

Comparative Efficacy of BioUD to Other Commercially Available Arthropod Repellents against the Ticks *Amblyomma americanum* and *Dermacentor variabilis* on Cotton Cloth

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Abstract. BioUD is an arthropod repellent that contains the active ingredient 2-undecanone originally derived from wild tomato plants. Repellency of BioUD was compared with five commercially available arthropod repellents against the ticks *Amblyomma americanum* (L.) and *Dermacentor variabilis* Say in two-choice bioassays on treated versus untreated cotton cheesecloth. Overall mean percentage repellency against both species was greatest for and did not differ significantly between BioUD (7.75% 2-undecanone) and products containing 98.1% DEET, 19.6% IR3535, and 30% oil of lemon eucalyptus. Products containing 5% and 15% Picaridin and 0.5% permethrin were also repellent compared with untreated controls but to a lesser degree than BioUD. The four most active repellents at the same concentrations used before were directly compared in head-to-head bioassays on cotton cheesecloth. BioUD provided significantly greater overall mean percentage repellency than IR3535 for *A. americanum* and *D. variabilis*. BioUD was significantly more repellent than oil of lemon eucalyptus for *A. americanum* but did not differ significantly in repellency against *D. variabilis*. No statistically significant difference in overall mean percentage repellency was found between BioUD and DEET for *A. americanum* or *D. variabilis*. In a 7-week time course bioassay, BioUD applied to cotton cheesecloth and held at room temperature provided 5 weeks of > 90% repellency against *A. americanum*.

INTRODUCTION

The lone star tick, *Amblyomma americanum* (L.), is an aggressive tick that bites humans during all post-embryonic life stages. *A. americanum* has expanded its range in the United States in recent years and is now distributed in some areas of the midwest, throughout the southeast, and along the east coast as far north as New York state.¹ This tick is the established vector of several human pathogens, including *Ehrlichia chaffeensis* and *E. ewingii*.¹ The American dog tick, *Dermacentor variabilis* Say, feeds on humans during the adult stage and is a known vector of *Rickettsia rickettsii* and *Francisella tularensis*, which are the pathogens that cause Rocky Mountain spotted fever, and tularemia, respectively.² *A. americanum* is commonly found attached to humans in the southern and Atlantic states of the United States, and *D. variabilis* frequently parasitizes humans in the eastern United States.³

One important protective measure against tick bites is the use of personal arthropod repellents. DEET (*N, N*-diethyl-*m*-toluamide) has been the most widely used arthropod repellent for personal protection for > 50 years. DEET is a broad-spectrum repellent that has been shown to be effective against mosquitoes and other biting flies, chiggers, and ticks.⁴ DEET is highly effective against several species of mosquitoes^{5–7} but is generally less repellent against ticks compared with other arthropod repellents, such as permethrin or piperidines.^{8–10} Additionally, although DEET has been widely used with few adverse health effects,^{11–13} the safety of this repellent has been questioned.¹⁴ Currently only two repellent alternatives to DEET are recommended by the Centers for Disease Control and Prevention (CDC) that are labeled for use on human skin against ticks by the US Environmental Protection Agency (EPA)¹⁵: IR3535 (3-[*N*-butyl-*N*-acetyl]-aminopropionic acid, ethyl ester) and the piperidine repellent Picaridin (2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester). The synthetic pyrethroid permethrin is also available as a repellent and acaricide for use on clothing

but can not be applied to human skin.² The protective action of permethrin against ticks has been attributed primarily to its toxic properties rather than to its repellency.¹⁶ Additional safe and efficacious repellent alternatives to DEET are needed to protect people that choose not to use DEET or other synthetic repellents.

BioUD is a new plant-based arthropod repellent registered by the US EPA for use on human skin and clothing against mosquitoes and ticks. The active ingredient in BioUD, 2-undecanone (methyl nonyl ketone), was originally isolated from the glandular trichomes of the wild tomato, *Lycopersicon hirsutum* Dunal f. *glabratum* C. H. Müll.¹⁷ In laboratory studies, BioUD was repellent to *D. variabilis* on cotton cheesecloth, filter paper, and human skin.¹⁸ In addition, BioUD applied to cotton cheesecloth was found to be highly repellent against *D. variabilis* for at least 8 days after treatment.¹⁸ BioUD also provided significantly greater percentage repellency than 98.1% DEET against adult *A. americanum* and blacklegged ticks, *Ixodes scapularis* Say, in choice bioassays on treated filter paper compared with untreated controls.¹⁹

One common use of repellents is their application to clothing. The longevity of arthropod repellents can be increased with application to clothing rather than to human skin.²⁰ This study was conducted to compare the efficacy of BioUD against *A. americanum* and *D. variabilis* on cotton cloth to that of repellents recommended by the CDC that are currently labeled for use against mosquitoes or ticks by the US EPA. Additionally, because BioUD is a new product and little is known about the longevity of its activity, the duration of tick repellency by BioUD on cotton cloth was examined over 7 weeks.

MATERIALS AND METHODS

Ticks. All ticks used in trials were naïve, unfed adults of mixed-sex exhibiting host-seeking behaviors (as indicated by raised forelegs in response to human breath). *A. americanum* were collected from wild populations in Sanford, NC, on 17 April and 13 June and in Wake County, NC, on 10 June 2008. *D. variabilis* were obtained from laboratory colonies of D. E. Sonenshine at Old Dominion University (Norfolk, VA)

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where they were reared as previously described²¹ from specimens originally collected near Richmond, VA. Before bioassays, ticks were maintained at ~28°C, ~75% relative humidity (RH), and a photoperiod of 14-hour light:10-hour dark, including dusk and dawn periods (60 minutes each).

Test substances. Repellency bioassays were conducted with seven commercially available arthropod repellent products (Table 1). All products were purchased at retail stores.

Choice trials (treated versus untreated surface). Trials were conducted at 25°C, 65% RH, and in complete darkness (except during the ~5 seconds needed to monitor tick distribution). Ticks were allowed to choose either a repellent-treated or untreated cheesecloth surface. Tests were conducted in 63.6-cm² Petri plate lids lined with two double-layered 31.8-cm² semi-circle pieces of cotton cheesecloth (type 11675; NCSU Central Stores, Raleigh, NC). Cloth was treated separately with 65 µL of repellent and allowed to dry for 3 hours at room temperature under a fume hood before beginning bioassays. Six ticks were placed in each arena at the junction where repellent-treated and untreated cloth met for all repellents except permethrin. To minimize the possible toxic effects of permethrin, ticks were placed on the untreated cloth surface just adjacent to the junction where treated and untreated cloth met in bioassays comparing permethrin-treated versus untreated cloth. Tick distribution was recorded every 5 minutes from 5 to 30 minutes after introduction of ticks to arenas. Arenas lined with two double-layered semi-circles of untreated cloth served as controls to measure the distribution of ticks in the absence of a repellent. All repellents were tested against both tick species with the exception of 5% Picaridin, which was not tested against *D. variabilis* because 15% Picaridin provided statistically lower repellency than BioUD against *D. variabilis*. Six replicates of each treatment were performed for trials using *A. americanum* and four replicates were performed for each treatment of trials using *D. variabilis*.

Choice trials (BioUD versus other commercial repellents). Head-to-head trials were conducted to directly compare products that exhibited the highest mean percentage repellency in the choice trials (treated versus untreated surface) just described and that did not differ significantly in repellency from that of BioUD against both tick species. Trials were conducted in the same manner as in choice tests (treated versus untreated surface) except that cheesecloth treated with BioUD was compared beside cheesecloth treated with DEET, IR3535, or

oil of lemon eucalyptus in the same test arena. Four replicates of each treatment combination were made. The same controls were used as described before.

Weekly time course trials. Trials were conducted to examine the longevity of BioUD repellency on cotton cloth over time against *A. americanum*. Six replicates were performed as described for choice trials (treated versus untreated surface) with cloth from each assay being re-assayed weekly for 7 weeks using naïve ticks for each assay. Untreated controls were conducted on weeks 1, 6, and 7.

Data analysis. Before analyses, mean percentage repellency data for *A. americanum* choice trials (treated versus untreated surface) were square root transformed to achieve approximate normality. Mean percentage repellency data for choice (treated versus untreated surface) and head-to-head trials were analyzed separately for each tick species by fitting a general mixed linear model to observed responses using the SAS procedure PROC MIXED²² with treatments, time, and their interaction as fixed-effect factors. Mean percentage repellency data for weekly time course trials were analyzed by fitting a general mixed linear model to observed responses using PROC MIXED with time, week, and their interaction as fixed-effect factors. Data for head-to-head, weekly time course, and mean percentage repellency for *D. variabilis* choice trials (treated versus untreated surface) were not transformed because a visual examination of scatter plots of predicted values against residuals²³ showed that the residuals were evenly distributed about a mean of zero, indicating that the response data exhibited homogeneity of variances and normality. Repeated observations on time within each replication were considered correlated measures, and the covariance structure for these repeated measures on time was modeled through a heterogeneous compound symmetry covariance. Pairwise mean comparisons ($P \leq 0.05$) were analyzed to determine statistical differences in mean repellency between repellents or between a repellent-treated and untreated surface across all time points and at each time point. χ^2 test for proportions was used to determine whether mean tick distribution for untreated sides in control trials differed significantly ($P = 0.05$) from the null hypothesis that the expected proportion in the absence of any repellent is 0.5 (H_0 : proportion = 0.5).

RESULTS

Choice trials (treated versus untreated surface). Mean percentage repellency at each observational time point for choice trials for *A. americanum* and *D. variabilis* are presented in Figures 1A and 2A, respectively. Overall mean percentage repellency averaged across all time points from 3 to 3.5 hours after repellent treatment are presented in Figures 1B and 2B for *A. americanum* and *D. variabilis*, respectively. Mean percentage repellency for each treatment did not change over time for *A. americanum* ($F = 0.34$; $df = 5,200$; $P = 0.89$) or *D. variabilis* ($F = 0.98$; $df = 5,105$; $P = 0.43$), and there was no significant interaction between each repellent treatment and time for *A. americanum* ($F = 0.85$; $df = 35,200$; $P = 0.70$) or *D. variabilis* ($F = 0.70$; $df = 30,105$; $P = 0.87$). All repellent treatments differed significantly in repellency compared with untreated controls ($P \leq 0.05$; pairwise comparison) from 3 to 3.5 hours after application against *A. americanum* (Figure 1B). Against *D. variabilis*, only permethrin did not differ significantly from untreated controls in mean percentage repellency

TABLE 1

Active ingredients and concentrations of repellent products used in tick bioassays

Active ingredient	Product
DEET (98.1%)	Jungle Juice; Sawyer Products, Safety Harbor, FL
IR3535 (19.6%)	Skin-So-Soft Expedition Bug Guard Plus; Avon Products, New York, NY
Oil of lemon eucalyptus (30%; ~65% p-menthane-3, 8-diol)	Cutter; Spectrum, St. Louis, MO
Permethrin (0.5%)	Premium Clothing insect repellent; Sawyer Products, Safety Harbor, FL
Picaridin (5%)	OFF! Familycare insect repellent II; S.C. Johnson & Son, Racine, WI
Picaridin (15%)	Cutter Advanced Outdoorsman; Spectrum, St. Louis, MO
2-undecanone (7.75%)	BioUD spray; HOMS, Clayton, NC

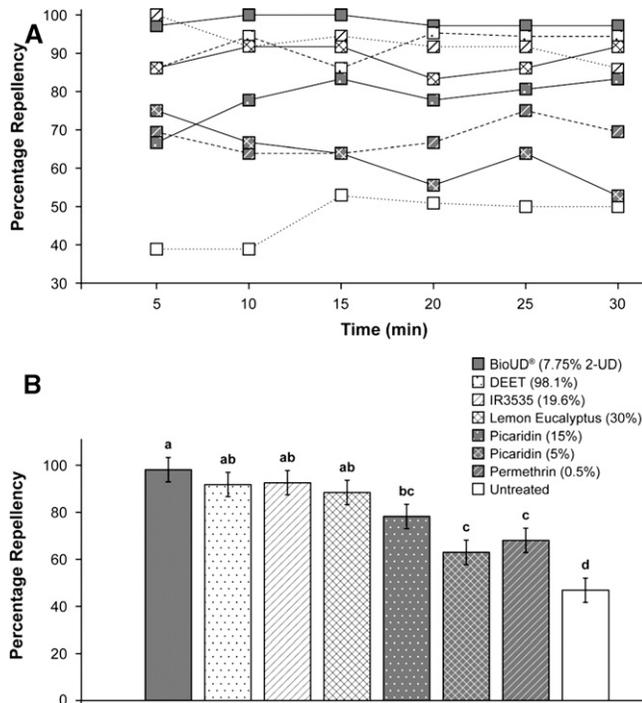


FIGURE 1. **A**, Mean percentage repellency of repellent-treated cotton cheesecloth compared with untreated controls against *A. americanum* at each time point from 3 to 3.5 hours after treatment (5–30 minutes after the addition of ticks to the arena) **B**, Overall mean percentage repellency (\pm SE; $N = 6$) of repellent-treated cotton cheesecloth compared with untreated controls against *A. americanum* from 3 to 3.5 hours after treatment (5–30 minutes after the addition of ticks to the arena). Different letters above means indicate a significant difference in repellency ($P \leq 0.05$ pairwise comparison; SAS Institute 2003).

($t = 1.03$; $df = 6,18$; $P = 0.31$) for the same time period (Figure 2B). Overall mean percentage repellency against *A. americanum* and *D. variabilis* was greatest for and did not differ significantly between BioUD, DEET, IR3535, and oil of lemon eucalyptus ($P \geq 0.05$; pairwise comparison). Overall mean percentage repellency also did not differ significantly between DEET, IR3535, oil of lemon eucalyptus, and the product with the highest Picaridin concentration (15%) for *A. americanum* or for *D. variabilis* ($P \geq 0.05$; pairwise comparison; Figures 1 and 2, respectively). Permethrin and the product with the lowest Picaridin concentration (5%) provided the lowest mean percentage repellency against *A. americanum* and did not differ significantly from each other in mean percentage repellency ($t = 0.69$; $df = 7,35$; $P = 0.49$; Figure 1B). Ticks were evenly distributed in the controls where cloth in both sides of the arena were untreated for *A. americanum* (χ^2 , $P = 0.71$) and *D. variabilis* (χ^2 , $P = 0.68$).

Choice trials (BioUD versus a different commercial repellent). Overall mean percentage repellency results from head-to-head trials are presented in Figure 3 (mean percentage repellency at each time point not shown). BioUD provided significantly greater overall mean percentage repellency than IR3535 for *A. americanum* ($F = 336.35$; $df = 1,3$; $P = 0.0004$) and *D. variabilis* ($F = 52.31$; $df = 1,3$; $P = 0.006$) from 3 to 3.5 hours after repellent application. BioUD was significantly more repellent than oil of lemon eucalyptus for *A. americanum* ($F = 307.04$; $df = 1,3$; $P = 0.0004$) from 3 to 3.5 hours after repellent application (Figure 3A). Overall mean percentage

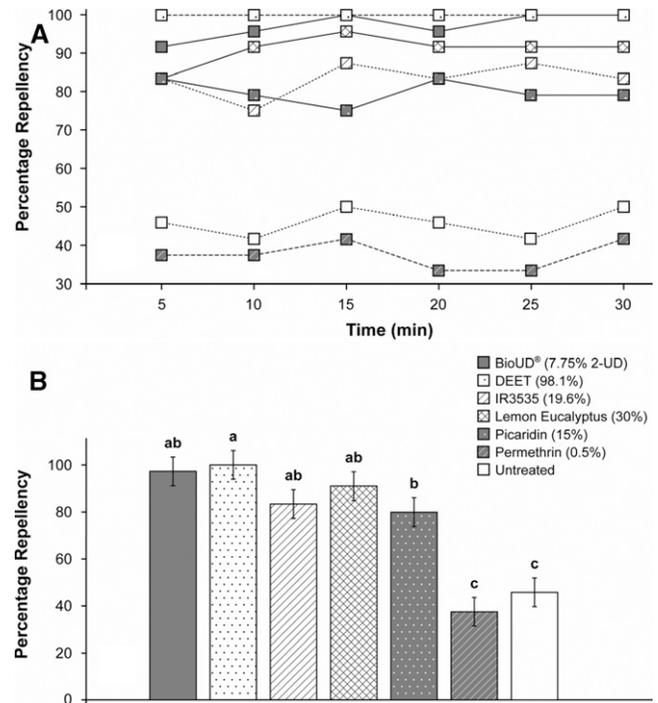


FIGURE 2. **A**, Mean percentage repellency of repellent-treated cotton cheesecloth compared with untreated controls against *D. variabilis* at each time point from 3 to 3.5 hours after treatment (5–30 minutes after the addition of ticks to the arena) **B**, Mean percentage repellency (\pm SE; $N = 4$) of repellent-treated cotton cheesecloth compared with untreated controls against *D. variabilis* from 3 to 3.5 hours after treatment (5–30 minutes after the addition of ticks to the arena). Different letters above means indicate a significant difference in repellency ($P \leq 0.05$ pairwise comparison; SAS Institute 2003).

reppency of BioUD from 3 to 3.5 hours after application did not differ significantly from that of oil of lemon eucalyptus for *D. variabilis* ($F = 7.79$; $df = 1,3$; $P = 0.07$; Figure 3B); however, a significant treatment by time interaction was observed ($F = 7.92$; $df = 5,30$; $P < 0.0001$) so that BioUD was significantly more repellent than oil of lemon eucalyptus at all time points except 5 minutes after introduction of ticks to test arenas. No statistically significant difference in overall mean percentage repellency was found between BioUD and DEET for *A. americanum* ($F = 1.3$; $df = 1,3$; $P = 0.37$) or *D. variabilis* ($F = 0.22$; $df = 1,3$; $P = 0.67$) for the same time period. Ticks were evenly distributed in controls for *A. americanum* ($P = 0.71$, χ^2) and *D. variabilis* ($P = 0.68$, χ^2 ; Figures 3–5, untreated). Figures 4 and 5 show the actual distribution of ticks in arenas 30 minutes after their introduction for all replicates combined. It is clear that BioUD was more repellent than IR3535 and oil of lemon eucalyptus at 30 minutes against *A. americanum* (Figure 4) and *D. variabilis* (Figure 5). It is also evident that BioUD was more repellent than DEET at 30 minutes for *A. americanum* ($t = 2.43$; $df = 5,30$; $P = 0.02$; Figure 4); however, there was no significant treatment by time interaction ($F = 1.84$; $df = 5,30$; $P = 0.14$), and there was no difference in repellency between BioUD and DEET at any time period other than 30 minutes. BioUD and DEET also did not differ significantly in repellency at 30 minutes for *D. variabilis* (Figure 5) for all of the replicates combined.

Weekly time course trials. Mean percentage repellency results for weekly time course trials are presented in Figure 6. Overall mean percentage repellency against *A. americanum*

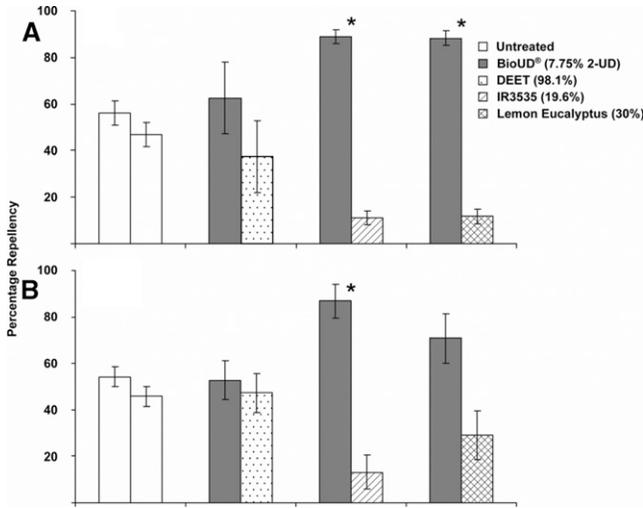


FIGURE 3. Mean percentage repellency (±SE; N = 4) for head-to-head assays comparing BioUD to DEET, IR3535, and oil of lemon eucalyptus on treated cotton cheesecloth surfaces against (A) *A. americanum* and (B) *D. variabilis* from 3 to 3.5 hours after treatment (5–30 minutes after the addition of ticks to the arena). The control represents a two-choice test in the absence of repellent. *Significant difference in repellency ($P \leq 0.05$ pairwise comparison; SAS Institute 2003).

was 98.6% on Week 1. Mean percentage repellency by BioUD did not decline significantly until Week 6 when it fell to 87.4%. Mean percentage repellency of BioUD over all 7 weeks of testing was 93.2%. Ticks were distributed evenly in arenas for untreated controls ($P > 0.05$, χ^2).

DISCUSSION

One common use of personal arthropod repellents is their application to clothing. This study was conducted to examine

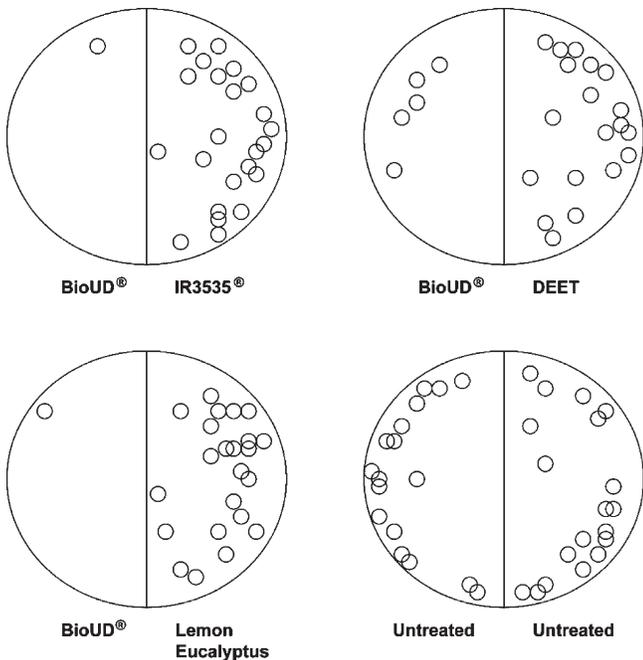


FIGURE 4. Pooled (over all replicates) distribution of *A. americanum* in test arenas 3.5 hours after treatment (30 minutes after addition of ticks to the arena) for head-to-head bioassays.

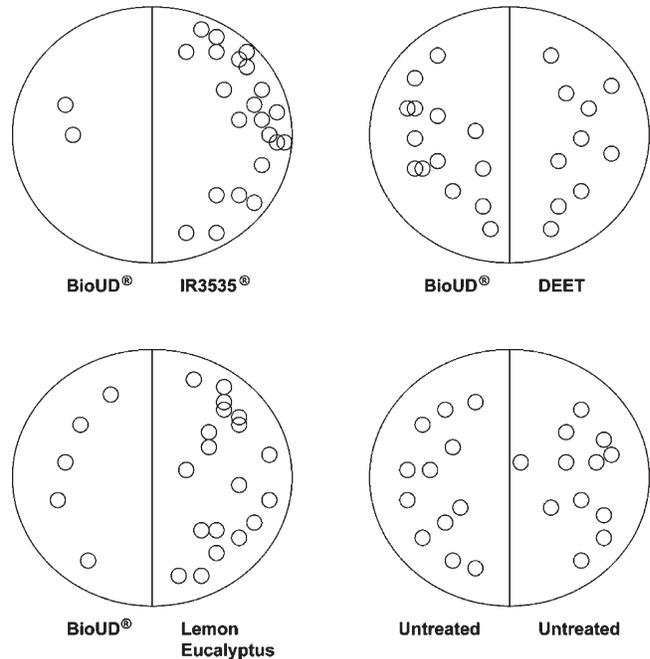


FIGURE 5. Pooled (over all replicates) distribution of *D. variabilis* in test arenas 3.5 hours after treatment (30 minutes after addition of ticks to the arena) for head-to-head bioassays.

the repellency of BioUD and other commercially available arthropod repellents containing EPA-registered active ingredients against the ticks, *A. americanum* and *D. variabilis*, on cotton cheesecloth. Previously we found that BioUD provided significantly greater mean percentage repellency than 98.1% DEET against *A. americanum* and equivalent repellency to 98.1% DEET against *D. variabilis* on treated filter paper compared with untreated controls.¹⁹ Similar results were found in this study when comparing BioUD to 98.1% DEET against *D. variabilis*, but mean percentage repellency did not differ between BioUD and DEET for *A. americanum*. Additionally, whereas BioUD was more repellent than DEET for both species in head-to-head trials on filter paper,¹⁹ no difference was found for either species in head-to-head trials on cotton cloth. This suggests that DEET may bind better to cotton cloth than to filter paper.

In choice trials (treated versus untreated surface), the repellency of BioUD did not differ from the products containing IR3535 or lemon eucalyptus for either tick species tested. However, in head-to-head trials, BioUD was more repellent

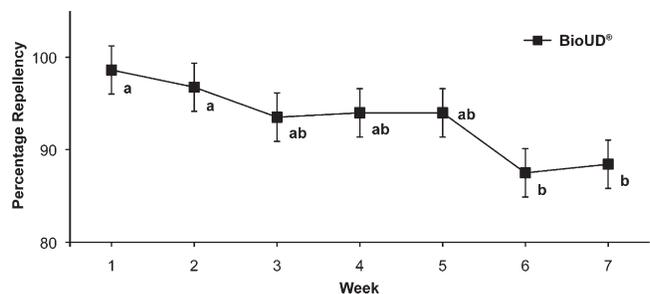


FIGURE 6. Weekly, overall mean percentage repellency (±SE; N = 6) of BioUD-treated compared with untreated cotton cheesecloth against *A. americanum*. Means for each time point followed by different letters are significantly different ($P \leq 0.05$ pairwise comparison; SAS Institute 2003).

than the product containing IR3535 against both *A. americanum* and *D. variabilis*. Mean percentage repellency against *D. variabilis* did not differ significantly between BioUD and the product containing oil of lemon eucalyptus in head-to-head trials, but BioUD was significantly more repellent than oil of lemon eucalyptus against *A. americanum*. BioUD was also significantly more repellent than Picaridin and permethrin products in the choice trials between a repellent and untreated surface for both tick species tested. It is important to note that permethrin is a toxicant. If sub-lethal effects occurred to reduce repellent detection and/or the ability to move away from a repellent surface, this might underestimate the repellent activity of permethrin in our assay format. The same could also be the case for the other repellents that were studied. No research was conducted to examine toxicity of the compounds tested.

A high concentration of active ingredient is often needed to elicit a repellent response against hematophagous arthropods from botanically based repellents.²⁴⁻²⁶ In this study, two botanically based repellents were tested: oil of lemon eucalyptus and BioUD. Both repellents provided high levels of repellency against the tick species tested; however, the amount of active ingredient in BioUD was 3.9 times less than the amount of active ingredient in the product containing lemon eucalyptus. Additionally, BioUD was less concentrated than most of the other repellents tested, containing 12.7 times less active ingredient than the DEET product, 2.5 times less active ingredient than the product containing IR3535, and 1.9 times less active ingredient than the most concentrated Picaridin product tested.

In addition to requiring a high concentration to be effective, the duration of repellency is often short-lived for botanically based repellents, largely because plant essential oils are highly volatile.²⁷ Previously we showed that BioUD applied to cotton cheesecloth was highly repellent against *D. variabilis* for at least 8 days after treatment.¹⁸ In this study, we examined repellency of BioUD against *A. americanum* for 7 weeks. Mean percentage repellency was > 90% for the first 5 weeks and did not decline significantly until the sixth week. These results indicate that BioUD could be an effective tick repellent on clothing for several weeks. Additional studies are needed to take into account the effects of body heat, perspiration, and abrasion on duration of repellency of BioUD.

Three repellent active ingredients approved for use on human skin against ticks by the US EPA are recommended by the CDC: DEET, IR3535, and Picaridin. In this study, DEET and IR3535 were highly repellent against both tick species tested, but 15% Picaridin provided a slightly lower level of repellency. Although highly concentrated DEET was repellent against both species of ticks in this study, some members of the public perceive DEET to be unsafe.¹⁴ Because of this, safe and efficacious botanically based repellents are needed to provide an alternative for the portion of the population that chooses not to use DEET-based products. Because the presence of a host, the specific field conditions under which a repellent might be used and/or the specific assay conditions used for repellent testing might affect repellent performance, more research is needed to fully understand the activity of BioUD compared with other available technologies. However, the studies described in this paper and others^{18,19} on and off the skin of human hosts thus far suggest that BioUD is an efficacious alternative to DEET and other commercially available arthropod repellents for use against ticks.

Received March 2, 2009. Accepted for publication July 12, 2009.

Acknowledgments: The authors thank Dr. Consuelo Arellano (NCSU Dept. of Statistics) for guidance in statistical analyses and Michael Bissinger (NCSU Department of Art and Design) for help with preparation of figures.

Disclosure: Dr. R. Michael Roe, a co-author on this paper, is the inventor of the US EPA registered active ingredient (2-undecanone) in BioUD. This statement is made in the interest of full disclosure and not because the author considers this to be a conflict of interest.

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