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Essential oils of *Cupressus funebris, Juniperus communis,* and *J. chinensis* (Cupressaceae) as repellents against ticks (Acari: Ixodidae) and mosquitoes (Diptera: Culicidae) and as toxicants against mosquitoes

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ABSTRACT: *Juniperus communis* leaf oil, *J. chinensis* wood oil, and *Cupressus funebris* wood oil (Cupressaceae) from China were analyzed by gas chromatography and gas chromatography-mass spectrometry. We identified 104 compounds, representing 66.8-95.5% of the oils. The major components were: α-pinene (27.0%), α-terpinene (14.0%), and linalool (10.9%) for *J. communis*; cuparene (11.3%) and δ-cadinene (7.8%) for *J. chinensis*; and α-cedrene (16.9%), cedrol (7.6%), and β-cedrene (5.7%) for *C. funebris*. The essential oils of *C. funebris*, *J. chine*nsis, and *J. communis* were evaluated for repellency against adult yellow fever mosquitoes, *Aedes aegypti* (L.), host-seeking nymphs of the lone star tick, *Amblyomma americanum* (L.), and the blacklegged tick, *Ixodes scapularis* Say, and for toxicity against *Ae. aegypti* larvae and adults, all in laboratory bioassays. All the oils were repellent to both species of ticks. The EC_{95} values of *C. funebris*, *J. communis,* and *J. chinensis* against *A. americanum* were 0.426, 0.508, and 0.917 mg oil/cm^2 filter paper, respectively, compared to 0.683 mg deet/cm^2 filter paper. All *I. scapularis* nymphs were repelled by 0.103 mg oil/cm^2 filter paper of *C. funebris* oil. At 4 h after application, 0.827 mg oil/cm^2 filter paper, *C. funebris* and *J. chinensis* oils repelled ≥80% of *A. americanum* nymphs. The oils of *C. funebris* and *J. chinensis* did not prevent female *Ae. aegypti* from biting at the highest dosage tested (1.500 mg/cm^2). However, the oil of *J. communis* had a Minimum Effective Dosage (estimate of ED_{99}) for repellency of 0.029 ± 0.018 mg/cm^2; this oil was nearly as potent as deet. The oil of *J. chinensis* showed a mild ability to kill *Ae. aegypti* larvae, at 80 and 100% at 125 and 250 ppm, respectively. ***Journal of Vector Ecology* 36 (2): 258-268. 2011.**

Keyword Index: Lone star tick, *Amblyomma americanum*, blacklegged tick, *Ixodes scapularis*, yellow fever mosquito, *Aedes aegypti*, repellency, toxicant.

INTRODUCTION

Mosquito and tick-borne illnesses exact a considerable toll in human misery and financial expenditure. Problems with ticks and tick-borne diseases have continued to grow during the past three decades (Parola and Raoult 2001). Lyme disease (caused by the spirochete *Borrelia burgdorferi*) and its principal vector, *Ixodes scapularis* Say (Spielman et al. 1985), have deservedly received significant attention in the United States. The lone star tick, *Amblyomma americanum* (L.), has acquired notoriety not only as a nuisance biter, but also as a vector of *Ehrlichia chaffeensis*, which causes human monocytic ehrlichiosis (Childs and Paddock 2003, Armstrong et al. 2001). Mosquitoes are vectors for many pathogens that cause serious diseases, such as malaria, dengue fever, yellow fever, Rift Valley fever, and Chikungunya, which can attain epidemic levels with high rates of human morbidity and mortality.

Repellents applied to skin or clothing are recommended as a means of personal protection against biting arthropods (e.g., CDC 2002). Toxicants can reduce the densities of mosquito and tick populations and the risk of disease transmission. The synthetic repellent deet (*N,N*-diethyl-3-methyl benzamide) has been used widely since the 1950s to defend against mosquitoes and ticks. Newer synthetics, such as picaridin and IR3535, now provide more options for users (Debboun et al. 2007).

Permethrin, a synthetic pyrethroid, is approved to be used as a repellent, but only when applied on clothing (Schreck et al. 1982). As toxicants, pyrethroids are used to control a wide range of arthropods that are agricultural and public health pests (Hoel et al. 2010). Repeated use of pyrethroids, or insecticides of any class, can lead to insecticide resistance. Consequently, there is an urgent

need to develop alternatives to chemical control of a wide variety of arthropod vectors of human diseases (Pridgeon et al. 2008, 2009). Many naturally occurring repellents and insecticides have potential for development into useful products because they combine efficacy, biodegradability, and limited risk to mammals and the environment (Dayan et al. 2009, Hoel et al. 2010, Carter 1989, Bissinger et al. 2009, Bissinger and Roe 2010). Plant essential oils and seed pressed oils comprise a significant portion of the market share for natural product-based insecticides, and some have served as the basis of commercial repellent formulations (Dayan et al. 2009, Nerio et al. 2010).

A great diversity of medicinal and aromatic plants occurs in China, where these natural products exert a strong impact on peoples' lives and culture (Li et al. 2006). Prominent among the useful taxa is the cypress family, Cupressaceae. Commercial cedarwood oils have been obtained from three genera of Cupressaceae: *Juniperus* (Texas, Virginia, and Africa), *Cupressus* (China), and *Cedrus* (Morocco, India) (Adams and Li 2008). *Cupressus funebris* Endl. is generally regarded as the botanical source of Chinese cedarwood oil, a well known perfume material (Adams and Li 2008), and its derivatives (Duquesnoy et al. 2006).

The studies of Panella et al. (1997, 2005), Dietrich et al. (2006), and Dolan et al. (2007, 2009) indicate that the Cupressaceae may be a rich source of anti-tick compounds and that further investigation of cypress species for repellent and insecticidal chemicals is warranted. We investigated Chinese weeping cedar, *C. funebris*, Chinese juniper, *Juniperus chine*nsis L., and common juniper, *J. communis* L., essential oils from China. One purpose of this study was to characterize the chemical composition of the essential oils of *C. funebris*, *J. chinensis*, and *J. communis* by analysis using gas chromatography (GC) and GC-mass spectrometry (GC-MS). The second purpose was to evaluate these oils as repellents against the ticks *A. americanum* and *I. scapularis* and as repellents and adult and larval toxicants against the yellow fever mosquito, *Aedes aegypti* (L.).

MATERIALS AND METHODS

Ticks

Larvae of *I. scapularis* from a colony at Oklahoma State University were fed as larvae on rats at the USDA, ARS, Beltsville Agricultural Research Center, Beltsville, MD in accordance with USDA, ARS, Beltsville Area Animal Care and Use Committee Protocol #08-013. The fed larvae and resultant nymphs were held at 23-24° C, ~97% RH and a photoperiod of 16:8 h (L:D). The *A. americanum* nymphs were from a colony at the USDA, ARS, Knipling-Bushland U. S. Livestock Insects Research Laboratory, Kerrville, TX and a colony at Oklahoma State University, Stillwater, OK (used in duration bioassay) and held at 23-24° C, ~97% RH and a photoperiod of 16:8 h (L:D). The *I. scapularis* and *A. americanum* nymphs were tested three to five, and three to six months after molting, respectively.

Mosquitoes

Pupae of *Ae. aegypti* from the Gainesville (CMAVE) colony were maintained in the laboratory at 28 ±1° C and 30-60% RH, and the resulting adults aged five to nine days were used for repellent testing. The *A. aegypti* mosquitoes (also from the CMAVE colony) used in the insecticide bioassays were held at 22-30° C, 80% RH, and a photoperiod of 14:10 (L:D) including 2 h of simulated dusk and dawn. The mosquitoes were maintained on 10% sucrose *ad libitum* and provided twice weekly with bovine blood in 1% heparin placed in pig intestine and warmed to 37° C.

Essential oils, repellent, and insecticide

Juniperus communis oil was purchased from Shanghai Qika Corporation, Shanghai, China, and *C. funebris* and *J. chine*nsis oils were purchased from Peking University Zoteq Co., Ltd., Beijing, China. Deet was purchased from Aldrich, Sigma-Aldrich, Inc., St. Louis, MO, and permethrin from Chem Service, West Chester, PA.

GC/FID and GC/MS analysis

The oils were analyzed by capillary GC-FID and GC/MS using an Agilent 5975 GC-MSD system (SEM Ltd., Istanbul, Turkey). A Hewlett Packard-Innowax FSC column (60 m x 0.25 mm, 0.25 μm film thickness) was used with helium as the carrier gas (0.8 ml/min). The GC oven temperature was held at 60° C for 10 min and ramped to 220° C at a rate of 4° C/min, then held constant at 220° C for 10 min with a final programmed ramp to 240° C at a rate of 1° C/min, and held a second time at 240° C for 20 min. The split ratio was adjusted to 40:1. The injector temperature was at 250° C. The mass spectrometer was operated with an electron energy of 70 eV. The mass range was *m/z* 35 to 425 at a scan rate of 3.46 scans/s. The GC analysis was carried out using an Agilent 6890N GC system equipped with a FID detector operated at a temperature of 300° C. To obtain the same elution order of peaks detected by GC/MS, simultaneous injection on the GC was performed using the same column and appropriate chromatographic conditions as those described for the GC/MS system.

Identification of the essential oil components was carried out by comparing their relative retention times with those of authentic samples or by comparing their relative retention index (RRI) to a series of *n*-alkanes. Computer matching identified compounds used as references (Wiley and MassFinder 3.1) (Koenig et al. 2004, McLafferty and Stauffer 1989). An in-house "Başer Library of Essential Oil Constituents" composed of genuine compounds and components of known oils, and MS literature data (Joulain and Koenig 1998, ESO 2000, Jennings and Shibamoto 1980) were also used for the identification. The relative concentrations of the separated compounds based on percentage were computed from chromatograms obtained with the GC/FID system.

Vertical filter paper bioassay

Host-seeking ticks of many species tend to climb vertical surfaces, particularly in the presence of host-

produced stimuli. This behavior was used to expose *A. americanum* nymphs to repellent treatments in an in vitro bioassay described in detail by Carroll et al. (2004). Briefly, a 4 X 7-cm rectangle of Whatman No. 4 filter paper was marked with a pencil into two 1 X 4-cm zones at the far ends of the paper strip and a central 4 X 5-cm zone. Using a pipettor, 165 µl of test solution was evenly applied to both sides of the central 4 X 5 cm of the filter paper. After drying for 10-15 min, the paper strip was suspended from a bulldog clip hung from a slender horizontal dowel held by an Aptex No. 10 double clip work holder (Aptex, Bethel, CT). A Petri dish (9 cm diameter) glued in the center of a 15 cm Petri dish created a moat when water was added between their walls (1.5 cm high). The moated Petri dishes were placed directly beneath the suspended filter paper. When *A. americanum* nymphs climbed to the rim of a storage vial opened in the center of the moated Petri dishes (5.5 and 9 cm diameters), the paper strip was removed from the dowel and held so that ten ticks crawled onto the lower untreated zone. Because nymphs of *I. scapularis* tended to be slower and more apt to drop from untreated filter paper, they were screened for readiness to crawl while the test solution dried on the filter paper (Schreck et al. 1995). As the treated filter paper dried, *I. scapularis* were transferred with forceps to an untreated vertical filter paper. Ten ticks that climbed >5 mm were selected for testing and placed in a moated petri dish until 10-15 min post-application had elapsed. Using forceps, the *I. scapularis* nymphs were then transferred individually from the moated Petri dish to the lower untreated zone of the filter paper. The locations of the ticks were recorded at 1, 3, 5, 10, and 15 min after the tenth *A. americanum* or *I. scapularis* nymph grasped the lower untreated zone of the filter paper. Ticks were considered repelled if they were in the lower untreated zone at 15 min or if they fell from the filter paper without having crossed the upper boundary of the treated zone.

Tick experimental design

Test solutions and ethanol controls were tested in random order. A control was run each day that the oils and deet were tested. Ticks were tested in replicates of ten ticks per combination of concentration and oil or deet. Essential oils from *C. funebris* and *J. chinensis* were tested against three replicates of *A. americanum* at 0.827, 0.413, 0.206, and 0.103 mg/cm^{2}filter paper, and *J. communis* at 0.827, 0.413, and 0.206 mg/cm^2 filter paper. A single concentration (0.103 mg oil/cm^2 filter paper) of each of *C. funebris*, *J. communis*, and *J. chinensis* essential oil was tested against three replicates (ten ticks per replicate) of *I. scapularis* nymphs.

To determine the duration of the repellency of *C. funebris* and *J. chinensis*, we tested their essential oils (0.827 mg oil/cm^2 filter paper) against *A. americanum* nymphs at 2, 4, and 6 h after application. Deet, at 0.827 mg oil/cm^2 filter paper, was included in the duration tests for comparison. Four replicates (ten ticks per replicate) were tested at 2 and 4 h post-application and three replicates (ten ticks per replicate) at 6 h.

Mosquito repellent assay

Female mosquitoes were selected from the stock cages by a hand-draw box (Posey and Schreck 1981). Approximately 500 (± 10%) mosquitoes, primarily females, were transferred into a test cage (capacity 59,000 cm^3, dimensions 45 cm x 37.5 cm x 35 cm) and held therein for 25 (± 2.5) min before initiating repellency assays (Barnard et al. 2007).

Extracts were weighed and placed in a 2-dram vial to which 2 ml acetone was added. The initial weight of extract was measured so that when one half (1 ml of solution) was removed and a 50 cm^2 muslin cloth was added to the vial, the remaining 1 ml solution would produce an initial concentration on cloth of 1.500 mg/cm^2. Serial dilutions were then made analogously such that the concentrations on cloth for the remaining 1 ml solution were: 0.750, 0.375, 0.187, 0.094, 0.047, 0.023, 0.011, and 0.005 mg/cm^2. Vials were sealed and stored at -4° C in a freezer until testing (normally <48 h). Each test involved removal of cloth from the vial and stapling it onto two sections of card stock (5 cm x 2.5 cm). Pieces of masking tape (2.5-5.0 cm long) secured the cloth onto the card stock. The card and cloth assembly was then placed on a drying rack for 3-5 min before testing.

A single test consisted of covering the hand of a volunteer with a soft-embossed long cuff poly glove (Atlantis Products, Mankato, MN), followed by a powder-free latex glove (Diamond Grip, Microflex Corporation, Reno, NV). A knee-high stocking (Leggs everyday knee highs, Winston-Salem, NC) was then placed over the gloved hand and arm. A plastic sleeve of polyvinyl was the final layer affixed over the stocking covered arm. The plastic sleeve was sealed around the arm by a Velcro™ strip. About half-way between the wrist and elbow a 4 x 8 cm opening in the sleeve allowed us to assess mosquito landing and biting behavior. Attractive odors emanated from the skin surface and attracted mosquitoes to the opening. During testing, this 32 cm^2 open area was covered with extract-treated muslin cloth. The order in which treated cloths were tested was randomized among volunteers and randomized to minimize any variation due to day-to-day effects.

A test started when the arm, sleeve, and cloth were inserted into the mosquito cage. If fewer than four bites were received during the 1 min, the dosage of repellent on cloth was considered to have "passed." A treatment in which five bites (out of 500 mosquitoes in the cage) occurred in 1 min was considered a failure. Normally, an intermediate dosage (e.g., 0.187 mg/cm^2) was tested first. Depending on whether this concentration passed or failed, higher or lower treatment concentrations were evaluated with all subjects until each had pinpointed their individual concentration that produced the 1% (five bite) failure point. If the 1.500 mg/cm^2 (or highest concentration) on cloth was not efficacious (>five bites in 1 min), then the minimum effective dosage (MED) was noted as ineffective at the highest concentration tested. Because the mosquitoes fatigue upon repeated exposure to repellent and attractant odors from the arm, a limit of ten successive tests were conducted, after which the caged mosquitoes were allowed a 15 min recovery period.

Three male volunteers (two tested twice) and a female volunteer participated in the studies of MED of the oils (USDA 1977). During a test, one or both volunteers wore a patch and tested each patch for 1 min intervals. Patches were rotated between the volunteers, thus, no patch was evaluated beyond 10 min after the 3-min drying period to avoid any bias that may result from evaporative loss of the treatment from the cloth during the duration of the test. The subjects provided written informed consent. The protocol was approved by the University of Florida Human Use Institutional Review Board-01 (Study # 636-2005).

Mosquito larvicidal assay

Larval bioassays were performed as described in Pridgeon et al. (2009). Briefly, five *Ae. aegypti* 1st instar larvae were placed into individual wells of a 24-well plate containing 950 µl deionized water and 40 µl of a 2:1 suspension of alfalfa:pot belly pig chow. Chemicals to be tested were resuspended in DMSO (Sigma Cat. D8418), for a stock concentration of 50 µg/µl. Six concentrations in a two-fold serial dilution series in DMSO were tested for mortality by adding 10 µl of each concentration into the wells. After 24 h, the number of dead larvae was recorded. Serial dilutions were continued until 0% mortality was observed for each chemical. All concentrations were tested in triplicate. Controls included negative (untreated), carrier (DMSO), and positive (permethrin).

Mosquito adult topical assay

Stock chemicals prepared from above were diluted into acetone for a final concentration of 6.25 µg/µl. Ten adult *Ae. aegypti* female mosquitoes, three to five days post-eclosion, were cold anaesthetized and placed on BioQuip chill table (Rancho Dominguez, CA) set at 4° C. Using a #1702 Gastight Hamilton syringe mounted onto a Hamilton PB600 repeating dispenser (Reno, NV), 0.5 µl of the test chemical was applied to the dorsal thorax of each insect, with a final dose of 3.125 µg per insect. After treatment, mosquitoes were placed in 3.5 oz (0.10 l) plastic cups, supplied with 10% sucrose solution, and maintained at 28° C and 80% relative humidity. All assays were performed in triplicate. Controls included negative (untreated), carrier (DMSO-acetone), and positive (permethrin).

Statistical analysis

The proportion of ticks in the vertical filter paper bioassay that were repelled was modeled in the generalized linear models framework (as binomial proportions) using the R statistical software glm function, with estimated EC_{50} and EC_{95} values computed using the dose.p function in the MASS package. A straight line relationship between the proportion repelled on the logit scale and concentration was obtained after a power transformation on concentration for some essential oils. When results from high concentrations were dropped, sometimes the proportion repelled was slightly lower in the highest concentration than at a previous lower concentration. With relatively few concentrations used, this artificially affects the modeling. The same methodology was used to model the mosquito larvicidal assay. Permethrin was compared to the essential oils in the adult mosquito topical assay data (also in a generalized linear models framework).

RESULTS

Essential oil composition

Essential oils from leaves of *J. communis* and wood oils from *J. chinensis* and *C. funebris* were characterized and identified by gas chromatography and gas chromatography/mass spectrometry. The relative percentages of the constituents are listed in Table 1. Forty-seven (95.5% total oil), 46 (72.6%), and 55 (66.8%) compounds were identified from *J. communis*, *J. chinensis*, and *C. funebris* essential oils, respectively. The major components of each species were: α-pinene (26.9%), α-terpineol (14.0%), linalool (10.9%), limonene (6.2%), and β-pinene (5.2%) for *J. communis*; cuparene (11.3%), δ-cadinene (7.8%), and α-cedrene (4.8%) for *J. chinensis*; α-cedrene (16.9%), cedrol (7.6%), β-cedrene (5.7%), and cuparene (5.4%) for *C. funebris*.

Tick repellency

All three essential oils were repellent to *A. americanum* and *I. scapularis* nymphs but were effective against *I. scapularis* nymphs at a much lower concentration than those needed to repel similar percentages of *A. americanum*. Figure 1 depicts dose-related responses of *A. americanum* nymphs to the three oils and to deet. The EC_{95} values of *C. funebris*, *J. chinensis*, and *J. communis* are 0.43, 0.92, and 0.51 mg oil/cm^2 filter paper, respectively, whereas the EC_{95} for deet is 0.68 mg oil/cm^2 filter paper (Table 2). Among the oils, the EC_{50} of *J. communis* (0.288 mg oil/cm^2 filter paper) was the highest, but that of deet was 0.394 (Figure 1). *Cupressus funebris* oil, at 0.103 mg oil/cm^2 filter paper, repelled all *I. scapularis* nymphs, and the same concentration of *J. chinensis* and *J. communis* oils repelled 90% and 73.3% respectively.

The oils of *C. funebris* and *J. chinensis*, at 0.827 mg oil/cm^2 filter paper, repelled ≥80% *A. americanum* 4 h after application (Table 3). Although repellent activity for both oils remained at 6 h post-application, it clearly showed diminished effectiveness.

Mosquito repellency

Neither the oil of *C. funebris* nor that of *J. chinensis* consistently repelled female *Ae. aegypti* mosquitoes from biting through cloth treated at the highest dosage tested (1.500 mg/cm^2). However, the oil of *J. communis* had a MED of 0.057 ± 0.013 mg/cm^2 (Table 4).

Mosquito toxicity

Mortalities of 100, 80, and 67% at 250 ppm and 80, 27, and 40% at 125 ppm in *Ae. aegypti* larvae were observed for *J. chinensis*, *C. funebris*, and *J. communis* oils, respectively. The concentrations required to produce these levels of mortality by these oils are significantly higher than the 0.25 ppb of the commonly used insecticide, permethrin

(synthetic pyrethroid), which produced 67% (SEM 5.2%) mortality. The LD_{50}s and LD_{95}s of the oils and deet for *Ae. aegypti* larvae are presented in Table 5. Mortality of adult *Ae. aegypti* was also negligible, 3.3% at a dose of 3.125 ug oil/insect, for all three oils.

DISCUSSION

α-Pinene predominates in most of the leaf oils of *J. communis* from countries of the eastern hemisphere (Butkiene et al. 2006). In contrast, major components of the oil of *J. chinensis* that we analyzed were cuparene (11.3%), δ-cadinene (7.8%), α-cedrene (4.8%), and cedrol (3.2%). The main components of wood essential oils of *J. chinensis* cultivated at Waco, TX, and collected from Japan were found by Adams and Li (2008) to be *cis*-thujopsene (28.4, 8.4%), cedrol (13.7, 39.4%), widrol (9.2, 2.0%), α-cedrene (3.6, 3.1%), and β-cedrene (3.5, 0.6%), respectively. These differences could be due to distillation variation or to ecological or climatic variables associated with the localities where the plants grew.

Cupressus funebris wood oil is characterized by the occurrence of four components: cedrol, α-cedrene, β-cedrene, and thujopsene. In our study, α-cedrene (16.9%), cedrol (7.6%), and β-cedrene (5.7%) were the major constituents of *C. funebris* oil. The chemical composition of our samples resembled those of samples originating in China or Vietnam (Adams and Li 2008, Duquesnoy et al.

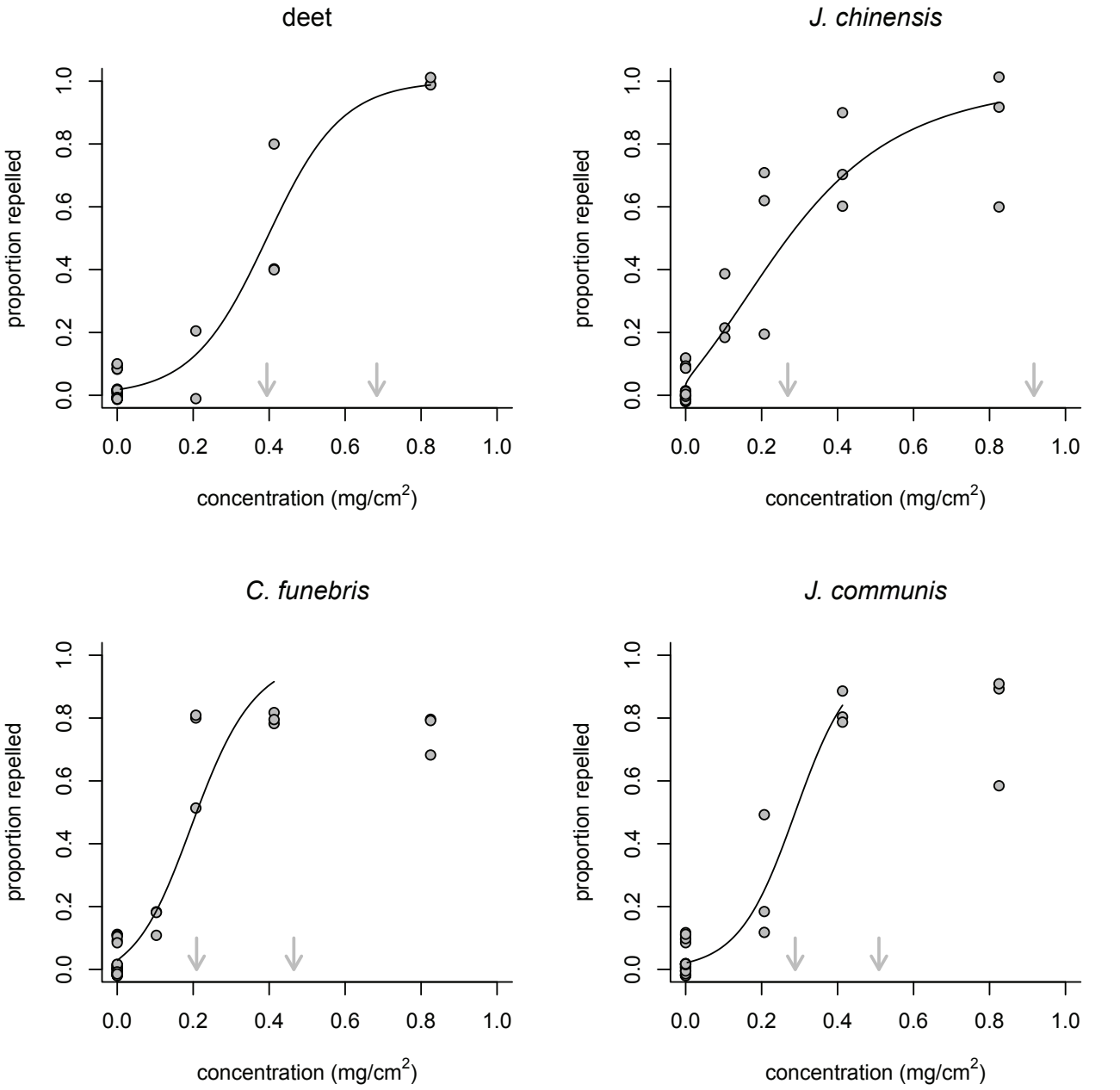

Figure 1. Responses of *A. americanum* nymphs to the essential oils of *C. funebris*, *J. chinensis*, and *J. communis*, deet, and an ethanol control (concentration of zero) in vertical filter paper bioassays. EC_{50} and EC_{95} indicated by arrows.

Table 1. Composition of essential oils of *Juniperus communis, J. chinensis* and *Cupressus funebris.*

RRI	Compound	Jc %	Jch %	Cf %
1014	Tricyclene	0.1		
1032	α-Pinene	27.0	0.1	
1035	α-Thujene	0.2		
1076	Camphene	1.2		
1118	β-Pinene	5.2	Tr	
1132	Sabinene	1.2		
1174	Myrcene	2.5		
1203	Limonene	6.2	0.1	
1213	1,8-Cineole	0.7		
1280	*p*-Cymene	0.8		
1384	α-Pinene oxide	0.8		
1406	α-Fenchone	0.1		
1429	Perillen	0.3		
1450	*trans*-Linalool oxide (*Furanoid*)	0.3		
1466	α-Cubebene		0.1	
1478	*cis*-Linalool oxide (*Furanoid*)	0.4		
1493	α-Ylangene		0.1	
1497	α-Copaene	0.4	0.4	
1500	Pentadecane			Tr
1513	Longicyclene	0.2		
1519	1,7-Diepi-α-Cedrene (=α-Funebrene)		0.1	0.4
1532	Camphor	2.2		
1538	7,8-Dihydrolinalool	0.1		
1541	Modhephene		0.1	
1553	Linalool	10.9		
1562	Isopinocamphone	0.1		
1568	*trans*-α-Bergamotene			0.1
1577	α-Cedrene		4.8	16.9
1583	Terpinen-1-ol	0.5		
1583	Longifolene (=*Junipene*)	2.5		
1591	Bornyl acetate	2.1		
1594	1,7-Diepi-β-cedrene (=β-Funebrene)		0.3	1.4
1600	β-Elemene		1.2	0.5
1611	Terpinen-4-ol	1.6		
1612	β-Caryophyllene		2.0	1.0
1613	β-Cedrene		1.1	5.7
1641	*cis*-β-Terpineol	1.9		
1644	Widdrene (=*Thujopsene*)		2.2	
1648	Myrtenal	0.5		
1661	Alloaromadendrene			0.4
1670	*trans*-Pinocarveol	0.6		
1677	*epi*-Zonarene		1.1	
1682	δ-terpineol	0.5		
1687	α- humulene	0.6	0.3	0.2
1688	Selina-4,11-diene (=*4,11-Eudesmadiene*)		0.4	0.9
1690	α-Acoradiene		0.2	0.4
1690	*trans*-β-terpineol	0.9		
1693	β-Acoradiene		0.3	1.2
1704	γ-Muurolene	0.3	0.8	1.4
1706	α-Terpineol	14.0		
1715	γ-Terpineol	1.4		
1718	β-Alaskene		0.4	1.0
1719	Borneol	0.6		

Continued on next page

Table 1. Continued.

RR1	Compound	Jc %	Jch %	Cf %
1725	Verbenone	0.3		
1725	β-=Chamigrene			0.9
1729	Zonarene		0.4	0.5
1740	α-Muurolene	0.4	1.6	2.0
1742	β-Selinene		0.8	1.5
1744	α-Selinene		1.0	1.7
1747	α-Alaskene		0.7	3.3
1751	Carvone	0.2		
1759	α-Cuprenene		0.3	1.1
1762	α-Chamigrene		0.4	0.6
1771	γ-Bisabolene			1.2
1773	δ-Cadinene		7.8	2.7
1776	γ-Cadinene	0.3	0.4	0.9
1786	*ar*-Curcumene		1.0	2.5
1801	β-Cuprenene		0.8	2.0
1804	Myrtenol	0.4		
1807	α-Cadinene		0.3	0.5
1845	*trans*-Carveol	0.2		
1849	Cuparene		11.3	5.4
1849	Calamenene		4.3	1.0
1864	*p*-Cymen-8-ol	0.4		
1918	β-Calacorene		0.7	0.1
1941	α-Calacorene		2.7	1.5
1949	(Z)-3-Hexenyl nonanoate	0.5		
2001	Isocaryophyllene oxide	0.8		
2008	Caryophyllene oxide	2.6		
2050	(*E*)-Nerolidol			0.1
2051	Gleenol		1.2	0.2
2071	Humulene epoxide-II	0.5		
2080	Cubenol		1.3	0.3
2080	Junenol (=Eudesm-4(15)-en-6-ol)			0.1
2088	1-*epi*-Cubenol		2.8	0.3
2143	Cedrol		3.2	7.6
2178	Widdrol			0.8
2185	γ-Eudesmol			0.8
2200	Pimara-8,15-diene		0.6	
2209	T-Muurolol		0.9	0.3
2217	α-Cedrenal		0.1	0.2
2219	δ-Cadinol (=alpha-muurolol)		0.6	
2232	α-Bisabolol		1.3	0.5
2250	α-Eudesmol			0.1
2255	α-Cadinol	0.1		
2256	Cadalene		1.8	0.4
2357	14-Hydroxy-β-caryophyllene		0.6	
2373	14-Oxo-α-muurolene		0.5	
2478	14-Hydroxy-α-humulene		0.4	
2501	8,13-Abietadiene		0.1	
2524	Abietatriene		0.2	
2568	14-Hydroxy-α-muurolene		0.3	
2607	14-Hydroxy-δ-cadinene		0.2	
2694	14-Hydroxy-calamenene		0.1	
	Total	95.5	66.8	72.6

Jc: *J communis;* Jch: *J. chinensis;* Cf: *C. funebris;* RRI: Relative retention indices calculated against n-alkanes; % calculated from FID data; Tr: trace (< 0.1 %).

Table 2. Concentrations of essential oils of *J. chinensis*, *J. communis*, *C. funebris,* and deet estimated to repel 50 and 95% of *A. americanum* nymphs in vertical filter paper bioassays.

Repellent	Concentration[a] (Power Transformation)	95% CI	Intercept (±SE)	Slope (±SE)
J. communis	EC_{50}0.288 (none)	0.243 – 0.334	-3.855 (±0.375)	13.362 (±1.544)
	EC_{95} 0.509 (none)	0.424 - 0.594		
J. chinensis	EC_{50} 0.268 (sq rt)	0.206 – 0.331	-3.477 (±0.305)	6.706 (±0.662)
	EC_{95} 0.917 (sq rt)	0.788 – 1.046		
C. funebris	EC_{50} 0.209 (none)	0.165 – 0.253	-3. 582 (±0.323)	11.589 (±1.258)
	EC_{95} 0.465 (none)	0.424 – 0.594		
Deet	EC_{50} 0.394 (none)	0.333 – 0.455	-4.011 (±0.403)	10.179 (±1.241)
	EC_{95} 0.683 (none)	0.567 – 0.800		

[a]Concentrations as mg oil or deet/cm^2 filter paper.

Table 3. Percent of *A. americanum* nymphs repelled by *C. funebris* and *J. chinensis* essential oils, and deet at 0.827 mg/cm^2 filter paper and ethanol control 2, 4, and 6 h after test solutions were applied to filter paper.

	2 h[a]	4 h[a]	6 h[b]
	% (mean ±SD)[c]	% (mean ± SD)	% (mean ± SD)
C. funebris oil	90.00 (9.00±1.41)	82.50 (8.25±1.26)	67.67 (6.67±2.31)
J. chinensis oil	95.00 (9.50±1.00)	80.00 (8.00±3.37)	46.67 (4.67±1.16)
Deet	97.50 (9.75±0.50)	97.50 (9.75±0.50)	90.00 (9.00±1.00)
Ethanol	5.00 (0.50±0.58)	0 (0±0)	3.33 (0.33±0.58)

[a]Four replicates tested. [b]Three replicates tested. [c]Mean per replicate of ten ticks.

Table 4. Minimum effective doses (MED)[a] of *J. chinensis*, *J. communis*, and *C. funebris* essential oils tested on human volunteers against *Ae. aegypti* mosquitoes.

Volunteer no.	*J. chinensis*	*J. communis*	*C. funebris*
1	>1.5[b]	0.011	>1.5
1	>1.5	0.047	>1.5
2	>1.5	0.047	>1.5
2	0.094	0.047	>1.5
3	>1.5	0.094	>1.5
4[c]	0.187	0.094	>1.5

[a]MED in mg/cm^2. [b]If ≥5 bites in 1 min exposure to 500 mosquitoes then that compound/dose was considered a failure. [c]Volunteer 4 was a female, the other three volunteers males (volunteers 1 and 2 tested twice).

Table 5. Doses of essential oils of *J. communis*, *C. funebris,* and permethrin estimated to kill 50 and 95% of *Ae. aegypti* larvae. There were not enough intermediate concentrations for *J. chinensis* to calculate the ED_{50} and ED_{95}. Intercept and slope values (±SE) are given for a generalized linear model (with proportion, the dependent variable, on the logit scale).

Toxicant	Concentration[a] (Power Transformation)	95% CI	Intercept (±SE)	Slope (±SE)
J. communis	LD_{50} 163.6 ppm (sqrt)	120.2 – 213.7	-4.596 (±0.870)	0.359 (±0.071)
	LD_{95} 440.3 ppm (sqrt)	282.6 – 632.9		
C. funebris	LD_{50} 263.2 ppm (none)	200.5 – 325.8	-3.726 (±0.718)	0.021 (±0.004)
	LD_{95} 298.9 ppm (none)	229.8 – 368.0		
Permethrin	LD_{50} 0.1894 ppb (sqrt)	0.146 – 0.239	-6.279 (±1.461)	14.426 (±3.381)
	LD_{95} 0.4088 ppb (sqrt)	0.273 – 0.571		

[a]Ten larvae tested for each of six concentrations for each oil, and seven concentrations for permethrin.

2006).

Among the major constituents of the three oils, α-terpineol (14.0% *J. communis*) and linalool (10.9% *J. communis*) are known to repel *A. americanum* nymphs, as do carveol and carvone, less plentiful *J. communis* components (Weldon et al. 2011). Terpinen-4-ol (1.6% in *J. communis*) has been found to be somewhat repellent to *I. scapularis* (Dietrich et al. 2006) and *A. americanum* nymphs (Weldon et al. 2011).

The oils of *C. funebris* and *J. communis* are comparable to deet in their effectiveness in repelling *A. americanum*, but have steeper dose response curves than deet and *J. chinensis* oil (Figure 1) and lower EC_{50}s and EC_{95}s than deet. The only oils, *C. funebris* and *J. chinensis*, tested for duration of repellency were effective (≥80% *A. americanum* repelled) for 4 h after application. Deet retained 90% repellency at 6 h post-application, at which time *C. funebris* and *J. chinensis* oils had declined to 67.7 and 46.7% repellency. The oils are clearly not ephemeral and formulation chemistry can extend the longevity of repellent activity should they be developed into commercial repellent products.

The tendency of *I. scapularis* nymphs to be repelled by considerably lower concentrations of repellent than *A. americanum* nymphs has been reported for several chemicals (Carroll et al. 2004, 2005, 2007), and was again observed in our bioassays with the three oils.

Because *C. funebris* and *J. chinensis* oils were not efficacious as repellents or toxicants against *Ae. aegypti* at the highest concentrations, neither of these oils nor their major constituents merit additional examination as repellents or insecticides for *Ae. aegypti*. The oil of *C. funebris* was one of 38 oils tested for repellency against *Ae. aegypti* (Trongtokit et al. 2005), and found to be ineffective at 30 min post-application for 10% and 50% dilutions; there was only one case where repellency was noted at 30 min post-application. Neither of the *Juniperus* species reported here was tested by Trongtokit (2005). Amer and Mehlkorn (2006) reported that in a study with human volunteers, the essential oil of *J. communis* (plant source Austria) repelled 43.2% of *Ae. aegypti* at 210 min after application.

In our study, the oil of *J. communis* (0.057 ± 0.013 mg/cm^2) was not quite as repellent as technical (85%) deet, whose MED typically ranged from 0.005-0.023 mg/cm^2 for volunteers participating in recent studies by Tabanca et al. (2010). Just over 50% of the composition of the *J. communis* oil is α-pinene (27%), α-terpineol (14%), and linalool (11%). Linalool has been well characterized as a repellent and attraction-inhibitor of mosquitoes (Hwang et al. 1985, Traboulsi et al. 2005, Park et al. 2005, Kline et al. 2003). Singh et al. (1984) reported the toxicity of cedarwood oil to mosquitoes. However, they tested oil from the deodar or Himalayan cedar, *Cedrus deodara* Roxb., which is classified in the Pinaceae, whereas *C. funebris*, *J. communis*, and *J. chinensis* are in the Cupressaceae. Both the Pinaceae and Cupressaceae belong to the order Pinales.

Perhaps most encouraging is that an oil (*J. communis*) comprising multiple compounds exhibited a MED against a mosquito, *Ae. aegypti*, and an EC_{95} against a tick, *A. americanum*, that resembled the values obtained for a single pure synthetic repellent (deet). This indicates the presence of at least one good repellent and probably multiple repellent compounds that act synergistically to produce the observed repellency. Panella et al. (1997) tested extracts from six species of the Cupressaceae for toxicity to *I. scapularis* larvae and nymphs, and found that the extract from Alaska yellow cedar, *Chamaecyparis nootkatensis* (D. Don) Spach, was the most effective extract against larvae, and the extract from eastern red cedar, *Juniperus virginiana* L., most effective against nymphs. Among the compounds identified from Alaska yellow cedar essential oil, the sesquiterpene nootkatone and monoterpene carvacrol were found notably toxic to *I. scapularis* nymphs (Panella et al. 2005). Dietrich et al. (2006) found that nootkatone, and carvacrol were similar to deet in their capacity to repel *I. scapularis*. In field tests, nootkatone effectively suppressed populations of *I. scapularis* and *A. americanum* (Dolan et al. 2009). The progress made in developing nootkatone and carvacrol as anti-tick compounds (Dolan et al. 2009) is an incentive for continued research on the constituent compounds of *J. communis*, and for further efforts in repellent and toxicant discovery among the essential oils (and constituents) of Cupressaceae.

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