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The Efficacy of Some Commercially Available Insect Repellents for *Aedes aegypti* (Diptera: Culicidae) and *Aedes albopictus* (Diptera: Culicidae)

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ABSTRACT. Reducing the number of host-vector interactions is an effective way to reduce the spread of vector-borne diseases. Repellents are widely used to protect humans from a variety of protozoans, viruses, and nematodes. DEET (N,N-Diethyl-meta-toluamide), a safe and effective repellent, was developed during World War II. Fear of possible side effects of DEET has created a large market for "natural" DEET-free repellents with a variety of active ingredients. We present a comparative study on the efficacy of eight commercially available products, two fragrances, and a vitamin B patch. The products were tested using a human hand as attractant in a Y-tube olfactometer setup with *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse), both major human disease vectors. We found that *Ae. albopictus* were generally less attracted to the test subject's hand compared with *Ae, aegypti*. Repellents with DEET as active ingredient had a prominent repellency effect over longer times and on both species. Repellents containing p-menthane-3,8-diol produced comparable results but for shorter time periods. Some of the DEET-free products containing citronella or geraniol did not have any significant repellency effect. Interestingly, the perfume we tested had a modest repellency effect early after application, and the vitamin B patch had no effect on either species. This study shows that the different active ingredients in commercially available mosquito repellent products are not equivalent in terms of duration and strength of repellency. Our results suggest that products containing DEET or p-menthane-3,8-diol have long-lasting repellent effects and therefore provide good protection from mosquito-borne diseases.

Key Words: repellents, *Aedes*, yellow fever mosquito, Asian tiger mosquito, DEET

Mosquitoes are hosts for an array of different protozoan parasites, nematodes, and viruses (Marquardt 2004, Enserink 2007, Murray et al. 2010). Controlling mosquito populations is an effective tool for the fight against such pathogens. Several different methods for mosquito control have been developed, e.g., source reduction, physical exclusion (nets, screens, etc.), pesticide application, biological control methods, sterile insect technique, and release of genetically modified mosquitoes (Rose 2001, Phuc et al. 2007, Alphey et al. 2010). Unfortunately and for a variety of reasons, these approaches can be difficult to implement in many locations (Peter et al. 2005). Widespread insecticide resistance in disease-carrying mosquito populations also poses a significant problem.

On an individual level, mosquito repellents are widely used to avoid disease exposure (Barnard 2000, Barnard and Xue 2004). Repellents, even though they can never guarantee complete protection, can significantly lessen the chance of contracting vector-borne diseases (Kahn et al. 1975, Barnard et al. 1998, Barnard and Xue 2004, Rowland et al. 2004, Hill et al. 2007). They are especially useful when used where human activity coincides with the diurnal activity patterns of mosquitoes, e.g., outdoor activities that take place at dusk and dawn, e.g., hunting and fishing.

The sense of smell is one of the most important senses that mosquitoes use for long range host seeking (Potter 2014). Insect olfaction has been extensively studied leading to the identification of the key proteins involved: odorant receptors, odorant receptor co-receptors, gustatory receptors, and odorant binding proteins (Kaupp 2010, Suh et al. 2014). The processing of olfactory information in different regions of the insect brain has also attracted a lot of research interest (Galizia 2014). Various mosquito attractants and repellents have been identified, many of which are produced by human metabolism or the bacterial degradation of sweat components. Lactic acid and 1-octen-3-ol are two components that act as strong mosquito attractants (Zwiebel and Takken 2004). Carbon dioxide from breath is another strong attractant that sensitizes mosquitoes to other odorants (Dekker et al. 2005). Studies have shown that different insect repellents use a similar mode-of-action. Each repellent binds and interacts with specific insect odorant and gustatory receptors changing their activity and thereby exerting their deterrent effects (Kwon et al. 2010, Dickens and Bohbot 2013, Xu et al. 2014). The most widely used insect repellent, DEET (N,N-diethyl-m-toluamide), has been in use for about 70 yr. DEET is considered a very safe repellent (Osimitz and Grothaus 1995, Koren et al. 2003, Sudakin and Trevathan 2003). Nevertheless, fear of possible side effects of DEET and general chemophobia has resulted in the development of a multitude of "DEET-free" mosquito repellents with a variety of active ingredients. Plant-based repellents usually contain essential plant oils as active ingredients.

There are several approaches for evaluating the efficacy of insect repellents. Some of the bioassays that have been used include spatial repellency assay, host attraction-inhibition assay, landing inhibition assay, effective dose and duration assays, taxis cage assays, etc. (Lorenz et al. 2013, Afify et al. 2014, Menger et al. 2014). Olfactometers are useful tools used in attraction-inhibition assays to test repellent efficacy. They allow the experimenter to perform the tests under very controlled conditions, thereby eliminating many variables that may alter experimental results in more open systems (McIndoo 1926).

Here, we report experiments performed with a Y-tube olfactometer that was constructed according to a blueprint published by the World

Health Organization in its publication "Guidelines for efficacy testing of spatial repellents" (World Health Organization [WHO] 2013). We performed host attraction-inhibition assays and tested the efficacy of eight commercially available mosquito repellent products, a perfume, a bath oil, and a vitamin B patch.

Materials and Methods

Mosquito Culture. *Ae. aegypti* UGAL strain and *Ae. albopictus* F10 strain were acquired from the Malaria Research and Reference Reagent Resource Center (ATCC 2015). Mosquito culture was executed as described in Marquardt(2004), using chicken for a blood meal source. Larvae were reared in 13" by 20" pans filled with 2 liters of deionized water held at 27°C. Larvae were given five dry cat food pellets (Special Kitty-Wal-Mart Stores, Inc. Bentonville, AR) every 3 d; water was changed after 5 d. The mosquitoes were allowed to mature for 5 d in a BugDorm-1 Insect Rearing Cage (30 by 30 by 30 cm, BugDorm Store, Taichung, Taiwan) before the experiment commenced. The adult mosquitoes were maintained on 20% sucrose solution, ad libitum, up to 24 h before the experiment began. The cages were placed in an incubation room that was maintained at 80% humidity and 27°C with a photoperiod of 14:10 (L:D) h. Mosquitoes were starved 24 h before each Y-tube assay.

Institutional Review Board Approval. This study has been approved by the New Mexico State University Institutional Review Board. Title: "Efficacy of different insect repellents," study no. 11505A.

Selection of Commercially Available Repellents. Repellents were purchased locally in Las Cruces, NM, or ordered online via Amazon. Table 1 lists the products tested, active ingredients, manufacturer and location, and the manufacturer's estimated protection times. The Mosquito Skin Patch was applied 2 h before the start of the experiment.

Attraction-Inhibition Assay. The Y-tube (see Fig. 1 and Supp Material 1 [online only]) was constructed according to the WHO schematic with modifications (WHO 2013). A constant air current was produced by a computer fan that was placed at the bottom of the Y-tube. The air flow was adjusted to 0.4 m/s in the base leg (0.2 m/s in each port) by placing the probe of an anemometer within the different shafts of the Y-tube and moving the fan in relation to the tube opening until the correct air flow speed was achieved. Experiments were all performed between 0800 and 1200 to avoid diurnal changes in mosquito activity. One of the co-authors was selected as the attractant for Y-tube assays based on preliminary attraction studies that found her to be a superior attractant. The volunteer was not allowed to wash her hands, wear perfume, or take a shower in the morning prior to the experiments. One of the attractant's hands was sprayed with approximately 0.5 ml of liquid repellent; the other hand was covered with a nitrile glove. The hand was sprayed on both sides and allowed to air dry. Trap doors 1 and 2 were opened and the mosquitoes were placed in the holding chamber of the Y-tube. The treated hand was then placed in one of the decision ports (from here on referred to as the "hand port"); the other, untreated gloved hand was inserted into the other port (control port). Alternating decision ports were used for the biological replicates to ensure there was no bias. The mosquitoes were given 30 s to acclimate to their environment while exposed to the odor on the hand before they were released from the holding chamber by opening trap door 3. The mosquitoes were given 2 min to relocate within the tube. After a 2-min period, all trap doors were closed. Three groups of mosquitoes were counted and recorded: the ones that stayed in the holding port, the ones that arrived in either decision port, and the ones that stayed in the shaft of the Y-tube. The mosquitoes that were not captured at either decision port or in the holding port were considered wandering. For each replicate, there were a total of 20 mosquitoes placed in the holding chamber. We evaluated efficacy of the repellent over a 4-h time period with evaluation points at: 0 min, 30 min, 120 min, and 240 min post application. The experiments commenced in the early morning and ran for 4 h, the time of commencement represented the laboratory mosquitoes' dawn. Five replicates were performed for each time point, and the experiments were performed over a 3-mo period. Attraction rate (%) was calculated as the number of mosquitoes in the treated hand port divided by the total number of mosquitoes in the replicate.

Statistical Methods. To evaluate the efficacy of each repellent, a one-way repeated measures analysis of variance was used. The dependent variable was the rank-transformed ratio of the number of mosquitoes that ended up in the port containing the hand or the holding port vs. the total number of mosquitoes in the test. One test was performed for the mosquitoes in the hand port and a second for those in the holding chamber. Dunnett's multiple comparison procedure was used, with the untreated time zero control group as the control.

Results

***Ae. aegypti* Attraction-Inhibition Assays.** Table 2 lists the overall attraction rates of *Ae. aegypti* females to an untreated control hand and

Table 1. Active ingredients, manufacturers, and estimated protection time of the repellents, fragrances, and patch

Product name	Product type	Active ingredient(s)	Manufacturer/distributor	Estimated protection time[a]
Repel 100 insect repellent	Repellent spray	DEET (98.11%)	WPC Brands, Inc.	10 h
OFF deep woods insect repellent VIII	Repellent spray	DEET (25%)	S.C. Johnson & Son, Inc.	Not provided
Cutter skinsations insect repellent	Repellent spray	DEET (7.0%)	Spectrum Division of United Industries Corporation	Not provided
Cutter natural insect repellent	Repellent spray	Geraniol (5%) Soybean oil (2%)	Spectrum Division of United Industries Corporation	2 h
EcoSmart organic insect repellent	Repellent spray	Geraniol (1.0%) Rosemary oil (0.5%) Cinnamon oil (0.5%) Lemongrass oil (0.5%)	EcoSMART Technologies Inc.	2 h
Cutter lemon eucalyptus insect repellent	Repellent spray	Oil of lemon eucalyptus (30%) This oil contains 65% p-menthane-3-8-diol	Spectrum Division of United Industries Corporation	6 h
Avon Skin So Soft Bug Guard	Repellent spray	Oil of citronella (10%)	Avon Products, Inc.	2 h
Avon Skin So Soft Bath Oil	Fragrance	Unknown	Avon Products, Inc.	Not Recommended
Victoria Secret Bombshell	Fragrance	Unknown	Victoria Secret	Not Recommended
Mosquito skin patch	Patch	Thiamin B1 (300 mg)	AgraCo Technologies International, LLC	36 h

[a] Manufacturer provided estimated protection time.

hands treated with various repellents. The overall attraction rate was determined as an average of five replicates, calculating the number of mosquitoes in the treated hand port out of the total mosquitoes in the replicate.

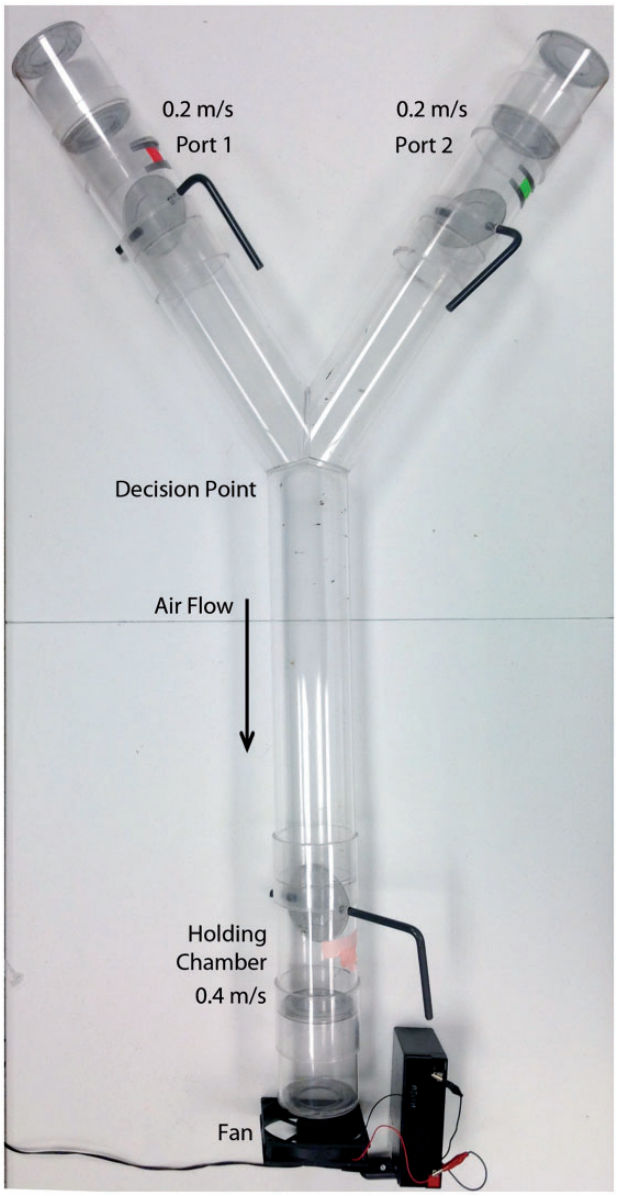

Fig. 1. The Y-tube used in the attraction-inhibition assays.

The control, bare hand, elicited an attraction rate of 61% (±1.6) from *Ae. aegypti* female mosquitoes during the initial evaluation. As expected, controls at time 30 min, 120 min, and 240 min were not significantly different from the control at time 0 min.

Application of sprays containing DEET (Repel 100 Insect Repellent, OFF Deep Woods Insect Repellent VIII, and Cutter Skinsations Insect Repellent) resulted in a strong reduction of attraction. At the 120 min, all three spray treatments still confer maximal protection, while at the 240 min, this effect starts to wear off with the lower DEET concentrations.

Application of DEET-free repellent sprays (Cutter Lemon Eucalyptus, Ecosmart Organic Insect Repellent, Cutter Natural insect repellent, and Avon Skin So Soft Bug Guard) produced different results. Two of the sprays (Cutter Natural insect repellent and Avon Skin So Soft Bug Guard) did not result in any reduction of attraction. Ecosmart Organic Insect Repellent resulted in a strong reduction of attraction at 0 min, but this effect did not persist after 30 min or any of the later time points.

Avon Skin So Soft bath oil resulted in significant reduction of attraction nearly half at the initial time point when compared with the control that had a 61% attraction rate. The effect was still significant at 120 min, but this effect was not seen at 240 min. Interestingly, Victoria Secret Bombshell repelled mosquitoes quite effectively 120 min post application. The Mosquito Skin Patch did not reduce attraction rates.

***Ae. albopictus* Attraction-Inhibition Assays.** Table 3 lists the overall attraction rates of *Ae. albopictus* females to an untreated control hand and hands treated with various repellents. The overall attraction rates were determined as described above.

The control, bare hand, elicited an initial attraction rate of 41% (±6.96). As expected, controls at 30 min, 120 min, and 240 min were not significantly different from the control at 0 min.

The DEET-containing sprays reduced attraction rates significantly for 120 min. The repellency effect was lost at 240 min resulting in no significant difference between the control and the treated hand with the two lower concentrated DEET sprays (OFF Deep Woods Insect Repellent VIII and Cutter Skinsations Insect Repellent).

The DEET-free repellent sprays produced varied results. Cutter Natural Insect Repellent produced no significant reduction in attraction. Avon Skin So Soft Bug Guard achieved a significant reduction in attraction of *Ae. albopictus* at 0 min and at 120 min after application.

Avon Skin So Soft bath oil had no effect on the attraction of *Ae. albopictus*. Victoria Secret Bombshell repelled mosquitoes quite effectively during the first 120 min after application. The Mosquito Skin Patch did not reduce attraction rates for both species.

Discussion

In the last centuries, mosquitoes of the genus *Aedes* have extended their range from tropical and subtropical habitats to more temperate climates (Lounibos 2002). Both *Ae. aegypti* and *Ae. albopictus*

Table 2. Average percentage of *Ae. aegypti* mosquitoes trapped in the port with the hand

Treatments ($N = 5$)	Initial (±SE)	30 min (±SE)	120 min (±SE)	240 min (±SE)
Control	61 (±4.30)	61 (±4.00)	58 (±2.00)	68 (±3.39)
Repel 100 insect repellent	10** (±1.58)	18** (±3.39)	15** (±5.24)	14** (±4.85)
OFF deep woods insect repellent VIII	6** (±1.87)	17** (±1.22)	14** (±1.00)	29** (±3.32)
Cutter skinsations insect repellent	11** (±3.67)	22** (±5.15)	17** (±2.55)	30** (±5.24)
Cutter natural insect repellent	57 (±3.39)	47 (±4.06)	64 (±1.87)	65 (±6.12)
EcoSmart organic insect repellent	9** (±1.87)	55 (±3.16)	68 (±2.00)	67 (±3.39)
Cutter lemon eucalyptus insect repellent	9** (±2.45)	8** (±3.00	13** (±4.64)	18** (±3.74)
Avon Skin So Soft Bug Guard	48 (±4.06)	42* (±2.55)	52 (±2.55)	67 (±5.15)
Avon Skin So Soft Bath Oil	31** (±1.00)	35** (±5.70)	43* (±6.04)	53 (±8.00)
Victoria Secret Bombshell	17** (±5.39)	15** (±5.24)	18** (±3.00)	47 (±5.39)
Mosquito skin patch	68 (±5.10)	67 (±5.61)	48 (±4.90)	68 (±5.15)

N, number of replicates.
*Significantly different than the control, $P < 0.05$.
**Significantly different than the control, $P < 0.01$.

Table 3. Average percentile of *Ae. albopictus* mosquitoes trapped in the port with the hand

Treatments ($N = 5$)	Initial (±SE)	30 (±SE)	120 (±SE)	240 (±SE)
Control	41 (±6.96)	50 (±4.18)	41 (±3.32)	47 (±7.68)
Repel 100 insect repellent	10** (±2.24)	7** (±3.39)	14** (±4.30)	14** (±2.45)
OFF deep woods insect repellent VIII	7** (±3.00)	13** (±3.74)	15** (±3.54)	27 (±2.55)
Cutter skinsations insect repellent	20** (±4.74)	18** (±5.39)	18** (±4.64)	30 (±4.74)
Cutter natural insect repellent	33 (±3.74)	26 (±2.92)	37 (±3.00)	50 (±4.47)
EcoSmart organic insect repellent	5** (±2.74)	15** (±2.74)	23* (±5.61)	15** (±3.16)
Cutter lemon eucalyptus insect repellent	9** (±2.92)	18** (±7.00)	23* (±4.06)	22* (±2.00)
Avon Skin So Soft Bug Guard	21** (±1.87)	27 (±3.39)	20** (±3.54)	38 (±6.44)
Avon Skin So Soft Bath Oil	31 (±3.67)	36 (±3.67)	36 (±2.92)	56 (±5.79)
Victoria Secret Bombshell	14** (±4.58)	24* (±3.32)	17**(±3.74)	35(±4.74)
Mosquito skin patch	40 (±4.18)	42 (±5.15)	34 (±1.87)	39 (±2.92)

N, number of replicates.
*Significantly different than the control, $P < 0.05$.
**Significantly different than the control, $P < 0.01$.

mosquitoes are vectors for human diseases (Brunkard et al. 2007, Shepard et al. 2011, Joy et al. 2012).

Mosquito repellents are an effective way to reduce vector/host contacts and incidence of vector-borne diseases on an individual level. The more effective and long-lasting the efficacy of a repellent is, the higher the level of protection it provides to the consumer. Therefore, information on the efficacy of repellents is extremely valuable for the individual consumer to make informed choices. A multitude of studies has addressed this problem using different techniques (Barnard et al. 1998, Kline et al. 2003, Barnard and Xue 2004, Trongtokit et al. 2005, Tsetsarkin et al. 2007).

For this study, we chose to test the efficacy of several commercially available repellents, two fragrances, and a mosquito repellent patch on two different *Aedes* species. We found that overall *Ae. albopictus* was not as strongly attracted to the control hand as *Ae. aegypti*. This result confirms the deduction by Lupi et al. which reviewed 102 publications on this topic (Lupi et al. 2013, Faherty 2015).

Ae. aegypti was strongly repelled by DEET-containing sprays (Table 1). Two of the DEET-free sprays produced little or no repellency effect (Cutter Natural Insect Repellent and Avon Skin So Soft Bug Guard). EcoSmart Organic Insect Repellent produced a strong but very short-duration repellency toward *Ae. aegypti*. One of the DEET-free sprays we tested (Cutter Lemon Eucalyptus Insect repellent) conferred long-lasting, strong repellency against this species. Avon Skin So Soft bath oil has been used as a home remedy for mosquito repellency and, in this study, has shown some level of protection for 120 min.

Interestingly, other studies have shown that floral scents attract mosquitoes, and it is suggested to avoid floral scented perfumes to reduce mosquito attraction (Beever 2006). Surprisingly, the perfume we tested, Victoria Secret Bombshell (Fragrance type: Fruity floral notes: Purple passion fruit, Shangri-la peony, Vanilla orchid [2015]) has shown to be a strong repellent with effects lasting longer than 120 min. It must be noted that the concentration of perfume we used in this test was rather high and that lower concentrations of the same fragrance might have different effects.

Mosquito repellent patches for the transdermal delivery of thiamine (vitamin B1) have been available to consumers for decades. The proposed mode-of-action is that the patch alters blood chemistry and human smell making the user less attractive to mosquitoes. Our results show that the Mosquito Skin Patch did not provide any repellency against *Ae. aegypti* or *Ae. albopictus*, which confirms a study from 1969 with similar results (Khan et al. 1969, Kahn et al. 1975).

Ae. albopictus females were also strongly repelled by DEET-containing sprays similar to *Ae. aegypti* females (see Tables 2 and 3). However; the results obtained from the DEET-free sprays differed from the results *Ae. aegypti* female repellency tests in two instances. While Cutter Natural Insect Repellent did not work in either species, Avon Skin So Soft Bug Guard reduced the number of *Ae. albopictus* mosquitoes attracted to the hand by almost 50%. Interestingly, EcoSmart Organic Insect Repellent lost its effect on *Ae. aegypti* after 30 min of exposure; however, the effect on *Ae. albopictus* was still prominent after 240 min of exposure.

Another notable result is that Avon Skin So Soft Bath Oil in contrast to our results with *Ae. aegypti* females did not confer any significant protection from *Ae. albopictus* females. The perfume (Victoria Secret Bombshell) had similar results in both species. Lastly, as has been shown in prior studies, the Mosquito Skin Patch had no effect on either species (Revay et al. 2013).

In summary, the results of this study show that not all commercially available mosquito repellents are effective in repelling mosquitoes and that efficacy is also dependent on the species of mosquito that is repelled. Overall, the results from this study confirm that DEET repellents are the most effective mosquito repellents in the market. Although, based on the results from this study, a lemon-eucalyptus oil containing p-menthane-3,8-diol has similar efficacy compared with DEET repellents. This DEET-free repellent had similar rates and duration of repellency as the commercially available DEET repellents. Our results challenge the notion that floral perfume-scented sprays, in general, attract mosquitoes. Floral fragrances may provide a masking odor resulting in low mosquito attraction rates but over a shorter duration of time. Our study has confirmed earlier results that vitamin B1 is not a systemic insect repellent.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

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