SHORT COMMUNICATION

Biocidal Activity of Three Wood Essential Oils Against *Ixodes scapularis* (Acari: Ixodidae), *Xenopsylla cheopis* (Siphonaptera: Pulicidae), and *Aedes aegypti* (Diptera: Culicidae)

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J. Econ. Entomol. 100(2): 622–625 (2007)

ABSTRACT The biocidal activity of three steam distilled wood essential oils—incense cedar, *Calocedrus decurrens* (Torr.) Florin; Port-Orford-cedar, *Chamaecyparis lawsoniana* (A. Murr.) Parl.; and western juniper, *Juniperus occidentalis* (Hook)—were evaluated against adult *Aedes aegypti* (L.) (Diptera: Culicidae) and *Xenopsylla cheopis* (Rothchild) (Siphonaptera: Pulicidae) and nymphal *Ixodes scapularis* Say (Acari: Ixodidae). In vitro laboratory bioassays were conducted to establish baseline dose–mortality data through 24 h. Incense cedar heartwood was the most toxic to all three vector species followed in order of activity by western juniper and Port-Orford-cedar based on LC_{50} and LC_{90} values. *Ae. aegypti* were substantially more susceptible to the oils than either *I. scapularis* or *X. cheopis*.

KEY WORDS essential oils, biocidal, *Ixodes scapularis*, *Xenopsylla cheopis*, *Aedes aegypti*

Arthropods are responsible for transmitting numerous etiological agents of public health importance throughout the world. In North America, the blacklegged tick, also known as the deer tick, *Ixodes scapularis* Say (Acari: Ixodidae), is the principal vector of Lyme disease spirochetes as well as the agents causing human granulocytic anaplasmosis and human babesiosis (Lane et al. 1991, Goodman et al. 1996, Stafford et al. 1999). Fleas transmit the plague bacteria and mosquitoes transmit West Nile virus and other arboviruses within the United States (Gage 1998, Komar 2003). In other more tropical regions of the world, the mosquito is a dangerous entity to human health, spreading malaria, yellow, and dengue fever (Gubler 2001).

The focus of present pest control operations to reduce transmission of vector-borne infectious diseases includes primarily areawide applications of synthetic pesticides. Whereas effective, the application of synthetic pesticides is not widely accepted due to growing public concerns of adverse environmental effects, toxicity, impact on nontarget organisms, and increased health problems associated with areawide spraying (Schulze et al. 2001, Chaton et al. 2002). The principal control agents used by pest management professionals and homeowners for ticks include liquid and granular formulations of carbaryl, cyfluthrin, diazinon, and chlorpyrifos (Schulze et al. 1991, Stafford 1991). The number of registered pesticides for use, however, was reduced when both diazinon and chlorpyrifos were removed from the market in 2004 due to health risks in humans and wildlife. For mosquito control, sumethrin and resmethrin are often used. Both of these pesticides are pyrethroid derivatives, which pose a lower health risk to humans overall, but they have come under scrutiny for being linked to breast cancer, endocrine disruptions, neurological damage, and childhood cancers (Matsumara 1975, Macan et al. 2006). Additionally, mosquito populations develop resistance to pesticides over time, decreasing susceptibility (Liu et al. 2005).

Steam distillation of plant essential oils has a long history of use in fragrances and flavorings in the perfume/cosmetic and food industries. Recently, essential oils have been suggested as an alternative source of materials for arthropod control due to their broad source of bioactive constituents (Isman 2006). This discovery has prompted focused research efforts on natural product extracts from plants as potential sources of commercial pest control agents. Well-documented records support this as evidenced by the nearly 276 registered plant species used as pesticides in China and Western Europe (Ibrahim et al. 2001). Some of the major botanical products currently used for insect control include pyrethrum, neem, and essential oils (Isman 2006). Panella et al. (1997) reported on the acaricidal activity of 13 essential oils extracted from various botanical species against immature *I. scapularis*, with Alaska yellow cedar, *Chamaecyparis nootkatensis* (D. Don) Spach., being the most effec-

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tive. In a subsequent study, Panella et al. (2005) isolated the individual compounds that make up the essential oil of Alaska yellow cedar and discovered many of the compounds had acaricidal activity >10 times that of the essential oil.

The current study was undertaken to determine the susceptibility of three medically important arthropods, *Aedes aegypti* (L.) mosquitoes, *I. scapularis* ticks, and *Xenopsylla cheopis* (Rothchild) fleas, to steam distilled essential oils extracted from the woods of incense cedar (*Calocedrus decurrens*) (Torr.) Florin, Port-Orford-cedar (*Chamaecyparis lawsoniana*) (A. Murr.) Parl., and western juniper (*Juniperus occidentalis*) (Hook), heartwood.

Materials and Methods

Crude Plant Extracts. Steam distilled essential oils were prepared from the ground heartwoods of incense cedar and western juniper. A commercial sample of Port-Orford-cedar essential oil prepared from wood shavings of downed logs was obtained from Rose City Archery, Myrtle Point, OR (Tucker et al. 2000). The oils were stored in glass vials in the dark at 4°C.

Tick Colonies. To avoid age-related variations in susceptibility to the essential oils being evaluated, nymphal *I. scapularis* (12–16 wk old) were used in all trials (Mount et al. 1970). Nymphal ticks were F_1 offspring of adult *I. scapularis* collected at Naval Weapons Station, Earle, NJ. Ticks were maintained at 21°C, 90% RH, and exposed to a photoperiod of 16:8 (L:D) h as described previously (Piesman 1993).

Flea Colonies. Adult *X. cheopis* (1–3 wk old) were used in all trials. This colony has been maintained for >8 yr, originating from adults received from Tom Schwan, Rocky Mountain Laboratories, Hamilton, MT. Colonies were maintained in glass jars containing a 4:1:1:1 ratio of sawdust, dried cow blood, powdered milk, and powdered mouse chow and were held at 23°C, 85% RH, and reared under a 24-h dark cycle.

Mosquito Colonies. Adult *Ae. aegypti* were obtained from an existing colony at the Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Fort Collins, CO. This colony has been maintained for >15 yr with no history of pesticide exposure. Mosquitoes were reared at 28°C, 85% RH, and a photoperiod of 14:10 (L:D) h. Larvae were reared in deionized water and fed ground liver powder solution ad libitum. Pupae and fourth instars were removed and placed in emergence cages, and adults were fed a 2% sucrose solution until assayed. Adults were exposed to test products at 5–7 d after emergence.

Tick and Flea Bioassays. Concentrations of the essential oils were prepared by two-fold serial dilutions of a 0.5% (wt:vol) solution of the extracts in acetone as described previously (Panella et al. 2005). Toxicity of individual oils was determined with a total of nine doses ranging from 0.002 to 0.5%. All three essential oils were run three times in duplicate for a total of six trials per essential oil. A control treated with acetone only was run with each series. Modifications made to the disposable pipet method reported by Panella et al. (2005) were used to evaluate tick and flea susceptibility.

Groups of 10 nymphal *I. scapularis* and adult *X. cheopis* were used in all tests. The inner surfaces of 2-dram friction cap vials (Kimbal, Vineland, NJ) were treated with an extract–acetone solution and left to air dry overnight as described previously by Panella et al. (2005). Three to four holes were made in the plastic cap for air exchange. Either ticks or fleas were introduced into vials by using forceps and vials were held in glass desiccators containing distilled water for 24 h at 21°C and 90% RH. Morbidity and mortality were recorded at 24 h. After 24 h, ticks and fleas were considered alive if they exhibited normal behavior when breathed upon or physically stimulated with wooden dowels. For each time point, if ticks and fleas were incapable of movement, maintaining normal posture, leg coordination, ability to right themselves, or any signs of life, they were considered moribund or dead. Efficacy of individual compounds was determined by calculating lethal concentration 50% (LC_{50}) and 90% (LC_{90}) using probit analysis.

Mosquito Bioassay. Modifications made from bottle bioassay methods developed by Brogdon and McAllister (1998) were used for adult testing. Natural product extracts were two-fold serially diluted for a total of nine concentrations ranging from 0.002 to 0.5% in 1.5 ml of acetone and added to 250-ml Wheaton glass bottles as described previously (Panella et al. 1997). Twenty-five to 50 adult male and female *Ae. aegypti* mosquitoes, 5–7 d old, were aspirated into the bottles. Mosquitoes were held in bottles for 24 h at 23°C and 85% RH. Morbidity and mortality were recorded at 24 h (total of six trials for each essential oil). One bottle for each trial was treated with acetone only and served as the control. The dose–mortality data were evaluated by probit analysis to determine LC_{50} and LC_{90} values.

Statistical Analyses. Dose–mortality (LC) data were evaluated using probit analysis (LdP line, copyright 2000 by Ehab Mostofa Bakr, Cairo, Egypt). The modified Abbott's formula was used for primary comparisons between LC_{50} values obtained for all three oils at 24 h. Statistical comparisons (chi-square) were based on observations for LC_{50} made at 24 h to determine significance ($P \leq 0.05$) between the three oils and susceptibility among the three test species (Panella et al. 2005).

Results

The susceptibility of adult *Ae. aegypti,* nymphal *I. scapularis,* and adult *X. cheopis* to three essential oils extracted from the woods of incense cedar, Port-Orford-cedar, and western juniper are presented in Table 1. The biocidal activity of the three essential oils was evaluated at 24 h in multiple trials at a dose range of 0.02–5.0 μg/ml by using probit analysis. Although all three oils exhibited some level of biocidal activity, on the basis of LC_{50} and LC_{90} values, incense cedar was

Table 1. Susceptibility of mosquitoes, ticks, and fleas to three wood-derived essential oils after 24-h exposure

Test species	Essential oil	Slope ± SEM	Concn (mg/ml)			
			LC_{50}	95% CI	LC_{90}	95% CI
Ae. aegypti[b]	Incense	4.28 ± 0.31	0.005[a]	0.002, 0.007	0.047	0.039, 0.054
	Port-Orford	1.69 ± 0.91	0.051	0.003, 0.06	0.17	0.08, 0.24
	Western juniper	2.32 ± 1.12	0.041	0.02, 0.05	0.11	0.09, 0.21
I. scapularis	Incense	2.69 ± 0.24	0.096[a]	0.079, 0.1	0.29	0.23, 0.38
	Port-Orford	1.32 ± 0.12	0.31	0.16, 0.53	2.68	1.75, 7.79
	Western juniper	1.39 ± 0.12	0.29	0.24, 0.4	2.58	1.72, 4.43
X. cheopis	Incense	3.41 ± 0.31	0.24[a]	0.09, 0.27	0.31	0.19, 0.39
	Port-Orford	6.11 ± 0.81	1.21	1.01, 1.34	1.85	1.61, 3.34
	Western juniper	11.6 ± 1.65	0.31	0.22, 0.26	0.93	0.28, 2.31

[a] LC_{50} values for incense cedar are statistically significant (chi-square test; $P < 0.001$) compared with LC_{50} values for Port-Orford cedar and western juniper generated against *Ae. agypti*, *I. scapularis*, and *X. cheopis*. The single instance where it is not significant is LC_{50} value (0.24) for incense cedar compared with western juniper (0.31) against *X. cheopis* (chi-square test; $P < 0.1$).

[b] *Ae. aegypti*, most susceptible among the test species to the three essential oils (chi-square test; $P < 0.001$).

the most toxic to all three arthropod test species, followed by western juniper and Port-Orford-cedar.

Incense cedar was >10× more effective than Port-Orford-cedar ($LC_{50} = 5 \times 10^{-3}$ and 5.1×10^{-2} µg/ml, respectively) against adult *Ae. aegypti* ($P \leq 0.001$). Incense cedar was also five times more potent against adult *X. cheopis* ($LC_{50} = 2.4 \times 10^{-1}$ µg/ml) than the least effective candidate, Port-Orford-cedar (LC_{50} = 1.2 µg/ml). Similar results were observed for nymphal *I. scapularis.* Incense cedar yielded an LC_{50} of 9.6×10^{-2} µg/ml compared with Port-Orford-cedar, $LC_{50} = 3.1 \times 10^{-1}$ µg/ml (>3 times less toxic; $P \leq 0.001$). Finally, *Ae. aegypti* were significantly ($P \leq 0.001$) more susceptible to the oils than either *I. scapularis* or *X. cheopis.*

Discussion

The wood-derived essential oils that were evaluated in this study have potential for controlling medically important arthropod species such as mosquitoes, ticks, and fleas. In addition, the prospective of these oils to be environmentally friendly, have less impact on non-target organisms, and have application strategies by using the same accepted methods as current commercial insecticides are intriguing. Many essential oils are known to exhibit repellent, antifeeding, and insecticidal activities against various arthropod species (Isman 1999, 2000, 2002). As an example, neem, *Azadirachta indica* A. Juss, oil has been linked to a number of biological activities, including feeding and oviposition deterrent, repellent, growth disruptor, fitness reducer, and sterilant in several species of insects (Schmutterer 1990, Kilonzo 1991, Naumann and Isman 1995). Additionally, Yang et al. (2004) reported on the pediculocidal activity of 54 plant essential oils against the head louse, *Pediculus humanus capitis* De Geer. Panella et al. (1997) reported on the biocidal activity of essential oils against *I. scapularis* by using the disposable pipet assay. Of the 13 plant extracts evaluated, Alaska yellow cedar was the most effective against nymphal ticks (0.151%, wt:vol) with eastern red cedar showing the greatest activity against larval ticks (0.001%, wt:vol). In the current study, potencies varied according to the tree species. Whereas all three extracts exhibited favorable levels of biocidal activity, incense cedar was the most toxic to all three arthropod test species. The order of increasing susceptibility of these oils against mosquitoes, ticks, and fleas was Port-Orford-cedar < western juniper < incense cedar.

In the United States, the commercial development of plant essential oils as insecticides and repellents has been greatly facilitated by the exemption of many essential oils from the Environmental Protection Agency (EPA) registration process. These typically include those oils commonly used as a flavoring or fragrance additive in consumables (Quarles 1996, Isman 2006). Cedar wood oil, such as the oils examined in this study, is among the many exempted essential oils as a pesticide active ingredient by the EPA (Quarles 1996). As a result, interest in these products has spurred the research and development of essential oil based insecticides, fungicides, and herbicides (Isman 2006). Moreover, natural products based on plant essential oils as insecticides may result in a relatively low-cost production of active ingredients. In addition, the use of natural products as alternatives to more toxic commercial pesticides provides an attractive solution for residents with environmental concerns. Finally, alternative control agents with novel modes of action due to resistance as well as low mammalian toxicity and environmental impact are in need.

Results of this study demonstrate that wood-derived essential oils such as incense and Port-Orford-cedar and western juniper may be useful in controlling medically important species of mosquitoes, ticks, and fleas. To determine the practical use of these oils, further research to determine the active components, mode of action, and environmental and human health safety issues is necessary. The results presented in this report represent data obtained from strictly crude oils and extracts. The isolation and purification of the active constituents may result in a product that is many times more lethal. The wood of trees used to prepare these oils is available as forest products residues and by-products. All three trees represent a potential source for new bio-based materials such as arthropod biocides.

Acknowledgments

We thank Joe Piesman for critical review of the manuscript.

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Received 8 August 2006; accepted 20 October 2006.