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INVESTIGATIONS INTO THE VOLATILITY OF FEMALE PHEROMONES AND THE AGGREGATION-INDUCING PROPERTY OF GUANINE IN *ARGAS (PERSICARGAS) WALKERAE*

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ABSTRACT

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During investigations into the volatility of female pheromones of *A. walkerae* in a Y-shaped olfactometer, engorged conspecific male ticks responded weakly both to female-contacted filter paper discs and to intact females enclosed in nylon bags. In tests in which female-contacted filter paper discs were separated from engorged male ticks by means of a screen, no aggregation was observed. However, replete male ticks showed a vigorous response to liquefied air and hexane-trapped volatiles collected from intact females.

Guanine showed a potent aggregation attraction for engorged and unfed larvae and 1st and 2nd stage nymphal ticks as well as for male and female imagines of *A. walkerae* over a wide concentration range.

It is concluded that at least 2 pheromones, 1 volatile and 1 non-volatile, are operative in the communication system of this tick species.

INTRODUCTION

The orientation, behaviour and maintenance of argasid ticks in their biotopes are determined and regulated by a communication system involving chemical messengers known as assembly or aggregation pheromones. In addition, messengers necessary for the establishment of a definite sexual relationship may possibly be designated as sex pheromones (Leahy, 1979; Gothe, 1983 a, b). Irrespective of their functions, pheromones are either primarily or possibly exclusively perceived via chemoreceptives located on the pedipalps (Leahy, Karuhize, Mango & Galun, 1975; Leahy, 1979; Gothe & Kraiss, 1982 b). It is not known, however, whether the receptive surface structures recognize and perceive the pheromone stimulus by olfactory or gustatory mechanisms.

The chemical structure of the pheromones of the argasids has not been investigated. However, because pheromone is primarily emitted through the anus (Gothe & Kraiss, 1982 b), from investigations into the nitrogenous excretory products of these ticks (Hamdy, 1972), the possibility that guanine, a natural excretory product, may serve as a chemical messenger with at least partial pheromone function must be considered. Sonenshine, Silverstein & Rechav (1982) have already mentioned this possibility and preliminary investigations by Gothe, Weck & Kraiss (1984) have shown that guanine induces a selective aggregation of 1st stage nymphs of *A. walkerae*.

The present study was therefore undertaken to investigate whether pheromones emitted by female *A. walkerae* ticks are volatile, and to determine to what extent, and at what concentrations, guanine may serve as an attractant for other post-embryonal stages of this tick species.

MATERIALS AND METHODS

Origin and rearing of A. walkerae

Laboratory colonies of *A. walkerae*, bred from ticks collected at Onderstepoort in 1966, were maintained and reared in an incubator at 27 °C and 90–95 % R.H. All stages were fed on LSL-Leghorn chickens and used within 2 days after engorgement.

1. *Investigations into the volatility of pheromones of female* A. walkerae

a. *Contact collection of female pheromones* Twenty engorged virgin female ticks were placed between 2 3 cm diameter sterile filter paper discs[1] in a small beaker. The top disc was pressed down firmly by means of a cotton-wool plug to ensure direct contact between ticks and discs. After incubation at 27 °C and 90–95 % R.H. for 6 days, the bottom disc was used as the pheromone source in various bioassay tests.

One bioassay method involved the use of a glass Y-shaped olfactometer similar to that described by Rechav, Parolis, Whitehead & Knight (1977), but with the following dimensions: internal diameter 12 mm, length of each of the short arms 18,5 cm, angle between short arms 40°, length of long lower arm 24,5 cm. An air flow of 35 cm^3/min through the tube was achieved by means of a vacuum pump connected to the long arm. For each test, a single replete male tick was introduced into the long arm, approximately 5 cm from the open end. Filter paper discs which had been in contact with females were pushed into the tip of 1 of the short arms. The 2nd short arm contained clean discs. In a further experiment involving the olfactometer, 12 replete female ticks enclosed in a fine nylon bag served as the source of pheromones.

Tests were performed in the dark at 25 °C and 85 % R.H. The movements of the ticks were observed at various timed intervals by means of a dim red light source. The number of replicates of each test performed is shown in Table 1. After each test the olfactometer was immersed in a cleaning agent[2] for 60 min, rinsed with distilled water and dried at 150 °C.

A 2nd bioassay was performed in a way similar to that described by Leahy (1979). Ten male ticks, either engorged or unfed, were placed in the centre of a 13,5 cm diameter circular screen (18 mesh/cm) located 5 mm above 4 filter paper discs. These were arranged in a Petri dish 0,5 cm from the rim and approximately 3 cm apart. One disc had previously been exposed to female ticks as described above, whilst 3 clean discs served as controls. In a further test the screen, with discs placed in a similar arrangement on it, was located 5 mm above the ticks. In an additional control, both ticks and discs were placed on the screen. Tests were performed in the dark at 27 °C and 90–95 % R.H. and the location of the ticks was observed at various time intervals up to 48 hours.

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b. *Collection of female pheromones by volatilization*
Two methods were employed, both performed at 20 °C and 60 % R.H. In the 1st method (Sonenshine, Silverstein, Collins, Saunders, Flynt & Homsher, 1977), 300 fed virgin female ticks were placed in a 40 cm^3 glass container with an inlet 10 mm in diameter opposite an outlet 3 mm in diameter. Air was drawn through the container at a flow rate of 40 cm^3/min by means of a vacuum pump connected to 2 25 cm^3 glass vials each containing 5 cm^3 hexane plus 5 cm^3 0,5 M NaOH. The vials were cooled with ice to minimize evaporation. Collection of effluent air was continued for 4 hours, then the hexane-NaOH mixtures were combined and neutralized with 0,5 M HC1. After vigorous shaking, the 2 phases were separated by means of a Pasteur pipette, and each was bioassayed for the presence of volatile pheromones. A control collection was performed as described above, omitting the ticks.

In the 2nd method (Browne, Birch & Wood, 1974), air was likewise drawn over 300 engorged virgin female ticks, then liquefied and trapped in a U-tube (total length 16 cm and internal diameter 10 mm) immersed in liquid nitrogen. Collection was continued for 6 hours, by which time the U-tube had become blocked with ice. The liquid air was then slowly evaporated. After the ice had thawed, the contents of the U-tube were salted with excess sodium chloride, then extracted with diethyl ether.

Aliquots of all phases were bioassayed immediately and again after incubation for 96 hours at 27 °C and 90–95 % R.H. for the presence of volatile pheromones. The bioassay was performed as follows: sterile filter paper discs 1,8 cm in diameter were separately impregnated with 10,30 or 100 $\mu\ell$ of each phase and each impregnated disc was placed in a 10 cm sterile Petri dish, 0,5 cm from its rim. Three clean discs were then arranged symmetrically in each dish and 10 engorged male ticks were placed in the centre. Finally the dishes were incubated at 27 °C and 90–95 % R.H. in the dark. The location of the ticks was observed at various time intervals up to 48 hours.

2. *Investigations into the aggregation response of* A. walkerae *to guanine*

Sterile filter paper discs, 1,8 cm in diameter, were impregnated with 0,01 cm^3 of an aqueous guanine solution[1] with concentrations of 1000; 100; 10; 1; 0,1; 0,01; 0,001; 0,0001 and 0,00001 ppm, respectively. Each impregnated disc was then placed in a 10 cm diameter sterile Petri dish, 0,5 cm from the rim. Three clean discs were arranged symmetrically in the dish and a collection of 10 ticks, either engorged or unengorged of all stages, was placed in the centre. The dishes were then incubated at 27 °C and 90–95 % R.H. in the dark. The location of the ticks was observed at various time intervals up to 48 hours.

The attraction of ticks to guanine-impregnated filter paper discs was also tested by the Y-shaped olfactometer method described above.

RESULTS

1. *Investigations into the volatility of pheromones of female* A. walkerae

In the Y-shaped olfactometer, only 20 % of the engorged male ticks responded either to female-contacted filter paper discs or to females enclosed in nylon bags (Table 1). The majority of the ticks tended to move downstream with the air flow within approximately 15 min. This tendency was also noted even when the air flow was reduced to 10 cm^3/min. Ticks moving upstream usually took 30–60 min to reach the end of 1 of the short arms of the olfactometer.

TABLE 1 Attraction of single engorged male *Argas (Persicargas) walkerae* to conspecific female pheromones and guanine in a Y-shaped olfactometer

Test material	Number of tests	% ♂♂ migrating to test material	% ♂♂ migrating to control	% ♂♂ migrating downstream
♀♀ contacted filter paper discs	50	20	15	65
♀♀ enclosed in nylon bag	20	20	20	60
Filter paper discs impregnated with 0,1 μg guanine	20	25	20	55
Filter paper discs impregnated with 10^{-6} μg guanine	20	20	20	60

In tests in which female-contacted filter paper discs were separated from engorged male ticks by means of a screen, no assembly of male ticks was observed either above or below the test discs during a period of 48 hours.

Replete male ticks showed a vigorous response to liquefied air and hexane-NaOH trapped volatiles when bioassays were performed immediately after collection (Fig. 1). The results indicate that 80 % of the ticks aggregated within 6 hours, and 90 % within 4 hours, at discs impregnated with 30 $\mu\ell$ of either the diethyl ether or the hexane phase, respectively. Impregnation with 100 $\mu\ell$ quantities gave similar results, whereas 10 $\mu\ell$ elicited a weak reaction only.

No responses were observed after incubation of the volatiles for 96 hours at 27 °C and 90–95 % R.H. prior to bioassay. A few ticks congregated on or under discs impregnated with the aqueous phases (Fig. 1), and all phases obtained from control collections.

2. *Investigations into the aggregation response of* A. walkerae *to guanine*

From the data presented in Fig. 2 it can be clearly deduced that guanine serves as a potent attractant for both engorged and unfed 1st and 2nd stage nymphal ticks as well as for male and female imagines over a wide concentration range. Nymphal ticks of both stages, either unfed or replete, reacted extremely vigorously,

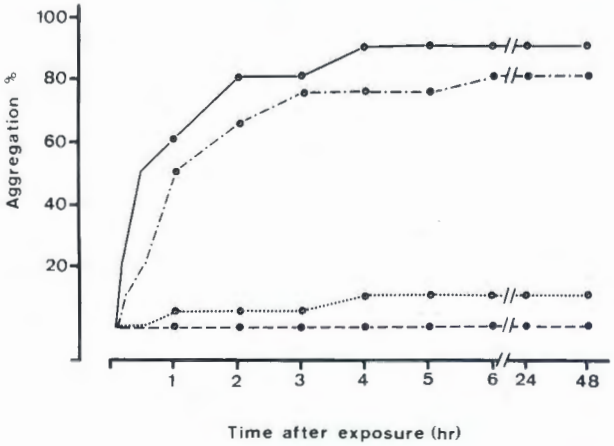

FIG. 1 Aggregation (%) of engorged male *Argas (Persicargas) walkerae* on filter paper discs impreganted with 30 $\mu\ell$ of female pheromone collected by volatilization

●——● hexane phase; ●-·-·-● diethyl ether phase;

●·····● aqueous phase from hexane-NaOH collection;

●– – –● aqueous phase from liquid air collection

[1] Merck, Darmstadt

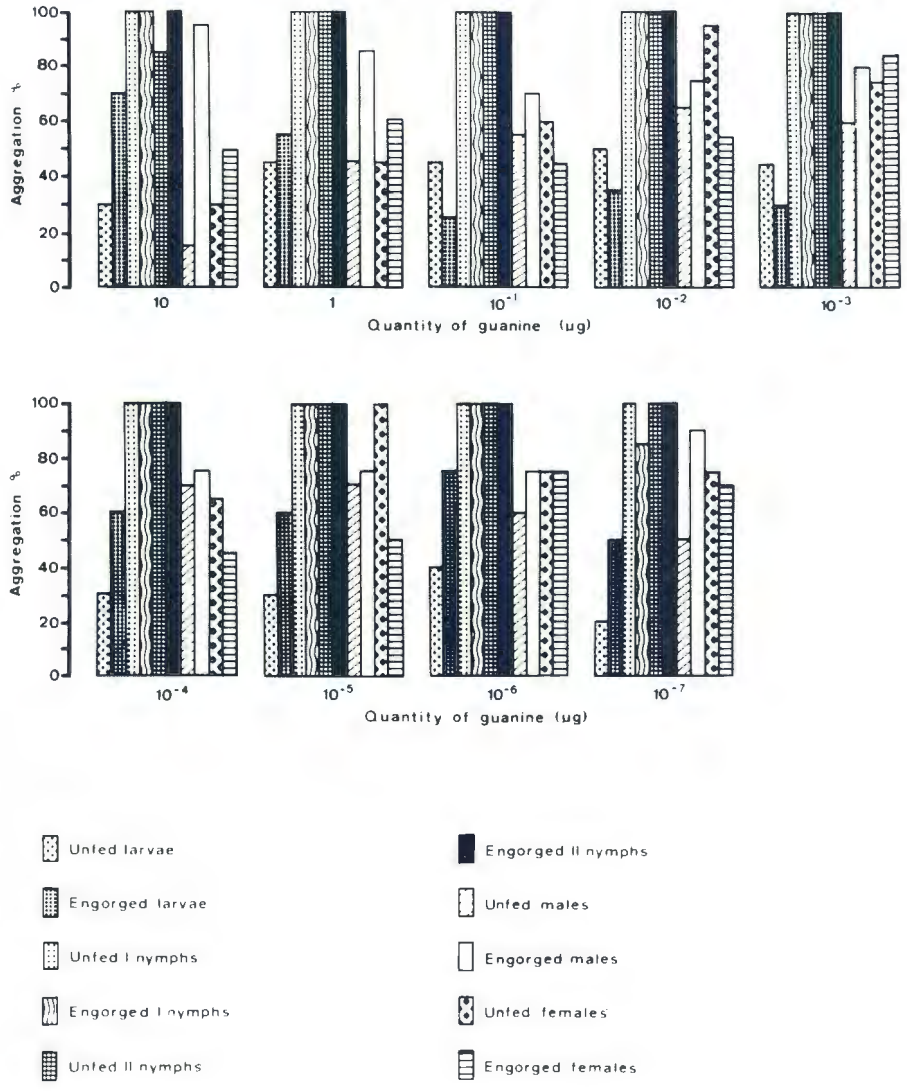

FIG. 2 Aggregation (%) of *Argas (Persicargas) walkerae* after 6 hours exposure to filter paper discs impregnated with guanine at various concentrations

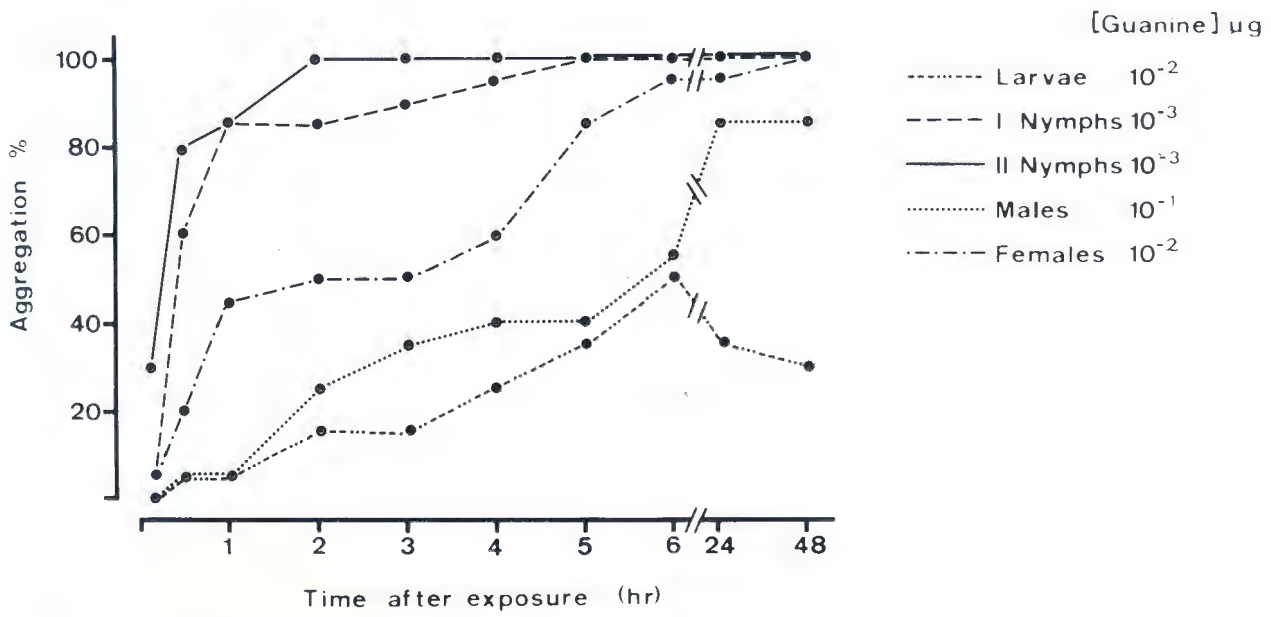

FIG. 3 Time-dependant aggregation of all stages of unengorged *Argas (Persicargas) walkerae* towards guanine at highly reactive concentrations

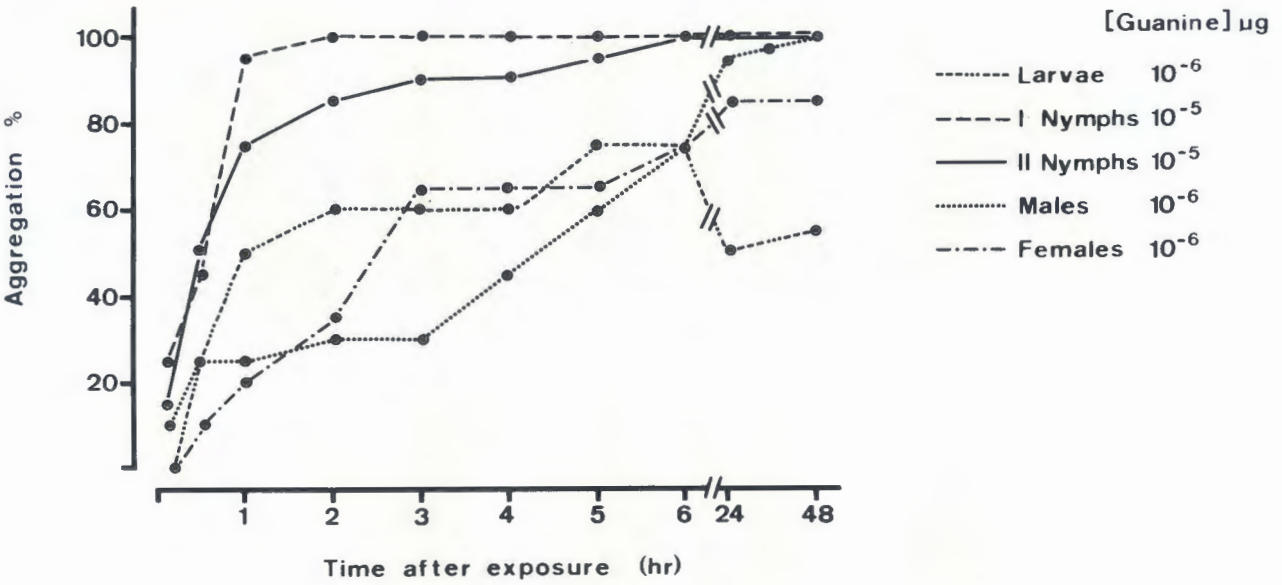

FIG. 4 Time-dependant aggregation of all stages of engorged *Argas (Persicargas) walkerae* towards guanine at highly reactive concentrations

with 100 % aggregation within 5–6 hours over almost the entire guanine concentration range tested. Engorged and unfed larvae, however, responded less readily, and demonstrated a greater dependence of migration on the guanine concentration.

Time-response curves (Fig. 3 & 4) indicate that both engorged and unfed 1st and 2nd stage nymphs respond most rapidly, whereas larvae, males and females, are much slower during the initial time period. In addition, both replete and unfed larvae show a slight tendency to disperse again after approximately 6 hours.

DISCUSSION

In the ecosystems of argasid ticks, intraspecific communication is *inter alia* regulated by chemical stimuli which, because they are information carriers, function as pheromones. In the case of *A. walkerae,* it has been demonstrated experimentally beyond doubt that during the entire premetamorphosal and moulting periods, as well as during the reproductive cycle, including preoviposition, embryonal development and larval hatching, stimulating pheromonal substances are produced and emitted. Furthermore, it has been shown that, irrespective of their degree of repletion, all postembryonal phases react to these pheromones to a greater or lesser extent, depending on the stage (Gothe & Kraiss, 1982a,b,c; Gothe, 1983b). The same pheromonal-dependent directing mechanisms also operate in any biotope in which this tick species happens to be present in an association. Both the initiation of movements and locality where they take place in the biotope are always determined by the female imagines. Their pheromonal signals first attract male ticks, after which the immature stages follow, probably because of a combined emmission and action of male and female pheromones (Gothe, Weck & Kraiss, 1984). The establishment and development of this association is, furthermore, a prerequisite for the regulation and maintenance of the abiotic conditions necessary for the viability and survival of larval ticks in particular and is therefore biologically significant (Gothe, Weck & Kraiss, 1984). However, as Gothe & Kraiss (1982a) postulated, a reaction to such stimulus, even over short distances, can only be guaranteed if these female pheromones, especially, are at least slightly volatile.

From the results obtained in the present work, it has now been proved that female pheromones are indeed volatile, since male imagines aggregate equally in response either to female-contacted filter paper discs (Gothe & Kraiss, 1982a) or to filter paper discs impregnated with volatiles drawn from the extra-integumental spaces surrounding female imagines and collected in hexane or liquid air. Accordingly, there seems no doubt that highly potent, volatile chemical substances are either involved in or exclusively responsible for the intersexual communication system of this tick species. These biosubstances, collected by passing air over intact living female *A. walkerea* ticks, are clearly pheromones as defined by Karlson & Lüscher (1959), since they are released extra-integumentally and male imagines react by migrating towards and subsequently aggregating on filter paper discs impregnated with them.

The only other studies on argasid ticks that can to some extent be compared are those on *Ornithodoros moubata* (Leahy, Karuhize, Mango & Galun, 1975; Leahy, 1979). This is because other investigations have involved only filter paper discs which had first been placed in direct contact with live ticks (Leahy, Vandehey & Galun, 1972, 1973; Leahy, Karuhize, Mango & Galun, 1975; Leahy, Sternberg, Mango & Galun, 1975; Leahy, 1979; Webb, 1979; George, 1981) or live ticks smeared with coxal fluid (Schlein & Gunders, 1981). Test ticks were then exposed to these pheromone sources. Although an olfactory response cannot be excluded in these investigations, perception of the pheromones was probably mainly gustatory. In an investigation in which the test ticks were separated by means of a screen from the impregnated filter paper discs, it could be shown that, with *O. moubata,* an olfactory response was possible over a distance of at least 0,5 cm (Leahy, Karuhize, Mango & Galun, 1975; Leahy, 1979).

That these results are contrary to our own analogous investigations may be due to species differences. They do not necessarily exclude the fact that the female pheromones of *A. walkerae* are volatile, as has clearly been proved by the isolation of these pheromones from effluent air, but rather emphasize the necessity of finding reliable bioassay methods. The negative responses obtained from the olfactometer may be primarily because

the ticks are disinclined to move upstream, not an expression of an accidental, exclusively gustatory, pheromone perception.

The chemical structure of pheromones of argasid ticks has not as yet been elucidated. nevertheless, through comparisons with and conclusions drawn from the investigations of Leahy, Vandehey & Galun (1973), Leahy, Karuhize, Mango & Galun (1975) and Leahy (1979), it can be deduced that 2 chemically different pheromones are involved in the communication system of this tick family. For *Argas (Persicargas) persicus* (Leahy, Vandehey & Galun, 1973; Leahy, Sternberg, Mango & Galun, 1975) and *O. moubata* (Leahy, Karuhize, Mango & Galun, 1975; Leahy, 1979) it was shown that the biosubstances under study remained active when they were extracted from impregnated filter paper discs with either physiological saline or water, but not with ether, benzene, acetone, pentane or methanol. The volatile pheromone of female *A. walkerae* can, however, be extracted with organic solvents.

In our investigations no aggregation was observed when test ticks were screened off by even very short distances from the source of stimulation. From deductions with respect to *A. persicus* and *O. moubata* (Leahy, Vanderhey & Galun, 1973; Leahy, Karuhize, Mango & Galun, 1975; Leahy, 1979), it can consequently be argued that filter paper discs impregnated by contact with the ticks bind 2 different pheromones. One of these pheromones is highly volatile and soluble in ether and hexane whereas the other is a stable, non-volatile substance which is soluble in water or physiological saline. The volatile pheromone most probably quickly loses its efficacy through rapid evaporation and is therefore detectable for a short period only after immediate exposure of ticks to the impregnated filter paper discs. There is also a possibility that the volatile pheromone is emitted in gaseous form, not as a solid or in a liquid from which it evaporates. The stable, non-volatile pheromone, however, remains active for days or even weeks and probably consists exclusively, or at least predominantly, of guanine, a natural excretory product of ticks (Hamdy & Sidrak, 1982).

In interactions between individuals of *A. walkerae*, guanine is known to induce migration towards and aggregation at the source of stimulation only. This phenomenon has already been mentioned in passing for soft ticks by Sonenshine, Silverstein & Rechav (1982). It has also been demonstrated for the closely-related mesostigmate mite *Dermanyssus gallinae* (Entrekin & Oliver, 1982) and also by Gothe, Weck & Kraiss (1984) for 1st stage nymphs of *A. walkerae*. Guanine is certainly not the only constituent of the pheromone because the response to it is lower than that to the naturally occurring pheromone. Furthermore, it can be deduced from its characteristics that it is a non-volatile substance (Chargaff & Davidson, 1955). Guanine can rather be regarded as a complementary substance, representing a highly potent fraction which initiates aggregation.

It may therefore be concluded, as was noted by Gothe, Weck & Kraiss (1984), that for the biological-chemical control of ticks the synthesis of the naturally occurring volatile pheromone is an important prerequisite. Only with the synthesized product, perhaps combined with acaricides, would it be possible to saturate the biotope. Then ticks that respond to the stimulus may either be killed immediately or at least be confused so that they cannot locate the natural source of pheromone or successfully trace the vertebrate host.

References

BROWNE, L. E., BIRCH, M. C. & WOOD, D. L., 1974. Novel trapping and delivery systems for airborne insect pheromones. *Journal of Insect Physiology*, 20, 183–193.

CHARGAFF, E. & DAVIDSON, J. N., 1955. The nucleic acids. Vol. I. New York: Academic Press.

ENTREKIN, D. L. & OLIVER, J. H., 1982. Aggregation of the chicken mite, *Dermanyssus gallinae* (Acari: Dermanyssidae). *Journal of Medical Entomology*, 19, 671–678.

GEORGE, J. E., 1981. The influence of aggregation pheromones on the behaviour of *Argas cooleyi* and *Ornithodoros concanensis* (Acari: Ixodoidea: Argasidae). *Journal of Medical Entomology*, 18 129–133.

GOTHE, R. & KRAISS, A., 1982a. Zur pheromonal induzierten Kommunikation im Entwicklungs- und Reproduktionszyklus von *Argas (Persicargas) walkerae* Kaiser und Hoogstraal, 1969. *Zentralblatt für Veterinärmedizin* B, 29, 540–557.

GOTHE, R. & KRAISS, A., 1982b. Zur Lokalisation der Pheromonemission und -perzeption bei *Argas (Persicargas) walkerae* Kaiser und Hoogstraal, 1969. *Zentralblatt für Veterinärmedizin* B, 29, 573–582.

GOTHE, R. & KRAISS, A., 1982c. On pheromone activity of *Argas (Persicargas) walkerae*. *Zentralblatt für Bakteriologie*, I. Abteilung *Referate*, 277, 123.

GOTHE, R., 1983a. Pheromones in ixodid and argasid ticks. Part 1: Ixodid ticks. *Veterinary Medical Review*, No. 1, 16–37.

GOTHE, R., 1983b. Pheromones in ixodid and argasid ticks. Part 2: Argasid ticks. *Veterinary Medical Review*, No. 2, 157–171.

GOTHE, R., WECK, P. & KRAISS, A., 1984. Zur pheromonal induzierten Kommunikation von *Argas (Persicargas) walkerae* und biologisch-chemischen Bekämpfung durch kombinierten Einsatz eines Pheromons und Pheromon-Analogons mit Flumethrin. *Zentralblatt für Veterinärmedizin* B. In press.

HAMDY, B. H., 1972. Biochemical and physiological studies of certain ticks (Ixodoidea). Nitrogenous excretory products of *Argas (Persicargas) arboreus* Kaiser, Hoogstraal & Kohls, and of other argasid and ixodid species. *Journal of Medical Entomology*, 9, 346–350.

HAMDY, B. H. & SIDRAK, W., 1982. Guanine biosynthesis in the ticks (Acari) *Dermacentor andersoni* (Ixodidae) and *Argas (Persicargas) arboreus* (Argasidae): fate of labelled guanine precursors. *Journal of Medical Entomology*, 19, 569–572.

KARLSON, P. & LÜSCHER, M., 1959. 'Pheromones': a new term for a class of biologically active substances. *Nature, London*, 183, 55–56.

LEAHY, M. G., VANDEHEY, R. & GALUN, R., 1972. Assembling in *Argas persicus*. *Abstracts of the 14th International Congress of Entomology, Canberra*, 271.

LEAHY, M. G., VANDEHEY, R. & GALUN, R., 1973. Assembly pheromone(s) in the soft tick *Argas persicus* (Oken). *Nature, London*, 246, 515–516.

LEAHY, M. G., KARUHIZE, G., MANGO, C. & GALUN, R., 1975. An assembly pheromone and its perception in the tick Ornithodoros *moubata* (Murray) (Acari: Argasidae). *Journal of Medical Entomology*, 12, 284–287.

LEAHY, M. G., STERNBERG, S., MANGO, C. & GALUN, R., 1975. Lack of specificity in assembly pheromones of soft ticks (Acari: Argasidae). *Journal of Medical Entomology*, 12, 413–414.

LEAHY, M. G., 1979. Pheromones of argasid ticks. In: RODRIGUEZ, J. G. (ed.) Recent advances in acarology, 2, 297–308. New York: Academic Press.

RECHAV, Y., PAROLIS, H., WHITEHEAD, G. B. & KNIGHT, M. M., 1977. Evidence for an assembly pheromone(s) produced by males of the bont tick, *Amblyomma hebraeum* (Acarina: Ixodoidea). *Journal of Medical Entomology*, 14, 71–78.

SCHLEIN, Y. & GUNDERS, A. E., 1981. Pheromone of *Ornithodoros* spp. (Argasidae) in the coxal fluid of female ticks. *Parasitology*, 82, 467–471.

SONENSHINE, D. E., SILVERSTEIN, R. M., COLLINS, L. A., SAUNDERS, M., FLYNT, C. & HOMSHER, P. J., 1977. Foveal glands, source of pheromone production in the ixodid tick *Dermacentor andersoni* Stiles. *Journal of Chemical Ecology*, 3, 695–706.

SONENSHINE, D., SILVERSTEIN, R. M. & RECHAV, Y., 1982. Tick pheromone mechanisms. *In:* OBENCHAIN, F.D. & GALUN, R. (eds.). Physiology of ticks, 439–468. Oxford: Pergamon Press.

WEBB, J. P., 1979. Host-locating behaviour of nymphal *Ornithodoros concanensis* (Acari: Argasidae). *Journal of Medical Entomology*, 16, 437–447.