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. 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.

Dengue fever is still the most important arboviral disease worldwide, causing 50–100 million cases and thousands of deaths every year. 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. Aedes aegypti (L.) is the only dengue virus vector in Martinique. On this island, where dengue occurs in an endemo-epidemic pattern, 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. Space spraying is used when source reduction has failed to limit the density of adult mosquitoes (i.e., high entomological 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, 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.

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.

Pyrethroid and organophosphate resistance in Ae. aegypti is now found worldwide 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. Resistance to pyrethroids caused by the kdr mutation has been reported in the Caribbean, South America, Africa, and Asia. 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. In Martinique, Rosine 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 sodium channel of domain II in the susceptible population and represents a serious obstacle for dengue vector control programs. Resistance is now found worldwide 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. Resistance to pyrethroids caused by the kdr mutation has been reported in the Caribbean, South America, Africa, and Asia. 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. In Martinique, Rosine 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 sodium channel, 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 pyrethroids 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 mosquitoes. Then, the WHO cage bioassay method 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
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 is 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 pyrethroids (AquaPy®, 3% [EW] [w/v]; Bayer Environmental Science, Lyon, France), and naled (Dibrom® 14 Concentrate, SL 87.4% [w/v]; 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 pyrethroids (AquaPy®, 3% [EW] [w/v]; Bayer Environmental Science, Lyon, France), and naled (Dibrom® 14 Concentrate, SL 87.4% [w/v] plus 13.5% PBO [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/ha for deltamethrin, 10 g ai/ha for pyrethroids (Pynet® and AquaPy®) and 114 g ai/ha 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. 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 ± 2°C and a relative humidity of 80 ± 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. 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.

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 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)
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 knockdown 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 exceeded 5 meters/second. 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 60Ni 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 Abbott27 (in case of control mortality > 5%) such that corrected mortality = [(X − Y)/X] × 100 where X = % survival in control cages and Y = % survival in treated cages. The data were then subjected to log-probit analysis28 to determine 50% lethal dose (LD50) and LD95 values and their 95% confidence intervals. Bora and Vauclin strains were considered as having different susceptibility to the insecticide used as having different susceptibility to a given pesticide when the ratio between their LD50 (resistance ratio [RR]50) values or LD95 (RR95) 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 LD50 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 (F1 progeny) showed high levels of resistance to deltamethrin (RR50 = 68) and to a lesser extent against pyrethrum (RR50 = 14) and naled (RR50 = 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.

Table 1

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Strain</th>
<th>Slope (SE)</th>
<th>LD50 % (95% CI)</th>
<th>LD95 % (95% CI)</th>
<th>RR50 (95% CI)</th>
<th>RR95 (95% CI)</th>
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<tr>
<td>Deltamethrin</td>
<td>Bora</td>
<td>4.22 (0.27)</td>
<td>0.0002 (0.00021–0.00023)</td>
<td>0.005 (0.00048–0.00061)</td>
<td>32 (29–35)</td>
<td>– –</td>
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<tr>
<td></td>
<td>Vauclin</td>
<td>2.3 (0.15)</td>
<td>0.071 (0.065–0.077)</td>
<td>0.36 (0.30–0.47)</td>
<td>15 (14–18)</td>
<td>14 (11–18)</td>
</tr>
<tr>
<td>Pyrethrum</td>
<td>Bora</td>
<td>4.1 (0.25)</td>
<td>0.22 (0.20–0.23)</td>
<td>0.55 (0.50–0.63)</td>
<td>4.9 (4.4–5.4)</td>
<td>12 (10–16)</td>
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<tr>
<td></td>
<td>Vauclin</td>
<td>8.71 (0.45)</td>
<td>0.021 (0.02–0.034)</td>
<td>0.032 (0.031–0.034)</td>
<td>– –</td>
<td></td>
</tr>
<tr>
<td>Naled</td>
<td>Bora</td>
<td>2.7 (0.17)</td>
<td>0.1 (0.094–0.11)</td>
<td>0.41 (0.35–0.51)</td>
<td>15 (14–18)</td>
<td>14 (11–18)</td>
</tr>
<tr>
<td></td>
<td>Vauclin</td>
<td>8.71 (0.45)</td>
<td>0.021 (0.02–0.034)</td>
<td>0.032 (0.031–0.034)</td>
<td>– –</td>
<td></td>
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</tbody>
</table>

* Values in bold are statistically significant. LD50 = 50% lethal dose; RR50 = 50% resistant ratio; CI = confidence interval.
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).

**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
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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, 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, 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 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 others33 reported good efficacy of Dibrom® 14 Concentrate against *Ae. sollicitans* 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/meter², 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 Gratza,37 space sprays can be an effective tool for adult mosquito control if they are correctly implemented. Conversely, some

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**Table 2**

<table>
<thead>
<tr>
<th>Source</th>
<th>N</th>
<th>DFnum</th>
<th>DFden</th>
<th>SS</th>
<th>F</th>
<th>P</th>
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<td>Between treatments</td>
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<td>Product (P)</td>
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*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.*

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**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 micrograms of active ingredient per cm² ± 95% confidence interval.
investigators suggest that adult control has limited efficacy during epidemics because of difficulties in targeting mosquito populations. This suggestion was partially supported by the study of Castle and others, 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, 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 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 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 and/or adulticides having slow down the evolution of insecticide resistance in Aedes spp. populations in the Caribbean and to characterize the physiologic mechanisms involved.

In the long term, innovative technologies such as the sterile insect technique, insect-pathogenic infection, genetically modified mosquitoes and viruses will become essential tools for the prevention and control of arthropod-borne diseases.

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