Limited Efficacy of Commercially Formulated Essential Oils on Vegetation against Female *Aedes albopictus* and *Culex quinquefasciatus*[1]

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ABSTRACT To address inquiries from the general public and Florida mosquito control programs on the effectiveness of natural products to control resting adult mosquitoes on vegetation, we conducted a study that evaluated the toxicity of several commercially available products containing plant essential oils against female *Aedes albopictus* (Skuse) and *Culex quinquefasciatus* Say (Diptera: Culicidae) when applied to the leaves of a common Florida landscape shrub, the southern wax myrtle, *Myrica cerifera* L. (Myricaceae). The products tested were EcoExempt MC©, Misting System Concentrate©, Mosquito Barrier©, No-See-Um Organic Repellent©, and Orange Guard©. All products were applied at maximum label rates via a hand pump sprayer and compared with a commercial formulation of synergized pyrethrins, Riptide©, as a standard. Excised leaf bioassays revealed that the essential oil formulations provided less than 10% mortality to both mosquito species with essentially no residual activity beyond 24 h. Riptide provided significantly greater mortality, approximately 25% and 41%, at 24 h for *Ae. albopictus* and *Cx. quinquefasciatus*, respectively, but this product did not exhibit residual activity beyond 24 h on treated leaves. We conclude that vegetative surfaces treated with the above products containing essential oils would not prove sufficiently effective to control mosquitoes when compared with conventional pyrethroid insecticides labeled for the same purpose.

KEY WORDS *Aedes*, botanical insecticides, *Culex*, essential oil, mosquito control, *Myrica cerifera*, southern wax myrtle

The application of insecticides to perimeter vegetation for the purpose of providing a zone or "barrier" of protection against adult mosquitoes continues to spark interest among the general public and pest control industry. This is because treating the surface of vegetation and other areas where mosquitoes rest with a toxicant can reduce the need (and cost) of repeated area-wide space-spray applications of insecticides that must also coincide with mosquito flight activity to be effective. Several synthetic pyrethroid insecticides are labeled for application to vegetation, as adulticides, and provide a modicum of reduction over time (Perich et al. 1993, Cilek & Hallmon 2006, Trout et al. 2007, Cilek 2008). However, there is growing resistance to the use these of chemicals due to

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perceived adverse environmental or health effects (Maguranyi et al. 2009). Moreover, increasing demands by the general public for alternative “natural products” for arthropod pest control, particularly those derived from botanical materials, continues to stimulate research for use against a variety of public health and urban arthropod pests (Ngoh et al. 1998, Barnard 1999, Phillips et al. 2010). Evaluation of the insecticidal properties of plant essential oils against mosquitoes have usually been in the form of larvicidal bioassays (Jacobson 1958, Sukumar et al. 1991, Barnard 1999) with sparse data on effectiveness as adulticides, especially under field conditions (Kang et al. 2009). To address inquiries from the general public and Florida mosquito control programs on the effectiveness of natural products against mosquitoes resting on vegetation, we conducted a study that evaluated the toxicity of several commercially available products, containing essential oils, for the control of female *Aedes albopictus* (Skuse) and *Culex quinquefasciatus* Say (Diptera: Culicidae) when applied to a common Florida landscape shrub species, the southern wax myrtle, *Myrica cerifera* L. (Myricaceae). This plant species is a native to Florida and is commonly used in southern residential landscapes (Rose 1975).

Materials and Methods

Five commercial products were tested as aqueous solutions at maximum label rates under operational conditions using a pressurized hand-pump sprayer (RL Flowmaster©, Model 1025HD, Root-Lowell Manufacturing Co., Lowell, MI). EcoExempt MC© (rosemary oil, 18% active ingredient (AI); cinnamon oil, 2% AI; lemongrass oil, 2% AI; “other ingredients” in wintergreen oil, 78% AI) (EcoSMART Technologies, Inc., Franklin, TN), was applied at 9 ml/L. According to the manufacturer’s specifications, EcoExempt Emulsifier© must be added to EcoExempt MC© at a 1:1 ratio in order to disperse the product’s essential oils fully into solution with water. Misting System Concentrate© (MSC) (oil of *Juniperus virginana*, 85% AI) (CedarCide Industries, Inc., Spring, TX) was applied at 4 ml/L. Mosquito Barrier© (garlic oil, 99.3% AI; and citric acid, 0.5% AI) (Garlic Research Labs, Inc., Glendale, CA) was applied at 31.1 ml/L. No-See-Um Organic Repellent© [lemongrass, 4% AI; citronella, 3% AI; castor oil, 3% AI; sodium laurate, 3% AI; and garlic (1% AI) in a 86% AI mixture of wintergreen oil, lecithin, and water] (Repellex USA, Inc., Niles, MI) was applied at 99.5 ml/L. Orange Guard© (D-limonene, 5.8% AI) (Orange Guard, Inc., Carmel Valley, CA) was applied undiluted. All products were compared with a commercial formulation of synergized pyrethrins [Riptide©, pyrethrins (5% AI) and piperonyl butoxide (25% AI)] that was applied at 9 ml/L as a standard botanical product of known toxicity.

Each product was separately applied to groups of ten southern wax myrtle shrubs in black plastic pots (28 cm diam., 25 cm tall). Leaves of each plant were thoroughly wetted but not to the point of runoff. Total volume of liquid applied to each plant averaged 140.0 ± 7.6 ml. The study was conducted for two weeks from 21 July through 4 August 2009. No rainfall occurred until after treated leaves had been evaluated for the one-week post treatment period; however, 8.5 ml of rain was recorded later in that week. During the second week, 2.5 to 14 ml of precipitation was recorded over 4 days. Precipitation was recorded using a Lamotte© rain gauge reservoir (Grainger, Lake Forest, IL).

Excised leaf bioassays were used to determine the activity (i.e., mortality) of treated foliage to adult mosquitoes using the methods of Cilek & Hallmon (2006). Treated leaves were exposed singly to approximately 15, non-blood fed, three- to four-day old, laboratory-reared, insecticide-susceptible female *Ae. albopictus* and *Cx. quinquefasciatus*. These two mosquito species were used because of their pestiferous nature around homes and their global importance as vectors of West Nile and dengue virus (Mullen & Durden 2009). Both mosquito species have been in continuous colonization at the Public Health Entomology Research & Education Center for over 20 years. Larvae were fed *ad libitum* a slurry mixture of powered liver and brewer's yeast (3:2), and reared at an air temperature between 32–34°C under ambient light and humidity.

For the bioassays, one leaf was placed in a 250 ml glass beaker for testing (average leaf area 14.1 ± 0.5 cm^2). Using the methods of Cilek & Hallmon (2008), mosquitoes were mouth aspirated into each beaker previously secured with cloth screen mesh and a rubber band; beakers were then placed horizontally on the laboratory bench counter. Although each species was exposed in different beakers, leaves were sampled from the same location of the same plant to minimize differences in leaf residues due to application. Leaves and mosquitoes remained in the beakers for 24 hours at which time mortality was recorded. Controls consisted of a similar set of untreated leaves. Mosquitoes that remained on the bottom of a beaker, and could not fly, were recorded as dead. All treatments and controls were replicated five times. Mosquitoes had access to 10% sugar-water soaked cotton balls placed on the top of the screened beaker. Leaves were removed from plants at 4 h, 24 h, 1 wk, and 2 wks post-treatment and exposed to mosquitoes at those time intervals.

Because pyrethrins and several essential oils may have been shown to exhibit some repellency to adult mosquitoes (Wright & Burton 1969, Barnard 1999, Snow & Cutler 2006), the resting and landing behavior of each mosquito species in the bioassays was observed at 1 h and 24 h. Repellency was considered present if mosquitoes in the treatment beakers avoided continuous contact with treated leaves during the entire exposure period for either time interval. Although some folk remedies suggest that crushed southern wax myrtle leaves may repel some arthropod pests, no published data exist to substantiate these claims. Furthermore, we have not observed repellency of excised untreated wax myrtle leaves in our beaker bioassays conducted in the past (Cilek 2008, Cilek & Hallmon 2006, 2008). Moreover, Bidlingmayer (1974) previously reported this shrub as a resting plant for mosquitoes, while Cilek & Hallmon (2006, 2008) directly observed female *Ae albopictus* and *Cx. quinquefasciatus* using these plants as diurnal harborage sites during their vegetative barrier studies.

Statistical analysis. Mean percent mortality from leaf bioassays for each species [corrected for natural mortality by Abbott's (1925) formula] was subjected to ANOVA (after arcsine transformation to normalize distribution of percentage data) then followed by a Student-Newman-Keuls test to compare treatments for each species and time interval (Cochran & Cox 1957, SAS Institute 2002). Differences were considered significant at $P \leq 0.05$. Table data shows untransformed means.

Table 1. Mean ± SE percent mortality (at 24 h) of female *Aedes albopictus* and *Culex quinquefasciatus* to leaves of *Myrica cerifera* treated with either Riptide©, Misting System Concentrate©, EcoExempt MC©, Mosquito Barrier©, No-See-Um Organic Repellent©, or Orange Guard© in laboratory bioassays at various time periods post treatment.

Mosquito species	Insecticide treatment	Post-treatment mortality at			
		4 h	24 h	1 wk	2 wk
Aedes albopictus	Riptide	17.4 ± 3.9Aa[a]	24.7 ± 7.5Aa	0.0	0.0
	Misting System Concentrate	1.1 ± 1.1Ab	2.6 ± 1.6Ab	1.1 ± 1.1A	0.0
	EcoExempt MC	0.0 b	0.0 b	0.0	0.0
	Mosquito Barrier	0.0 b	0.0 b	0.0	0.0
	No-See-Um Organic Repellent	0.0 b	0.0 b	0.0	0.0
	Orange Guard	1.1 ± 1.1Ab	1.3 ± 1.3Ab	0.0	0.0
Culex quinquefasciatus	Riptide	18.3 ± 5.8Aa	40.7 ± 8.0Ba	0.0	0.0
	Misting System Concentrate	10.6 ± 1.1Ab	5.4 ± 2.0Ab	0.0	0.0
	EcoExempt MC	0.0 b	0.0 b	0.0	0.0
	Mosquito Barrier	0.0 b	0.0 b	0.0	0.0
	No-See-Um Organic Repellent	0.0 b	0.0 b	0.0	0.0
	Orange Guard	1.2 ± 1.2Ab	6.5 ± 2.5Ab	0.0	0.0

[a]Means in each row followed by a different uppercase letter are significantly different ($P \leq 0.05$), Student Newman-Keuls multiple mean test within each mosquito species. Means in each column (within species) followed by a different lowercase letter are significantly different ($P \leq 0.05$), Student Newman-Keuls multiple mean test within each mosquito species.

Results and Discussion

Riptide (synergized pyrethrins) was significantly more toxic to both mosquito species than any of the essential oil formulations (Table 1). However, mortality to Riptide in this bioassay was relatively low and did not exceed 25% for *Ae. albopictus* and 41% for *Cx. quinquefasciatus* at 24 h. MSC (oil of cedar) and Orange Guard were the only essential oil products that showed any toxicity to either species (Table 1). The poor performance of Orange Guard (less than 7% mortality) may be the result of the product containing only about 6% D-limonene. Hink & Fee (1986) reported that 92% mortality of adult cat flea, *Ctenocephalides felis* Bouché, could be achieved when exposed to 98% D-limonene on treated filter paper. In addition, Kassir et al. (1989) reported that this essential oil was toxic to *Cx. quinquefasciatus* larvae with an LC_{90} of 53.8 ppm. Interestingly, MSC, Orange Guard, and Riptide appeared to be slightly more toxic to *Cx. quinquefasciatus* than *Ae. albopictus* when exposed to treated leaves 24 h post treatment. For both mosquito species, no appreciable residual toxicity to any of the treatments was observed beyond 24 h with the exception of MSC, where about 1% mortality to *Ae. albopictus* occurred one week post treatment. Because there was no rainfall during the study until after the excised leaf bioassays had been conducted for the 1 week sample, we are confident that our results are an accurate reflection of the toxicokinetics of these products.

Although some of the essential oil products we evaluated may have some repellent properties (Wright & Burton 1969, Barnard 1999, Snow & Cutler 2006), we did not observe any repellency in the bioassays. Both mosquito species were observed to contact treated leaves repeatedly during the 1 h and 24 h exposure periods. As illustrated by Rutledge & Day (2009), we acknowledge that the mosquito species, the size of the exposure container, as well as the concentration and mode of delivery of the product are factors to consider when evaluating the manifestation of repellency of these materials. However, some botanical oils that exhibit repellent properties can also be toxic. For example, Xue et al. (2003) reported that commercial products with supposed repellent substances containing 0.1% or 10% citronella (one of the active ingredients in No-See-Um Organic Repellent) gave complete mortality to adult *Ae. albopictus* after direct spray application. Similarly, Kang et al. (2009) stated that oil of rosemary produced nearly 50% mortality to caged *Cx. pipiens pallens* Coquillett, while citronella and lemongrass oil gave only 20% kill, when all were applied in aerosol form at 1000 ppm; these oils were part of the active ingredients in EcoExempt MC and No-See-Um Organic Repellent in our study. Moreover, George et al. (2010) found that filter paper treated at a rate of 0.21 mg/cm^2 with garlic and lemongrass oil (the active ingredients in Mosquito Barrier and No-See-um Organic Repellent) provided complete mortality against adult poultry red mites, *Dermanyssus gallinae* (DeGeer). In addition, citronella and rosemary resulted in about 95% and 20% control, respectively, at the same rate for this species.

In summary, the commercial products containing essential oils applied to southern wax myrtle leaves at the rates in our study were not operationally useful to control resting female mosquitoes. Indeed, Kang et al. (2009) has stated that most essential oils are not effective against adult mosquitoes, primarily due to their high volatility and LD_{50}s compared with conventional adulticides. It should be pointed out that several synthetic pyrethroid insecticides, currently labeled for application to vegetation as barriers against mosquitoes, can be applied at much lower rates than essential oil formulations while achieving levels of greater than 80% mortality for up to 12 weeks on treated leaves (e.g., Perich et al. 1993, Cilek & Hallmon 2006, Trout et al. 2007, Cilek 2008). As a result, we conclude that vegetative surfaces treated with the botanical products we tested would not prove sufficiently effective against resting mosquitoes when compared with conventional pyrethroid insecticides labeled for the same purpose.

References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Barnard, D. R. 1999. Repellency of oils to mosquitoes. J. Med. Entomol. 36: 625–629.

Bidlingmayer, W. L. 1971. Mosquito flight paths in relation to the environment. I. Illumination levels, orientation, and resting areas. Ann. Entomol. Soc. 64:1121–1131.

Cilek, J. E. 2008. Application of insecticides to vegetation as barriers against host-seeking mosquitoes. J. Am. Mosq. Cont. Assoc. 24: 172–176.

Cilek, J. E. & C. F. Hallmon. 2008. Residual effectiveness of three pyrethroids on vegetation against adult *Aedes albopictus* and *Culex quinquefasciatus* in screened field cages. J. Am. Mosq. Cont. Assoc. 24: 263–269.

Cilek, J. E. & C. F. Hallmon. 2006. Residual effectiveness of pyrethroids-treated foliage against adult *Aedes albopictus* and *Culex quinquefasciatus* in screened field cages. J. Am. Mosq. Cont. Assoc. 22: 725–731.

Cochran, W. G. & G. M. Cox. 1957. Experimental designs, 2nd ed. John Wiley & Sons, New York, 617 pp.

George, D. R., O. A. E. Sparagano, G. Port, E. Okello, R. S. Shiel & J. H. Guy. 2010. Environmental interactions with the toxicity of plant essential oils to the poultry red mite *Dermanyssus gallinae*. Med. Vet. Entomol. 24: 1–8.

Hink, W. F. & B. J. Fee. 1986. Toxicity of D-limonene, the major component of citrus peel oil, to all life stages of the flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae). J. Med. Entomol. 23: 400–404.

Jacobson, M. 1958. Insecticides from plants: A review of the literature 1941–1953. USDA Handb., Washington DC, 154 pp.

Kang, S. H., M. K. Kim, D. J. Noh, C. Yoon & G.H. Kim. 2009. Spray adulticidal effects of plant oils against house mosquito, *Culex pipiens pallens* (Diptera: Culicidae). J. Pestic. Sci. 34: 100–106.

Kassir, J. T., A. H. Mohsen & N. S. Mehdi. 1989. Toxic effects of limonene against *Culex quinquefasciatus* Say larvae and its interference with oviposition. Anz. Schälingskde., Pflanzenschutz Umweltschutz 62: 19–21.

Maguranyi, S. K., C. E. Webb, S. Mansfield & R. C. Russel. 2009. Are commercially available essential oils from Australian native plants repellent to mosquitoes? J. Am. Mosq. Cont. Assoc. 25: 292–300.

Mullen, G. R. & L. A. Durden. 2009. Medical and veterinary entomology, 2nd ed. Academic Press, New York, 637 pp.

Ngoh, S. P., L. E. W. Choo, F. Y. Pang, Y. Huang, M. R. Kini & S. H. Ho. 1998. Insecticidal and repellent properties of nine volatile constituents of essential oils against the American cockroach, *Periplaneta americana* (L.). Pestic. Sci. 54: 261–268.

Perich, M. J., M. A. Tidwell, S. E. Dobson, M. R. Sardelis, A. Zaglul & D. C. Williams. 1993. Barrier spraying to control the malaria vector *Anopheles albimanus*: laboratory and field evaluation in the Dominican Republic. Med. Vet. Entomol. 7: 363–368.

Phillips, A. K., A. G. Appel & S. R. Sims. 2010. Topical toxicity of essential oils to the German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol. 103: 448–459.

Rose, S. A. 1975. Wax myrtle. Univ. Fla., Inst. Food Agric. Sci., Hort. Fact Sheet 13, 2 pp.

Rutledge, C. R. & J. F. Day. 2009. Mosquito repellents. Univ. Fla., Inst. Food Agric. Sci., EDIS #ENY-671. <http://edis.ifas.ufl.edu/in419>.

SAS Institute. 2002. SAS procedures guide, version 9. SAS Instit., Cary, North Carolina.

Snow, K. & R. Cutler. 2006. A preliminary note on the evaluation of garlic as a mosquito repellent. Europ. Mosq. Bull. 21: 23.

Sukumar, K. M., J. Perich & L. R. Boobar. 1991. Botanical derivatives in mosquito control: a review. J. Am. Mosq. Cont. Assoc. 7: 210–237.

Trout, R. T., G. C. Brown, M. F. Potter & J. L. Hubbard. 2007. Efficacy of two pyrethroid insecticides applied as barrier treatments for managing mosquito (Diptera: Culicidae) populations in suburban residential properties. J. Med. Entomol. 44: 470–477.

Wright, R. H. & D. J. Burton. 1969. Pyrethrum as a repellent. Pyrethrum Post 10: 14–16, 21.

Xue, R., A. Ali & D. R. Barnard. 2003. Laboratory evaluation of toxicity of 16 insect repellents in aerosol sprays to adult mosquitoes. J. Am. Mosq. Cont. Assoc. 19: 271–274.