Efficacy of Plant-Derived and Synthetic Compounds on Clothing as Repellents Against *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae)

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ABSTRACT We conducted field trials to compare the relative repellent activity of two natural product compounds (nootkatone and carvacrol) with commercially available plant-derived (EcoSMART organic insect repellent) and permethrin-based (Repel Permanone) repellents against adult *Ixodes scapularis* Say and *Amblyomma americanum* (L.) (Acari: Ixodidae) by using treated coveralls. One day after treatment, nootkatone and carvacrol provided 100% repellency of *I. scapularis* adults, with nootkatone maintaining complete protection through 3 d, whereas carvacrol showed steadily declining repellency against *I. scapularis* during the 7-d course of the trials. Nootkatone was at least as effective against host-seeking *A. americanum* as against *I. scapularis* through 3 d. Carvacrol provided little protection against *A. americanum* adults. Both natural compounds performed well initially in comparison with the commercial products. After 7 d, nootkatone was the most effective against both species followed in order of activity by Permanone, EcoSMART, and carvacrol. Nootkatone seems to have offer considerable potential as a clothing repellent against both *I. scapularis* and *A. americanum*.

KEY WORDS repellent testing, nootkatone, carvacrol, EcoSMART, permethrin

Human tick-borne diseases are a growing worldwide concern (Gratz 1999). Lyme disease, caused by the spirochete *Borrelia burgdorferi,* is the most commonly reported vector-borne disease in the United States, and the incidence of Lyme disease continues to increase. In the past 5 yr, an average of >20,000 cases have been reported annually, whereas the number of reported Lyme disease cases reached an all-time high of >35,000 in 2008 (CDC 2010). The blacklegged tick, *Ixodes scapularis* Say, the principal vector of *B. burgdorferi* in the Northeast, also transmits the causative agents of human babesiosis and human granulocytic anaplasmosis (Piesman et al. 1987, Goodman et al. 1996, Stafford et al. 1999). In addition, the locally sympatric and much more aggressive lone star tick, *Amblyomma americanum* (L.), transmits the agent of human monocytic ehrlichiosis and may serve as the vector for several other emerging tick-borne pathogens (Childs and Paddock 2003, Mixson et al. 2006, Apperson et al. 2008).

Repellents remain the primary method of personal protection against tick bites (Piesman and Eisen 2008). *N,N*-Diethyl-*m*-toluamide (DEET) has been the most widely used repellent for personal protection against arthropod-borne diseases. However, although DEET has been used for >50 yr with few adverse health effects (Sudakin and Trevathan 2003), there continue to be persistent safety concerns about its use (Robbins and Cherniack 1986, Clem et al. 1993, Aquino et al. 2004). In addition, DEET is generally less effective against ticks compared with other repellents, such as permethrin or piperidines (Evans et al. 1990, Schreck et al. 1995, Solberg et al. 1995).

People who choose not to use DEET or other synthetic repellents require a safe and effective alternative. In particular, there is increasing interest in natural repellents that can substitute for existing synthetic compounds (Peterson and Coats 2001, Krajick 2006, Moore et al. 2007). Recently many studies have examined biologically based repellents for use against ticks and other arthropods (Bissinger and Roe 2010). Plants produce numerous secondary compounds and essential oils that are repellent or toxic to phytophagous and hematophagous insects and arthropods, including ticks (Levin 1976, Isman 2006, Bissinger and Roe 2010). The majority of phytochemicals that have been tested for repellency against ticks are terpenoids, a structurally diverse assembly of compounds that make up the largest group of secondary plant chemicals (Langenheim 1994) and are involved

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in plant defense mechanisms against herbivorous arthropods and pathogens (Kappers et al. 2008). Research on various terpenes as tick repellents was recently reviewed by Bissinger and Roe (2010).

Dietrich et al. (2006) reported on the repellent activity of natural compounds isolated from essential oil components extracted from the heartwood of Alaska yellow cedar, *Chamaecyparis nootkatensis* (D. Spach), in in vitro laboratory bioassays against *I. scapularis* nymphs. In particular, the terpenes nootkatone and carvacrol demonstrated repellent activity similar to that of DEET. Carroll et al. (2008) suggested that repellent efficacy is best tested under field conditions. Unfortunately, field testing these compounds as skin repellents is complicated by the potential allergenic and irritant properties of terpenes and their autoxidation products (Weinzierl and Henn 1994, Bråred-Christensson et al. 2009). However, application of terpenes to clothing rather than directly to skin may resolve these problems, while increasing their persistence (Smith and Gouck 1946).

We conducted field trials to compare the relative repellent activity of two natural product compounds (nootkatone and carvacrol) with commercially available plant-derived (EcoSMART organic insect repellent) and permethrin-based (Repel Permanone) repellents. We tested the repellents applied to coveralls because use of protective clothing is recommended as a way to prevent ticks from reaching skin and attaching (Stafford 2005, Schulze and Jordan 2006, Clark and Hu 2008) and because walking surveys most accurately reflect human exposure to questing adults ticks (Ginsberg and Ewing 1989, Schulze et al. 1997). We report on trials against adult *I. scapularis* and *A. americanum* and hypothesize that plant-derived natural products will demonstrate repellent activity similar to permethrin against host-seeking ticks.

Materials and Methods

Site Description. Trials were conducted in April 2010 at Naval Weapons Station (NWS) Earle, a military facility located in central Monmouth County, NJ, where both tick species are abundant (Schulze et al. 1997, 2005, 2008). The forest canopy is dominated by pitch pine, *Pinus rigida* Mill.; white oak, *Quercus alba* L.; red oak, *Qercus rubra* L.; and chestnut oak, *Quercus prinus* L. The understory and shrub layer consist of a sparse cover of seedlings and saplings of the dominant canopy species; greenbriar, *Smilax glauca* Walt.; highbush blueberry, *Vaccinium corymbosum* L.; and huckleberries (*Gaylussacia* spp.).

Coverall Treatment. Coveralls were treated with either nootkatone, carvacrol, EcoSMART organic insect repellent (EcoSMART Technologies, Inc., Alpharetta, GA), a commercial permethrin-based clothing repellent (Repel Permanone, Wisconsin Pharmacal Co., Inc., Jackson, WI), or left untreated. To create a 10% solution, 10 ml of nootkatone (Frutarom, North Bergen, NJ) was added to 90 ml of histological grade reagent alcohol comprised of 90% ethanol, ≈5% methanol, and ≈5% isopropanol (Fisher, Fair Lawn, NJ). A 9.5% carvacrol solution was prepared by mixing 10 ml of 95% carvacrol (TCI America, Portland, OR) with 90 ml of reagent alcohol. We sprayed (model S-11686, 16 oz spray bottle, ULINE, Chicago, IL) the entire outer surface of both legs (crotch to cuff) of light-colored coveralls (5-oz, 65% polyester/35% cotton poplin; WearGuard, Norwell, MA), or ≈1 m^2 of fabric, with 100 ml of prepared nootkatone and carvacrol solutions to yield a dosage rate of ≈1.0 mg (AI)/cm^2. The EcoSMART product was provided as a premixed solution with the instructions that the entire 300 ml of finished solution were to be applied, yielding an application rate of 0.25 mg (AI)/cm^2. Active ingredients on the commercially available product label include rosemary, cinnamon leaf, and lemongrass oils (all 0.5%) and geraniol (1.0%) in solution with other ingredients (isopropyl alcohol, isopropyl myristate, and wintergreen oil). Following label instructions, 100 ml of the commercial 0.5% (AI) Permanone was applied to coveralls at 0.05 mg/cm^2. Separate sprayers were used for each repellent. The narrow applicator head of the sprayer used minimized measurable over-spray. Control coveralls received no treatment to simulate typical conditions. Coveralls were allowed to air dry overnight indoors on separate clothing hangers at room temperature and stored in separate sealed drum liners for 24–48 h until initial use. Thereafter, treated coveralls were stored separately on hangers in an unheated garage between uses.

Field Trials. All trials on a given day involved three treated coverall replications per treatment and four 100-m transects per replication. All field trials were conducted by the authors and no other human volunteers were used. The observers were randomly assigned coveralls at the start of each trial. Observers slowly walked through forested areas with substantial shrub layer vegetation known to support both tick species. Observers counted paces and kept a minimum of 20 m between them, pausing every 10 m to count ticks adhering to coveralls. We observed ticks on treated coveralls for up to 3 min (the previously observed time taken for *A. amblyomma* to crawl from cuff to waist on untreated coveralls; data not shown) and recorded the time it took for individual ticks to drop off the treated fabric in 15-s intervals. All ticks that dropped off within 3 min were considered repelled. Ticks remaining on coveralls after 3 min were gently removed and returned to the transect. Trials were conducted at 1, 2, 3, and 7 d after treatment. All trials were conducted by the same individuals when conditions favored questing by both species: between 0800 and 1200 hours on days when vegetation was dry, wind speed was <10 kph, and temperature was 10–15.5°C (Ginsberg and Ewing 1989, Schulze et al. 1997, Schulze et al. 2001).

Statistical Analysis. Pre- and posttreatment comparison of host-seeking *I. scapularis* and *A. americanum* abundance between trial dates were made using separate Friedman's test for each repellent and species (Sokal and Rohlf 1995). Post hoc comparisons between dates were made using Mann–Whitney tests using Bonferroni-corrected levels of significance. Re-

Table 1. Retention of adult *I. scapularis* on repellent-treated coveralls under field conditions, NWS Earle, Colts Neck, NJ, April 2010

Treatment	Time after treatment				Friedman test result
	1 d	2 d	3 d	7 d	
Untreated	9.1 ± 0.8[a]	8.3 ± 0.8	7.8 ± 0.6	9.5 ± 0.8	$\chi^2_{(n = 12, df = 3)} = 5.81; P = 0.12$
EcoSMART	0.5 ± 0.2a (94.5)[b]	0.3 ± 0.1a (96.4)	0.7 ± 0.2a (91.0)	3.0 ± 0.4b (68.4)	$\chi^2_{(n = 12, df = 3)} = 22.53; P < 0.01$
Permanone	0.2 ± 0.2a (97.8)	0.2 ± 0.2a (97.6)	2.4 ± 0.8b (69.2)	2.6 ± 0.4b (72.6)	$\chi^2_{(n = 12, df = 3)} = 20.38; P < 0.01$
Carvacrol	0a (100)	0.7 ± 0.2a (91.6)	2.8 ± 0.7b (64.1)	6.1 ± 0.6c (35.8)	$\chi^2_{(n = 12, df = 3)} = 29.09; P < 0.01$
Nootkatone	0a (100)	0a (100)	0a (100)	2.3 ± 0.5b (75.8)	$\chi^2_{(n = 12, df = 3)} = 30.00; P < 0.01$

[a] Values are mean ± 1 SE of 12, 100-m transects. All ticks adhering to treated coveralls were observed for up to 3 min or until they dropped off. Reported values are for ticks adhering after 3 min. Values in the same row followed by the same letter are not significantly different (Mann–Whitney tests using Bonferroni-corrected levels of significance).

[b] Percentage of repellency = 100 × (no. of ticks on untreated coveralls − no. of ticks on treated coveralls) / (no. of ticks on untreated coveralls).

pellent efficacy was measured as percentage of repellency = 100 × (no. ticks counted on untreated sample − no. of ticks counted on treated sample) / (no. of ticks on untreated sample). All statistical tests were performed using STATISTICA analysis packages (StatSoft, Inc. 2005).

Results

Daily encounter rates (n = 12 100-m transects) on untreated coveralls averaged 9.0 ± 0.4 (SE) *I. scapularis* adults per 100 m per d over 7 d (n = 93–126 total ticks collected on untreated coveralls per d) and did not differ among trial dates [$\chi^2_{(n = 12, df = 3)} = 5.81; P = 0.12$] or observer [$\chi^2_{(n = 12, df = 3)} = 7.78; P = 0.06$] (Table 1). Similarly, encounter rates on untreated coveralls averaged 4.1 ± 0.4 *A. americanum* adults per 100 m per d (n = 31–61 total ticks per d) over the course of the trials and did not differ among trial dates [$\chi^2_{(n = 12, df = 3)} = 6.70; P = 0.08$] or observer [$\chi^2_{(n = 12, df = 3)} = 1.96; P = 0.58$] (Table 2). Numbers of ticks of both species on untreated coveralls were comparable to what we have historically encountered in similar habitats at NWS Earle in April (Schulze et al. 1997, 2005, 2008).

One day after treatment, observers counted in total 22 *I. scapularis* adults on nootkatone-treated coveralls (mean = 1.8 ± 0.6 ticks per transect), with no ticks remaining on coveralls after 3 min (100% repellency). We counted only two *I. scapularis* adults on carvacrol-treated coveralls (mean = 0.2 ± 0.1 ticks per transect), with no ticks remaining on coveralls after 3 min (100% repellency). In contrast, we observed in total 126 ticks on untreated coveralls (mean = 10.5 ± 1.1 ticks per transect) with all ticks remaining on coveralls longer than 3 min. Nootkatone provided 100% protection against *I. scapularis* adults through 3 d. The repellent efficacy of carvacrol declined steadily during the course of the trials and provided only 35.8% protection by 7 d. One day after treatment, we observed a total of seven *I. scapularis* adults (mean = 0.6 ± 0.4 ticks per transect) on Permanone-treated coveralls, with two ticks (mean = 0.2 ± 0.2 ticks per transect) remaining after 3 min (97.8% repellency). Permanone demonstrated ≥97% repellency through 2 d, but lost effectiveness by 3 d (69.2% repellency). We observed in total 27 *I. scapularis* adults (mean = 2.3 ± 0.3 ticks per transect) on EcoSMART-treated coveralls, with six ticks (mean = 0.5 ± 0.2 ticks per transect) remaining after 3 min (94.5% repellency). The EcoSMART product continued to provide >90% protection after 3 d before losing effective repellency at 7 d (68.4% repellency) (Table 1).

At 1 d after treatment, both nootkatone and the EcoSMART product provided 100% repellency against *A. americanum* adults (Table 2). We counted six (mean = 0.5 ± 0.2 ticks per transect) and 10 (mean = 0.8 ± 0.2 ticks per transect) *A. americanum* adults on nootkatone- and EcoSMART-treated coveralls, respectively, with no ticks remaining on coveralls after 3 min. Nootkatone continued to provide protection ≥89% through 7 d, but EcoSMART showed a monotonic decline in protection through the course of the trial (65.2% repellency at 7 d). In total, 13 (mean =

Table 2. Retention of adult *A. americanum* on repellent-treated coveralls under field conditions, NWS Earle, Colts Neck, NJ, April 2010

Treatment	Time after treatment				Friedman test result
	1 d	2 d	3 d	7 d	
Untreated	2.6 ± 0.5[a]	4.0 ± 0.9	5.1 ± 0.6	4.6 ± 0.8	$\chi^2_{(n = 12, df = 3)} = 6.70; P = 0.08$
EcoSMART	0a (100)[b]	0.4 ± 0.2b (90.0)	0.7 ± 0.2bc (86.3)	1.6 ± 0.3c (65.2)	$\chi^2_{(n = 12, df = 3)} = 19.10; P < 0.01$
Permanone	0.4 ± 0.3 (84.6)	0.5 ± 0.3 (87.5)	0.4 ± 0.1 (92.2)	0.7 ± 0.3 (84.7)	$\chi^2_{(n = 12, df = 3)} = 2.62; P = 0.45$
Carvacrol	1.2 ± 0.4 (53.8)	1.3 ± 0.3 (67.5)	1.6 ± 0.5 (68.6)	2.9 ± 0.6 (36.9)	$\chi^2_{(n = 12, df = 3)} = 3.97; P = 0.26$
Nootkatone	0 (100)	0.1 ± 0.1 (97.5)	0.2 ± 0.2 (96.0)	0.5 ± 0.2 (89.1)	$\chi^2_{(n = 12, df = 3)} = 5.55; P = 0.14$

[a] Values are mean ± 1 SE of 12, 100-m transects. All ticks adhering to treated coveralls were observed for up to 3 min or until they dropped off. Reported values are for ticks adhering after 3 min. Values in the same row followed by the same letter are not significantly different (Mann–Whitney tests using Bonferroni-corrected levels of significance).

[b] Percentage of repellency = 100 × (no. of ticks on untreated coveralls − no. of ticks on treated coveralls) / (no. of ticks on untreated coveralls).

1.1 ± 0.5 ticks per transect) *A. americanum* adults were observed on Permanone-treated coveralls, with five (mean = 0.4 ± 0.3 ticks per transect) remaining after 3 min (84.6% repellency). On average Permanone provided >84% protection for 7 d. Carvacrol-treated coveralls yielded a total of 20 (mean = 1.7 ± 0.5 ticks per transect) *A. americanum* adults 1 d after treatment, with 14 (mean = 1.2 ± 0.4 ticks per transect) remaining after 3 min (53.8% repellency). Carvacrol provided poor protection (<68.6% repellency) against *A. americanum* through 7 d (Table 2).

With the exception of nootkatone-treated coveralls, there was no significant difference between treatments in daily mean numbers of *I. scapularis* adults retained on treated coveralls through 3 d (Table 1, within column Mann–Whitney U test comparisons, Bonferroni-corrected $\alpha = 0.017$), whereas mean *A. americanum* adults retained was greater on carvacrol-treated coveralls than on all other treatments ($\alpha = 0.008$). After 7 d, nootkatone was the most effective repellent against both species followed in order of activity by Permanone, EcoSMART, and carvacrol.

Discussion

Results of these field trials suggest that nootkatone and carvacrol that have shown significant repellency against nymphal *I. scapularis* in the laboratory (Dietrich et al. 2006) also may have considerable potential as clothing repellents for field applications against both *I. scapularis* and *A. americanum.* Nootkatone applied to coveralls provided very good protection against host-seeking ticks and was at least as effective against *A. americanum* adults as against *I. scapularis* adults through 3 d. We expected that the two tick species would respond differently to repellents and that the more aggressive *A. americanum* would exhibit greater resistance to the repellents tested (Flor-Weiler et al. 2011). Although Schreck et al. (1995) suggested that nymphal *I. scapularis* were less sensitive than *A. americanum* nymphs to 11 of 29 compounds (including DEET) tested, subsequent studies have shown that *A. americanum* is substantially more tolerant of DEET and terpenoids than *I. scapularis* in laboratory trials (Carroll et al. 2004, 2007; Zhang et al. 2009). However, nootkatone seems to offer effective repellency against *A. americanum* adults for 7 d as applied to the fabric used here. Carvacrol provided good initial (2-d) protection against *I. scapularis,* but repellency declined substantially after 2 d and carvacrol provided little protection overall against *A. americanum* adults.

Both natural compounds performed well initially in comparison to the two commercial products. The EcoSMART product used here is marketed as a skin repellent and may perform differently and more effectively as such. Rather than work as a repellent, permethrin's (the active ingredient in Permanone) predominant effect is as a contact toxicant and true repellency as distinct from acaricidal activity may be short-lived (Lane and Anderson 1984), which may account for the inconsistent results we observed. Neither nootkatone nor carvacrol was formulated beyond solution in alcohol for application to coverall fabric and, consequently, were applied at higher concentration of active ingredient than the commercial products, which may have contributed to observed differences in repellency. Proper formulation of repellents can substantially increase effectiveness against tick sensory receptors, their duration of activity, and human acceptance and perception of safety (Bissinger and Roe 2010). Salafsky et al. (2000) showed that effectiveness of DEET varied with formulation and Faulde et al. (2003) showed that different methods of impregnation affected the repellent performance of permethrin on clothing.

In addition, the outcome and interpretation of repellent bioassays can be influenced by environmental factors (including light, temperature, and humidity) and their interaction with repellent loss through evaporation and abrasion (Barnard 2005). We attempted to control for environmental effects by replicating trials under the same conditions, but essential oils and terpenes are highly volatile (Connolly and Hill 1991) and the very low vapor pressure of carvacrol, without any additional formulation (Karlsen 2010) may account for its rapid loss in effectiveness through evaporation. We have found that coveralls treated as described here and kept sealed in Ziploc bags provided 91.2% (EcoSMART), 96.5% (Permanone), 100% (nootkatone), and 84.2% (carvacrol) repellency after 5 mo (R.A.J., unpublished data). Any future formulation of these natural products should provide sufficient vapor concentration to be repellent, whereas not evaporating so rapidly that they lose effectiveness (Rueda et al. 1998).

The relative responses of the two tick species to the repellents tested here also may have reflected their seasonal activity patterns. It is likely that large numbers of *I. scapularis* adults questing in April may have been seeking hosts since the previous October–November, and because they are chronologically and physiologically old, may be less vigorous than newly molted adults (Schulze et al. 1986). Similarly, April is the beginning of the seasonal activity period for *A. americanum* adults in our study area and these warmer weather ticks may have been less aggressive than they typically are later in the season. Consequently, the relative questing vigor of the two species may have affected our results. Although we conducted these trials to assess repellent efficacy against *I. scapularis* and *A. americanum* when both were active and potentially encountering human hosts (Armstrong et al. 2001, Schulze et al. 2001), similar trials conducted during different seasons might be fruitfully explored to more fully characterize the responses of the two species to chemical repellents.

These results suggest that nootkatone has considerable potential as a clothing repellent against both *I. scapularis* and *A. americanum.* In a case-controlled study of personal protection measures, Vázquez et al. (2008) demonstrated that the use of protective clothing was more effective at reducing acquisition of Lyme disease than either use of repellents or routine tick checks. Wearing protective clothing, especially when augmented with repellents, is effective in reducing human exposure to ticks and disease transmission when avoidance of tick habitat is unrealistic. Public health professionals should continue to stress the use of proper attire in conjunction with personal repellents, when going into tick habitat.

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