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EVALUATION OF THE EFFICACY OF LAMBDA-CYHALOTHRIN APPLIED BY THREE SPRAY APPLICATION METHODS FOR EMERGENCY CONTROL OF *AEDES AEGYPTI* IN COSTA RICA[1]

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ABSTRACT. An extended duration formulation of lambda-cyhalothrin (Icon™ CS) applied as either an ultra-low volume (ULV) or thermal fog spray from a new hand-held sprayer (Twin-Fog™) or as a low-volume spray (LV) from a backpack mist blower against *Aedes aegypti* was evaluated in Costa Rica. Spray applications were made at the front door for 1 min or to each room for 15 sec for the ULV and LV, and thermal fog applications were made to houses in separate blocks for each treatment. The efficacy and duration of effectiveness of the spray was determined from sentinel caged mosquito mortality and mosquito collections from within houses using hand-held, battery-powered aspirators. Sentinel caged mosquito mortality in both open and sequestered locations was 97–100% for the ULV and thermal fog spray treatments, with control mortality less than 2%. Both ULV applications (front door and each room) provided 3 wk of significant control ($P < 0.05$) based on adult *Ae. aegypti* house collections.

KEY WORDS *Aedes aegypti,* ultra-low volume, thermal fog, low volume, emergency control, lambda-cyhalothrin

INTRODUCTION

The threat of dengue and dengue hemorrhagic fever (DHF) in Costa Rica has been increasing, with the number of serologically confirmed cases of dengue rising from 2,628 in 1998 to 6,041 in 1999, with 1,082 of those cases from the Puntarenas area in 1999 (Costa Rican Ministry of Health). Dengue and DHF are mosquito-borne viral diseases that coincide with the distribution of *Aedes aegypti* (L.), the primary vector throughout the tropical and semitropical world, including Costa Rica (Halstead and Gomez-Dantes 1992). *Aedes aegypti* is an urban mosquito that has adapted to utilizing man-made containers for breeding, feeds almost exclusively on humans (Christopher 1960), and rests in places inside houses where outdoor traditional insecticide spraying is ineffective.

Sanitation and clean-up in which potential mosquito breeding containers are eliminated or made mosquito proof is the primary method for long-term control of *Ae. aegypti.* However, not all breeding sites can be eliminated or made totally mosquito proof and not all individuals cooperate in clean-up campaigns. In addition, natural disasters (hurricanes, earthquakes, and floods) and man-made disasters (war and economic decline) create conditions for rapid and massive increase in *Ae. aegypti* populations. All these scenarios can lead to outbreaks of dengue/DHF and require emergency suppression of *Ae. aegypti* to break the transmission cycle (PAHO 1994).

Adult *Ae. aegypti* control using either ground vehicle or aerially applied insecticide is, at best, fair in reducing *Ae. aegypti* populations (Chadee 1985, Hudson 1986, Perich et al. 1990). This is primarily due to the seclusive resting behavior of *Ae. aegypti* within homes (Perich et al. 2000), where traditionally applied insecticide sprays do not penetrate (Perich et al. 1992). In a prior study in Honduras (Perich et al. 2001), the new extended duration insecticide formulation (Icon™ CS) applied as either an ultra-low volume (ULV) or thermal fog spray application, when applied either through the front door or in each room, significantly suppressed the *Ae. aegypti* population. This study was designed to evaluate the efficacy of such methodology; in addition, we evaluated the effectiveness of low volume (LV) application of lambda-cyhalothrin in the suppression of *Ae. aegypti* populations in Costa Rica.

MATERIALS AND METHODS

Study site

Field tests were conducted from May to July 1999 in 2 neighborhoods (Juanita Mora and Fray Casiano) in Puntarenas, Costa Rica (16°S, 88°W), a city historically with cases of dengue and high *Ae. aegypti* populations. Seven city blocks were selected in each neighborhood for the tests. Blocks were separated from each other by a minimum of

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100 m. Houses were 1 story with 3–4 rooms and they were constructed of either stucco/cement block or wood. Each house was approximately 250 m^2, and each city block contained approximately 30 houses. Six houses within the contour of each block were randomly selected for sampling and received the same treatment assigned to that block. The most upwind block, in relation to the other 6 blocks at both Juanita Mora and Fray Casiano, were designated as the untreated control block. The other 6 blocks in the 2 neighborhoods received one of the following treatments: 1) ULV at the front door, 2) ULV in each room, 3) thermal fog at the front door, 4) thermal fog in each room, 5) LV at the front door, or 6) LV in each room.

Mosquito sampling

Two mosquito sampling criteria were used to determine the efficacies of the treatments: 1) adult mosquito collections within houses and 2) sentinel mosquito mortality. Mosquitoes were collected using hand-held, battery-powered aspirators (Hausherr's Machine Works, Tom's River, NJ) operated by 2 Costa Rican Ministry of Health (MOH) personnel while walking throughout the house and aspirating within each house for 15 min. Mosquitoes were identified to species and the number recorded. Mosquito collections were initiated 2 wk prior to spraying and then weekly thereafter for 7 wk posttreatment. An initial collection was made 24 h after spraying.

For sentinel cage tests, twenty-five 3–5-day-old, nonbloodfed female *Ae. aegypti* reared from eggs collected from the test site were placed into 20-cm-diameter cardboard, disposable sentinel cages covered with fine-mesh screen (Clarke Mosquito Control Inc., Roselle, IL). Sentinel cages were transported to the field in coolers with damp paper towels on top of the cages. Two sentinel cages were placed in 3 houses in each of the 7 blocks (1 control and 6 treatments) at both neighborhood test sites just prior to spraying. One sentinel cage was placed in an open location on top of a table, and the other sentinel cage was placed in a hidden location beneath a bed. Sentinel cages were orientated perpendicular to the spray. Sentinel cages were retrieved 30 min after spray application. Exposed mosquitoes were transferred to clean holding cages and a water-soaked cotton ball was placed on each cage. Mortality was recorded at 3, 12, and 24 h posttreatment.

Spray treatment

The ULV and thermal fog spray applications were made using a new handheld spray generator, Twin-Fog™ (Clarke Mosquito Control Inc.), formerly known as the Terrier™ (Vectec Engineering Division, Rogers, MN). The Twin-Fog can be operated either in the ULV or thermal fog mode by flipping a switch to the desired mode of application. The Twin-Fog was calibrated to disperse at a flow rate of 100 ml/min and a ULV droplet size of 10–25 μm, determined prior to spray applications at study sites. The LV spray applications were made using a MD 155DX backpack mist blower (Maruyama U.S., Inc., Redmond, WA) and was calibrated to disperse at the same flow rate of 100 ml/min as the Twin-Fog sprayer. All spray applications (ULV, LV, and fog) made at the front door were done for 1 min, and for those designated for room treatment, spraying was done for 15 sec in each room. Spraying was conducted by Costa Rican MOH personnel, who wore rubber gloves, eye and ear protection, respirators, and protective clothing.

The insecticide applied in this study was lambdacyhalothrin CS (Icon™ CS) (Zeneca Public Health, Surrey, UK, now Syngenta AG, Basel, Switzerland), a new micro-encapsulated formulation. The Icon CS used in this study contained 2.5% active ingredient (AI). The final applied concentration of 0.5% AI was obtained by mixing with water for all applications (ULV, LV, and thermal fog).

Prior to spraying, insecticide susceptibility in the *Ae. aegypti* populations in Juanita Mora and Fray Casiano neighborhoods in Puntarenas was determined. Ovitraps were set out 1 wk prior to spray applications at houses within both neighborhoods. Oviposition strips were collected 3 days after ovitrap placement, allowed to dry, labeled with site location, and sent to the Entomology Branch at the Center for Disease Control and Prevention (CDC) in Atlanta, GA. At the CDC, lab eggs were hatched, reared to adults, and insecticide susceptibility determined by microplate and bottle assay (Brogden and McAllister 1998).

Statistical analysis

Treatment mortality for the sentinel cage data was corrected using Abbott's (1925) formula. Corrected sentinel mortality is reported in Table 1. The experimental design of this study was a 3- × 2-factor experiment using analysis of variance techniques for repeated measurements (Winer 1991). The factors were sprayed treatment (ULV or LV or thermal fog) and application site (at front door or inside each room). Because each sampling period included data from 6 control houses, Dunnett's multiple comparison procedure was used to compare shifts from pretreatment levels for each treatment combination with corresponding changes in the untreated (control) group (StatXact Turbo 1994). Additional comparisons of treatment groups (e.g., front door versus each room and ULV versus LV versus fogging) were examined using F tests (StatXact Turbo 1994).

RESULTS

The *Ae. aegypti* populations in both Juanita Mora and Fray Casiano neighborhoods of Puntarenas,

Table 1. Mean percent mortality of sentinel caged *Aedes aegypti* females from open and hidden locations from houses in 2 neighborhoods in Puntarenas, Costa Rica.[1]

Treatment	Cage location	Postspray time mean % mortality 3 h	12 h	24 h
Juanita Mora				
ULV front door	Open	98	100	100
	Hidden	94	98	100
ULV each room	Open	100	100	100
	Hidden	96	99	100
Thermal fog front door	Open	97	99	100
	Hidden	96	100	100
Thermal fog each room	Open	98	98	100
	Hidden	90	96	100
LV front door	Open	55	74	74
	Hidden	35	57	57
LV each room	Open	65	69	69
	Hidden	67	78	78
Fray Casiano	Open	100	100	100
	Hidden	95	100	100
ULV each room	Open	100	100	100
	Hidden	90	94	100
Thermal fog front door	Open	90	98	100
	Hidden	92	96	100
Thermal fog each room	Open	100	100	100
	Hidden	100	100	100
LV front door	Open	20	27	27
	Hidden	7	7	7
LV each room	Open	17	20	20
	Hidden	2	2	2

[1] Mean mortality based on 3 sentinel cages per treatment per location with 25, 3–5-day-old, nonbloodfed *Ae. aegypti* females in each cage.

Costa Rica, were 100% susceptible to lambda-cyhalothrin based on the resistance tests conducted prior to the spray applications in this study. Mixing and applications of the lambda-cyhalothrin CS insecticide using the Twin-Fog sprayer were executed with no difficulties. The flow rate was maintained for all ULV and thermal fog applications. Problems were encountered with adjusting and maintaining the flow rate (100 ml/min) with the backpack mist blower for the LV applications. This problem was probably due to the fact that this type of sprayer is not designed to normally operate at such a low flow rate.

Sentinel cage mortality was highly significant (Table 1) ($P < 0.001$) for the ULV and thermal fog spray treatments in both open and hidden locations in both neighborhoods (Juanita Mora and Fray Casiano) compared with the untreated controls. Sentinel cage mortality for the LV spray applications was not significant in either the open or hidden locations in either neighborhood (Table 1). Mortality in the untreated control site cages was always less than 2%. Initial mortality (3 h postspray) for the ULV and fog applications was 97–100% for the open sentinel cage locations and 90–100% for the hidden locations, while the initial mortality for the LV applications was significantly less at 17–65% for the open locations and 2–67% for the hidden locations. Within 24 h, mortality was 100% in all the sentinel mosquitoes both from open and hidden locations for the ULV and thermal fog spray application methods (Table 1). The 24-h mortality for the LV spray applications again was significantly less (2–78%) compared with the ULV and thermal fog spray applications. This indicates that the insecticide applied by any of the ULV or thermal fog spray application methods used in this study reached both open and secluded potential resting sites of *Ae. aegypti* in houses in lethal amounts. However, the LV spray applications did not achieve this same level of delivering a lethal dose of insecticide to either open or hidden potential resting sites of *Ae. aegypti.*

There were no significant differences ($P > 0.05$) in the mean number of *Ae. aegypti* collected from houses between the 7 test blocks in either neighborhood (Juanita Mora and Fray Casiano) prior to spray treatment (Table 2). This indicated that the mosquito population was relatively consistent throughout the 7 test block sites and would allow for treatment comparison between the blocks within each neighborhood (Juanita Mora or Fran Casiano).

Twenty-four hours after spraying, no *Ae. aegypti* were collected in most of the houses that received either the ULV or thermal fog spray at the front door or in each room in both Juanita Mora and Fray Casiano neighborhoods (Table 2). The houses that received the LV spray treatment continued to have *Ae. aegypti* collected from them and the number collected was not significantly different ($P > 0.05$) from that collected in the untreated control homes in the respective neighborhoods (Table 2). Through 3 wk posttreatment, those houses that had received either the ULV or thermal fog spray application from the Twin-Fog sprayer at either the door or in each room had significantly ($P < 0.05$) fewer *Ae. aegypti* collected inside them compared with the untreated control houses in each of the 2 respective neighborhoods (Table 2). Again, those houses that received the LV spray application with the mist blower had similar numbers of *Ae. aegypti* collected inside when compared with the untreated control houses in each neighborhood (Table 2). During the 4th week of posttreatment monitoring, houses from the ULV and thermal fog treatment blocks had an increase in the mean number of mosquitoes collected. However, in Juanita Mora they still remained twice as low when compared with the number collected from houses in the untreated control block (Table 2).

There was a significant difference between the number of *Ae. aegypti* collected from the houses that received the ULV or the thermal fog treatment at either the front door or each room for the entire 7-wk posttreatment sampling at both Juanita Mora and Fray Casiano neighborhoods ($P < 0.0001$).

Table 2. Mean number of *Aedes aegypti* collected per house for various spray applications of lambda-cyhalothrin CS in 2 neighborhoods in Puntarenas, Costa Rica.

	Mean number of *Ae. aegypti* collected[1,2]									
	Pretreatment		Posttreatment							
Treatment	1 wk	2 wk	24 h	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	7 wk
Juanita Mora										
ULV front door	13.6^{a}	8.0^{a}	0.0^{a}	0.5^{a}	1.5^{a}	2.2^{a}	4.7^{a}	5.2^{a}	8.8^{a}	7.6^{a}
ULV each room	5.3^{a}	7.7^{a}	0.3^{a}	0.7^{a}	1.6^{a}	2.0^{a}	2.8^{a}	2.8^{a}	6.7^{a}	5.2^{a}
Thermal fog front door	4.8^{a}	5.0^{a}	0.0^{a}	0.3^{a}	1.2^{a}	1.2^{a}	4.5^{a}	6.2^{a}	5.5^{a}	6.0^{a}
Thermal fog each room	8.3^{a}	11.6^{a}	0.0^{a}	0.6^{a}	2.0^{a}	2.0^{a}	2.7^{a}	5.7^{a}	5.0^{a}	5.0^{a}
LV front door	9.6^{a}	9.0^{a}	6.8^{b}	7.3^{b}	9.8^{b}	12.8^{b}	10.8^{b}	11.7^{b}	9.0^{a}	5.2^{a}
LV each room	6.8^{a}	5.5^{a}	7.0^{b}	7.0^{b}	9.0^{b}	13.5^{b}	6.0^{a}	7.8^{a}	8.1^{a}	6.8^{a}
Control	6.6^{a}	7.8^{a}	5.7^{b}	3.3^{b}	6.8^{b}	12.4^{b}	9.0^{b}	13.0^{b}	9.2^{a}	6.8^{a}
Fray Casiano										
ULV front door	8.3^{a}	12.0^{a}	0.3^{a}	0.9^{a}	1.7^{a}	2.8^{a}	4.7^{a}	9.6^{a}	9.7^{a}	6.8^{a}
ULV each room	5.6^{a}	9.5^{a}	0.0^{a}	0.6^{a}	1.0^{a}	2.8^{a}	7.5^{a}	4.6^{a}	13.0^{a}	8.4^{a}
Thermal fog front door	8.8^{a}	8.0^{a}	0.0^{a}	1.2^{a}	1.2^{a}	3.0^{a}	6.4^{a}	7.6^{a}	10.8^{a}	7.2^{a}
Thermal fog each room	8.0^{a}	13.1^{a}	0.0^{a}	0.6^{a}	1.8^{a}	3.2^{a}	8.0^{a}	12.3^{a}	11.8^{a}	9.2^{a}
LV front door	8.8^{a}	11.2^{a}	4.5^{a}	15.6^{b}	10.4^{b}	11.6^{b}	15.2^{b}	16.3^{b}	10.0^{a}	13.3^{a}
LV each room	6.0^{a}	11.0^{a}	6.0^{b}	10.3^{b}	11.2^{b}	13.0^{b}	14.0^{b}	13.0^{ab}	13.1^{a}	14.3^{a}
Control	6.5^{a}	9.8^{a}	6.4^{b}	9.3^{b}	7.0^{b}	11.5^{b}	8.5^{a}	15.0^{b}	8.8^{a}	12.3^{a}

[1] Based on collections made by 2 collectors sampling 6 houses for 15 min per house.

[2] Means in a column from the same neighborhood with different superscript letters are significantly different ($P < 0.05$).

However, there was no significant difference when comparing between the number of *Ae. aegypti* collected in houses from these 4 treatments (ULV at front door, ULV in each room, thermal fog at front door, thermal fog in each room). There was no significant difference between the mean number of *Ae. aegypti* collected from the houses that received the LV spray treatment at either the front door or in each room for the entire 7-wk posttreatment sampling in either neighborhood.

DISCUSSION

The lambda-cyahalothrin CS formulation as applied in our study using the Twin-Fog sprayer significantly affected the *Ae. aegypti* within houses in 2 neighborhoods in Puntarenas, Costa Rica. These results are similar to those obtained in a previous Honduran study (Perich et al. 2001), with the exception that the duration of significant suppression of the *Ae. aegypti* in Costa Rica was shorter. This indicates the importance of testing new vector control methods in several geographical and ecological areas.

The failure of the LV application of the Icon CS using the backpack mist blower to affect significantly *Ae. aegypti* within houses in this study most likely was associated with the difficulties in obtaining and maintaining a flow rate of 100 ml/min. This rate is extremely low for this type of sprayer and may have adversely affected its performance in applying the Icon CS in this study. In the event a backpack mist blower sprayer is employed for this type of vector control methodology, we suggest that it be operated at a higher flow rate. However, higher flow rates may increase the amount of Icon CS applied within each house to achieve effective control.

Two criteria necessary for adulticide space spraying to be effective in emergency dengue vector suppression are 1) an initial kill of adult vectors present and 2) provision of a significant level of residual control to allow for other implemented suppression interventions (source reduction, larviciding) to take affect. Both criteria have been met for use in Costa Rica when using the lambda-cyhalothrin CS applied from the Twin-Fog sprayer under the conditions described in our study. The 100% sentinel cage mosquito mortality in both the open and hidden locations and the absence of mosquitoes in houses 24 h after either ULV or thermal fog indicates successful completion of the first criterion. The second criterion was met by the significant ($P < 0.05$) suppression of the adult *Ae. aegypti* within houses for 3 wk posttreatment. The LV spraying of lambda-cyhalothrin directly into houses either at the front door or inside each room using a backpack mist blower may be as effective as the other ULV and thermal fog spray treatments if an increased flow rate is used. Lambda-cyhalothrin, when applied as a residual insecticide to wooden huts in Malaysia, was reported to produce 100% knockdown of *Ae. aegypti* through 28 days (Sulaiman et al. 1993). The residual effect found in this study, as with the prior Honduran study (Perich et al. 2001), is most likely due to the capsule-suspension formulation of the lambda-cyhalothrin CS used in these studies. This formulation allows for a slow release of the lambda-cyhalothrin, which explains the several weeks of significant effect not found with other ULV or thermal fog insecticide applications.

The exact level and duration of vector suppression required to suppress a dengue outbreak is unknown. However, our study reveals the potential use of lambda-cyhalothrin CS applied as either a ULV or thermal fog using the hand-held Twin-Fog sprayer for use in emergency control of *Ae. aegypti* in Costa Rica. When the ULV application at the front door was utilized, we determined that one spray operator could treat 75 houses in a day. The front-door spray applications were the fastest and most cost effective, although treatment of a large area of several hundreds to thousands of homes would be labor intensive. The ineffectiveness of either vehicle-mounted spray equipment or aerial application of adulticides makes the described methods in our study a true alternative for emergency *Ae. aegypti* suppression in Costa Rica. Further evaluation of these vector control methodologies in other geographical areas where the threat of dengue/DHF exists should be performed to determine the level of effect and duration against dengue vectors in those areas.

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