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USE OF CARBON DIOXIDE AND NATURAL OR SYNTHETIC AGGREGATION-ATTACHMENT PHEROMONE OF THE BONT TICK, AMBLYOMMA HEBRAEUM, TO ATTRACT AND TRAP UNFED ADULTS IN THE FIELD

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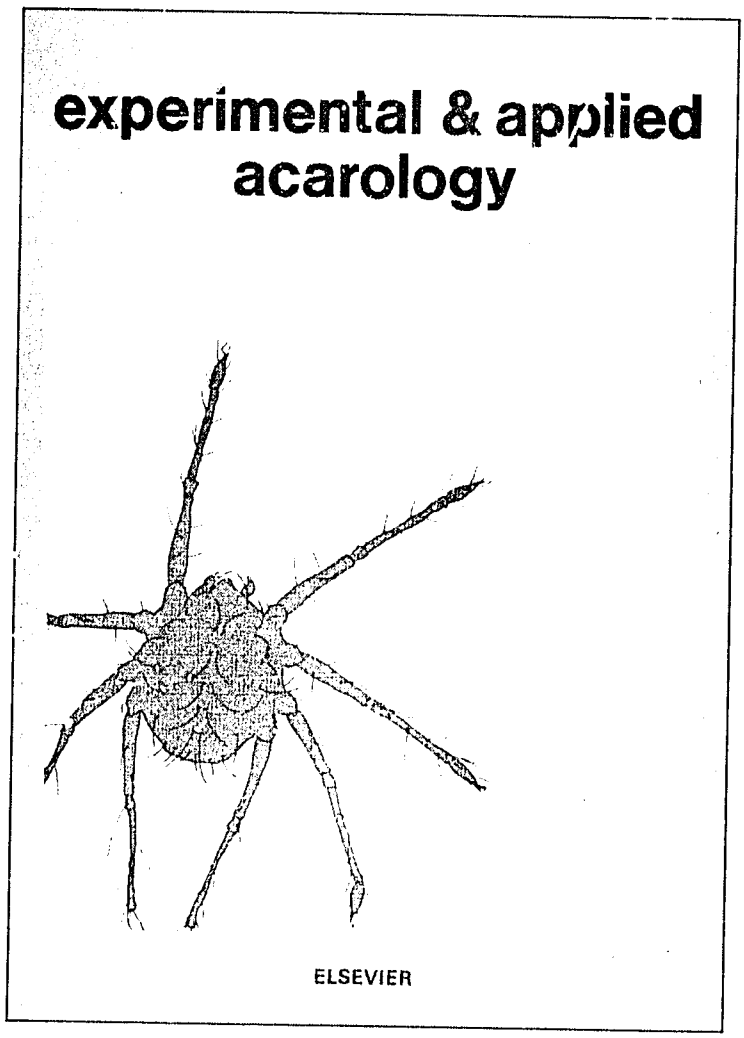

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Use of Carbon Dioxide and Natural or Synthetic Aggregation-Attachment Pheromone of the Bont Tick, *Amblyomma hebraeum*, to Attract and Trap Unfed Adults in the Field

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ABSTRACT

Norval, R.A.I., Butler, J.F. and Yunker, C.E., 1989. Use of carbon dioxide and natural or synthetic aggregation-attachment pheromone of the bont tick, *Amblyomma hebraeum*, to attract and trap unfed adults in the field. *Exp. Appl. Acarol.*, 7: 171-180.

It was determined previously that unfed adults of *Amblyomma hebraeum* become active in response to carbon dioxide but are not attracted by it. We have now shown in field experiments that males and females which are activated by carbon dioxide are attracted to the aggregation-attachment pheromone produced by males after a period of feeding on the host. Unfed adults were attracted to *o*-nitrophenol, a component of the aggregation-attachment pheromone of *Amblyomma variegatum*, pre-fed males of *A. hebraeum*, and extracts of such males. Live males were more attractive than the extracts which, in turn, were more attractive than *o*-nitrophenol. The ticks were attracted to sources of *o*-nitrophenol from as far away as 11 m in 90 min. Simple traps, baited with carbon dioxide and aggregation-attachment pheromone (from pre-fed males or extracts of pre-fed males), were used to capture unfed adults that had been released in the field. The response of unfed adults to carbon dioxide and the aggregation-attachment pheromone presents an obvious host-location mechanism and a means for the ticks to discriminate between suitable hosts (those with males already attached) and potentially unsuitable hosts (those with no males attached).

INTRODUCTION

Heartwater, caused by *Cowdria ruminantium*, is regarded as one of the most important tick-borne diseases of domestic ruminants in southern Africa (Van

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de Pypekamp and Prozesky, 1987). It is transmitted by several species of the genus *Amblyomma*, with the bont tick, *A. hebraeum* Koch, 1844, being the main southern African vector.

At present, little is known about the epidemiology of heartwater, including infection rates in vector populations. The main reason for the latter has been our inability to sample unfed nymphs and adults of African *Amblyomma* species. These ticks are not collected by conventional methods such as flagging, blanket-dragging or static carbon-dioxide traps. However, it has recently been shown that adults and nymphs of *A. hebraeum* respond to carbon dioxide by emerging from shelter beneath soil and litter to become active on the soil surface (Norval et al., 1987, 1988). The ticks are not attracted to the source of the carbon dioxide but, when active, adults can be collected from the soil surface by hand (Norval et al., 1987) and the less conspicuous nymphs by either vacuum or flag-sampling (Norval et al., 1988). Hess and de Castro (1986) have shown that unfed females of the closely related tropical bont tick, *A. variegatum*, are attracted to *o*-nitrophenol, a component of the male-produced aggregation-attachment pheromone (Schöni et al., 1984).

In view of the above findings, we hypothesized that host location in adults, and possibly nymphs, of *A. hebraeum* involves two main responses: firstly, a response to carbon dioxide initiates searching activity in the ticks but is non-directional, and secondly, a directional response to components of the aggregation-attachment pheromone and/or other host odours.

In the present study we tested the responses of adults of *A. hebraeum* in the field to carbon dioxide plus the components of the aggregation-attachment pheromone of *A. variegatum* described by Schöni et al. (1984; *o*-nitrophenol, methyl salicylate and pelargonic acid), the aggregation-assembly pheromone of fed males of *A. hebraeum*, and extracts of such males. Simple attractant traps for collecting adults of *A. hebraeum* in the field also are described.

MATERIALS AND METHODS

The work was carried out in April and August 1987 at Mbizi Quarantine Area in southeastern Zimbabwe, previously described by Norval et al. (1987). Different methods were used to test the attractiveness of the various compounds or mixtures of compounds to adult ticks.

Candles

Compounds of the *A. variegatum* aggregation-attachment pheromone (Schöni et al., 1984) and *o*-nitrophenol alone were incorporated at a concentration of 0.05% into slow-burning candles* and used in the initial experiments in April 1987.

*Supplied by International Flavours and Fragrances Inc., 1515 Highway 36, Union Beach, NJ 07735, U.S.A.

Individual candles were covered with large metal cans (diameter 18 cm) in which ventilation holes had been punched, and were placed on wooden platforms in the centres of 1×1-m flannelette squares whose edges were covered with soil. Known numbers of laboratory-reared adults of *A. hebraeum* were released at various distances from the candle traps, and the numbers reaching the flannelette squares were recorded at fixed time-intervals after the candles were lit.

Ticks released at different distances from the candles were marked with different-coloured fluorescent dusts to enable us to identify them (Butler et al., 1984). Where carbon dioxide was used in association with the candles, blocks of dry ice (approximately 0.25 kg) were placed on 0.5-m-high concrete bricks next to the candles.

Carbon dioxide passed over fed male ticks and other compounds and mixtures

Fed males of *A. hebraeum* were used as a natural source of the aggregation-attachment pheromone. This, and the following experiments, were performed in August 1987. Ticks were removed by hand from a herd of undipped cattle at the experimental site and used on the day of collection. Carbon dioxide from a compressed-gas cylinder was blown over batches of approximately 50 males

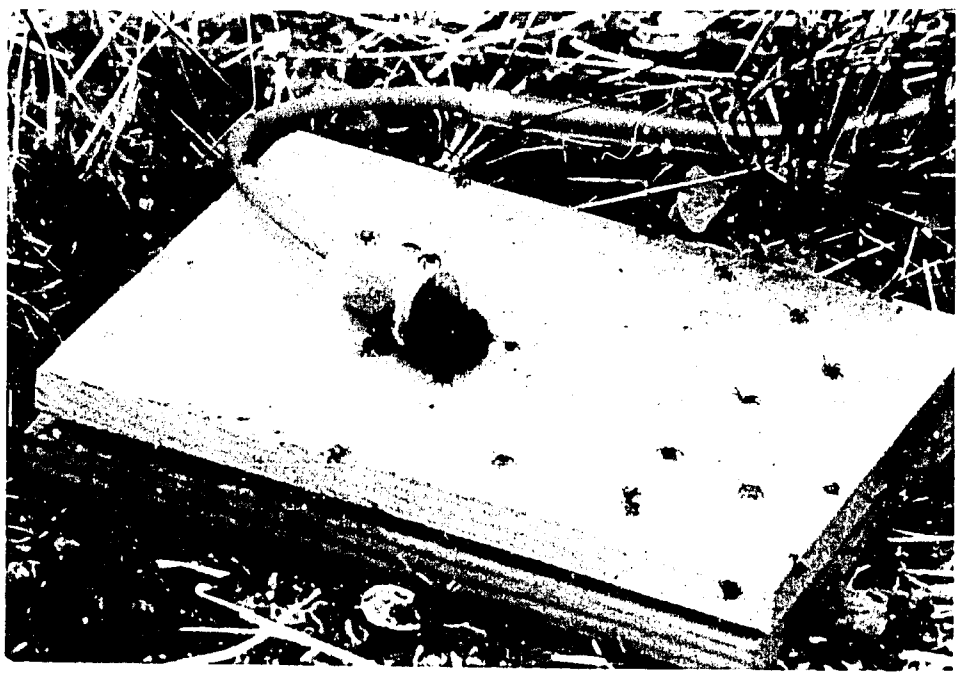

Fig. 1. Unfed *Amblyomma hebraeum* ticks attracted to a stream of carbon dioxide passing over partially fed male ticks held in plastic vial.

TABLE 1

Mixtures of compounds tested for attractiveness to unfed adults of *A. hebraeum* relative to fed males in Experiment 3

Mixture	Components	mg/ml in final dilution*
A	*o*-nitrophenol	4.0
	methyl salicylate	2.0
	pelargonic acid	1.6
B	*o*-nitrophenol	4.0
	methyl salicylate	2.0
	pelargonic acid	1.6
	benzyl alcohol	8.0
C	*o*-nitrophenol	4.0
	methyl salicylate	2.0
	pelargonic acid	1.6
	benzyl alcohol	8.0
	hexane extract from 1 l of bovine blood dried to 2 ml and diluted to 10 μl	–
D	*o*-nitrophenol	4.0
	methyl salicylate	2.0
	pelargonic acid	1.6
	benzyl alcohol	8.0
	guanine	2.0
E	*o*-nitrophenol	—
F	Extracts of fed males of *A. hebraeum***	—

*All components diluted in diethylether: hexane (1:1) and applied in 5 μl amounts.
**Ten ml of diethylether: hexane (1:1) were used to extract pheromones from 50 prefed males.

held in plastic vials with gauze-covered stoppers (Fig. 1). The rate of gas flow from the cylinder was adjusted so as to be barely audible. The attractiveness of these vials was compared with that of vials in which strips of filter paper that had been treated with 5-μl amounts of 1:100 dilutions of various compounds and mixtures of compounds (Table 1) in diethylether: hexane (1:1) (DEE:H), had been placed. In another experiment, the attractiveness of an extract of 50 fed males was compared with 6 μl *o*-nitrophenol diluted 1:100. The extract was made by soaking 50 fed males for 24 h in 10 ml DEE:H and then allowing 5 μl of the extract to evaporate on a piece of filter paper.

For testing, the vials were connected by a Y-tube to a CO_2 cylinder and placed 2 m apart on wooden platforms (600 cm^2) resting on the ground in areas that had been heavily seeded with adult ticks (approximately 150 adults released 3

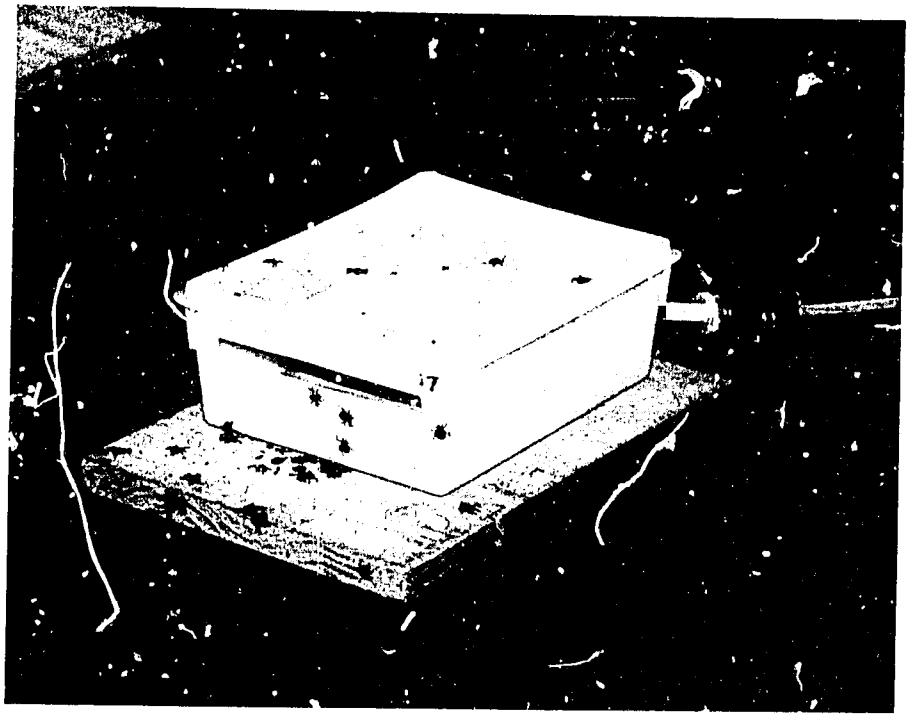

Fig. 2. Tick trap made from a plastic refrigerator box connected to apparatus shown in Fig. 1. Ticks entering slot in front of box are anaesthetized by the CO_2.

m downwind). The numbers of adults arriving at the two treatments were recorded every 30 min for 90 min and compared with those arriving on a blank wooden platform between them.

Trap

The tick trap, modified from Butler et al. (1984), consisted of a 22×18×8-cm plastic 'lunch box' through which CO_2 was blown after it had passed over approximately 50 fed males, or an extract of 50 fed males. The attracted ticks entered the box by a 1-cm-wide slit across one end (Fig. 2) and tended to be anaesthetized by CO_2 upon entering. During testing, ticks were collected every 30 min for 90 min in, on, or in the immediate vicinity of the traps.

RESULTS

Experiments were conducted during daylight hours when the soil temperature was in the range 20–35 °C and wind activity was low. The experiments are discussed below in the order in which they were conducted.

Experiment 1

Fifty marked adults of *A. hebraeum* were released, in the absence of CO_2, 3 m downwind from a candle emitting the synthetic *A. variegatum* aggregation-attachment pheromone. The ticks remained largely inactive and, after 60 min, none had reached the flannelette square on which the candle was located. A block of dry ice was then placed next to the candle, causing an immediate increase in tick activity, and within 30 min 6 (12%) had reached the flannelette square.

In the presence of carbon dioxide and the pheromone, the ticks were observed to quest periodically and then to move towards the source of the compounds. Questing involved the lifting and lateral movement of the forelegs to locate the source of the odours, and was frequently observed when ticks were on small elevations such as sticks, rocks or dried cow-pats.

Experiment 2

Four *o*-nitrophenol candles were set up at 10-m intervals across the prevailing wind. Adult ticks marked with a different-coloured dust for each release point were released downwind at distances of 1, 6 and 11 m from each candle. The numbers of adults released per candle were 100 at 1 m, 25 at 6 m and 25 at 11 m. Blocks of dry ice were placed immediately behind the lit candles at the start and the numbers of adults on the flannelette squares were recorded every 30 min for 150 min (Table 2). The ticks released from 1 and 6 m were first recorded on the flannelette squares at 30 min, and those released from 11 m at 90 min. The proportion of ticks recovered was very much higher from those released from 1 m (14–34%) than from those released from 6 m (0–24%) or 11 m (0–8%). The experiment was started at 0700 h and terminated at 0930

TABLE 2

Recovery of adults of *A. hebraeum* in Experiment 2 where marked ticks were released at different distances from 4 candles emitting *o*-nitrophenol*

Time (min)	Mean no. adults recovered from different release points. (Range in parentheses)		
	1 m	6 m	11 m
30	14.5(8-21)	0.5(0-1)	0.0
60	17.3(12-22)	2.5(0-4)	0.0
90	23.3(14-34)	4.5(3-6)	0.5(0-2)
120	16.5(9-24)	4.0(3-6)	0.8(0-1)
150	15.0(9-22)	3.3(2-5)	0.5(0-1)

No. ticks released per candle: 100 at 1 m, 25 at 6 m and 25 at 11 m.
*Blocks of dry ice were placed upwind of the candles. The experiment was run from 0070 until 0930 h on 23 April 1987.

h, but there was very little adult activity after 0830 h when soil temperatures were as high as 35°C.

Experiment 3

In this series the attractiveness of pre-fed males of *A. hebraeum* was compared with 6 mixtures of compounds listed in Table 1 that were known or suspected to be attractive to the ticks.

The ticks were attracted to all of the mixtures A–F but not nearly to the extent that they were attracted to the fed males (i.e., natural pheromone). In these choice experiments, there tended to be a 'winner take all' factor as clumps of ticks formed at the source of the most attractive mixture of compounds, and the ticks generally ceased 'searching' behaviour once they joined a clump. The recovery rate of released ticks at the source of the carbon dioxide/fed-male pheromone release source was high, ranging from 28.7% to nearly 100%.

TABLE 3

Numbers of unfed adults of *A. hebraeum* attracted to sources of carbon dioxide passed over fed males, compared to sources of carbon dioxide passed over mixtures of the various compounds listed in Table 1 and blanks

Time (min)	Mixture	Mixture $+CO_2$	Blank	Fed males $+CO_2$
30		7	0	13
60	A	9	0	33
90		5	0	46
30		15	0	64
60	B	8	0	101
90		7	0	127
30		16	0	24
60	C	15	0	36
90		18	0	47
30		1	3	48
60	D	4	2	86
90		1	0	163
30		3	0	11
60	E	10	0	26
90		6	0	43
30		18	0	54
60	F	15	1	68
90		11	0	71

TABLE 4

Numbers of unfed adults of *A. hebraeum* attracted to a source of carbon dioxide passed over an extract of fed males compared to a source of carbon dioxide passed over *o*-nitrophenol and a blank

Time (min)	Fed-male extract $+CO_2$	Blank	*o*-nitrophenol $+CO_2$
30	35	0	14
60	38	1	10
90	49	0	9

Experiment 4

The design was the same as in experiment 3 except that an extract of fed males was used. Its attractiveness was compared with that of *o*-nitrophenol. Both attracted adults, but greater numbers aggregated around the vial containing the fed male extract (Table 4).

Traps

An experiment on the recovery of unfed adults using traps was repeated on four occasions. On each occasion 100 marked ticks were released 5 m downwind of each trap. The percentages recovered were 30, 47, 61 and 83. The lowest recoveries were in cool (20–22°C), fairly windy early morning conditions, and the highest recoveries were in warm (25–27°C), calm afternoon conditions.

In a comparative experiment on the percentage recovery of ticks from traps baited by fed males and extracts of fed males, two traps (appropriately baited) were set up 2 m apart, and 100 marked adults were released 5 m downwind of each. The recovery of ticks from the trap baited with fed males was 52% while that from the trap baited with extract of fed males was 19%.

DISCUSSION

This study showed that adults of *A. hebraeum* are not attracted to a pheromone source in the absence of carbon dioxide. This supports the initial hypothesis that activity on the soil surface is dependent on the presence of carbon dioxide but that the directional response is determined by a male-produced aggregation-attachment pheromone. In nature, therefore, the ticks should become active in the presence of large mammals, which give out large amounts of carbon dioxide (Norval et al., 1987), but which will be attractive only if they have pheromone-emitting fed males attached to them. This is apparently an adaptation to prevent loss of adults through attachment to unsuitable hosts (e.g., effective groomers). It will also mean that the ticks will be less likely to attach to cattle that are regularly treated with acaricides, and hence male-free, than untreated, infested cattle. Presence of the pheromone also brings the sexes

together and ensures that they attach on parts of the host that are suitable for feeding and are protected from grooming.

Fed males of *A. hebraeum*, emitting the complete aggregation-attachment pheromone and possibly host odours, were extremely attractive to unfed males and females of the species. Ether: hexane extracts of fed males were less attractive than the males themselves, but were more attractive than *o*-nitrophenol. There was no apparent difference between the attractiveness of *o*-nitrophenol alone or mixed with the other components of the synthetic *A. variegatum* aggregation-attachment pheromone (Schöni et al., 1984). This supports the findings of Hess and de Castro (1986) on *A. variegatum*. The addition of other substances, including benzyl alcohol, guanine and bovine blood extract, to the aggregation-attachment pheromone mixture also failed to increase its attractiveness. It is possible that the greater attractiveness of fed males is due to the presence of another substance or substances which are not soluble in ether: hexane, although factors relating to concentration could also be of importance.

The response of adults of *A. hebraeum* to *o*-nitrophenol, a known component of the *A. variegatum* aggregation-attachment pheromone, indicates the similarity of the pheromone systems between these species. Interspecific responses to the aggregation-attachment pheromones of the species have already been recorded by Norval and Rechav (1979) and Rechav et al. (1982). It is thus likely that both the response of *A. variegatum* to carbon dioxide and its method of host location are similar to those of *A. hebraeum*.

This study has provided a method for sampling field populations of *A. hebraeum*, and probably related *Amblyomma* species, which should be of value in future ecological and epidemiological studies. The use of the aggregation-attachment pheromone of *A. hebraeum* to increase attachment to acaricide-treated areas of hosts has already been described by Rechav and Whitehead (1978). It may now also be possible to consider the use of carbon dioxide and pheromone-baited traps to assist in the eradication of *Amblyomma* species from small areas following introductions (e.g. *A. variegatum* from certain Caribbean islands).

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