Ability of Two Natural Products, Nootkatone and Carvacrol, to Suppress *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) in a Lyme Disease Endemic Area of New Jersey

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ABSTRACT We evaluated the ability of the natural, plant-derived acaricides nootkatone and carvacrol to suppress *Ixodes scapularis* Say and *Amblyomma americanum* (L.) (Acari: Ixodidae). Aqueous formulations of 1 and 5% nootkatone applied by backpack sprayer to the forest litter layer completely suppressed *I. scapularis* nymphs through 2 d. Thereafter, the level of reduction gradually declined to ≤50% at 28 d postapplication. Against *A. americanum* nymphs, 1% nootkatone was less effective, but at a 5% concentration, the level of control was similar or greater to that observed with *I. scapularis* through 21 d postapplication. Initial applications of 0.05% carvacrol were ineffective, but a 5% carvacrol formulation completely suppressed nymphs of both species through 2 d and resulted in significant reduction in *I. scapularis* and *A. americanum* nymphs through 28 and 14 d postapplication, respectively. Backpack sprayer applications of 5% nootkatone to the shrub and litter layers resulted in 100% control of *I. scapularis* adults through 6 d, but the level of reduction declined to 71.5% at 28 d postapplication. By contrast, high-pressure applications of 2% nootkatone to the litter layer resulted in 96.2–100% suppression of both *I. scapularis* and *A. americanum* nymphs through 42 d, whereas much lower control was obtained from the same formulation applied by backpack sprayer. Backpack sprayer application of a 3.1% nootkatone nanoemulsion resulted in 97.5–98.9 and 99.3–100% reduction in *I. scapularis* and *A. americanum* nymphs, respectively, at 1 d postapplication. Between 7 d and 35 d postapplication, the level of control varied between 57.1% and 92.5% for *I. scapularis* and between 78.5 and 97.1% for *A. americanum* nymphs. The ability of natural products to quickly suppress and maintain significant control of populations of these medically important ticks at relatively low concentrations may represent a future alternative to the use of conventional synthetic acaricides.

KEY WORDS *Ixodes scapularis*, *Amblyomma americanum*, nootkatone, carvacrol, tick control

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 27,699 in 2007 (CDC 2008). The nymphal stage of the blacklegged tick, *Ixodes scapularis* Say, serves as the principal vector of *B. burgdorferi* in the Northeast and also transmits the causative agents of human babesiosis and human granulocytic anaplasmosis (Piesman et al. 1987, Lane et al. 1991, 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). Developing strategies that are effective and environmentally acceptable for the control of these vector ticks has become an important public health issue (Stafford and Kitron 2002, Hayes and Piesman 2003, Dolan et al. 2004).

Various approaches have been attempted to reduce disease incidence by limiting human exposure to infected ticks. In evaluating the effectiveness of educational interventions, Hallman et al. (1995) reported that although 84% of participants were aware of at

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Table 1. Formulation summary for applications of the selected natural products nootkatone and carvacrol (2006–2008)

Yr	Location	Stage	No. plots treated/control	Application/100 m^2			
				Formulation	% (AI)[a] g/m^2	Diluent (liters)	Method
2006	NWSE[b]	Nymph	2/2	Nootkatone 75 g d-Limonene 37.5 g EZ-Mulse 37.5 g	1.0/0.75	7.57	Backpack
		Nymph	2/2	Carvacrol 3.785 g Calamide C 1.9 g	0.05/0.038	7.57	Backpack
		Adult	2/2	Nootkatone 378 g d-Limonene 189 g EZ-Mulse 189 g	5.0/3.78	11.36	Backpack
2007	NWSE	Nymph	5/5	Nootkatone 568 g EZ-Mulse 284 g	5.0/3.94	15.14	Backpack
		Nymph	5/5	Carvacrol 568 g Calamide C 568 g HM-9114 Soy Vegetable oil 568 g	5.0/3.94	15.14	Backpack
2008	NWSE	Nymph	5/5[c]	Nootkatone 152 g d-Limonene 75 g EZ-Mulse 75 g	2.0/0.76	15.14	Backpack
		Nymph	5/5[d]	Nootkatone 152 g d-Limonene 75 g EZ-Mulse 75 g	2.0/0.76	45.42	High Pressure
	PLP[c]	Nymph	5/5	Nootkatone 237 g Corn oil 369 g Transcutol 863 g Cremophor El 811 g	3.1/1.19	7.57	Backpack
		Nymph	5/5	Nootkatone 237 g Corn oil 369 g Transcutol 863 g Cremophor EL 811 g	3.1/1.19	15.14	Backpack

[a] Refers to percentage of active ingredient in mixture only, before diluent being added.
[b] NWSE, Naval Weapons Station Earle, Coltsneck, NJ.
[c] PLP, Perrineville Lake Park, Perrineville, NJ.
[d] Treated and control plots were divided into 100-m^2 core and 100-m^2 edge plots.

least one precaution to reduce exposure to ticks, only 43% reported taking any precaution. Therefore, actual reduction of the tick population may be more effective in mitigating transmission risk (Hayes et al. 1999, Hayes and Piesman 2003, Piesman and Eisen 2008). Conventional chemical control directed against host-seeking ticks has proven to be the most reliable means of suppressing tick populations (Stafford and Kitron 2002; Schulze et al. 2005a, 2008). The broad-scale application of area-wide pesticides, however, is not widely accepted due to growing public concerns about adverse environmental effects (Ginsberg 1994, Schmidtmann 1994, Schulze et al. 2001b, Gould et al. 2008). Community surveys conducted in four northeastern states (Connecticut, Massachusetts, New Jersey, and New York) revealed that <25% of residents have used acaricides on their properties to control ticks (Piesman 2006, Schulze et al. 2007). In contrast, a majority of residents claimed they would consider controlling ticks if alternatives to conventional chemical control were available.

Botanical acaricides may provide one such alternative. Laboratory bioassays using steam-distilled extracts from Alaska yellow cedar (AYC), *Chamaecyparis nootkatensis* (D. Don) Spach, were found to be biocidally active against subadult *I. scapularis* (Panella et al. 1997). In a subsequent study, Panella et al. (2005) isolated the individual compounds that comprise the essential oil of AYC and discovered many of the compounds, including nootkatone and carvacrol, had acaricidal activity >10 times that of the essential oil. The current study presents the results of field trials conducted during 2006–2008 to test the efficacy of several formulations of the plant-derived acaricides nootkatone and carvacrol against *I. scapularis* and *A. americanum*.

Materials and Methods

Site Description. Naval Weapons Station (NWS) Earle, a military facility located in central Monmouth County, NJ, served as the treatment site for four trials conducted between 2006 and 2008. In 2008, a fifth trial was conducted at Perrineville Lake Park, located in western Monmouth County, NJ. Sympatric populations of *I. scapularis* and *A. americanum* exist in both locations (Schulze et al. 1997, 2005b, 2007). The forest canopy at the NWS Earle site used for trials against nymphs is dominated by pitch pine (*Pinus rigida* Mill.), white oak (*Quercus alba* L.), red oak (*Quercus 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, greenbrier (*Smilax rotunifolia* L.), highbush blueberry (*Vaccinium corymbosum* L.), and huckle berries (*Gaylussacia* spp.). The site used in the trial against *I. scapularis* adults is an early successional forest with a sparse canopy consisting of black cherry (*Prunus se-*

rotina Ehrh.), eastern redcedar (*Juniperus virginiana* L.), sassafras (*Sassafras albidum* [Nuttall] Nees), and white oak. The shrub layer is dominated by greenbrier and saplings and seedlings of the canopy species, along with patches of highbush blueberry, huckle berries, and grasses. The Perrineville Lake Park site is a mid-successional forest with a mixed canopy including black cherry, sassafras, black locust (*Robinia pseudoacacia* L.), red maple (*Acer rubrum* L.), and sweetgum (*Liquidambar styraciflua* L.). The sparse understory and shrub layer is comprised of seedlings and saplings of the dominant canopy species and greenbrier.

Acaricide Applications. Selected formulations of nootkatone were applied during each of the 3 yr of the study, whereas carvacrol was only applied in 2006 and 2007. In 2006 and 2008, 98.5% nootkatone crystals (Frutarom, North Bergen, NJ) were dissolved in 93% d-limonene, an all natural, biodegradable solvent extracted from orange peels (Florida Chemical Co., Winter Haven, FL) and added to the spray tank containing water and 95% EZ-Mulse, a proprietary blend of nonionic surfactants used to emulsify terpene-specific compounds, citrus extracts, and natural oils (Florida Chemical Co., Winter Haven, FL). The solution was thoroughly mixed and immediately applied. In 2007, d-limonene was not included in the formulation (Table 1).

In 2008, a "nanoemulsion" formulation containing nootkatone and food grade corn oil (King Soopers Kroeger, Fort Collins, CO) was developed by Legacy BioDesign, LLC (Loveland, CO) by using a 1:1 ratio of the surfactants transcutol (also known as carbitol or diethylene glycol monoethyl ether) (batch 18703CEO, Sigma-Aldrich, St. Louis, MO) and cremephor EL (Polyoxyl 35 Castor Oil ([USP/NF]) (batch 037L0213, Sigma-Aldrich) mixed in a 1:5 (corn oil/cosurfactant) ratio (Shafiq-un-Nabi et al. 2007). The resulting nanoemulsion facilitates dissolving a poorly water-soluble compound in an oil phase and then distributing that oil throughout the vehicle.

The carvacrol formulations consisted of 95% carvacrol (TCI America, Portland, OR) and 92% Calamide C, prepared from highly refined coconut oil used as an emulsifier, detergent, and stabilizer (Pilot Chemical Co., Red Bank, NJ). For all applications, the premix was added to water in the spray tank, mixed, and immediately applied.

In 2006 and 2007, all nootkatone and carvacrol applications were made using SP2 Knapsack Sprayers (SP Systems, Santa Monica, CA). In 2008, half of the nootkatone-treated plots at NWS Earle were treated using SP2 sprayers (maximum 80 psi) and half were treated using a truck-mounted, high-pressure (maximum, 600 psi) sprayer. All nanoemulsion plots at Perrineville Lake Park were treated using SP2 sprayers. Applications directed against nymphs at NWS Earle were made on 31 May 2006, 30 May 2007, and 2 June 2008, whereas the application at Perrineville Lake Park was conducted on 1 June 2008. The application against *I. scapularis* adults was performed on 1 No-

Table 2. Mean ± SD number of *I. scapularis* and *A. americanum* nymphs collected at 100 m^2 treatment and control plots at NWS Earle, Colts Neck, NJ, May–June 2006, before and after application of nootkatone and carvacrol

Nootkatone (1.0%)								
Treatment[a]	Pretreatment[b]	Days posttreatment[c]						Kruskal-Wallis test[d]
		1 d	2 d	10 d	14 d	21 d	28 d	
I. scapularis								
Untreated	6.7 ± 1.4d	13.5 ± 9.2	11.5 ± 6.4	10.5 ± 2.1	11.0 ± 4.2	9.0 ± 4.2	8.5 ± 10.6	$H = 5.07$; df = 6, 18; $P = 0.54$
Nootkatone (1.0%)	8.0 ± 1.5a	0c (100%)[e]	0c (100%)	1.5 ± 0.7c (88.0%)	3.0 ± 2.8b,c (77.2%)	4.0 ± 0.0b (62.7%)	6.0 ± 4.2b (40.9%)	$H = 14.41$; df = 6, 18; $P < 0.03$
Carvacrol (0.05%)	7.0 ± 1.5	6.5 ± 3.5 (53.9%)	12.0 ± 9.0	12.0 ± 1.4	11.0 ± 4.2	8.5 ± 4.9	11.0 ± 4.2	$H = 4.88$; df = 6, 18; $P = 0.56$
A. americanum								
Untreated	10.8 ± 9.8	18.5 ± 0.7	16.0 ± 9.9	9.0 ± 0.0	14.0 ± 12.7	14.5 ± 7.8	14.5 ± 2.1	$H = 3.24$; df = 6, 18; $P = 0.78$
Nootkatone (1.0%)	32.0 ± 33.6	6.5 ± 6.4 (88.1%)[e]	10.0 ± 5.6 (78.9%)	48.5 ± 61.5	89.5 ± 103.9	76.5 ± 77.1	83.5 ± 85.6	$H = 8.02$; df = 6, 18; $P = 0.24$
Carvacrol (0.05%)	8.0 ± 4.6	33.0 ± 19.8	15.5 ± 4.9	19.0 ± 5.7	23.5 ± 14.8	38.5 ± 19.1	50.0 ± 26.8	$H = 12.77$; df = 6, 18; $P < 0.05$

[a] Sampling of $n = 2$ plots per treatment.

[b] Mean ± SD of three sample dates; numbers of host-seeking *I. scapularis* and *A. americanum* nymphs did not differ between treated and untreated plots before acaricide application (Kruskal-Wallis tests: $H = 2.46$; df = 2, 18; $P = 0.29$ and $H = 3.07$, df = 2, 18; $P = 0.22$, respectively.

[c] Posttreatment means for acaricide-treated and control plots based on $n = 2$ for each date.

[d] Means in the same row followed by the same letter are not significantly different (Kruskal-Wallis test).

[e] Percentage of control, after Henderson's equation: $100 - (T/U \times 100)$, where T and U are the mean after treatment/mean before treatment in treated plots and untreated plots, respectively.

Table 3. Number of questing *I. scapularis* adults (mean ± SD) collected at treatment and control plots (n = 2 each) at NWS Earle, Colts Neck, NJ, before and after nootkatone application October–November 2006

Location	Pretreatment[a]	Days posttreatment						Kruskal–Wallis test[b]
		1 d	2 d	6 d	15 d	21 d	28 d	
Untreated	11.7 ± 1.9	12.5 ± 0.7	11.5 ± 2.1	11.0 ± 0.0	17.0 ± 1.4	4.0 ± 1.4	15.5 ± 7.8	H = 9.52; df = 8, 18; P = 0.30
Nootkatone (5.0%)	9.3 ± 3.9a	0c (100%)[c]	0c (100%)	0c (100%)	4.0 ± 1.4b (70.4%)	1.0b,c (68.5%)	3.5 ± 3.5b (71.5%)	H = 15.50, df = 8, 18; P < 0.05

[a] Plots were sampled 3 times: 19–25 October 2006. Pretreatment adult abundance not significantly different between control and treatment plots (Mann–Whitney test: U = 13.00; df = 6, 6; P = 0.42).
[b] Percentage of control (modified Henderson's equation).
[c] Means in the same row followed by the same letter are not significantly different (Kruskal–Wallis test).

vember 2006. The exact specifications for each application are summarized in Table 1.

Experimental Design. The experimental design for the initial 2006 pilot study incorporated two 100-m^2 (10 by 10 m) plots spaced ≥30 m apart each for nootkatone and carvacrol with paired controls. After reviewing the results of the 2006 trials, we increased the amount of active ingredient (AI) and the number of paired treatment and untreated plots for both formulations in 2007. However, despite increases in active ingredient, the 2007 results continued to show a lack of residual control of host-seeking ticks, suggesting either recolonizing of sprayed plots from surrounding areas or failure to adequately treat the litter column. Although *I. scapularis* does not seem to exhibit significant lateral movement (Falco and Fish 1991, Goddard 1993), the more aggressive *A. americanum* is known to move substantial distances in response to certain environmental cues (Schulze et al. 1997, 2001a). We monitored the movement of ticks into treated plots by increasing the size of the plots at NWS Earle from 100 to 200 m^2 (14.14 by 14.14 m) in 2008. The centrally located 100-m^2 core and 100-m^2 border plots were sampled separately. To more effectively treat ticks that are not questing and may be sequestered lower in the leaf litter at the time of application, we used two approaches to enhance penetration of the litter column. At NWS Earle, we compared backpack spray applications with applications made using a truck-mounted high-pressure sprayer, which required three times the amount of diluent to achieve full coverage of the plots. At Pèrrineville Lake Park, we used only backpack sprayers, but doubled the amount of diluent in half the plots, theorizing that the extra volume would help to more fully saturate the litter column (Table 1).

Tick Sampling. Plots were treated and sampled during the peak activity periods of *I. scapularis* and *A. americanum* nymphs and *I. scapularis* adults (Schulze et al. 1986). Weather conditions permitting, plots were scheduled to be sampled three times before each application, at 24 and 48 h postapplication, and at weekly intervals thereafter in all years. Plots were sampled using a combination of dragging and walking survey techniques conducted by the same individuals between 0800 and 1200 hours on days when vegetation was dry and wind speed was <10 kph (Ginsberg and Ewing 1989, Schulze et al. 1997). The entire surface of each plot was sampled each time. Drags and coveralls were examined at 10-m intervals, and all ticks adhering to drags and coveralls were counted and returned to their respective plots. Rare samples of *Dermacentor variabilis* Say were not included in the analysis.

Statistical Analysis. Pretreatment comparison of host-seeking *I. scapularis* and *A. americanum* abundance between treatment and control plots was made using either Mann–Whitney U tests or Kruskal–Wallis tests (Sokal and Rohlf 1981). Separate Kruskal–Wallis tests were used to compare tick abundance between postapplication sampling dates for treatment and control plots. An algebraic variation of Henderson's method was used to calculate percentage of control of ticks on acaricide-treated plots: $100 - (T/U \times 100)$, where T and U are the mean after treatment/mean before treatment in treated plots and untreated plots, respectively (Henderson and Tilton 1955, Mount et al. 1976). All statistical tests were performed using STATISTICA analysis packages (StatSoft 2005).

Results

2006 Trials. Numbers of host-seeking *I. scapularis* and *A. americanum* nymphs did not differ between treated and untreated plots before acaricide application (Kruskal–Wallis tests: H = 2.46; df = 2, 18; P = 0.29 and H = 3.07; df = 2, 18; P = 0.22, respectively) (Table 2). Application of a 1% (AI) formulation of nootkatone applied to forest leaf litter against *I. scapularis* nymphs resulted in 100% control, relative to untreated plots, after 2 d and 88% control after 10 d. Observed control declined steadily thereafter as numbers of questing nymphs began to decline in the untreated plots. The same application achieved 88.1% control of *A. americanum* nymphs after 1 d and provided no suppression of host-seeking *A. americanum* nymphs after 2 d. An application of 0.05% (AI) carvacrol seemed to have little or no suppressive effect against either *I. scapularis* or *A. americanum* nymphs.

Pretreatment abundance of *I. scapularis* adults was not significantly different between control and treatment plots (Mann–Whitney test: U = 13.00; df = 6, 6; P = 0.42) (Table 3). Application of 5% (AI) formulation of nootkatone applied to forest shrub layer against host-seeking *I. scapularis* adults in November seemed to provide 100% control for 7 d and control exceeded 68% for 28 d.

2007 Trials. Numbers of host-seeking *I. scapularis* nymphs did not differ between treated and untreated plots before acaricide application (Kruskal–Wallis tests: $H = 3.77$; df = 2, 45; $P = 0.15$). However, there were significantly fewer *A. americanum* nymphs at the carvacrol plots before applications ($H = 9.48$; df = 2, 45; $P < 0.01$) (Table 4). Application of a 5% (AI) formulation of nootkatone against *I. scapularis* nymphs resulted in 100% control, relative to untreated plots, after 2 d. Observed control fell to 79.2% after 14 d and declined steadily as numbers of host-seeking nymphs in the treated plots did not differ from pretreatment abundance after 21 d. The effect of nootkatone applications seemed to be more successful against *A. americanum* nymphs, achieving 100% control after 2 d and >95% control after 14 d (Table 4). Nymphal abundance rebounded to twice the pretreatment levels after 28 d. Application of a 5% formulation of carvacrol also provided 100% control of *I. scapularis* nymphs after 2 d, with >85% control provided after 21 d. Numbers of host-seeking nymphs remained reduced relative to pretreatment abundance after 28 d. The carvacrol application provided 100% control of *A. americanum* nymphs after 2 d (Table 4). Numbers of host-seeking *A. americanum* seemed to remain reduced after 14 d but recovered to numbers exceeding pretreatment levels after 21 d.

2008 Trials. At NWS Earle, we found no significant difference in abundance of host-seeking *I. scapularis* ($H = 2.17$; df = 2, 60; $P = 0.34$) or *A. americanum* ($H = 0.39$; df = 2, 60; $P = 0.82$) nymphs between treatments before acaricide application (Table 5). Application of a 2% (AI) formulation of nootkatone to forest leaf litter by using backpack sprayers resulted in 100% control of *I. scapularis* in core areas of treated plots after 1 d, but the level of control dropped to 82.1% after 7 d, rebounding somewhat to 84.5% after 14 d. Trends in levels of control seemed to be similar to those observed in 2007. In contrast, backpack sprayer-applied nootkatone resulted in 96.6% control of *A. americanum* nymphs in core areas of treated plots after 1 d, but observed levels of control dropped to 80.8% after 7 d and 53.3% after 14 d (Table 6). Because of an increase in numbers of *A. americanum* nymphs in the untreated plots, there seemed to be a rebound in control after 21 d, but levels remained <50% after 28 d.

The application of the same 2% (AI) formulation of nootkatone by high-pressure truck-mounted sprayer provided substantially better control of both species over a much longer period. After 1 d, we collected no *I. scapularis* nymphs from high-pressure sprayer-treated plots and control of *I. scapularis* nymphs remained between 98.1 and 100% at 42 d postapplication (Table 5). Similar results were observed in the control of *A. americanum*, with 100% control observed after 1 d and control ranging between 96.2 and 100% throughout the postapplication study period (Table 6).

We found no significant difference in tick numbers between the core and border areas at either the backpack or high-pressure sprayer-treated plots at any time during the trials. It seemed that movement of ticks

Table 4. Mean ± SD number of *I. scapularis* and *A. americanum* nymphs collected at 100 m^2 treatment and control plots at NWS Earle, Colts Neck, NJ, May–June 2007, before and after application of nootkatone and carvacrol

Treatment	Pretreatment[a]	Day posttreatment					Kruskal-Wallis test[b]
		1 d	2 d	7 d	14 d	21 d	28 d	
I. scapularis								
Untreated	4.9 ± 3.0a	7.4 ± 1.8b	7.4 ± 1.1b	9.0 ± 3.1b	9.8 ± 1.9b	11.8 ± 3.6c	8.4 ± 1.5b	$H = 19.16$; df = 6, 45; $P < 0.01$
Nootkatone (5.0%)	7.7 ± 3.9a	0b (100%)	0b (100%)	3.8 ± 2.6c (73.1%)	3.2 ± 2.4c (79.2%)	5.2 ± 4.9a,c (71.9%)	6.6 ± 4.2a,c (50%)	$H = 26.74$; df = 6, 45; $P < 0.01$
Carvacrol (5.0%)	6.3 ± 2.8a	0b (100%)[c]	0b (100%)	2.0 ± 1.0c (82.7%)	2.0 ± 1.4c (84.1%)	2.2 ± 2.3c (85.5%)	2.4 ± 2.8c (77.8%)	$H = 30.93$; df = 6, 45; $P < 0.01$
A. americanum								
Untreated	6.3 ± 3.6a	7.8 ± 3.7a	11.0 ± 9.9b	12.2 ± 5.9b	24.8 ± 17.1c	33.8 ± 38.8d	22.8 ± 22.2c	$H = 15.54$; df = 6, 45; $P = 0.02$
Nootkatone (5.0%)	8.5 ± 10.9a	0b (100%)	0b (100%)	1.4 ± 3.1b (91.5%)	1.6 ± 1.1b (95.2%)	7.8 ± 9.1a (82.9%)	16.0 ± 23.6c (47.9%)	$H = 27.91$; df = 6, 45; $P < 0.01$
Carvacrol (5.0%)	2.7 ± 1.9a	0b (100%)[c]	0b (100%)	1.8 ± 1.1b (65.6%)	1.0 ± 1.2b (90.6%)	5.0 ± 3.5c (65.5%)	6.0 ± 3.2c (38.6%)	$H = 25.41$; df = 6, 45; $P < 0.01$

Plots/100-m^2 plots, five plots per treatment.

[a] Mean ± SD of three sample dates; Numbers of host-seeking *I. scapularis* nymphs did not differ between treated and untreated plots before acaricide application (Kruskal–Wallis test: $H = 3.77$; df = 2, 45; $P = 0.15$). However, numbers of *A. americanum* nymphs were significantly less at the carvacrol plots before applications ($H = 9.48$; df = 2, 45; $P = 0.01$).

[b] Means in the same row followed by the same letter are not significantly different (Kruskal–Wallis test).

[c] Percentage of control, after Henderson's equation: $100 - (T/U \times 100)$, where T and U are the mean after treatment/mean before treatment in treated and untreated plots, respectively.

Table 5. Mean ± SD number of *I. scapularis* nymphs collected at treated and control plots at NWS Earle, Colts Neck, NJ, May–June 2008 before and after application of nootkatone (2.0%) by using backpack or high-pressure sprayers

Treatment	Pretreatment[a]		Days posttreatment					
			1 d		7 d		14 d	
	Core	Border	Core	Border	Core	Border	Core	Border
Untreated	9.4 ± 5.9	8.5 ± 2.8	14.2 ± 9.5	8.6 ± 3.8	21.6 ± 12.0	14.4 ± 7.2	20.2 ± 7.6	17.4 ± 7.7
Backpack	7.8 ± 3.3a	7.9 ± 3.3a	0b (100%)[c]	0.4 ± 0.9b (94.9%)	3.2 ± 2.2a,b (82.1%)	1.4 ± 0.9a,b (89.5%)	2.6 ± 1.5a,b (84.5%)	3.4 ± 2.1a,b (78.9%)
High-Pressure	10.8 ± 4.6a,b	9.1 ± 3.8a,b	0c (100%)	0c (100%)	0.4 ± 0.9b,c (98.4%)	1.2 ± 1.8a,b,c (92.2%)	0.4 ± 0.9b,c (98.3%)	0.4 ± 0.9b,c (97.8%)

21 d		28 d		35 d		42 d		Kruskal–Wallis test[b]
Core	Border	Core	Border	Core	Border	Core	Border	
14.8 ± 6.9	18.6 ± 10.1	14.8 ± 5.5	12.6 ± 7.5	9.4 ± 3.0	11.2 ± 6.1	6.4 ± 3.6 a	4.8 ± 3.9a	$H = 31.33$; df = 15, 90; $P < 0.01$
5.8 ± 3.7a (52.8%)	4.6 ± 3.4a,b (73.4%)	4.8 ± 5.3a,b (60.9%)	4.6 ± 2.6a,b (60.7%)	4.6 ± 1.9a,b (41.0%)	4.0 ± 3.9a,b (61.6%)	1.2 ± 1.1a,b (77.4%)	2.2 ± 1.9a,b (59.1%)	$H = 47.26$; df = 15, 90; $P < 0.01$
0c (100%)	0c (100%)	0c (100%)	1.0 ± 0.7a,b,c (92.6%)	0.2 ± 0.4b,c (98.1%)	1.4 ± 0.9a,b,c (88.3%)	0c (100%)	0.6 ± 0.9a,b,c (88.3%)	$H = 71.38$; df = 15, 90; $P < 0.01$

Plots/200-m^2 (includes 100-m^2 Core area surrounded by separately sampled 100-m^2 border area). There are five randomly assigned plots per treatment.

[a] Mean ± SD of two sample dates; no significant difference between treatments ($H = 2.17$; df = 2, 60; $P = 0.34$) before applications.

[b] Means in the same row followed by the same letter are not significantly different (Kruskal–Wallis test).

[c] Percent control, after Henderson's equation: $100 - (T/U \times 100)$, where T and U are the mean after treatment/mean before treatment in treated plots and untreated plots, respectively.

from surrounding areas played no appreciable part in the decline in observed levels of control of either tick species in backpack sprayer-treated plots (Tables 5 and 6).

In 2008 at Perrineville Lake Park, there was no significant difference in abundance of host-seeking *I. scapularis* ($H = 1.74$; df = 2, 30; $P = 0.42$) or *A. americanum* ($H = 1.91$; df = 2, 30; $P = 0.38$) nymphs between treatments before acaricide application (Table 7). Application of the 3.1% nootkatone nanoemulsion formulation, made using 7.57 and 15.14 liters of water diluent produced 98.9 and 97.5% control of host-seeking *I. scapularis* nymphs, respectively, after 1 d (Table 7). Control declined substantially after 7 d. There seemed to be no difference in efficacy against *I. scapularis* nymphs between

Table 6. Mean ± SD number of *A. americanum* nymphs collected at treated and control plots at NWS Earle, Colts Neck, NJ, May–June 2008, before and after application of nootkatone (2.0%) by using backpack or high-pressure sprayers

Treatment	Pretreatment[a]		Days postteatment					
			1 d		7 d		14 d	
	Core	Border	Core	Border	Core	Border	Core	Border
Untreated	22.6 ± 33.8	29.6 ± 42.0	19.0 ± 24.0	34.2 ± 42.4	69.0 ± 81.8	44.6 ± 54.9	31.2 ± 37.3	29.2 ± 43.7
Backpack	21.1 ± 27.3a	17.6 ± 22.4a	0.6 ± 1.3b (96.6%)[c]	2.4 ± 2.9a,b (88.2%)	12.4 ± 12.5a (80.8%)	17.0 ± 19.5a (35.9%)	13.6 ± 16.1a (53.3%)	8.6 ± 10.5a,b (50.5%)
High-Pressure	22.6 ± 24.9a	16.2 ± 19.4a,b	0c (100%)	0.2 ± 0.4b,c (98.9%)	1.2 ± 2.7b,c (98.3%)	0.6 ± 0.9b,c (97.5%)	1.2 ± 1.6b,c (96.2%)	0.4 ± 0.9b,c (97.5%)

21 d		28 d		35 d		42 d		Kruskal–Wallis test[b]
Core	Border	Core	Border	Core	Border	Core	Border	
56.4 ± 57.6	72.2 ± 76.8	54.6 ± 66.6	58.4 ± 47.3	47.0 ± 37.2	60.6 ± 47.9	44.8 ± 49.4	48.0 ± 44.9	$H = 16.86$; df = 15, 90; $P = 0.33$
22.0 ± 23.9 (67.5%)	41.2 ± 50.3 (4.0%)	28.2 ± 33.7 (44.6%)	48.8 ± 62.2 (0%)	27.8 ± 31.9 (36.6%)	41.0 ± 49.7 (0%)	21.4 ± 27.2 (48.8%)	40.0 ± 44.1 (0%)	$H = 20.03$; df = 15, 90; $P = 0.17$
0c (100%)	1.4 ± 2.1b,c (96.5%)	0.4 ± 0.5b,c (99.3%)	1.8 ± 1.3b,c (94.4%)	0.2 ± 0.4b,c (99.6%)	2.8 ± 3.6b,c (91.6%)	0c (100%)	2.2 ± 2.9b,c (91.6%)	$H = 62.04$; df = 15, 90; $P < 0.01$

Plots/200-m^2 (includes 100-m^2 core area surrounded by separately sampled 100-m^2 border area). There are five randomly assigned plots per treatment.

[a] Mean ± SD of two sample dates; no significant difference between treatments before applications: $H = 0.39$; df = 2, 60; $P = 0.82$.

[b] Means in the same row followed by the same letter are not significantly different (Kruskal–Wallis test).

[c] Percentage of control, after Henderson's equation: $100 - (T/U \times 100)$, where T and U are the mean after treatment/mean before treatment in treated plots and untreated plots, respectively.

Table 7. Mean ± SD number of *I. scapularis* and *A. americanum* nymphs collected at 100 m^2 treated and untreated plots at Perrineville Lake Park, Millstone Township, NJ, May–June 2008, after application of nootkatone (3.1% nanoemulsion) by using a backpack sprayer

Treatment	Pretreatment[a]	Days posttreatment							Kruskal–Wallis test[b]
		1 d	7 d	14 d	21 d	28 d	35 d	42 d	
I. scapularis									
Untreated	15.8 ± 4.7	19.2 ± 10.3	29.6 ± 14.8	18.0 ± 8.1	12.2 ± 3.5	15.6 ± 7.9	12.8 ± 6.5	4.2 ± 2.9	$H = 20.03$; df = 7, 45; $P = 0.09$
1X diluent	14.8 ± 5.2a	0.2 ± 0.4b (98.9%)[c]	5.8 ± 1.8a,b (79.1%)	7.0 ± 2.7a,b (58.5%)	3.2 ± 1.5a,b (71.9%)	2.2 ± 1.1a,b (84.9%)	2.6 ± 0.9a,b (78.3%)	3.6 ± 2.9a,b (8.5%)	$H = 36.29$; df = 7, 45; $P < 0.01$
2X diluent	13.1 ± 3.2a	0.4 ± 0.5b (97.5%)	4.0 ± 2.0a,b (83.7%)	6.4 ± 2.9a,b (57.1%)	3.0 ± 1.8a,b (70.3%)	1.8 ± 0.8b (86.1%)	0.8 ± 1.3b (92.5%)	2.0 ± 0.7b (42.6%)	$H = 35.90$; df = 7, 45; $P < 0.01$
A. americanum									
Untreated	6.5 ± 4.5a	21.4 ± 9.7a,b	42.2 ± 19.6b	23.6 ± 18.7a,b	22.0 ± 16.8a,b	21.1 ± 16.3a,b	25.0 ± 19.8a,b	22.8 ± 14.4a,b	$H = 17.47$; df = 7, 45; $P < 0.01$
1X diluent	6.4 ± 2.5a	0b,c (100%)	1.2 ± 0.4a,b,c (97.1%)	5.0 ± 3.5a,b (78.5%)	3.4 ± 1.5a,b (84.3%)	1.0 ± 1.2b,c (95.2%)	3.4 ± 1.5a,b (86.2%)	9.4 ± 5.8a (58.1%)[5]	$H = 32.42$; df = 7, 45; $P < 0.01$
2X diluent	9.2 ± 5.5a,b	0.2 ± 0.4b (99.3%)	2.0 ± 2.4a,b (96.6%)	5.2 ± 5.1a,b (84.4%)	3.6 ± 4.2a,b (88.4%)	2.6 ± 3.6a,b (91.3%)	4.8 ± 4.1a,b (86.4%)	10.2 ± 4.3a,b (68.4%)	$H = 23.71$; df = 7, 45; $P < 0.01$

[a] Mean ± SD of two sample dates; no significant difference between treatments prior to applications for *I. scapularis* ($H = 1.74$; df = 2, 30; $P = 0.42$) or *A. americanum* ($H = 1.91$; df = 2, 30; $P = 0.38$).
[b] Means in the same row followed by the same letter are not significantly different (Kruskal–Wallis test).
[c] Percentage of control, after Henderson's equation: $100 - (T/U \times 100)$, where T and U are the mean after treatment/mean before treatment in treated plots and untreated plots, respectively.

applications of nootkatone by using 7.57 or 15.14 liters of water diluent. Nevertheless, numbers of host-seeking ticks remained significantly depressed in all treated plots through 35 d postapplication (Table 7).

The nootkatone nanoemulsion seemed to be somewhat more effective against questing *A. americanum* nymphs, providing >96% control in all treated plots at 7 d postapplication (Table 7). Again, different rates of diluent seemed to have no difference in efficacy. Although numbers of host-seeking *A. americanum* nymphs did not vary between sampling dates to the degree that numbers of questing *I. scapularis* did, very high variability in numbers of ticks among treatment plots resulted in apparent fluctuation in calculated percentage of control values for *A. americanum* nymphs. However, numbers of host-seeking ticks were significantly depressed in all treated plots, relative to untreated plots, throughout the study period, remaining at <50% of the abundance observed in the untreated plots at 42 d postapplication (Table 7).

Discussion

Results of trials conducted in 2006 suggested that nootkatone provided excellent short-term control of host-seeking *I. scapularis* nymphs and adults but that the level of control declined through 28 d postapplication. Nootkatone had little effect on *A. americanum* nymphs after 2 d, whereas carvacrol was ineffective against both species. In 2007, the five-fold increase in concentration of nootkatone did not appreciably affect the level of control against *I. scapularis* nymphs but markedly improved the level of control of *A. americanum* nymphs. Similarly, the substantial increase in the concentration of carvacrol resulted in considerably better levels of control of both tick species. However, we did not observe acceptable residual activity of either compound beyond 7–14 d postapplication. We postulated that this may have been the result of ticks recolonizing treated plots from adjacent untreated areas or our failure to adequately treat the entire litter column. In the latter case, any ticks in the lower portions of the litter that were inactive might not have been affected by the initial application. As these ticks became active and the plant-derived compounds degraded over time, ticks could become active at the litter surface and account for the observed 20–40% decline in the level of control. However, nootkatone trials conducted in 2008 showed that horizontal migration of ticks into treated plots did not account for the observed decline in levels of control. Also, use of additional diluent alone did not seem to improve residual control of host-seeking ticks. In fact, backpack sprayer applications of nootkatone at NWS Earle and Perrineville Lake Park resulted in levels of control of *I. scapularis* nymphs similar to those observed in earlier trials. However, the backpack sprayer application of the nootkatone nanoemulsion at Perrineville Lake Park seemed to improve the level of control of *A. americanum* nymphs compared with other trials. In contrast, percent control of both *I. scapularis* and *A.*

americanum nymphs was substantially greater in plots treated via high-pressure sprayer compared with backpack sprayer-treated plots, providing ≥98.1 and ≥96.2% control, respectively, for the entire 42-d trial. The volatile nature of botanical oils and both mono and sesquiterpene compounds have been exploited as natural sources of flavor, fragrance, and repellent chemicals (Moore et al. 2006). Dietrich et al. (2006) reported on the repellent efficacy of nootkatone and carvacrol against nymphal *I. scapularis*. This repellent effect may have accounted for early tick suppression and "recovered" tick abundance in backpack versus high-pressure-treated plots.

The results of these trials suggest that both nootkatone and carvacrol can provide relatively quick knockdown (1 d) and significant short-term (7–14 d) control of both *I. scapularis* and *A. americanum* but seem to have little residual activity when applied by backpack sprayer. However, by treating the entire litter column using a high-pressure sprayer, we were able to demonstrate that nootkatone provided high levels of control throughout the nymphal activity period, similar to those achieved with conventional acaricides (Schulze et al. 2001c, 2005a, 2007). These preliminary trials suggest that, when applied with the proper equipment, these compounds may provide long-term control without reliance on environmental persistence. Coupled with the relative absence of mammalian toxicity (Panella et al. 1997, 2005) of food-grade natural products such as nootkatone, this feature may provide the kind of alternative to conventional acaricides sought by the public (Piesman 2006, Schulze et al. 2007). Additional studies include the identification of other all natural botanically derived candidate compounds, development and testing of formulations to provide residual activity as well as exploring modes and times of application for maximizing acaricidal efficacy, and development of alternative sources or methods of production to make the use of these plant-derived acaricides cost-effective.

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