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1-OCTEN-3-OL ISOLATED FROM BONT TICKS ATTRACTS *Amblyomma variegatum*

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Abstract—Volatiles from various life-stages of the bont ticks *Amblyomma variegatum* and *A. hebraeum* were collected by using solid-phase microfibers and charcoal traps. An octenol isomer was found to be a major constituent of most of the tick material sampled and was identified as 1-octen-3-ol by gas chromatography–mass spectrometry and by using antenna of the tsetse fly *Glossina brevipalpis* in gas chromatography-linked antennogram detection. Release of this compound increased during molt to adulthood and following mechanical disturbance of adult ticks. (*R*)-(−)-1-Octen-3-ol and racemate 1-octen-3-ol both induce an increase in upwind walk to the odor source from *A. variegatum* in an airstream on a servosphere. Volatiles from tick exuviae plus feces and from dead ticks also attracted *A. variegatum*, suggesting that 1-octen-3-ol may contribute to the aggregation response of *Amblyomma* spp. on such substrates. 2,6-Dichloroanisol and 2,5-dimethylpyrazine also were detected in volatiles from the ticks but induced no behavioral responses on the servosphere. The suspected tick pheromone component, 2,6-dichlorophenol, was detected from *A. variegatum* adults cut into pieces but had no effect on the behavior of *A. variegatum* on the servosphere at a range of doses.

Key Words—1-Octen-3-ol, ixodid tick, *Amblyomma variegatum*, tick attractant.

INTRODUCTION

Secretions from dermal glands of both prostriate and metastriate ticks have been widely reported (Lees, 1947; Balashov, 1972, 1983; Yoder et al., 1993a; Walker et al., 1996). Particular attention has been paid to the aggregation–attachment pheromone of some *Amblyomma* spp. (Gladney et al., 1974; Apps et al., 1988;

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Diehl et al., 1991) and the suspected tick pheromone product 2,6-dichlorophenol (De Bruyne and Guerin, 1994). It is known that several metastriate ticks transiently release cuticular droplets in response to physical disturbance, for which a defensive role has been suggested. Yoder et al. (1993a) found that *Dermacentor andersoni*, *D. variabilis*, *A. americanum* and *A. maculatum* release a "pungent odor" associated with the appearance of droplets on the cuticle. Pavis et al. (1994) observed the release of droplets by *A. variegatum* after disturbance, and Walker et al. (1996) reported the release of droplets with a "sour, nut-like smell" from unfed and fed *Rhipicephalus appendiculatus* adults after handling. Behavioral tests with these exudates were carried out by Yoder et al. (1993b) on *D. variabilis* and by Pavis et al. (1994) on *A. variegatum*. Each demonstrated that the exudate gave significant protection to ticks for a day in a Petri dish assay against fire ant predation. It is likely that protection was afforded in these studies by nonvolatile components of the exudates and not by the transient odor associated with them upon release from the cuticle.

Here, we collected odor volatiles released by both resting and physically disturbed *Amblyomma variegatum* (Fabricius) and *A. hebraeum* (Koch) by using solid-phase microfibers and charcoal. The volatiles were analyzed by gas chromatography-linked mass spectrometry (GC-MS) and by gas chromatography-linked electroantennogram detection (GC-EAD). A servosphere apparatus was used to examine the behavioral responses of *A. variegatum* adults to the headspace of ticks, to 1-octen-3-ol and other volatiles identified.

METHODS AND MATERIALS

Ticks. The two tick species examined, *A. variegatum* and *A. hebraeum* (Acari, Ixodidae), have been reared at the Centre de Recherche Santé Animale S.A., Novartis, St. Aubin, Switzerland since 1981. All stages (immature and adult) are fed on the tails of Simmental calves at 22–24°C and kept under constant darkness during molt at 28°C/80–90% relative humidity. For our experiments, engorged females, unfed adults of both *A. variegatum* and *A. hebraeum*, and unfed nymphs of *A. variegatum* were maintained in a 10L : 10D cycle with 2-hr ramps of dawn and dusk at 19°C/90% relative humidity. *A. variegatum* adults of either sex, between 1 and 9-months old, were prepared for behavioral tests on the servosphere as in McMahon and Guerin (2000). Each tick was tested only once.

Odor Sampling with SPME. Two solid-phase microextraction (SPME) stationary phases (Supelco) were used: Carbowax/divinylbenzene (Carbowax/DVB), which can adsorb volatile alcohols, and Carboxen/polydimethylsiloxane (Carboxen/PDMS), which has an affinity for hydrocarbons after several hours with the analyte. The latter phase with its lower affinity for water is more suit-

able for sampling over biological material in humid environments. The fibers were preconditioned for 2 hr at 300°C in a N_2 stream.

Odor Sampling over Undisturbed Ticks. Volatiles from resting or dead ticks were sampled by using the Carboxen/PDMS fiber that was inserted without disturbing the resting ticks into a horizontally held glass tube (12 cm long, 2 cm diam.) containing 200 ticks and sealed at both ends with perforated plastic stoppers. The control consisted of placing a fiber in an empty glass tube within 50 cm of the test tube. The collection fibers were left to stand overnight. In this manner, volatiles from the following tick substrates were sampled: 9-month-old *A. variegatum* nymphs, 1- and 9-month-old *A. variegatum* adults, *A. variegatum* adults that had died in the previous two months, and 12-month-old *A. hebraeum* adults.

Odor Sampling over Manipulated Ticks. The Carbowax/DVB fiber was used to sample volatiles from disturbed unfed *A. variegatum* adults of both sexes by holding five unfed adults in a 2-ml vial and squeezing the ticks individually with forceps. The vial was sealed immediately with Parafilm, and a Carbowax/DVB fiber was inserted through the seal and held over the ticks for 1 hr. The sampling was repeated overnight. The control for this test was to hold the fibers above undisturbed ticks for the same time intervals. The Carbowax/DVB fiber also was used to collect volatiles from four *A. variegatum* females killed by freezing and immediately cut into four pieces.

Odor Sampling with Charcoal. Air was sucked with a membrane pump (200 ml/min) over groups of five gently shaken engorged *A. hebraeum* or *A. variegatum* females (<1 week after drop-off) held in a 500-ml gas-wash bottle. Volatiles from the ticks were trapped on a 5-mg charcoal trap held in a 60-mm long × 4-mm-diam. glass tube in the closed-loop stripping apparatus of Grob and Zürcher (1976). As water condensation can reduce the effectiveness of charcoal as an adsorbant, the charcoal trap was heated to 10°C above room temperature during odor entrainment. After collection of volatiles for 10 min, the flask was gently shaken again to induce the release of more droplets from the dermal glands, and odors were collected onto the charcoal trap for another 10 min. The charcoal trap was extracted with 12 μl dichloromethane, of which 2 μl were analyzed (below).

Volatiles from 9-month-old unfed *A. variegatum* adults were entrained onto a charcoal trap (above) from air (200 ml/min) passing over 200 ticks held in a 50-ml gas-wash bottle and shaken to induce release of volatiles from dermal glands. The odor collected was extracted with dichloromethane (as above).

Gas Chromatography-Linked Mass Spectrometry Analysis. The SPME fibers were withdrawn into the hollow needle of the holding syringe after odor sampling and reexposed for 1 min to desorb the trapped volatiles in a split/splitless injection port (injection temperature, 280°C) of a gas chromatograph (Hewlett-Packard 5890 GC) linked to a HP 5971A mass selective detector

(MSD). The analyte was swept from the injector port onto a DB-Wax high-resolution fused-silica capillary column (30 m long, 0.25 mm ID, 0.25-μm film thickness, J&W Scientific) with a precolumn (1 m deactivated fused silica). The column was connected via a 1-m deactivated fused-silica capillary (0.25 mm ID) to the MSD ionization chamber (temperature 160°C, ionization energy 70 eV). The MSD, in the EI mode, scanned for masses m/z 20–300. Helium was used as carrier gas at 50 kPa head pressure (but was set at a constant flow, 30 cm/sec for on-column injection of the charcoal trap extract, see above). Components of the desorbed material were identified by comparing the mass spectra of unknowns in the analyte with those of standards in a library of the HP Chemstation software and by matching retention times with synthetic equivalents.

Gas Chromatography-Linked Electroantennogram Detection. Tick volatiles were tested for the presence of 1-octen-3-ol by using the antenna of the tsetse fly *Glossina brevipalpis* in GC-linked electroantennogram detection (GC-EAD) (Arn et al., 1975). The antenna of *G. brevipalpis* is sensitive to subnanogram levels of this compound eluting from a GC column. Furthermore, by using the specific response of the antenna of *G. brevipalis* to 1-octen-3-ol (Ujváry et al., 2000), the product can be identified by its retention time and by comparing the response of the antenna to the analyte with that to a similar amount of the naturally occurring pure (*R*)-(−) isomer. This was achieved by comparing the ratio of the EAG to FID peak heights for the extract and natural (*R*-(−)-1-octen-3-ol.

The procedure for electroantennogram detection briefly described is as follows. Volatiles were delivered on-column to a Carlo Erba 5160 GC with a flame ionization detector (FID at 260°C). The extract or headspace was injected in splitless mode onto a 25-m fused-silica SE-54 column (Macherey-Nagel), 0.25 mm ID, 0.35-μm film thickness with H_2 as carrier gas (27 cm/sec). The column effluent was split (glass Y-splitter) so that 60% was directed to the FID and 40% to the electrophysiological preparation. The latter was swept by a conditioned airstream (see below) to the electrophysiological preparation from a heated transfer line (240°C) in the wall of the chromatograph in such a way that the column effluent was simultaneously monitored by the FID and fly antenna. The method of mounting the fly head for EAG recording was as in Guerin and Visser (1980). Briefly, the fly head was excised after momentarily anaesthetizing it with CO_2. A chloridized silver wire was placed in a drawn-out capillary (10-μm tip diameter) filled with 0.1% KCl that was inserted through the occipital opening reaching into the pediculus and served as the reference electrode. This preparation was then mounted under a microscope (WILD Kombistereo M3Z) where the antenna was bathed in a humidified airstream (90–100% relative humidity, 23 ± 2°C) flowing at 1 m/sec via a glass water-jacketed tube (6 mm ID) whose outlet was about 1 cm from the preparation. The unbroken tip of the recording electrode was brought into contact with the funiculus surface upon which the tip

broke. Funiculus surface contact was enough to record the EAG. Micromanipulators (Leitz) permitted accurate positioning of the preparation and recording electrode. The EAG signal was captured via a silver wire in the electrolyte-filled (0.1 M KCl) glass electrode connected to a high-impedance preamplifier and an AC/DC amplifier (UN-03, Syntech) and recorded on the hard disk of a PC with the FID signal via a 16-bit analog–digital IDAC card (Syntech) by using the GC-EAD software package (Syntech), and monitored simultaneously with an oscilloscope (Tektronix 5103).

G. brevipalpis antennae were employed in GC-EAD to test for the presence of 1-octen-3-ol in volatiles collected on charcoal from 9-month-old disturbed *A. variegatum* adults as described above. *G. brevipalpis* EAD responses also were used to monitor directly for the presence of 1-octen-3-ol in the air above tick material. For this, *A. variegatum* exuviae (0.3 g; 5 days after molt) from 150 individuals were placed in a closed 5-ml vial with a Teflon-coated rubber seal. One day later, 2 ml of air was withdrawn from this vial with a gas-tight syringe and injected on to the DB-Wax column in splitless mode (1 min). To facilitate the large-volume injection, the column head pressure was reduced manually for 5 sec during injection and then raised to 65 kPa. Analysis for the presence of 1-octen-3-ol over 10 unmolted *A. variegatum* ticks was carried out in the same manner. The influence of molting on the release of 1-octen-3-ol in the headspace was analyzed by sealing 10 unmolted *A. variegatum* ticks in a 5-ml vial as before. After two animals had molted, 2 ml of this headspace was withdrawn in the gas-tight syringe without disturbing the animals and injected in splitless mode as above. In addition, odor puffs from groups of five undisturbed and just disturbed *A. variegatum* adults were delivered to the antenna of *G. bevipalpis* from the barrels of 5-ml plastic syringes in which the ticks were held to test for electroantennogram responses (Steullet and Guerin, 1992).

Behavior. A locomotion compensator or servosphere (Kramer, 1976) was used to evaluate the behavioral responses of walking ticks to volatiles from tick material borne in an airstream (18 cm/sec, 70% relative humidity, 23–25°C) (McMahon and Guerin, 2000). Only the responses to *A. variegatum* adults were tested. Briefly, an animal is maintained at the apex of a Perspex sphere (50 cm diam.) to which the airstream is directed. Displacements of the sphere caused by the moving animal are monitored by pulse generators allowing the reconstruction of the tracks described by the arthropod (Kramer, 1976). This permits an evaluation of several parameters such as speed, angular velocity, the duration of the walk, and direction. The latter parameters can be used to calculate upwind (attractive) responses to volatiles. Two estimates of the upwind responses elicited by a treatment in the test period (1 min) compared to a control period of equal duration were employed in this study: (1) the percentage time spent walking in a cone 60° either side of due upwind, and (2) the change in "target vector" (Jones, 1977). The target vector provides an estimate of the efficiency with which a par-

ticular direction is followed. It is independent of the arbitrarily chosen upwind cone and is calculated by multiplying the cosine of the mean direction (∅) by the path straightness (circular statistics after Batschelet, 1981). Values for target vector range from −1 (downwind direction) to +1 (upwind direction).

Walks of the ticks also were monitored for a further minute (end-control period) following the withdrawal of test vapors to determine if they executed any off-responses. Such off-responses consist of the tick describing small circles, abruptly turning downwind, or both, within 10 sec of the loss of chemical. These behaviors never occur during control runs (McMahon and Guerin, 2000). Intertreatment comparisons of the proportion of ticks displaying such off-responses were carried out by using the Fischer exact test.

Tick Odors Tested on the Servosphere. Vapors from the following biological substrates were presented to walking *A. variegatum* adults on the servosphere from 500-ml gas-wash bottles (after McMahon and Guerin, 2000): (1) air at 150 ml/min swept over 200 dead *A. variegatum* adults, (2) air at 10 ml/min passed over 0.6 g of exuviae plus feces and of newly molted (<2 weeks) *A. variegatum* adults, (3) air at 75 ml/min over 20 unmolted *A. variegatum* adults, (4) air at 75 ml/min over 10 freshly molted *A. variegatum* adults (<48 hr after emergence), (5) air at 75 ml/min over five engorged *A. hebraeum* females, and (6) air at 75 ml/min over five engorged *A. variegatum* females. During experiments 5 and 6, the bottle containing engorged females was shaken gently after every two tests to provoke the release of more droplets from the dermal glands. The blank controls for the above experiments consisted of passing air through empty gas-wash bottles at the same flow rate as used in the tests.

Synthetic Volatiles Tested on the Servosphere. Vapors of the following compounds were delivered separately to *A. variegatum* adults on the servosphere from 500-ml gas-wash bottles at 150 ml/min (after McMahon and Guerin, 2000): 2,5-dimethyl pyrazine, 2,6-dichloroanisol (Aldrich; source doses 10 ng and 1 μg), 2,6-dichlorophenol (2,6-DCP, Supelco; source doses 100 fg to 10 ng in log steps), (*R*)-(−)-1-octen-3-ol (Robertet; source dose 10 ng); racemate 1-octen-3-ol (Merck; source dose 10 ng). All compounds were >97% pure as indicated by GC and were prepared in dichloromethane (Merck, analytical grade). The racemate was a 50 : 50 mixture of (*R*)-(−)-1-octen-3-ol and *S*)-(+)-1-octen-3-ol (separated on a 0.25-μm film of the chiral phase 6-TBDMS-2,3-diacetyl-β-cyclodextrin 100% in OV-1701 coated on a 30-m × 0.32-mm-ID capillary column with He as carrier gas).

RESULTS

Volatiles identified from A. hebraeum *and* A. variegatum. An octenol isomer matching the mass spectrum and retention time of racemate 1-octen-3-ol

was identified by GC-MSD over engorged *A. variegatum* (6 ng) and *A. hebraeum* females (0.2 ng) collected onto charcoal (Figure 1a, Table 1). This product also was identified in all the headspace samples of either species sampled with the Carboxen/PMDS fiber (Table 1) at levels between 0.2 and 2 ng. Furthermore, GC-MSD analysis of the volatiles over five unfed, undisturbed, and disturbed *A. variegatum* adults with the Carbowax/DVB fiber demonstrated that release of octenol (0.6 ng) was associated with squeezing *A. variegatum* (Figure 1b, Table 1). No octenol was detected after sampling the headspace for a further 13 hr, or in the headspace of the undisturbed control ticks with this SPME fiber.

The octenol isomer was identified as 1-octen-3-ol since it elicited the same response as the natural product from the antenna of *G. brevipalpis* in GC-EAD analysis of the vapors over disturbed unfed *A. variegatum* collected on charcoal (Figure 2). In the other GC-EAD analyses that used *G. brevipalpis* antennae, 1-octen-3-ol was the predominant chemical detected over freshly molted ticks at levels of 1 ng/tick. However, 1-octen-3-ol was not found over unmolted ticks or exuviae, the only tick headspace samples in which this compound was not detected. Direct stimulation of the *G. brevipalpis* antennae with volatiles from undisturbed and disturbed tisks held in syringes corroborated the GC-MSD and GC-EAD finding, i.e., the release of 1-octen-3-ol by ticks is enhanced by physical manipulation. Air passing over undisturbed ticks to the antenna failed to induce an EAG response, whereas the air over disturbed ticks caused a strong depolarization of the antennal receptors.

Other compounds, including short-chain aliphatic aldehydes and alcohols were recovered from both the charcoal trap extract and the SPME fibers in the vapors over engorged *A. variegatum* and *A. hebraeum* females. However, few of these volatiles were recovered in sufficient quantities to permit identification (the MSD has a detection limit for a full mass spectrum at ca. 0.1 ng). Apart from 1-octen-3-ol, only three compounds above the MSD threshold were identified over ticks (Table 1). 2,6-Dichlorophenol was detected only in the headspace of *A. variegatum* females cut into pieces (ca. 1 ng), and 2,6-dichloroanisol and 2,5-dimethyl pyrazine were identified only over 9-month-old or dead *A. variegatum* and *A. hebraeum* adults and, as such, may be degradation products. These three tick products had matching mass spectra and retention times with synthetic equivalents. Retrospective analysis for the presence of the major ion of each of these three compounds in the other tick headspace samples analyzed by GC-MSD failed to indicate the presence of any of these products at their respective retention times.

Behavior. Odors from tick material elicited behavioral responses in *A. variegatum* adults walking on the servosphere. Exuviae plus feces from freshly molted *A. variegatum* adults induced a significant increase in the time spent walking in the upwind cone ($P < 0.05$; Table 2). Volatiles of dead conspecifics attracted *A. variegatum* adults ($P < 0.01$; Table 2) and also induced a significant

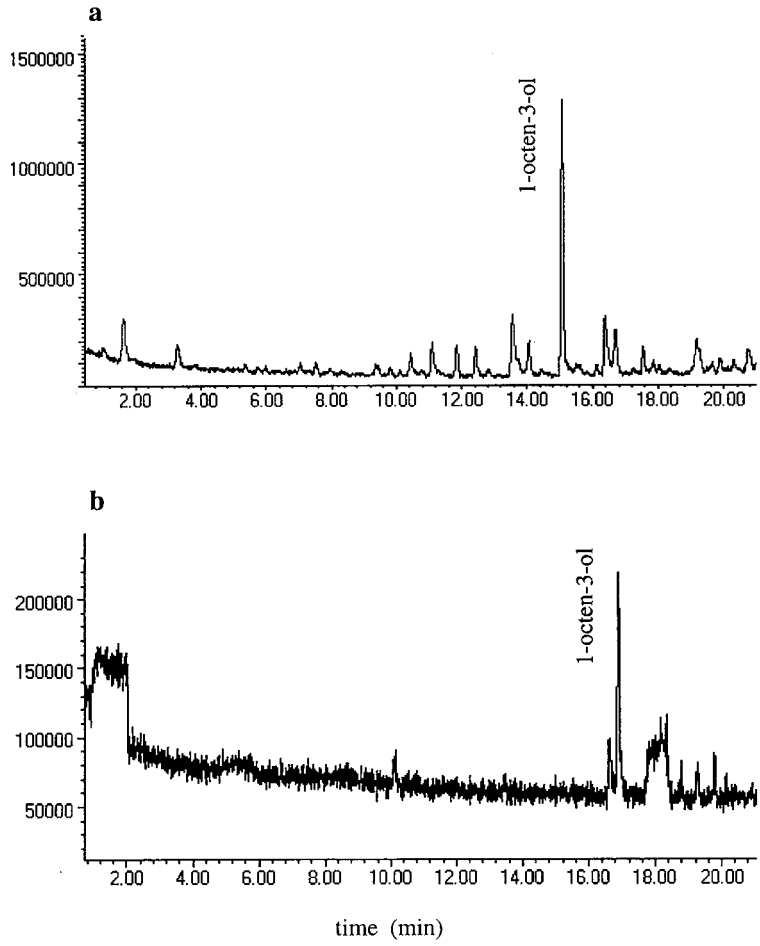

FIG. 1. Total ion chromatograms of volatiles collected over (a) disturbed engorged *Amblyomma variegatum* females by using a charcoal trap and (b) over unfed disturbed *A. variegatum* adults sampled by solid-phase microextraction with a Carbowax/divinylbenzene coated fiber (see text). Each 2-min interval (abscissa on the chromatogram) is equivalent to a 10°C rise in the oven temperature, starting at 40°C after 5 min. 1-Octen-3-ol predominates in both analyses occurring at a level of ca. 6 ng in a and ca. 0.6 ng in b in the 5–135°C elution temperature range presented. It was identified by matching the retention time and mass spectrum with a synthetic standard. The shift in the retention time of 1-octen-3-ol between the two chromatograms is due to a difference in column head pressure employed for the two analyses (on-column for the charcoal trap extract, splitless desorption for SPME fiber).

TABLE 1. COMPOUNDS IDENTIFIED IN HEADSPACE OVER *A. variegatum*

Compound	Identification by GC-MS			Identification by GC-EAD with *Glossina* antenna as detector	
	SPME		Charcoal entrainment	Direct headspace sampling	Charcoal entrainment
	Carboxen/PDMS	Carbowax/DVB			
1-Octen-3-ol	Disturbed adults Engorged females[a] Nymphs Dissected females Dead ticks Exuviae plus feces Undisturbed adults (1 month old)[a] Undisturbed adults (9 months old)[a]	Disturbed adults	Disturbed adults Engorged females[a]	Newly moulted adults	Disturbed adults
2,6-Dichlorophenol	Dissected females				
2,6-Dichloroanisol	Dead ticks Undisturbed adults (9 months old)[a]				
2,5-Dimethyl pyrazine	Dead ticks Undisturbed adults (9 months old)[a]				

[a]Compounds were also detected in the headspace of the same life stage of *A. hebraeum*.

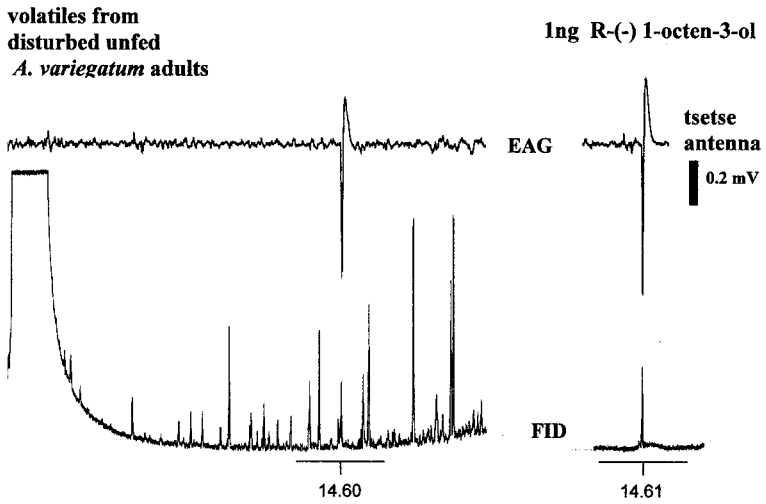

FIG. 2. Analysis of volatiles collected on a charcoal trap from disturbed unfed *Amblyomma variegatum* adults with the antenna of the tsetse fly, *Glossina brevipalpis*, as a biological detector in gas chromatography coupled electroantennogram detection (EAD). 1-Octen-3-ol eluted at 14.60 min. The amplitude of the EAG response (right) to 1 ng of (*R*)-(−)-1-octen-3-ol matched the response of a similar amount of the biologically active product in the extract. Several of the FID peaks are laboratory pollutants present in controls.

decrease in speed ($P < 0.01$). Odors from freshly cut females and unmolted and freshly molted *A. variegatum* adults did not attract conspecifics or influence their walking behavior. Odors from disturbed engorged *A. hebraeum* or *A. variegatum* adult females were unattractive, but *A. variegatum* responded to the odor of disturbed engorged *A. hebraeum* with a significant decrease in speed ($N = 14$; $P < 0.05$) and to disturbed engorged conspecifics with a decrease in angular velocity ($N = 24$; $P < 0.01$). No off-response occurred upon the withdrawal of tick odor.

The response to 1-octen-3-ol on the sphere was typified by an increase upwind walk (Figures 3 and 4) at a reduced speed and increased angular velocity. Both racemate 1-octen-3-ol and (*R*)-(−)-1-octen-3-ol attracted male and female *A. variegatum*. Nonetheless, (*R*)-(−)-1-octen-3-ol was a weaker attractant than any other treatment (Table 2), eliciting an increase in the walk in the upwind cone of just 8% and a moderate but insignificant increase in target vector. Although racemate 1-octen-3-ol was a strong attractant, evoking an increase in the walk in the upwind cone of 47%, no other differences in the behavior of the ticks were detected to these treatments. Furthermore, both 1-octen-3-ol

TABLE 2. UPWIND AND OFF-RESPONSES OF *A. variegatum* ADULTS ELICITED BY TICK ATTRACTANTS ON SERVOSPHERE[a]

Treatment (source dose)	*N*	% Change in time spent walking in upwind cone			Change in target vector			Off-responses	
		Median upwind response (interquartile range[b])	Wilcoxon signed rank test for a treatment (two-tailed *P* values)	Significance of change in duration of walk in upwind cone between treatments $P < 0.05$)	Median upwind response (interquartile range[b])	Wilcoxon signed rank test for a treatment (two-tailed *P* values)	Significance of change in target vector between treatments ($P < 0.05$)	Proportion of ticks showing an off-response	Significance of proportion of ticks showing off responses between treatments (Fisher exact test, two-tailed, $P < 0.05$)
Exuviae plus faeces (0.6 g)	18	11 (−1 to 31)	0.05	a	0.26 (−0.00 to 0.62)	0.01	a	1/18	b
Dead ticks (200)	13	21 (2–41)	0.01	a	0.23 (−0.05 to 0.64)	0.05	a	0/13	b
(*R*)-(−)-1-octen-3-ol (10 ng)	20	8 (−6 to 54)	0.05	ab	0.04 (−0.23 to 0.87)	0.23 (NS)	a	8/20	a
Racemate 1-octen-3-ol (10 ng)	21	47 (22–62)	0.001	b	0.71 (0.42 to 1.06)	0.001	b	13/20	a

[a] The index of the upwind response presented is the percentage difference in time spent walking in an upwind cone 60° either side of the air stream in the test period compared to the preceding control period. Intertreatment comparisons of the upwind responses were carried out by applying the Wilcoxon-Mann-Whitney test (two-tailed) to both the percentage change in the time spent walking in the upwind cone and to the change in target vector (values not shown) elicited by the treatments. Intertreatment comparisons of the proportion of off-responses induced by loss of stimulus were made with the Fisher Exact test (two-tailed). NS: Vapors of (*R*)-(−)-1-octen-3-ol did not elicit a significant change in target vector. Within a behavioral category, responses followed by the same letter are not significantly different.

[b] The interquartile range represents the 25 and 75 percentiles of the upwind response.

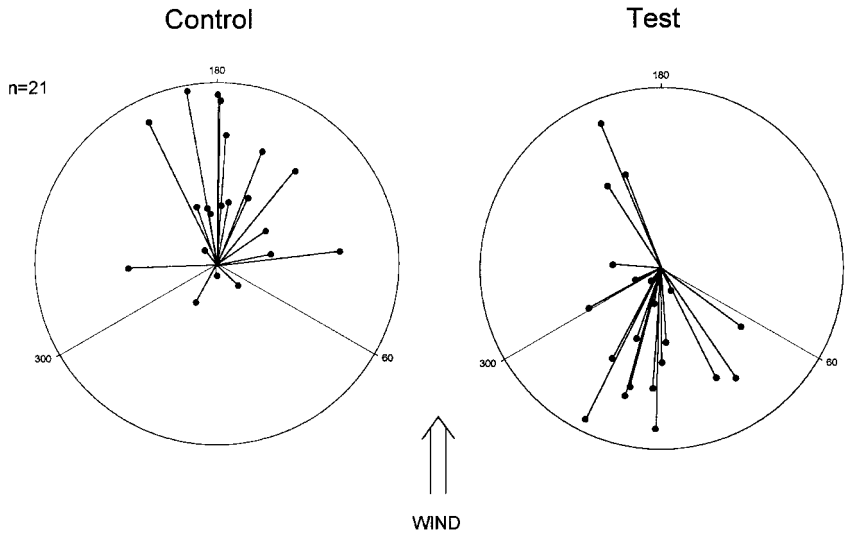

FIG. 3. Polar plot of the distribution of the mean direction (ϕ) of walks of *A. variegatum* adults ($N = 21$) on the servosphere during a 1-min control period followed by a 1-min test period where the same ticks were presented with vapors of a racemic mixture of 1-octen-3-ol (source dose 10 ng). The nearer the vector weighting (r) to the perimeter, the straighter the walk in that direction [circular statistics, after Batschelet (1981)]. Note the shift in mean walking directions in the presence of racemic 1-octen-3-ol.

samples elicited off responses (Table 2 and Figure 4) in 21 of a total of 41 *A. variegatum* adults tested, where the ticks reacted to the loss of the attractant either by describing small circles, abruptly turning downwind, or both in the 10 sec after loss of stimulus. This off-response was unique to 1-octen-3-ol. No such responses were observed for any other chemicals or tick volatiles presented to *A. variegatum* adults (Table 2).

The synthetic equivalents of the other products identified from ticks, i.e., vapors of 2,6-chloroanisol and 2,5-dimethyl pyrazine (source doses of 10 ng and 1 μg) presented separately to *A. variegatum* adults ($N = 15$, $N = 20$, respectively) failed to elicit any behavioral responses from *A. variegatum* on the servosphere, nor did 2,6-DCP have any effect on *A. variegatum* behavior on the servosphere in several tests spanning a source dose range with 10-fold increases from 100 fg to 10 ng ($N > 10$ for each dose).

DISCUSSION

1-Octen-3-ol is present in volatiles over various life-stages of both *A. variegatum* and *A. hebraeum*. Moreover, this compound attracts unfed *A. variegatum*

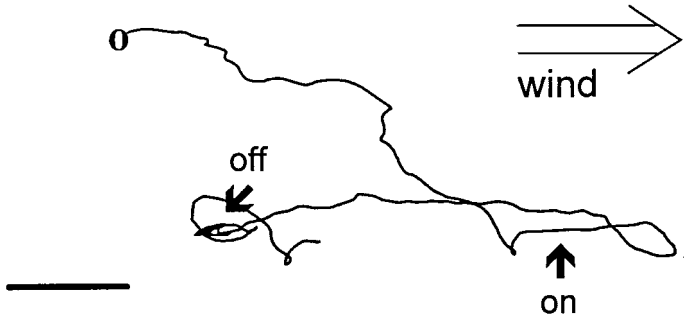

FIG. 4. Track described by an *Amblyomma variegatum* adult on the servosphere in response to vapors from racemate 1-octen-3-ol (source dose 10 ng). The track started (o) with the tick walking downwind (open arrow) in the control period. Note the off-response on loss of the attractant at odor off. The arrows on the track indicate odor on and odor off, and the bar beneath the track represents a displacement of 20 cm. Control, test, and end control periods of 1 min each.

adults on the servosphere. Whereas 1-octen-3-ol is a known semiochemical for hematophagous insects such as tsetse flies (Hall et al., 1984) and mosquitoes (Braverman et al., 1991), to our knowledge, this is the first report of 1-octen-3-ol being attractive to ticks. Although Norval et al. (1987) recorded activation of *A. hebraeum* in the field by odors of cattle and sheep, these authors recorded no such response to an open vial containing neat 1-octen-3-ol in the same study. Osterkamp et al. (1999) demonstrated that this compound enhanced the questing behavior of *Boophilus microplus*, albeit at the source dose of 3 μg compared to the 10-ng source dose used here. The level tested in this study is equivalent to about one twentieth that in bovid breath (Vale and Hall, 1985), and within the range of concentrations found attractive in wind-tunnel tests to the tsetse fly *Glossina morsitans morsitans* (Paynter and Brady, 1993) and the stable fly *Stomoxyes calcitrans* (Schofield and Brady, 1997).

The identity of the 1-octen-3-ol isomer released by the two *Amblyomma* tick species in our study is unknown. (*R*)-(−)-1-octen-3-ol predominates in bovid breath (Hall et al., 1984), probably arising from the enzymatic oxidation of linoleic acid (Tressl et al., 1982), but the racemate can arise from the nonenzymatic degration of this polyunsaturated fatty acid (Porter et al., 1980). In behavior, both 1-octen-3-ol samples tested elicited a similar proportion of off-responses upon withdrawal of the stimulus for *A. variegatum*, responses that were all but absent from the other treatments. Nonetheless, we found racemate 1-octen-3-ol to be a superior attractant to (*R*)-(−)-1-octen-3-ol, suggesting that the (*S*)-(+) isomer in the racemate might exert an independent effect. However, it is probably in the context of other as yet unidentified volatiles from ticks that

1-octen-3-ol exerts its effect. A difference in the efficacy of single compounds as attractants compared to the mixtures in which they are released has already been reported for this tick species (McMahon and Guerin, 2000).

The exact biological role of 1-octen-3-ol in the biology of *A. variegatum* and *A. hebraeum* remains to be determined. The greater amounts of 1-octen-3-ol recovered over disturbed ticks is consistent with reports such as that of Yoder et al. (1993b), wherein even bright light was sufficient to provoke the secretion of droplets by engorged *D. variabilis* females. Nonetheless, odors from tick material releasing relatively high amounts of 1-octen-3-ol in this study (newly molted ticks and engorged female *A. hebraeum* and *A. variegatum*) did not induce either attraction or repellency in *A. variegatum* adults on the servosphere. This suggests that ticks are either insensitive to the strong odor released or that some essential components of the odor did not reach the ticks on the servosphere. In contrast, attraction was recorded to samples emitting lower levels of volatiles, such as from dead ticks and exuviae plus feces. In the latter treatment, fecal volatiles are suggested as the potential source of the attractants, as 1-octen-3-ol was not detected over *A. variegatum* exuviae by using the sensitive electroantennogram detection method. The contribution of fecal attractants to "self-odor" in arthropods has long been recognized, particularly in aggregation. Ammonia has been implicated in the aggregation responses of arthropods, such as kissing bugs (Taneja and Guerin, 1997) and flour mites (Levinson et al., 1991) to their own feces, but fecal volatiles and odors released from the arthropods themselves may interact. Torto et al. (1996) demonstrated that phenols released from nymphal feces of the desert locust *Schistocerca gregaria* combined with several fatty acids and aldehydes released from nymphs themselves synergized the aggregation response. A similar role may exist for the several unidentified aldehydes present over engorged females (a particularly rich source of volatiles) in this study. Moreover, 1-octen-3-ol and several short chain aldehydes also are emitted by the host (Hall et al., 1984; Steullet and Guerin, 1994), and host volatiles attract *A. variegatum* (McMahon, 1999). It appears that ticks may be making parsimonious use of 1-octen-3-ol as a cue for aggregation with conspecifics and for host finding when in search of a blood meal.

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