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In vitro assays for repellents and deterrents for ticks: differing effects of products when tested with attractant or arrestment stimuli

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Abstract. Most *in vivo* and *in vitro* tests with repellents or deterrents against ticks have not considered which sensory channel is being targeted. We have recorded the responses of two hard tick species (Acari: Ixodidae) *in vitro* to determine if such products can disrupt the perception of an attractant in a repellent assay or the perception of an arrestment stimulus in a deterrent assay. Ethyl butylacetylaminopropionate (EBAAP), *N*,*N*-diethyl-methyl-benzamide (deet), permethrin and indalone were chosen to test their capacity to inhibit the attraction of *Amblyomma variegatum* Fabricius to its aggregation-attachment pheromone. Vapours of each test product plus those from a synthetic blend of the pheromone were delivered to the walking tick in an air stream on a locomotion compensator. Neither EBAAP, deet, permethrin nor indalone could inhibit attraction of *A. variegatum* even when each of the test products was delivered at 10^6 times the pheromone. Indalone did decrease the attraction of *A. variegatum* to the pheromone and induced repulsion of *A. variegatum* when presented on its own in the air stream. The effect of permethrin, a sodium channel blocker, was also tested in a deterrent assay measuring the arrestment of *Ixodes ricinus* (L.) adults on its own faeces and faecal constituents. Permethrin deterred arrestment at doses of 670 fg/cm^2 to 67 ng/cm^2, i.e. at levels five times lower than the dose of chemostimuli present in the arrestment stimulus. This sensitivity to permethrin suggests that it acts via the contact chemoreception channel.

Key words. *Amblyomma variegatum*, *Ixodes ricinus*, Ixodidae, attraction, butopyronoxyl, deet, *N*,*N*-diethyl-methyl-benzamide, deterrent, ethyl butylacetylaminopropionate, indalone, permethrin, repellent, tick.

Introduction

Ticks typically feed for lengthy periods on vertebrate hosts, causing anaemia and enabling the transmission of more diseases than vectored by any other group of haematophagous arthropods (Obenchain & Galun, 1982). While acaricides are extensively used in the control of these ectoparasites, resistance to such chemicals is of concern (Mekonnen *et al.*, 2002). Repellents and deterrents offer a different approach, where the goal is simply to prevent ticks attaching on a host and so reduce acaricide use. To achieve this, such products must interact with the chemosensory systems that ticks use to locate and attach to hosts (Waladde & Rice, 1982). To date, tests with repellents and deterrents for ticks have been made predominantly *in vivo* where ticks' responses to the topical application of test products on the host are recorded, but it has not been clear whether it is via the olfactory or gustatory (if any) sensory channel that such products exert their effect.

Fortunately, the chemosensory apparatus with which ticks perceive olfactory and gustatory stimuli is now well understood compared to other arthropods (Guerin *et al.*, 2000). The olfactory capability of ticks resides in some 20 wall-pore sensilla borne on the first leg tarsi (Hess & Vlimant, 1986), which serve as functional antennae to detect

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volatiles from hosts and conspecifics. Studies have identified stimuli that excite specific chemoreceptor cells housed in these sensilla: in the tropical bont tick, *Amblyomma variegatum* Fabricius, olfactory receptor cells responding to components of host odour and to the aggregation-attachment pheromone (AAP) have been characterized (Steullet & Guerin, 1992a,b, 1994a,b). The pheromone is composed of *o*-nitrophenol and methyl salicylate and is secreted by feeding males in order to attract conspecifics (Schöni *et al*., 1984; Norval *et al*., 1989; Diehl *et al*., 1991). Vapours of these compounds presented in an air stream attract adults of this species, both individually and in binary blends (McMahon & Guerin, 2000a). Gustation is also known to mediate several aspects of tick biology, such as mating (de Bruyne & Guerin, 1998), feeding (Waladde *et al*., 1979) and aggregation (Leahy *et al*., 1973; Dusbábek *et al*., 1991). Recently, Grenacher *et al*. (2001) demonstrated that the arrestment behaviour in the sheep tick, *Ixodes ricinus* (Linnaeus), on its own faeces is mediated by gustatory receptor cells housed in terminal wall-pore sensilla on the first leg tarsus. These authors also established that the purines guanine, xanthine and uric acid present in tick faeces also act as arrestment stimuli for this species.

To date, *in vitro* tests of repellents and deterrents for ticks have consisted of comparing the effect of the treatment vs. an inert control and include tests on the repellency of certain grasses (Malonza *et al*., 1992; Mwangi *et al*., 1995) and synthetic products (Matthewson *et al*., 1981; Lane & Anderson, 1984). These experiments nevertheless suffer from the same limitations as *in vivo* experiments where it is impossible to ascertain on which sensory channel they operate. Moreover, results from such *in vitro* tests may not be reproducible on the host (Matthewson *et al*., 1981). To attempt to overcome these difficulties, we have adopted another *in vitro* experimental design where the ticks' responses to products are recorded in the presence of air-borne attractants or contact stimulants. Using our well studied tick species *A. variegatum* and *I. ricinus* as models, we have made tests to determine if products with documented activity against ticks can disrupt (i) the attraction of *A. variegatum* to its AAP perceived via olfactory receptors and (ii) arrestment by *I. ricinus* on conspecific faecal products perceived via gustatory receptor cells. For the purposes of this study, we define a repellent as a compound whose vapour inhibits the response to an attractant and a deterrent as a compound (independent of its vapour pressure) that inhibits the response to an arrestment stimulus. The test products we have chosen are, ethyl butylacetylaminopropionate (EBAAP), *N*,*N*-diethyl-3-methyl-benzamide (deet), formerly *N*,*N*-diethyl-*m*-toluamide, (3-phenoxyphenyl)methyl (±)-*cis*, *trans*-3-(2,2-dichloroethenyl)-2, 2-dimethyl cyclopropanecarboxylate (permethrin), and butyl 3,4-dihydro-2, 2-dimethyl-4-oxo-2*H*-pyran-6-carboxylate (butopyronoxyl or indalone). These four compounds have been documented as having effects on ticks either by reducing the presence of ticks on hosts (e.g. Staub *et al*., 2002), by eliciting avoidance of a treated substrate (e.g. Matthewson *et al*., 1981; Dautel *et al*., 1999) or by reduced feeding on hosts (e.g. Mehr *et al*., 1986; Kumar *et al*., 1992).

Materials and methods

Ticks

One to nine-month old *A. variegatum* of both sexes were prepared for behavioural tests on the servosphere as in McMahon & Guerin (2000a). Each tick was only tested once. *Ixodes ricinus* adults reared in the laboratory were stored in glass vials at 15°C, 95% r.h. under L : D 16 : 8 h for 6 months.

Test products

The pheromone products *o*-nitrophenol (Merck, Dietikon, Switzerland) and methyl salicylate (Fluka, Buchs SG, Switzerland) and test products, EBAAP (SieberHegner, Zürich, Switzerland), deet (Sigma, Buchs SG, Switzerland), and indalone (Aldrich, Buchs SG, Switzerland) solutions were prepared in dichloromethane (DCM). All the above products were > 97% pure as indicated by gas chromatography, except indalone (92%). Permethrin in diethylene glycol monomethyl ether was also dissolved in DCM for repellency tests.

Faeces for the arrestment stimuli were obtained from faecal deposits on filter paper of >600 newly moulted male and female *I. ricinus* held in vials for 6 months. The filter paper strips were sonicated for 5 min in nanopure water (50 mL) and the resulting faecal wash was stored at −20°C until use. To supplement the faecal extract, two blends of synthetic faecal constituents known to induce arrestment (Grenacher *et al*., 2001) were prepared in nanopure water: a 0.25 mM solution of guanine, xanthine, uric acid and the guanine breakdown product 8-azaguanine (2-amino-6-hydroxy-8-azapurine (solution 1) and a solution of xanthine (0.3 mM), uric acid (0.03 mM) and 8-azaguanine (0.03 mM) (solution 2). One part of either solution 1 or 2 was then mixed with two parts of the faecal wash. Solution 2 of xanthine, uric acid and 8-azaguanine was also used as an arrestment stimulus on its own. The purines guanine, xanthine and uric acid were supplied by Fluka and the purine analogue 8-azaguanine was supplied by Aldrich (all chemicals >98% pure). Permethrin (>98%; Riedel de Haën, Buchs SG, Switzerland) solutions (31.10^{-15} to 31.10^{-6} M) were prepared in DCM.

Repellent assays

The effect of test products on the attraction of *A. variegatum* adults to its AAP products (a 1 : 1 mixture of *o*-nitrophenol and methyl salicylate) were measured using a locomotion compensator (Kramer, 1976; McMahon & Guerin, 2000a). Three factors in particular recommend this experimental set-up for testing odours on arthropods walking in an air stream. Firstly, the tick has complete freedom of movement in a circle at the apex of a sphere yet cannot adjust its position relative to the odour dose. Secondly, the onset and cessation of the odour is completely under the control of the operator, allowing a temporal

presentation of the treatment. Thirdly, the co-ordinates of the sphere's displacement while compensating for the movement of the tick can be recorded by computer and the vectors used to reconstruct the tracks described.

To test the responses of ticks to the pheromone, 0.4 mL from 1 : 1 *o*-nitrophenol and methyl salicylate solutions (25 ng/mL or 25 μg/mL) was placed on filter paper (7 cm diameter) to give source doses of 10 ng and 10 μg, respectively. After evaporation of the solvent, the filter paper was placed upright at the bottom of a 500-mL gas-wash bottle. The same procedure was followed for the control using 0.4 mL of solvent. Gas-wash bottles were then left to equilibrate for at least 5 min before presenting the evaporated vapours (150 mL/min) to the tick walking in an air stream (3.5 L/min, cross-sectional area 4.6 cm^2) of constant temperature and humidity (interday variation 23–25°C, 70–80% r.h.; McMahon & Guerin, 2000a) on a Perspex® sphere (diam. 50 cm). Each experiment consisted of a control period (1 min, solvent control) immediately followed by the test period (1 min, presentation of the test stimulus), in turn followed by an end-control period (1 min, re-presentation of the solvent control). Filter papers for the gas-wash bottles were replaced after every two tests. Recordings of the response of *A. variegatum* to the pheromone were made over several weeks in tandem with the tests for the repellency of EBAAP, deet, permethrin or indalone.

The repellent tests were carried out in the following manner. Solutions of the four products were prepared in DCM, 0.4 mL of which was applied to filter paper and introduced into gas-wash bottles as above. During the 1-min test period, vapours from such bottles were co-presented (150 mL/min) with vapours from the pheromone gas-wash bottle (150 mL/min) to the walking tick. The flow through the gas-wash bottle containing the solvent control was adjusted to 300 mL/min. Filter papers for the control gas-wash bottle were replaced after each test and filter papers in the gas-wash bottles containing either the pheromone or test product were replaced after every second test. The responses of *A. variegatum* were recorded to vapours of deet, permethrin and indalone at source doses of 1 μg to 10 mg in log steps, and EBAAP at a source dose of 10 mg (in one case permethrin was tested at 150 mg) in the presence of the pheromone at a 10 ng source dose. The responses to vapours of deet and indalone at source doses of 1 mg and 10 mg were also recorded in the presence of a 10^3 higher source dose of the pheromone (10 μg). We also investigated if an increased dose of indalone vapour could inhibit attraction to the pheromone. After equilibrating the solvent control and test gas-wash bottles at 37°C in a water bath, indalone vapour at a source dose of 100 mg was presented to *A. variegatum* in the presence of the pheromone at a source dose of 10 ng. Tests were also made to record the responses of *A. variegatum* presented with indalone vapour on its own. Indalone was delivered to the walking tick from a source dose of 10 mg at 24°C and also from a 100-mg source dose at 37°C in a water bath. The respective solvent controls for both tests were held at the same temperature as indalone.

Although the locomotion compensator can record several parameters of a tick's walk, we focused on three measures of walking direction that best summarize the orientated response of a tick to an attractant or repellent in the test period compared with the preceding control period: (i) the percentage change in time walking upwind, (ii) the percentage change in distance walked upwind, and (iii) the change in target vector. The percentage change in time or distance walking upwind (defined as the walk in a cone 60° either side of due upwind) gives an estimate of attraction towards the source. The change in target vector [a multiple of the path straightness and the cosine of the mean direction, both of which can be calculated from the vectors described by the tick using circular statistics (Batschelet, 1981)] gives a measure of the overall walking direction taken by the tick that is independent of the arbitrarily chosen upwind cone and can range from +1 to −1 (McMahon *et al.*, 2001). As the data for each measure of walking direction are not normally distributed, the significance of the difference between test and control for a given treatment was analysed using the Wilcoxon Signed Rank test (two-tailed). To estimate significance of differences between treatments we used the Mann–Whitney-U (MWU) test (two-tailed).

The following procedure was adopted to guard against false negatives and false positives in repellent assays. A test only started if the tick was walking downwind or crosswind at the beginning and at the end of the control period. When quantifying the response to the pheromone, only ticks that walked upwind $\leq$50% of the time in the initial control period were taken into account, as upwind walking ticks frequently exhibit initial downwind turns even in response to an attractant. To test the influence of indalone on its own, only ticks that walked upwind 15–50% of the time in the control period were tested. This 15% threshold was necessary to ensure that ticks undertook some walk towards the source in the control period so that any avoidance of indalone could be quantified. To determine if the consecutive presentation of the control followed by test treatment might bias results, ticks ($n = 12$) were presented with controls for two consecutive 1-min periods but no difference in the three measures of the walking direction between the first and second min was apparent ($P > 0.4$, Wilcoxon signed rank test).

Deterrent assay

Ixodes ricinus adults of both sexes were placed in Petri dishes (80 mm diam. and 15 mm high, Grenacher *et al.*, 2001) with six 1.5-cm^2 filter paper strips. One strip was treated with one of three arrestment stimuli in 30 μL nanopure water in positive controls amounting to a total dose of synthetic faecal constituents at least 400 ng/cm^2 (see above). An *I. ricinus* adult introduced into the middle of each dish walked around for some hours and settled eventually with a preference for the filter paper strip treated with the arrestment stimulus (Grenacher *et al.*, 2001). In the deterrent assay, one filter paper strip was first treated with the arrestment stimulus (as above). After drying, the paper

strip was treated with permethrin in 10 μL DCM (see above) to give a dose of 67 ag/cm^2 to 67 ng/cm^2. Solvent only was applied to control strips. The dried paper strips were then placed equidistant on the Petri dish floor. Tests with the positive controls were carried out in parallel with each deterrent assay to assure that each batch of ticks used could respond to the contact chemostimuli. All handling of ticks and filter paper strips was done with clean forceps to avoid contamination (Grenacher & Guerin, 1994). The experiments were started in the evening, kept overnight in the dark, and the position of each tick was recorded the next morning. The sum of ticks on the treated filter paper strips vs. the sum on the five control strips was compared with a random distribution (H_0-hypothesis) using the unilateral binomial test; ticks occurring elsewhere in the Petri dishes were ignored.

Results

Response to the pheromone

The walk of adult *A. variegatum* in an air stream alternates between a slow six-legged walk with both forelegs constantly reaching to sample the air and a faster eight-legged walk. These ticks respond to their pheromone with an upwind walk within the first 30 s and usually continue walking towards the source for some time after the pheromone is withdrawn (McMahon & Guerin, 2000a). Attraction is reduced at higher doses of the pheromone: a 10 ng source dose induces an increase (median +42%) in the time spent walking upwind (Table 1), significantly higher ($P < 0.05$, MWU test) than the increase (median +24%) induced by a 10 μg source dose of the pheromone (Table 2).

Repellent assays

In preliminary experiments, admixture of deet, permethrin and indalone delivered from source doses of 1 μg to 1 mg failed to influence the attraction of *A. variegatum* to the pheromone at a 10 ng source dose (data not shown). Further, admixtures of 10 mg source doses of EBBAP, deet and indalone, and of a 150 mg source dose of permethrin to a 10 ng source dose of the pheromone did not inhibit attraction (Table 1; Fig. 1). Nevertheless, the test products did have different effects on the responses of the ticks. Taking each measure of the walking direction into account, neither deet nor permethrin influenced attraction of *A. variegatum* to the pheromone source in any respect (Table 1; Fig. 1). EBAAP enhanced attraction to the pheromone (median increase 47–49%, $P < 0.05$ MWU test; Table 1, Fig. 1). Indalone was the only product to reduce attraction (median decrease 69–74%, $P < 0.05$, MWU test; Table 1; Fig. 1). This effect of indalone was rather limited, with only five of the 14 animals tested showing a reduced walk towards the source. Admixture of indalone at the higher source dose of 100 mg at 37°C failed to inhibit attraction of *A. variegatum* to its pheromone (data not shown). Two of the four products, deet and indalone at source doses 1 and 10 mg were also tested in the presence of a 10^3 higher source dose of the pheromone, i.e. 10 μg, but neither affected attraction of the ticks towards the pheromone source (Table 2). Indalone at a source dose of 10 mg on its own did, nevertheless, elicit reduced upwind walking (median change in time and distance walked upwind of −15% and −18%, respectively; Table 3; Fig. 2). The magnitude of this repulsion was not increased when indalone was presented at a 100-mg source dose at 37°C (Table 3; Fig. 2) and after indalone vapour was removed from the air stream the effect disappeared (Fig. 2).

Deterrent assays

Data of the responses of *I. ricinus* to the three mixtures of arrestment stimuli are pooled (Table 4). Individual male and female *I. ricinus* showed significant arrestment on these conspecific faecal products in the Petri dish assay: of 102 ticks

Table 1. Measures of walking direction by *Amblyomma variegatum* adults on a locomotion compensator presented with its pheromone alone (a 1 : 1 binary mixture of *o*-nitrophenol plus methyl salicylate) at a source dose 10 ng and the pheromone plus EBAAP, deet and indalone at source doses of 10 mg and permethrin at a source dose of 150 mg. Parameters, expressed as medians, are the change in target vector, percentage change in distance walked upwind and percentage change in time walked upwind (see text) in the test period compared to the preceding control period. Asterisks indicate attraction for a treatment ($^{NS}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; Wilcoxon's signed rank test). Treatments sharing the same letter for a particular measure of walking direction are not significantly different ($P > 0.05$; Mann–Whitney-U test). Note the enhanced upwind responses in the presence of EBAAP and the reduced upwind responses in the presence of indalone (see also Fig. 1)

	Measures of walking direction						
Treatment (source dose)	Ticks tested	Change in target vector		% change in distance walked upwind		% change in time spent walking upwind	
Pheromone (10 ng)	26	+0.72***	b	+47***	e	+42***	h
+EBAAP (10 mg)	10	+1.06**	a	+70**	d	+62**	g
+deet (10 mg)	10	+0.76**	ab	+58**	de	+45**	hi
+permethrin (150 mg)	10	+0.46*	bc	+37*	ef	+35*	hi
+indalone (10 mg)	14	$+0.22^{NS}$	c	+12*	f	+13*	I

Table 2. Measures of walking direction by *Amblyomma variegatum* adults on a locomotion compensator presented with its pheromone alone at a source dose of 10 µg and the pheromone plus deet or indalone at source doses of 1 and 10 mg. For further explanation see Table 1

	Measures of walking direction						
Treatment (source dose)	Ticks tested	Change in target vector		% change in distance walked upwind		% change in time spent walked upwind	
Pheromone (10 µg)	20	+0.31**	a	+20***	b	+24***	c
+deet (1 mg)	14	$+0.28^{NS}$	a	+24**	b	+23**	c
+deet (10 mg)	11	+0.37**	a	+24**	b	+30**	c
+indalone (1 mg)	13	+0.30*	a	+23*	b	+25**	c
+indalone (10 mg)	10	$+0.28^{NS}$	a	+31*	b	$+21^{NS}$	c

tested in the different positive controls 48% settled on filter papers treated with the arrestment stimulus ($H_0 = 17\%$, $P = 0.0001$, binominal test; Table 4). Permethrin applied at 670 fg/cm^2 to 67 ng/cm^2 on the filter paper strips treated with the arrestment stimulus (>400 ng/cm^2 for the combined synthetic faecal constituents) inhibited arrestment such that the distribution of ticks was no longer different from random ($P > 0.05$, ns, binominal test, Table 4). At the highest dose (67 ng/cm^2) of permethrin no tick settled on the paper treated with the arrestment stimulus and no mortality was observed within 12 h of the test. Permethrin no longer inhibited arrestment on the filter paper strips at the lowest doses tested (0.67 ag/cm^2 and 67 fg/cm^2; Table 4).

Discussion

We have tested the effects of EBAAP, deet, indalone and permethrin on the attraction of *A. variegatum* to its aggregation-attachment pheromone. Each of the four products was delivered from a 10 mg source dose in the presence of the synthetic pheromone at a source dose of 10 ng (equivalent to ≤5% of the amount released by a single male feeding on the host in one minute; Diehl *et al.*, 1991; McMahon & Guerin, 2000b). Taking into account the dimensions of the air stream used in this study, we estimate that the 10 mg source dose of test products used here is 10 times higher than in a similar *in vitro* experiment demonstrating a negative effect of deet on the upwind response of the yellow

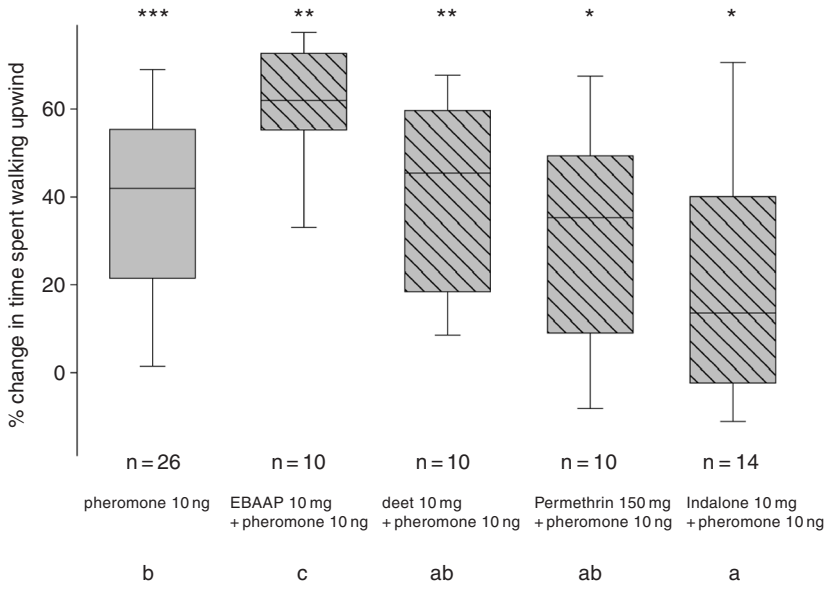

Fig. 1. Change in percentage time spent walking upwind (see text) over the blank control by *Amblyomma variegatum* adults on the locomotion compensator in the presence of its pheromone (a 1 : 1 mixture of *o*-nitrophenol and methyl salicylate, source dose 10 ng) and in the presence of the pheromone plus EBAAP, deet and indalone at source doses of 10 mg and permethrin at a source dose of 150 mg. The line within a box plot marks the median, the lower and upper boundaries of a box indicate the 25th and 75th percentiles, error bars below and above a box indicate the 10th and 90th percentiles. Asterisks above the treatments indicate a significantly higher time spent walking upwind in the test period compared to the preceding control period (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; Wilcoxon signed rank test). Treatments sharing the same letter (a, b or c) are not significantly different ($P > 0.05$; Mann–Whitney-U test). Note the varying upwind responses due to the admixture of the different test products with the pheromone.

Table 3. Measures of walking direction by *Amblyomma variegatum* adults on a locomotion compensator presented with indalone on its own from a source dose 10 mg at room temperature and from a source dose of 100 mg at 37°C. Note the similarity of the downwind responses elicited by both treatments. For further explanation see Table 1

	Measures of walking direction						
Treatment (source dose)	Ticks tested	Change in target vector		% change in distance walked upwind		% change in time spent walked upwind	
Indalone (10 mg)	12	−0.18*	a	−15**	b	−17**	c
Indalone (100 mg) at 37°C	14	−0.20*	a	−15*	b	−16*	c

fever mosquito, *Aedes aegypti* (L) to the host stimuli CO_2 and lactic acid (Boeckh *et al.*, 1996). This inhibition of attraction in *A. aegypti* to lactic acid by deet was also demonstrated *in vitro* by Dogan *et al.* (1999), who recorded the vertical distribution of flying mosquitoes in the presence of lactic acid and deet solutions placed at the bottom of a test chamber. In yet another *in vitro* test, human hair treated with deet impeded the arrival of the human body louse, *Pediculus humanus humanus* L., on hair treated with ammonium bicarbonate to which they are normally attracted (Mumcuoglu *et al.*, 1996). However, the latter authors do not report if the effect of deet was due to contact or perception of deet vapour by the lice. In this study we found no repellent activity for deet or for permethrin. EBAAP even enhanced the attraction of *A. variegatum* to its pheromone. Indalone, the only one of the four products to show any repellent effect, showed no activity when the pheromone dose was increased from 10 ng to 10 µg. However, this product did elicit an avoidance behaviour by the ticks when tested on its own. It seems clear that for

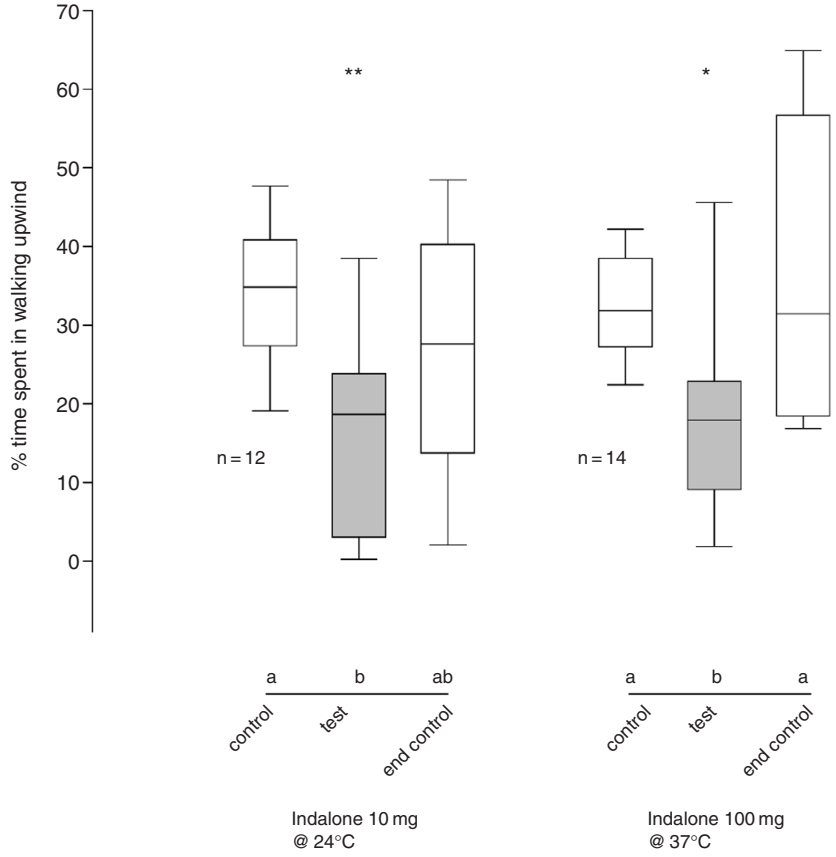

Fig. 2. Plots of the time spent walking upwind (see text) as a percentage of the total spent walking by *Amblyomma variegatum* adults on the locomotion compensator in response to delivery of indalone in the air stream. Indalone was presented from a source dose of 10 mg at room temperature (as in Fig. 1) and from a source dose of 100 mg at 37°C. Responses to each dose are preceded and followed by control periods, presented left and right of the central box. Note the reduced upwind walk in the presence of indalone and the lack of downwind walk in the end-control period after indalone was withdrawn. For further explanation see Fig. 1.

Table 4. Individual *Ixodes ricinus* adults are arrested on filter paper strips treated with their own faeces constituents (positive control; $P < 0.0001$). This arrestment is disrupted by application of permethrin at doses of 67 ng to 670 fg to the stimulus, resulting in a distribution of ticks settling on filter papers that is no longer different from random (ns). Note, at the highest dose of permethrin (67 ng/cm^2) no tick settled on the filter paper strips treated with the arrestment stimulus. Doses of 67 ag and 67 fg/cm^2 permethrin no longer inhibited arrestment; $*P < 0.05$; $****P < 0.0001$, unilateral binomial test

Test substance	Amount/cm^2	Ticks arrested on treated filter papers	Total no. of ticks on filter papers	P_{binom}
Arrestment stimulus†	–	49	102	****
+permethrin	67 ng	0	7	ns
+permethrin	670 pg	2	12	ns
+permethrin	67 pg	8	23	*
+permethrin	6.7 pg	3	10	ns
+permethrin	670 fg	3	22	ns
+permethrin	67 fg	4	12	*
+permethrin	67 ag	5	11	*

†Numbers represent pooled data from positive controls with different mixtures of arrestment stimuli (see Materials and methods).

A. variegatum the test products we have used cannot be considered as repellents whose vapours can inhibit responses to the tick attractant in an air stream. This is not to suggest that these chemicals are not of value in limiting the presence of ticks on hosts, but a direct effect on olfaction cannot be supported based on our results.

Permethrin, inactive in the repellent assay, successfully deterred arrestment by *I. ricinus* adults on three different mixtures of faecal constituents. Electrophysiological responses to faeces and faecal constituents used here as arrestment stimuli have been recorded from contact chemoreceptor cells in sensilla on the ventral side of the tarsus of *I. ricinus*, clearly implicating these sensory organs in the arrestment behaviour (Grenacher *et al.*, 2001). We have reason to believe that permethrin is a true deterrent that acts directly on this sensory modality. Our video recordings show ticks walking across the filter paper strips treated with permethrin (6.7 ng/cm^2), inconsistent with an effect mediated by volatiles even at close range. Furthermore, the dose of permethrin that completely inhibited arrestment (67 ng/cm^2) was at least five times lower than that of the combined synthetic constituents of the arrestment stimuli applied to the filter paper strips. By comparison, the ratio of test product to attractant was $\geq 10^2$ times higher in our repellent tests. The low doses of permethrin found active here are consistent with other studies measuring the effectiveness of this product against ticks: *in vitro* tests show that permethrin at doses of 90 ng/cm^2 deters the aggregation of first instar *Argus persicus* (Oken) on guanine hydrochloride (Dusbábek *et al.*, 1997) and a dose of 5–12 µg/cm^2 elicits avoidance behaviour in adult *Dermacentor occidentalis* Marx and *Ornithodoros coriaceus* Koch (Lane & Anderson, 1984). We did not test the effect of EBAAP, deet or indalone on the arrestment response of *I. ricinus* but reports from the literature indicate that the minimum concentration of two of these (deet and indalone) required to inhibit attachment or feeding by ticks is in the range of >17% (w/v) (Mehr *et al.*, 1986; Kumar *et al.*, 1992). This is over 100 times higher than the minimum effective concentration of permethrin (Mehr *et al.*, 1986). At such high doses it cannot be excluded that EBAAP, deet or indalone might influence tick behaviour via another mode (for example via the respiratory system) rather than by an effect on a specific sensory channel. Moreover, the mode of action of permethrin is well known, whereas no data exist on the mode of action of the other compounds. Permethrin blocks sodium channels (Vijverberg *et al.*, 1982; Soderlund & Bloomquist, 1989), so it may well be inducing deterrency via effects on the contact chemoreceptor cells in the tarsal sensilla that contacted the treated substrate.

The focus of this study was to quantify the influence of candidate repellents and deterrents on olfactory and gustatory mediated responses of ticks. Only by making experiments that test for effects of products on a specific sensory system can we begin to design repellents and deterrents that disrupt such a channel. Although some of our results may seem surprising (the increased upwind response to EBAAP, the lack of an effect of deet), earlier tests with known doses of repellent vapours in an air stream are rare.

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