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AN INVESTIGATION INTO THE EMISSION SITES OF THE VOLATILE PHEROMONE PRODUCED BY FEMALES OF *ARGAS (PERSICARGAS) WALKERAE*

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ABSTRACT

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By leaving open in turn the genital orifice, coxal apertures and anus, the present study has shown that engorged female *Argas walkerae* release at least 1 volatile pheromone mainly, or maybe exclusively, from the genital aperture. Although this aggregation-inducing pheromone is perceived by all post-larval stages, engorged males of *A. walkerae* react most strongly. A possible, but only slight, emission through the anus and the coxal aperatures is also indicated.

Most probably, this airborne biosubstance is the primary directing and orienting pheromone in the interrelations between members of this tick species.

INTRODUCTION

The various messenger compounds emitted by *Argas walkerae* into the extra-integumental space differ from each other both functionally and in chemical structure. At least 4 pheromones can be distinguished. One, which acts as an aggregation pheromone, is a stable, water-soluble, non-volatile compound that can be demonstrated only by contact-impregnation of filter paper discs (Gothe & Kraiss, 1982a,b; Gothe, 1983). This biosubstance, like the 2nd active compound, guanine, is emitted by all post-embryonal stages and the response to it is reciprocal, principally by means of the same reaction patterns (Kraiss, Gothe & Weck, 1983; Kraiss, Neitz & Gothe, 1984; Gothe, Weck & Kraiss, 1984; Neitz & Gothe, 1984). A 3rd stimulating substance produced by female imagines, which is also obtained only by contact-impregnation of filter paper discs, is most probably a sex pheromone because it has a specific attraction for male ticks (Gothe & Kraiss, 1982b,c). The 4th pheromone is a potent, volatile, unstable substance which is soluble in diethyl ether or hexane (Neitz & Gothe, 1984).

The emission sites of the non-volatile aggregation and sex pheromones have been established by selectively leaving open the anus, coxal apertures or genital opening, subsequent contact-impregnation of filter paper discs by the treated ticks and exposure of 2nd nymphal stage and male ticks to these discs. It was shown that the non-volatile aggregation-inducing biosubstance is released through the anus and possibly, but only very slightly, also through the coxal apertures. The sex pheromone is emitted through the genital orifice (Gothe & Kraiss, 1982b,c). The site from which the volatile, water soluble aggregation pheromone is released was not detected during these investigations.

The present study was undertaken, therefore, to determine which organs and surface structures of female *A. walkerae* are functionally involved in the production and emission, respectively, of the unstable, volatile and water-insoluble pheromone. The study was carried out by leaving open in turn the genital orifice, coxal apertures and anus and blocking the other body openings, then collecting and concentrating the messenger substances contained in the air drawn from the extra-integumental space surrounding the treated ticks. Futhermore, through separate evaluation of the aggregation intensity of the pre-imaginal stages as well as those of female and male imagines after they had been in contact with the pheromones, we tried to establish whether stage or sex-dependent demarcations existed and to what extent differences could be correlated with the site of emission.

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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. For the study of the localization of pheromone emission by replete female *A. walkerae*, the genital orifice, anus or coxal apertures of the ticks before infestation were blocked off with nail varnish either simultaneously or in various combinations so as to permit emission through 1 opening only. After engorgement, the ticks were inspected and freshly blocked. From these ticks volatile pheromones were collected.

Collection and bioassay of volatile pheromones of replete female A. walkerae

Two collection methods were used, both performed at 25 °C and 70 % R.H. In the 1st method (Browne, Birch & Wood, 1974; Neitz & Gothe, 1984), a stream of air was passed through a 40 cm^3 capacity glass receptacle containing 120 engorged females. The effluent air was trapped in a U-tube (total length: 16 cm and internal diameter: 10 mm) which was immersed in liquid nitrogen. A vacuum pump on the outlet of the U-tube was used to raise the liquid air that was formed above the liquid nitrogen level. Thus a continuous, regulated evaporation of the air on the outlet and condensation on the inlet side, as well as an airflow, were maintained. After 6 hours, the inlet became blocked with ice. The liquid air was then cautiously evaporated, the ice allowed to thaw and the U-tube contents salted with excess NaCl. After extraction with diethyl ether, the organic phase was bioassayed for the presence of pheromones as described below.

In the 2nd method (Sonenshine, Silverstein, Collins, Saunders, Flynt & Homsher, 1977; Neitz & Gothe, 1984), air was also drawn over 120 engorged female ticks and then passed through 2 25 cm^3 capacity glass vials, each containing 5 cm^3 hexane and 5 cm^3 NaOH. To minimize evaporation, the vials were cooled with ice. After 4 hours, the hexane-NaOH mixtures were combined and neutralized with 0,5 M HCl. After vigorous shaking the hexane phase was bioassayed for the presence of pheromones. Control collections were performed for the above methods by replacing the ticks with a small filter paper disc, coated with nail varnish.

In the bioassay of organic phases for the presence of volatile pheromones, sterile filter paper discs[1] 1,8 cm in diameter were separately impregnated with 0,1 cm^3 of each phase. Each disc was placed in a Petri dish 10 cm in diameter, 0,5 cm from its rim. Three clean discs were

[1] Schleicher and Schüll, Marke Selecta

TABLE 1 Aggregation (%) of post-larval stages of *Argas (Persicargas) walkerae* on filter paper discs impregnated with diethyl ether-trapped volatile pheromones from conspecific, replete female ticks

Body openings blocked	Emission possibility	Aggregation (%)*							
		1st stage nymphs		2nd stage nymphs		Males		Females	
		Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed
—	Genital orifice Coxal aperture Anus	25	45	60	100	50	85	55	75
Coxal apertures Anus	Genital orifice	20	60	60	80	60	90	50	70
Genital orifice Coxal apertures	Anus	30	20	30	60	20	40	30	40
Genital orifice Anus	Coxal apertures	20	40	35	30	20	40	25	30
Genital orifice Coxal apertures Anus	—	20	20	20	10	10	20	10	10
Control: nail varnish in the absence of donor ticks		5	0	10	0	10	5	10	10

* Number of ticks per assay: 20

TABLE 2 Aggregation (%) of post-larval stages of *Argas (Persicargas) walkerae* on filter paper discs impregnated with hexane-trapped volatile pheromones from conspecific, replete female ticks

Body openings blocked	Emission possibility	Aggregation (%)*							
		1st stage nymphs		2nd stage nymphs		Males		Females	
		Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed
—	Genital orifice Coxal aperture Anus	40	50	50	95	50	95	50	60
Coxal apertures Anus	Genital orifice	20	30	60	65	60	90	60	60
Genital orifice Coxal apertures	Anus	50	20	30	40	50	40	40	30
Genital orifice Anus	Coxal apertures	20	25	25	20	10	35	10	20
Genital orifice Coxal apertures Anus	—	10	10	20	20	20	20	10	0
Control: nail varnish in the absence of donor ticks		0	0	0	0	0	0	0	0

* Number of ticks per assay: 20

arranged symmetrically in the dish and a collection of 10 ticks, either engorged or unengorged of all post-larval stages, was placed in the centre. A total of 20 ticks was tested per assay. The dishes were then incubated at 27 °C and 75–85 % R.H. in the dark. The location of the ticks was observed at 1 hour intervals for 5 hours and again after 24 hours.

RESULTS

From the data presented in Tables 1 and 2, it is evident that *A. walkerae* females emit a volatile aggregation-inducing pheromone either mainly or exclusively through the genital aperture. This pheromone was perceived by all post-larval stages, particularly after engorgement. Engorged males, however, showed the highest response, with a 90 % aggregation on filter paper discs impregnated with either diethyl ether or hexane-trapped volatiles. The corresponding value for unfed males was 60 %. Engorged 2nd stage nymphs reacted with 80 % and 65 % respectively, whereas fed females responded with 70 % and 60 % towards the ether and hexane phases respectively. Up to 20 % weaker responses were recorded for these unengorged stages. The 1st stage nymphs, either fed or unfed, reacted at least 20 % less readily, compared with other stages.

A possible, but only slight, emission through the anus is also indicated, since responses of 60 % for replete 2nd stage nymphs and between 20 % and 50 % for all other stages, fed or unfed, was observed in the case of donor ticks in which the coxal apertures and genital orifice were blocked.

A slight emission through the coxal apertures was also evident. Although in general, similar aggregation percentages were produced with pheromones obtained from totally blocked ticks and from ticks in which the anus and genital orifice had been blocked, engorged post-larval stages reacted with between 30 % and 40 % towards volatiles collected in the ether phase obtained from ticks with open coxal apertures.

DISCUSSION

The first and most important conclusion that can be drawn from our investigations on the volatile pheromone of *A. walkerae* is that this biosubstance is mainly, or possibly even exclusively, emitted through the genital aperture. This water-insoluble, unstable, airborne pheromone induces aggregation by all the post-larval stages, particularly the engorged males. It is most probably the pheromone that primarily controls the interrelations between members of this tick species in their biotope.

This assumption is substantiated by the results obtained both in this study and also by Gothe *et al.* (1984) in their investigations involving a simulated biotope. It was shown that replete females first determine the colonizing point in the natural habitat and then release their aggregating pheromone. This stimulates the males to migrate towards the females. Later they are followed by the nymphae. Ultimately, therefore, a multistadial association is established (Gothe *et al.*, 1984).

The demonstrated emission of the volatile pheromone through the genital opening, anus and possibly through the coxal apertures, by selectively leaving these orifices open 1 at a time, is difficult to interpret. It either reflects definite quantitatively graduated, extra-integumental liberation of the same pheromone through all 3 body openings, or it is an expression of chemically different pheromones. Surface contamination of the female ticks, resulting in a persistent residual activity by only 1 pheromone, which is emitted solely through the genital aperture, should also be considered. The latter possibility seems the most likely because a substantially higher aggregation percentage is induced by ticks that could only emit the pheromone through this opening.

The distinctly stronger reaction by replete male ticks towards the volatile pheromone emitted through the genital aperture of female imagines can also be discussed hypothetically only. It can be explained either by a higher perception ability by sexually competent male ticks, enabling them to copulate immediately, or by a pheromone mixture. In the latter case 1 component may function as an aggregation pheromone which attracts nymphae as well as males, and the other as a sex pheromone which selectively stimulates replete, sexually active males.

Functional differentiation and assignment as well as chemical characteristics cannot be deduced from the present investigation. Elucidation of these aspects were not intended in the studies and are reserved for exact chemical investigations and specific bioassays with isolated and synthetic pheromones.

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