more time examining large nymphs per visit compared to the examination time of small nymphs. The same is true for the examination times and frequencies of unfed and fed nymphs. These findings show that the parasitoids try longer to gain access to more profitable hosts detected visually without leaving the area frequently. However, the opposite is true for the examination times and frequencies of *A. variegatum* and *R. appendiculatus* nymphs tested against each other (Table 5.1). Although the parasitoids examined the vials containing *A. variegatum* nymphs significantly longer compared to vials containing *R. appendiculatus* nymphs, the parasitoids left and re-visited the vials containing *A. variegatum* nymphs more frequently than the vials containing *R. appendiculatus* nymphs (Table 5.1). These conflicting findings suggest that the time *I. hookeri* females stay, leave or return to an area containing profitable hosts (represented by vials) is variable if the parasitoids are denied access to these hosts although they can see them. It is known that parasitoids remain longer in areas containing hosts and increase search intensity if they are allowed

to oviposit in the hosts (Nelson & Roitberg, 1995).

The findings on visual cues used by *I. hookeri* females support the suggestion in Chapter 4 that *I. hookeri* females need visual as well as chemical cues from the host in order to retain the parasitoids in the olfactometer arm carrying infochemicals.

The finding that significantly more host nymphs (*A. variegatum*, 96.7%) were attacked by the parasitoids (ovipositor inserted into nymphs) than the non-hosts nymphs (*R. appendiculatus*, 16.7%)(Table 5.2) and that the parasitoids spent significantly more time attacking host nymphs compared to non-host nymphs demonstrates the special relationship between *I. hookeri* and *A. variegatum*. Furthermore, the parasitoids laid eggs in all *A. variegatum* nymphs attacked but not in every *R. appendiculatus* nymph attacked during this study. Although 16.7% *R. appendiculatus* nymphs were attacked, eggs were laid in 6.7% of these nymphs showing that the parasitoids do not accept *R. appendiculatus* nymphs for oviposition (Table 5.2). Parasitoids are known to insert their ovipositors into non-hosts without laying eggs (Godfray, 1994). The ovipositor is normally covered with sensillae and it seems likely that the parasitoids reject the non-hosts or low quality hosts after perceiving that they are unsuitable for oviposition (Vinson, 1976; Godfray, 1994). Thus the oviposition of the parasitoids in 6.7% of *R. appendiculatus* nymphs can be regarded as accidental.

Conspecific superparasitism was frequently observed in the laboratory. Sometimes, up to three parasitoids were seen parasitizing a host simultaneously. Despite the observed superparasitism, this study showed that *I. hookeri* females can discriminate between parasitized and unparasitized nymphs and lay fewer eggs in parasitized hosts (provided the oviposition time is an accurate predictor of the number of eggs laid)(Fig. 5.2). The first female parasitoid which encountered an unparasitized host spent significantly more time ovipositing

(141.2 seconds) compared to superparasitizing females (i.e. females 2 (82.9 seconds), 3 (62.5 seconds) and 4 (44.9 seconds)). In many parasitoids, it has been found that the eggs laid by the first female (primary clutch) are more than those laid by superparasitizing females. The first evidence that secondary and tertiary egg clutches of superparasitizing parasitoids are smaller than the primary clutches was obtained by Wylie (1965) working on the pteromalid, *Nasonia vitripennis*, a parasitoid of dipteran pupae. Conspecific superparasitism in *I. hookeri* may occur in the field as the distribution of *A. variegatum* nymphs on cattle is patchy and seasonal. Superparasitism could be an adaptive strategy for *I. hookeri* as the parasitoids seem to avoid over-parasitization through controlling the number of eggs they lay in parasitized hosts. Through such control, the parasitoids prevent the death of immature insects due to inadequate food resources in the nymphs. The decreasing oviposition time with increasing superparasitization could be due to a marking pheromone left by former females on the surface of the host or the sensing of the eggs of conspecifics or a changed internal state of the nymph upon the insertion of the ovipositor (Salt, 1937; Lloyd, 1939; Wylie, 1965).

Superparasitism in *I. hookeri* might also be advantageous in some hosts as 16.7% of *A. variegatum* nymphs attacked after feeding and 36.7% of the nymphs fed after being attacked moulted into adults (Table 5.4). It could either be that the parasitoids did not lay eggs in these nymphs or that the parasitoid immatures were killed by the internal defenses of the nymphs. Mwangi & Wabwoba (unpublished) found that *I. hookeri* females need to oviposit a minimum of eight eggs in *A. variegatum* nymphs to secure the successful development of these eggs. It is also known that small egg clutches of gregarious species (species which lay more than one egg per host) may have problems developing in large hosts. DeLoach and Rabb (1972) found that immature mortality of the tobacco hornworm (*Manduca sexta*) tachinid

parasitoid, *Winthemia manducae*, was very high in small clutches of one to three eggs compared to the normal clutches of ten to twenty or higher. Thus, females with smaller clutches due to earlier host encounters or because of having fewer eggs at emergence will need to superparasitize instead of parasitizing unparasitized hosts. This hypothesis needs to be verified (DeLoach & Rabb, 1972).

The first *I. hookeri* female presented with an unparasitized *A. variegatum* nymph started ovipositing in the nymph after a brief period (26.0 seconds) while the second female started oviposition after a significantly longer period (47.9 seconds). This could again be due to a marking pheromone. However, the effect of any marking pheromone did not deter superparasitizing parasitoids from ovipositing as the pre-oviposition time between the second, third and fourth superparasitizing females were similar. The function of marking is to protect the eggs of the first female during the period when the eggs of superparasitizing females could still win the competition for the host (van Alphen & Visser, 1990). This could explain the observed simultaneous oviposition of up to three parasitoids in the same host. All fed *A. variegatum* and *R. appendiculatus* nymphs presented to the parasitoids were used on the day they dropped from the rabbit ears since the integuments of these nymphs hardened after about three days post drop in preparation for the moult. As the parasitoids found it difficult to oviposit in these nymphs it could influence host acceptance since the thickness of the host cuticle have been shown to be important in host acceptance in parasitoids laying eggs in hosts (Godfray, 1994).

On the other hand, *A. variegatum* nymphs produce allomones (repellents) to keep the parasitoids at bay. These allomones which are believed to be secreted by the dermal glands (Pavis *et al.*, 1994) have a significant short-term (about 10 minutes) repellent effect on the parasitoids. *I. hookeri* females stayed for an average of 72.7 seconds waiting for the highly volatile secretions to

evaporate off the body surface of disturbed nymphs before contacting them compared to the 21.0 seconds delay before contacting undisturbed nymphs. The observed ten minute repellent effect of the secretions could be even shorter under field conditions as wind could facilitate the quicker evaporation of the secretions.

A few parasitoids (5%) were seen ovipositing in nymphs which still had visible droplets of dermal gland secretions on them while others retreated and groomed repeatedly after coming into contact with the secretions. Pavis *et al.* (1994) also found that *A. variegatum* dermal gland secretions repel the fire-ant, *Solenopsis geminata*. Yoder *et al.* (1993) found that the dominant component of defensive secretions from several ticks was squalene (biological precursor of steroids).

Furthermore, this study demonstrated that *I. hookeri* females can be induced to attack *R. appendiculatus* nymphs through employing *A. variegatum* derived signals (Table 5.3). The presence of *A. variegatum* nymphal washes and integument pieces increased the percentage of *R. appendiculatus* nymphs attacked and the mean attack time significantly, however, the percentages of *R. appendiculatus* nymphs oviposited into in the presence of *A. variegatum* signals were not significantly different from the percentage oviposited into in the absence of these signals (Table 5.3). This shows again that the parasitoids get additional information on the suitability of the *R. appendiculatus* nymphs upon the insertion of the ovipositor and can reject the nymphs without laying eggs although external examination of these conditioned nymphs may reveal that they are 'hosts'.

The differences in the texture of the scuta of *A. variegatum* and *R. appendiculatus* nymphs as seen in the micrographs (Plates 5.1 & 5.2), other differences which might not be clear in the micrographs and infochemicals might govern the discrimination of *I. hookeri* females between nymphs of the two tick

species.

The more numerous dorsal setae of *A. variegatum* nymphs compared to *R. appendiculatus* nymphs could be one of the cues used by *I. hookeri* females to discriminate between the two tick species. It has been shown that hairiness and odour were important in the initiation of oviposition in *Apanteles melanoscelus* (Weseloh, 1974). Furthermore, the concentration of the dorsal setae along the margins of the dorsa of *A. variegatum* nymphs (Plate 5.1) compared to the absence of such arrangement in *R. appendiculatus* nymphs (Plate 5.2) could also help in host recognition as the parasitoids ran along the dorsal margins of the nymphs upon encountering them. Setation has been used in the identification of immature ticks. For example, dorsal setation of *A. variegatum* nymphs are known to consist of nine pairs of marginodorsal setae, one pair of centrodorsal setae and one to two pairs of premarginal setae (Van der Borght-Elbl, 1977).

The length and texture of the palps together with possible secretions could also provide additional information about the host nymphs. During this study, parasitoids were seen attempting to oviposit on the palps of *A. variegatum* nymphs. The parasitoids always visited the bases capituli and mouthparts of the nymphs before, in between or after oviposition(s), but definitely before leaving the nymphs. The hexagonal bases capituli with sharp lateral angles and the short, blunt-ended palps (Walker, 1970) could also have contributed to the rejection of *R. appendiculatus* nymphs. Through coevolution, the parasitoids could have preferred subtriangular bases capituli with rounded lateral angles and longer palps which are some of the attributes of *A. variegatum* nymphs.

Furthermore, the slightly rugose scutal surface of *A. variegatum* nymphs with its numerous evenly spaced medium-sized punctations (Van der Borght-Elbl, 1977, Plate 5.1) and rounded v-shaped posterior margin compared to the smoother scuta of *R. appendiculatus* nymphs with their bell-shaped posterior margins could also have aided in the discrimination of *I. hookeri* females between

the two tick species. While running on the scuta of the nymphs, the parasitoids could have obtained visual, tactile and/or chemotactile information leading to the rejection of *R. appendiculatus* and acceptance of *A. variegatum* nymphs.

The parasitoids could also have obtained host-related information while running along the posterior area of the dorsum since *A. variegatum* nymphs have more festoons with shorter grooves separating them, while the opposite is true for *A. variegatum* nymphs. When running along the dorsal margin of nymphs, especially *A. variegatum*, the parasitoids usually completed a circle(s) on the body surface of the nymphs, gaining information from the anterior, lateral and posterior regions of the nymphs.

All *R. appendiculatus* nymphs moulted into adults and did not yield any parasitoid progeny despite 83.3% being attacked by the parasitoids (Table 5.4). This explains why the parasitoids are reluctant to lay eggs in these nymphs. *R. appendiculatus* ticks are believed to have a strong immune system that kills off the parasitoid eggs through encapsulation (Bengaly, pers. comm.).

Taking into account the lack of attraction of *I. hookeri* females to cattle ear odours, the chemical, visual and tactile discrimination against *R. appendiculatus* nymphs and the failure of these nymphs to support the development of *I. hookeri* immatures, it is clear that *I. hookeri* females will not parasitize these nymphs in the field. Thus, the biocontrol of *R. appendiculatus* in Kenya rests with the other components of biocontrol such as predators, pathogens, anti-tick botanicals and immunity of cattle to East Coast Fever for the foreseeable future if classical biocontrol (importation of other tick parasitoid species or different strains of *I. hookeri*) is not considered.

Furthermore, unfed *A. variegatum* nymphs (not fed before or after parasitoid attack) are also not suitable for the development of *I. hookeri* immatures, as suggested in Chapter 4, since a bloodmeal is needed for the development of the eggs. *A. variegatum* nymphs fed after being attacked by the

parasitoids had difficulty in feeding and almost half of them (43.3%) died before engorgement (Table 5.5). From those which engorged 20% yielded progeny although the number of progeny yielded (average of 5.8 progeny/female) was significantly lower than the number of progeny yielded by nymphs parasitized after feeding (average of 72.3 progeny/female)(Table 5.4). Parasitized unfed nymphs died rapidly with mortality starting on the fifth day after the attack. After 30 days, 10% of the unfed nymphs were still alive, while the unparasitized nymphs from the colony lived for more than six months under the same conditions. Similar observations were made by Graf (1979) on the effects of an undescribed tick parasitoid on *A. nuttalli* in the Ivory Coast.

This study highlights two findings which have serious implications for tick biocontrol utilizing *I. hookeri*. The finding that *I. hookeri* targets feeding on-host *A. variegatum* nymphs and not the questing unfed nymphs which are not suitable for the immature parasitoids suggests that the risk of declining parasitoid numbers in the field is minimized.

On the other hand, the host specificity of *I. hookeri* suggests that there are no alternative host(s) to sustain high parasitoid population levels when there is a decline in the host tick populations. The lack of alternative hosts will result in a time lag in the build-up of parasitoid field populations compared to tick population build-up.

CHAPTER 6: FIELD AND LABORATORY OBSERVATIONS ON THE HOST SEEKING AND DISPERSAL BEHAVIOUR

6.1. Introduction

The ultimate goal of biological control is to increase the effectiveness of natural enemies in the field. However, the small size of many parasitoids makes observation of their field behaviour often difficult if not impossible. This applies particularly to the monitoring of the movement of individuals, for example between patches (i.e. units of hosts/prey spatial distribution or limited areas in which natural enemies search for hosts/prey (van Alphen & Jervis, 1996)). The upwind flight of various parasitoids in response to odours has been studied in flight or wind tunnels (Drost *et al.*, 1986; Hérard *et al.*, 1988; Zanen *et al.*, 1989), leaving us with no comprehensive literature on the field flight ranges of parasitoids. The release of parasitoids during biological control programmes offers unparalleled opportunity for studying the geographical dispersal. Sadly, most programmes are undertaken in response to an agricultural emergency, leaving little time for monitoring spread beyond checking that establishment has occurred on geographical scales (Godfray, 1994).

Most ticks actively seek hosts only during certain well-defined periods of the year. These well-defined seasonal activity periods are the weeks or months during which ticks transmit diseases (Sonenshine & Mather, 1994). Ixodid ticks feed for 6-8 days and drop off in synchrony with the animal host's behaviour patterns. This tends to disperse the fed ticks in optimal habitats where they can develop, reproduce or find a host again (Sonenshine & Mather, 1994). This strategic dispersal of ticks in the habitats of their vertebrate hosts will enhance the finding of these vertebrate hosts and the ticks feeding on them by emerging parasitoids.

Parasitization rates are traditionally used to assess the performance of the parasitoids in the field. However, researchers interested in optimal behaviour, should consider encounter rates because not every encounter will be followed by an oviposition (van Alphen & Jervis, 1996).

Moreover, since some females superparasitize hosts already parasitized by conspecifics, field parasitization rates will not truly reflect the number of hosts encountered and parasitized per unit time. Furthermore, the feeding status (unfed, partially fed or fed) at which the ticks are parasitized is important for the reproductive success of the parasitoid and the sustained regulation of the ticks in the field since a blood meal is essential for the successful development of the parasitoid immatures in the tick hosts.

The first objective of this study was to carry out field experiments to investigate the behaviour of *I. hookeri* between release and finding of the tick hosts. Mated naive *I. hookeri* females were released on the shoulders of calves and the searching time of these parasitoids recorded per front leg region searched.

To investigate the field parasitization rates of on-host *A. variegatum* nymphs encountered by the parasitoids on various body regions of cattle, nymphs were collected from free ranging cattle in Kuja River and Trans-Mara areas of Kenya (Appendix 2). The front legs, hind legs, bellies, scrota, udders and dewlaps and ears of the cattle were inspected for nymphs.

The second objective of the study was to study the dispersal behaviour of the parasitoids. In order to achieve this, mated naive parasitoids were released in the field as well as in a wind tunnel.

6.2 Results

6.2.1. Parasitoid releases on cattle

Female parasitoids were released singly high up on the shoulders of tethered calves to investigate the searching behaviour of mated naive *I. hookeri* females on cattle (section 3.10.1.1.1, Fig. 3.4). As the majority of the released parasitoids searched on the front legs for hosts, searching time was recorded for various parts of the front legs searched.

The total search time of *I. hookeri* females was significantly longer on the lower leg (Fig. 3.4) than on the other parts of the front leg (Table 6.1).

Table 6.1. Searching time of mated naive *I. hookeri* parasitoids released on the shoulders of calves

Body region	Length(cm)	Total search time (sec). [1]Mean ± SE	Search time per unit length (sec/cm)
shoulder	36	4.8 ± 0.03[c]	1.2 ± 0.03[c]
upper leg	24	5.4 ± 0.06[b]	2.2 ± 0.06[b]
middle leg	17	4.9 ± 0.03[c]	2.1 ± 0.03[b]
lower leg	11	6.1 ± 0.05[a]	3.7 ± 0.05[a]

Values followed by different superscript letters in the same column are significantly different (SNK, $P<0.05$). [1]Means are log-transformed

The second longest total search time was spent on the upper leg. The total search times on the middle leg and shoulder were not significantly different (Table 6.1). When the total searching time was related to the lengths of each of the front leg regions searched, the searching time at the lower leg was again significantly longer than those of the other regions. The upper and middle leg searching times per unit length were not significantly different, while that of the shoulder was the shortest (Table 6.1).

6.2.2. Parasitization of *A. variegatum* nymphs per feeding site on cattle

Unfed, partially fed and fed *A. variegatum* nymphs were collected from free ranging cattle in Kuja River and Trans-Mara areas of Kenya to investigate the field parasitization rates of on-host *A. variegatum* nymphs on various body parts (front legs, hind legs, bellies, scrota, udders, dewlaps and ears) of cattle. The nymphs were dissected or kept in the laboratory until signs of parasitism appeared or moulting occurred (section 3.10.1.1.2.).

In the Trans-Mara collection of September 1995, most of the parasitized close-to-repletion nymphs were collected from the front and hind legs (50% each) (Table 6.2), followed by the bellies (25%). None of the nymphs collected from the scrota were parasitized. No nymphs were collected from the udders, dewlaps and ears. In the Kuja River collection of September 1995, most of the parasitized close-to-repletion nymphs were collected from the front legs (66.7%), followed by the bellies (50%), scrota (40%) and hind legs (25%)(Table 6.2). No nymphs were collected from the udder, dewlap and ear.

In the Kuja River collection of January 1997, most of the parasitized unfed nymphs were collected from the bellies (61.5%), followed by the front legs (59.0%), hind legs (55.6%), scrota (48.5%) and udder (48.1%)(Table 6.2). None of the nymphs collected from the dewlaps were parasitized while no nymphs were collected from the ears.

In the Kuja River collection of January 1997, most of the parasitized partially fed nymphs were collected from the front legs (85.6%), followed by the hind legs (79.6%), bellies (73.3%), dewlaps (73.1%), scrota (67.1%) and udders (60.4%)(Table 6.2). No nymphs were collected from the ears.

In the Kuja River collection of January 1997, most of the parasitized close-to-repletion nymphs were collected from the bellies and scrota (80.0% each), followed by the front legs (76.5%), hind legs (75.0%) and udders (50.0%)(Table 6.2). No nymphs were collected from the dewlaps and ears.

The overall percentage of *A. variegatum* nymphs collected from the front legs in Kuja River and Trans-Mara (26.9%) was significantly more compared to the percentages of nymphs collected from the other body regions (Table 6.3). The bellies yielded the second highest percentage of nymphs (20.9%), followed by the udders (19.0%), scrota (17.4%), hind legs (11.7%) and the dewlaps (4.1%). No *A. variegatum* nymphs were found on cattle ears during field collections (Table 6.3). The percentages of nymphs collected from the different body regions were significantly different from each other (Table 6.3).

Table 6.2. Field parasitization rates of *A. variegatum* nymphs feeding on various body regions of cattle

Body region	No. collected	No. parasitized	% Parasitization
Trans-Mara (24 - 29 September, 1995)			
Close-to-repletion nymphs			
Front leg	4	2	50.0
Hind leg	6	3	50.0
Belly	4	1	25.0
Scrotum	2	0	0.0
Udder	0	0	0.0
Dewlap	0	0	0.0
Ear	0	0	0.0
Kuja River (24 - 29 September, 1995)			
Close-to-repletion nymphs			
Front leg	9	6	66.7
Hind leg	4	1	25.0
Belly	2	1	50.0
Scrotum	5	2	40.0
Udder	0	0	0.0
Dewlap	0	0	0.0
Ear	0	0	0.0

Table 6.2 continued

Body region	No. collected	No. parasitized	% Parasitization
Kuja River (23 - 27 January, 1997)			
Unfed nymphs			
Front leg	39	23	59.0
Hind leg	9	5	55.6
Belly	39	24	61.5
Scrotum	33	16	48.5
Udder	27	13	48.1
Dewlap	2	0	0.0
Ear	0	0	0.0
Partially fed nymphs			
Front leg	118	101	85.6
Hind leg	54	43	79.6
Belly	90	66	73.3
Scrotum	76	51	67.1
Udder	101	61	60.4
Dewlap	26	19	73.1
Ear	0	0	0.0

Table 6.2 continued

Body region	No. collected	No. parasitized	% Parasitization
Close-to-repletion nymphs			
Front leg	17	13	76.5
Hind leg	8	6	75.0
Belly	10	8	80.0
Scrotum	5	4	80.0
Udder	4	2	50.0
Dewlap	0	0	0.0
Ear	0	0	0.0

Table 6.3. Overall numbers and parasitization rates of *A. variegatum* nymphs collected from Kuja River and Trans-Mara areas of Kenya

Body region	No. coll.	%Coll.	No. paras.	%Paras.
Front leg	187	26.9^{a}	145	77.5^{a}
Hind leg	81	11.7^{e}	58	71.6^{b}
Belly	145	20.9^{b}	100	69.0^{bc}
Scrotum	121	17.4^{d}	73	60.3^{d}
Udder	132	19.0^{c}	76	57.6^{d}
Dewlap	28	4.1^{f}	19	67.9^{c}
Ear	0	0^{g}	0	0^{e}

Values followed by different superscript letters in the same column are significantly different (SNK, $P<0.05$).

Furthermore, the overall percentage of parasitized *A. variegatum* nymphs collected from the front legs in Kuja River and Trans-Mara (77.5%) was significantly more compared to the percentages of parasitized nymphs collected from the other body regions (Table 6.3). The second most parasitized group of nymphs were collected from the hind legs (71.6%), followed by the bellies (69.0%), dewlaps (67.9%) scrota (60.3%) and the udders(57.6%)(Table 6.3).

The percentage of parasitized nymphs collected from the hind legs was significantly more than that of nymphs collected from the scrota, udders and dewlaps but was not significantly different from that of nymphs collected from the bellies (Table 6.3). The percentage of parasitized nymphs collected from the bellies was significantly more than that of nymphs collected from the scrota and udders but was not significantly different from that of nymphs collected from the hind legs and dewlaps (Table 6.3). The percentages of parasitized nymphs collected from the scrota and udders were not significantly different from each other and were the lowest of all the body regions from which nymphs were obtained (Table 6.3).

Significantly more partially fed nymphs (70.7%) were collected from Kuja River between the 23rd and the 27th of January 1997 compared to unfed (22.6%) and close-to-repletion (6.7%) nymphs (Table 6.4). Significantly more unfed nymphs were collected than the close-to-repletion nymphs (Table 6.4).

Parasitized partially fed and close-to-repletion nymphs were significantly more than the parasitized unfed nymphs (Table 6.4). There was no significant difference between the percentages of parasitized partially fed and close-to-repletion nymphs (Table 6.4).

6.2.3. Field dispersal behaviour

Female parasitoids were released at 50cm, 100cm, 200 cm and 300cm from tethered white calves to study the movement of these parasitoids from the point of release to cattle. The behaviour of the parasitoids at the release site, the number of parasitoids dispersing within 10 minutes, numbers reaching the calves

Table 6.4. Overall numbers and parasitization rates of the various feeding states of *A. variegatum* nymphs collected from Kuja River, Kenya

Feeding state	No. coll.	%Coll.	No. paras.	%Paras.
Unfed	149	22.6[b]	81	54.4[b]
Partially fed	465	70.7[a]	341	73.3[a]
Close-to-repletion	44	6.7[c]	33	75.0[a]

Values followed by different superscript letters in the same column are significantly different (SNK, $P<0.05$).

and those still at the release site after 1 hour were recorded (section 3.10.1.1.3).

All the females released ran up and down the grass blades (Table 6.5). Between runs, the females stopped and cleaned their antennae with front legs and the wings with the hind legs (Table 6.5).

The percentages of female parasitoids dispersing within 10 minutes from the release site were not significantly different when released at 50cm, 100cm, 200cm and 300cm (Table 6.6).

The percentages of females reaching the calves were not significantly different when the parasitoids were released at the above-mentioned distances (Table 6.6).

The percentages of females still at the release site 1 hour after release did not differ significantly when the parasitoids were released at the above-mentioned distances (Table 6.6). The parasitoids employed crawling, jumping and flight to get onto the calves (Table 6.7). Significantly more parasitoids (53.7%) crawled up the legs of calves compared to those which jumped and landed on the legs (29.8%) or flew and landed on the legs or bellies (16.5%) of the calves (Table 6.7). Furthermore, significantly more parasitoids jumped

Table 6.5. Behaviour exhibited by mated naive *I. hookeri* females at the field release site

Behaviour	Description	% Females exhibiting behaviour
Running	Running up and down the grass blades	100
Antenna cleaning	Cleaning antennae with front legs	100
Wing cleaning	Cleaning wings with hind legs	100

Table 6.6. Field dispersal of mated naive *I. hookeri* females

Distance(cm)	No. females released	% Dispersing within 10 mins	% Reaching calves	% At release site after 1 hour
50	60	66.7[a]	60.0[a]	10.0[a]
100	60	63.3[a]	56.7[a]	13.3[a]
200	60	75.0[a]	48.3[a]	13.3[a]
300	60	65.0[a]	45.0[a]	16.7[a]

Values followed by same superscript letters in the same column are not significantly different (SNK, $P<0.05$)

Table 6.7. Different modes used by mated naive *I. hookeri* females to get onto calves

Mode	No. of females	% Females using mode of dispersal
Crawling up legs	65	53.7[a]
Jumping and landing on legs	36	29.8[b]
Flying and landing on legs or belly	20	16.5[c]

Values followed by different superscript letters in the same column are significantly different (SNK, $P<0.05$).

(29.8%) compared to those which flew (16.5%).

6.2.4. Wind tunnel dispersal behaviour

To test the medium and long-range attraction of mated naive *I. hookeri* females to cattle odours, parasitoids were released 50cm or 100cm away from animal odour swabs in a wind tunnel. The modes of dispersal, the amount of time spent on each as well as the distances covered were recorded (section 3.10.2.1.).

When released at 50cm, the parasitoids covered a significantly longer distance through flight (24.5cm) compared to the distances covered through jumping and running (Table 6.8). The second longest distance was covered through jumping (18.2cm) while the shortest distance was covered through running (13.5cm).

Parasitoids released at 50 cm stayed on the platforms (vials) significantly longer before dispersing (54.6 seconds) compared to the time spent running (33.1 seconds), jumping (7.4 seconds) or flying (4.5 seconds)(Table 6.8). After leaving the platform, the parasitoids spent a significant amount of time (492.7 seconds) sitting on the wind tunnel floor. For dispersal, the parasitoids ran most of the time.

Table 6.8. Upwind dispersal of mated naive *I. hookeri* females in a wind tunnel in response to calf front heel and urine odours

Parameter	Test distance: 50cm [1]Mean ± SE		Test distance: 100cm [1]Mean ± SE	
	Distance covered(cm)	Time taken(sec)	Distance covered(cm)	Time taken(sec)
Platform	NA	54.6 ± 1.21[b]	NA	60.3 ± 1.21[b]
Run	13.5 ± 1.12[c]	33.1 ± 1.16[c]	14.9 ± 1.17[b]	14.9 ± 1.31[c]
Jump	18.2 ± 1.08[b]	7.4 ± 1.17[d]	49.4 ± 1.05[a]	11.0 ± 1.11[c]
Flight	24.5 ± 1.09[a]	4.5 ± 1.08[e]	40.4 ± 1.16[a]	4.5 ± 1.13[d]
Sit	NA	492.7 ± 1.06[a]	NA	601.8 ± 1.04[a]

Values followed by different superscript letters in the same column are significantly different (SNK, $P<0.05$). [1]Means are back-transformed

Jumping was the second most used mode of dispersal while flight was rare and brief (Table 6.8).When released at 100cm, the parasitoids covered the longest distance through jumping (49.4cm). The second longest distance was covered through flight (40.4cm) while running accounted for a distance of 14.9cm (Table 6.8). The distances covered through jumping and flight were not significantly different.

Furthermore, parasitoids released at 100cm also spent a significant amount of time (60.3 seconds) on the vials before responding to the odour sources compared to the time spent running (14.9 seconds), jumping (11.0 seconds) and flight (4.5 seconds)(Table 6.8). Parasitoids also spent a significant amount of time on the tunnel floor (601.8 seconds) compared to the times spent on the platform, running, jumping and flight. When parasitoids were released at 100cm, the amounts of time spent running and jumping were not significantly different. For dispersal, the parasitoids spent most of the time running. The second most used mode of dispersal was jumping while flight was brief (Table 6.8).

When parasitoids were released 50cm from the odour source, significantly more parasitoids jumped (86.7%) compared to those which ran (60.0%) or flew (56.7%)(Table 6.9). The percentages of running and flying parasitoids were not significantly different at 50cm. The percentage of females coming within 20cm of the odour source (86.7%) was significantly more than those reaching the target (56.7%) (Table 6.9).When parasitoids were released at 100cm, the percentage of females jumping (90.0%) were significantly more than those running (80.0%) or flying (60.0%) (Table 6.9). The percentages of running parasitoids were significantly more than those which flew. The percentage of females coming within 20cm of the odour source (76.7%) was significantly different from those reaching the target (53.3%)(Table 6.9).

Table 6.9. Modes of dispersal in the wind tunnel and the percentage of mated naive *I. hookeri* females using them to reach the target

Parameter	Test distance: 50cm	Test distance: 100cm
% Females running	60.0[b]	80.0[b]
% Females jumping	86.7[a]	90.0[a]
% Females flying	56.7[b]	60.0[d]
% Females getting within 20cm from target	86.7[a]	76.7[c]
% Females reaching target	56.7[b]	53.3[e]

Values followed by different superscript letters in the same column are significantly different (SNK, $P<0.05$).

6.3. Discussion

Searching time of parasitoids released on the calf shoulders revealed that the parasitoids spent significantly more time searching around the heels for *A. variegatum* nymphs (lower leg, Fig. 3.4 & Table 6.1). This significantly longer searching time once again underlines the importance of odours from the heel areas. It was observed that upon reaching the lower leg, the parasitoids frequently visited the area between the hooves.

Interdigital glands are located between the hooves in most species of deer (Cervidae), antelope (Bovidae) and pronghorns (Antilocapridae)(Wood *et al.*, 1995) and are known to arrest host-seeking ticks (Caroll *et al.*, 1995). A similar retaining mechanism from cattle on host-seeking *I. hookeri* females may operate in conjunction with waste product contamination around the hooves. The heels might play an important part as the converging zones of both the ticks and the parasitoids.

Although the parasitoids were released high up on the shoulders (Fig. 3.4), the parasitoids moved down to the heels. Some parasitoids were swept off by the tail while searching in areas easily reached by the tail. Avoidance of the tail and sun, orientation to dung and urine odours contaminating the bellies and legs of cattle as they lie down in contaminated cattle sheds (as mentioned in Chapter 4), as well as orientation toward the heel odours could be responsible for the downward movement of the parasitoids. The search time on the lower leg was significantly more than the time spent on the middle leg, upper leg and shoulder (Table 6.1).

Field collections from Kuja River and Trans-Mara demonstrated that *A. variegatum* nymphs fed more in certain body regions of cattle. For example, a significant proportion of the nymphs (26.9%) was collected from the front legs during this study of which 77.5% was parasitized (Table 6.3). This parasitization rate was significantly higher than the rates for the other body regions from which nymphs were obtained (Table 6.3). This finding further supports the above suggestion that cattle heels might act as converging zones for both the tick nymphs and the parasitoids.

Although a significant percentage of the parasitoids (53.7%) crawled up the legs of calves compared to those which flew and landed on the legs or bellies (16.5%)(Table 6.7), there was no significant difference in the overall parasitization rates of nymphs collected from the hind legs and bellies in Kuja River and Trans-Mara (Table 6.3). This finding could suggest that the parasitoids do not have to fly and land on the bellies to parasitize nymphs feeding there, but that the parasitoids actively crawl from the legs onto the bellies in search for hosts. On the other hand, it is possible that although flight was not observed frequently during the field studies, the parasitoids could fly more under a different set of environmental conditions. For example, Roberts & Irving-Bell (1996) found that the main factor affecting flight activity in four Nigerian blackfly species was the circadian change in light intensities. Wind velocity is also known to affect insect flight (Cooter, 1982).

The crawling habit of the parasitoids could be an adaptation to the crawling of questing ticks onto the legs and heads of grazing animals. In the field, the crawling of the parasitoids onto cattle legs bring the parasitoids in contact with cattle legs and the host nymphs feeding on them.

Of further interest are the lowest parasitization rates recorded for nymphs collected from the scrota (60.3%) and udders (57.6%)(Table 6.3). Although more than half of the nymphs feeding on the scrota and udders were parasitized, it is not clear from this study why nymphs feeding at these two sites (which are in the same location - between the hind legs) were less parasitized than the other feeding sites. On the other hand, the parasitization rate for nymphs collected from the bellies was significantly higher (Table 6.3).

The parasitized nymphs collected from the scrota of cattle in Kuja River (Table 6.2) support the finding in Chapter 4 that although tick-free scrota did not attract the parasitoids significantly in the Y-tube olfactometer, odours from tick-infested scrota attract the parasitoids leading to the parasitization of nymphs.

The absence of *A. variegatum* nymphs from the ears of cattle inspected for the collection of nymphs (Tables 6.2 & 6.3). supports the observed lack of attraction of the parasitoids to ear odours in the Y-tube olfactometer (Chapter 4).

Furthermore, the parasitization rates of the partially fed and close-to-repletion nymphs collected from Kuja River between the 23rd and 27th of January 1997 were significantly different from that of the unfed nymphs collected (Table 6.4). Although the parasitization rate for close-to-repletion nymphs was slightly higher than that of the partially fed nymphs, the two rates were not significantly different (Table 6.4). The observed general trend was that the parasitization rates of the various feeding states (unfed, partially fed and close-to-repletion) of the nymphs collected from Kuja River increased with increased blood feeding (January collections, Table 6.4). Mwangi *et al.* (1994) also found that parasitization rates in unfed *A. variegatum* nymphs collected from cattle were lower than in the engorged nymphs collected from the same animals.

These findings could be due to the different number of days the various nymphs (unfed, partially fed and close-to-repletion) were present on the cattle. Since the feeding cycle (unfed to repletion) of *A. variegatum* nymphs lasts about six days, the close-to-repletion nymphs could have been on cattle for about 5-6 days, partially fed nymphs for about 2-4 days and unfed (recently attached) nymphs for about 1-2 days. Thus, since this study has shown that nymphs are located and parasitized while on the cattle (Chapter 4), the close-to-repletion nymphs were on the cattle long enough to be located by a female parasitoid. The unfed nymphs were on cattle for a shorter time, hence parasitization of these nymphs was not yet intense. However, it can be expected that the parasitization rates of these nymphs will increase the longer they stay on the cattle.

The variation in parasitization rates of close-to-repletion nymphs collected from Kuja River in January 1997 and the rates of the nymphs collected in September 1995 (Table 6.2) could suggest a possible seasonal or monthly fluctuation in the parasitization of these nymphs by *I. hookeri* in the field. In Kenya, September is the transition from the cool dry season to the warm wet season. January is usually a warm month signalling the beginning of the long rains which usually last up to May. Thus, the tick load on cattle and the subsequent parasitization of these ticks by *I. hookeri* can be expected to fluctuate with changing temperature and moisture (soil and atmospheric) levels as both the ticks and the parasitoids will be affected by these changes. After observing similar variations in the number of nymphs collected from various locations at different times, Stafford *et al.*, (1996) suggested that seasonality in the parasitization of these nymphs by *I. hookeri* could account for these variations. Stafford *et al.* (1996) found a lower parasitization rate in host-seeking off-host nymphs parasitized by *I. hookeri* and collected in July 1994 compared to the parasitization rates of nymphs collected in early June 1993.

However, the observed parasitization rates could also be due to the low numbers of close-to-repletion nymphs collected. Close-to-repletion nymphs engorge rapidly, detach and drop from cattle, hence their low numbers compared

to unfed and partially fed nymphs collected during this study.

The observed low parasitization rates of recently attached unfed nymphs support the finding in Chapter 4 that grass odours were not attractive to the parasitoids. Mwangi *et al.* (1994) did not find any parasitization in the 274 *A. variegatum* nymphs they collected from grasses and subsequently fed on rabbits (196 of these ticks fed to repletion). On the other hand, the finding that host-seeking nymphs collected from the vegetation by Stafford *et al.* (1996) were parasitized suggests that the Kenyan and Connecticut *I. hookeri* parasitoids could be different strains as mentioned in Chapter 4.

The finding that *A. variegatum* nymphs feeding at all sites were parasitized indicates that *I. hookeri* females search all the feeding sites of *A. variegatum* on cattle as suggested in Chapter 4. No *A. variegatum* nymphs were collected from the ears (predilection sites of *R. appendiculatus* ticks) of cattle (Tables 6.2 & 6.3).

On the other hand, the observed field parasitization rates might not truly reflect the host finding success of *I. hookeri* females due to superparasitism (Chapter 5), since each parasitized nymph, regardless of possible superparasitism was recorded as one unit. As pointed out by van Alphen & Jervis (1996) more *I. hookeri* females could have located *A. variegatum* nymphs but some might have located parasitized nymphs which they superparasitized due to the scarcity of unparasitized hosts or for the advantages of superparasitism pointed out in Chapters 2 & 5.

Field observations on the host seeking behaviour of *I. hookeri* females revealed that the females ran up and down the grass blades and groomed themselves before jumping or flying off (Table 6.5).

The distances (50cm, 100cm, 200cm & 300cm) at which the parasitoids were released from the calves in the field did not influence the percentages of nymphs reaching the calves significantly (Table 6.6). This finding suggests that parasitoids foraging for hosts after emerging 300cm from cattle could be just as successful in locating the cattle and the tick hosts feeding on them as parasitoids

emerging 50cm away from cattle. The potential of *I. hookeri* as an efficient forager for host ticks is highlighted by the above finding although the parasitoids appear to be weak fliers under laboratory conditions. The dispersal capacity of *I. hookeri* over longer distances still need to be established in the field to get a clearer picture of the dispersal potential of these parasitoids.

Furthermore, the distances at which the parasitoids were released from the calves also did not influence the percentages of female parasitoids dispersing within 10 minutes from the release site significantly (Table 6.4). This finding could suggest that the dispersal of the parasitoids from the release or emergence site is not influenced by the proximity of the host or the host habitat. On the other hand, the proportion of the parasitoids remaining at the release site after one hour is also not influenced by the proximity of the host or host habitat (Table 6.6). These results suggest that some females have a higher urge to look for hosts or food than others and that some females are not in a hurry or in a position to look for hosts or food. This could be due to the physiological state of these females (e.g lower egg-load, virginity etc.), environmental conditions (e.g. temperature, humidity, windspeed etc.) or some other factors. It is known that egg-load (number of eggs in a female) is one of the factors prompting female parasitoids to search for hosts (Potting, 1996). It could be possible that although males were present at the emergence site, some of the females were not mated. Forsse *et al.* (1992) suggested that virgin *Trichogramma minutum* females are less likely to fly and disperse. However, Parra *et al.* (1996) feel that the suggestion by Forsse *et al.* (1992) may depend on the mating strategies of the parasitoid species in question.

In the wind tunnel, the parasitoids spent some time on the platform (vial) before responding to the calf odours presented (Table 6.8). On the platform, the parasitoids exhibited the same behaviour mentioned in Table 6.5. After leaving the platform, the parasitoids spent additional time sitting on the tunnel floor. This observation is consistent with the observed similar response times (time between the introduction of the parasitoids in the Y-tube and the crossing of the finishing

line) of tests and controls as reported in Chapter 4. Although the parasitoids tested in the wind tunnel were conditioned with calf odours before the test, they still took some time before moving towards the target. Since the wind tunnel experiments were designed to shed light on the dispersal behaviour of the parasitoids and were not bioassays to test for the attractiveness of odours like the Y and T-tube bioassays, the parasitoids were conditioned. Conditioning with test odours has been shown to give improved responses in other parasitoids. For example, conditioned (experienced) *Habrobracon hebetor* (parasitoids of several pyralids) responded better in the wind tunnel to odours from the host, *Ephestia kuehniella* as compared to naive parasitoids (Parra *et al.*, 1996).

When released 50cm away from the odour source in the wind tunnel, the parasitoids covered most of the distance through flight, while jumping was used to cover most of the distance when the parasitoids were released 100cm away from the odour source. This could suggest that the distance of the these weak flying parasitoids from the odour source determines the mode of dispersal employed by the parasitoid. Although flight was short-lived (4.5 seconds), at both 50cm and 100cm, the parasitoids covered long distances in the wind tunnel (Table 6.8). Jumping was frequent both in the field and the wind tunnel.

The commonly used mode of dispersal in the wind tunnel was jumping at both 50cm and 100cm (Table 6.9). During field observations, parasitoids used jumping to escape from predatory ants (*Monomorium* sp., Appendix 1) attacking parasitoids sitting on grass blades. The second most commonly used mode of dispersal was running followed by flight. More than half of the parasitoids released reached the target at both 50cm and 100cm. The percentages of parasitoids which came within 20cm of the target were significantly more than those reaching the target (Table 6.9). According to Hendry *et al.* (1973), from 20cm onwards, short-range cues like feeding *A. variegatum* faeces acting over a distance of 2-20cm from the nymphs should be more attractive than the long-range attractants like calf front heel and urine swabs. However, in spite of the absence of any short-range cues from *A. variegatum* nymphs, more than half of

the parasitoids released still reached the targets from both 50cm and 100cm, implying that *I. hookeri* females can be attracted to cattle even in the absence of *A. variegatum* nymphs as mentioned in Chapter 4.

CHAPTER 7: GENERAL DISCUSSION

This study has shown that the host selection strategy of *I. hookeri* females are similar to those of the parasitoids of herbivorous pests as outlined in Chapter 2. Just as plant odours play an important role as long-range attractants for parasitoids of herbivorous pests, this study indicated that cattle odours play an important role as long-range attractants. Grass (plant) odours, however, were not attractive to *I. hookeri* females as these parasitoids parasitize *A. variegatum* nymphs feeding on cattle and not the questing unfed ticks on the grasses as shown by this study and by Mwangi *et al.* (1994). Unfed nymphs parasitized in the laboratory died rapidly with mortality starting on the fifth day after the attack (Chapter 5), while unparasitized unfed nymphs lived for more than six months under the same conditions. The specifity of the Kenyan *I. hookeri* strain to *A. variegatum* nymphs, the short lifespan of *I. hookeri* and the long period unparasitized *A. variegatum* nymphs survive without taking a blood meal suggest that the parasitoid populations in the field can decline while *A. variegatum* nymphs are still abundant in an area if the nymphs stay off-host due to acaricidal treatment of cattle or other factors. The host specificity of *I. hookeri* suggests that the parasitoids cannot be sustained by any other tick species feeding on cattle when *A. variegatum* nymphs are not present on cattle. The pick-up rate of ticks in a paddock by cattle is complex (Hassan, pers. comm.). Although many ticks may be present in an area, only some of them may feed on animals. During this study, not all the nymphs applied to rabbit ears fed. Furthermore, in Kenya, *I. hookeri* is absent from areas where acaricides have been used extensively by farmers. The parasitoid colonies in such areas could have died out due to contact with the acaricides while searching for hosts and/or the absence of on-host ticks which could sustain the development of parasitoid immatures. Pesticide-resistant parasitoid strains need to be reared to be more compatible with other methods of IPM (Potting, 1996)

Findings of this study suggest that cattle waste odours (dung and urine) may help in the initial orientation of the parasitoids towards cattle while odours from the feeding sites of *A. variegatum* ticks on cattle (dewlap, belly, heels, scrotum & udder) may help in 'mapping out' the areas to be searched to keep the parasitoids from searching less rewarding areas such as the ear which is the predilection site of *R. appendiculatus* ticks (non hosts). The mapping out of the micro-habitats (feeding sites) of *A. variegatum* nymphs on cattle saves the parasitoids time and energy since cattle body surfaces are huge relative to the size of the parasitoids. Searching the entire body would require the parasitoids to expend much energy and time which the parasitoids do not have due to their short lifespan (live for 1-2 days in the laboratory). However, the finding that the parasitoids respond to tick-free cattle odours show that naive *I. hookeri* parasitoids still waste time and energy. It can be expected that with increasing experience with hosts, this wastage will be reduced.

The role cattle odours play in the host location process of *I. hookeri* suggests that there might also be a close association between the parasitoids and cattle and not between the parasitoids and *A. variegatum* nymphs only. Stafford *et al.* (1996) found that more *I. scapularis* nymphs collected from white-footed mice were parasitized by *I. hookeri* compared to those collected from white-tailed deer. However, since the parasitoid occurs naturally in Kenya, it could have survived for this long on ticks feeding on various hosts. Work has begun on the evaluation of parasitization rates in ticks feeding on wildlife in Kenya (E. Mwangi, pers comm.).

In contrast to the Connecticut strain which parasitizes both off-host and on-host *I. scapularis* nymphs, the Kenyan strain of *I. hookeri* parasitizes on-host *A. variegatum* nymphs only. However, the eggs of the Connecticut strain overwinter in *I. scapularis* nymphs and develop only after the nymphs had fed on vertebrates implying that *I. scapularis* nymphs are still capable of transmitting diseases to cattle before being killed by the parasitoids. So far, the control of off-host nymphs relies on methods which destroy the vegetation the ticks find

themselves in between meals. These methods include bush canopy clearance and the subsequent exposure of the soil to sunlight and burning of paddocks. However, due to the problems mentioned earlier and the shortage of adequate grazing for livestock in arid regions of Africa, the destruction of available grazing is not an option for small-scale resource-poor farmers.

Off-host *A. variegatum* nymphs are potential reservoirs of tick-borne diseases and if these ticks prefer to feed on wildlife in areas were cattle share pastures with wildlife, the tick numbers and the transmission of heartwater in such areas will be difficult to contain using the parasitoid, if nymphs feeding on wildlife are not parasitized at similar or higher rates than those feeding on cattle.

Furthermore, this study showed that the presence of ticks actively feeding on cattle boosts the attractiveness of cattle to the parasitoids. Unfortunately, these nymphs have to feed on cattle first, transmitting diseases before they are parasitized by *I. hookeri*. Nymphs or adult ticks transmit *C. ruminantium* to susceptible hosts without losing the infection (Bezuidenhout *et al.*, 1994). Newly hatched larvae do usually not transmit heartwater as transovarial transmission (from the female to her eggs - from which the larvae hatch) is probably rare in nature (Bezuidenhout & Jacobsz, 1986). *I. hookeri* can decrease the transmission of heartwater by feeding nymphs as follows. If *I. hookeri* parasitizes an uninfected nymph (not carrying *Cowdria ruminantium*) feeding on an infected animal, this nymph will not get a chance to feed on another healthy animal to transmit the disease since it will die before moulting into an adult. Hence the parasitoids eliminate the chances of such a nymph transmitting heartwater as well as the numerous offspring such a nymph could have if it had moulted into a female.

In Y-tube olfactometer bioassays, field releases and wind tunnel experiments, the parasitoids sat for some time after introduction or release, probably to orientate themselves, before responding to calf odours. This was contrary to the expectations that these 'time-tight' parasitoids would start looking for hosts, the moment they get a chance to do so.

However, once they started to respond in Y-tube olfactometer bioassays, *I. hookeri* females searched for hosts and not just for attractive odours as shown by the lack of significant retention of these parasitoids in attractive host odours as compared to their retention in control air. This apparent purposefulness in the search for hosts reflects the 'time-tight' host searching schedule of the parasitoids with more than host odours needed to retain the females in a particular area.

Furthermore, during field dispersal behaviour studies, it was found that some females were still at the release site at the end of the observation period (1 hour). This observation suggests that not all females look for hosts upon emergence, perhaps due to their physiological state, environmental conditions or some other unknown factors. It was suggested that *Trichogramma minutum* virgin females are less likely to fly and disperse than mated ones (Forsse *et al.*, 1992). Ants (*Monomorium* sp.) were seen attacking and carrying away parasitoids which were slow to disperse from the release site. It is likely that parasitoids are pre-disposed to predation if they do not disperse quickly after emergence.

Moreover, tortoises, ants, spiders, lizards, rodents and shrews are known to prey on ticks (reviewed by Mwangi *et al.*, 1991) and may indirectly influence the parasitoid population dynamics through devouring parasitized ticks. Future studies into the impact of the predation of *I. hookeri* by predatory ants as well as the predation of parasitized ticks on the parasitoid population dynamics are needed.

The finding that the parasitoids utilize visual cues in close-range host evaluation and recognition is advantageous to biocontrol in that the parasitoids can make decisions at a distance without contacting potentially aggressive non-hosts. Visual cues help the parasitoids to differentiate between sizes, feeding status (replete or unfed) and the species of the host. All these parameters play an important role in parasitoid fitness, hence the female has to make crucial decisions concerning a host before laying eggs (Godfray, 1994).

The ability of the parasitoids to discriminate visually between hosts shows that the parasitoids optimize on fitness by choosing good quality hosts which will sustain the development of the immatures. That could be how *I. hookeri* survived all these years. The choice of good quality and the subsequent laying of eggs in them is good for the biological control of ticks if such control relies on the natural parasitoid population alone since the numbers of parasitoids in the field will not dwindle as long as good quality hosts are present on the cattle. Visually detected qualities of the hosts helps with some very important decisions in parasitoid females, for example whether to lay eggs, how many eggs to lay and what sex the offspring should be (Godfray, 1994).

Visual discrimination by the parasitoids, especially between *A. variegatum* and *R. appendiculatus* ticks show that the parasitoids can avoid accidental oviposition in non-hosts by being able to reject these hosts at a distance. Since *R. appendiculatus* ticks are unsuitable hosts, the lack of attraction of the parasitoids to *R. appendiculatus* washes and faeces as well as the reluctance of the parasitoids to oviposit in these nymphs is clearly advantageous for the thriving of the parasitoid population in the field. So far the Kenyan *I. hookeri* strain has been found to be naturally specific to *A. variegatum* nymphs and to discriminate against nymphs of other *Amblyomma* ticks (E. Mwangi, pers. comm.). The association between *I. hookeri* and *A. variegatum* ticks seems not to be limited to chemical information the parasitoids perceive from the body surface of the tick or other host-related products like faeces only. Upon the insertion of the ovipositor, the parasitoids get additional information on the internal state of the host before eggs are laid.

This study found that *R. appendiculatus* nymphs conditioned with *A. variegatum* odours and integument and subsequently attacked by the parasitoids did not yield any progeny although eggs were recovered from a few nymphs. *R. appendiculatus* ticks are believed to have a strong immune system which kills off the parasitoid eggs through encapsulation (Bengaly, pers. comm.), and cannot be controlled by *I. hookeri*.

Thus, this study demonstrated that the application of *A. variegatum* odours on *R. appendiculatus* nymphs do not lead to the successful parasitization of these non-host nymphs by *I. hookeri* parasitoids although they did increase the percentage oviposition. However, *A. variegatum* nymphal washes need to be evaluated for their role in boosting the efficiency of parasitoids in the field through their application to *A. variegatum* infested cattle. Devices permitting the slow release of concentrated *A. variegatum* nymphal washes can be attached to *A. variegatum* feeding sites on cattle in order to attract the parasitoids to on-host nymphs. The finding by Sonenshine *et al.* (1985) that sex pheromone-impregnated microcapsules and a pesticide applied on the fur of dogs could confuse and kill mate-seeking males before they could inseminate female ticks, shows that tick infochemicals can work in the field.

It has been shown that attractive plant extracts can improve the parasitization of *Helicoverpa zea* and *Anagasta kuehniella* by *Trichogramma* wasps under soyabean and greenhouse conditions (Altieri *et al.*, 1981; Altieri & Letourneau, 1982; Altieri *et al.*, 1993). Cuticular components (obtained through washes) are species-specific and have been used to solve a variety of taxonomic problems in insects (Phillips *et al.*, 1988) and ticks (Hunt, 1986; Estrada-Peña *et al.*, 1992; 1994a,b). Thus, the specificity of *I. hookeri* parasitoids to *A. variegatum* ticks could be governed by specific cuticular components of these ticks. It was demonstrated that host acceptance in *Brachymeria intermedia*, a parasitoid of lepidopterous pupae, is mediated by a cuticular kairomone (Tucker & Leonard, 1977). Visual inspection of chromatograms of *A. variegatum* and *R. appendiculatus* nymphal washes revealed differences in the chemical concentration and composition of substances washed from the bodies of these tick species. Further analyses of these washes may reveal the components responsible for the special relationship between *A. variegatum* and *I. hookeri*.

Although movement of the host detected visually by the parasitoid frequently guide parasitoids in the final stage of host location (Monteith, 1956, 1963), movement of *A. variegatum* nymphs is not a prerequisite for the

recognition of these nymphs by *I. hookeri* parasitoids. This could be due to the sessile habit of feeding *A. variegatum* ticks. On the other hand, the insignificant role movement of hosts play in host recognition and acceptance lead to the probing of mummies in the laboratory. This can be very costly to the parasitoids in terms of time and energy.

Furthermore, the parasitoids will not gain in fitness by wasting time and energy on mummies. This study further demonstrated that colour was just as unimportant as movement in host recognition. If naive female parasitoids spent time at the oviposition site trying to oviposit in mummies in the field it will take some time out of their 'tight' time schedule (1-2 days) in which they have to locate hosts.

Tick-free front heel odours were the most attractive of all the odours from the feeding sites of *A. variegatum* on cattle. This attraction of the parasitoids to front heel odours explains why most of the *A. variegatum* nymphs on free ranging cattle were found on the heels during field collections. Furthermore, the field collections of on-host *A. variegatum* nymphs showed that the front legs were the topmost parasitized body regions.

Interdigital glands (located between the hooves) are found in most species of deer (Cervidae), antelope (Bovidae) and pronghorns (Antilocapridae)(Wood *et al.*, 1995). Secretions of these glands contaminate the heels and have been found to arrest host-seeking *I. scapularis* ticks (Caroll *et al.*, 1995). A similar arrestment mechanism from cattle heels may be responsible for the feeding of the majority of *A. variegatum* nymphs on cattle heels. The same mechanism may also be responsible for the attraction of the parasitoids to cattle heels.

Scanning electron microscopy of the integument surface textures of unfed *A. variegatum* and *R. appendiculatus* nymphs also revealed differences in the texture of the scuta, length of the grooves separating the festoons, number of festoons, lengths of palps, shapes of bases capituli and the distribution of setae on the integument surfaces. These physical differences together with chemical ones could mediate in the discrimination between *A. variegatum* and

R. appendiculatus nymphs by the parasitoid after external examination of these ticks while the internal states of these ticks can lead to the abortion of egg-laying after the insertion of the ovipositor into the nymphs. Parasitoid(s) of *R. appendiculatus* ticks need to be identified in areas where *I. hookeri* suppresses *A. variegatum* tick populations.

Although conspecific superparasitism occurs frequently in *I. hookeri*, females can discriminate between parasitized and unparasitized nymphs. This discrimination could be due to marking pheromones left by the former female and/or the sensing of the eggs of conspecifics or the changed internal state of the nymph upon the insertion of the ovipositor (Salt, 1937; Lloyd, 1939; Wylie, 1965). Superparasitizing *I. hookeri* females spent less time ovipositing in parasitized hosts, probably laying less eggs and hence avoiding the overloading of the nymphs with more eggs than the food resources inside the nymphs allow. By overloading the nymphs, the parasitoids risk losing their offspring since parasitoid immatures die if food resources are used up before development is complete (Godfray, 1994).

The observed discrimination between parasitized and unparasitized hosts by *I. hookeri* females suggests that parasitoids can 'sense' the amount of food reserves available for their offspring in parasitized host and adjust their clutches accordingly. Furthermore, a female parasitoid superparasitizing a nymph formerly parasitized by a female with few eggs (due to being born with fewer eggs or previous parasitization of another host) will need to lay more eggs in such a nymph since about eight eggs are needed for the successful development of parasitoid eggs in nymphs according to Mwangi & Wabwoba (unpublished). Since the distribution of *A. variegatum* nymphs on cattle is patchy and seasonal, superparasitism may be an adaptive strategy for *I. hookeri*.

Unlike aphids which kick violently when attacked (Kouamé & Mackauer, 1991), or many lepidopterans which deliberately jump off plants but remain attached by a silk thread (Yeargan & Braman, 1986), no active defense or avoidance behaviour was mounted by *A. variegatum* nymphs attacked by

I. hookeri females in the laboratory. Unfed nymphs became restless and ran around, while replete nymphs could only walk about. Unlike unfed nymphs, attached nymphs could not run to avoid parasitoid attacks due to their sessile habit while feeding. When lying on their backs nymphs were seen waving their legs but this did not keep the parasitoids from ovipositing. On the other hand, mechanically disturbed *A. variegatum* nymphs (legs twisted with forceps) produce an allomone (repellent) from dermal glands (Pavis, *et al.*, 1994). During this study, nymphs attacked by the parasitoids did not produce visible secretions like those handled with forceps. This study demonstrated that this allomone repels *I. hookeri* parasitoids, however, the effect of this allomone is short-lived (active for about 10 minutes). Moreover, these allomones are produced after repletion only (Pavis *et al.*, 1994) and thus will not hamper the parasitization of feeding nymphs by the parasitoids.

This study also revealed that unfed *A. variegatum* nymphs (not fed after parasitization), just like *R. appendiculatus* nymphs were not suitable for the development of *I. hookeri* immatures. The difference was, however, that 90% of parasitized unfed *A. variegatum* nymphs died within 30 days while all replete *R. appendiculatus* nymphs parasitized moulted and were all alive after 30 days. The importance of a blood meal for the development of the parasitoid immatures was demonstrated by the failure of unfed *A. variegatum* nymphs to yield progeny, while nymphs parasitized after repletion yielded the highest number of progeny (72.3 parasitoids/female).

Almost half (43.3%) of the nymphs parasitized before feeding died before engorgement. Furthermore, those which engorged yielded a significantly lower number of progeny (5.8 parasitoids/female) as compared to nymphs parasitized after repletion. Similar findings were made by Graf (1979) on the effects of an undescribed tick parasitoid on *A. nuttalli* in the Ivory Coast. Although *I. hookeri* eggs were reported to overwinter in moulting *Dermacentor andersoni* larvae and started to develop only after the nymphs had engorged in the following spring in the United States (Cooley & Kohls, 1934), this study and the study by Graf

(1979) have shown that the laying of *I. hookeri* eggs in unfed *A. variegatum* nymphs affects and kills the majority of these nymphs before they get a chance to feed.

Parasitization of questing unfed nymphs in the vegetation would mean a decline in the population numbers of the parasitoids in the field, but since this study has demonstrated that *I. hookeri* targets on-host feeding nymphs, that risk is excluded. Fluctuations in population numbers may still occur due to heavy rains, strong winds or seasonal abundance of the ticks etc. There might also be a seasonal or monthly fluctuation in the parasitization rates of *A. variegatum* nymphs in the field. However, the findings on the field parasitization rates in this study are not conclusive, since collections were not done long enough on a monthly or seasonal basis, as this was not one of the objectives of this study. Nevertheless, future studies may concentrate on the possible changes in field parasitization rates of *A. variegatum* nymphs as populations of these ticks fluctuate seasonally or monthly. From the January field collections it was also apparent that the percentages of parasitized nymphs increased with increased blood feeding. Close-to-repletion nymphs were parasitized the most since they were on the cattle much longer than partially engorged nymphs (second most parasitized) and recently attached unfed nymphs (least parasitized). These findings suggest that the parasitoids parasitize all physiological stages (unfed, partially fed or close-to-repletion) of *A. variegatum* nymphs present on cattle and are consistent with findings by Mwangi *et al.* (1994).

Due to superparasitism, field parasitization rates do not truly reflect the host location ability of parasitoids (van Alphen & Jervis, 1996). For example, if 66.7% of nymphs collected from the front legs are parasitized, it does not mean that only 66.7% of the parasitoids in the field locate nymphs feeding on the front legs of cattle. More parasitoids (e.g. 80%) could have located these nymphs and superparasitized them. Therefore, ecological studies will be needed to observe the actual numbers of parasitoids locating unparasitized nymphs and those superparasitizing in order to find out the real efficiency with which

I. hookeri females locate hosts.

In both the field releases and wind tunnel experiments, flight was rarely observed in *I. hookeri* females. Relative humidity, temperature and wind could have been responsible for this. The experiments were conducted at 65-90% r.h. and 24-32°C. Furthermore, strong afternoon winds were experienced in the field release site. Wind velocity is known to affect insect flight (Cooter, 1982). However, the parasitoids also did not fly frequently during morning hours when the wind was absent. The main factor affecting the flight activity in Nigerian blackflies is the circadian change in light intensities (Roberts & Irving-Bell (1996). Adults of the aphid parasitoid, *Praon abjectum*, often fly and disperse under favourable weather conditions, but otherwise prefer to run under less favourable conditions (Stary, 1970). This observation could also be due to the adaptation of the parasitoids to the dispersal behaviour of the ticks, which do not fly themselves, as it is known that the dispersal range of a parasitoid depends on the dispersal of its host. Stary (1970) found that aphid parasitoids which parasitize earlier wingless instars do not need to disperse as far as those which attack winged instars which are capable of long-range dispersal.

It seems as if the distribution of *I. hookeri* is much narrower than that of the host, since *I. hookeri* is not found in some parts of Kenya where *A. variegatum* nymphs exist, although cattle movement occurs due to cattle trade and dowry exchanges (E. Mwangi, pers. comm.). If cattle are sold or exchanged from an area, like Kuja River, where the parasitoid occurs naturally, these cattle are expected to carry some parasitized nymphs. The apparent failure of *I. hookeri* to establish in some areas could be due to environmental conditions. Studies are needed to find the optimum conditions for the survival of these parasitoids since the utilization of these parasitoids in broader biological control programmes will need the introduction of the parasitoids in areas where they do not exist.

Another behaviour exhibited by the parasitoids and which could also be an adaptation to the behaviour of ticks is the crawling of most (53.7%) of the parasitoids up the legs of calves. Questing ticks also crawl up the legs and heads of grazing animals. Crawling up the legs brought the parasitoids in contact with the heels, the importance of which has been stressed already.

Although significantly less time was spent on flight in the wind tunnel as compared to times spent on jumping and running, most of the distance was covered through flight (although distances covered through flight and jumping were not significantly different at 100cm), suggesting short-lived but relatively long flights. In both the field and the wind tunnel experiments, the distance at which the parasitoids were released did not influence the numbers of parasitoids reaching the calves (field) or the calf heel and urine swabs (wind tunnel). The most common mode of dispersal in the wind tunnel was jumping, followed by running and finally flying.

More than 75% of the females released in the wind tunnel came within 20cm of the target. This observation shows how efficient the parasitoids utilize calf odours in long distance location of the host habitat. Although short-range cues (acting over a distance of 2-20cm; Hendry *et al.* 1973), like feeding nymphal *A. variegatum* faeces were not present in the wind tunnel, more than half of the parasitoids reached the target, suggesting that the parasitoids can be attracted to cattle regardless of the presence of feeding nymphs.

7.1. Importance of the findings of this study

The findings of this study fill a very important void in the understanding of the host selection process of *I. hookeri* parasitoids, especially host habitat location, host location, host acceptance and suitability. Moreover, this is the first ever report on the host finding behaviour of a tick parasitoid. Information from this study is expected to provide crucial baseline information in the planning of biological tick control programmes involving *I. hookeri*.

The importance of cattle odours as the habitat of *A. variegatum* nymphs has been highlighted. Schmidtmann (1994) suggested that the failure of Larrousse's attempted control of *Dermacentor variabilis* ticks with *I. hookeri* might have been due to the host searching behaviour favouring the location of immature *Ixodes* ticks on white-footed mice, *Peromyscus leucopus*, that frequent woodlands, rather than immature *D. variabilis* on meadow voles in grasslands. This suggestion is based on reports indicating that 27% of host-seeking nymphal *I. dammini* (not *D. variabilis* which was targetted) on Naushon Island, near the release site of Larrousse in 1926, were infected with *I. hookeri* (Mather *et al.*, 1987a). The findings of this study and the suggestion by Schmidtmann (1994) stress the importance of host habitat odours in the location of the ticks, since odours released by the ticks are short-range cues which are difficult to detect at long-range.

The findings of this study further stressed the host specificity of *I. hookeri* which seems to be mediated by visual as well as chemotactile cues. These findings might generate mixed feelings in farmers. Initially, the farmers will be happy to hear that the parasitoids are not harmful to their livestock or any other organism which is not a tick, however, when farmers realize that the parasitoid can only parasitize a single tick species, they might not be impressed, especially in areas where livestock are infested with multiple tick species. Whenever *A. variegatum* and *R. appendiculatus* ticks occur on the same animal, like in Kuja River and Trans-Mara, Kenya, *R. appendiculatus* ticks can be controlled through acaricide-impregnated ear tags while *I. hookeri* controls *A. variegatum* ticks. This help from the parasitoids will cut down the expenditure of the farmer on acaricides since whole body treatments of cattle with acaricides will no longer be necessary.

This study also showed that the parasitoids do not employ flying very often for dispersal. Although this could be because of the environmental conditions at the time of the studies, this finding suggests that *I. hookeri* does not disperse very far.

This finding will have serious implications for the biological control programme whenever the farmers in an area where these parasitoids occur decide to dip their cattle. As the parasitoids seem not to be able to disperse far, they will be killed off by the acaricides whenever they search for hosts on cattle. This is shown by the absence of the *I. hookeri* in areas where farmers use acaricides frequently (E. Mwangi, pers. comm.). It would be interesting, however, to see whether the parasitoids also migrate to wildlife together with the host ticks since these ticks have been found to feed on wildlife, such as buffaloes, whenever cattle were treated with acaricides (Norval *et al.*, 1994). Sublethal concentrations of insecticides have been found to alter the behaviour patterns of parasitoids and predators (Haynes, 1988; Elzen, 1989; Croft, 1990). For example, when *Aphidius rhopalosiphi* reduced its search time on insecticide-treated plants (Longley & Jepson, 1996). If *I. hookeri* females reduce their search time on acaricide-treated cattle it will grossly reduce their efficiency as biocontrol agents while increasing their chances of survival. However, if this 'time-tight' insects do not get acaricide-free cattle within a day it will lead to a decline and the subsequent elimination of the parasitoid population in that area.

In conclusion, it is apparent from field collections that *I. hookeri* does not parasitize all *A. variegatum* nymphs found on cattle. The residual population of unparasitized nymphs on cattle and in grasses is important for the existence of the parasitoid population in the field. This residual tick population is also important in bringing about endemic stability in cattle vaccinated against heartwater (D.T. de Waal, pers. comm.). The challenge from feeding pathogen-carrying ticks will manifest longer lasting resistance in vaccinated cattle compared to cattle vaccinated in the absence of a tick challenge (D.T. de Waal, pers. comm.). Norval *et al.* (1992c, 1994) argue that the survival of some ticks is important in certain geographic regions for the maintenance of endemic stability of bovine diseases, and that tick control in conjunction with the maintenance of endemic stability is a more realistic objective than total eradication of tick-borne diseases of livestock in Africa.

7.2. Suggestions for further studies

1. Investigate the possible amplification and use of *A. variegatum* odours to boost the location of these nymphs by *I. hookeri*
2. Carry out field studies on the effects of environmental factors on the survival of *I. hookeri*
3. Investigate the impact of predators, such as ants, on the field population dynamics of *I. hookeri*
4. Determine the effects of superparasitism on sex ratios, size and the host location ability of *I. hookeri*
5. Study the parasitization rates of *A. variegatum* nymphs feeding on wildlife. Close cooperation between the ministry responsible for wildlife and the researcher will be required to enable him to collect ticks from animals darted or killed by officials or from animals in sanctuaries. The rates obtained before and after cattle dipping in areas where wildlife and livestock share grazing should be compared to determine whether the parasitoids prefer wildlife over cattle during acaricidal treatment of cattle.

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APPENDIX I

Ants (*Monomorium* sp.) found attacking *I. hookeri* at Mbita Point Field Station, Kenya

APPENDIX 2

Map of Kenya showing the field study sites