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INTERSPECIFIC ATTRACTION TO MALE-PRODUCED PHEROMONES OF TWO SPECIES OF  
AMBLIOMMA TICKS (ACARI: IXODIDAE)

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Yunker, C. E., Andrew, H. R., Norval, R. A. I. and Keirans, J. E. 1990. Interspecific Attraction to Male-Produced Pheromones of Two Species of Amblyomma Ticks (Acari:Ixodidae). J. Insect Behav. 3:557-555.

**ABSTRACT:-** Adults of *Amblyomma hebraeum* Koch and *A. variegatum* (Fabricius), both important vectors of human and animal diseases (e.g., heartwater disease of ruminants), were tested for their degree of attraction to the aggregation-attachment pheromones (AAP's) of either species. In selected field sites of Zimbabwe, marked adults were released 4 meters downwind of filter paper targets treated with conspecific or heterospecific AAP and CO<sub>2</sub> vaporizing from dry ice, or of untreated targets and CO<sub>2</sub>. The numbers of ticks migrating to the targets in 1 hour were counted and removed from the experiment. The AAP's of both species were markedly attractive to adults of either species. Up to 81.4% of ticks migrated to the heterospecific pheromones, up to 80% migrated to conspecific pheromones, while not more than 12.5% migrated to CO<sub>2</sub> alone. No statistically significant differences in responses between sexes of a single species or between species were seen. This study indicates that a single pheromone preparation may serve to attract either species of tick to acaricide-treated sites for purposes of control. Alternatively, natural populations of heartwater-infected ticks might be lured to animals with waning immunity by means of such a preparation, thus increasing transmission levels.

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*Short Communication*

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**Interspecific Attraction to Male-Produced  
Pheromones of Two Species of *Amblyomma* Ticks  
(Acari: Ixodidae)****C. E. Yunker,<sup>1</sup> H. R. Andrew,<sup>1</sup> R. A. I. Norval,<sup>1</sup> and J. E. Keirans<sup>2</sup>***Accepted January 25, 1990; revised April 2, 1990***KEY WORDS:** Acari; Ixodidae; *Amblyomma*; ticks; pheromone; interspecific attraction.

The male-produced aggregation-attachment pheromones (AAP) of ticks are known only from species of the ixodid genus *Amblyomma* that parasitize wild and domestic ruminants of Africa and North America (D. Sonenshine, in press). Volatile and effective over long distances, AAPs serve to attract unfed ticks to hosts parasitized by conspecific males (Norval *et al.*, 1989a,b) and to the sites of attachment of males on the hosts (Rechav *et al.*, 1976, 1977). These pheromones are generally believed to be highly species specific (Rechav, 1978; Sonenshine *et al.*, 1985; Goethe, 1987), thereby promoting mating success with conspecific individuals and limiting interspecific matings that might result in the production of sterile eggs or infertile progeny. However, in two African species, *A. variegatum* (Fabricius) and *A. hebraeum* Koch, a weak interspecific response has been observed (Norval and Rechav, 1979; Rechav *et al.*, 1982) and the AAPs of both are known to share a common component, *o*-nitrophenol (Schöni *et al.*, 1984; Apps *et al.*, 1988), which is attractive to the two species (Hess and de Castro, 1986; Norval *et al.*, 1989b). These observations led us to investigate the attractiveness of the AAP of one species for adults of the other in field situations in Zimbabwe.

In preliminary experiments, we compared the response of marked, released *A. hebraeum* males and females both to their conspecific pheromone and to that of *A. variegatum*. Pheromone-producing males of both species were transported

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to the government quarantine station at Mbizi, in the lowveld of Southeast Zimbabwe, where *A. hebraeum* is established and *A. variegatum* is absent. The male ticks were confined in muslin bags taped to the ears of caged rabbits, on which hosts they had fed for the preceding 8 days. The experimental sites were in unoccupied paddocks on level ground (elevation, 500 m) in areas of sandy soil and sparse, short grass that were shaded by a cover of mopane (*Colophospermum mopane*) trees. Three rabbits, in separate cages, were placed on the ground 20 m apart in the early morning of 29 December 1987, when temperatures were mild (19–25°C) and wind was light. Each ear of one of the rabbits was infested with 25 *A. hebraeum*, and those of a second rabbit with 25 *A. variegatum*. The third, unparasitized rabbit served as a control. Equal numbers of laboratory-reared *A. hebraeum* adults were marked with fluorescent dusts (Butler *et al.*, 1984) and released approximately 4 m downwind of each cage. Counts were made of the numbers of marked ticks moving onto (or within 25 cm of) the cages after 60–90 min. The test was repeated under similar conditions a month later. In both tests, the majority of released ticks was seen to wander nondirectionally in and near the release site for a few minutes, after which they sought shelter beneath ground litter and became immobile. In the first trial, of 200 adults released, 3 females and 1 male moved to the rabbit parasitized by *A. hebraeum*, while 2 females and 4 males moved to the rabbit parasitized by *A. variegatum*; none sought out the unparasitized rabbit. In the second trial, of 400 adults released, 14 females and 14 males moved to the *A. hebraeum*-parasitized rabbit, 11 females and 6 males moved to the *A. variegatum*-parasitized rabbit, and 1 of each sex moved to the control rabbit. Some of those responding to the parasitized rabbits moved on to the ear bags, where they attempted to probe through the cloth (Fig. 1). Although attraction to the source of the pheromones was obvious, the proportion of ticks responding (2.0–11.3%) was small. It was concluded that the majority of released ticks had been insufficiently stimulated to exhibit host-seeking behavior.

Our previous studies of attraction of *A. hebraeum* to AAP extracts and artificially generated CO<sub>2</sub> (Norval *et al.*, 1987) or to cows treated with these extracts (Norval *et al.*, 1989a) suggested that large volumes of the gas (such as those exhaled by large ruminants) are required to elicit host-seeking behavior in quiescent forms. Hence, in our subsequent experiments we omitted rabbits, substituting blocks of dry ice as CO<sub>2</sub> sources. Also, ether extracts of AAP were used as attractants in lieu of live, pheromone-emitting male ticks. In the experiments that followed, the responses of both marked, released *A. hebraeum* and *A. variegatum* adults to AAPs of either species, in combination with CO<sub>2</sub>, were tested.

*Amblyomma hebraeum*. These assays were carried out at the Mbizi field station during the early morning hours of 15 February and 25 April, when temperatures were mild (22–25°C) and the wind was light and steady. A short stick



Fig. 1. Attraction of *Amblyomma hebraeum* adults to bags taped to rabbit ears, in which pheromone-emitting male *A. variegatum* were feeding. Note splayed palpi, indicative of cheliceral probing, of uppermost tick.

was forced into the soil and a filter-paper "target" of 150-mm diameter was impaled on it approximately 100 mm above the surface. A block of dry ice ( $\pm 1$  kg) was placed next to and upwind of the stick. To activate the target, 5 ml of AAP extract was poured on the filter paper. The extract had previously been prepared by soaking 100 fed males of either species in 100 ml diethyl ether. Controls consisted of dry ice and untreated filter paper. Marked adults of *A. hebraeum* (approximately 50–100 of either sex) were released 4 m downwind of each target and those moving onto or within 50 cm of the source in 1 h were counted and removed from the experiment.

In three trials, an average of 43.3% (range, 32.7–56.8%) of males and 54.1% (range, 44.7–80.0%) of females of *A. hebraeum* was attracted to the conspecific pheromone (Table I), whereas only 3.8% (range, 0.0–12.5%) of males and 2.3% (range, 0.0–4.9%) of females moved to the control target. The response of *A. hebraeum* to the AAP of *A. variegatum* was even more pronounced (Table I). In two trials (in which the same controls as above were used) an average of 67.5% (range, 52.5–81.4%) of males and 62.2% (range, 45.5–78.3%) of females was attracted to the pheromone source.

*Amblyomma variegatum*. The responses of adults of this species to conspecific and heterospecific pheromone were assayed at the Lake Kariba Research

**Table I.** Attraction of Released *Amblyomma hebraeum* Adults to Sources of Male Aggregation-Attachment Pheromone (AAP) and CO<sub>2</sub><sup>a</sup>

Trial no.	Conspecific attraction, <i>A. hebraeum</i> AAP + CO <sub>2</sub>				Interspecific attraction, <i>A. variegatum</i> AAP + CO <sub>2</sub>				Control, CO <sub>2</sub>			
	Females		Males		Females		Males		Females		Males	
	<i>n/N</i>	% <i>n</i>	<i>n/N</i>	% <i>n</i>	<i>n/N</i>	% <i>n</i>	<i>n/N</i>	% <i>n</i>	<i>n/N</i>	% <i>n</i>	<i>n/N</i>	% <i>n</i>
1	44/94	47.8	32/98	32.7	NT	—	NT	—	0/93	0.0	0/93	0.0
2	17/38	44.7	25/44	56.8	20/44	45.5	21/40	52.5	2/42	4.8	1/42	2.4
3	32/40	80.0	24/45	53.3	36/46	78.3	35/43	81.4	2/41	4.9	6/48	12.5
Total ( $\bar{X}$ )	93/172*	(54.1)	81/187*	(43.3)	56/90**	(62.2)	56/83**	(67.5)	4/176	(2.3)	7/183	(3.8)

<sup>a</sup>Ticks moving from 4 m away to within 50 cm of test substance in 1 h were counted and removed from experiment. *N* = No. of ticks released; *n* = No. of ticks recovered; NT = not tested.

\*Adjusted  $\chi^2$  value for difference between females and males = 3.73; *P* > 0.05 (df = 1).

\*\*Adjusted  $\chi^2$  value for difference between females and males = 0.78; *P* > 0.25 (df = 1).

Station operated by the University of Zimbabwe. This station, approximately 500 m in elevation, is located in the lowveld of Northern Zimbabwe, where *A. variegatum* occurs and *A. hebraeum* is absent. Release sites were selected in a shaded area similar in soil texture and vegetation to that of the Mbizi Quarantine Station. Temperatures were mild (19–26°C) and winds were light and variable during the early morning hours of February 8 and 9 and April 27, 1989, when tests were performed. Assay procedures were identical to those for the preceding experiment.

Significant attraction of *A. variegatum* adults to the AAP of either species was seen. Average values for ticks attracted to the conspecific pheromone were 48.0% (range, 20.8–74.0%) for males and 59.6% (range, 49.0–71.1%) for females (Table II), whereas an average of only 1.2% (range, 0.0–2.3%) of males and 2.6% (range, 0.0–4.3%) of females was attracted to CO<sub>2</sub> alone. Similarly, for those attracted to the heterospecific target, average values were 40.3% (range, 20.5–69.2%) for males and 45.1% (range, 26.6–72.1%) for females (Table II). Average values for attraction of these ticks to the controls were low: 5.1% (range, 2.2–11.4%) for males and 2.8% (range, 0.0–8.3%) for females.

No significant differences were seen between responses of male and responses of female ticks of either species to conspecific or heterospecific pheromones when data were analyzed by 2 × 2 contingency tables with  $\chi^2$  adjusted for continuity (Tables I and II). Likewise, there was no significant difference between the two species in their response to either of the pheromones. Of the ticks responding, 174 of 286 *A. hebraeum* (Table I) and 150 of 257 *A. variegatum* (Table II) moved to the AAP of *A. hebraeum*, and the remainder moved to that of *A. variegatum* (adjusted  $\chi^2 = 0.017$ ,  $P > 0.75$ ,  $df = 1$ ).

These experiments reveal a marked attractiveness of the AAPs of *A. hebraeum* and *A. variegatum* for adults of either species. Large proportions of males and females were attracted indiscriminately to both pheromones. That this interspecific attraction is due, at least in part, to *o*-nitrophenol, which is common to fed males of both species (Schöni *et al.*, 1984; Apps *et al.*, 1988), is apparent from field tests involving *A. variegatum* (Hess and De Castro, 1986) and *A. hebraeum* (Norval *et al.*, 1989b). However, the possibility that additional volatile components are involved in the phenomenon cannot be excluded, because *o*-nitrophenol has been found to be less attractive to *A. hebraeum* than fed conspecific males or their extracts (Norval *et al.*, 1989b).

Earlier reports suggesting that the AAP of *Amblyomma* males was species specific (Rechav *et al.*, 1977; Rechav, 1978) or, at most, weakly interspecific (Norval and Rechav, 1979) may have been influenced by the method of preparation of the pheromone. This method, which included concentration by vacuum distillation, might have reduced the potency of the volatile component(s), including *o*-nitrophenol. Nevertheless, it is possible that, once the ticks have

**Table II.** Attraction of Released *Amblyomma variegatum* Adults to Sources of Male Aggregation-Attachment Pheromone (AAP) and CO<sub>2</sub><sup>a</sup>

Trial no.	Conspecific attraction								Interspecific attraction							
	<i>A. variegatum</i> AAP + CO <sub>2</sub>				Control (CO <sub>2</sub> )				<i>A. hebraeum</i> AAP + CO <sub>2</sub>				Control (CO <sub>2</sub> )			
	Females		Males		Females		Males		Females		Males		Females		Males	
	<i>n/N</i>	% <i>n</i>	<i>n/N</i>	% <i>n</i>	<i>n/N</i>	% <i>n</i>	<i>n/N</i>	% <i>n</i>	<i>n/N</i>	% <i>n</i>	<i>n/N</i>	% <i>n</i>	<i>n/N</i>	% <i>n</i>	<i>n/N</i>	% <i>n</i>
1	24/49	49.0	10/48	20.8	0/32	0.0	0/39	0.0	25/94	26.6	18/88	20.5	0/95	0.0	2/93	2.2
2	32/45	71.1	37/50	74.0	2/46	4.3	1/44	2.3	31/43	72.1	27/39	69.2				
3	—	—	—	—	—	—	—	—	17/25	68.0	36/75	48.0	4/48	8.3	5/44	11.4
Total (X)	56/94*	(59.6)	47/98*	(48.0)	2/78	(2.6)	1/83	(1.2)	73/162**	(45.1)	81/202**	(40.3)	4/143	(2.8)	7/137	(5.1)

<sup>a</sup>See footnote *a*, Table I.

\*Adjusted  $\chi^2$  value for difference between females and males = 2.16;  $P > 0.05$  (df = 1).

\*\*Adjusted  $\chi^2$  value for difference between females and males = 0.72;  $P > 0.25$  (df = 1).

gained access to a host, semiochemicals other than AAP may operate to favor association of conspecific adults, as seen by Rechav *et al.* (1982). Our studies, which were carried out over relatively great distances, would not have involved such a mechanism. The possibility also exists that CO<sub>2</sub> vapors may have synergistically enhanced the attractiveness of the AAPs to heterospecific forms.

The two tick species studied here are the principal vectors to domestic ruminants of heartwater disease (*Cowdria ruminantium* infection) in sub-Saharan Africa. *Amblyomma variegatum* is also the major vector of the disease in African offshore islands and certain islands of the Caribbean (Walker and Olwage, 1987; Provost and Bezuidenhout, 1987). We recently observed that calves parasitized by pheromone-emitting male *A. hebraeum* (or treated with an extract of this substance) were significantly more attractive to both marked and wild *A. hebraeum* than were unparasitized or untreated control calves (Norval *et al.*, 1989a). We then proposed that immunity to heartwater in young calves, which tends to wane without repeated exposure, might be augmented by treating them with AAP, thus making them attractive to infected *A. hebraeum*. The present study indicates that a single compound may serve to attract disease-bearing ticks of either species and thus increase transmission levels wherever heartwater exists.

Opportunities for selective control of ixodid ticks through the use of attractant pheromones have long been considered feasible. *Amblyomma maculatum* Koch and *A. hebraeum* were attracted to acaricide-treated sites on cattle by the respective AAP extracts, where the ticks attached and died (Gladney *et al.*, 1974; Rechav and Whitehead, 1978). Mating of *Dermacentor variabilis* on tick-infested dogs was significantly reduced by dusting these hosts with slow-release preparations of the female sex pheromone, 2,6-dichlorophenol. The preparation lured mate-seeking males away from attached females and disrupted mating (Ziv *et al.*, 1981). Later it was shown that, by combining the dust containing sex pheromone with a pesticide, mating of *D. variabilis* could be further reduced due to the significantly greater kill of male ticks (Sonenshine *et al.*, 1985). The interspecific attractiveness of *A. hebraeum* and *A. variegatum* shown here may also hold promise for control. These two species have a distribution that is essentially mutually exclusive (Walker and Olwage, 1987). Although some overlap has been recorded, hybrids have not been found (Norval, 1983). Rechav *et al.* (1982) observed interspecific mating of the two species under laboratory conditions, which resulted in the production of eggs that were essentially nonviable. Hence, a potential for biological control, through introduction on ruminants of males of one species into the distributional range of the other, deserves consideration. In areas where alternate wild ruminant hosts coexist with domestic stock, such control efforts would conceivably act to reduce tick populations. In less complex foci (e.g., *A. variegatum*-infested Caribbean islands), such reproductive interference might facilitate eradication.

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## REFERENCES

- Apps, P. J., Viljoen, H. W., and Pretorius, V. (1988). Aggregation pheromones of the bont tick *Amblyomma hebraeum*: Identification of candidates for bioassay. *Onderstepoort J. Vet. Res.* **55**: 135-137.
- Butler, J. F., Holscher, K. H., Adeyeye, O., and Gibbs, E. P. J. (1984). Sampling techniques for burrow dwelling ticks in reference to potential African swine fever vectors. In Griffiths, D. A., and Bowman, C. E. (eds.), *Acarology VI, Vol. 2*, Ellis Horwood, Chichester, pp. 1065-1074.
- Goethe, R. (1987). Tick pheromones. *Onderstepoort J. Vet. Res.* **54**: 439-441.
- Hess, E., and de Castro, J. J. (1986). Field tests of the response of female *Amblyomma variegatum* (Acari: Ixodidae) to the synthetic aggregation-attachment pheromone and its components. *Exp. Appl. Acarol.* **2**: 249-255.
- Norval, R. A. I. (1983). The ticks of Zimbabwe. VII. The genus *Amblyomma*. *Zimbabwe Vet. J.* **14**: 3-18.
- Norval, R. A. I., and Rechav, Y. (1979). An assembly pheromone and its perception in the tick *Amblyomma variegatum* (Acarina: Ixodidae). *J. Med. Entomol.* **16**: 507-511.
- Norval, R. A. I., Yunker, C. E., and Butler, J. F. (1987). Field sampling of unfed adults of *Amblyomma hebraeum* Koch. *Exp. Appl. Acarol.* **3**: 213-217.
- Norval, R. A. I., Andrew, H. R., and Yunker, C. E. (1989a). Pheromone-mediation of host-selection in bont ticks (*Amblyomma hebraeum* Koch). *Science* **243**: 364-365.
- Norval, R. A. I., Butler, J. F., and Yunker, C. E. (1989b). 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.
- Provost, A., and Bezuidenhout, J. D. (1987). The historical background and global distribution of heartwater. *Onderstepoort J. Vet. Res.* **54**: 165-169.
- Rechav, Y. (1978). Specificity in assembly pheromones of the tick *Amblyomma hebraeum* (Acarina: Ixodidae). *J. Med. Entomol.* **15**: 81-83.
- Rechav, Y., and Whitehead, G. B. (1978). Field trials with pheromone-acaricide mixtures for control of *Amblyomma hebraeum*. *J. Econ. Entomol.* **71**: 149-151.
- Rechav, Y., Whitehead, G. B., and Knight, M. M. (1976). Aggregation response of nymphs to pheromone(s) produced by males of the tick *Amblyomma hebraeum* Koch. *Nature* **259**: 563-564.
- Rechav, Y., Parolis, H., Whitehead, G. B., and Knight, M. M. (1977). Evidence for an assembly pheromone(s) produced by males of the bont tick, *Amblyomma hebraeum* (Acarina: Ixodidae). *J. Med. Entomol.* **14**: 71-78.
- Rechav, Y., Norval, R. A. I., and Oliver, J. H., Jr. (1982). Interspecific mating of *Amblyomma hebraeum* and *Amblyomma variegatum* (Acari: Ixodidae). *J. Med. Entomol.* **19**: 139-142.
- Schöni, R., Hess, E., Blum, W., and Ramstein, K. (1984). The aggregation-attachment pheromone of the tropical bont tick *Amblyomma variegatum* Fabricius (Acari: Ixodidae): Isolation, identification and action of its components. *J. Insect Physiol.* **30**: 613-618.

- Sonenshine, D. E., Taylor, DeM., and Corrigan, G. (1985). Studies to evaluate the effectiveness of sex pheromone-impregnated formulations for control of populations of the American dog tick, *Dermacentor variabilis* (Say) (Acari: Ixodidae). *Exp. Appl. Acarol.* **1**: 23-24.
- Walker, J. B., and Olwage, A. (1987). The tick vectors of *Cowdria ruminantium* (Ixodoidea, Ixodidae, genus *Amblyomma*) and their distribution. *Onderstepoort J. Vet. Res.* **54**: 353-379.
- Ziv, M., Sonenshine, D. E., Silverstein, R. M., West, J. R., and Gingher, K. H. (1981). Use of sex pheromone, 2,6-dichlorophenol, to disrupt mating by American dog tick *Dermacentor variabilis* (Say). *J. Chem. Ecol.* **7**: 829-840.