

## FIELD EVALUATION OF FOUR SPATIAL REPELLENT DEVICES AGAINST ARKANSAS RICE-LAND MOSQUITOES

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**ABSTRACT.** Four commercially available spatial repellent devices were tested in a rice-land habitat near Stuttgart, AR, after semi-field level assessments had been made at the Center for Medical, Agricultural, and Veterinary Entomology, Agricultural Research Service, US Department of Agriculture in Gainesville, FL. OFF! Clip-On® (metofluthrin), Mosquito Cognito® (linalool), No-Pest Strip® (dichlorvos), and ThermaCELL® (d-*cis/trans* allethrin) were selected for this study from >20 candidate products. The units based on metofluthrin, linalool, or d-*cis/trans* allethrin significantly reduced captures of 1 or more of the mosquito species at surrogate human sites (unlit Centers for Disease Control and Prevention traps with CO<sub>2</sub> and octenol). Among the mosquito species analyzed statistically (*Anopheles quadrimaculatus*, *Culex erraticus*, and *Psorophora columbiae*), there were significantly different responses (up to 84% reduction) to individual products, suggesting that combinations of certain spatial repellents might provide significantly greater protection.

**KEY WORDS** CO<sub>2</sub>, octenol, surrogate human, *Anopheles quadrimaculatus*, *Culex erraticus*, *Psorophora columbiae*

### INTRODUCTION

The ultimate objective of mosquito control strategies is to prevent or eliminate mosquito–host contact. To accomplish this objective often requires 2 areas of responsibility: individual and community. Most mosquito problems cannot be controlled by individual efforts alone because of the extent of the problem. However, in a localized area an individual can have a large impact through the use of personal protection measures. The most commonly employed individual approach is the use of chemical repellents applied to either skin and/or clothing. Usually, the general public will use a topical repellent applied to the skin, such as *N,N*-diethyl-3-methylbenzamide (deet), which has been regarded as the standard topical repellent for the past several decades (Fradin and Day 2002). The use of these topical repellents is often complicated by an unpleasant smell, oily residue, and/or dermal irritation (Lloyd et al. 2013). These complications make many people uncomfortable when they use a topical repellent, causing them to seek alternative ways to achieve personal protection. One alternative that has recently gained in popularity is the use of spatial repellent devices. The use of the word “spatial” to classify repellents was defined by Gouck et al. (1967) as a compound or agent that can produce repellency at a distance. Nolen et al. (2002) further defined a spatial repellent as a compound dispensed into the atmosphere of a

3-dimensional space that inhibits the ability of mosquitoes to locate a host. There are many commercially available spatial repellent products currently on the market. These products include impregnated plastic or paper strips (Argueta et al. 2004; Kawada et al. 2005, 2008), coils (Jensen et al. 2000, Ogoma et al. 2012), candles (Muller et al. 2008), fan emanators (Zollner and Orshan 2011), and heat-generating devices (Alten et al. 2003, Collier et al. 2006). Although much interest exists regarding the efficacy of these products, very few scientific studies, especially in the field against natural populations of mosquitoes, have been conducted. Therefore, the major objective of this study was to compare collections from traps provided with 4 commonly used spatial repellent devices to untreated control traps in an area known to produce large populations of important species of nuisance mosquitoes.

### MATERIALS AND METHODS

#### Study site

These field studies were conducted at the University of Arkansas Rice Research and Education Center (RREC), near Stuttgart, AR, with equipment and technical support from the Center for Medical, Agricultural and Veterinary Entomology (CMAVE), in Gainesville, FL. The selected field site was located in a large rice growing area where late-spring and summer agricultural irrigation generates dense mosquito populations.

#### Spatial repellent devices evaluated

Four commercially available spatial repellent devices were tested over a 2-year period. In July

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## Report Documentation Page

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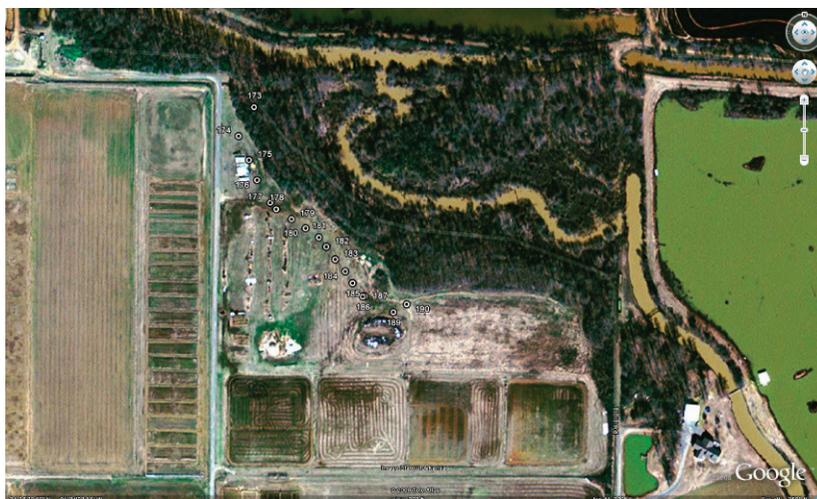


Fig. 1. Aerial photo of spatial repellent sites 174–177 at northwest end of the trap line.

2009, the OFF! Clip-On® (31.2% metofluthrin, fan-emanated; S. C. Johnson & Son, Racine, WI), ThermaCELL® (21.97% *d-cis/trans* allethrin, heat-generated; The Schwabel Corporation, Bedford, MA), and No-Pest Strip® (18.6% dichlorvos, plastic strip; United Industries, Inc., St. Louis, MO) were used. In July 2010, the No-Pest Strip was replaced with the Mosquito Cognito® (95.5% linalool, fan-emanated; Conceal® Metagel formulation; BioSensory, Inc., Putnam, CT). These devices were configured to release the volatilized repellents at the same position on each trap in each replication.

### Experimental design

A  $4 \times 4$  Latin square experimental design was used for each test period. A requirement of this design was that each trap location be provided with a different treatment daily without repeating any treatment combination at that location during any single replication. This design enabled removal of the differences among rows (days) and columns (trap locations) from the experimental design and allowed for a more precise analysis of the effects of treatment combinations on mosquito collections. A trap line was established (Fig. 1) within an ecotone located between forested swampland and irrigated rice, cultivated soybean, and corn fields. Four distinct trap sites were established located approximately 30 m apart. Traps, equipped with a plastic collection cup (Model 512 Centers for Disease Control and Prevention [CDC]-type trap; J. W. Hock Company, Gainesville, FL) and baited with CO<sub>2</sub> and a 1-octen-3-ol (octenol) lure, were used to collect mosquitoes and served as surrogate hosts. In 2009 (Fig. 2a), the CO<sub>2</sub> was metered at the desired rate from a 9-kg compressed gas cylinder fitted with a

fixed output regulator (release rate of 200 ml/min). A Gilmont no. 12 flow meter was used to periodically check the accuracy of the regulator. The metered gas was delivered to its release point approximately 5 cm from the top trap entrance via polyethylene tubing. In 2010, although not shown in Fig. 2b, CO<sub>2</sub> was supplied at the same point at trap lid level from an opening at the bottom of an insulated container (Igloo®) (J. W. Hock Company, Gainesville, FL) filled with 2 kg of dry ice nuggets. The octenol lure was the commercially available standard lure produced by BioSensory Inc., which consisted of 3.75 g of a 50:50 *R:S* racemic blend of octenol formulated in 12 g of a patented blend of waxes, which slowly released the octenol from a patented plastic dispenser. This apparatus was hung at trap lid level and the traps were hung from shepherd's hooks so that the trap entry was approximately 1.8 m above ground level. Treatments consisted of 1 unit of a spatial repellent device placed at each site. The treatment locations were randomly chosen for the 1st night. On each subsequent night the treatments were rotated clockwise to the next location, so that each treatment occupied each position only once during the 4-night experiment. The experiment was replicated 3 times each year. Each year this design resulted in 12 trap-nights for each treatment with 2 exceptions: in 2009, 1 night a ThermaCELL treatment trap failed and, in 2010, 1 night a Mosquito Cognito trap failed.

At the time of this testing there were no standard methods for field testing spatial repellents, such as those published recently by WHO (2013). Thirty minutes prior to trap activation, the spatial repellent devices were turned on so that the volatiles they release could form a protective barrier around the trap. In both years,

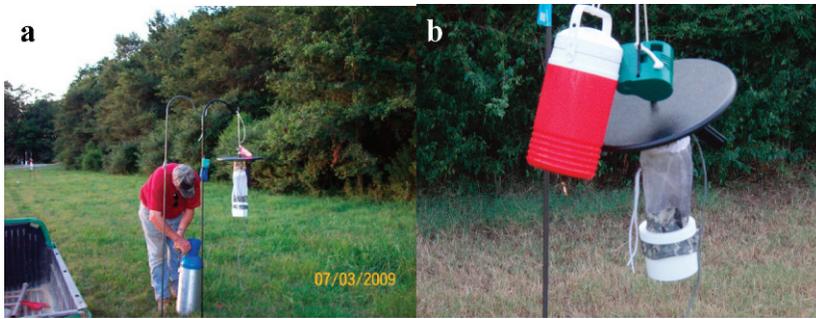


Fig. 2. (a) Centers for Disease Control and Prevention (CDC) trap with compressed CO<sub>2</sub> (2009). (b) CDC trap with pelletized dry ice (2010).

the traps were activated between 7:45–8:00 p.m. depending on the time of civil sunset. In 2009, the traps were then operated for 3.5 h before the collection cups were retrieved and the traps deactivated. In 2010, the traps were operated all night long. Collection cups were retrieved between 8:00–9:30 a.m. and the traps were deactivated. Both years all traps were baited similarly with CO<sub>2</sub> + octenol, while those without a spatial repellent device were used as untreated controls.

In both years, all collection cups were labeled and placed in a freezer (−18°C) immediately after collection. After 3–4 h, the collection containers were removed from the freezer and the contents were weighed, then returned to the freezer and stored until further processing. In 2009, the individual collections or aliquots thereof were identified to species and counted. For aliquots, the abundance for each species was extrapolated based on species proportions in the aliquot and the size of the collection. In 2010, the entire collection was counted and identified to species.

Meteorological conditions were monitored hourly at the RREC West weather station (34.464°N, 91.401°W) located approximately 900 m southwest of the trap sites (34.280°N, 91.241°W).

### Statistical analysis

Data were subjected to the general linear models procedure (SAS Institute 2003) to determine the effects of treatment (spatial repellent devices) and mosquito species. Means were separated with the Least Significant Difference test (SAS Institute 2003) and, unless otherwise stated,  $P = 0.05$ .

## RESULTS

Large numbers of mosquitoes were collected nightly each year. Both years >99% of the collections consisted of 3 species: *Anopheles quadrimaculatus* Say, *Psorophora columbiae* (Dyar and Knab), and *Culex erraticus* Dyar and Knab. Ten other species were caught in small numbers.

In order of decreasing abundance they were: *Aedes vexans* (Meigen), *Cx. territans* Walker, *An. crucians* Wiedemann, *Coquillettidia perturbans* Walker, *Ae. fulvus-pallens* Ross, *Ps. howardii* Coquillett, *Uranotaenia sapphirina* (Osten-Sacken), *Cx. salinarius* Coquillett, *Ps. cyanescens* (Coquillett), and *Ps. ciliata* (Fabricius).

Meteorological conditions were similar in both years, with sparse rainfall and variable RH and winds during the test periods, which were slightly warmer during the 1st few nights and cooler in the final few nights of 2009 than in 2010 (Table 1).

In 2009, ThermaCELL (d-*cis/trans* allethrin) means for *An. quadrimaculatus*, *Ps. columbiae*, *Cx. erraticus*, and Total Mosquitoes categories were all significantly lower than the control means (Table 2). These means were numerically lower, but not significantly so, than the means for the OFF! Clip-On (metofluthrin) and No-Pest Strip (dichlorvos) treatments for *An. quadrimaculatus* and *Cx. erraticus*, but significantly lower than these treatments for *Ps. columbiae* and Total Mosquitoes. OFF! Clip-On and No-Pest Strip collection means for *Cx. erraticus* were also significantly lower than control means. However, the OFF! Clip-On and No-Pest Strip units failed to significantly reduce captures of either *An. quadrimaculatus* or *Ps. columbiae*. In fact, the mean collection of *Ps. columbiae* increased with the No-Pest Strip treatment. The observed percentage reduction in captures for *Cx. erraticus* was 84%, 69%, and 65%, respectively, with the ThermaCELL, OFF! Clip-On, and No-Pest Strip spatial repellent devices. For *An. quadrimaculatus*, the percent reduction was 41% (ThermaCELL), 23% (No-Pest Strip), and 13% (OFF! Clip-On). For *Ps. columbiae*, the percent reduction was greatest with the ThermaCELL (52%) followed by the OFF! Clip-On (8%); use of the No-Pest Strip treatment actually resulted in a 30% increase for this species. The percent reduction for Total Mosquitoes was greatest with the ThermaCELL (47%), followed by the OFF! Clip-On (13%) and only 3% with the No-Pest Strip.

Table 1. Hourly meteorological conditions recorded during the test periods in 2009 and 2010.

Conditions	2009 (3.5 h)			2010 (12 h)		
	Total	Mean	Range	Total	Mean	Range
Rainfall (inches)	1.00	0.08	0.00–1.00	0.10	0.01	0.00–0.10
Temperature (°F)						
Mean		79.5	73.9–86.9		82.4	78.1–87.7
Max.		80.8	75.1–88.0		83.7	78.8–89.6
Min.		78	72.6–85.7		81.2	77.5–86.1
Wind						
Velocity (mph)		3.4	2.0–4.4		4.4	2.6–7.5
Direction (°)		145	78–269		174	107–291
RH (%)						
High		79	71–91		87	77–98
Low		69	52–83		77	65–85

In 2010, ThermaCELL and Mosquito Cognito significantly suppressed collection means of *An. quadrimaculatus*, *Cx. erraticus*, and Total Mosquitoes (Table 2), but not *Ps. columbiana*. Use of the OFF! Clip-On significantly reduced *Cx. erraticus* captures, but not *An. quadrimaculatus*, *Ps. columbiana*, or Total Mosquitoes. None of the treatments significantly reduced the collection means for *Ps. columbiana*. The percentage reduction observed in 2010 was 82%, 78%, and 65% for *Cx. erraticus*, respectively, with ThermaCELL, OFF! Clip-On, and Mosquito Cognito, but <40% for *An. quadrimaculatus*, *Ps. columbiana*, and Total Mosquitoes. The largest percent reduction for *An. quadrimaculatus* was achieved with the Mosquito Cognito (50%), followed by the ThermaCELL (36%) and OFF! Clip-On (16%). These treatments had the least impact on *Ps. columbiana*, resulting in 32%, 19%, and 12% reduction, respectively, with ThermaCELL, OFF! Clip-On, and Mosquito Cognito. Mosquito Cognito had the greatest impact on Total

Population, resulting in a 38% reduction followed by ThermaCELL with a 36% reduction; the mean collection in the traps with the OFF! Clip-On actually resulted in a 2% increase.

Although there was considerable variability in nightly collection size each year, no statistically significant differences were found between trap sites and replications either year. Notwithstanding this variability, the analyses revealed which spatial repellents were most likely ( $P \leq 0.05$ ) to have reduced the total capture and produced statistically meaningful levels of protection.

## DISCUSSION

None of the products tested were completely effective, i.e., none totally protected the human surrogate (CDC trap with CO<sub>2</sub> and octenol) from sizable numbers of blood-seeking mosquitoes. The high level of variability in mosquito abundance within and between the collection sites from night to night may have partially obscured

Table 2. Mean<sup>1,2</sup> collection (SE) from traps treated with spatial repellent devices.

Treatment	Species			
	<i>Anopheles quadrimaculatus</i>	<i>Psorophora columbiana</i>	<i>Culex erraticus</i>	Total mosquitoes
2009				
ThermaCELL	1,103 (196) B	641 (201) C	17 (16) B	1,766 (301) B
OFF! Clip-On	1,643 (337) AB	1,237 (293) B	33 (13) AB	2,917 (469) A
No-Pest Strip	1,441 (240) AB	1,742 (401) A	37 (12) AB	3,222 (444) A
Control	1,879 (352) A	1,338 (171) AB	107 (60) A	3,344 (391) A
2010				
ThermaCELL	1,640 (423) B	887 (200) A	20 (5) B	2,551 (480) B
OFF! Clip-On	2,972 (503) A	1,056 (366) A	25 (12) B	4,058 (620) A
Mosquito Cognito	1,284 (277) B	1,151 (246) A	39 (10) B	2,482 (311) A
Control	2,552 (649) A	1,302 (405) A	112 (43) A	3,975 (981) A

<sup>1</sup>  $n = 12$  except for ThermaCELL in 2009 where  $n = 11$  and Mosquito Cognito in 2010 where  $n = 11$ .

<sup>2</sup> Least Significant Difference test means comparison; means within a column for each year, separately, with the same letter are not significantly different,  $P \leq 0.05$ .

the full impact of the spatial repellents that we tested. Nevertheless, the observed significant reductions with ThermaCELL, Mosquito Cognito, and OFF! Clip-On suggest that use of these products could form a 1st line of defense against penetration of one or more species into a zone inhabited by humans if additional methods of protection, e.g., an insecticide barrier, were available to protect individuals within the zone from the (reduced) number of penetrating mosquitoes. In areas of high mosquito density, such as where these tests were conducted, the observed level of protection might afford a meaningful buffer for inhabitants that are additionally protected by other measures, such as contact repellents.

Perhaps equally important, these results reveal potential species specificity of the active ingredients. This finding points to a need to assess the ability of combinations of spatial repellents to protect against multiple species and those that are not responsive to a single active ingredient.

Few published field studies have been conducted against natural populations of mosquitoes or other biting dipterans with any of these devices. Lloyd et al. (2013) conducted a study with several repellent devices, including the ThermaCELL and OFF! Clip-On in a suburban neighborhood in northeastern Florida with BGS traps (baited only with BG-Lure, Biogents AG, Regensburg, Germany) as surrogate hosts against *Ae. albopictus* (Skuse). The ThermaCELL was significantly better than all the other repellent devices tested except the OFF! Clip-On at reducing mosquito collections. When compared to the control trap, ThermaCELL reduced trap capture of *Ae. albopictus* by 76% and the OFF! Clip-On reduced trap capture by 64%.

Alten et al. (2003) conducted a field evaluation of the ThermaCELL with human volunteers against phlebotomine sand flies and mosquitoes in Turkey. The ThermaCELL provided highly significant protection for up to 4 h from attack by *Phlebotomus papatasi* (Scopoli) and *Ochlerotatus caspius* (Pallas). Reduction for the sand flies and mosquitoes ranged from 87.5% to 97.7% (mean protection 92%) and 90.2% to 97.4% (mean protection 93%), respectively. Overall, the number of bites by *P. papatasi* and *Oc. caspius* was reduced by >11-fold and 13-fold, respectively, by the ThermaCELL device.

Collier et al. (2006) conducted a 14-m study in Louisiana using a variety of spatial repellent devices and systems to determine their impact on backyard mosquito population levels. Efficacy was determined by placing dry ice-baited American Biophysics Corporation (ABC) traps in the treatment yards and comparing trap counts. The ThermaCELL was the most effective device in reducing mosquito populations in this yard. They also used an S. C. Johnson OFF! Mosquito Lantern (AI, *cis-trans*-allethrin), which was the

2nd most effective device. A Mosquito Cognito device was paired with a Dragonfly® Biting Trap (BioSensory Inc.). The ABC traps placed in yards with the Dragonfly–Mosquito Cognito system caught the highest number of mosquitoes. This study was different from ours in that the Mosquito Cognito device was not placed in combination with the ABC trap.

Two studies have been published which evaluated the OFF! Clip-On. In the 1st study, Zollner and Orshan (2011) evaluated this device against phlebotomine sand flies in Israel. They used both suction and sticky traps. In trials with unbaited suction traps, similar numbers of sand flies were collected in traps with a metofluthrin device, blank device, or no device (i.e., suction only). In suction traps baited with CO<sub>2</sub>, higher numbers of *P. sergenti* Parrot males and bloodfed females were collected in traps with a blank device compared with a metofluthrin device. In sticky traps baited with CO<sub>2</sub>, there was no significant difference between catches in traps with a metofluthrin device, blank device, or no device. They concluded that their results suggested that metofluthrin from the device was not repellent against sand flies in the field environment despite showing insecticidal activity against flies collected in suction traps. We noticed that at the time of trap cup collection nearly all the mosquitoes that had entered all the traps except those with a linalool device, or that served as our controls, were dead. In the linalool and control traps, nearly all the mosquitoes were alive at pickup. In the 2nd study, Xue et al. (2012) evaluated the repellent efficacy of the OFF! Clip-On device on human landing counts against *Ae. albopictus* and *Ae. taeniorhynchus* (Wiedemann). They found that this repellent device was 70% and 79% effective at repelling *Ae. albopictus* and *Ae. taeniorhynchus* from landing on human volunteers.

In conclusion, linalool, *d-cis/trans* allethrin, and metofluthrin were found to significantly protect human surrogates in this study from one or more mosquito species. Complete protection was not achieved, but the probability of packaging several spatial repellents together to enhance protection levels and perhaps yield a synergistic effect provides guidance for further study.

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