SEMIOCHEMICAL – A NOVEL THOUGHT FOR TICK CONTROL

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Ticks and tick borne diseases pose enormous problems to man and domestic animals. The impact of ticks and tick-borne diseases on the livelihood of resource poor farming communities have been ranked high (Perry *et al*., 2002). These problems are closely associated with domestic animals and pets in the tropical and subtropical regions of the world. The direct and indirect effects of ticks are either through their role as vectors of disease or by their feeding activities. Direct losses include reduced weight gain, damaged hides, reduced milk production, loss due to tick toxicosis and tick paralysis (Graf *et al*., 2004). Indirect losses are due to the vector potentiality of the tick. In India, the economic losses due to tick and tick-borne diseases are estimated to be US$ 498.7 million per annum (Ghosh *et al*., 2007) with a global annual loss of $109 billion (Jabbar *et al*., 2007).

Currently the mainstay of tick control measure relies on the use of chemical acaricides. However the use of acaricides is often accompanied by serious drawbacks such as chemical pollution of the food chain and environment (Dipeolu and Ndungu, 1991), apart from the worrisome selection of acaricide resistant ticks. The rapid development of tick resistance to new compounds discourages efforts to discover new acaricides due to high cost of research, development and registration of new drugs. Effective control is possible only when acaricides are applied constantly and this makes the method labour intensive and very costly. Failure to continue the strict application of acaricides results in a rapid propagation of tick populations (Imamura *et al*., 2007). Biological control becomes impractical due to less number of natural enemies for ticks. The development of vaccines against tick infestations although offers a cost effective environmentally sound control strategy, multiantigenic vaccines are needed to target a broad range of species and to prevent the transmission of pathogens. These limitations point to an urgent need for novel tick control measure to reduce or to replace the use of acaricides, especially in the regions where extensive tick resistance has occurred.

One of the new approaches proposed to improve tick control is the use of semiochemicals in combination with acaricides to attract and kill ticks.

SEMIOCHEMICALS

In ticks as in most animals, chemical mediators guide behaviour. These information bearing compounds are called semiochemicals. These are secreted external to the body and when recognized will result in a specific behavioural response such as food finding, mate finding, escape and other such behaviour. Semiochemicals are thus chemical signal vehicles of host/tick origin which are secreted into the external environment that mediate tick behaviour. Chemical signalling using semiochemicals remains the dominant form of communication between animals. They are secreted in an ordered hierarchy of specific compounds and are perceived in a precise sequential order to the desired end result. At times a single semiochemical is

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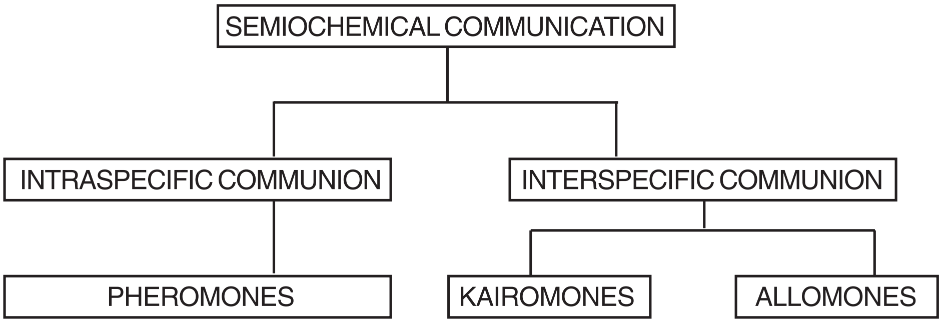
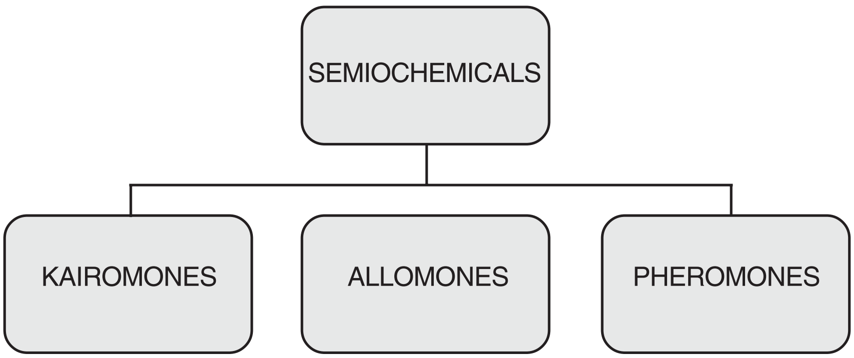
sufficient to have the desired effect e.g. squalene while in other cases two or more compounds mixed in a specific proportion may be required to induce maximum behavioural response e.g. hematin/guanine/xanthine.

Semiochemical communication in nature can be divided based on the type of behaviour they mediate and not based on the compounds that mediate behaviour.as shown below (Regnier, 1970).



These information containing compounds can be classified as follows (Sonenshine, 2004)



One more group of semiochemicals, synommones have been reported by Sonenshine (2006) which evoke a favourable response in both emitter and the receiver and but this group is of less importance in ticks.

1. KAIROMONES

Kairomones are information bearing compounds or mixtures released by individuals of one species, detected by individuals of other species that benefit the recipient (Sonenshine, 2003). Locating a host for blood meal is one of the most formidable challenges for ticks (Sonenshine, 1991). The questing behaviour of ixodid ticks enable for identification and localisation of approaching hosts and these are evoked by chemical cues known as kairomones (Osterkamp *et al.*, 1999). In order to obtain blood for their nutritional needs, ticks need to detect the presence of the host, which is facilitated by kairomones. The most notable host cue is carbon dioxide produced by host respiration which functions predominately as a non-specific, general excitant and can activate ticks from distances as great as 30 meters (Norval *et al.*, 1989). Additional cues that guide ticks to host include water vapour emitted via host respiration and evaporative losses, lactic acid present on skin, mammalian skin lipid, nitrogen containing host excretory- secretory products, ammonia and urea and the volatile fatty acids emanating from ruminants (Yoder and Stevens, 2000).

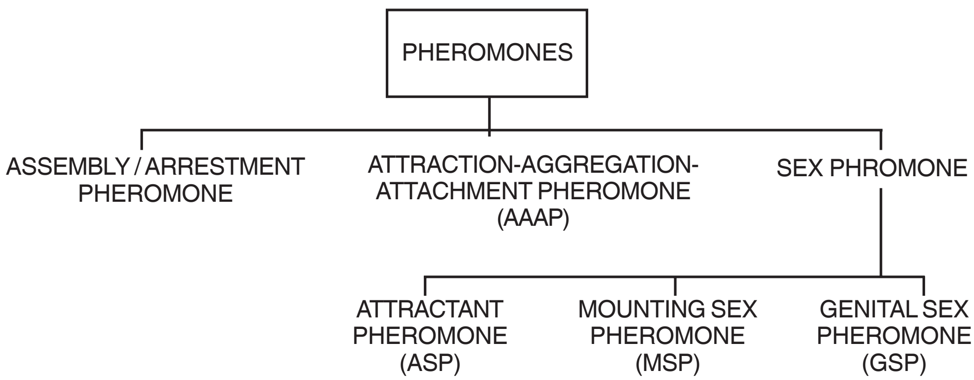
Ruminants regularly eruct gases from the foregut to relieve excess pressure and maintain a chemical equilibrium, which inadvertently signal their presence to hard ticks. Rumen fluid odour is the product of a stable bioreactor whose major chemical constituents do not vary between ruminant species. Behavioural responses were recorded to entrained rumen odour, acetic, propionic, isobutanoic acids, 1-octen-3-ol and several short chain aldehydes (Hall *et al*., 1984; Steullet and Guerin, 1994). Among these the parsimonious use of 1-octen-3-ol was explained as a cue for aggregation with conspecifics and for host finding when in search for a blood meal (McMahon *et al*., 2001). Studies conducted at Madras Veterinary College, Chennai on the larval stages of *Rhipicephalus sanguineus, R.microplus, R.haemaphysaloides, Hyalomma marginatum* and *Haemaphysalis bispinosa* using volatile fatty acids like butyric acid and propionic acid in combination and separately revealed that 32-43 per cent ruminant ticks were attracted to these while *R. sanguineus* larvae did not exhibit any attraction or evince any behavioural response towards the volatile fatty acids. Amongst the ruminant tick larvae tested with 1-octen-3-ol, maximum attraction was seen in *H.marginatum* (72%) and *R.microplus* (71%) while *H.bispinosa* and *R.haemaphysaloides* showed 52 and 50 per cent attraction respectively. The dog tick failed to respond. Five percent carbon dioxide also elicited an attraction of 72 per cent among the partially fed adult ticks of *R.sanguineus.*

2. ALLOMONES

Allomones are information bearing compounds or mixtures emitted by individuals of one species that affect the behaviour of individuals of a different species for the benefit of the emitter (Sonenshine, 2003). This group evoke behavioural or physiological changes in a receiving organism that adaptively favour the emitter e,g., ticks produce hydrocarbons like squalene as defence secretions against ant predators (Regnier, 1970). Ticks lack the ability to synthesise squalene from precursor compounds and so acquire it from their host during blood feeding. Subsequently, the ticks sequester large quantities of this compound in large dermal glands. When a tick is attacked especially by fire ants, it secretes a hydrocarbon based fluid that is rich in squalene. Other hydrocarbons identified in the tick defence secretion include $C_{20,}$ C_{24} and a methyl branched C_{25} alkane. These allomones do not have any obnoxious repellent odour, the avoidance is subtle and it serves to temporarily neutralize predator aggressiveness.

3. PHEROMONES

Pheromones are the best known, intensively studied group of semiochemicals. The need to recruit mates for sexual reproduction is critical to the success of any population of sexual animals. An impressive variety of pheromones are seen in ticks including those used for food finding, arrestment, alarm, nest building and sex pheromones. In ticks the first pheromone discovered was 2, 6-dichlorophenol (2, 6-DCP), a sex pheromone of the lone star tick, *A. americanum*. Different chemicals serve as pheromones ranging from the high volatile molecules like substituted phenols namely methyl salicylate, o-nitrophenol or 2, 6-DCP to cholesteryl esters as non-volatile contact pheromones (Sonenshine, 2004). Pheromones can be classified as follows.



Of the different pheromones identified in ticks, research has favoured use of arrestment / assembly pheromone, Attraction–Aggregation – Attachment pheromones (AAAP) and sex pheromones for tick control (Sonenshine, 2004, 2006).

A. Assembly /Arrestment Pheromones

Arrestment is the cessation of kinetic activity, a response that reduces the distance between individuals that perceive the stimulus in their environment (Carde and Baker, 1984) and leads to clusters of individuals in their natural environment. This pheromone is seen both in argasid and ixodid ticks. Arrestment behaviour is best known in soft ticks and it was first described from *Argus* ticks by Leahy *et al.* (1973). Later this behaviour was reported from 14 species of soft ticks, as well as several hard tick species including *Ixodes ricinus, Hyalomma dromedari* (Leahy *et al*., 1981), *R. appendiculatus, A. cohaerans* (Otieno *et al.*,1985).

Waste products of nitrogen metabolism eliminated in tick excreta, comprise the assembly pheromone in Argasid ticks (Dusbabek *et al.,* 1998). The response to this pheromone was in the form of clustering of ticks in caves, under ledges, cracks and crevices which enhanced the mating and host finding success (Sonenshine, 1985).

In prostriate ticks like *I.ricinus* and *I.scapularis,* such hungry tick clusters were found on vegetations awaiting the passing hosts (Graf, 1974). However no arrestment behaviour was observed in American dog ticks, *Dermacentor variabilis* and *D. andersoni.* The main compound of the pheromone is the purine, guanine, but xanthine, hypoxanthine and uric acid were also identified in the excreta of some ticks (Hamdy 1972; Otieno *et al*., 1985; Dusbabek *et al*., 1991).

The mixture of guanine, xanthine and adenine in 25:1:1 ratio elicited an attractive response ranging from 25-40 per cent in adults to 16 per cent in the nymphal stages of *I.ricinus* ticks. Hematin elicited an attractive response of 26 per cent (Allan *et al*., 2002). Bioassays conducted by Sonenshine *et al.* (2003) revealed a strong, positive arrestment response to cast skins which contained guanine and xanthine and also to black faecal/ excretory exudates containing guanine, adenine xanthine, and the putative 8–azaguanine and hematin. *In vitro* evaluation was made at Madras Veterinary College, Chennai using five ixodid tick larvae and the adults of *R.sanguineus* and *R.microplus* to evaluate the efficacy of assembly pheromone, assembly pheromone-deltamethrin complex and deltamethrin. Maximum attraction was seen in all the ticks except *H.bispinosa* to assembly pheromone. When deltamethrin was used alone it resulted in 18 - 32 per cent death while when it was combined with assembly pheromone it resulted in 90 - 95 per cent death of ruminant tick larvae and 70 per cent death in dog tick larvae after one hour. In adult *R.sanguineus* and *R.microplus* the assembly pheromone-deltamethrin complex caused around 90 per cent death whereas deltamethrin alone caused only 4 - 6 per cent death with in one hour. The study revealed that combining pheromone with the regularly used acaricide aided in attracting the ticks to the acaricide and killing them.

B. Attraction–Aggregation–Attachment Pheromone (AAAP)

The Attraction-Aggregation-Attachment pheromones attract hungry ticks to a tick infested host and induce them to cluster together at a single location probing and attaching to the host skin. This group includes a mixture of organic volatiles secreted from the dermal glands of **feeding males** but is attractive to both male and female ticks. This pheromone has been reported from the genus *Amblyomma* (Obenchain, 1984; Sonenshine, 1993) with a remarkable amount of production per tick per hour (Diehl *et al.,* 1991; Pavis and Barre, 1993 and Price *et al*., 1994). It elicits attraction for up to 3 meters from a tick infested host with maximum activity in the presence of carbon dioxide. Females rarely attach to uninfested hosts, unless they encounter the male originated pheromone (Norval *et al.,* 1989).

Schoni *et al.* (1984) first elucidated the chemical composition of AAAP in the tropical bont tick *A. variegatum* and showed that it consists of a mixture of three organic volatiles namely o-nitrophenol, methyl salicylate and nonanoic acid in a ratio of 2:1:8 respectively. Further studies revealed the presence of additional compounds like 2, 6 dichlorophenol and benzaldehyde (Lusby *et al*., 1991; Price *et al.,* 1994). Among these o-nitrophenol, methyl salicylate and 2, 6–dichlorophenol were found to be long range attractants and strong stimulants for inducing the aggregation response by unfed female (Norval *et al*., 1991). Allan *et al.* (1998) used AAAP from natural tick extracts for preparation of tail tags to attract *A.variegatum* ticks on cattle. Tick levels observed on pheromone/pheromone-acaricides treated animals were significantly lower than cattle with acaricide treatment alone. Experiments conducted by Nchu *et al.* (2009) to explore the use of AAAP as an attractant for *A.variegatum* in fields along with Kairomones like 1-octen-3-ol, butyric acid and carbon dioxide helped to attract up to 94.0 $\pm$ 6 per cent of adult ticks from a distance of 6 metres and up to 24.0 $\pm$ 5.1 per cent from 8 metres. Experiments conducted in Madras Veterinary College, Chennai using AAAP along with carbon dioxide showed 38 per cent attraction among *R.sanguineus* ticks. However AAAP alone attracted only 20 per cent of ticks. Two ticks of the same species were capable of differentiating differences in the composition of natural pheromone and this could be the possible reason for the variation obtained in the experiments. This probably helps to avoid interspecific mating and explain species specific aggregation that occurs in nature.

C. SEX PHEROMONES

By definition, sex pheromones are compounds or mixtures of compounds secreted by individuals of one sex that are attractive to individuals of the opposite sex. Sex pheromones are the compounds or mixtures of compounds that mediate the various phases of mate recruitment, mate selection and ultimately insemination and fusion of gametes between the mating partners (Sonenshine, 2006). Most knowledge regarding sex pheromones are based on studies on ixodid ticks where all sexual activity occur on the host. When the nymphs moult and begin to feed, spermatogenesis and oogenesis are initiated and the sex pheromone secretion follows. There are three types of sex pheromones each of which mediate different aspects of courtship process.

- Attractant sex pheromone(ASP)
- Mounting sex pheromone(MSP)
- Genital sex pheromone(GSP)

a. Attractant Sex Pheromone (ASP)

2, 6–dichlorophenol (2, 6-DCP) is the only proven attractant sex pheromone in metastriate ticks and has been reported from 15 species including six genera of ticks. Only fed or feeding males can detect this compound (Sonenshine, 2003, 2004, 2006). Unfed males can detect the pheromone but do not respond to it (Haggart and Davis, 1981). Female ticks produce this pheromone which intiates mate finding process in most ixodid tick species. Females commence biosynthesis of pheromone soon after emerging from nymphal moult. The pheromone is stored as oily droplets within foveal glands. When the female tick attach and commence feeding the pheromone is secreted. In *D. variabilis*, 2, 6–DCP may also be produced by the males and stored in their pheromone glands, but not secreted. *Amblyomma variegatum* and *A.habraeum* males produce and secrete this pheromone for attraction and attachment (Norval *et al.,* 1992; Price *et al*., 1994). It also acts as an attractant stimulus for immature ticks (Yoder and Stevens, 2000). A curious anomaly was the finding of 2, 6-DCP in the cattle tick *R.microplus* in all its different active life stages. However, de Bruyne and Guerin (1994) could not find any orientation or searching response in *R. microplus* males in response to 2, 6–DCP. The observation of 2, 6–DCP in various stages of this tick is consistent with the hypothesis of Yoder *et al.* (2002) linking chlorophenol production in ticks to water conservation. In an environment saturated with 2, 6 –DCP males were not able to detect females and did not mate (Ziv *et al*., 1981). Thus the control became possible by confounding males or mimicking females. Males were attracted to inanimate objects coated with 2, 6-DCP. Sonenshine *et al.* (1985) observed that the mating frequencies in the dog ticks that received pheromone were reduced to 11.4 per cent. A complete behaviour sequence of orientation, location, mounting and ventral positioning on the dummies impregnated with 50, 500 and 5000 ng of 2, 6–DCP suggest the role of 2, 6-DCP as an attractant and mounting sex pheromone in these species (Borges *et al.*, 2002).

Studies conducted in Madras Veterinary College, Chennai, using 0.1M and 0.05M concentrations of 2,6-DCP on the larval stages of *R.sanguineus, R.microplus, R.haemaphysaloides, H.marginatum* and *H.bispinosa* revealed maximum attraction with 0.1 M concentration. Trials with 0.1 M ASP showed highest per cent of attraction in *R.sanguineus* larvae (71%) followed by *H.bispinosa* (55%) and *R.microplus* (55%). With 0.1 M ASP *R.haemaphysaloides* and *H.marginatum* showed least attraction (39%). However the per cent of attraction of *R.haemaphysaloides* was higher (46) with 0.05 M ASP. The larvae also exhibited behavioural responses such as feeding, probing, resting and questing posture. Mounting posture, feeding posture, orientation, ventral positioning and resting posture were the behavioural responses exhibited by the adult ixodid ticks of *R.sanguineus* and *R.microplus*. Adult *R. sanguineus* exhibited 64 per cent attraction whereas *R.microplus* showed 60 per cent attraction with 0.1 M ASP.

b. Mounting Sex Pheromone (MSP)

MSP is a non volatile contact sex pheromone produced by the feeding females which enables the males to identify the females as suitable mating partners (Sonenshine, 2003). Male ticks even when attracted by ASP, will never mate with a female unless it recognises MSP. Recognition of MSP mediates dorsal mounting, tip over (ventral turning) and genital searching behaviour (Hamilton and Sonenshine, 1988). Chemical studies showed that the same class of compounds function as the mounting sex pheromone in the brown dog tick, *R.sanguineus* and the camel tick, *H.dromedarii* (Sobby *et al.,* 1994). In most of the hard ticks, females secrete ASP, which acts as an excitant, and provides directional information for feeding or fed males to detach and seek mates (Sonenshine, 1985). Mounting follows if the males perceive the mounting sex pheromone with the foreleg claw sensilla (Phillips and Sonenshine, 1993). Male contacts the female, detects the MSP, mounts, turns and crawls over the female·s opisthosoma to the ventral surface. The male locates the female gonopore and with the help of GSP spermatophore is formed. Although ixodid ticks have cholesteryl esters as components of their mounting sex pheromone, differences in concentration as well as composition appear to alter the mating ability of the male with conspecific females (Sonenshine, 2004, 2006). In Madras Veterinary College, Chennai, the effect of MSP in combination with ASP was assessed using tick decoys. *Rhipicephalus sanguineus* and *R.microplus* showed 10 and 20 per cent attraction to the decoy respectively. Mounting response was observed only in dog ticks.

c. Genital sex pheromone(GSP)

This little known pheromone occurs in a few species of closely related ticks, e.g. *D. variabilis* and *D. andersoni*. During the courtship process, identification of this pheromone by the sexually excited males stimulates synthesis and eversion of the spermatophore and subsequent insemination. Identification of this pheromone minimizes the occurrence of interspecific matings when individuals of both species infest the same host. In *D.variabilis*, GSP consists of a mixture of long chain (C14–20), saturated fatty acids, and the steroid, 20-hydroxyecdysone. Courting males crawling over the female's ventral body are guided to the genital pore when they detect these compounds with their chelicerae. Identification of the pheromone stimulates the males to insert their chelicerae into the vestibular vagina. In this location, the males soon receive further positive reinforcement as they detect even greater concentrations of the pheromone components. This in turn stimulates the males to synthesize and transfer the sperm-filled spermatophore into the female's vulva, using their chelicerae to complete the process.

SEMIOCHEMICAL ASSISTED TICK CONTROL METHODS

The earliest recorded attempt to use tick pheromones to assist in tick control was by Gladney *et al*. (1974). The extract of AAAP from male Gulf coast ticks, *A. maculatum* along with acaricides (Isobenzan) was applied on bovine. The female ticks, attached nearby were lured to the treated site, and were killed. Similar results were obtained by the combined application of the extract of fed male *A.habraeum* with an acaricide (taxophene) on cattle. The combination lured nymphal and adult ticks to the treated areas where they attached and died (Rechav and Whitehead, 1978). The efficacy of these two treatments however was low due to the discontinuous delivery of pheromone. According to Sonenshine (2003, 2004, 2006), manufacture of a long lived control device required the continuous delivery of pheromone source by a slow-release device. Three specific types of pheromone assisted tick control devices have been developed in recent years namely arrestments, confusants and attract and kill devices (Sonenshine, 2004, 2006).

1. Arrestment pheromone impregnated devices

A patented device incorporating purines from the faecal wastes of the prostriate tick, *I.scapularis* into oily droplets released from a pump sprayer was designed for delivery to vegetation. The oily droplets adhered to vegetation where *I.scapularis* quest for hosts (Allan *et al*., 2002). The arrestment pheromone components like guanine and xanthine along with acaricide, permethrin caused the ticks that encounter the droplets to cling to the contaminated surfaces where they acquire a lethal dose of acaricide (Sonenshine, 2006). An increased mortality from 70 per cent for the device with acaricide alone to 95 per cent for arrestment pheromone– acaricides mixtures were observed in laboratory studies using *I.scapularis* ticks (Sonenshine, 2004, 2006).

2. 2, 6-DCP as confusants

The male ixodid ticks move freely on the same animal or in between the animals housed together (Little *et al., 2007*). They became excited by the female sex attractant 2, 6–DCP, produced by a feeding female, detach and crawl over the surface searching for the females. A confusant exploits this mate searching behaviour of the male by minimizing their ability to locate females as the emitting source. A sex pheromone–pesticide combination was used to confuse mate seeking male, causing them to acquire more pesticide as they wander through the pheromone and pesticide treated fur (Ziv *et al*., 1981; Sonenshine, 2004, 2006).

3. Tick decoys

a. Sex pheromone tick decoys

Hamilton and Sonenshine (1988) developed a device to attract mate seeking males to bead shaped plastic spherules using ASP and MSP. The attracted ticks were killed by using small quantities of toxicant in the plastic spherules. Micro capsules, plastic decoys, or a trap using rubber septum, hollow fibres, capillary filaments, poly ethylene or gelatine capsules or multi layer tags made of natural or synthetic polymer resins served as the female mimics or the decoys. Any one of these devices was impregnated with 2, 6-DCP and propoxur. Cholesteryl oleate (MSP) was also coated on to the decoy. These decoys were attached to the hair coat of the tick infested rabbit with cement at a rate of 10 decoys per naturally attached female ticks. The males that were found in the mating posture on the decoys were 89 per cent; the remaining 11 per cent were attached to the skin of the animal adjacent to these devices. This resulted in the death of all males that were attached. The female ticks failed to engorge to repletion and most of them died. Engorged female ticks which dropped off the host failed to lay eggs.

b. AAAP tick decoys

The decoy strategy was modified to suit use on cattle and other livestock attacked by the African bont tick, *A.variegatum* and *A. habraeum*. Decoy was made by using AAAP components o-nitrophenol, methyl salicylate and 2, 6-DCP along with a proven artificial attractant phenyl acetaldehyde (Norval *et al*., 1992). Either cyfluthrin or flumethrin was used as the source of acaricide. Pheromones and acaricides were impregnated into plastic tags which were then attached to the tails of cattle. Ticks which aggregated on the animal body adjacent to or near the decoys acquired lethal doses of acaricide and died (Sonenshine, 2004, 2006).

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