

## Repellency of two terpenoid compounds isolated from *Callicarpa americana* (Lamiaceae) against *Ixodes scapularis* and *Amblyomma americanum* ticks

John F. Carroll · Charles L. Cantrell · Jerome A. Klun ·  
Matthew Kramer

Received: 15 December 2006 / Accepted: 26 January 2007  
© Springer Science+Business Media B.V. 2007

**Abstract** Callicarpenal (13, 14, 15, 16-tetranor-3-cleroden-12-al) and intermedeol [(4*S*,5*S*,7*R*,10*S*)-eudesm-11-en-4-ol], isolated from American beautyberry, *Callicarpa americana* (Lamiaceae), were evaluated in laboratory bioassays for repellent activity against host-seeking nymphs of the blacklegged tick, *Ixodes scapularis*, and lone star tick, *Amblyomma americanum*. A strip of organdy cloth treated with test solution was doubly wrapped (treatment on outer layer) around the middle phalanx of a forefinger and ticks released on the fingertip. Callicarpenal and intermedeol, at 155 nmole/cm<sup>2</sup> cloth repelled 98 and 96% of *I. scapularis* nymphs, respectively. Dose response tests with *I. scapularis* nymphs showed no difference in repellency among callicarpenal, intermedeol and Deet (*N,N*-diethyl-3-methylbenzamide), however, SS220 ((1*S*,2'*S*)-2-methylpiperidinyl-3-cyclohexene-1-carboxamide) was significantly more repellent than the other compounds. Callicarpenal, at 155 nmole/cm<sup>2</sup> cloth, repelled 100 and 53.3% of *I. scapularis* nymphs at 3 and 4 h, respectively, after the cloth was treated, whereas intermedeol repelled 72.5% of *I. scapularis* nymphs 3 h after treatment. In comparison with the results obtained with *I. scapularis*, callicarpenal, intermedeol, Deet and SS220 were less effective against *A. americanum*. Only intermedeol and SS220 repelled significantly more *A. americanum* than

---

J. F. Carroll (✉)  
USDA, ARS, Animal Parasitic Diseases Laboratory, Beltsville Agricultural Research Center,  
Bldg. 1040, Beltsville, MD, 20705, USA  
e-mail: jcarroll@anri.barc.usda.gov

C. L. Cantrell  
USDA, ARS, Natural Products Utilization Research Unit, University, MS, 38677, USA

J. A. Klun  
USDA, ARS, Chemicals Affecting Insect Behavior Laboratory, Beltsville, MD, 20705, USA

M. Kramer  
USDA, ARS, Biometrical Consulting Service, Beltsville, MD, 20705, USA

ethanol controls at 155 nmole compound/cm<sup>2</sup> cloth. At 1,240 nmole/cm<sup>2</sup> cloth, callicarpenal and intermedeol repelled 20 and 40% of *A. americanum* nymphs.

**Keywords** American beautyberry · Blacklegged tick · Lone star tick · Deet · SS220

## Introduction

In Mississippi, crushed leaves of American beautyberry, *Callicarpa americana* L. (Lamiaceae), were placed under the harnesses of draft animals as a traditional means to protect the animals from hematophagous insects (Cantrell et al. 2005; Krajick 2006). Beautyberry leaves have been used as recently as the 1980s to repel arthropods (Charles Bryson, pers. comm.). Cantrell et al. (2005) studied terpenoid compounds isolated from American and Japanese beautyberry, *C. japonica* Thunb., and discovered that two of these compounds, callicarpenal and intermedeol, had significant repellent activity against two species of mosquitoes. This discovery prompted us to speculate that these natural products might also have repellent activity against ticks.

Ticks and tick-borne diseases seriously affect the health of humans and domesticated animals throughout the habitable world (Sonenshine 1991). Species of *Amblyomma*, *Dermacentor*, *Rhipicephalus* (*Boophilus*) and *Ixodes* ticks transmit a variety of bacterial, viral and protozoan pathogens. Tick-borne diseases in the US include Rocky Mountain spotted fever, bovine anaplasmosis, ehrlichioses, Lyme disease, tularemia and human babesiosis (Sonenshine 1993). The US has experienced an upsurge in tick-borne diseases in recent years (Gratz 1999). The blacklegged tick, *Ixodes scapularis* (Say), principal vector of the causative agent of Lyme disease (Spielman et al. 1985), and the lone star tick, *Amblyomma americanum* (L.), which transmits pathogens causing ehrlichioses (Childs and Paddock 2003), are especially troublesome.

Repellents provide a last line of protection against tick bite and pathogen transmission (CDC 2002). Permethrin-based products are marketed as repellents for use on clothing and have proven effective against *A. americanum* and *I. scapularis* (Schreck et al. 1982, 1986; Lane and Anderson 1984). For use on skin, products containing *N,N*-diethyl-3-methylbenzamide (Deet) have been widely used for decades to protect against ticks and biting flies. Recently developed arthropod repellents, such as 1-methyl-propyl-2-(hydroxyethyl)-1-piperidinecarboxylate (picaridin) and (1*S*,2'*S*)-2-methylpiperidinyl-3-cyclohexene-1-carboxamide (SS220) have also shown promise against ticks (Pretorius et al. 2003; Carroll et al. 2004, 2005).

The purpose of this study was to ascertain the potential of callicarpenal and intermedeol as novel natural product tick repellents by evaluating their efficacy against host-seeking nymphs of *I. scapularis* and *A. americanum*.

## Materials and methods

### Ticks

Larvae of *I. scapularis* were obtained from Oklahoma State University and fed on rats (in compliance with USDA, ARS, Beltsville Area Animal Care and Use Committee Protocol #05-022). After the fed *I. scapularis* larvae dropped from the rats, they were held in vials at 24°C, ≈97% R. H. and a photoperiod of 16:8 h (L:D).

The *I. scapularis* nymphs were used in repellent bioassays 7–16 wk after eclosion. Nymphs of *A. americanum* were from a colony at the United States Department of Agriculture, Agricultural Research Service, Knipling-Bushland Livestock Insects Research Laboratory, Kerrville, TX and held at 24°C, ≈97% R. H. and a photoperiod of 16:8 h (L:D).

## Test compounds

### *Instrumentation*

<sup>1</sup>H- and <sup>13</sup>C-NMR spectra were recorded in CDCl<sub>3</sub> on a Bruker Avance 400 MHz spectrometer (Billerica, MA). High-resolution mass spectra were obtained using an Agilent 1100 HPLC coupled to a JEOL AccuTOF (JMS-T100LC) (Peabody, MA). Column chromatography was performed using a Biotage, Inc. Horizon<sup>TM</sup> Pump (Charlottesville, VA) equipped with a Horizon<sup>TM</sup> Flash Collector.

### *Gas chromatography-mass spectrometry analysis*

Callicarpenal and intermedeol were analyzed by GC-MS on a Varian CP-3800 GC coupled to a Varian Saturn 2000 MS/MS. GC was equipped with a DB-5 column (30 m × 0.25 mm fused silica capillary column, film thickness of 0.25 μm) operated using the following conditions: injector temperature, 240°C; column temperature, 60–240°C at 3°C/min then held at 240°C for 5 min; carrier gas, He; injection volume, 1 μl (splitless). MS ionization energy set to 70 eV.

### *Plant material*

Leaves of *C. americana* were collected on September 2, of 2005 from a single large plant (4 m tall × 5 m wide) growing in Lafayette County, Mississippi at latitude 34°20'25''N and longitude 89°40'17''W. A voucher specimen was previously deposited in the Pullen Herbarium in Oxford, Mississippi and assigned voucher number MISS #71,495 (Cantrell et al. 2005).

### *Essential oil preparation*

Fresh cut leaves of *C. americana* were immediately frozen in sealed plastic bags upon collection until needed. A Clevenger-type volatile oil distilling apparatus (Wilmad Labglass, Buena, NJ) was attached to a 12 l round bottom flask containing *C. americana* leaves (900.5 g fresh weight) in 6 l of deionized H<sub>2</sub>O. Upon heating to boiling, the distillate was continuously extracted during a 96-h distillation with 6 ml of pentane providing 747 mg of crude essential oils. This process was repeated as needed to obtain additional oil for fractionation.

### *C. americana* oil fractionation

A portion (693 mg) of the *C. americana* essential oil was subjected to silica gel (25 × 150 mm, 60 Å, 40–63 μm) column chromatography. A hexane/EtOAc linear gradient consisting of the following steps was used: 100/0 to 90/10, 1,200 ml; 90/10 to 80/20, 600 ml; 80/20 to 50/50, 360 ml; 50/50 to 0/100, 1,008 ml. A total of 132, 24-ml

test tubes were collected and combined into six fractions [Fr. A 322 mg, Fr. B 48 mg (callicarpenal), Fr. C 35 mg, Fr. D 37 mg, Fr. E 45 mg (intermedeol), Fr. F 157 mg] based on TLC similarity. This process was repeated as needed to obtain sufficient quantities of callicarpenal and intermedeol for bioassays.

### *Callicarpenal and intermedeol*

Callicarpenal and intermedeol were identified using mass spectrometry and  $^1\text{H}$ - and  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy (NMR) data which was in complete agreement with that which had been reported previously (Cantrell et al. 2005).

### *Deet and SS220*

Deet was purchased from Morflex, Inc. (Greensboro, NC). SS220 was prepared earlier at the USDA, ARS, Chemicals Affecting Insect Behavior Laboratory (Klun et al. 2003). Both compounds were 98% pure chemically according to gas chromatographic analyses.

### Bioassay

In vivo bioassays of repellents generally are preferable to in vitro tests. Although the foliage of *Callicarpa americana* has been applied to animal integument without discernible ill effects, the consequences of application of concentrated callicarpenal and intermedeol on human skin are unknown. Therefore, we used a fingertip bioassay similar to one described and depicted by Carroll et al. (2005) in which the test repellent solution was applied to a strip of cloth that was wrapped around the finger. A strip of organdy cloth ( $7 \times 7$  mesh/mm) (Hancock Fabrics, Laurel, MD) was cut in the shape of a hockey stick (9 cm long section, 4.5 cm short section, 4–4.5 cm wide) so that it could be wrapped twice around the index finger with only the outer layer receiving test solution. An area of each cloth strip corresponding to the area bounded by the first and second joints of the index finger was marked with a lead pencil and served as the treatment area. All compounds tested in the bioassays were applied to the cloth using ethanol solutions of appropriate concentrations to generate desired doses of compounds/cm<sup>2</sup> cloth.

The volume of solvent applied was based on the dimensions of the left index finger of JFC. The volume required to give the desired nmoles/cm<sup>2</sup> cloth was calculated from the average of the circumferences of the two finger joints multiplied by the distance between the deepest crease of each joint.

An organdy strip was placed in a glass Petri dish (9 cm diam.) and 52  $\mu\text{l}$  of test solution was evenly distributed on the treatment area with a pipettor. After allowing 10–15 min for the solution to dry, the cloth was doubly wrapped around the index finger. The treated portion of the cloth completely encircled the finger and covered the entire second phalanx of the finger. An untreated portion of cloth extended 5–6 mm beyond the first joint (toward the base of the finger). The cloth was held in place by three small dabs of beeswax smeared on the upper surface of the inner layer of cloth where the layers overlapped and pressure from another finger applied for  $\approx 10$  s to adhere the layers. A vial containing nymphs was opened in a Petri dish (9 cm diam., 1 cm high) that had been glued in the center of a larger Petri dish (15 cm diam., 1.5 cm high) with water added to the intervening space to form a moat. The index

finger was held horizontally and 10 nymphs placed with forceps on its dorsal surface between the edge of the cloth and the base of the fingernail. Once all the ticks were clinging to the finger, it was tilted slowly until vertical with the tip downward.

While the locations of the ticks were recorded 1, 3, 5, 10 and 15 min after the last tick was released on the fingertip, we scored repellency based on the 15 min count. Ticks were considered repelled if they fell from the finger without having crossed the cloth strip or were on the untreated fingertip distal to the cloth. Because *I. scapularis* nymphs were more apt to fall from untreated skin and had a greater tendency to remain immobile for extended periods on untreated skin than *A. americanum* nymphs, we screened the former for tenacity and readiness to climb (Schreck et al. 1995). While the test solution was drying on the cloth, *I. scapularis* nymphs were placed on the tip of an untreated finger until 10 nymphs climbed  $\approx 0.5$  cm, and those ticks that climbed were used in the bioassay. Before each bioassay, the corresponding author thoroughly washed his index finger with soap and rinsed with water.

### Experimental design

We conducted four experiments. Test solutions and ethanol controls were tested in random order. A full block of compounds was generally completed in 1 day. Controls were always run in a block with repellent compounds, even if the block was incomplete. Therefore, more ticks were tested with the ethanol control than with any individual compound. In these experiments, we scored groups (replicates) of 10 ticks, with several replicates for each compound–dose–species combination.

Experiment 1 compared the response of *I. scapularis* and *A. americanum* to a fixed 155 nmole compound/cm<sup>2</sup> cloth dose of callicarpenal, intermedeol, Deet, SS220 and an ethanol control using the fingertip bioassay. This experiment was designed to compare the efficacy of callicarpenal and intermedeol to two proven tick repellents, Deet and SS220. Five replicates of *I. scapularis* and *A. americanum* were used for each compound–dose combination.

In Experiment 2, we estimated the EC<sub>50</sub> and EC<sub>95</sub> (EC is effective concentration) against *I. scapularis* nymphs using concentrations of 155, 78, 39, 19.5, 0 nmole compound/cm<sup>2</sup> (109 replicates total).

Because these doses were too low to establish reliable estimates for *A. americanum*, this species was tested at higher concentrations in Experiment 3, using callicarpenal and intermedeol at 620 and 1,240 nmole compound/cm<sup>2</sup> and three replicates for each compound–dose combination.

In Experiment 4, we investigated the efficacy of callicarpenal and intermedeol several hours following application. In this experiment, callicarpenal and intermedeol at 155 nmole compound/cm<sup>2</sup> were tested against four replicates of *I. scapularis* nymphs 3 h after application to the cloth strips, and callicarpenal was tested against three replicates at 4 h after application.

### Statistical analysis

Since the data were binomial in nature (an individual tick was scored as either repelled or not repelled), we used a generalized linear model with a binomial link (McCullagh and Nelder 1989) to model the logit of the proportion of ticks repelled ( $\log [p/(1 - p)]$ , where  $p$  is the proportion of ticks repelled) using the R software package (Free Software Foundation, Boston, MA, <http://www.gnu.org/>).

A preliminary analysis of the data suggested that there might be a block (trial) effect, so we included block as a random effect, making the model a generalized linear mixed model.

For experiments comparing compounds or comparing efficacy several hours after application, we performed multiple comparisons using methodology producing results similar to a closed test procedure using max-T-type statistics, available in the add-on multcomp R package.

For callicarpenal and intermedeol, dose–response relationships were based on the linear relationship between the square root of the concentrations and the logit of the proportion of the ticks repelled. For Deet and SS220, we used untransformed concentrations, as this yielded a better fitting curve based on the AIC criterion (Burnham and Anderson 1998). We used a “quasibinomial” distribution to account for the overdispersion introduced by conducting Experiment 2 in blocks, rather than explicitly modeling the block effect.  $EC_{50}$  and  $EC_{95}$  were estimated using “inverse” regression, and the standard errors for the concentration at these points using fiducial limits (Draper and Smith 1981); these are implemented in the R software with the MASS library developed by W. N. Venables and B. D. Ripley (<http://www.lib.stat.cmu.edu/R/CRAN/>). Because graphs on the original scale (proportion repelled) are easier to interpret, the modeled concentration response relationship was back transformed from the logit scale for visually displaying the concentration–response relationship (corresponds to dose–response). However, the theoretical straight line relating the logit of the proportion repelled to the concentration then becomes a curve.

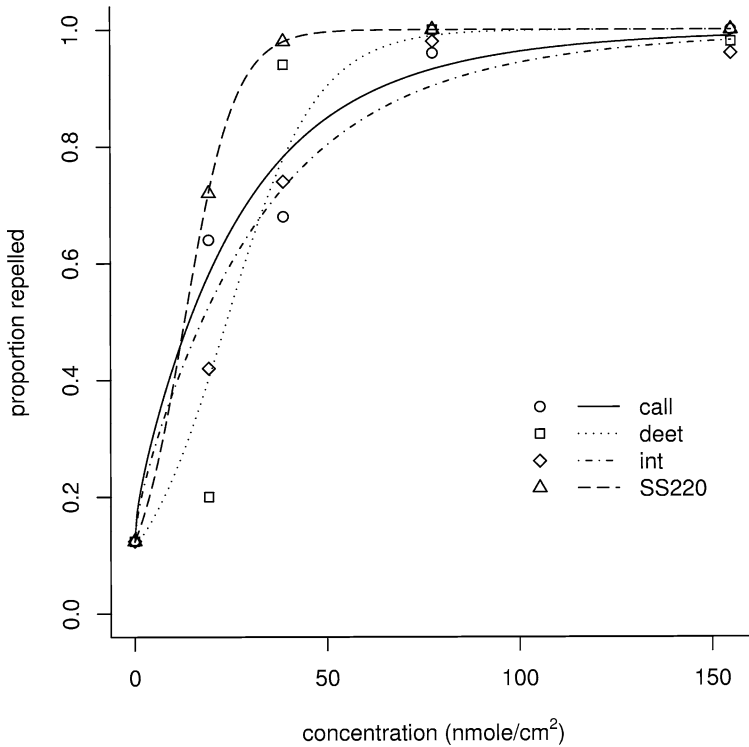
## Results

### Experiment 1

We scored repellency based on the locations of ticks at 15 min after they were released on the fingertip. Ticks that were on the untreated fingertip or had dropped off were considered repelled. In Experiment 1, at 155 nmol/cm<sup>2</sup>, callicarpenal and intermedeol repelled highly significant proportions ( $P < 0.001$  for both compounds) of the *I. scapularis* nymphs (98 and 96%, respectively), as did Deet and SS220 (100% repelled). At this concentration, there was no significant difference between any of the repellent compounds for *I. scapularis* nymphs. Against *A. americanum* nymphs, 155 nmol/cm<sup>2</sup> intermedeol repelled a significantly greater proportion of the ticks than the ethanol control ( $P < 0.003$ ), but callicarpenal did not ( $P = 0.117$ ). SS220 was more repellent than the other compounds against *A. americanum*. At 155 nmol/cm<sup>2</sup>, intermedeol repelled 28% *A. americanum* nymphs. In contrast, SS220 at 155 nmol/cm<sup>2</sup>, repelled 84% of *A. americanum* nymphs. Interestingly, the block effect was significant only for *A. americanum* ( $P < 0.001$ ).

### Experiment 2

Dose response curves for callicarpenal and intermedeol against *I. scapularis* were nearly indistinguishable (Fig. 1). SS220 was more repellent ( $P < 0.05$ ) than the other three compounds against *I. scapularis* except at high concentrations, when it was indistinguishable from Deet (Fig. 1). SS220 had the lowest estimated  $EC_{50}$  value



**Fig. 1** Responses of *I. scapularis* nymphs in fingertip bioassays (treated-cloth strip wrapped around finger) to four concentrations of callicarpal (call), intermedeol (int), Deet and SS220 and an ethanol control (compound concentration of zero)

(13 nmole/cm<sup>2</sup>). The estimated EC<sub>50</sub> values for callicarpal and intermedeol were 14 and 17 nmole/cm<sup>2</sup>, respectively, lower than the estimated EC<sub>50</sub> of Deet (23 nmole/cm<sup>2</sup>). However, the estimated EC<sub>95</sub> values for callicarpal and intermedeol (89 and 105 nmole/cm<sup>2</sup>, respectively) were higher than those of Deet (58 nmole/cm<sup>2</sup>) and SS220 (33 nmole/cm<sup>2</sup>) (Table 1). Note that the calculated standard errors in Table 1 for the estimated EC<sub>50</sub> and EC<sub>95</sub> for all compounds except SS220 are relatively large, suggesting that a straight line (on the logit scale) is not the best functional form to model dose–response relationships for these compounds. However, attempts to fit lines with curvature (on only 5 points) could rightly be criticized for over parameterization (i.e. many different kinds of curves would yield equivalently good fits to our data without shedding light on the “true” relationship). In particular, note the steep rise in Deet efficacy going from 19 to 39 nmole/cm<sup>2</sup> (Fig. 1), which the estimated curve is unable to capture. Many additional points in this region would be necessary to determine the shape of the curve in this region.

### Experiment 3

At 1,240 nmoles/cm<sup>2</sup>, callicarpal and intermedeol repelled 20 and 40% of *A. americanum* nymphs, significantly more ticks ( $P < 0.05$ ) than the controls, but there was not a significant difference from controls at 622 nmole/cm<sup>2</sup> ( $P > 0.05$ ).

**Table 1** Effective concentrations (EC) of callicarpenal, intermedeol, Deet and SS220 in fingertip bioassays with *I. scapularis* nymphs<sup>a</sup>

	Estimated concentration (nmole/cm <sup>2</sup> )	
	EC <sub>50</sub> (SE)	EC <sub>95</sub> (SE)
Callicarpenal	14.2 (12.3)	88.7 (27.9)
Intermedeol	17.4 (14.3)	105.3 (32.7)
Deet	23.9 (25.5)	58.4 (62.4)
SS220	13.0 (1.7)	32.6 (3.9)

<sup>a</sup> Five groups of 10 nymphs each were tested at each of five concentrations, including 0 nmole/cm<sup>2</sup> (ethanol control), for each compound (SE = standard error)

**Table 2** Percent of *I. scapularis* nymphs repelled by callicarpenal and intermedeol at 3 and 4 h after test solution of 155 nmole/cm<sup>2</sup> was applied to cloth strip

	3 h <sup>a</sup>	4 h <sup>b</sup>
Callicarpenal	100	53.3
Intermedeol	72.5	–
Control	25	16.7

<sup>a</sup> Four groups of 10 nymphs each tested

<sup>b</sup> Three groups of 10 nymphs each tested, no data collected for intermedeol

## Experiment 4

When callicarpenal and intermedeol were tested at 155 nmole/cm<sup>2</sup> cloth against *I. scapularis* nymphs 3 h after their application to the cloth strips, callicarpenal repelled all the ticks tested ( $n = 40$ ) and intermedeol repelled 72.5% of the ticks ( $n = 40$ ) (Table 2). At 4 h post-treatment, callicarpenal repelled 53.3% of the ticks ( $n = 30$ ), significantly more than the control ( $P < 0.05$ ).

## Discussion

Overall callicarpenal and intermedeol were similar to one another in their repellent properties. These two compounds were considerably more effective against *I. scapularis* than *A. americanum*, against which SS220 was the most effective compound tested. This has been a common result seen in experiments with these two species (Carroll et al. 2004, 2005, Carroll unpublished data). Our data suggest that *A. americanum* may be far less sensitive to repellents than *I. scapularis*, and that it may be practical for repellent evaluation programs to use *A. americanum* as the standard for efficacy.

In the dose–response experiments, responses to Deet differed somewhat from those of the other compounds, with a more abrupt change from low to high repellency. As depicted in Fig. 1, Deet repelled smaller proportions of *I. scapularis* nymphs than callicarpenal and intermedeol at lower concentrations, but greater proportions than callicarpenal and intermedeol at higher concentrations. Except at higher concentrations, Deet was estimated to be less effective than SS220. Similarly,



in bioassays in which Deet and racemic 220 were applied to filter paper, racemic 220 was more effective than Deet against *A. americanum*, and Deet also had a steep dose–response curve with *I. scapularis* (Carroll et al. 2004). The inability of the standard straight line relationship (on the logit scale) to adequately model repellency responses to these compounds (other than SS220) suggests that future studies include more concentrations in areas of transition between low and high repellency (perhaps offset by fewer replicates per concentration) in an effort to better model the shape of the curve in this region. However, we note that most interest in compounds tested for repellent activity will be in the region near the  $EC_{95}$ , so this region of the curve should also be well supported by empirical data, requiring many concentrations here as well. We suggest that in future experimentation, small pilot runs be used to determine where the true  $EC_{50}$  and  $EC_{95}$  lie, as the relationship between the logit of proportion repelled and dose does not appear to follow a (standard) straight line.

In fingertip bioassays, when 1,600 nmole/cm<sup>2</sup> of SS220 or Deet was applied to the skin of the middle phalanx of an index finger or to an equivalent area of organly wrapped around the middle phalanx, Carroll et al. (2005) found similar percentages of *I. scapularis* were repelled by treated skin and treated cloth. With *A. americanum*, they found that 98% of the ticks were repelled by Deet on skin and 85% by treated cloth, and that SS220 repelled 94 and 100% on skin and cloth, respectively. Therefore, it is not unreasonable to expect that fingertip bioassays using callicarpenal and intermedeol-treated cloth provide at least a rough approximation of their effectiveness on skin.

Interestingly, 3 h after application, callicarpenal repelled all *I. scapularis* tested and retained >50% repellency at 4 h after application. Formulation of callicarpenal in the proper carrier could potentially extend the persistence of its efficacy.

Inasmuch as callicarpenal and intermedeol were present in concentrations that effectively repelled biting arthropods when beautyberry foliage was applied topically to farm animals as a traditional husbandry practice (Cantrell et al. 2005) and did not ostensibly harm the treated mammals, we believe that callicarpenal and intermedeol have potential for human use. Since sustained activity is also a desirable quality in a repellent, the demonstrated effectiveness of callicarpenal and intermedeol against *I. scapularis* 3–4 h after application further strengthens the potential usefulness of these natural products as repellents. The results we report and those of Cantrell et al. (2005) with mosquitoes dictate further evaluation of callicarpenal and intermedeol, their analogs and related compounds as repellents.

## References

- Burnham KP, Anderson DR (1998) Model selection and inference: a practical information-theoretic approach. Springer, NY, p 353
- Cantrell CL, Klun JA, Bryson CT, Kobaisy M, Duke SO (2005) Isolation and identification of mosquito bite deterrent terpenoids from leaves of American (*Callicarpa americana*) and Japanese (*Callicarpa japonica*) beautyberry. J Agric Food Chem 53:5948–5953
- Carroll JF, Solberg VB, Klun JA, Kramer M, Debboun M (2004) Comparative activity of deet and AI3-37220 repellents against the ticks *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) in laboratory bioassays. J Med Entomol 41:249–254
- Carroll JF, Klun JA, Debboun M (2005) Repellency of two species of ticks (Acari: Ixodidae) by deet and SS220 applied to skin involves olfactory sensing. Med Vet Entomol 19:101–106

- CDC (2002) Lyme disease. Department of Health and Human Services, Centers for Disease Control and Prevention, Ft. Collins, CO, p 12
- Childs JE, Paddock CD (2003) The ascendancy of *Amblyomma americanum* as a vector of pathogens affecting humans in the United States. *Annu Rev Entomol* 48:307–337
- Draper NR, Smith H (1981) Applied regression analysis. Wiley, New York
- Gratz NG (1999) Emerging and resurging vector-borne disease. *Annu Rev Entomol* 44:51–75
- Klun JA, Khirimi A, Margaryan A, Kramer M, Debboun M (2003) Synthesis and repellent efficacy of a new chiral piperidine analog: comparison of Deet and Bayrepel activity in human volunteer laboratory repellent assays against *Aedes aegypti* and *Anopheles stephensi*. *J Med Entomol* 40:293–299
- Krajcik K (2006) Keeping bugs away. *Science* 313:36–38
- Lane RS, Anderson JR (1984) Efficacy of permethrin as a repellent and toxicant for personal protection against the Pacific coast tick and the pajaruelo tick (Acari: Ixodidae and Argasidae). *J Med Entomol* 21:692–702
- McCullagh P, Nelder JA (1989) Generalized linear models, 2nd edn. Chapman and Hall/CRC, London
- Pretorius A-M, Jensenius M, Clarke F, Ringertz SH (2003) Repellent activity of DEET and KBR 3023 against *Amblyomma hebraeum* (Acari: Ixodidae). *J Med Entomol* 40:245–248
- Schreck CE, Mount GA, Carlson DA (1982) Wear and wash persistence of permethrin used as a clothing treatment for personal protection against the lone star tick (Acari: Ixodidae). *J Med Entomol* 19:143–146
- Schreck CE, Snoddy EL, Spielman A (1986) Pressurized sprays of permethrin or deet on military clothing for personal protection against *Ixodes dammini* (Acari: Ixodidae). *J Med Entomol* 23:396–399
- Schreck CE, Fish D, McGovern TP (1995) Activity of repellents applied to skin for protection against *Amblyomma americanum* and *Ixodes scapularis* ticks (Acari: Ixodidae). *J Am Mosq Contr Assoc* 11:136–140
- Sonenshine DE (1991) Biology of ticks, 1st edn. Oxford University Press, New York
- Sonenshine DE (1993) Biology of ticks, 2nd edn. Oxford University Press, New York
- Spielman A, Wilson ML, Levine JF, Piesman JF (1985) Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. *Annu Rev Entomol* 30:439–460