2013

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A. L. Carr

R. M. Roe

C. Arellano

D. E. Sonenshine
Old Dominion University, dsonensh@odu.edu

C. Schal

See next page for additional authors

Original Publication Citation

Carr, A. L., Roe, R. M., Arellano, C., Sonenshine, D. E., Schal, C., & Apperson, C. S. (2013). Responses of *Amblyomma americanum* and *Dermacentor variabilis* to odorants that attract haematophagous insects. *Medical and Veterinary Entomology, 27*(1), 86-95. doi:10.1111/j.1365-2915.2012.01024.x

Medical and Veterinary Entomology

Medical and Veterinary Entomology (2013) **27**, 86–95 doi: 10.1111/j.1365-2915.2012.01024.x

Responses of *Amblyomma americanum* and *Dermacentor variabilis* to odorants that attract haematophagous insects

A. L. CARR[1], R. M. ROE[1], C. ARELLANO[2], D. E. SONENSHINE[3], C. SCHAL[1] and C. S. APPERSON[1]

[1]Department of Entomology, North Carolina State University, Raleigh, NC, U.S.A., [2]Department of Statistics, North Carolina State University, Raleigh, NC, U.S.A. and [3]Department of Biological Sciences, Old Dominion University, Norfolk, VA, U.S.A.

Abstract. Carbon dioxide (CO_2), 1-octen-3-ol, acetone, ammonium hydroxide, L-lactic-acid, dimethyl trisulphide and isobutyric acid were tested as attractants for two tick species, *Amblyomma americanum* and *Dermacentor variabilis* (Acari: Ixodidae), in dose–response bioassays using Y-tube olfactometers. Only CO_2, acetone, 1-octen-3-ol and ammonium hydroxide elicited significant preferences from adult *A. americanum*, and only CO_2 was attractive to adult *D. variabilis*. Acetone, 1-octen-3-ol and ammonium hydroxide were separately evaluated at three doses against CO_2 (from dry ice) at a field site supporting a natural population of *A. americanum* nymphs and adults. Carbon dioxide consistently attracted the highest number of host-seeking ticks. However, for the first time, acetone, 1-octen-3-ol and ammonium hydroxide were shown to attract high numbers of *A. americanum*. Further research is needed to determine the utility of these semiochemicals as attractants in tick surveillance and area-wide management programmes.

Key words. *Amblyomma americanum*, *Dermacentor variabilis*, attractant, behaviour, bioassay, field trial, kairomone, tick.

Introduction

Ixodid ticks transmit bacteria, viruses and protozoa that cause severely debilitating diseases in humans (Goodman *et al.*, 2005). Identifying the tick species that vector pathogens is a significant aspect of disease surveillance (Moore & Gage, 2005; Jameson & Medlock, 2011). Tick collections made in a systematic fashion provide the necessary information on relative abundances and spatial distributions of ticks to assess the risk for infection from tick-borne pathogens (Diuk-Wasser *et al.*, 2006; Walk *et al.*, 2009; Eisen & Eisen, 2011; Reis *et al.*, 2011). Estimates of local abundance enable evaluation of the efficacy of acaricidal and other methods of control (Mount, 1981; Davidson *et al.*, 1994; Pound, *et al.*, 2000; Bissinger *et al.*, 2011). Carbon dioxide (CO_2) is a universal attractant for blood-feeding arthropods and, used as dry ice, is the main attractant used for collecting ticks and conducting tick surveillance (Garcia, 1962; Oliveira *et al.*, 2000; Guedes *et al.*, 2005). Traps baited with dry ice have been reported to collect significantly more adults of *Ixodes scapularis* Say (= *Ixodes dammini* Spielman, Clifford, Piesman Corwin), adults and nymphs of *Amblyomma americanum* L. and adults of *Dermacentor variabilis* Say (Acari: Ixodidae) than flagging vegetation (Kinzer *et al.*, 1990; Solberg *et al.*, 1992; Petry *et al.*, 2010). In addition, significantly more *A. americanum* adults and nymphs were collected in dry ice-baited traps than in rabbit-baited traps (Koch & McNew, 1981). Similarly, dry ice-baited traps placed in domestic landscapes collected more *I. scapularis* larvae and nymphs than were found on *Peromyscus* mice trapped in the same habitats (Falco & Fish, 1992). However, dry ice may not

Correspondence: Charles S. Apperson, Department of Entomology, Campus Box 7613, North Carolina State University, Raleigh, NC 27695-7613, U.S.A. Tel.: +1 919 515 4326; Fax: +1 919 515 3748; E-mail: apperson@ncsu.edu

be available in some locales, is often costly to purchase in the quantities needed for area-wide sampling, and is burdensome to transport. The effective collection range of traps baited with dry ice is unknown because CO_2 attracts ticks over an area of indeterminate size (Moore & Gage, 2005).

Although CO_2 is also an attractant for a variety of haematophagous insects, including mosquitoes, tabanid flies, tsetse flies, *Culicoides* midges, bedbugs and triatomine bugs (Mands *et al.*, 2004; Krcmar, 2005; Milne *et al.*, 2009; Wang *et al.*, 2009; Jawara *et al.*, 2011; Torr *et al.*, 2011), there are other host-derived kairomones that are attractive to blood-feeding arthropods. Sonenshine (2004) and Logan & Birkett (2007) provide informative reviews of semiochemicals that attract ticks and insects that bite humans and animals. As well as CO_2, some host chemicals that have been reported to be attractive to a variety of biting arthropods include L-lactic acid, other carboxylic acids, acetone, nitrogenous wastes, sulphides and 1-octen-3-ol.

In the present investigation, we evaluated the responses of two species of ixodid tick, *A. americanum* and *D. variabilis*, in laboratory bioassays to some semiochemicals that were reported to be attractive to blood-feeding arthropods. The chemicals that elicited behavioural responses were subsequently tested with a natural tick population in field trials. Our objective was to identify chemicals for potential use in tick surveillance and control programmes.

Materials and methods

Ticks

Amblyomma americanum adults were purchased from the Oklahoma State University Tick Rearing Facility (Stillwater, OK, U.S.A.). Adult *D. variabilis* were obtained from laboratory colonies maintained by DES at Old Dominion University (Norfolk, VA, U.S.A.). Ticks were housed in an insectary maintained at approximately 28 °C and relative humidity (RH) of 75% with a photoperiod of LD 14 : 10 h, with dawn and dusk periods of 1 h each at the beginning (05.00 hours) and end (19.00 hours) of the scotophase. After receipt, ticks were held in the insectary for 72 h prior to use in laboratory bioassays.

Response to CO_2 in olfactometer assays

Preliminary behavioural assays were conducted with CO_2 using a glass Y-tube olfactometer (Fig. 1) to establish that this bioassay system could be used to measure the response to a

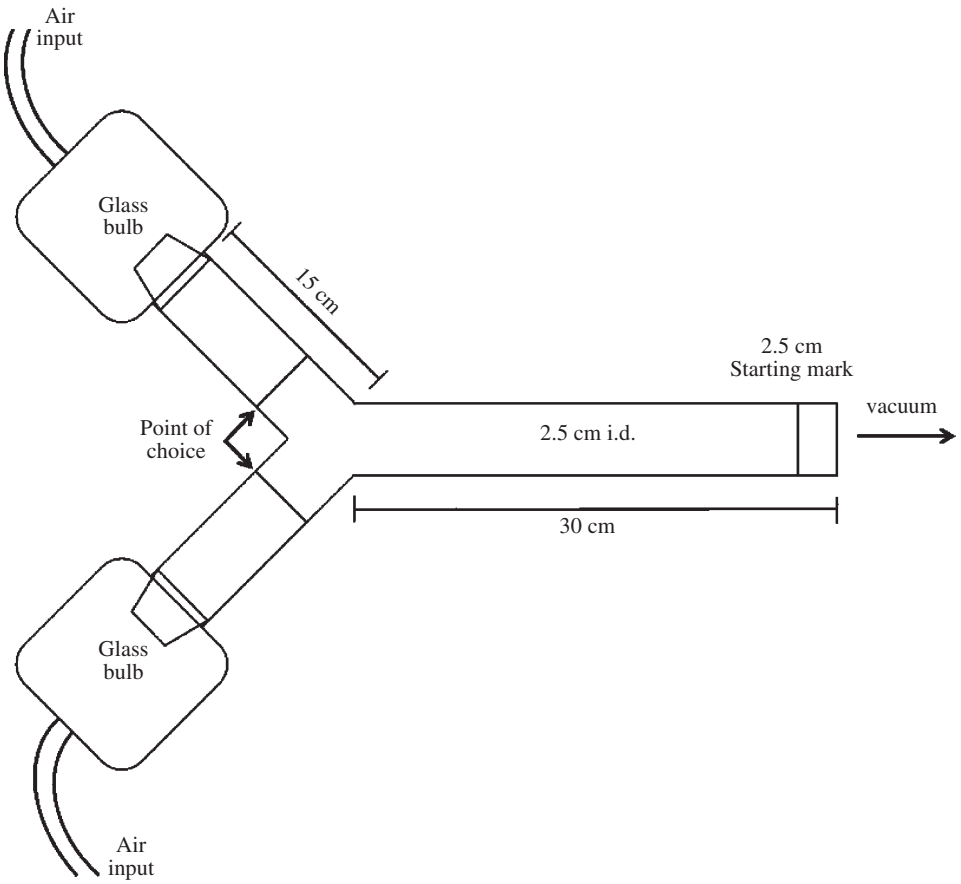

Fig. 1. Diagram of Y-tube apparatus used in laboratory bioassays of candidate attractants.

known tick attractant. Tests were conducted during the daytime between 11.00 hours and 15.00 hours at 23 ± 1 °C and RH 40% under ambient (fluorescent) lighting. Carbon dioxide (3% by volume in breathing-quality air) was administered into one port of the Y-tube from a compressed gas tank (Airgas, Inc., Charlotte, NC, U.S.A.). Breathing-quality air was introduced into the second port of the Y-tube from another compressed gas tank (Airgas, Inc.). Gas flow rates were regulated with #11 Compact Shielded flow meters (Gilmont Instruments, Inc., Barrington, IL, U.S.A.). The responses of *A. americanum* and *D. variabilis* adults to a series of different flow rates were separately evaluated. Thus, as the flow rate increased, the concentration of CO_2 remained the same (3%), but the flux of CO_2 over the ticks increased. Flow rates of breathing-quality air released into the second port were symmetrically adjusted to match those of the first port. A vacuum pump was used to remove gases from the downwind end of the Y-tube at a rate equal to the total flow through the olfactometer. Gases removed from the olfactometer were exhausted out of the test area. Test odorants were rotated between the two ports to compensate for potential positional response bias. All equipment was washed with hot tap water and 95% ethanol in distilled water between trials to eliminate contaminants and dried at least overnight at room temperature.

All ticks were acclimated to the experimental setting 30 min prior to being transferred into the olfactometer. Six unfed adult ticks of mixed sex of one species were placed into the Y-tube exhaust outlet at the 2.5-cm start mark (Fig. 1). Each bioassay was conducted for 5 min and replicated six times on separate dates with ticks not previously tested. Ticks were recorded as positive responders if they made a choice in Y-tube bioassays (i.e. they moved 2.5 cm into one of the arms of the Y-tube) (Fig. 1).

Because the concentration of CO_2 in the tank was fixed, the requirements of a dose–response study indicated that the flow rates be adjusted. To eliminate the possibility that the response of ticks was stimulated by increased air flow rate and not by the increased flux of CO_2, ticks were tested for responses to flow rates of 25 mL, 50 mL, 75 mL, 100 mL, 125 mL and 150 mL per min of only breathing-quality air from both arms of the olfactometer.

Candidate attractants

The following American Chemical Society (ACS) reagent grade chemicals (Sigma Aldrich Corp., St Louis, MO, U.S.A.) were screened: 1-octen-3-ol (octenol, >98%); acetone (>99.9%); L-lactic-acid (lactic acid, >98%); isobutyric acid (99%); ammonium hydroxide (28–30% aqueous solution), and dimethyl trisulphide (>98%). The chemicals were further diluted with distilled water for bioassay. Dilutions (1-mL volumes) were made (w/v) 1 h prior to bioassay.

Y-tube assay of candidate attractants

Bioassays were conducted using the glass Y-tube olfactometer as described previously for CO_2. All compounds were first evaluated with *A. americanum* ticks. As ticks must be reared on animals, in order to reduce animal use, only chemicals eliciting significant responses were then tested with *D. variabilis*. Airflow, based on optimum results from CO_2 assays, was set to 100 mL/min/port for *A. americanum* bioassays and to 75 mL/min/port for *D. variabilis* trials. Distilled water was used as a control chemical and was applied to a piece of cellulose filter paper (1.5 cm^2, Whatman #1), which was immediately placed into one of the Y-tube ports with forceps. A standard volume (22.5 µL) of each chemical dilution was applied to another piece of cellulose filter paper (1.5 cm^2), which was immediately placed into the second Y-tube port. The control filter paper was always placed into the Y-tube port first to prevent cross-contamination by chemicals during handling and assay set-up. Treatments and controls were rotated between the two ports to prevent positional response bias. All ticks that made a choice in the Y-tube bioassays were recorded as positive responders.

Chemicals eliciting significant attraction of *A. americanum* were separately tested at their optimally active concentrations in combination with CO_2. All pairwise combinations of the chemicals were then tested at their optimally active concentrations in ratios (v/v) of 1 : 2, 1 : 1 and 2 : 1 without CO_2 against a distilled water control. Following these assays, all three chemicals were combined at their optimally active concentrations in a 1 : 1 : 1 ratio and tested without CO_2, using distilled water as a control. These bioassays were conducted using the same number of ticks per replicate assay and the same number of assays as previously described.

Field trials

Semiochemicals exhibiting statistically significant attraction in laboratory assays were tested at a field site supporting a population of *A. americanum*. Accordingly, octenol, acetone and ammonium hydroxide were compared with CO_2 (from dry ice) and water controls. Field trials were conducted in Harris Lake County Park in Wake County, Newhill, NC, U.S.A. (35°37′30.92″ N, 78°55′35.24″ W) during June 2011. Field trials were set up between 11.00 hours and 12.00 hours and conducted from 12.00 hours to 13.00 hours. Air temperatures ranged from 32.2 °C to 35.0 °C and RH ranged from 40% to 70%. The ground cover at the test site consisted of oak leaf and pine needle litter. An overstory of hardwoods of mixed species and pine trees shaded the test site. Trials were never conducted on days with wet or damp ground cover. The three chemicals and water were dispersed from open-mouthed glass vials (1.4 cm internal diameter, 5.5 cm high) containing 2 mL of undiluted chemical. The amount of each chemical released was increased by raising the number of vials used. Carbon dioxide was dispersed using 0.68 kg of dry ice pellets. Vials and dry ice pellets were placed onto individual 60 × 60-cm white cloth sheets (60% cotton, 40% polyester) that were set on the ground at the field site. Prior to use, all cloth sheets were cleaned in a washing machine without detergent, in hot water on a regular cotton cycle and dried at high heat in a standard household clothing dryer. After washing, sheets were

stored in plastic bags until used. Nitrile gloves were worn when handling cloths.

At the field site, wire surveyor's flags were used to mark out a 5×5 grid of sampling cells (each cell measured 7.6 $\times$ 7.6 m). For each chemical, one trial with test and control compounds was conducted per day along a transect line of contiguous cells. Because it was likely that more ticks would be collected than could be counted in a timely manner, all ticks collected on each cloth sheet were transported to the laboratory for processing rather than being released back into the field. To avoid any bias caused by the removal of ticks, the test and control chemicals were shifted to the adjacent transect line when another replicate trial was initiated the next day (Table 1). Five trials were completed for each chemical in the same grid following a Latin square experimental setting, with trial (*Day*) as the row blocking factor and columns (*Position*) as the second blocking factor. All treatments were included each day so that all treatments had been delivered in each column by the end of the trials. The grid for each chemical was located in a different area of the field site. When a trial was conducted, one sheet was placed on the ground in the geometric centre of each grid cell and vials containing a chemical, water or dry ice were placed onto the centre of each sheet. Nitrile gloves were worn during trial set-up to prevent contamination of vials and sheets with skin odorants. One hour after placement of vials and dry ice on each cloth, ticks were collected with forceps and stored in labelled 50-mL conical tubes (BD Falcon, Inc., Franklin Lakes, NJ, U.S.A.). Subsequently in the laboratory, ticks were identified to species and life stage and counted. Release rates of test chemicals, water and CO_2 were determined by weighing the vials or dry ice immediately before and after field trials.

Statistical analysis

Laboratory bioassays. Olfactometer assays in which fewer than two of the six ticks responded and made a choice were excluded from analysis. Conditional to the total number of ticks making a choice of test or control substances, the frequency response for each chemical was analysed using a chi-squared test of homogeneity of proportions (the null hypothesis stated that the expected proportion for either Y-tube choice would be 0.5). The PROC FREQ procedure in SAS for Windows Version 9.2 (SAS Institute, Inc., Cary, NC, U.S.A.) was used.

Field trials. Results for each chemical were analysed separately with the PROC GLIMMIX procedure in SAS. Response was modelled through a Poisson regression with an overdispersion parameter to account for larger variation than expected under the assumption that tick counts would follow a Poisson distribution. The statistical model assumes that the expected mean of each tick count observed was a function of *Treatment*, *Day* (= grid row or trap transect line) and *Position* (= grid column). *Day* and *Position* were blocking factors for the Latin square experimental design used to investigate *Treatment* effect on the number of ticks attracted to each chemical. The Poisson regression assumes that the expected mean value of the number of ticks observed in a plot (experimental unit) at a particular position and on a given day receiving a given treatment is represented as a linear model for the natural log of the expected mean: log (μ) = Overall constant + Day effect + Position effect + Treatment effect + uncontrolled random effect. Treatment effect differences were analysed with Tukey's multiple range test for pairwise least squares means at a 0.05 significance level, and Dunnett–Hsu test to compare treatment least squares means with water (negative control) least squares means and also to compare treatment least squares means with CO_2 (positive control) at a 0.05 significance level.

Results

Response to CO_2 in olfactometer assays

Air flow rate did not affect tick response (Figs 2B and 3B), which indicated that all observed movements of *A. americanum* and *D. variabilis* adults up the Y-tube olfactometer were in response to CO_2. *Amblyomma americanum* exhibited significant attraction to 3% CO_2 at all flow rates except 25 mL/min, with the highest response to a flow rate of 100 mL/min ($\chi^2 = 21.16$, d.f. = 1, $P < 0.0001$) (Fig. 2A). By contrast, *D. variabilis* was significantly attracted to 3% CO_2 at flow rates of 50 mL/min ($\chi^2 = 13.76$, d.f. = 1, $P = 0.0002$) and 75 mL/min ($\chi^2 = 17.19$, d.f. = 1, $P < 0.0001$) (Fig. 3A).

Response to chemicals in olfactometer assays

Of the six chemicals tested, only acetone, octenol and ammonium hydroxide elicited movement of *A. americanum* towards the odorants (Fig. 4). When octenol was tested against distilled water, more ticks chose octenol at all concentrations tested except the 10% concentration, which repelled ticks towards the water control ($P = 0.001$) (Fig. 4). However, the preference for octenol was statistically significant only for the 2.5% concentration ($P = 0.003$). Ticks showed a significant preference for acetone at concentrations of 5% ($P = 0.020$), 1% ($P = 0.007$) and 0.5% ($P = 0.052$) (Fig. 4). No preference between distilled water and acetone was evident at concentrations of 10% ($P = 0.560$) and 0.1% ($P = 0.590$). Significant preference for ammonium hydroxide was exhibited for concentrations of 12.5% ($P = 0.032$), 10% ($P = 0.012$), 5% ($P = 0.007$) and 1% ($P = 0.035$), but not for concentrations of 25% ($P = 0.150$) and 0.1% ($P = 0.410$) (Fig. 4).

Ticks did not show a preference for isobutyric acid at any of the tested concentrations, and the higher concentrations repelled them towards distilled water. The choice of distilled water was significant at 0.05% ($\chi^2 = 10.89$, d.f. = 1, $P = 0.001$) and 10% ($\chi^2 = 22.53$, d.f. = 1, $P < 0.0001$) isobutyric acid, but not at 1% ($\chi^2 = 2.58$, d.f. = 1, $P = 0.110$). Lower concentrations of isobutyric acid were less repellent to ticks, although no preference was observed for either distilled water or isobutyric acid (0.1%: $\chi^2 = 0.11$, d.f. = 1, $P = 0.74$; 0.01%: $\chi^2 = 0.82$, d.f. = 1, $P = 0.370$).

Table 1. Rotation of candidate tick attractants and control chemicals in the 5 × 5 plot used in field trials. For each candidate chemical, five trials were carried out with each trial completed on a different day.

		Position				
		A	B	C	D	E
Day	1	Dry ice	Distilled water	1 vial	2 vials	3 vials
	2	3 vials	Dry ice	Distilled water	1 vial	2 vials
	3	2 vials	3 vials	Dry ice	Distilled water	1 vial
	4	1 vial	2 vials	3 vials	Dry ice	Distilled water
	5	Distilled water	1 vial	2 vials	3 vials	Dry ice

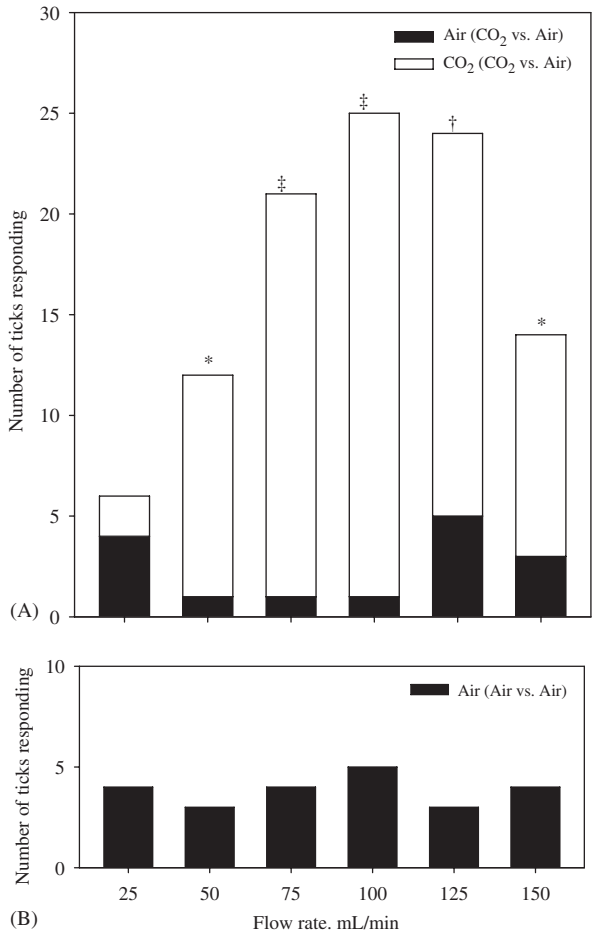

Fig. 2. Cumulative response of *Amblyomma americanum* adults to 3% CO_2 and humidified air in a Y-tube olfactometer. Six ticks were tested in each of five assays (30 ticks in total) in which ticks were given a choice between (A) air and CO_2, and (B) air entering both arms of the olfactometer as a control for varying the flow rate. The frequency response was analysed using a chi-squared test of homogeneity of proportions under the null hypothesis that the expected proportion for either Y-tube choice was 0.5. Response to CO_2 was significant at $0.05 \geq P > 0.01$ (*), $0.01 \geq P > 0.001$ (†), $P \leq 0.001$ (‡).

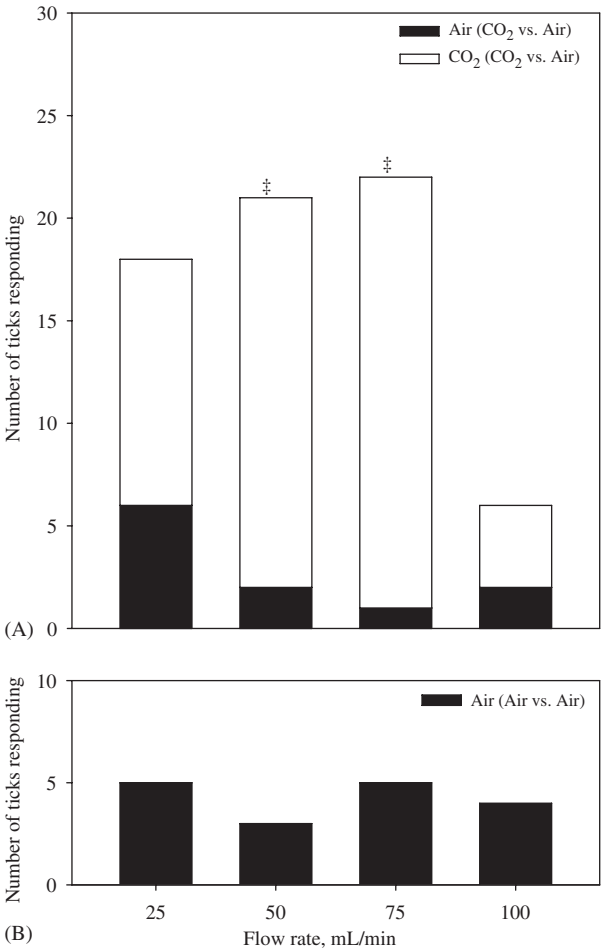

Fig. 3. Cumulative response of *Dermacentor variabilis* adults to 3% CO_2 and humidified air in a Y-tube olfactometer. Six ticks were tested in each of five assays (30 ticks in total), in which ticks were given a choice between (A) air and CO_2, and (B) air entering both arms of the olfactometer as a control for varying the flow rate. The frequency response was analysed using a chi-squared test of homogeneity of proportions under the null hypothesis that the expected proportion for either Y-tube choice was 0.5. Response to CO_2 was significant at $0.05 \geq P > 0.01$ (*), $0.01 \geq P > 0.001$ (†), $P \leq 0.001$ (‡).

Responses to dimethyl trisulphide were similar to those seen for isobutyric acid. Ticks showed a significant preference for distilled water over 10% dimethyl trisulphide ($\chi^2 =$ 9.94, d.f. $= 1$, $P = 0.002$). Lower concentrations were less repellent, but differences in responses to distilled water and dimethyl trisulphide were not statistically significant (1%:

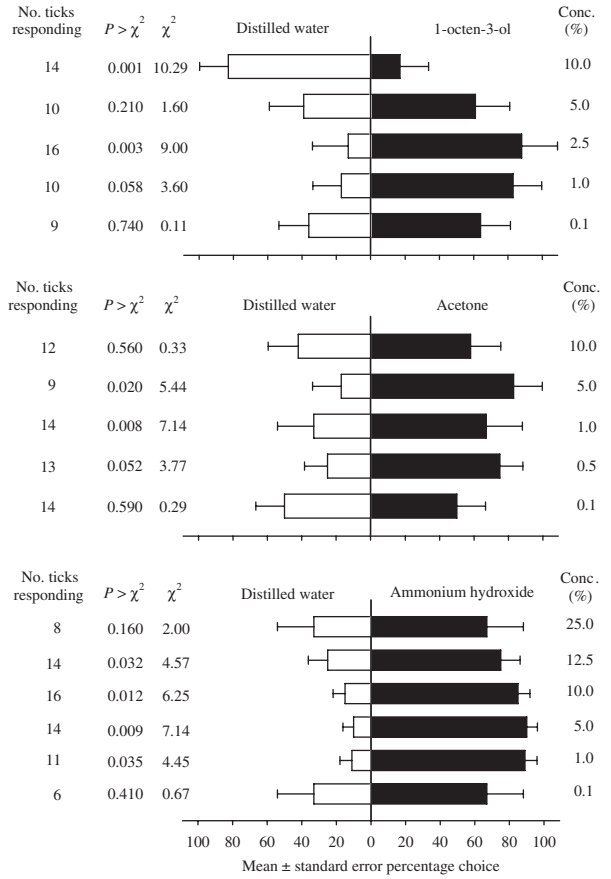

Fig. 4. Responses of *Amblyomma americanum* adults to 1-octen-3-ol, acetone and ammonium hydroxide in a Y-tube olfactometer. Thirty ticks were assayed at each concentration.

$\chi^2 = 0.20$, d.f. $= 1$, $P = 0.650$; 0.1%: $\chi^2 = 0.11$, d.f. $= 1$, $P = 0.740$; 0.01%: $\chi^2 = 0.11$, d.f. $= 1$, $P = 0.740$).

Ticks did not exhibit a preference for any of the L-lactic acid concentrations tested against distilled water. Significant repellency was observed for 1% lactic acid ($\chi^2 = 11.27$, d.f. $= 1$, $P = 0.0008$). More ticks chose distilled water over lower concentrations of lactic acid, but none of the responses were statistically significant (10%: $\chi^2 = 0.14$, d.f. $= 1$, $P = 0.71$; 5%: $\chi^2 = 2.00$, d.f. $= 1$, $P = 0.160$; 0.05%: $\chi^2 = 1.14$, d.f. $= 1$, $P = 0.290$; 0.01%: $\chi^2 = 1.60$, d.f. $= 1$, $P = 0.210$).

Dermacentor variabilis was not attracted to any of the chemicals examined. Ticks showed a significant repellency from octenol at concentrations of 10% ($\chi^2 = 9.80$, d.f. $= 1$, $P = 0.002$) and 1% ($\chi^2 = 9.53$, d.f. $= 1$, $P = 0.002$). No preference for octenol or water was observed at concentrations of 0.5% ($\chi^2 = 0.69$, d.f. $= 1$, $P = 0.410$) and 0.1% ($\chi^2 = 0.00$, d.f. $= 1$, $P = 1.000$). Ticks showed a preference for distilled water over acetone for both concentrations of acetone tested; however, their preferences at 10% ($\chi^2 = 3.60$, d.f. $= 1$, $P = 0.058$) and 1% ($\chi^2 = 0.50$, d.f. $= 1$, $P = 0.480$) acetone were not statistically significant. A higher percentage of *D. variabilis* preferred ammonium hydroxide over distilled water although none of the ammonium hydroxide concentrations elicited statistically significant responses (10%: $\chi^2 = 1.33$, d.f. $= 1$, $P = 0.250$; 1%: $\chi^2 = 2.91$, d.f. $= 1$, $P = 0.088$; 0.5%: $\chi^2 = 1.14$, d.f. $= 1$, $P = 0.290$; 0.1%: $\chi^2 = 2.78$, d.f. $= 1$, $P = 0.960$).

Additional bioassays were conducted with *A. americanum*. Octenol, acetone and ammonium hydroxide were tested individually at their optimum concentrations in combination with 3% CO_2 (100 mL/min). Three different ratios of each pairwise combination of these chemicals and a mixture of all three chemicals were tested without CO_2. There was no significant increase in responses ($P > 0.05$) to the chemicals when they were added separately to CO_2 or combined and tested without CO_2 against distilled water controls.

Field trials of selected chemicals

Based on results of laboratory olfactometer experiments for *A. americanum*, three chemicals (octenol, acetone and ammonium hydroxide) were chosen for evaluation in the field. In these field trials, only *A. americanum* was collected. Nymphs and adults comprised 90% and 10%, respectively, of the collections. Statistical analyses were carried out for candidate attractants using combined data for nymphs and adults. *Treatment* was a significant main effect variable for all three chemicals. There was no significant difference between transect lines (= *Day*) for any of the chemicals. However, *Position* was significant for the acetone and ammonium hydroxide field trials (Table 2).

Octenol was tested in field assays at mean ± standard error (SE) release rates of 10.0 ± 0.8 mg/h (one release vial), 20.0 mg/h (two release vials) and 30.0 mg/h (three release vials) separately against distilled water (10.1 ± 7.0 mg/h) and CO_2 from dry ice (547 ± 23 g/h) controls. The response to octenol was highly variable under field conditions (Fig. 5). There was no significant difference between the numbers of ticks attracted to distilled water and octenol at 10 mg/h ($t = 1.57$, d.f. $= 16$, $P = 0.137$) and 20 mg/h ($t = 1.06$, d.f. $= 16$, $P = 0.307$), but higher numbers of ticks were attracted to octenol at a release rate of 30 mg/h ($t = 2.19$, d.f. $= 16$,

Table 2. Type 3 test of fixed effects* for response of *Amblyomma americanum* ticks to candidate attractants in field trials.

Source	d.f.	*F*-value	$P > F$
1-octen-3-ol			
Treatment	4, 12	5.08	0.015
Position	4, 12	0.49	0.743
Day	4, 12	0.39	0.811
Acetone			
Treatment	4, 12	6.90	0.004
Position	4, 12	5.23	0.011
Day	4, 12	2.54	0.094
Ammonium hydroxide			
Treatment	4, 12	18.37	<0.0001
Position	4, 12	5.67	0.008
Day	4, 12	2.76	0.077

*Poisson regression, GLIMMIX procedure in SAS Version 9.2.

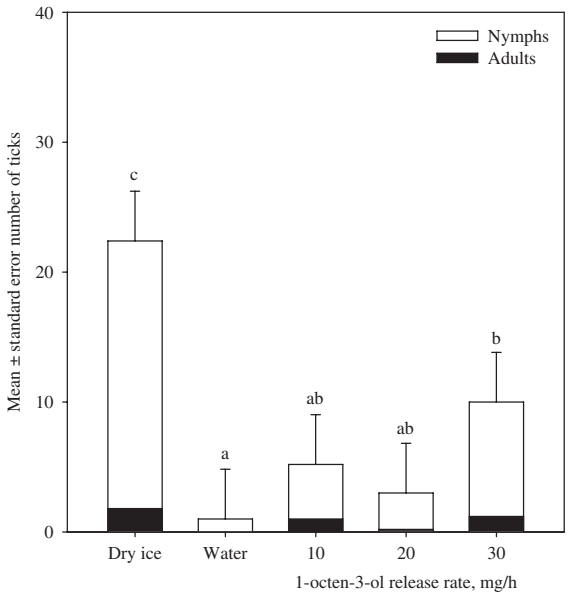

Fig. 5. Mean ± standard error number of *Amblyomma americanum* ticks collected in field trials of 1-octen-3-ol with water and carbon dioxide controls. Treatments were compared with pairwise *t*-tests. Outcomes for treatments marked with the same letter are not significantly different ($P > 0.05$).

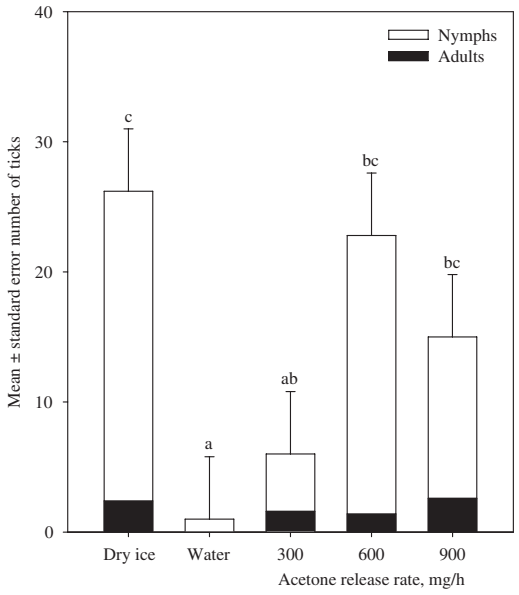

Fig. 6. Mean ± standard error number of *Amblyomma americanum* ticks collected in field trials of acetone with water and carbon dioxide controls. Treatments were compared with pairwise *t*-tests. Outcomes for treatments marked with the same letter are not significantly different ($P > 0.05$).

$P = 0.044$). In addition, the numbers of ticks attracted to the three release rates of octenol did not differ significantly ($P > 0.05$). Significantly more ticks were attracted to CO_2 than to distilled water ($t = 2.94$, d.f. $= 16$, $P = 0.009$) and to all three octenol release rates (10 mg/h: $t = -2.81$, d.f. $= 16$, $P = 0.015$; 20 mg/h: $t = -2.94$, d.f. $= 16$, $P = 0.012$; 30 mg/h: $t = -2.13$, d.f. $= 16$, $P = 0.049$). Nevertheless, a surprisingly high number of ticks were attracted to the highest release rate of octenol, suggesting that even higher rates might be more attractive.

Acetone was also tested at three release rates (300 ± 18.8 mg/h, 600 mg/h and 900 mg/h) and was considerably more attractive than octenol (Fig. 6). However, the mean numbers of ticks responding to the three acetone treatments did not differ significantly ($P > 0.05$). Significant differences were observed for the number of ticks responding to octenol at 600 mg/h ($t = 2.42$, d.f. $= 16$, $P = 0.028$) and 900 mg/h ($t = 2.71$, d.f. $= 16$, $P = 0.019$) and to CO_2 from dry ice ($t = 2.53$, d.f. $= 16$, $P = 0.022$) compared with distilled water. The numbers of ticks attracted to acetone at 300 mg/h and to distilled water did not differ significantly ($t = 1.31$, d.f. $= 16$, $P = 0.208$). Significantly more ticks responded to CO_2 than to acetone at a release rate of 300 mg/h ($t = -2.57$, d.f. $= 16$, $P = 0.020$), but not when acetone was released at 600 mg/h ($t = -0.38$, d.f. $= 16$, $P = 0.706$) or 900 mg/h ($t = -1.36$, d.f. $= 16$, $P = 0.192$) (Fig. 6), indicating that acetone has good potential as a tick attractant.

Ammonium hydroxide in water was tested at three release rates (0.6 ± 0.013 g/h, 1.2 g/h and 1.8 g/h). Similarly to acetone, ammonium hydroxide attracted *A. americanum* in the field (Fig. 7). Comparisons of the three release rates of ammonium hydroxide showed that only the highest release rate attracted significantly more ticks than the intermediate ($t = -2.54$, d.f. $= 16$, $P = 0.022$) and lowest ($t = -2.18$, d.f. $= 16$, $P = 0.045$) release rates. More ticks were attracted by the highest release rate of ammonium hydroxide ($t = 2.52$, d.f. $= 16$, $P = 0.023$) and CO_2 from dry ice ($t = 2.96$, d.f. $= 16$, $P = 0.009$) than by distilled water. No difference was observed between the number of ticks attracted by distilled water and the numbers attracted by low ($t = -0.52$, d.f. $= 16$, $P = 0.610$) and intermediate ($t = 0.09$, d.f. $= 16$, $P = 0.932$) release rates of ammonium hydroxide. Significantly more ticks were attracted to CO_2 than to ammonium hydroxide at release rates of 0.6 g/h ($t = -2.445$, d.f. $= 16$, $P = 0.026$) and 1.2 g/h ($t = -3.01$, d.f. $= 16$, $P = 0.008$), but not when ammonium hydroxide was released at 1.8 g/h ($t = -1.19$, d.f. $= 16$, $P = 0.253$), indicating that ammonium hydroxide has some potential as an attractant for ticks.

Discussion

Laboratory bioassays

For the first time, several host-associated kairomones were shown to be attractive to *A. americanum*. By contrast, *D. variabilis* adults were not attracted to any of the chemicals tested other than CO_2. Differences in the responses of *A. americanum* and *D. variabilis* to the host-associated semiochemicals may be partially explained by dissimilarities in their host preferences. Preferred hosts of *Amblyomma* species include large ungulate species, such as deer and cattle, and medium-sized mammals, such as raccoons (Ouellette *et al.*,

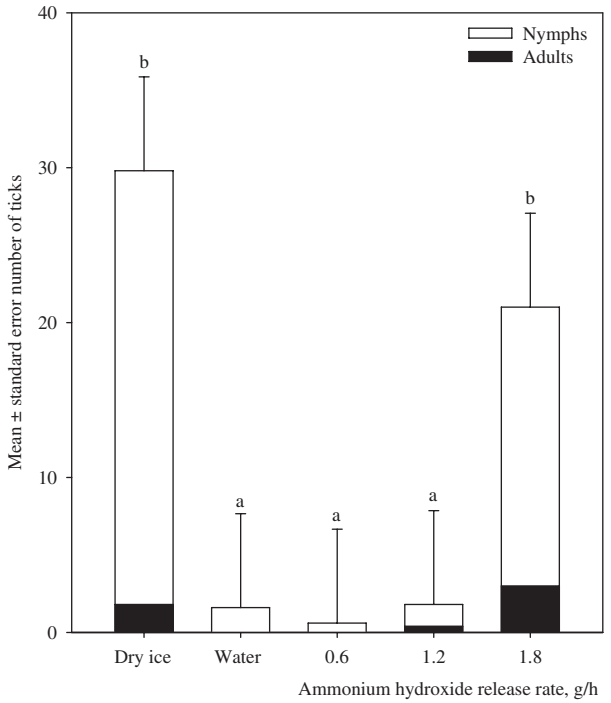

Fig. 7. Mean ± standard error number of *Amblyomma americanum* ticks collected in field trials of ammonium hydroxide with water and carbon dioxide controls. Treatments were compared with pairwise *t*-tests. Outcomes for treatments marked with the same letter are not significantly different ($P > 0.05$).

1997; Cohen *et al.*, 2010). Acetone and octenol are components of the breath of ruminant animals (Vale & Hall, 1985; Osterkamp *et al.*, 1999) and ammonia is found on the skin (Geier *et al.*, 1999) and in the breath of humans (Norwood *et al.*, 1992) and is produced from bacterial decomposition of tick faeces and animal carcasses (McMahon & Guerin, 2002; Sonenshine *et al.*, 2003). By contrast, *D. variabilis* adults do not feed on large ungulate hosts. *Dermacentor variabilis* adults prefer to feed on medium-sized mammals, such as dogs, coyotes and raccoons (Kollars *et al.*, 2000; Foster *et al.*, 2003; Cohen *et al.*, 2010).

How broadly attractive the three chemicals are for other tick species merits additional research effort. Similar attractant behaviour to acetone was reported for *Amblyomma variegatum* and *Rhipicephalus sanguineus* (Acari: Ixodidae) (Haggart & Davis, 1981; McMahon & Guerin, 2002). The attraction of *A. americanum* to a narrow range of concentrations of octenol is similar to that reported by McMahon & Guerin (2002) for *A. variegatum.* Octenol has also been reported to be attractive to *Amblyomma hebraeum* and *Rhipicephalus microplus* (Norval, 1987; Norval *et al.*, 1988; Osterkamp *et al.*, 1999).

Field trials

The activities of octenol, acetone and ammonium hydroxide in attracting *A. americanum* ticks were tested for the first time under field conditions. All three semiochemicals attracted high numbers of ticks in the field trials. The intermediate release rate of acetone (600 mg/h) attracted a mean of approximately 22.8 ticks/h, which was comparable with and not significantly different ($P > 0.05$) from the mean of 26.0 ticks/h attracted by CO_2 released from dry ice. The highest release rate of ammonium hydroxide (in water) (1.8 g/h) attracted a mean of about 21.0 ticks/h, which was not significantly ($P > 0.05$) different from the mean of 29.0 ticks/h attracted by CO_2. Despite positive laboratory bioassays, octenol attracted a mean of 10.0 ticks/h at the high release rate (30 mg/h), which was five times greater than but not significantly different ($P > 0.05$) from the mean number attracted to water. It should be noted that the release rate of CO_2 from dry ice (547 g/h) was 300, 900 and 18 000 times greater than the release of ammonium hydroxide (in water), acetone and octenol, respectively, based on the loss of weight of these chemicals during the field trials. On the basis of chemical molarity, the three semiochemicals were more potent tick attractants than CO_2. The latter captured an average of 2.3 ticks per mole of gas released per hour (ticks/mol/h) compared with an extrapolated estimate of 736, 2800 and 42 735 ticks/mol/h for ammonium hydroxide (in water), acetone and octenol, respectively. The approximation for ammonium hydroxide (in water) is almost certainly an underestimation as a portion of the measured ammonium hydroxide weight loss should be attributed to water loss. In addition, these extrapolations may be significantly constrained by the shapes of the extrapolated dose–response curves, particularly for acetone.

Results for the dose–response trials suggest that higher release rates of octenol and ammonium hydroxide would attract more ticks. As the intermediate release rate of acetone captured the largest number of ticks, it is unlikely that releasing acetone at a higher rate would increase the catch of ticks. It is likely, however, that a longer exposure time would increase the number of ticks attracted to all three of these semiochemicals. Although CO_2 did not synergize the activity of acetone, ammonium hydroxide or octenol against *A. americanum* adults in laboratory bioassay, under field conditions CO_2 has been shown to synergize the activity of haematophagous insect traps baited with the semiochemicals used in our investigation (Kline, 2007; Logan & Birkett, 2007). Consequently, these semiochemicals should be re-evaluated in combination with CO_2 against field populations of *A. americanum*. Similarly to CO_2, the volatility and dispersion of these chemicals is likely to be affected by local meteorological conditions, principally temperature, wind speed and wind direction.

Our field sampling design, which involved a grid of sampling cells, was effective in evaluating the activity of the test chemicals. However, the aggregated nature of tick populations was probably a factor in the variability of our data and increased replication would be beneficial in future field trials using our technique. In addition, we removed ticks from each sampling grid on a daily basis because ticks were too numerous to count at the field site at the end of each assay period. Reintroduction of these disturbed ticks would be expected to affect the research results. We attempted to avoid any bias that would be introduced by 'sampling with removal' by shifting the treatments each day to an adjacent transect line. Koch & McNew

(1982) used a mark–release–recapture method to determine that only 10–20% of marked *A. americanum* nymphs were recovered from a distance of 3.1 m on dry ice-baited traps 2 h after the ticks had been released. Thus, it is unlikely that on a given day, removing ticks from the sampling grid affected the numbers of ticks collected on subsequent days because our sampling period was 1 h and the centre point of each grid cell was 3.8 m from its edge.

Field trials of candidate attractants could also be conducted by releasing cohorts of marked ticks into woodlands rather than testing semiochemicals against wild tick populations. This approach would be likely to reduce the variability in numbers of ticks collected between trials as tick populations are distributed in a clustered manner in the environment (Carroll *et al.*, 2006). It would also compensate for differences in the numbers of *A. americanum* adults and nymphs collected. In our field trials, it is likely that differences in the numbers of nymphs and adults collected reflected disparities in their abundances rather than differences in the behaviour of the two stages. In a survey conducted in a county adjacent to the field site used in the present investigation (Apperson *et al.*, 2008), flagging vegetation yielded the same ratio of *A. americanum* adults to nymphs. Future work should be conducted during variant seasons and in different habitats to compare the activities of the semiochemicals in other tick species and life stages.

Conclusions

Octenol, acetone and ammonium hydroxide were shown for the first time to attract *A. americanum* adults in laboratory bioassays and nymphs in field trials. Additional research is warranted to determine the performances of these semiochemicals in different habitats and against other tick species. Our results indicate that these semiochemicals are promising lures, which, on a molar basis, are more effective than CO_2, and could potentially be combined with traps to develop focused lure-and-kill strategies in home landscapes. In this regard, Nchu *et al.* (2009) recently used an attraction–aggregation–attachment pheromone to lure *A. variegatum* to traps containing the entomopathogenic fungus *Metarhizium anisopliae*. The semiochemicals tested in the present study have the potential to fit effectively into this type of control application. The lures could also be used to assess tick distribution in the landscape so that acaricides could be focused rather than generally applied. These semiochemicals would also facilitate the collection of ticks for pathogen testing in order to assess the risk for infection from tick-borne agents. As attractants for ticks, these semiochemicals would be less costly and burdensome to use relative to dry ice.

Acknowledgements

ALC was supported by a teaching assistantship from the Department of Entomology at North Carolina State University, Raleigh, NC, U.S.A. Her research was also supported in part by the Blanton J. Whitmire Endowment at North Carolina State University. RMR and DES received grants from the National Science Foundation (IBN-0315179 and IOS-0949194, respectively). The authors thank Sydney Jarrett, Department of Entomology, North Carolina State University, for her help in the field trials.

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Accepted 21 April 2012
First published online 11 June 2012