Reduced Efficacy of Pyrethroid Space Sprays for Dengue Control in an Area of Martinique with Pyrethroid Resistance

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Abstract. In the Caribbean, insecticide resistance is widely developed in *Aedes aegypti* and represents a serious obstacle for dengue vector control. The efficacy of pyrethroid and organophosphate ultra-low volume space sprays was investigated in Martinique where *Ae. aegypti* has been shown to be resistant to conventional insecticides. In the laboratory, a wild-field caught population showed high levels of resistance to deltamethrin, organophosphate (naled), and pyrethrum. Simulated-field trials showed that this resistance can strongly reduce the knock-down effect and mortality of deltamethrin and synergized pyrethrins when applied by thermal-fogging. Conversely, the efficacy of naled was high against insecticide-resistant mosquitoes. Chemical analyses of nettings exposed to the treatments showed a decrease in residues over distance from release for the pyrethroids, and naled was not detected. This finding has important implications for dengue vector control and emphasizes the need to develop innovative strategies to maintain effective control of resistant *Ae. aegypti* populations.

INTRODUCTION

The past 30 years have witnessed a dramatic resurgence of several infectious diseases across the globe, especially those caused by dengue virus and chikungunya virus, which have resulted in major public health problems.[1] The main factors involved are human population growth, lack of effective mosquito control, geographic spread of viruses along with their vectors, and genetic variation of these viruses.[2–4]

Dengue fever is still the most important arboviral disease worldwide, causing 50–100 million cases and thousands of deaths every year.[5] In the past 10 years, Martinique (French West Indies) experienced four major dengue outbreaks in 1997, 2001, 2005, and 2007 with 17,000, 27,000, 14,000, and 18,000 reported cases, respectively.[6,7] *Aedes aegypti* (L.) is the only dengue virus vector in Martinique.[8] On this island, where dengue occurs in an endemo-epidemic pattern,[9] larval source reduction by cleaning of water-holding containers that serve as the larval habitats for *Aedes* mosquitoes in the domestic environment and by using larvicides (temephos [Abate®] and *Bti* [Vectobac®]) in permanent water containers is implemented routinely.[10] Space spraying is used when source reduction has failed to limit the density of adult mosquitoes (i.e., high entomologic indices) or when the risk of dengue transmission is high (dengue cases). Organophosphates (malathion, fenitrothion) have been used for space treatments for more than 20 years,[11] but there is now a trend to switch to pyrethroids because they have high insecticidal properties at low application rates, relatively short persistence in the environment, and no bioaccumulation and low mammalian toxicity.[12] Deltamethrin, a pyrethroid insecticide (ultra-low volume [ULV] and emulsifiable concentrate [EC] formulations), is the mainstay of adult control program and is sprayed at a rate of 1 g of active ingredient (ai)/hectare every 3 days during dengue epidemics.[6]

Pyrethroid and organophosphate resistance in *Ae. aegypti* is now found worldwide[13,14] and may represent an increasing obstacle for dengue vector control programs. Resistance is associated with either alterations in the sequence of the target protein, the sodium channel that confers resistance to pyrethroids (the knockdown resistance [kdr] mutation) and/or an increase in metabolic rates through the involvement of detoxification enzymes.[15] Resistance to pyrethroids caused by the kdr mutation has been reported in the Caribbean, South America, Africa, and Asia.[16,17] In addition, higher activity of P450 monooxygenases, glutathione-S-transferases, and esterases has been shown to be associated with moderate to high level of resistance to pyrethroids, organophosphates, and carbamates in Latin America countries.[18–20] In Martinique, Rosine[21] reported high level of resistance of *Ae. aegypti* to temephos, deltamethrin. Other molecular assays showed the presence of the kdr mutation (Val to Gly substitution) at the 106 position in the S6 hydrophobic segment of domain II in the sodium channel,[16] which suggested resistance of field mosquitoes to pyrethroids. The impact of insecticide resistance on the efficacy of space spraying operation has not yet been tested.

In this context, we carried out a simulated field trial (phase II) in an area of insecticide resistance (Martinique) to compare the performance of deltamethrin versus synergized natural pyrethrins and organophosphate against laboratory susceptible and wild-field caught *Ae. aegypti* mosquitoes. First, a World Health Organization (WHO) filter paper test was used to determine the level of resistance of *Ae. aegypti* (Vauclin population) to deltamethrin (pyrethroid), pyrethrum (natural pyrethrins), and naled (organophosphate) in comparison with the susceptible (Bora) strain. Then, the WHO cage-bioassay method[22] was used to evaluate the efficacy of pyrethroid and organophosphate ULV-space sprays in terms of knock-down effect 20 minutes post-treatment and on mortality 24 hours later by using a 4 × 4 mounted vehicle thermal fogger. We also determined by chemical analyses the insecticide residues remaining on nettings after treatment to obtain information on the actual amount of active substance received by

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mosquitoes. This purpose of this study was to provide mosquito control services with practical information to implement more effective vector control and resistance management strategies in the future.

MATERIALS AND METHODS

Biological material. Two strains of *Ae. aegypti* were used in this study. The susceptible reference Bora strain, originating from Bora-Bora in French Polynesia, has been colonized for many years and is free of any detectable insecticide resistance mechanisms. It is checked regularly for resistance mechanisms (e.g., kdr mutation and detoxification enzyme activity) as part of our laboratory routine. The Vauclin strain, which was our resistant strain, is a colony of *Ae. aegypti* established from wild field-caught mosquito larvae collected from individual houses in the locality of Vauclin, Martinique. Adults obtained from the F_1 progeny were used for bioassays (phase I) and field experiments (phase II).

Insecticides and formulations. Laboratory bioassays were carried out by using technical grades of deltamethrin (100% [w/w]; AgrEVO, Herts, United Kingdom), pyrethrum (25.44% [w/w]; Pyrethrum Board of Kenya, Nakuru, Kenya), and naled (97.2% [w/w]; Sigma-Aldrich, Seelze, Germany). For the field experiment, formulations of pyrethrum (a mixture of six pyrethrins with the synergist piperonyl butoxide [PBO]; Pynet®, 5% EC [w/v] plus 20% PBO [w/v]; Pyrethrum Board of Kenya), synergized pyrethrins (AquaPy®, 3% [EW] [w/v] plus 13.5% PBO [w/v]; Bayer Environmental Science, Lyon, France), and naled (Dibrom® 14 Concentrate, SL 87.4% [w/v] + dichlorvos < 2% [w/v]; AMVAC Chemical Corporation, Los Angeles, CA) were evaluated in comparison with two formulations of deltamethrin mixed with water (Aqua K-Othrine®, EW 2% [w/v]) or gasoil (K-Othrine® 15/5 ULV, UL 15% [w/v] + 0.5% esbiothrine [w/v], both from Bayer Environmental Science). K-Othrine® 15/5 ULV is the reference formulation that has been used for many years in Martinique for the control of *Ae. aegypti* populations. Application rates were 1 g ai/hectare for deltamethrin, 10 g ai/hectare for pyrethrins (Pynet® and AquaPy®) and 114 g ai/hectare for naled. K-Othrine® 15/5 ULV, Dibrom® 14 Concentrate and Pynet® were mixed with gasoil, and Aqua K-Othrine® and AquaPy® were mixed with water according to the manufacturers' recommendations. Each insecticide and their formulations have been reported in the European Directive 98/8/EC of 16 February 1998 concerning the placing of biocidal products on the market.

Tarsal contact with treated filter paper. Tarsal contact tests were run using filter papers treated with a technical grade of each insecticide. Filter papers were treated following a WHO protocol using acetone solutions of insecticide and silicone oil as the carrier.[23] Impregnation was conducted by dripping evenly onto paper 2 mL of technical grade chemical dissolved in acetone and silicone oil. Concentrations were expressed in w/w percentage of the active ingredient in silicone oil. The paper was dried for 24 hours before the test.

Mortality resulting from tarsal contact with treated filter papers was measured using WHO test kits against adult mosquitoes of the Bora and Vauclin strains. Five batches of 20 non-blood fed females (2–5 days of age) were introduced into holding tubes and maintained for 60 minutes at $27 \pm 2°C$ and a relative humidity of $80 \pm 10\%$. Insects were then transferred into the exposure tube and placed vertically for 60 minutes

under subdued light. Mortality was recorded 24 hours after exposure. Each test was replicated twice (n = 200 per dose).

Field experiment. The efficacy of synergized pyrethrins, deltamethrin and naled was evaluated against Bora and Vauclin strains according to the WHO cage bioassay method.[22] The efficacy of each insecticide was measured by performing space spray applications using a 4×4 vehicle-mounted with a MaxiPro4 thermal fogger (Curtiss-Dynafog Ltd., Westfield, IN). Trials were conducted early in the morning (7:00 AM to 9:00 AM) in central southwestern Martinique in the locality of Ducos at Pays-Noyer in an open field setting. Before each treatment, the spraying system was calibrated (i.e., flow rates were 580 mL water/minute and 587 mL gasoil/minute). During application, the speed of the vehicle was 10 km/hour, and the volume of mixture applied was 700 mL/hectare. Cylindrical steel frame cages (90 mm diameter × 153 mm height) covered with a mosquito net (1-mm mesh) was used to house groups of 20 adult female mosquitoes. Cages were hung on steel poles 1 meter above the ground 15 minutes before spraying treatments began and were placed at increasing distances from the point of treatment (10, 20, 30, and 50 meters) and in five transects separated by 10 meters along the path of the vehicle releasing the insecticide (Figure 1). This configuration and position of the cages has been shown to enable maximum penetration of aerosol into the cages.[24]

A typical trial involved 40 cages being exposed to a one insecticide. Two cages, one containing Bora females and the other containing Vauclin females, were placed at each of the four distances from insecticide release for each of the five transects along the path of spray release (Figure 1). Individual trials were conducted on separate days. However, there were exceptions, in that the first trials with K-Othrine® and Aqua K-Othrine® were carried out separately for the two strains and these trials only involved 20 cages. Twelve trials were performed, eight involving both strains and four involving only one strain.

In each trial, the knock-down[25] effect was measured by counting the number of knocked-down females and/or dead females 20 minutes post-treatment. All mosquitoes from a particular cage were transferred into cages (20 cm × 20 cm × 20 cm)
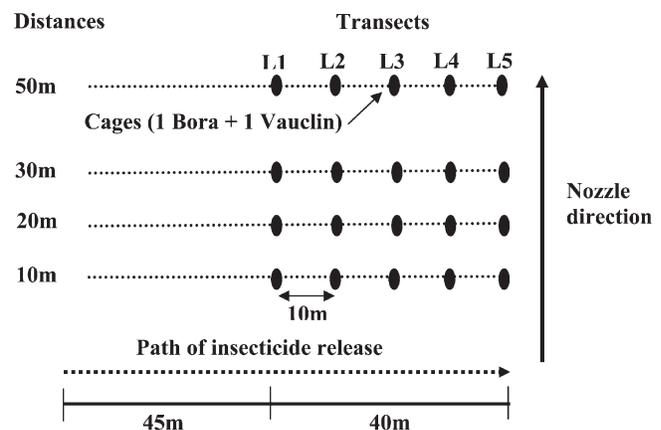

FIGURE 1. Layout of distance test experimental set-up with cages showing path of treatment from 10 meters to 50 meters using thermal fogger equipment. A total volume of 297 mL was applied over an area of 4,250 meters² where 40 cages of adults mosquitoes were placed on 5 transects. Each plot represents 2 cages (each cage containing 20 females of either the Bora or Vauclin strain) fixed on poles.

provided with sugar-soaked cotton (sugar diluted to a concentration of 10% in tap water) and brought back to the laboratory for assessment of post-treatment mortality 24 hours later. In each trial and for each strain, 5 cages containing 20 females were placed as controls 30 meters from the insecticide application area and in the opposite direction of the nozzle and wind direction. These cages were also assessed for the knock-down effect 20 minutes post-treatment and for mortality 24 hours later, and their values were used to correct for mortality observed in treatment cages.

During each trial, wind velocity, direction, temperature, and relative humidity were recorded using an anemometer (Sylva®) and a portable meteorological station (Testo175®). Trials were carried out when the wind direction was in the direction of the nozzle. No assays were carried out when the wind velocity exceed 5 meters/second.[26] The people spraying and those involved in recording the knock-down effect on mosquitoes were instructed about safety precautions. The people involved in spraying used protective clothing, shoes, and facemasks to reduce the risk of exposure to insecticide.

Chemical analysis of pesticide residues on nettings. One netting sample from each distance of the transect L3 (Figure 1) that had been exposed to each insecticide treatment was sent to the WHO collaborating center for the quality control of pesticides in Gembloux, Belgium, to determine the content of active substances. Two sub-samples of four pieces of 5 cm × 5 cm were cut randomly from each net to obtain two composite samples representative of the treatment. The Analytical method MEREPYRE of the Pesticides Research Department of the Walloon Agricultural Research Center was used. Detection of pyrethroids and pyrethrins was conducted by capillary gas chromatography with ^{63}Ni electron capture detection, and detection of naled was conducted by capillary gas chromatography with mass spectrometry detection using the external standard calibration.

Statistical analysis. Mortality recorded in laboratory bioassay (WHO test kits) was corrected for control mortality by the formula of Abbott[27] (in case of control mortality > 5%) such that corrected mortality = $[(X – Y)/X] \times 100$ where X = % survival in control cages and Y = % survival in treated cages. The data were then subjected to log-probit analysis[28] to determine 50% lethal dose (LD_{50}) and LD_{95} values and their 95% confidence intervals. Bora and Vauclin strains were considered as having different susceptibility to a given pesticide when the ratio between their LD_{50} (resistance ratio [RR_{50}]) values or LD_{95} (RR_{95}) values had confidence intervals (CIs) excluding the value 1.

Data from the field experiment were analyzed as a split-plot analysis of variance in a repeated measures design following the procedure of Milliken and Johnson.[29] The repeated measure was the knock-down effect on mosquitoes in each cage at 20 minutes post-treatment and mosquito mortality 24 hours

post-treatment. In each case, the observed effect was corrected for mortality in control cages. The largest experiment unit, or whole-plot, involved 12 separate trials of insecticide application. Individual trials were nested within a particular insecticide (product [P]). The next experimental units were the groups of cages at a particular distance from the point of product release (distance [D]). The next units were cages of mosquitoes classed by the strain of mosquito they held (strain [S]). The smallest experimental units were the individual cages. The whole experiment involved a total of 400 cages, with two measures of mortality being taken from each cage. Data were arcsine square-root transformed before analysis. The analysis was performed using JMP version 5.1.2.[30]

RESULTS

Insecticide resistance status of *Ae. aegypti* in Martinique. Results obtained from WHO tube tests are shown in Table 1. For each strain and each insecticide tested, the dose-mortality relationships were fitted by straight lines ($P > 0.05$). With the Bora strain, the LD_{50} of deltamethrin (0.002, 95% CI = 0.0021–0.0023) was significantly lower than those of naled (0.021, 95% CI = 0.02–0.023) and pyrethrum (0.22, 95% CI = 0.2–0.23), thus indicating the higher toxicity of deltamethrin against susceptible *Ae. aegypti* mosquitoes. However, the mosquitoes collected from Vauclin (F_1 progeny) showed high levels of resistance to deltamethrin ($RR_{95} = 68$) and to a lesser extent against pyrethrum ($RR_{95} = 14$) and naled ($RR_{95} = 12$).

Efficacy of insecticide space sprays against resistant mosquitoes. Trial experiments were made from June through July 2007. During this period, the temperature ranged from 28°C to 39°C, the relative humidity ranged from 47% to 68%, and the wind speed ranged from 0 meters/second to 4 meters/second. Data from the field experiment and the corresponding statistical analysis are shown in Figure 2 and Table 2, respectively.

Significantly fewer mosquitoes were dead 24 hours post-treatment than the number found knocked-down 20 minutes post-treatment (effect time; Table 2). This finding shows that some mosquitoes were able to recover from being knocked-down. Furthermore, the extent of recovery depended on the insecticide used (effect T.P.; Table 2). Recovery almost exclusively occurred in treatments involving the two pyrethrin-based products (Pyrethrum® and AquaPy®). Approximately 42% and approximately 48% recovered, respectively. This recovery was less than 7% in treatments with the three other products.

The significant difference found among insecticides (effect P; Table 2) was caused mainly by naled having a greater effect on knock down and mortality than the other four products (naled versus others; F[1,7] = 4.150, $P < 0.001$). Furthermore, a strong strain-by-product interaction showed that naled was the only product causing comparable knock down and mortality in

TABLE 1
Resistance status of *Aedes aegypti* from Martinique (Vauclin) to deltamethrin, pyrethrum, and naled in the World Health Organization tube test*

Insecticide	Strain	Slope (SE)	LD_{50}, % (95% CI)	LD_{95}, % (95% CI)	RR_{50} (95% CI)	RR_{95} (95% CI)
Deltamethrin	Bora	4.22 (0.27)	0.002 (0.0021–0.0023)	0.005 (0.0048–0.0061)	–	–
	Vauclin	2.3 (0.15)	0.071 (0.065–0.077)	0.36 (0.30–0.47)	**32 (29–35)**	**68 (54–84)**
Pyrethrum	Bora	4.1 (0.25)	0.22 (0.20–0.23)	0.55 (0.50–0.63)	–	–
	Vauclin	4.62 (0.27)	3.53 (3.34–3.73)	8.01 (7.23–9.07)	**15 (14–18)**	**14 (11–18)**
Naled	Bora	8.71 (0.45)	0.021 (0.02–0.034)	0.032 (0.031–0.034)	–	–
	Vauclin	2.7 (0.17)	0.1 (0.094–0.11)	0.41 (0.35–0.51)	**4.9 (4.4–5.4)**	**12 (10–16)**

* Values in **bold** are statistically significant. LD_{50} = 50% lethal dose; RR_{50} = 50% resistant ratio; CI = confidence interval.
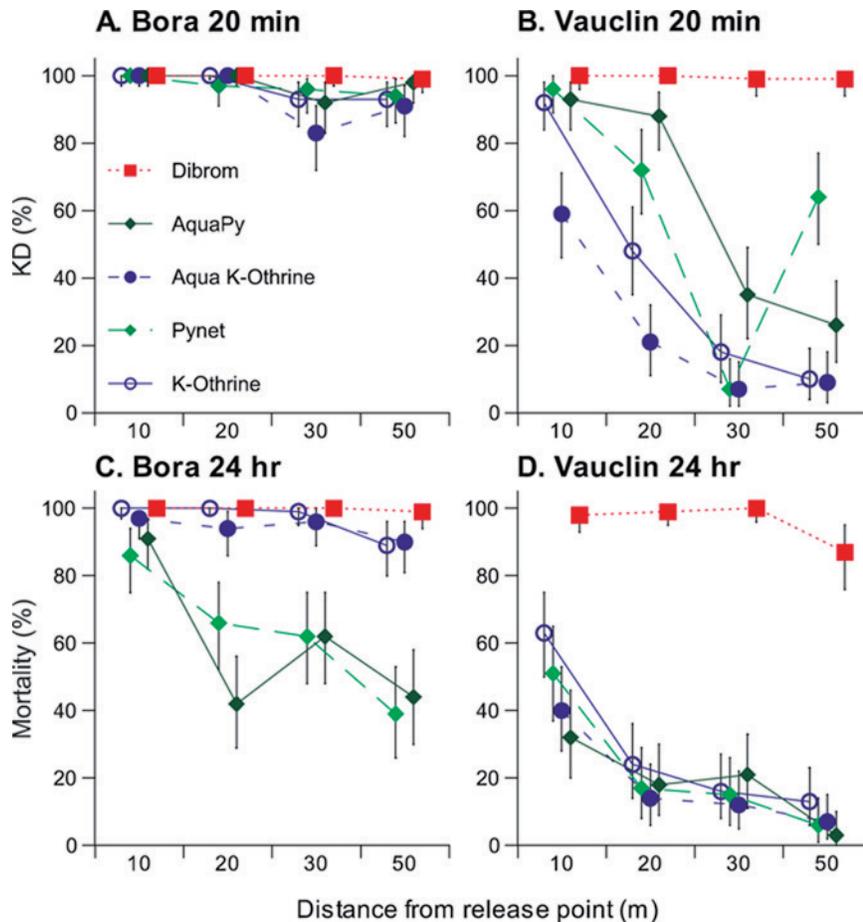
FIGURE 2.   Effect of insecticides with distance from the point of release on the knock-down effect 20 minutes post-treatment (**A** and **B**), and mortality 24 hours post-treatment (**C** and **D**) for the susceptible reference Bora strain (**A** and **C**) and the locally caught wild-strain Vauclin (**B** and **D**). Means and standard errors are back-transformed arcsine square-root estimates from the analysis in Table 2. This figure appears in color at www.ajtmh.org.

both mosquito strains, whereas the four other products had a much weaker effect on the wild-caught Vauclin strain (effect S.P.; Table 2).

There was less knock down and mortality as distance from the point of chemical release increased (effect D; Table 2). However, this trend depended on the strain concerned (effect S.D.; Table 2). Knock-down and mortality were generally higher for the Bora strain (effect S; Table 2) and did not decrease significantly over distance treatments. In contrast, the combined effect of knock down and mortality for the Vauclin strain was generally less and tended to decrease with distance from the point of product release. However, this pattern also depended on whether knock down or mortality after 24 hours was being considered because the knock-down effect on the Vauclin strain was relatively weak at distances of 30 meters and 50 meters (effect T.D.S.; Table 2). Interestingly the knock-down effect of the two pyrethrins against the Vauclin strain in the 10-meter and 20-meter treatments was significantly greater than for the two deltamethrins (knock-down effect of Pynet® and AquaPy® versus K-Othrine® and Aqua K-Othrine® for the Vauclin strain in the 10-meter and 20-meter treatments; $F[1,358] = 13.871$, $P < 0.001$).

Determination of pesticide content on nettings. Results showed an important loss in the amount of active substance with distance from release (Figure 3). With K-Othrine® 15/5, 42%, 38%, 25%, and 31% of the total ai sprayed was captured

at distances of 10, 20, 30 and 50 meters, respectively (operational rate = 1.03 g/hectare or 103 μg/meter² ai). With Aqua K-Othrine®, 25% and 34% of the total active substance was found at 10 meters and 20 meters, respectively, whereas less than 10% ai was detected at 30 meters and 50 meters (operational rate = 1.2 g ai/hectare). A much more important loss of insecticide was observed with natural pyrethrins, with less than 10% of the active substance detected regardless of the distance (operational rates = 9.95 g/hectare and 14 g ai/hectare for Pynet® and AquaPy®, respectively). More surprising was the lack of detection of naled on netting samples (limit of the quantification technique = 250 μg/meter² ai) despite the far greater amount of active ingredient sprayed per hectare (operational rate = 157 g ai/hectare).

DISCUSSION

The purpose of this study was to evaluate the impact of insecticide resistance of *Ae. aegypti* on the efficacy of pyrethroid and organophosphate ULV-space sprays. Bioassays first showed that the Vauclin strain was strongly resistant to deltamethrin ($RR_{95} = 68$) and to a lesser extent against pyrethrum ($RR_{95} = 14$) and naled ($RR_{95} = 12$). This finding confirms previous results obtained with other insecticides of the same chemical classes.[21,31] The simulated-field trial carried out in Martinique showed that pyrethroid resistance can strongly

TABLE 2
Split-plot repeated measures analysis of variance for treatment effects on mosquito knock-down/mortality*

Source	N	DFnum	DFden	SS	F	P
Between treatments						
Product (P)	4	4	7	4.347	14.289	0.002
Error (a)	12	7	21	0.489		
Distance (D)	3	3	21	4.062	17.803	< 0.001
D.P.	12	12	21	0.809	0.887	0.573
Error (b)	48	21	12	1.404		
Strain	1	1	12	26.130	343.530	< 0.001
S.P.	4	4	12	6.749	22.182	< 0.001
S.D.	3	3	12	1.047	4.590	0.023
S.P.D.	12	12	12	1.319	1.445	0.267
Error (c)	80	12	320	0.790		
Error (d)	400	320	358	9.854		
Within treatments						
Time (T)	1	1	358	10.324	135.729	< 0.001
T.P.	4	4	358	9.186	30.192	< 0.001
T.D.	3	3	358	2.569	11.259	< 0.001
T.S.	1	1	358	0.060	0.790	0.375
T.P.D.	12	12	358	1.344	1.473	0.132
T.P.S.	4	4	358	0.372	1.222	0.301
T.D.S.	3	3	358	0.759	3.328	0.020
T.P.D.S.	12	12	358	1.583	1.734	0.058

* N = number of parameters; DFnum = degrees of freedom numerator; DFden Degrees of freedom denominator; SS = sums of squares. Errors a, b, c, and d accounted for 4.6%, 8.3%, 4.3%, and 13.5% of the total variance explained, respectively.

reduce the efficacy of deltamethrin (K-Othrine® 15/5 ULV, Aqua K-Othrine®) and synergized pyrethrins (Pynet® and AquaPy®) relative to naled (Dibrom® 14 concentrate) when applied by ULV thermal fogging. Our experiments were conducted in an open setting, and it is likely that in field conditions of use, i.e., in urban areas with vegetation and resting places for mosquitoes, the efficacy of pyrethroids would be even worse. However, it was interesting to note that pyrethrins caused a greater knock-down effect than deltamethrin up to 20 meters from their release point. If one considers that knocked-down mosquitoes are rapidly eliminated or preyed upon in tropical areas,[32] formulations of AquaPy® or Pynet® may be more appropriate for controlling resistant *Aedes* spp. mosquitoes than synthetic pyrethroids.

In contrast to pyrethroids and pyrethrins, and despite the presence of moderate levels of organophosphate resistance
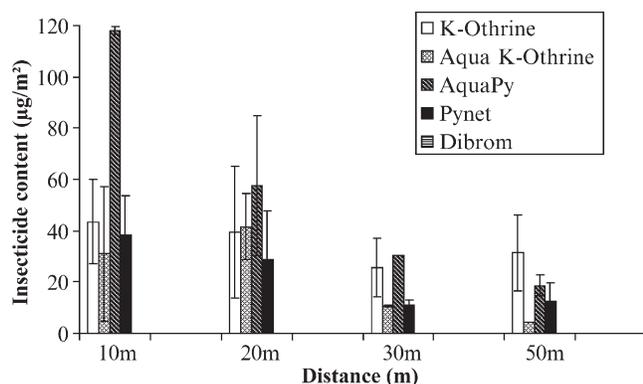

FIGURE 3. Insecticide content in mosquito nets after space sprays of K-Othrine® 15/5 ultra-low volume, Aqua K-Othrine®, AquaPy®, Pynet®, and Dibrom® 14 Concentrate according to distance. Data are expressed in microgams of active ingredient per cm^2 ± 95% confidence interval.

in mosquitoes, naled (157 g ai/hectare) was highly effective in terms of its knock-down effect and mortality. However, one should note that lower application rates (e.g., 24 and 60 g ai/hectare) were less effective against both susceptible and resistant mosquito strains. Ham and others[33] reported good efficacy of Dibrom® 14 Concentrate against *Ae. sollictans* and *Ae. taeniorhynchus* either by aerial (112 g ai/hectare) or ground spraying (22 g ai/hectare), although no information was provided on the insecticide resistance status of the mosquitoes tested. Despite controversy related to the use of organophosphates for adult control (i.e., their lower safety profile), these chemicals may represent at the current time the sole alternatives to pyrethroids in areas where pyrethroid-resistance is present.

Chemical analysis performed on nets showed an important loss of insecticide content with distance from its release. This finding can be explained by the nature of the substrate (netting with 1-mm mesh) that could not capture all of the insecticide sprayed. Despite the same conditions of storage and higher application rates, the content of natural pyrethrins on nets was much lower than that of deltamethrin. This finding is probably caused by the lower stability of pyrethrins that persist only for a short period after treatment.[34] Surprisingly, no residues of naled were found on mosquito nets regardless of the distance considered. Our lower limit to quantify this compound was 250 μg ai/meter^2, which corresponds to approximately 10% of the total amount that had been sprayed (i.e., 157 g ai/hectare). The lack of detection of the active substance may be explained by an important loss of the insecticide during solvent evaporation (Pigeon O, unpublished data). Nevertheless, rapid metabolic transformation of naled to its common active metabolite dichlorvos cannot be excluded.[35]

Vector control remains extremely difficult to implement because it requires a large budget, skilled staff, commitment, and active community participation.[36] According to Gratz,[37] space sprays can be an effective tool for adult mosquito control if they are correctly implemented. Conversely, some

investigators suggest that adult control has limited efficacy during epidemics because of difficulties in targeting mosquito populations.[36] This suggestion was partially supported by the study of Castle and others,[38] who demonstrated that malathion-space sprays did not have any impact on adult reduction and dengue transmission. If one considers these data, it would be interesting to conduct a small-scale field trial in Martinique to determine the impact of insecticide space sprays on mosquito density and longevity.

Pyrethroid resistance in Martinique is associated with the kdr mutation,[16] but we now have evidence that metabolic resistance play a key role in both pyrethroid and organophosphate resistance (Marcombe S and others, unpublished data). Strode and others[39] have recently identified several candidate genes of P450s mono-oxygenase and glutathione-S-transferase families in *Ae. aegypti* from Mexico that could be involved in this resistance. In addition, the first detection of an insensitive acetylcholinesterase in an *Ae. aegypti* population from Cuba[40] is worrying and strengthens the need to pursue the monitoring of insecticide resistance in *Aedes* spp. populations in the Caribbean and to characterize the physiologic mechanisms involved.

With resistance increasing on a worldwide scale and the dramatic reduction in the number of insecticides available for public health (due to environmental and toxicologic considerations, costs of development, and registration), there is an urgent need to develop innovative vector control strategies to maintain effective control of resistant mosquitoes and to slow down the evolution of insecticide resistance. The two-in-one strategy of mixing larvicides[41] and/or adulticides[42] having different modes of action may be useful in the short term. In addition, the pull-to-kill strategy, which consists of attracting adult mosquitoes to specific habitats containing an insecticide, may be promising means of targeting *Aedes* spp. populations.[43,44] In the long term, innovative technologies such as the sterile insect technique,[45] insect-pathogenic infection,[46] genetically modified mosquitoes[47] and viruses[48] will become essential tools for the prevention and control of arthropod-borne diseases.

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REFERENCES

1. Gubler DJ, 2002. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends Microbiol 10:* 100–103.
2. Gubler DJ, 2004. The changing epidemiology of yellow fever and dengue, 1900 to 2003: full circle? *Comp Immunol Microbiol Infect Dis 27:* 319–330.
3. Pages F, Corbel V, Paupy C, 2006. *Aedes albopictus:* chronical of a spreading vector. *Med Trop 66:* 226–228.
4. Vazeille M, Moutailler S, Coudrier D, Rousseaux C, Khun H, Huerre M, Thiria J, Dehecq JS, Fontenille D, Schuffenecker I, Despres P, Failloux AB, 2007. Two chikungunya isolates from the outbreak of La Reunion (Indian Ocean) exhibit different patterns of infection in the mosquito, *Aedes albopictus. PLoS One 2:* e1168.
5. World Health Organization, 2006. *Report of the Scientific Working Group on Dengue.* Document WHO/TDR/SWG/08. Geneva: World Health Organization.
6. Chaud P, Yebakima A, 2006. Programme de surveillance, d'alerte et de gestion des épidémies de dengue (PSAGE Dengue) en Martinique. *Rapport InVS:* 65.
7. Point Epidémiologique, 2008. Surveillance de la dengue, Bilan de l'épidemie 2007/2008. *PEP 2008.3:* 2.
8. Yebakima A, Failloux AB, 2002. Compétence vectorielle et génétique des populations d'*Aedes aegypti* à la Martinique. *Rapport Intermediaire Centre de Démoustication de la Martinique.*
9. Gubler DJ, 1998. The global pandemic of dengue/dengue haemorrhagic fever: current status and prospects for the future. *Ann Acad Med Singapore 27:* 227–234.
10. Corriveau R, Philippon B, Yebakima A, 2003. *Le Dengue dans les Départements Français d'Amérique. Comment Optimiser la Lutte Contre cette Maladie? IRD Édition.* Paris: Expertise Collégiale.
11. Yebakima A, 1991. Recherche sur *Aedes aegypti* et *Culex pipiens* en Martinique. Ecologie Larvaire, Résistance aux Insecticides, Application à la Lutte. Thèse de Doctorat d'Etat es Sciences, Université Montpellier II: 210.
12. World Health Organization, 2006. *Pesticides and their Application for the Control of Vectors and Pests of Public Health Importance.* Document WHO/CDS/WHOPES/GCDPP/2006.1. Geneva: World Health Organization.
13. Rawlins SC, 1998. Spatial distribution of insecticide resistance in Caribbean populations of *Aedes aegypti* and its significance. *Rev Panam Salud Publica 4:* 243–251.
14. Rodriguez MM, Bisset JA, Fernandez D, 2007. Levels of insecticide resistance and resistance mechanisms in *Aedes aegypti* from some Latin American countries. *J Am Mosq Control Assoc 23:* 420–429.
15. Hemingway J, Hawkes NJ, McCarroll L, Ranson H, 2004. The molecular basis of insecticide resistance in mosquitoes. *Insect Biochem Mol Biol 34:* 653–665.
16. Brengues C, Hawkes NJ, Chandre F, McCarroll L, Duchon S, Guillet P, Manguin S, Morgan JC, Hemingway J, 2003. Pyrethroid and DDT cross-resistance in *Aedes aegypti* is correlated with novel mutations in the voltage-gated sodium channel gene. *Med Vet Entomol 17:* 87–94.
17. Saavedra-Rodriguez K, Urdaneta-Marquez L, Rajatileka S, Moulton M, Flores AE, Fernandez-Salas I, Bisset J, Rodriguez M, McCall PJ, Donnelly MJ, Ranson H, Hemingway J, Black WC IV, 2007. A mutation in the voltage-gated sodium channel gene associated with pyrethroid resistance in Latin American *Aedes aegypti. Insect Mol Biol 16:* 785–798.
18. Bisset J, Rodriguez M, Molina D, Díaz C, Soca L, 2002. Esterasas elevadas como mecanismo de resistencia a insecticidas organofosforados en cepas de *Aedes aegypti. Rev Cubana Med Trop 53:* 37–43.

19. Rodriguez MM, Bisset J, Ruiz M, Soca A, 2002. Cross-resistance to pyrethroid and organophosphorus insecticides induced by selection with temephos in *Aedes aegypti* (Diptera: Culicidae) from Cuba. *J Med Entomol 39:* 882–888.

20. Macoris M, Andrighetti MT, Takaku L, Glasser CM, Garbeloto VC, Bracco JE, 2003. Resistance of *Aedes aegypti* from the state of Sao Paulo, Brazil, to organophosphates insecticides. *Mem Inst Oswaldo Cruz 98:* 703–708.

21. Rosine J, 1999. *Resistance d'Aedes aegypti et de Culex pipiens quinquefasciatus aux Insecticide Organophosphorés, Biologique et aux Pyréthrinoides en Martinique et en Guadeloupe.* Diplôme d'Etudes Approfondies. Paris: Université Pierre et Marie Curie (Paris VI): 51.

22. World Health Organization, 2001. *Guidelines for Assessing the Efficacy of Insecticidal Space Sprays for Control of the Dengue Vector Aedes aegypti.* Document WHO/CDS/CPE/PVC/2001.1. Geneva: World Health Organization.

23. World Health Organization, 2005. *Guidelines for Testing Mosquito Adulticides for Indoor Residual Spraying and Treatment of Mosquito Nets.* Document WHO/CDS/NTD/WHOPES/ GCDPP/2006.3. Geneva: World Health Organization.

24. Bunner BL, Posa FG, Dobson SE, Broski FH, Boobar LR, 1989. Aerosol penetration relative to sentinel cage configuration and orientation. *J Am Mosq Control Assoc 5:* 547–551.

25. World Health Organization, 1996. *Report of the WHO Informal Consultation on the "Evaluation and Testing of Insecticides."* Document WHO/CDT/WHOPES/IC/96.1. Geneva: World Health Organization.

26. World Health Organization, 2003. *Space Spray Application of Insecticides for Vector and Public Health Pest Control. A Practitioner's Guide.* Document WHO/CDS/WHOPES/ GCDPP/2003.5. Geneva: World Health Organization.

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

28. Raymond M, Prato G, Ratsira D, 1997. *Probit and Logit Analysis Program Version 2.0, Praxème: R&D.* Montepellier, France: Centre National de la Recherche Scientifique.

29. Milliken GA, Johnson DE, 1992. *Analysis of Messy Data. Volume I: Designed Experiments.* London: Chapman & Hall.

30. *JMP, Version 5.1.2,* 1989–2004. Cary, NC: SAS Institute Inc.

31. Yebakima A, Charles C, Mousson L, Vazeille M, Yp-Tcha MM, Failloux AB, 2004. Genetic heterogeneity of the dengue vector *Aedes aegypti* in Martinique. *Trop Med Int Health 9:* 582–587.

32. Mouchet J, Carnevale P, Julvez J, Manguin S, Richard-Lenoble D, Sircoulon J, 2004. *Biodiversité du Paludisme dans le Monde.* Paris: John Libbey Eurotext.

33. Ham C, Meisch M, Meek C, 1999. Efficacy of Dibrom, Trumpet, and Scourge against four mosquito species in Louisiana. *J Am Mosq Control Assoc 15:* 433–436.

34. World Health Organization, 2000. *Repellents and Toxicants for Personal Protection.* Document WHO/CDS/WHOPES/ GCDPP/2000.5. Geneva: World Health Organization.

35. Jokanovic M, 2001. Biotransformation of organophosphorus compounds. *Toxicology 166:* 139–160.

36. Gubler DJ, Kuno G, 1997. *Dengue and Dengue Hemorrhagic Fever.* New York: CAB International Press.

37. Gratz NG, 1991. Emergency control of *Aedes aegypti* as a disease vector in urban areas. *J Am Mosq Control Assoc 7:* 353–365.

38. Castle T, Amador M, Rawlins S, Figueroa JP, Reiter P, 1999. Absence of impact of aerial malathion treatment on *Aedes aegypti* during a dengue outbreak in Kingston, Jamaica. *Rev Panam Salud Publica 5:* 100–105.

39. Strode C, Wondji CS, David JP, Hawkes NJ, Lumjuan N, Nelson DR, Drane DR, Karunaratne SH, Hemingway J, Black WC IV, Ranson H, 2008. Genomic analysis of detoxification genes in the mosquito *Aedes aegypti. Insect Biochem Mol Biol 38:* 113–123.

40. Bisset J, Rodriguez MM, Fernandez D, 2006. Selection of insensitive acetylcholinesterase as a resistance mechanism in *Aedes aegypti* (Diptera: Culicidae) from Santiago de Cuba. *J Med Entomol 43:* 1185–1189.

41. Darriet F, Corbel V, 2006. Laboratory evaluation of pyriproxyfen and spinosad, alone and in combination, against *Aedes aegypti* larvae. *J Med Entomol 43:* 1190–1194.

42. Chung YK, Lam-Phua SG, Chua YT, Yatiman R, 2001. Evaluation of biological and chemical insecticide mixture against *Aedes aegypti* larvae and adults by thermal fogging in Singapore. *Med Vet Entomol 15:* 321–327.

43. Perich MJ, Kardec A, Braga IA, Portal IF, Burge R, Zeichner BC, Brogdon WA, Wirtz RA, 2003. Field evaluation of a lethal ovitrap against dengue vectors in Brazil. *Med Vet Entomol 17:* 205–210.

44. Darriet F, Corbel V, 2008. Influence des engrais de type NPK sur l'oviposition d'*Aedes aegypti. Parasite 15:* 89–92.

45. Benedict MQ, Robinson AS, 2003. The first releases of transgenic mosquitoes: an argument for the sterile insect technique. *Trends Parasitol 19:* 349–355.

46. Scholte EJ, Takken W, Knols BG, 2007. Infection of adult *Aedes aegypti* and *Ae. albopictus* mosquitoes with the entomopathogenic fungus *Metarhizium anisopliae. Acta Trop 102:* 151–158.

47. Ito J, Ghosh A, Moreira LA, Wimmer EA, Jacobs-Lorena M, 2002. Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. *Nature 417:* 452–455.

48. Bonning BC, Hammock BD, 1996. Development of recombinant baculoviruses for insect control. *Annu Rev Entomol 41:* 191–210.