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ACTIVITY OF REPELLENTS APPLIED TO SKIN FOR PROTECTION AGAINST *AMBLYOMMA AMERICANUM* AND *IXODES SCAPULARIS* TICKS (ACARI: IXODIDAE)[1]

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ABSTRACT. Twenty-nine repellents were tested on human skin for duration of activity as protectants against nymphal lone star ticks (*Amblyomma americanum*) and against black-legged or deer ticks (*Ixodes scapularis* Say). Eleven of these repellents, including deet, provided >2 h of protection against the lone star tick. One repellent, 1-(3-cyclohexenyl-ylcarbonyl) piperidine, was effective ≥4 h. Four repellents (2 pyridines and 2 piperidines with protection lasting 2.3–3 h) showed acaricidal activity to more than half of the ticks tested after 9–12 min of exposure. Seven repellents that were most effective against *A. americanum,* including deet, were tested against the black-legged tick. None was effective and no knockdown was observed. These results suggest that the black-legged tick is less sensitive to repellents than the lone star tick.

INTRODUCTION

In less than 2 decades, Lyme disease spirochetosis, caused by *Borrelia burgdorferi,* has emerged as the most frequently transmitted tick-borne infection in North America (Barbour and Fish 1993). Thousands of cases are reported annually. Humans are also at risk for contracting other tick-borne diseases, which include Rocky Mountain spotted fever, Colorado tick fever, babesiosis, tularemia, and ehrlichiosis.

Personal protection recommendations for those exposed to ticks include long pants and long-sleeved shirts, closing and sealing openings in clothing, and use of the repellent deet (*N,N*-diethyl-3-methyl-benzamide) on skin and clothing (Schreck 1992). Currently, the most effective treatment for clothing is permethrin in a pressurized spray (Schreck et al. 1982, Mount and Snoddy 1983, Lane and Anderson 1984, Schreck et al. 1986, Lane 1989, Evans et al. 1990). This safe, very effective protectant kills/repels ticks when they crawl on treated garments. In warm weather, however, when people wear abbreviated clothing, arms and legs are exposed to ticks and the risk of tick bites is intensified. During these times it is also necessary to use a repellent on the skin.

Nearly all commercial repellent formulations registered by the Environmental Protection Agency for skin application to repel ticks contain deet. Nevertheless, published data on the repellency of deet on skin to various tick species is unknown. Unpublished laboratory and field data (C. E. Schreck, unpublished data), indicate that deet on skin is ≥80% effective against the lone star tick *Amblyomma americanum* (Linn.) a vector of tularemia and Rocky Mountain spotted fever (Benenson 1990). Laboratory tests with the black-legged (deer) tick, *Ixodes scapularis* Say (= *Ixodes dammini*) (Oliver et al. 1993), the major vector of Lyme disease in North America, demonstrated various concentrations of deet to be 75–87% effective (Anonymous 1993).

Here, we report the results of tests of the effectiveness of 29 compounds to repel lone star and black-legged ticks. The purpose of this investigation was to identify a number of new tick repellents that are effective on skin and have promise for future study.

MATERIALS AND METHODS

Ticks: Unfed nymphs of lone star and black-legged ticks were used in the study. Lone star ticks were from uninfected colonized stocks at the USDA/ARS Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, TX. Black-legged ticks were from uninfected colonized stocks at the Medical Entomology Laboratory, New York Medical College, Valhalla, NY. Prior to testing, ticks were held separately in small groups in vials at 90% RH and 21°C. *Amblyomma* nymphs were tested at ca. 10 wk postmolt and *Ixodes* nymphs at 12 wk postmolt.

Chemicals: The 29 compounds selected for these tests were synthesized (T. P. McGovern)

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at the USDA/ARS Insect Chemical Ecology Laboratory, Beltsville, MD. These chemicals originated from a long-term program to develop better insect repellents. Each was chosen because: 1) it repelled *Aedes aegypti* (Linn.) mosquitoes for 11–21 days, or more, and *A. americanum* nymphs for 2–17 wk in screening tests on cloth, and 2) each had been approved by the Toxicology Division of the U.S. Army Environmental Hygiene Agency, Aberdeen, MD, for experimental entomological testing on the skin of volunteers. Deet was included as a standard of comparison.

Test procedure: Nymphs of the lone star tick are difficult to handle in laboratory tests because of their small size, rapid movement, tendency to escape, and aggressive biting behavior. Although not as aggressive as *A. americanum, I. scapularis* will bite readily. These ticks are small and easily overlooked in a large arena or on a dark background.

Initial trials showed that nymphs of both tick species would attempt to bite on untreated fingers of volunteers (human subjects participating in this study gave free and informed voluntary consent). Falco and Fish (1988) reported the arms and hands were one of 3 areas of the body most frequently infested by *I. scapularis* nymphs. Thus, we used the index finger as a repellent treatment site; doing so minimized the exposure of volunteers to tick bites, and simplified the observation of tick responses to each repellent. On each of 3 male volunteers, to determine the skin area that would be treated, we measured the distance from the 1st distal skin fold on the ventral side of both index fingers (1st joint), to the 3rd skin fold at the edge of the palm, and the circumference of the finger at the 1st and 3rd skin folds. The average area to be treated on each finger was calculated as the area of a cylinder and ranged from 38.3 to 55.2 cm^2.

As a testing arena for the ticks, we used 2 inverted 60 × 15-mm glass Petri dish covers, spaced 6 cm apart, and attached with hot-melt glue to the bottom of a 19 × 30 × 5-cm porcelain pan. In the pan we placed water about 1 cm deep to prevent tick escape.

To test the effect of each repellent against *A. americanum,* groups of 10 nymphs were narcotized with CO_2, transferred into each Petri dish cover and allowed 15 min to recover before testing began. A separate testing arena (porcelain pan) was used for each of the 3 volunteers.

Before the skin on an index finger was treated with repellent, each volunteer pretested groups of 10 nymphs to determine tick activity. This was done by placing the finger into the cover vertically so that the finger tip touched the bottom of the dish. The volunteer recorded the number of nymphs crawling to the 3rd joint in 3 min. Nymphs crawling beyond this point (usually all responded) were returned to the dish cover with forceps and used for the tests. If fewer than 85% of the ticks responded (index of rejection), they were replaced by a new group of 10 ticks. This pretest data served as control data, with each approved group serving as its own control.

Fingers were treated at the rate of 0.3 mg of repellent (AI)/cm^2. We used a 100-μl digital pipette to dispense the correct amount of repellent as formulated in 25% stock solutions in ethanol. The disposable tip of the pipette was used to dispense and spread the repellent evenly over the skin, except for the 1st joint of the finger (Fig. 1, area #1), which was left untreated. This application procedure permitted ticks to crawl up onto the untreated skin of the 1st joint before encountering the repellent (Fig. 1, areas #2 and #3). Continued movement by ticks up and onto the treated skin, to the 3rd finger joint, indicated a lack of repellency. Ticks that moved to the treated skin, but stopped, reversed direction, or dropped off, were considered repelled.

Repellent treatments were aged 10 min before the 1st trial; thereafter they were tested hourly for 4 h or until failure, which was denoted as <90% protection in 2 successive tests. Percent protection was calculated as:

$$\frac{(\text{no. on control} - \text{no. on treatment})}{\text{no. on control}}$$

Between each test, volunteers protected the treated fingers from rubbing, touching, or other action, which might prematurely reduce or remove the repellent from the skin.

To test the effect of repellents against *I. scapularis,* 3 modifications were made to the test procedure. First, an 80% index of rejection was used for ticks that responded in the pretest (control) because preliminary tests showed these nymphs did not climb readily onto an untreated forefinger. The 2nd modification consisted of using the left fingers only and dividing the finger into 3 nearly equal parts. The 1st, 2nd, and 3rd joints of the left index finger were marked by circling the finger with a black wax pencil (see arrows, Fig. 1). The right hand was kept free to manipulate the ticks and to record data. The 3rd modification consisted of pretesting the nymphs on the untreated, horizontally held index finger of each volunteer. These data were used as the control. The 4th modification consisted of reducing the number of nymphs to 5/trial and they were placed on area 2 of a treated finger (Fig. 1, area #2) to determine if they crawled into areas 1 and/or 3 of an untreated finger within 3 min. If <80% responded, the nymphs were replaced and the

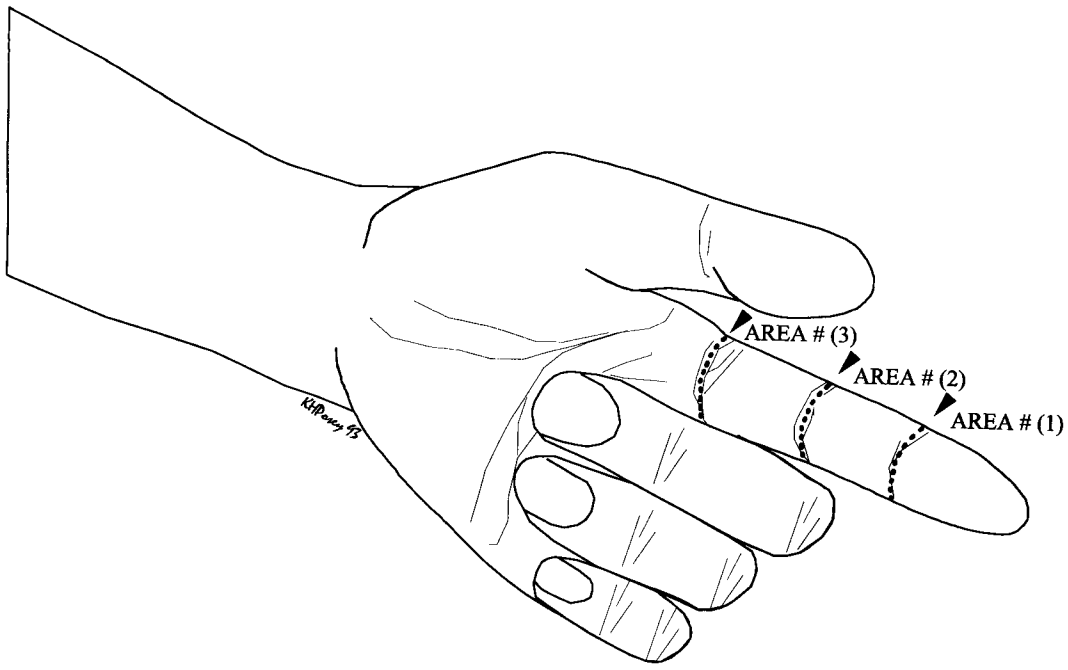

Fig. 1. Areas of the index finger used in evaluating tick repellents.

test repeated. When each volunteer obtained 2 groups of 5 ticks that responded to the untreated finger, areas 1 and 3 of the finger were treated with the candidate repellent. Repellents were applied at the rate of 0.3 mg (AI)/cm^2. After 10 min, 5 nymphs were placed on untreated area 2. Ticks that remained in this area during the test period were considered to be repelled. Lack of repellency was indicated by the ticks crawling onto treated skin. Results were recorded after 3 min, the nymphs removed, and the test repeated with a 2nd group of 5 nymphs. Tests were made hourly thereafter, or until <90% repellency was recorded in 2 successive tests. We tested 7 repellents against *I. scapularis.*

Due to the preliminary nature of these investigations and the test design, a data analysis was not performed.

RESULTS AND DISCUSSION

Ten of the 29 tested repellents, and deet, provided a duration of protection averaging >2 h against *A. americanum* (Table 1). Six of these were about equal to deet at 2.3–2.7 h of protection, 3 were slightly longer lasting at 3 h and one, 1-(3-cyclohexenyl-ylcarbonyl) piperidine (code number, AI3-35765), protected for 4 h (1.5× longer than deet). Of the remaining compounds, 15 provided average protection <1 h and 4 provided <2 h of protection. One unforeseen occurrence was the acaricidal effect compounds AI3-36564, 38354, 38361, and 39683 had on the nymphs of this species after 3 or 4 3-min exposures.

Seven of the repellents that were effective against *A. americanum,* including deet, provided <1 h of protection against *I. scapularis.* None gave complete protection at 10 min posttreatment. The most effective protection at 1 h posttreatment was 77% with deet. No acaricidal activity was observed against *I. scapularis* for the repellents we tested.

In summary, 11 of the compounds studied here repelled *A. americanum* for >2 h; 4 showed acaricidal activity. None, including the deet standard, repelled *I. scapularis* or showed acaricidal activity against this tick. This was unexpected because *I. scapularis* is less aggressive than *A. americanum* in seeking a feeding site on the host. The apparent absence of a repellent effect on *I. scapularis* also may be the result of the brief exposure of these ticks to the repellent; the failure of repellency early on meant that no additional test exposures would be made.

Success in repelling *A. americanum* with chemicals applied to skin was expected because these compounds were effective against this tick in tests on cloth. The results obtained earlier in studies with deet applied to clothing to repel *I. scapularis* (Schreck et al. 1986, Evans et al. 1990),

Table 1. Duration of effectiveness of candidate tick repellents and deet in laboratory tests on human skin. Duration based on percent protection (means of 3 replications) from crawling nymphs at hourly intervals posttreatment.

USDA code no.	Chemical name	Protection time (h)	
		Amblyomma americanum	*Ixodes scapularis*
35765	1-(3-cyclohexenyl-ylcarbonyl)piperidine	4.0	<1
36331	*N,N*-dibutyl-6-methyl-3-cyclohexene-1carboxamide	0.5	
36347	3-hydroxybutyl cyclohexanecarboxylate	2.3	
36374	1-hexanoyl-1,2,3,4-tetrahydroquinoline	0.2	
36424	1-(2-ethylbutyryl)-1,2,2,4-tetra hydroquinoline	0.2	
36562	1-(2-methylcyclohexane carbonyl)-2-methyl piperidine[1]	2.3	<1
36564	2-methyl-1-[(6-methyl-3-cyclohexen-1-yl)carbonyl] piperidine	2.7	
36566	4-methyl-1-[(6-methyl-3-cyclohexen-1-yl)carbonyl] piperidine	3.0	
36570	1,2,3,6-tetrahydro-1-[(6methyl-3-cyclohexen-1-yl) carbonyl] pyridine	0.7	
37175	2-(2-ethoxyethoxy)ethyl 4-ethylphenyl ether	0.2	
37346	4-heptyn-2-yl mandelate	0.2	
37349	2-pentynyl madelate	0.5	
37543	1-(cyclopropylcarbonyl)-1,2,3,4-tetrahydro-quinoline	0.5	
37572	2-hydroxypropyl cyclohexaneacetate	1.1	
38010	cyclohexanecarboxylic acid, 2-hydroxybutyl ester	0.5	
38011	2-hydroxybutyl 3-cyclohexene-1-carboxylate	0.5	
38193	6-methyl-2-hydroxy-1-methyl propyl *trans*-3-cyclohexencarboxylate	0.5	
38196	2-hydroxy-1-methylpropyl 4-cyclohexylbutyrate	0.2	
38269	3-hydroxybutyl bicyclo [2.2.1]hept-5-ene-2-carboxylate	1.1	
38273	4-hydroxy-2-butenyl cyclohexanecarboxylate	0.5	
38350	1,2,3,6-tetrahydro-1(2-methylbenzoyl)pyridine	2.3	
38354	1,2,3,6-tetrahydro-1-phenylacetyl-pyridine[1]	2.7	<1
38355	1,2,3,6-tetrahydro-1-(1-oxo-3-phenylpropyl)-pyridine	0.5	
38360	1,2,3,6-tetrahydro-1-(3-chlorobenzoyl)-pyridine	0.7	
38361	1,2,3,6-tetrahydro-1-(4-chlorobenzoyl)-pyridine[1]	2.7	<1
39673	*N,N*-di(2-methylpropyl) cyclopentane carboxamide	1.4	

Table 1. Continued.

USDA code no.	Chemical name	Protection time (h) *Amblyomma americanum*	Protection time (h) *Ixodes scapularis*
39679	1-cyclopentylcarbonlyl-3,5-dimethylpiperidine	3.0	<1
39683	1-(*trans*-2-methylcyclohexane-1-carbonyl-3,3-dimethylpiperidine[1]	3.0	<1
39684	1-(3-cyclohexene-*trans*-6-methyl-1-carbonyl)-3,3-dimethyl piperidine	1.7	
22542	*N,N*-diethyl-meta-toluamide (deet)	2.7	<1

[1] Ticks observed to be knocked down after 3rd and 4th 3-min exposure to these compounds.

suggested the possible failure of deet applied to skin to repel this tick species. It also may be that *Amblyomma* and *Ixodes* tick genera have varied sensitivities to repellents, as is the case in different genera of mosquitoes (Schreck 1985) or that the test method used with *I. scapularis* does not measure response to repellents applied to the skin. It is further possible that the response to repellents of *I. scapularis* and *A. americanum* populations from different geographic areas is variable.

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