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Tick Pheromones and Their Use in Tick Control

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Table of Contents.

Abstract. Tick pheromones that regulate assembly, attraction/aggregation/attachment and mating behavior have been described. Most of the compounds regulating these behaviors are purines, substituted phenols, or cholesteryl esters. Other pheromonal compounds include organic acids, hematin, or ecdysteroids. Novel devices have been developed that combine the specific compounds comprising these pheromones with an acaricide. When applied to tick-infested vegetation or directly to the body surfaces of livestock or companion animals, these devices are effective for tick control. This review summarizes the current state of knowledge of tick pheromones. In addition, this review also presents examples illustrating how devices using tick pheromones can offer effective alternatives to conventional methods for achieving tick control.

INTRODUCTION

Pheromones are but one class of a broad category of information bearing compounds, known as semiochemicals. More specifically, pheromones are emitted by one individual to influence the behavior of other individuals of the same species. Other types of semiochemicals influence behavior between individuals of different species, e.g., chemical signals that repel predators (allomones) or attract them (kairomones). Pheromones comprise two major categories, (*a*) primer pheromones, which are slowly acting compounds that initiate complex physiological events that affect development and/or reproduction; and (*b*) releaser pheromones, which are fast-acting compounds that elicit immediate behavioral

responses by the recipients(62). Primer pheromones are especially important in the social insects, e.g., bees, wasps and ants, where development of queens and different castes play an essential role in the success of the colonies(62, 63). Only one example of a primer pheromone has been reported in ticks (21), although delayed mating and reduction in female fecundity was believed to reflect a pattern of density dependent fertility in the tick *Ixodes triangulíceps* (41). Most pheromones, however, fall into the latter category. Releaser pheromones can be subdivided further on the basis of the type of behaviors that they mediate, specifically, sex pheromones, food-finding pheromones, assembly and aggregation pheromones, alarm pheromones among others.

Diverse chemicals serve as pheromones. These may range from highly volatile molecules, e.g., substituted phenols such as methyl salicylate, *o*-nitrophenol, or 2, 6-dichlorophenol (2,6-DCP), to nonvolatile contact pheromones such as ecdysteroids, cholesteryl esters among others. In some insects, volatile pheromones may be secreted in sufficient amounts and high volatility, and thus, detected hundreds of meters from the secreting individual (e.g., the moth pheromones). In ticks, however, the longest distances recorded range from 10 to 15 meters (32) while others are limited to only a few centimeters (53). A pheromone may consist of a single compound or a mixture of several different compounds in a distinct ratio characteristic of that species. Often, these different compounds guide specific components of the behavior in a precise sequence of events. Other species may use the same compounds, e.g., certain cholesteryl esters or selected purines, but in a different ratio, thereby facilitating species-specific recognition (47). One of the best known examples is the sequential recognition of the compounds that regulate mating in ticks. The tick must recognize, in a precise hierarchy of detection, specific compounds from the foveal pores,

others from the female body surface, and, finally, yet another set of compounds in the genital aperture before copulation can occur (47). In other cases, the pheromone is a complex mixture, e.g., the compounds secreted by feeding male *Amblyomma variegatum*, utilized to attract conspecific males and females to the same host and form aggregations for feeding and mating success (44). Another example is the mixture of purines and other compounds that induces off-host assembly in prostriate ticks (18, 49). Whether alone or in combination, these chemical signals are effective in regulating food finding, assembly (= clustering), aggregation and mate searching behavior, as well as mate discrimination, copulation and other activities that function at the population level of a species. This mix of chemical compounds forms a simple chemical communication system, or chemical language. Although a single compound may suffice to regulate a particular behavior, complex behaviors such as mating often require several different compounds that must be secreted and perceived in a precise, sequential order in order to achieve the end result.

Research on pheromones has benefited greatly by improvements in the sensitivity and reliability of chemical instrumentation, especially nuclear magnetic resonance, coupled gas chromatography--ion trap mass spectrometry, chemical ionization, and other systems used for identifying these bioactive compounds. Pheromone research has also been advanced by improvements in electrophysiological methods, e.g., electrotarsograms and, more recently, single-cell recordings for determining the tick's sensory responses to these compounds, as well as new methods for bioassay and recording of observations, e.g., computerized video tracking (31) olfactometers (37, 68) and computerized motion detectors such as the servosphere (18). Studies using these improved techniques have provided

researchers with a greater understanding of how these bioactive substances regulate the behavior of ticks and other ectoparasites.

This review explores the diversity of tick pheromones and their use as aids in tick control. A table listing the major categories of tick pheromones, their function(s) and relevant references is provided here to assist the reader (**Table 1**). Primary emphasis throughout this review is directed to advances in basic knowledge within the past 10 to15 years and their application for pheromone-assisted tick control.

<COMP: PLEASE INSERT TABLE 1 HERE>

2. DIVERSITY OF TICK PHEROMONES

2.1. Arrestment (Assembly) pheromones. Arrestment pheromones induce a cessation of locomotor activity. When ticks come in contact with other conspecific individuals, or waste material deposited by such individuals, they cease activity and remain quiescent. Often, clusters of arrested individuals occur in vegetation or, in the case of nidicolous species, in the duff on the floor of caves or burrows. Arrestment pheromones, originally known as assembly pheromones, were first described to occur in ticks of the genus *Argas* (24). Arrestment pheromones from numerous species in both ixodid and argasid families have been reported (48). The most common arrestants are purines (5, 16, 49), especially guanine and xanthine, commonly found in tick excreta, although several other purines may also have an effect. Low levels of ammonia emanating from ticks or tick fecal wastes as well as products resulting from bacterial digestion of these wastes (e.g., 8-azaguanine) are also reported to contribute to the clustering response by attracting ticks to the purine deposits (18). Volatiles also have been reported to attract other species of ixodid ticks, e.g., *Rhipicephalus evertsi,* and even argasid ticks, e.g., *Argas walkerae* (30) to form

clusters when exposed to extracts from conspecific adults. In *Ixodes scapularis,* mixtures of guanine, xanthine and adenine in a ratio of 25:1:1 were most effective for inducing arrestment in nymphs (5). Chemical analysis of acidified extracts of cast skins from molting larvae and nymphs showed the presence of guanine and xanthine (ratio 10.6: 1), hematin, but no adenine. In addition, low levels of ammonia ($\sim 10^{-6}$ mols L^{-1}) also were detected in saline extracts of excreta from feeding ticks. Although nymphal ticks assembled in response to the purines, adults responded most strongly to hematin, one of the primary components in their fecal wastes. Using computer-assisted video tracking (31), my students and I followed the rate of assembly over time in response to different concentrations of these compounds (49), as shown in the group of three graphs available on line (follow the Supplemental Material link in the on line version of this chapter or at http://www.annualreviews.org). The use of video tracking made it possible to characterize features of the arrestant response that would not have been possible otherwise. Once the ticks arrested in contact with the hematin: guanine: xanthine mixtures, they tended to remain in contact for 24 h or longer **(Table 2)**. The clusters induced by arrestment behavior are thought to enhance host finding opportunities and possibly even protect the ticks against desiccation. Other tick species respond to different purines. *Argas persicus* showed strong (>55%) responses to xanthine, hypoxanthine and inosine (16). For a more detailed review of arrestment pheromones and tick assembly behavior, the reader is referred to Reference 48).

<COMP: PLEASE INSERT TABLE 2 HERE>

Knowledge of the arrestment pheromones of ticks may be useful for tick control. By combining the components of the arrestment pheromone with an acaricide (i.e., toxicants used to kill ticks) in a slow-release delivery system, a substantial increase in tick control was

achieved (49). This novel device for controlling ticks before they can attack humans or animals is described in more detail below.

Attraction-aggregation-attachment pheromone (AAA Pheromone).

The attraction-aggregation-attachment pheromone is actually a mixture of three or more specific compounds that mediate different behaviors leading to the formation of species-specific feeding clusters on a bovine or other large ungulate host. In contrast to other pheromones, the AAA pheromone is produced exclusively by males, but attractive to both unfed males and females of the same species. It is secreted by unusually large glands, the Type 2 dermal glands located on the ventral surfaces of the feeding males. Both *o*-nitrophenol and methyl salicylate were identified by high-pressure liquid chromatography (HPLC) in extracts of these glands dissected from fed male ticks (12). AAA pheromones have been reported in a small subset of the genus *Amblyomma* that feeds on these large animals. The pheromone is secreted by fed males, but is attractive to unfed males, females, and even nymphs. The best-known example is the AAA pheromone of the tropical bont tick, *Amblyomma variegatum*, comprising a mixture of two substituted phenols, methyl salicylate and *o*-nitrophenol and a fatty acid, nonanoic acid (44). An additional component, 2,6-dichlorophenol, (2,6-DCP), identified in the tick extracts by Price et al. (40) also attracts unfed ticks (32) but its precise role in the aggregating or attachment response is unclear. Another volatile, 1-octen-3-ol, also was found to attract *A. variegatum* adults that formed aggregations around tick waste material or molted skins. This compound was a major constituent of secretions from *A. variegatum* adults, as well as from tick exuviae, fecal wastes, or even dead ticks (28). 1-octen-3-ol is also found on the skin of humans (and some other mammals) and may be a contributing factor to human infestations with these ticks.

Differences in the relative abundance of methyl salicylate and *o*-nitrophenol, as well as the presence or absence of benzaldehyde, are believed to contribute to the formation of species-specific aggregations in which *A. variegatum* and its close relative, *A. hebraeum* are sympatric (40). Despite the wide distribution of *A. variegatum* , geographic range does not appear to have any effect on the efficacy of the AAA pheromone; male extracts (or live feeding males) from several Caribbean islands, Kenya, Zimbabwe and other localities at different parts of the tick range were equally attractive (50). In Africa, this pheromone is found in several other ungulate-infesting *Amblyomma* ticks, including *A. marmoreum, A. lepidum* and *A. gemma.* In North America, an aggregation-attachment pheromone occurs in the Gulf Coast tick, *A. maculatum,* but its composition is unknown. The unusually large amounts of pheromone secreted by feeding male ticks (12, 36, 40), combined with CO_2 emissions from their large ungulate hosts, enable the pheromone to attract unfed males and females from distances as great as 5 to10 meters (32). Large amounts of CO_2 are essential for effective stimulation and attraction of these ticks. According to Barré et al. (7) the amount of CO_2 produced over 8 h from a 500-g block of dry ice (equivalent to the amount emitted by one 600-kg bovine) attracted adults from 2 to 8 m away, depending upon wind direction. Incorporation of the pheromone components increased the attraction effectiveness as much as 70-fold. This detailed knowledge of the chemical composition of the AAA pheromone and the various attributes of adult tick responses to its presence has been exploited in a novel tick control device, the bont-tick decoy, to control bont tick infestations in Africa and the Caribbean. This is described below.

Male-produced pheromones also occur in other species of *Amblyomma*, but their role is uncertain. For example, a male-produced attachment pheromone was found in the cayenne

tick, *A. cajennense*, an important pest of cattle and other livestock in South and Central America. Although attractive to other unfed ticks, there is no aggregation response and it is not known how the sexes find one another for mating (42).

Sex Pheromones

Mating in ticks follows a ritualistic pattern that is characteristic of all species. A male that contacts a sexually active female mounts onto its dorsum, applies its legs and mouthparts to the body surface, turns, and crawls over the posterior edge onto the female's ventral surface. Once the male locates the female's genital pore, it inserts its mouthparts into the female's vulva and begins to form a spermatophore. When ready, the copulating male inserts the spermatophore to inseminate the female. Most of our knowledge of tick courstship behavior has been obtained from studies of mating in the metastriate ticks of the family Ixodidae (all Ixodidae except the genus *Ixodes*).

SEX PHEROMONES OF THE METASTRIATE IXODIDAE. The courtship process in this group has been reviewed previously (47, 48). Consequently, only the general outlines of the courtship process in the metastriate ticks are described here (although the most recent discoveries are noted).

In the metastriate Ixodidae, mating is delayed until the adults have commenced blood feeding on their vertebrate hosts. The sexually immature males and females seek hosts and commence feeding independent of one another. However, once feeding begins, spermatogenesis and oogenesis are initiated, and within 1 or 2 days (54), females begin to secrete the attractant sex pheromone (ASP) 2, 6-DCP. Males feeding nearby are stimulated to detach and commence searching for the pheromone-secreting females. This compound, which has been reported from seven genera of ticks, including at least 16 different species, is

highly volatile (47). Males detect the pheromone and crawl over the host until they contact the emitting female. A related compound, 2, 4-dichlorophenol (2, 4-DCP) also was found in extracts of fed female *Dermacentor variabilis,* although at a 1:9 ratio of 2, 4-DCP to 2, 6-DCP. 2, 4-DCP was also found to be attractive to mate-seeking males (20). The range of detection of 2, 6-DCP is limited, usually only 3 or 4 cm. Following male – female contact, recognition is facilitated when the males detect cholesteryl esters on the female's body. The mixture of compounds, which comprise the mounting sex pheromone (MSP), stimulates the male to mount the dorsal surface, clasp the female, turn to the female's venter, and search for the genital pore. Variations in the composition of the cholesteryl esters on the females of different species facilitate species recognition and determine whether courtship continues. MSP is essential for the mating process. If the female is washed with a lipid solvent, mating is aborted. However, when lipid extracts (made by washing fed females) were applied to the carcasses of delipidized females, the mounting response was restored. Males could even be induced to mount inanimate objects, e.g., plastic beads, coated with a mixture of 2, 6-DCP and the cholesteryl esters. The chemical composition of the MSP is also critical to the completion of the mating process. Different mixtures of cholesteryl esters have been identified in different species and genera of these ticks. Even though attracted by ASP from a feeding female, males will not mate with a female unless it recognizes the MSP (specific for that species) upon contact with the female body surface. However, species-specific discrimination with this pheromone is imperfect. Male ticks showed the greatest ability to distinguish between ticks of different genera, but only a limited ability to distinguish between the MSP extracts or artificial mixtures of species within the same genus. Knowledge of this aspect of tick courtship behavior led to the development of a novel device for controlling

ticks, i.e., the tick decoy, in which inanimate "dummies" containing both pheromones and an acaricide are used to attract and kill male ticks. By disrupting mating and reproduction, tick eradication is theoretically possible to achieve, at least in restricted environments. The use of this novel technology for tick control is discussed in detail below.

In some species, a third pheromone, the genital sex pheromone (GSP), located in the female's vagina and genital pore, is essential for copulation to occur. GSP occurs in *D. variabilis* and *D. andersoni* and possibly in species of other genera. Males probing the female's genital pore identify the components of this pheromone with sensilla located on their chelicerae. In *D. variabilis*, GSP consists of a mixture of long-chain, saturated C_{14-20} fatty acids and the ecdysteroid 20-hydroxyecdysone (4, 57). Pheromone identification stimulates the male to form the spermatozoa-filled spermatophore and insert it into the vagina. Males that mount conspecific females copulate successfully. However, males that mount heterospecific females abort their courtship after probing the female's vulva and depart in search of conspecific mates. Thus, in those species where it occurs, GSP acts as a final filter to insure successful conspecific matings (3).

Exceptions to this general pattern exist. In the tropical horse tick, *Anocentor nitens,* a one-host tick, 2, 6-DCP administered at concentrations of 50 ng per tick induced males to carry out the entire sequence of mate searching and mounting behavior described above. The same response was observed with inanimate objects ("dummies") treated with this compound, indicating that cholesteryl esters were not necessary to induce mounting once the males had located the object (8). However, no attempt was made to correlate the amounts tested with the natural production of 2, 6-DCP in females, although the authors suggest that it may have been much lower than the amounts used in their bioassay. Moreover, no effort was made to

determine the presence of cholesteryl esters on the females of this species. Thus, the possible role of other compounds such as cholesteryl esters cannot be excluded. Another anomaly was the finding of 2, 6-DCP in the southern cattle tick, *Boophilus microplus*, in all of its active life stages, but there was no evidence that it functions as a sex pheromone. In this species, male ticks do not show the characteristic orientation and searching responses seen in males of other metastriate ticks during their courtship activities. Just how the males identify the females in the cattle ticks is unknown (10).

SEX PHEROMONES OF THE PROSTRIATE IXODIDAE. In the Prostriata, i.e., ticks of the genus *Ixodes*, mating occurs among unfed adults, often beginning soon after emergence from the nymphal molt. In contrast to the metastriate genera, the ovaries and testes of *Ixodes* spp. mature during the nymphal molt or soon after adult emergence. Mating in prostriate ticks appears generally similar to that found in the metastriate ticks, i.e., males exhibit the same pattern of dorsal mounting, turning and ventral probing for the female genital aperture. These sexual contacts may occur among unfed ticks in the vegetation or in the host's nest or burrow as well as during feeding. The latter is a curious response despite the apparent existence of a copulation-inhibiting pheromone (see below), as attempted courtship with a fully engorged female is a futile effort. Aside from the apparent similarity in sexual behavior, little else is known. No evidence of 2, 6-DCP has been reported. Studies done in my laboratory using gas chromatography and HPLC also failed to show any evidence of this compound in the blacklegged ticks (D.E. Sonenshine unpublished data). However, an unknown volatile sex pheromone was reported to occur in the taiga tick *I. persulcatus* (13). This is the first report of a volatile sex pheromone from unfed females of a prostriate tick. More than 100 organic compounds were found

on the external female body surfaces of specimens of this tick, including several known to be pheromones in other species, but none proved to be components of the *I. persulcatus* sex pheromone (59).

In the sheep tick, *Ixodes ricinus,* females begin mating in the vegetation where they wait for hosts. Gradually, as they age, an ever-increasing proportion of the female population is found to have been mated (17). A volatile sex pheromone was also reported to occur, but it was secreted only by feeding or fed females. Among the volatiles recovered in extracts from the volatile emissions of these ticks were diterpenoids derived from cembrene as well as mono- and dichloro- derivatives of methoxybenzoic acid. However, none were attractive to males in bioassays (15). Of special interest was the occurrence of an unidentified copulation-inhibiting compound that deterred mating attempts by mate-seeking males (9, 15). Studies of sexual behavior in the sheep tick show that males mate readily with unengorged females, and also mate with semi-engorged females at high frequencies. Significant differences were noted between wild-caught versus laboratory reared tick populations. For wild-caught females, copulations occurred most frequently with semi-engorged (virgin) and engorged (mated) females; for laboratory-reared females, however, copulations occurred most frequently with the unmated females (69). Mating occurs primarily among unfed ticks, i.e., preprandially, but can also occur among feeding ticks, i.e., perprandially (22). Preprandial matings are restricted solely to feeding ticks.

One explanation for the differences in mating patterns between the prostriate versus metastriate ticks is related to preprandial versus perprandial mating behavior. Most species of *Ixodes* are nidicolous and the males, which never seek hosts, mate preprandially. Males of the non-nidicolous species *I. ricinus* and *I. scapularis* can also mate with partially fed virgins,

i.e., perprandially (22, 23). However, partially fed virgin *I. scapularis* that have fed for more than 4 or 5 days (engorged more than 10 X their pre-engorgement weight) are no longer attractive to males, perhaps because they have exceeded their critical engorgement weight (64).

Although the subject of extensive study, to date the sex pheromone of the prostriate ticks has not been identified. Ticks of this genus (*Ixodes*) lack foveal glands and, as noted previously, no evidence of 2, 6-DCP has been found in extracts. In a recent study of the lipophylic compounds extracted from the body surface of adult *I. persulcatus*, no evidence of substituted phenols was found, although cholesterol, cholesterol derivatives, and other lipids were identified. However, whether any of these compounds act as sex pheromones for regulating *I. persulcatus* mating behavior was not determined (59). Obviously, knowledge of the sex pheromones in this and other important species of the genus *Ixodes* may be of use for developing pheromone-assisted technologies for their control. This is discussed further below.

Most species of the genus *Ixodes* are nest parasites, and mating strategies in these ticks have evolved in response to the pressures of local environmental constraints. In the seabird tick *I. uriae*, mating among adults occurs in the nest. Males do not feed, a common feature of nest-inhabiting ticks, and they will copulate with unfed and fed females. Moreover, multiple matings of the same female with different males often occur, leading to increasingly genetically diverse offspring (27).

SEX PHEROMONES OF ARGASID TICKS. Sex pheromone mediation of mating behavior has also been described in argasid ticks. The behavioral pattern, with dorsal mounting, reversal, tip over and ventral searching by the males, appears similar to that of the ixodids. The

pheromone, of unknown identity, is secreted in the coxal fluid of adult females several days after feeding but only attracts males if the females are moving. The pheromone attracts males of other argasid ticks, i.e., it is interspecific in character, but does not attract other males or nymphal ticks (29, 43). No new information has been reported regarding the pheromones of these highly secretive ticks.

PHEROMONE GLANDS AND DYNAMICS OF PHEROMONE SECRETION.

This section reviews what is known about the glands that produce the tick pheromones and the regulation of their secretion. Knowledge of how and where the pheromones are made is important to gain insights into pheromone biosynthesis and physiology that may be exploited for tick control.

Fecal and Excretory Wastes

No specific glands appear to be involved in the production of the purines, e.g., guanine, xanthine and similar compounds that serve as the arrestment pheromone for many species of *Ixodes* and argasid ticks. These compounds are formed in the Malpighian tubules as the nitrogenous wastes resulting from metabolism of the blood meal, accumulate in the rectal sac, and exit via the anal pore. Similarly, hematin wastes, which were reported to function as an arrestment pheromone in *I. scapularis* (49), also exit via the rectal sac and anal aperture.

Dermal Glands

The AAA pheromone found in large ungulate-parasitizing *Amblyomma* spp. is secreted by the type II dermal glands. The giant cells that comprise these unusual glands are located on the ventral surface of the body of the adult males, and become active during blood feeding. At first, the amount of pheromone secreted during the first 10 days of attachment is less than 10 ng/tick/day, but secretion of the AAA pheromone accelerates rapidly to more than 2

µg/tick/day for ticks attached for up to 80 days. During peak production, the highest amounts extracted from a single male were 223 ng methyl salicylate, 1435 ng *o*-nitrophenol and 1941 ng nonanoic acid, i.e., more than 3 µg of pheromone components (12). Secretion of such large quantities, approximately 1000-fold greater than the level of pheromone production in *D. variabilis*, is understandable in relation to the role of pheromone-mediated communication in the African bont ticks. In contrast to *D. variabilis*, *A. americanum, R. sanguineus,* and other species that rely on the female-originated sex pheromone to attract males cofeeding on the same animal host, the AAA pheromone of *A. variegatum* and *A. hebraeum* is secreted by feeding males and attracts unfed females and males from as far away as 5 to10 m in the surrounding vegetation.

Type I dermal glands, considerably smaller than the AAA pheromone-secreting Type II glands, occur over most of the tick's body. These ubiquitous glands may be the sites of secretion of the MSP. Wax glands (dermal glands) on the anterior margins of the scutum of *D. variabilis* were reported to be the site of secretion of squalene, which is used as a repellent (allomone) against predatory ants (66).

Foveal Glands

In metastriate ticks, these glands produce the volatile ASP, 2,6-DCP. The pheromone is stored in oily droplets in the cells of the foveal glands, and is secreted during blood feeding. In *D. variabilis,* feeding females secrete minute amounts of 2,6-DCP, ranging from 2- 3 ng/tick/day(53). These amounts are sufficient to excite males feeding nearby, e.g., 2 – 3 cm away, and guide them to the emitting source. Release rates are important, as 2,6-DCP is an irritant when present in high concentrations. Moreover, attraction of males in several closely related species is concentration dependent, e.g., the camel tick, *Hyalomma anatolicum*

excavatum is repelled when 2,6-DCP is secreted in amounts that are attractive to its close relative *Hyalomma dromedarii* (45).

Unfortunately, virtually nothing is known about the hormonal stimulation of pheromone secretion, the genes responsible for pheromone biosynthesis, or the physiology of 2,6-DCP production. Dees et al. (11) reported evidence suggesting that the ecdysteroid 20-hydroxyecdysone stimulated increased production, although not the necessarily secretion, of this pheromone. Other studies suggested that pheromone secretory activity is mediated, at least in part, by the neurosecretory pathway (54).

Lobular and tubular accessory glands

A cluster of oil-filled cells, the lobular accessory gland, surrounds the anterior region of the vagina in sexually active *D. variabilis* females. Also present is a pair of tubular accessory glands at the junction of the vestibular and cervical regions of the vagina. These glands are likely sources of the GSP, although other possible sources cannot be excluded.

PHEROMONE BIOSYNTHESIS

Nothing is known about the biosynthetic pathway for 2, 6-DCP in ticks. The presence of this molecule in ticks is unusual. No other examples of chlorinated organic compounds in land animals have been reported. However, microbial biosynthesis of halometabolites in plants and microbes is well known (61). Chlorometabolites are predominantly produced by terrestrial microbes, primarily by enzymes such as chloroperoxidases, which in combination with FADH2 coenzymes, catalyze the formation of carbon-halogen bonds. Chloroperoxidases synthesize a variety of chlorinated hydrocarbons in plants such as the kidney bean (67), various fungi (46) and bacteria (39). An intriguing possibility is that the biosynthesis of 2, 6-DCP may have originated with a commensal or mutualistic microbe,

perhaps by capture and incorporation of one or more microbial genes into the tick's genome. *D. variabilis* also produces trace amounts of 2, 4-DCP (20), which may represent an intermediate in a biosynthetic pathway with 2, 6-DCP as the final product. Ticks harbor a variety of microorganisms within their body tissues (66) and one such microbe, the fungus *Scopulariopsis brevicaulis*, can also synthesize 2, 6-DCP (J.A. Yoder, personal communication). However, none are known to occur in the pheromone glands. Nevertheless, this intriguing possibility certainly merits further study.

Even less is known about the biosynthesis of the AAA pheromone, secreted by the ventrally located Type II dermal glands. The GSP is believed to be synthesized in and secreted by the lobular accessory gland surrounding the vestibular region of the vagina. These glands fill with lipid droplets during blood feeding. The tubular accessory glands, located at the junction of the vestibular and cervical regions of the vagina, may also contribute to this pheromone (52). The source of the sex pheromone in the few argasid ticks that have been studied is believed to be the coxal gland fluids secreted during or immediately after blood feeding (43).

PHEROMONE RECOGNITION AND INTERPRETATION

This brief review addresses the most important aspects of the sensory systems that enable ticks to recognize and respond to pheromones and other semiochemicals they may encounter. For more detailed descriptions of this subject, the reader is referred to earlier reviews (47, 48).

The most important organ for pheromone recognition is the Haller's organ located on the dorsal surface of the tarsus of the forelegs. Multiporose sensory hairs located within and in front of the anterior capsule function as olfactosensilla, detecting odors and chemicals from a distance. Several multiporose sensilla also occur in the Haller's organ capsule. They play an important role in responding to chemical cues, NH_3, CO_2 and H_2S, that enable ticks

to recognize when hosts are present. These olfactosensilla are innervated by numerous mechanosensory and chemosensory neurons. Some of these sensilla detect 2, 6-DCP and other substituted phenols, i.e., the volatile sex pheromones of many metastriate ixodid ticks. For example, in the lone star tick, *Amblyomma americanum*, 2, 6-DCP is detected by a single, large, multiporose olfactory sensilla in the anterior pit. Other tip-pore and wall-pore sensilla also detect compounds on contact or at very short ranges. The Haller's organ sensilla serve also to detect mechanical changes as well as changes in temperature, air currents and other physical events. In the prostriate tick *I. ricinus*, the multiporose olfactosensilla in the Haller's organ can detect selected substituted phenols, including 2, 6-DCP, 2, 6-dibromophenol, *o*-chlorophenol, *o*-bromophenol and *o*-methylphenol, even though these ticks do not synthesize or secrete these compounds. It is claimed that detection of these compounds enables the ticks to recognize hosts at distances of 10 to 15 m (25). However, there is no evidence that they play any role in mating behavior. A similar arrangement of sensilla occurs in the Haller's organ of argasid ticks. A more specialized group of tip-pore sensilla located around the apotele (claw apparatus) can detect the cholesteryl esters that make up the MSP of many metastriate ixodid ticks (38). Gustatory sensilla on the terminal segment of the palps enable the ticks to detect surface compounds on the host skin. In *B. microplus*, electrophysiological recordings demonstrated that these gustatory sensilla could detect cholesteryl esters on the female cuticle (10), i.e., the familiar components of the MSP in many metastriate ixodid ticks.

The chelicerae also contain sensilla that can detect the GSP. In this case, the gustatory sensilla reside in pores on the cutting spines of the inner digit of each chelicera. The pores are innervated by chemosensory as well as mechanosensory neurons. In addition to the usual

phagostimulants (e.g., ATP), these sensilla also respond to the ecdysteroids ecdysone and 20-hydroxyecdysone, components of the GSP (57). When the males detect these and other compounds, especially long-chain polyunsaturated fatty acids (4, 6), they form a spermatophore and inseminate the female.

PHEROMONE-ASSISTED TICK CONTROL.

The purpose of tick control is to reduce the number of ticks infesting humans or other vertebrate animals to the lowest acceptable level. Ignoring tick control is not an option. In many tropical and subtropical regions of the world, raising livestock is impossible without it. Despite its obvious importance, this has proved to be a daunting task. For more than 100 years, tick control has been done almost exclusively by the treatment of animals with massive amounts of acaricides delivered to their body surfaces. A variety of methods have been developed to accomplish this, e.g., dipping the animals in vats (e.g., cattle dips), spraying, pour-on or spot-on treatments and powders. In recent years, improvements in the development of toxicants with exceptionally low mammalian toxicity (e.g., pyrethroids and avermectins) have enhanced the efficacy of such treatments but at greatly increased cost. These improvements have even made it possible to use the toxicants as systemics, although under strict supervision. In some instances, when approved by governmental agencies, acaricides have also been used to treat roadsides, trails, lawns, gardens and other habitats where ticks are likely to encounter humans and/or their companion animals. However, the rapid development of tick and insect resistance to these new toxicants, the enormous cost for each new product registration, and the ever-increasing opposition from environmental activists have limited their usefulness for tick control. As a result, there is now considerable

interest in novel alternatives, such as the use of pheromones, hormones or other natural products, to assist in controlling ticks.

Pheromones alone cannot control ticks. Rather, they must be used in combination with a toxicant. For effective use, these compounds must be incorporated into a slow-release delivery device. Otherwise, any improvement in efficacy is short-lived. A variety of technologies are available to retard emergence of pheromone compounds, e.g., incorporation into plastic, adhesive materials, paraffin, or gelatin microcapsules. Therefore, to develop a device or system for tick control using pheromone-acaricide combinations, the investigator must first determine how it will be applied, e.g., to vegetation in the host environment or onto the host itself. In addition, the solubility of the pheromone components in the delivery device, the range (in centimeters or meters) of attraction expected, and the duration of activity also must be considered. Here we review a few of the more promising techniques that have been developed in recent years for pheromone-assisted tick control. In addition, we also consider some of the options for future research.

Pheromone-assisted Matrix for Application to Vegetation

Blacklegged ticks (also known as deer ticks) form clusters when they encounter one another in the natural environment and recognize components of the arrestment pheromone, namely guanine, xanthine and hematin. Guanine and xanthine are necessary for nymphal assemblies (5), while guanine, xanthine and hematin are necessary for stimulating adult assemblies (49). By incorporating these compounds and an acaricide (permethrin) into oily droplets, Allan et al. (6) developed a patented technology that, when applied to vegetation, kills ticks that cluster onto the droplets. This type of device also offers the opportunity to control ticks with a single application, since the active compounds emerge gradually over long periods (slow

release technology). Formulations of the oily material with and without pheromone were tested against female *I. scapularis* in a laboratory trial. The addition of the pheromone components increased the efficacy of the oily droplets from 70% for droplets with acaricide alone to 95% for Last Call™, with both acaricide and pheromone (**Table 3**). Similar increases in efficacy were observed when the oily droplets were tested against nymphs (data not shown). Encouraged by these results, these compounds were incorporated into a commercial device, Last Call™ (IPM Technology, Inc., Portland, Oregon), a product comprising small adhesive droplets that can be delivered in large quantities from a hand-held pump sprayer. A field trial using this technology showed significant reduction in *I. scapularis* nymphal activity in pheromone-Last Call™-treated plots versus control plots in a field site near Armonk, New York (T. Daniels & R. Falco, personal communication). Field tests with hematin-purine-permethrin mixtures to control *I. scapularis* adults are also planned. Incorporation of the pheromone-acaricide mixtures into microfibers, which adhere to vegetation by electrostatic attraction, is also being considered as an alternative to the Last Call droplets.

<COMP: PLEASE INSERT TABLE 3 HERE>

Tick Decoy

To disrupt tick reproduction rather than merely killing ticks, a pheromone-acaricide-impregnated device was developed to attract and kill male ticks before they could mate with feeding females (19). The ASP, 2, 6-DCP and an organophosphorus acaricide, propoxur were mixed with polyvinylchloride resin and molded (with a ceramic mold) into numerous small plastic spherules resembling feeding female ticks. Following heating (curing) at 100^0-C for 20 min, the spherules were coated with an oily extract containing cholesteryl oleate, the MSP

of *D. variabilis*. When completed, the devices (14 mm long x 10 mm wide, weight 0.36 grams) mimicked the shape and pheromonal composition of a partially fed female tick. 2, 6-DCP emission from these plastic decoys was estimated by gas chromatography analysis at ~ 0.9 ng min^{-1} and acaricide emission at 152.5 ng min^{-1}. Thus, they could act as decoys to attract and quickly kill mate seeking *D. variabilis* males. Decoys were attached to the hair coat of a rabbit host with cement and dispersed at a ratio of 10 decoys per each live female tick. At this ratio, the decoys were highly effective in killing male ticks before they could mate with females. Within 30 min after application to a tick-infested host, 89% of the males released onto the animal were killed after attempting to mate with the decoys; the remainder died while trying to attach nearby. However, decoys without MSP, i.e., with just 2, 6-DCP alone, were much less effective. Fewer males attempted to mate with the single-pheromone decoys or attach beside them; most attached elsewhere or mated with live females. Within 30 min after release, only 36% of the males had died. Treatment with single-pheromone decoys required up to 48 h for a 98% kill rate (**Table 4**). Most females on the tick-infested animal also died; the remainder either failed to engorge or dropped off and died without laying eggs. Tick reproduction was completely disrupted.

<COMP: PLEASE INSERT TABLE 4 HERE>

When the decoy/live tick ratio was reduced to 5: 1, fewer males mated with the two-pheromone-containing decoys (66.0%), although more males attached beside the devices (30.0%). Nevertheless, the kill rate was similar to that observed with the higher ratio, namely, 96.0 ± 3.6 % within 30 min. All remaining males were dead within 24 h. As in the preceding experiment, decoys without MSP were much less effective, requiring 48 h for a 98% kill rate (**Table 4**). Again, no reproduction occurred.

Similar results were obtained when the tick decoys were applied to tick-infested cows in a barn. In this case, treatment was administered at a ratio of 10 decoys to each live female on three Guernsey calves and the response of *D. variabilis* males was observed. Although the kill rate was somewhat slower than that on rabbits, all of the male ticks died within 90 min after release onto the calves. The pheromone/acaricide-impregnated decoys were also effective against other tick species. Tests by these same researchers showed the decoys attracted more than 90% of the mate-seeking males of three other species, *A. maculatum, D. andersoni,* and *H. dromedarii,* to *mate* with or attach beside these devices. Here again, the kill rate was 100% within 60 min. Subsequently, this technology was patented under U.S. patent no. 4884361 (19). Additional improvements described in a later patent (51) were made following unequivocal identification of the cholesteryl esters, thereby enabling coating of the plastic decoys with inexpensive, authentic compounds rather than tick extracts. This same technique was also used in Egypt to control camel ticks, *H. dromedarii,* on camels. For this purpose, the 2, 6-DCP- and acaricide-impregnated decoys were mixed with cholesteryl esters specific for this tick species before they were cemented to the hair coat of the animals. The response of the males was similar to that seen with *D. variabilis*, i.e., they selected the numerous decoys in preference to the feeding females, attempted to mate with these devices, and died soon afterwards. Test results showed an efficacy of 85.3% as well as eventual kill of the unmated females (1). Clearly, this technology offers the opportunity to eradicate a tick population in a semi-enclosed environment, e.g., farm, ranch, home or kennel, with just a few applications, since reproduction is completely disrupted. This same goal has been achieved rarely with acaricides alone, and then only after enormous effect and expense. Because of the tick decoy's long-term efficacy (up to 3 months), tick control can be achieved with only two

or three applications per year. This is especially useful in tropical and sub-tropical regions where conventional tick control must be administered frequently (e.g., once per week). However, no commercial development of this patented technology has occurred. Consequently, it has not been made available for sale to the livestock industry or for use on companion animals.

6.3. Bont Tick Decoy. A specialized modification of the decoy concept was adapted for use on cattle to control the African bont ticks, *Amblyomma hebraeum* and *A. variegatum.* These ticks are the major vectors of *Anaplasma ruminantium* (= *Cowdria ruminantium*), the agent of deadly heartwater disease. Ticks of these species form aggregations on their hosts in response to a male-originated AAA pheromone, as described above. In this case, methyl salicylate, *o*-nitrophenol, 2,6-DCP, and phenylacetaldehyde, previously shown to induce attraction and aggregation and attachment in this species (32-34) were incorporated into a plastic strip attached to the animal's tail. Also included in the tail tags was a pyrethroid acaricide. Laboratory trials showed that the volatile pheromone components emerged gradually, at a rate of approximately 1% per day, and could provide continuous tick control for more than 3 months. AAA pheromone was most effective in attracting bont ticks from their shelters in the surrounding rough grazings when combined with CO_2. CO_2 was produced in large amounts by the herds of cattle to which the tail tag decoys were attached. In a three-month field trial involving hundreds of animals, including a control group, efficacy for cattle treated with cyfluthrin plus pheromone-impregnated tags was 94.9% and efficacy for cattle treated with the flumethrin plus pheromone-impregnated tags was 87.5%. When the test was repeated for a second three-month trial period, long-term efficacy for the same treatments increased to 99.3% and 95.1%, respectively **(Figure 1).** However, tags

Figure caption for Fig. 1 "Control of bont ticks by tick decoys"

impregnated with pheromones plus α-cypermethrin were considerably less effective (35). Loss of the tags from the treated animals was noted initially, but improvements made in the selection of adhesives resolved this problem. In more trials recent trials using deltamethrin, control of *A. hebraeum* ranged from 83 to 95% during a 12 week trial. When applied to the neck as well as the tail, the tags proved effective against brown ear ticks, *Rhipicephalus appendiculatus*, with efficacy ranging from 98% to 100% during the same trial period (M. J. Burridge, unpublished data).

The legend for Figure 1 is located at the end of the document

<COMP: PLEASE INSERT FIGURE 1 HERE>

The high level of efficacy and the ability to provide effective treatment with a single application make this technology of great interest. The ability to apply the tick control devices without the need for large amounts of water is also important, especially during the periodic droughts common in southern and eastern Africa. The tail tags use relatively inexpensive ingredients, are easy to manufacture and require no special training to apply to the target animals. Because of the small amount of acaricide used in the devices, the overall costs for the normally expensive acaricides required to treat cattle herds are greatly reduced. The pheromone-impregnated decoys also were tested against tropical bont ticks, *A. variegatum*, on cattle on Guadeloupe, an island in the Caribbean Sea, using the same pheromone components but different acaricides (cyfluthrin and deltamethrin). In this case, the tags were applied to the neck and tail of the treated animals. Improvements in attachment techniques led to greater than 98% and 90% retention of the tags over a 13-week study period. At the end of the trial period, tick infestations increased on untreated cattle (311.7%) but were greatly reduced on cattle treated with the neck and tail tags. Tick infestation was reduced by similar amounts on animals treated with pheromone/acaricide-impregnated tags versus acaricide-only-impregnated tags. However, cattle with pheromone tags had greater

proportions of tropical bont ticks on the hind and neck regions of the body (81% and 18.2%) versus animals without pheromone tags (62.5% and 8.2%), indicating that ticks aggregated in response to the pheromones. Analysis of hair samples showed that both acaricide components were detectable all over the body throughout the 13-week trial period. Emission rates for the pheromone components from the tags were most rapid during the first 4 weeks after the tags were installed but diminished thereafter. However, all of the tags still retained pheromone components at the time of study termination (2). Further studies are planned to enhance the efficacy of the these devices for tropical bont ticks by modifying the composition of the pheromone components, e.g., incorporation of nonanoic acid, or adjusting the proportions of the other volatiles to more closely reflect the natural abundance of these compounds. Despite the demonstrated success of this technology under field conditions, no commercialization of the bont tick decoy technology has occurred and none are available for sale (although several companies have shown interest in licensing it).

Instead of applying the decoy to cattle, Maranga et al. (26) used this concept to attract *A. variegatum* to a treated site in the center of a circular field plot. Different doses of the AAA pheromone were dissolved in paraffin oil and the response of ticks released from varying distances was determined. When combined with a source of CO_2, within 3 h up to 90% of ticks released into the field plot were attracted to the treated site. When used in combination with an acaricide in a corral, feedlot or other animal enclosure, this method offers the opportunity to attract and kill large numbers of bont ticks before they can attack valuable livestock.

Confusants

Another means of disrupting mating among metastriate ixodid ticks is to "confuse" the males by saturating their environment with an excess of pheromone. With 2,6-DCP all over the host, males are unable to discriminate differences in concentration and locate the feeding females. The longer they move in search of mates, the greater the likelihood that they will acquire a lethal dose of acaricide and die. To accomplish this goal, Sonenshine et al. (56) dispensed a water emulsion of 2,6-DCP and acaricide impregnated into gel microcapsules onto tick-infested dogs. Microscopic observations showed numerous microcapsules attached to the hair of these animals, providing continuous release of the active ingredients for up to 3 weeks. Most of the male ticks were killed by the treatment and the few surviving females laid less than 10% of the eggs produced by the controls.

FUTURE GOALS.

Knowledge of genes encoding the enzymes involved in pheromone biosynthesis offers researchers opportunities for novel methods for disrupting mating in ticks. Considerable information is now known about the pheromone biosynthesis-activating neuropeptide (PBAN) in insects and even crustaceans. This same protein also regulates diapause in these animals. In several cases, the complete gene sequence and predicted amino acid sequence have been determined (14, 60, 65). Other proteins play a critical role in odorant capture and transfer to the sensory dendrites. A considerable body of knowledge has accumulated concerning pheromone- and odorant-binding proteins in the sensillum liquor of the chemosensory sensilla in insects. These proteins carry the specific stimulating compounds across the sensillum lymph (58). Esterases and/or other enzymes degrade the ligand-carrier protein linkage. With such information in hand, it will soon be possible to design new interventions using

transgenic techniques or RNAi to disrupt the natural biosynthesis of the insect pheromones. For ticks, similar studies may soon be possible as a result of efforts currently in progress to sequence the entire tick genome of *I. scapularis*.

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Table 1. List of tick pheromones.

Representative Tick species	Pheromone Category	Identified Compounds	Behavioral Function (s)	References Cited
Argas (P.) arboreus	Primer	Unknown	Reduce fecundity in overcrowded populations	21
Argas persicus, Ornithodoros spp Ixodes scapuaris	Assembly	Guanine, xanthin hematin, ammoni	Clustering of unfed individuals in the natural environment	3,16, 18 30, 49
Amblyomma variegatum	Attraction-Aggregation-attachment pheromone	*o*-nitrophenol, methyl salicylate	Feeding clusters formed by ♂♂ and ♀♀ in response to feeding ♂♂	7, 12, 26, 32-36, 44 40, 50
Dermacentor variabilis	Attractant Sex pheromone	2,6-dichloro-phenol	Volatile compound secreted by feeding ♀♀ that attracts fed/ feeding ♂♂ for mating	8, 45, 48, 50
D. variabilis	Mounting sex pheromone	Mixture of specif cholesteryl esters	Non-volatile surface compound(s) on surface of feeding females that enables males to recognize	47, 48, 51
D. variabilis	Genital sex pheromone	Mixture of specif Fatty acids + 20-hydroxyecdysone		4, 11, 47, 48, 52, 57

TABLE 2. Video tracking responses of *Ixodes scapularis* adults to Last Call™ impregnated with hematin, guanine and xanthine versus untreated controls in a petri dish bioassay.

	Hematin: guanine:xanthine		Control	
Trial #	On disk	Hidden under disk	On disk	Hidden under disk
1	749.1 ± 115.2	25.6 ±23.3	8.0 ±2.8	0.0
2	676.8 ± 65.7	1006.7 ± 465.9	67.0 ±2.8	19.3 ±18.4

From Sonenshine et al. 2003 (49) with permission from the Entomological Society of America.

Table 3. Kill of *I. scapularis* females by permethrin-Last Call™ matrix with or without arrestment pheromone.

Treatment	Mean % Ticks in treatment sector (± SE)*			% Mortality
Type	0.5 h	1 h	2 h	24 h
Permethrin/Last Call	10.0 (6.9) a	10.0 (6.9)a	10.0 (6.9)a	70
Permethrin and pheromone Last Call	40.0 (11.2) bc	35.0 (10.9)b	35.0 (10.9)b	95
Pheromone/Last Call	25.0 (9.9)ac	20.0 (9.2) a	40.0 (11.5)a	0
Last Call (No pheromone or permethrin)	10.0 (6.9) a	10.0 (6.9) a	5.0 (5.0) b	0

* Means within each column followed by a different letter are significantly different (paired *t*-test, $p < 0.05$)

From Sonenshine et al. 2003 (49) with permission from the Entomological Society of America

Table 4. Ability of pheromone-pesticide treated plastic decoys to kill male *Dermacentor variabilis* and prevent mating.[1]

	TYPE OF TREATMENT											
	Ratio 10 decoys per female						Ratio 5 females per decoy					
	Both pheromones + acaricide			2, 6-dichlorophenol alone + acaricide			Both pheromones + acaricide			2, 6-dichlorophenol alone + acaricide		
	Hours after ♂♂ released			Hours after ♂♂ released			Hours after ♂♂ released			Hours after ♂♂ released		
Location of ♂♂	0 – 0.5 hours	24 hours	48 hours	0 – 0.5 hours	24 hours	48 hours	0 – 0.5 hours	24 hours	48 hours	0 – 0.5 hours	24 hours	48 hours
Mating with decoys	89.0* ±3.3	---	---	20.0 ±4.0	0.0	0.0	66.0 ±11.5	0.0	----	26.0 ±3.8	8.0 ±7.2	6.0 ±5.4
Attached besides decoys	89.0 ±3.3	---	---	24.0 ±3.8	11.0 ±3.6	2.0 ±2.0	30.0 ±10.2	4.6 ±3.6	----	24.0 ±6.7	6.0 ±5.4	4.0 ±3.6
Mating with live females	0.0	---	---	6.0 ±1.6	3.0 ±1.5	0.0	4.0 ±3.6	0.0	----	6.0 ±6.7	2.0 ±1.8	4.0 ±2.2
Attached elsewhere	0.0	---	---	50.0 ±0.4	24.0 ±4.0	2.0 ±5.5	0.0	0.0	----	44.0 ±8.3	30.0 ±2.8	8.0 ±5.2
Dead	100.0	----	----	36.0 ±4.3	73.0 ±4.3	98.0 ±1.5	96.0 ±3.6	4.0 ±4.3	----	40.0 ±11.3	78.0 ±4.4	98.0 ±1.8

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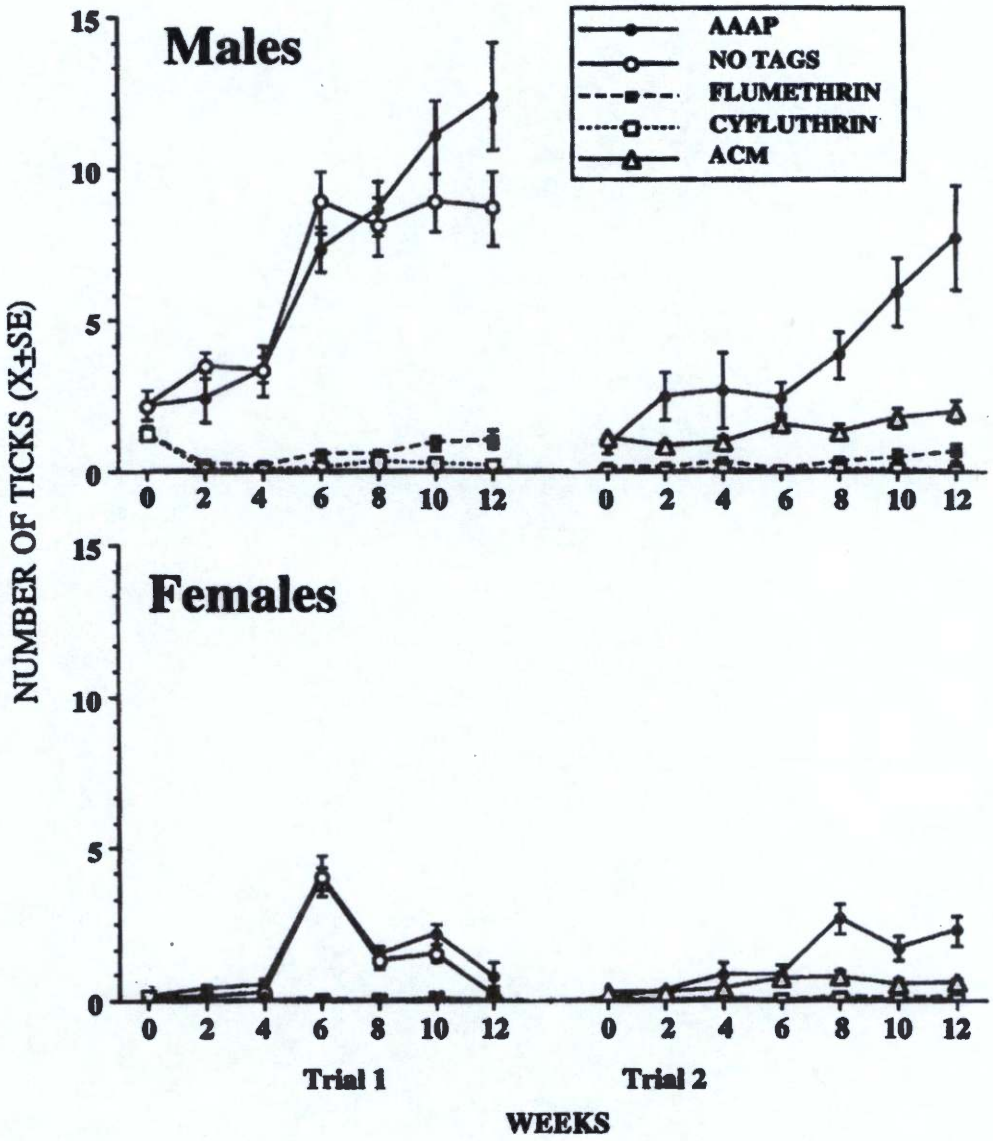
[1] Data from reference 19.

* Two males deposited spermatophores on the decoys before dieing.

DESCRIPTION OF FIGURES

Figure 1. Control of bont ticks by tick decoys. Numbers of bont ticks on cattle during two field trials in Zimbabwe. Cattle were untreated (no tags) or treated with tail tags containing pheromone only (AAAP), Flumethrin and pheromone, Cyfluthrin and pheromone or α-cypermethrin and pheromone. From Norval et al. 1996 (35) with permission from Kluwer Academic Publishers, The Netherlands.


Males
AAAP
NO TAGS
FLUMETHRIN
CYFLUTHRIN
ACM
Females
NUMBER OF TICKS (X±SE)
15
10
5
0
0 2 4 6 8 10 12
Trial 1
Trial 2
WEEKS