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DEVELOPMENT OF A NOVEL ALL NATURAL TICK AND INSECT REPELLENT, BIOUD, AS A DEET REPLACEMENT AND FOR USE ON COTTON FABRIC

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Abstract

Novel aromatic and aliphatic organic acids, esters and ketones were synthesized and assayed as repellents for ticks. *E*-7-(cyclohexyl)hept-4-enoic acid (CHEA), *E*-7-phenylhept-4-enoic acid (PHEA), ethyl *E*-7-(cyclohexyl)hept-4-enoate (CHEN) and ethyl *E*-7-phenylhept-4-enoate (PHEN) had repellent activity against the soft tick, *Ornithodoros parkeri* (Acari: Argasidae) in a two-choice bioassay. PHEN, an aromatic organic ester, was the most active. 2-undecanone, a natural product found in the trichomes of wild tomatoes, was found to mimic our lead chemistry and was active as a repellent at 50 µg/cm^2. Since this compound is an already known natural botanical with a proven safety record including being approved as a food additive, formulation studies were conducted to optimize its volatility and maximize its repellent activity against ticks and mosquitoes when applied to human skin. Combining undecanone with proprietary emulsions from HOMS LLC, we were able to develop BioUD. BioUD30 and BioUD8 (30 and 8% undecanone, respectively) was a repellent to the American dog tick, *Dermacentor variabilis* (Acari: Ixodidae), 2.5 h after applications on the skin of human subjects. In field studies in two different habitats (forest and marsh), 99% repellency was found 4.5 h after application to the skin of human subjects for a population of mosquitoes comprised of mixed species. BioUD8 at 6 h after application was better than OFF Botanicals with 10% PMD and equivalent to OFF with 15% DEET. Application of 20 µl BioUD30 silicon (5.88mg undecanone) to 19.63 cm^2 of cotton fabric repelled on average >90% of *D. variabilis* ticks in a two-choice bioassay 5 d after application, suggesting that it effectively binds to cotton fabric. The repellent was active on cloth up to 7 d. The submission of BioUD for US EPA registration is expected in early 2006. The product will be marketed by HOMS LLC as a DEET alternative. BioUD will be all natural and non-flammable unlike many DEET products, with the potential of being organically certified.

Introduction

Blood feeding arthropods vector a number of diseases including West Nile virus, Rocky Mountain Spotted fever, several forms of encephalitis, Lyme disease, malaria, yellow fever and Dengue, among others (Parola and Raoult 2001; Durden and Mullen 2002). The threats that hematophagous arthropods pose to human health can be mitigated with avoidance, physical barriers, mechanical devices, chemoprophylaxis and repellents. Repellents have the distinct advantage of being topically applied to human skin and/or clothing, are generally suitable for use in misting systems, and are relatively inexpensive and portable. Obvious disadvantages are that they require reapplication for continued protection against biting arthropods. A wide variety of insect and tick repellents are commercially available in sprays, creams and lotions, most often containing DEET (N,N-diethyl-3-methylbenzamide).

Used by millions of people worldwide on an annual basis, DEET has become the standard by which all other repellents are measured (Fradin 1998). It is the active component in 225 EPA-registered products and is used by approximately 30% of the US population (Veltri et al. 1994; EPA 1998). The safety of DEET has been questioned after reports of seizures (Oransky et al. 1989), possibly due to misuse of the product and failure to follow product labels. These reports however cannot confirm the direct connection between DEET overexposure and neurological disorders. Nevertheless, the EPA did recommend improved label warnings and restrictions for DEET use, especially with children. A recent survey by Menon and Brown (2005) showed that nearly 33% of 301 respondents admitted to not reading or following label instructions, indicating that improved labeling of DEET containing products is not sufficient for improving its safety. Considering these findings, use and development of alternative insect and tick repellents remains an attractive option.

We have synthesized four compounds [*E*-7-(cyclohexyl)hept-4-enoic acid (CHEA), *E*-7-phenylhept-4-enoic acid (PHEA), ethyl *E*-7-(cyclohexyl)hept-4-enoate (CHEN) and ethyl *E*-7-phenylhept-4-enoate (PHEN)] and tested them against mosquitoes. Methyl nonyl ketone or 2-undecanone was found to mimic the structure of PHEN, our most active compound against ticks. 2-undecanone, a naturally occurring allelochemical found in the trichomes of tomato plants in the genus *Lycopersicon*, has been patented (Roe 2002) for use as an insect and tick repellent (Scott 2002). The technology was recently licensed by HOMS, LLC (Clayton, NC) and has been incorporated into a proprietary emulsion under the trade name BioUD in order to reduce the evaporation rate of 2-undecanone and prolong its effectiveness as an insect and tick repellent.

The objectives of the current study were to test the efficacy of BioUD applied to skin against mosquitoes and ticks under lab and field conditions and its use as a repellent on cotton cloth.

Materials and Methods

Preparation of ethyl E-7-phenylhept-4-enoate (PHEN) and E-7-phenylhept-4-enoic acid (PHEA)

E-7-Phenylhept-4-enoic acid was prepared from commercially available 3-phenylpropanal (dihydrocinnamaldehyde) in four steps as follows. Dihydrocinnamaldehyde was added to an excess of vinyl magnesium bromide in tetrahydrofuran (THF) at 0^{o}C and allowed to warm to room temperature and remain there for 2 h. The reaction was quenched with saturated ammonium chloride solution and extracted twice with ether. The combined ether extracts were dried over anhydrous sodium sulfate and after evaporation of the ether *in vacuo*, the residue was purified by column chromatography on silica gel to yield 5-phenyl-3-hydroxypent-1-ene. The allylic alcohol was then dissolved in triethyl orthoacetate with a catalytic amount of propionic acid and heated at 140^{o}C for 6 h (following distillation of the ethanol). After cooling to room temperature, the excess triethyl orthoacetate was removed under reduced pressure, and the resulting residue purified by flash chromatography to give E-7-phenylhept-4-enoate at >95% purity. Ethyl E-7-Phenylhept-4-enoate was saponified in methanolic sodium hydroxide at room temperature. The resulting acid, E-7-phenylhept-4-enoic acid, was obtained at >95% purity after chromatography. The structures of the final products were verified by the appropriate spectral analysis (infrared and nuclear magnetic spectroscopy) and combustion analysis. CHEN and CHEA were prepared by similar means.

Ticks

The American dog tick, *Dermacentor variabilis* (Say), was reared as previously described (Sonenshine, 1993) on New Zealand White Rabbits (*Oryctolagus cuniculus*) at the Department of Biological Sciences at Old Dominion University (ODU), Norfolk, VA. Rearing conditions were 26±1°C, 92±1% relative humidity and a photoperiod of 14:10 h (L:D). The strain was colonized from wild specimens collected near Williamsburg and Chincoteague, VA in 1995. The soft tick *Ornithodoros parkeri* Cooley (Acari: Argasidae) were also obtained from the Sonenshine lab at ODU and were maintained on white mice (*Mus musculus*) at 25±2°C, 85±2% RH, a photoperiod of 15:8 (L:D) and 0.5 h of crepuscular light before and after the photophase. The colony was established from individuals provided by J. H. Oliver, Georgia Southern University, Statesboro, GA.

Tick bioassays

Repellent activity of 2-undecanone, CHEA, PHEA, CHEN and PHEN against O. parkeri. To determine if 2-undecanone, CHEA, PHEA, CHEN and PHEN have repellent activity against soft ticks, a two-choice bioassay was conducted using unfed *O. parkeri* nymphs and adults. Two 48 mm diameter half-circle test papers (9.04 cm^2 each) were prepared from Whatman filter paper (#2 Qualitative, Whatman Inc., Clifton, New Jersey). Half-circle test papers were saturated with different doses of 2-undecanone, CHEA, PHEA, CHEN or PHEN (5-100 µg/cm^2) diluted in 100 µL acetone. A test paper saturated with 100 µL of acetone served as the control. The solvent was allowed to evaporate at room temperature in a fume hood for 1 h prior to testing. The arenas for the two-choice test were prepared by placing one half-circle of paper treated with the test compound (in acetone) and one half-circle of paper treated with acetone only into a small glass petri dish (diameter = 60 mm, height = 15 mm). The two half-circle test papers completely covered the bottom of the petri dish. Ten ticks were transferred into the center of each arena and then covered with the lid of the glass petri dish. For each arena, the number of ticks in contact with the treatment and the acetone control were recorded 2 h after the introduction of ticks into the arena. Experiments with each dose of 2-undecanone, CHEA, PHEA, CHEN, PHEN and the no-choice control were replicated four times.

Repellent activity of BioUD against the American dog tick on human skin. Test area was 4 cm in diameter (12.56 cm^2) on the left leg just above the knee of a human (male) subject. The control was conducted 30 min prior to the application of repellent. For the treatment application, 20 µl of BioUD30 (30% undecanone) or 20 µl of BioUD8 (8% undecanone) was applied to the left half of the test area. Ticks (unfed mixed sexes of the American dog tick, *D. variabilis*) were added to the test apparatus just prior to the application of the repellent. The apparatus was a plastic petri plate top with an opening covered with aluminum screening; inside between the plate and the screen were two layers of cotton cheese cloth. Bioassays were conducted at room temperature with the testing apparatus on human skin covered with dark cloth. The apparatus containing ticks was applied to skin 2 h after the application of the repellent.

Repellent activity of BioUD30 silicon against the American dog tick on cotton cloth. A two-choice bioassay with unfed mixed sex adult American dog ticks was conducted using cotton fabric as a substrate. Six ticks per replicate were placed in a 5 cm diameter (19.63 cm^2) arena on top of two half-circle pieces of cotton gauze; one side was untreated cloth and the other side was treated with BioUD30 silicon (HOMS, LLC, Clayton, NC). To prepare the fabric samples, 20 µl of BioUD were evenly spread onto half of a 5 cm diameter petri dish lid (Becton Dickinson Labware, Franklin Lakes, NJ) with a plastic pipet tip and a half-circle-shaped piece (9.81 cm^2) of 2-ply cotton gauze (Type #11675, NCSU Central Stores, Raleigh, NC) was placed on top to evenly absorb the BioUD30. The samples were incubated for 2 h at room temperature prior to beginning the experiment. After placing the ticks in the center of the arena containing untreated and BioUD treated cloth, a clear plastic lid with a rubber tube lined edge was placed over the ticks and fabrics to prevent the ticks from climbing off of the cotton fabric, thereby limiting them to either the untreated or treated surface. The distribution of ticks was checked at 5, 10, 15, 30, 60 and 90 min after the start of each assay. Ticks that were in contact with both the untreated and treated fabric at the center margin where the two cloths meet, were not counted in determining percent repellency. During each assay, five separate testing arenas were used, with one containing only untreated cloth in order to observe the distribution of ticks without the presence of a repellent. Each experiment was conducted with no light except during monitoring of tick distribution. Fabric samples were stored at room temperature and the same samples re-assayed over the course of 8 d.

Mosquito bioassays

North Carolina (USA). The test area was the surface of the arm from just distal to the elbow to the most distal end of the hand. The following test conditions were used: (a) Control (no treatment) and (b) 1.5 ml of BioUD8 (8% undecanone). The application of the repellent to all subjects was conducted within a 10 min time period in the air-conditioned reception area of HOMS, LLC. Landing counts in the field were conducted at 2, 3, 4 and 4.5 h after application of the repellent with the 4.5 h assay conducted at dusk. The repellent volume to be applied was measured with a P5000 Gilson Pipetman, directly to the subject's skin, which was then spread with the subjects' free hand to the entire area to be treated. Subjects were asked to remain in the reception area until about 1 h prior to the first field test (the 2 h post-treatment test). Each replicate was one person (control, 1 male and 1 female; BioUD8 (1.5 ml), 2 males and 1 female), and the same person was tested at each time (total number of human subjects = 5). At approximately 1 h before the field test, all subjects traveled by car for about 40 min to the parking lot of the visitor center at Howell Woods where the tests were conducted. All mosquito tests were conducted with wild populations on a nature trail at the Howell Woods Environmental Education Center, Bentonville (Johnston County), North Carolina (USA). Two specific study locations were chosen: (a) a 3 meter wide trail through a heavily wooded area (Location: forest) and (b) on a 1.2 meter wide plank bridge, approximately 0.6 meters above the surface of a heavily wooded marsh (Location: marsh).

All subjects were dressed in their personal clothing of choice with only the treated or control area of their forearm, their hands and their head exposed. Each subject wore at least two shirts. The head of each subject was covered with a hat and mosquito net, and the hand on their untreated arm was covered with a latex disposable glove. The only exposed skin for mosquito landings was the control or treated surface of the forearm and hand of one arm. The pants for both legs of each subject was either taped tight against the leg or inserted into their socks and each subject had the option of receiving a permethrin treatment to their ankles to control for possible ticks or chiggers. Each subject was provided a pencil and data form to record landing counts and then all test subjects walked together about 0.25 miles to the test location.

Two test locations (forest and marsh) were used. Each test location covered a linear area of 37 meters. Two to three measurements were made at a different site in the same test location (forest or bridge) at each time (2-4.5 h post-treatment). Changes in the site within a location were achieved by asking subjects to randomly exchange position with other subjects. After each test time (2, 3 and 4 h), the subjects all returned together to the parking lot of the Howell Woods Visitor's Center. Between the 4 and 4.5 h reading, the subjects remained in the forest location. Subjects were asked to count the number of mosquito landings over a given observation period which was initiated and ended by voice communication from one of the control subjects. Landings were defined as a mosquito on the subject's forearm/hand for at least 2 sec and/or after observing probing. The subjects were asked to physically remove the mosquito from their arm with their free hand using at least a brushing motion to prevent mosquito bites. The estimated skin surface area for the control and treatments was 900 square cm each. All landing count measurements were taken simultaneously across Reps at each location and at different sites within a location.

Guelope, Ontario (Canada). Mosquito bioassays were conducted in Guelope, Ontario, Canada under natural field conditions using human subjects by the company, Arcturus, Guelph Canada. The mean biting pressure over 3 nights in these studies were 79.7 mosquitoes per 30 min. Treatment effects were calculated based on nightly means. BioUD8 was provided by HOMS. The other repellents tested were obtained from commercial sources.

Results and Discussion

Synthesis of TMOF analogs

The synthesis of PHEN and PHEA is shown in Fig. 1. Similar approaches were used for the synthesis of CHEA and CHEN. The compounds were stored in the cold before use and dissolved in reagent grade acetone for assay.

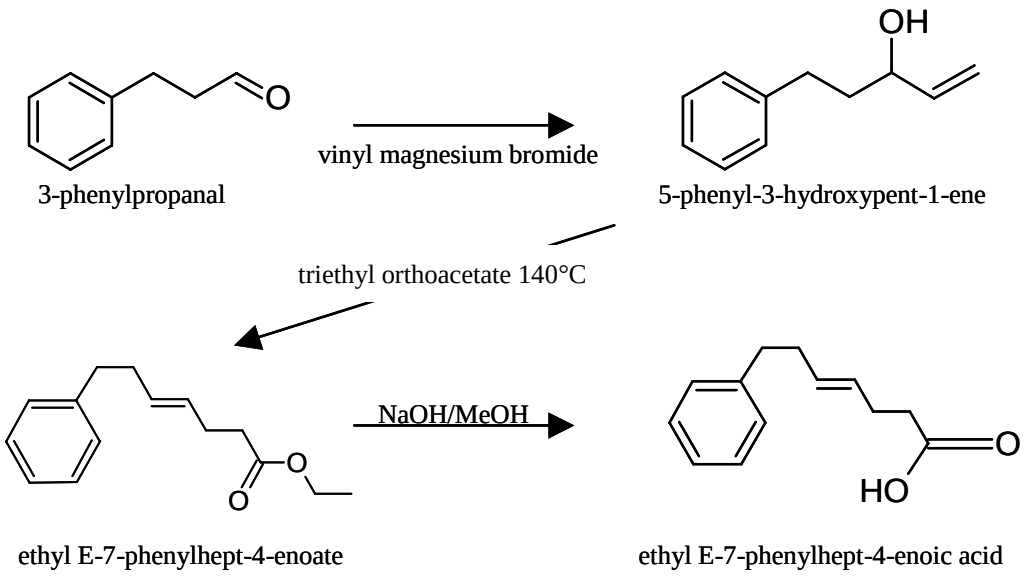

Fig. 1. Preparation of ethyl E-7-phenylhept-4-enoate (PHEN) and E-7-phenylhept-4-enoic acid (PHEA). Dihydrocinnamaldehyde was added to an excess of vinyl magnesium bromide in tetrahydrofuran (THF) at 0°C and allowed to warm to room temperature and remain there for 2 h. The reaction was quenched with saturated ammonium chloride solution and extracted twice with ether. The combined ether extracts were dried over anhydrous sodium sulfate and after evaporation of the ether *in vacuo*, the residue was purified by column chromatography on silica gel to yield 5-phenyl-3-hydroxypent-1-ene. The allylic alcohol was then dissolved in triethyl orthoacetate with a catalytic amount of propionic acid and heated at 140°C for 6 h (following distillation of the ethanol). After cooling to room temperature, the excess triethyl orthoacetate was removed under reduced pressure, and the resulting residue purified by flash chromatography to give E-7-phenylhept-4-enoate at >95% purity. PHEN was saponified in methanolic sodium hydroxide at room temperature producing E-7-phenylhept-4-enoic acid (PHEA) (>95% purity).

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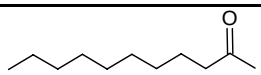
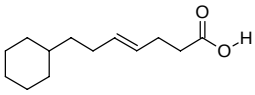
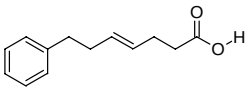
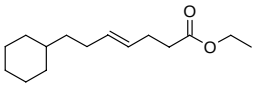
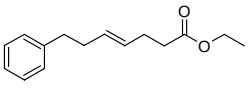
Repellency of CHEA, PHEA, CHEN, PHEN and 2-undecanone in a two-choice filter paper bioassay

No differences were found in our no-choice bioassay between the control (C) and treated (T) filter paper samples for the soft tick, *O. parkeri* (t-test, alpha = 0.05; Table 1). These results indicated that tick movement in our two-choice assay was random. All of the compounds showed insect repellency. The best compound was PHEN, which was repellent at 5 micrograms of compound tested per square cm. CHEA, PHEA and CHEN showed repellent activity at levels as low as 50, 50 and 10 µg of compound tested per square cm, respectively. PHEN, CHEA, PHEA and CHEN represent novel chemistries with unknown toxicology profiles. The compound, 2-undecanone, demonstrates reasonable structural similarities to these compounds both in the carbon chain length and double bond oxygen-functional group as shown in Table 1. This compound has never been examined for its repellent activity against ticks and mosquitoes. However, it is a natural product found in the trichomes of wild tomato plants, is currently commercialized as an animal repellent, is non-toxic and is used as a food additive. Its repellent activity against the soft tick was significantly less than that of PHEN and CHEN and the same as that for CHEA and PHEA. However, the potential development of 2-undecanone (UD) as a commercial insect repellent is much greater than that of our lead synthetic chemistries because of its favorable and well-established toxicology profile and its potential use as an all-natural, organically certified insect tick and mosquito repellent for children and adults.

Table 1. Results of repellency assays with *Ornithodoros parkeri*. Structures of TMOF mimics are also shown.

				µg/cm^2			
Compound				100	50	10	5
1.		(2-undecanone)	C =	9.0 ± 0.4a[1]	9.5 ± 0.3a	5.5 ± 0.3a	-
			T =	1.0 ± 0.4b[1]	0.5 ± 0.3b	4.5 ± 0.3a	-
2.		(CHEA)	C =	10.0 ± 0.0a	10.0 ± 0.0a	4.5 ± 0.3a	-
			T =	0.0 ± 0.0b	0.0 ± 0.0b	5.5 ± 0.3a	-
3.		(PHEA)	C =	10.0 ± 0.0a	9.2 ± 0.3a	4.8 ± 0.3a	-
			T =	0.0 ± 0.0b	0.8 ± 0.3b	5.2 ± 0.3a	-
4.		(CHEN)	C =	-	9.8 ± 0.2a	7.8 ± 0.6a	6.0 ± 1.0a
			T =	-	0.2 ± 0.2b	2.2 ± 0.6b	4.0 ± 1.0a
5.		(PHEN)	C =	-	10.0 ± 0.0b	10.0 ± 0.0b	8.5 ± 0.6a
			T =	-	0.0 ± 0.0b	0.0 ± 0.0b	1.5 ± 0.6b
	no-choice		C =	4.8±0.5 a			
			T =	5.3±0.5 a			

[1]Mean number of ticks on the untreated (C) versus the treated surface (T) ± 1 SEM. Means followed by different letters were significantly different as determined by a paired t-test. (α=0.05).

Repellency of BioUD on human skin against ticks

One problem with 2-undecanone as a tick and mosquito repellent was its high volatility. HOMS LLC obtained an exclusive license for the commercialization of UD as a repellent. Their formulation, which contains either 30 or 8%UD, is being developed as BioUD30 and BioUD8, respectively. As shown in Table 2, in a two choice assay on human skin 2 h after the application of the repellent, BioUD30 Spray and BioUD8 Lotion were both repellent to the American dog tick, a vector of Rocky mountain spotted fever.

Repellency of BioUD on human skin against mosquitoes in natural field conditions

BioUD was also highly repellent on human skin against mosquitoes (Fig. 2). Assays were conducted in a forest habitat and in two marsh locations in Johnston County, NC. The number of mosquito landing counts typically exceeded 7 per min and ranged from 7.33-22.00 per min. Percent repellency was calculated based on a control as follows: [(mean landing counts per min for control)-(landing counts per min for Rep)/mean landing counts per min for control] X100. Mean repellency at different times post treatment for all habitats combined declined 99.73 to 98.85% from 2 to 4.5 h, respectively for BioUD8; however, based on the 95% confidence intervals, there was no statistically significant difference in repellency at any time post treatment. The individual results for each habitat examined is shown in Fig. 2. The study was ended at 4.5 h because of lack of natural light, which was needed to observe mosquito landings. Mosquitoes were collected off of subjects at the end of the assays. The mosquitoes collected were identified as follows: 12 *Ochlerotatus atlanticus*/*tormentus*, 4 *Psorophora ferox*, and 1 *Psorophora columbiae*. Based on these studies, it was concluded that BioUD8 was a highly effective repellent against mosquitoes under natural field conditions with high mosquito densities.

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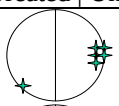
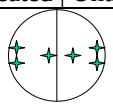
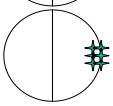
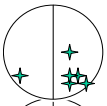
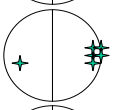
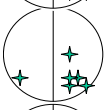
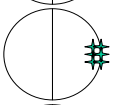
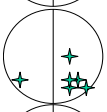
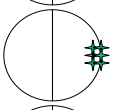
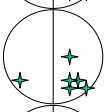
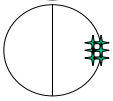
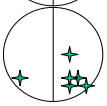
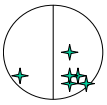
Table 2. Two-choice test for 20 µL BioUD30 Spray (30% undecanone) and 20 µL BioUD8 Lotion (8% undecanone) on untreated human skin against the American dog tick.

Time (h:min)	BioUD30 Spray Treated | Untreated	BioUD8 Lotion Treated | Untreated
2: 0		
2: 5		
2 : 10		
2 : 15		
2 : 20		
2 : 25		
2: 30	not recorded	

Test area was 4 cm in diameter (12.56 cm^2) on the left leg just above the knee of the human (male) subject. Control, 30 min prior to the application of repellent. Applied 20 µl of BioUD30 Spray (30% undecanone) or 20 µl of BioUD8 Lotion (8% undecanone) to left half of test area. Ticks (unfed mixed sexes of the American dog tick, *Dermacentor variabilis*) were added to the test apparatus just prior to the application of the repellent. Apparatus was a plastic petri plate top with the opening covered with aluminum screening and the inside between the plate and screen containing two layers of cheese cloth. Assay conducted at room temperature with apparatus on skin covered with dark cloth. Ticks with apparatus were applied to skin 2 h: 0 min after the application of repellent.

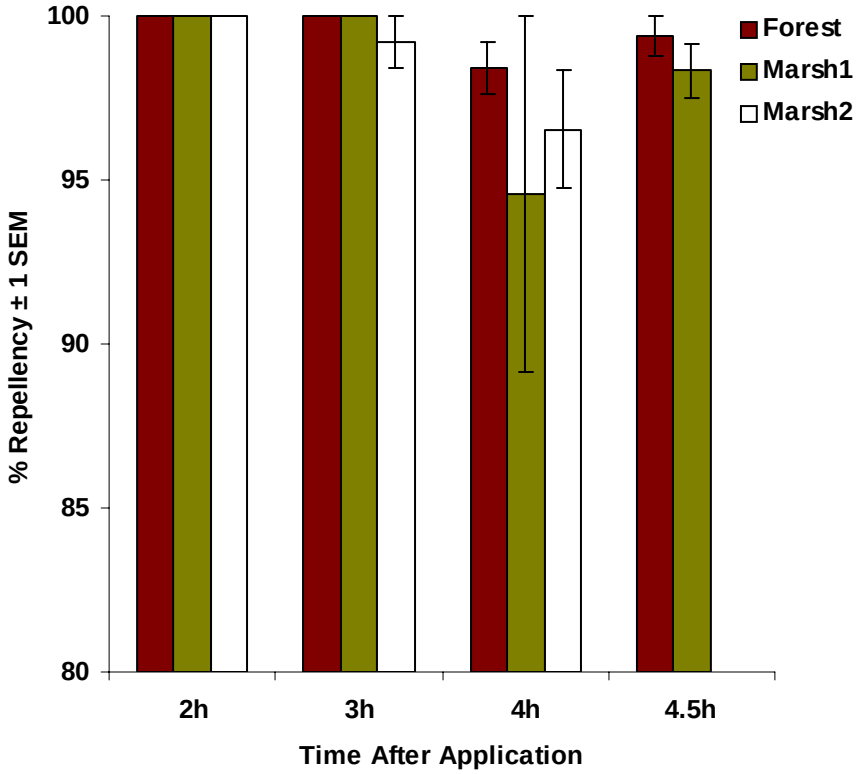


Figure 2. Percent repellency of BioUD8 Spray (8% undecanone) against mosquitoes on the surface of the arm of human subjects from just distal to the elbow to the most distal end of hand [Johnston county, NC (USA)]

Independent studies were contracted with the company Arcturus in Guelph, Ontario, Canada to compare the mosquito repellent activity of BioUD8 against OFF Botanicals with 10% PMD (p-menthane-3,8-diol) and Off with 15% DEET. These studies were conducted under field conditions with a mean biting pressure over 3 nights of 79.7 mosquitoes per 30 min. The results are shown in Table 3. The percent reduction of mosquito biting 6 h after application to human skin for BioUD8 and Off with 15% DEET was similar (68.2 and 67.4%, respectively) as compared to 31.7% for OFF Botanicals with 10% PMD. These studies suggest that BioUD8 was an effective mosquito repellent with activity similar to a commercial DEET product.

Repellency of BioUD applied to cotton fabric against ticks

American dog ticks, *D. variabilis*, were subjected to a two choice test of cotton fabric without repellent and cotton fabric treated with BioUD30 silicon. The fabric was assayed over the course of 8 d to evaluate the binding of BioUD to cloth and assess whether the duration of repellency of BioUD against ticks could be lengthened over that of human skin (Table 2). While undecanone alone was found to repel the soft tick, *O. parkeri* at 50 µg/cm^2 (Table 1), the volatility of undecanone limits the duration of its effectiveness. The proprietary emulsion, BioUD, was developed by HOMS, LLC, to overcome this problem. Previous preliminary data (not shown) suggested that undecanone can also bind to cotton fabric, decreasing its volatility.

Table 3. Mean number[1] (±1 SD) and percent reduction of mosquitoes biting human subjects[2] during 30 min mosquito biting counts in field trials conducted near Guelph, Ontario.

Treatment	Hours post-application	Mean[1] (±1 SD) # of mosquitoes per 3.5 min	Percent reduction[3]
Experiment 1			
Control	-	9.30 ± 4.41	-
BioUD8 Lotion 8% undecanone	6 h	3.05 ± 2.70	68.2
OFF Botanicals 10% PMD	6 h	7.73 ± 6.08	31.7
Experiment 2			
Control	6 h	7.95 ± 4.96	-
OFF 15% DEET	6 h	2.59 ± 2.73	67.4

[1]n=5 replicates
[2]Mean biting pressure over 3 nights equaled 79.7 mosquitoes per 30 min
[3]Calculated from nightly means, not from data in column three; PMD (p-menthane-3,8-diol; Testing performed by Arcturus, Guelph Canada. The HOMS BioUD8 Lotion provided significantly (P<0.05) better protection from biting mosquitoes at 6 h post-application than OFF Botanicals.

BioUD30 applied to cotton fabric significantly repelled American dog ticks at 10, 15, 30, 60 and 90 min, 2 h after application with the exception of the 5 min time point (paired *t*-test, P<0.5). During the 5 min time interval, 100% of the ticks were repelled in 3 of the 4 replicates; in the fourth replicate only 50% of the ticks were repelled. In the 15 and 30 min time intervals, greater than 95% of the ticks were significantly repelled (P<0.001), while 100% of the ticks were repelled during the 10, 60 and 90 min time intervals (Fig. 3). One day after application, greater than 90% of the ticks were repelled at all time intervals (P<0.003). Results of the bioassays 2 d after application of BioUD were highly variable with tick repellencies of 35±47.26 (5 min), 55±35.85 (10 min), 58.33±21.52 (15 min), 75±21.52 (30 min), 83.33±19.25 (60 min) and 83.33±19.25% (90 min). Ticks were significantly repelled at the 60 and 90 min time intervals only (P<0.05). Interestingly, the same fabric samples tested the following day against naïve ticks, repelled 90±11.5 (5 min), 87.5±8.33 (10 min), 87.5±8.33 (15 min), 91.67±9.62 (30 min), 95.83±8.33 (60 min) and 95.83±8.33% (90 min), all of which were found to be statistically significant (P<0.05). The cause for the variability during the bioassay on d 2 is unknown. Control samples with untreated cotton fabric were conducted simultaneously to ensure that the ticks would not preferentially move to one side of the test area. Comparison of the control samples during all bioassays did not result in false positives (*t*-test, P>0.05), thereby validating the bioassay. Ticks were significantly repelled at all time intervals during d 4, 5 and 6 (with the exception of d 6, 5 min)(P<0.05). On d 7, ticks were significantly repelled at 10, 15 and 30 min after the start of the assay (P<0.05), and not at all on d 8 (Fig. 3).

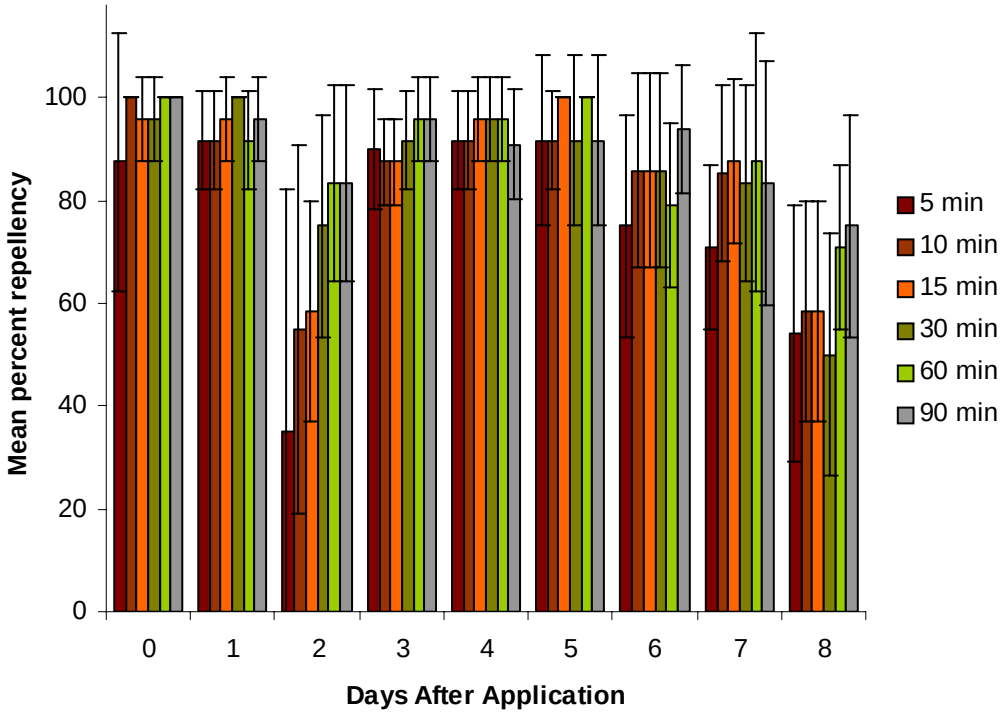


Fig. 3. Two-choice test for 20 µl BioUD30 silicon (30% undecanone by weight; 5.88 mg) applied to cotton cloth against the American dog tick, *Dermacentor variabilis*. Six ticks per assay were placed in a 5 cm diameter (19.63 cm^2) arena on top of two half-circle pieces of cotton gauze; one side untreated cloth and the other side treated with 20 µl BioUD30 silicon (previously incubated at room temperature for 2 h after the application of BioUD30 prior to assay). Fabric samples were stored at room temperature and the same samples reassayed over the course of 8 d, using naïve ticks for each assay. The distribution of the mixed sex adult ticks was checked at 5, 10, 15, 30, 60 and 90 min. Each assay was conducted with no light except during monitoring of tick distribution. Error bars in graph are ±1 standard deviation; lack of error bars represents no variability.

Summary

Novel repellent chemistry was developed for ticks. Based on this lead chemistry, 2-undecanone was discovered as a structural mimic of our lead chemistry and when tested was found to have tick repellent activity. To regulate the volatility of UD, HOMS formulated undecanone into BioUD. BioUD when tested on human skin was repellent to the American dog tick 2 h after application. BioUD in natural field studies was an effective repellent (demonstrated 99% repellency) on human skin against mosquitoes after 4.5 h. When compared to other commercial repellents, BioUD8 was as effective as OFF with 15% DEET against mosquitoes in field studies 6 h after application to human skin and both of these repellents performed better than OFF Botanicals with 10% PMD. BioUD silicon applied to cotton cloth was effective as a repellent for 7 days after treatment against the American dog tick. HOMS LLC is developing BioUD as an all natural insect repellent with the potential of being organically certified. The repellent is formulated to be nonflammable. Our studies also indicate BioUD8 is as effective as OFF with 15% DEET and can be used on cloth. The EPA submission of BioUD as a repellent for use on children and adults is planned for early 2006.

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References cited

Durden, L. A. and G. R. Mullen. 2002. Chapter 1; Introduction. *In* G. R. Mullen and L. A. Durden [eds.], Medical and Veterinary Entomology, pp. xv + 597. Academic Press, San Diego, California.

Fradin, M. S. 1998. Mosquitoes and mosquito repellents: a clinician's guide. Ann. Intern. Med. 128: 931-940.

Oransky, S., B. Roseman, D. Fish, M. S. Gentile, J. Melius and M. L. Carter. 1989. Seizures temporally associated with use of DEET insect repellent - New York and Connecticut. Mortal. Morbid. Week. Rev. 38:678-680.

Parola, P. and D. Raoult. 2001. Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. Clin. Infect. Dis. 32: 897-928.

Roe, R. M. 2002. Method of repelling insects. United States patent 6,437,001. August 20, 2002.

Scott, A. 2002. Insect repellent designed from tomatoes. Chem. Week, June 26, 2002. p. 30.

Veltri, J.C., T. G. Osimitz, D. C. Bradford and B. C. Page. 1994. Retrospective analysis of calls to Poison Control Centers resulting from exposure to the insect repellent N,N-diethyl-m-toluamide (DEET) from 1985-1989. Clin. Toxicol. 32:1-16.

United States Environmental Protection Agency [EPA]. 1998. Reregistration eligibility decision (RED): DEET. September 1998. Office of Prevention, Pesticides and Toxic Substances. pp. vi + 118.