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RESEARCH COMMUNICATION

A BIOASSAY METHOD FOR THE PHEROMONE(S) OF THE BONT TICK *AMBLYOMMA HEBRAEUM* KOCH

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ABSTRACT

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A bioassay was developed which enabled the detection of the pheromones of the bont tick (*Amblyomma hebraeum* Koch) within 2 hours with a reproducibility of 82 ±13%. Dried, solvent-extracted ticks were glued onto the shaved backs of rabbits. Those ticks to which pheromone extract was subsequently applied strongly attracted females of the same species.

In a 2-way choice test a cork disc impregnated with pheromone extract was even more attractive to female ticks than a treated dried tick.

Résumé

TECHNIQUE DE BIOTEST POUR LA (LES) PHÉROMONE(S) DE LA TIQUE AMBLYOMMA HEBRAEUM *KOCH*

On a mis au point un biotest qui permet de déceler les phéromones de la tique Amblyomma hebraeum *Koch dans les deux heures avec une reproductibilité de* 82 ± 13%. *Des tiques desséchées, ayant subi l'extraction par des solvants, ont été collées sur des dos rasés de lapins. Celles de ces tiques auxquelles on a ensuite appliqué de l'extrait de phéromone ont exercé une forte attraction sur des femelles de la même espèce.*

Dans une expérience de choix à deux possibilités, un disque de liège imprégné d'extrait de phéromone s'est même révélé plus attirant pour les tiques femelles qu'une tique desséchée et traitée.

INTRODUCTION

When unfed female *Amblyomma* ticks are placed on a host on which males have already been attached for some days, they quickly attach alongside the males (Gladney, Ernst & Grabbe, 1974; Rechav, Parolis, Whitehead & Knight, 1977). Rechav *et al.* (1977) have also shown that pheromone extracts prepared by washing *A. hebraeum* male ticks with organic solvents are highly attractive to males and females of the same species. They obtained approximately 80% attachment of females within 2 hours in treated areas on the shaved backs of rabbits and scrota of calves. On the other hand, the authors found that when an aliquot of *A. hebraeum* pheromone prepared by condensation of the volatile components of male ticks was placed on the back of a rabbit, onto which unfed *A. hebraeum* females were released, the ticks either moved about or remained stationary around the site where the pheromone had been applied for up to 18 hours before attaching. They were clearly attracted by the pheromone, but appeared to need a further stimulus to induce rapid attachment. As it was thought that the required stimulus was a tactile one, the conventional bioassay for detecting the presence or absence of pheromone activity was revised and the following procedure adopted.

BIOASSAY METHOD

Pheromone collection

The volatile components with which pheromone activity is associated were collected over a period of 4 days from male ticks which had fed for at least 7 days, using a cryogenic trap similar to that used by Browne, Birch & Wood (1974). The ether washing of the trap contents was dried over anhydrous magnesium sulphate and concentrated in a rotary evaporator at −10 °C until the concentration of the volatile components in the ether extract was equal to that of approximately 100 ticks/mℓ. This solution constituted the pheromone extract referred to below.

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Bioassay of the pheromone extract

Dead *A. hebraeum* males, which had been preserved in 80% ethyl alcohol for several months, were extracted approximately 100 times with chloroform in a 100 mℓ Soxhlet extractor and then dried overnight at 90 °C. Two of the dried ticks were attached 50 mm apart onto the shaved back of a rabbit, using a quickset clear epoxy resin glue. The rabbit was then left for an hour to allow the glue to dry. A 100 µℓ aliquot of pheromone extract was placed on one of the dried ticks as well as on the skin of the rabbit, 50 mm from each of the dried ticks, this forming the apex of an equilateral triangle. Unfed *A. hebraeum* females were released onto the rabbit which had been fitted with a canvas jacket to prevent the ticks from escaping. After 2 hours the rabbit was inspected and it was found that 100% of the females had attached around the dried tick that had been treated with pheromone extract (Fig. 1). The results of the bioassay, which was repeated with a number of extracts are shown in Table 1.

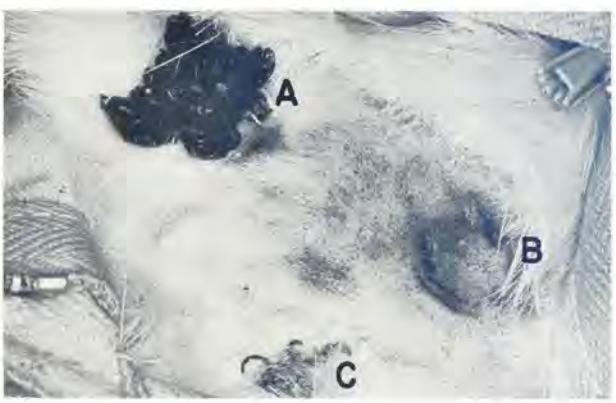

FIG. 1 Bioassay of pheromone extract No. 1
A Unfed females attached around pheromone-treated dried tick
B Pheromone applied to skin of host
C Untreated dried tick

TABLE 1 Results of 9 bioassays using the method described

Pheromone extract No.	Number of unfed *A. hebraeum* females					Percentage unfed females attached around treated tick within 2 h
	Used	Attached		Unattached		
		Around treated dried tick	Around treated area on skin of host	Sitting on top of ticks attached around treated dried ticks	Not sitting on top of attached ticks	
Control: ether only	20	0	0	0	20	0
1.....	20	20	0	0	0	100
2.....	20	17	0	3	0	85
3.....	10	8	0	2	0	80
4.....	10	8	0	2	0	80
5.....	20	15	0	5	0	75
6.....	20	19	0	1	0	95
7.....	20	16	0	4	0	80
8.....	20	11	1	0	8	55
9.....	16	14	0	2	0	88
Mean ±SE	82 ±13					

In one case, of the 20 unfed females used, 20 were attached around the treated dried tick. More often, however, not all the unfed females were attached but were found sitting on top of those attached.

In a 2-way choice test, in which a cork disc (10 mm diam, 3 mm thick) impregnated with 100 $\mu\ell$ pheromone extract was tested against a similarly treated dried tick, 80% of the unfed females attached around the cork disc and 20% around the dried tick.

It was also shown that extracted, dried, female ticks could be used instead of the males.

Discussion

A rapid and reproducible bioassay for detecting the pheromone(s) of the bont tick *Amblyomma hebraeum* Koch was developed. This practical method enabled the detection of bont tick pheromone(s) within 2 h with a reproducibility of 82 ±13%. The method involves the use of a chloroform-extracted tick or cork disc impregnated with pheromone(s). The use of treated discs, which allow a slow release of the pheromone could very likely increase the effective period of pheromone-pesticide mixtures in field trials beyond the 4 days reported by Rechav & Whitehead (1978).

The rapid attachment of the unfed females around the pheromone-treated dried tick in preference to the pheromone-treated area on the skin of the host clearly indicated that some form of tactile stimulus is necessary. This would also explain the observation of Rechav *et al.* (1977) that females required more time for attachment than males on treated hosts. In the natural situation this stimulus is supplied by the male tick, but in an artificial situation, where this stimulus is not present, the results obtained may be spurious. The nature of the tactile stimulus does not appear to be very specific, since pheromone-treated cork discs proved more attractive to the unfed females than treated dried ticks. This is probably due to the fact that the pheromone is released from the cork disc more slowly and over a longer period. On the other hand, the rapid attachment of unfed females to areas treated with male washings (Rechav *et al.*, 1977) suggests that a separate non-volatile component of the pheromone(s) may also be involved.

Acknowledgements

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