

Field Efficacy of New Larvicide Products for Control of Multi-Resistant *Aedes aegypti* Populations in Martinique (French West Indies)

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Abstract. World-wide dengue vector control is hampered by the spread of insecticide resistance in *Aedes aegypti*. We report the resistance status of a wild *Ae. aegypti* population from Martinique (Vauclin) to conventional larvicides (*Bacillus thuringiensis* var *israeliensis* [*Bti*] and temephos) and potential alternatives (spinosad, diflubenzuron, and pyriproxyfen). The efficacy and residual activity of these insecticides were evaluated under simulated and field conditions. The Vauclin strain exhibited a high level of resistance to temephos, a tolerance to insect growth regulators, and full susceptibility to spinosad and *Bti*. In simulated trials, pyriproxyfen and *Bti* showed long residual activities in permanent breeding containers (28 and 37 weeks), whereas under field conditions they failed to curtail *Ae. aegypti* populations after four weeks. Conversely, diflubenzuron and spinosad showed a residual efficacy of 16 weeks, suggesting that these chemicals may be promising alternatives to *Bti* and temephos for controlling insecticide-resistant *Ae. aegypti* populations.

INTRODUCTION

Dengue virus exists as four antigenically distinct serotypes (DEN-1, DEN-2, DEN-3, and DEN-4) and causes 50–100 million cases of dengue and thousands of deaths every year.¹ Because of global changes (environment, climate) and increasing transportation, recent decades have seen a dramatic resurgence of dengue throughout regions where dengue vectors are present and this has led to major public health problems.² Because there is still no vaccine or specific treatment available against the virus, vector control remains the only strategy for reducing dengue transmission. Effective vector control measures rely on active community participation, health education programs, and environmental management that include improvement of water supplies and storage, solid waste management, and modification of human-made larval habitats.³ During inter-epidemic periods or when the elimination of breeding habitats of the mosquito is not easily achievable, insecticide application in larval habitats is routinely conducted by public health services.^{4–6}

Unfortunately, many dengue vector control programs are threatened by development of insecticide resistance in *Aedes aegypti* (L.), the main vector of dengue virus.^{7,8} Recent field trials carried out in Martinique (French West Indies) showed that the level of pyrethroid resistance in adult mosquitoes is high⁹ and can drastically reduce the efficacy of space spray applications.¹⁰ The organophosphate temephos was widely used as larvicide for the control of dengue vectors in Martinique. Strong levels of resistance to organophosphates have also been detected in Southeast Asia,¹¹ South America,¹² and the Caribbean.⁹ In Martinique, temephos was replaced in 2009 by *Bacillus thuringiensis* var *israeliensis* (*Bti*) after European Biocide legislation (European Directive 98/8EC, February 1998). *Bti* has desirable properties for mosquito control because of its fast killing effect, a good toxicologic profile,¹³ and the absence of cross-resistance with conventionally

used pesticides. Unfortunately, *Bti* shows low residual activity in natural breeding sites¹⁴ and needs frequent re-application, especially in polluted habitats.^{15,16}

There is now a strong consensus among scientists and public health workers that alternative tools (insecticides) and strategies are urgently needed to ensure effective and sustainable control of dengue in the tropics.¹⁷ The new tools need to be specific, cost-effective, and safe for the environment and non-target fauna. Unfortunately, the research and development of new chemical classes for use in public health control has been rather limited in the past 20 years because it involves a long, expensive, and non-profitable process for private companies.

The rare new chemicals available for dengue vector control at the larval stage (e.g., diflubenzuron, spinosad, pyriproxyfen) have all arisen from the agricultural market.¹⁸ Diflubenzuron is an insect growth regulator (IGR) that belongs to the family of benzoylurea and acts by disrupting chitin synthesis and deposition.¹⁹ Several studies reported the potent insecticidal activity of diflubenzuron against several mosquito species, especially *Ae. aegypti*.²⁰ Spinosad belongs to the class of natural products that are based on natural metabolites (spinosine A and D) derived from the actinomycetale *Saccharopolyspora spinosa* and, as such, may be considered as a bioinsecticide.²¹ Because of its unique mode of action involving the post-synaptic nicotinic acetylcholine and γ -aminobutyric acid receptors,²² spinosad shows promising potential for the control of dengue vectors.²³ Pyriproxyfen is an IGR that mimics the action of juvenile hormones and is a potent inhibitor of embryogenesis, metamorphosis, and adult formation.²⁴ Pyriproxyfen is safe for non-target organisms and effective at low doses against *Ae. aegypti*.²⁵ All of the above insecticides are recommended by the World Health Organization (WHO) for use for vector control in drinking water sources and containers and may be used routinely by mosquito control services.^{26–28}

The purpose of this study was to characterize the resistance status of *Ae. aegypti* larvae from Martinique to conventional (*Bti* and temephos) and alternative insecticides (pyriproxyfen, diflubenzuron, and spinosad) and to assess their efficacy and residual activity under simulated and field conditions. The value of these tools for vector control is discussed.

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MATERIALS AND METHODS

Mosquito strains. Two strains of *Ae. aegypti* were used in trials conducted in the laboratory and in semi-field conditions. The susceptible reference Bora strain, which originated in Bora-Bora (French Polynesia), has been colonized for many years and is checked regularly as part of our laboratory routine for resistance mechanisms (e.g., knockdown resistance mutation, detoxification enzyme activity).⁹ The Vauclin strain was established from larvae caught in natural breeding sites of the community of Vauclin in Martinique (14°54'N, 60°84'W). Larvae of the first generation (F₁) were used for the laboratory and semi-field assays. This population showed strong resistance to commonly used pesticides because of the V1016I knockdown resistance mutation and increased metabolic detoxification.^{9,29}

Insecticides. For larval bioassays, technical grades of temephos (97.3%, Pestanal®; Sigma-Aldrich, Riedel-de Haën, Germany), pyriproxyfen (98.7%; Sumitomo Chemical Co., Ltd. First, Tokyo, Japan), diflubenzuron (99.5%, Pestanal®; Sigma-Aldrich), spinosad (90.4%; Dow AgroScience, Indianapolis, IN), and *Bti* Vectobac®12AS (Abbott Laboratories, Abbott Park, IL) (1.2%, 1,200 international toxic units/mg) were used. For the semi-field trials, temephos (Abate®; 1% granules [GR]), *Bti* (Vectobac®, water dispersible granule (WG), 3,000 UTI/mg, 37.4%), spinosad (7.48%, dispersible tablets [DTs], 0.5% GR), and pyriproxyfen (Sumilarv®, 0.5% GR) were used. For field trials, *Bti* (Vectobac® DT, 3,400 international toxic units/mg), spinosad (0.5% GR), pyriproxyfen (Sumilarv®, 0.5% GR), and diflubenzuron (Dimilin® TB-2, 2%) were used.

Laboratory evaluation. Larval bioassays were carried out using technical grades of temephos, *Bti*, spinosad, pyriproxyfen, and diflubenzuron according to WHO guidelines.³⁰ Bioassays were performed using late third and early fourth-instar larvae of the Bora and Vauclin strains. For each bioassay, 20 larvae of each strain were transferred to cups containing 99 mL of distilled water and 1 mL of the insecticide tested at the desired concentration. Five cups per concentration (100 larvae) and 5–8 concentrations in the activity range of each insecticide were diluted in ethanol, except for *Bti*, which was diluted in water. Control treatments consisted of the addition of 1 mL of ethanol to 99 mL of water (distilled water for *Bti* assays). Larval mortality was recorded after an exposure of 24 hours to temephos, spinosad and *Bti*. Because of the delayed action of diflubenzuron and pyriproxyfen, larval, pupal, and adult mortality was assessed every day until emergence. In these cases, larvae were fed every day with dry cat food at a concentration of 100 mg/L. For each bioassay, temperature was maintained at 27°C with a 12-hour light:12-hour dark photoperiod.

Simulated field trial. The trial was carried out in Fort de France, Martinique according to WHO procedures.³⁰ The effects of spinosad, temephos, *Bti*, and pyriproxyfen were evaluated and compared until the efficacy, i.e., the emergence inhibition rates (EIs) decreased to < 80%. Blue plastic containers (drums) with a capacity of 175 liters were used because they are widely used for water storage in Martinique and have been shown to be the most productive breeding habitats for *Ae. aegypti*.³¹ These containers were filled with 145 liters of domestic water and covered with a mosquito net to prevent oviposition by wild female mosquitoes in the area and to prevent the deposition of debris. Containers were placed

under a shelter to prevent direct exposure to rain and sunlight. All insecticides were tested at the WHO-recommended dosages for the control of mosquito larvae.^{32,33} A total of 24 containers (3 replicates per insecticide) were put in rows and allocated to insecticide treatments at random: 6 drums were treated with spinosad GR at concentrations of 0.1 mg/L and 0.5 mg/L, 3 with spinosad DT (1 tablet/145 liters, equivalent to 0.67 mg/L active ingredient [AI]), 6 with pyriproxyfen at concentrations of 0.02 mg/L and 0.05 mg/L AI, 3 with *Bti* (5 mg/L AI), 3 with temephos (1 mg/L AI), and 3 were left untreated (controls). Groups of 100 third-instar larvae of the F₁ generation of the Vauclin strain were added to each container with one gram of food (dry cat food) at time 0, and then every 10 days until the end of the experiment. The containers were replenished every 10 days to maintain the initial level of water.

Emerging adults were collected in each container by using electric aspirators. In each container, the temperature and pH were checked every 10 days with a portable tester (HI98128; Hanna Instruments, Ontario, NY) to detect any discrepancies between replicates and/or treatments. External temperature and hygrometry were recorded by using a meteorologic unit (Auria 4®; Degreane Horizon, Cuers, France) provided by the General Council of Martinique (Château Paille). Temephos, *Bti*, and pyriproxyfen were evaluated during February–December 2007. Spinosad GR and DTs were evaluated in September 2007–May 2008 because we received these formulations later in the year.

Large-scale field trials. The efficacy and the residual activity of the larvicides against natural populations of *Ae. aegypti* were evaluated in three sites in the commune of Vauclin, which is located in southeastern Martinique. This commune has a dry tropical climate with a rainy season during May–November and an annual precipitation of 2,000 mm. The three study sites were selected because they were isolated locations at least 2 km from each another (to avoid possible migration of *Ae. aegypti* between locations). The first site was Anse Maroquet, which is a group of houses located near a creek on the coast of the Atlantic Ocean, the second site was Château Paille, a classical housing estate next to the ocean, and the third site was Cadette, a hamlet situated approximately 4 km from the ocean in a more humid area (Figure 1). The areas of the treated areas were 0.02 km², 0.12 km², and 0.11 km² for Anse Maroquet, Château Paille, and Cadette, respectively.

Formulation and dosages used in the field trial were Vectobac® DT (1 tablet/50 liters, 5 mg/L), Sumilarv® 0.5% GR (0.05 mg/L), Dimilin® TB-2, 2% (1 tablet/200 liters, 0.2 mg/L), and Spinosad DT (1 tablet/200 liters, 0.5 mg/L). The DT formulations were preferred when possible because they were more convenient for field applications than WG formulations. In each location, 5 breeding sites of *Ae. aegypti* per insecticide were selected at random (i.e., 15 containers per insecticide in total and 15 untreated controls). The application rate of each larvicide was based on the capacity of the container and not the amount of water present at the time of application. Each container was positive for *Ae. aegypti* larvae before the trial began and their physical coordinates were recorded one week before treatments were applied (Figure 1).

Each container was sampled to determine the density of *Ae. aegypti* larvae and pupae prior to treatment (D0), two days post treatment (D2), and then every week thereafter (e.g., D7, D14, D21). The larval sampling method consisted of 3 dips using small fish nets (15 × 15 cm) that were conducted

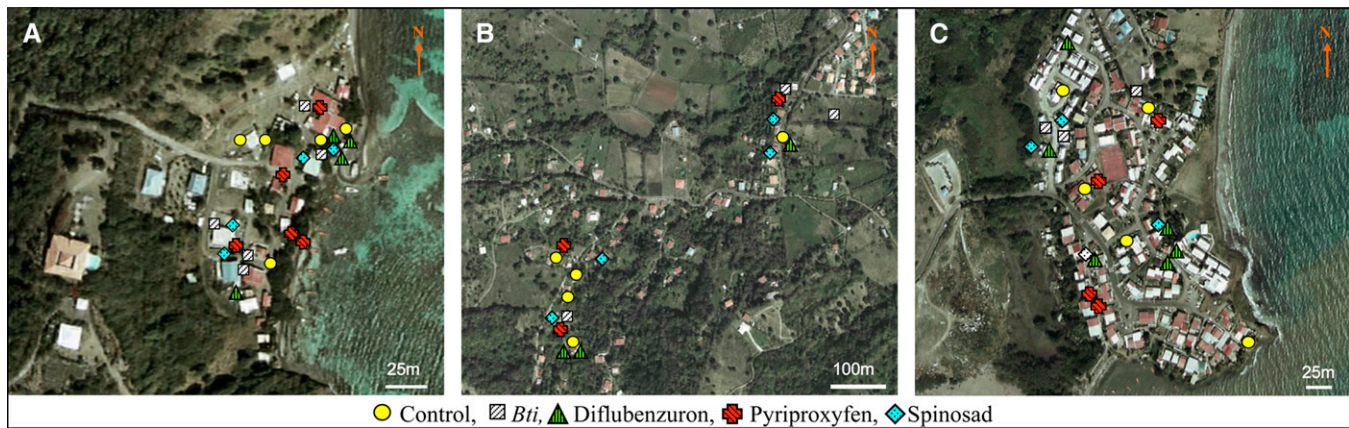


FIGURE 1. Satellite picture of the three field studies chosen for the evaluation of the insecticide formulations, Martinique. **A**, Anse Maroquet (14°33'N, 60°49'W). **B**, Cadette (14°33'N, 60°52'W). **C**, Château Paille (14°33'N, 60°50'W). The breeding habitats of *Aedes aegypti* were plotted by using a global positioning system. Different treatments of the containers are represented by symbols. This figure appears in color at www.ajtmh.org.

by the same operator in each location. Each fish net was allocated for use with one insecticide to avoid contamination among the containers. To compare the relative density (RD) of *Ae. aegypti* populations before and after treatment, third and fourth instars and pupae were counted per dip. The first, second, and third instars were replaced in their habitats whereas the fourth instars and pupae were brought back to the laboratory, put in 200 mL of water from their original container, and checked for adult emergence. Temperature and pH in each container were measured weekly. Meteorologic data were obtained by a meteorologic unit of the General Council of Martinique (Château Paille).

Statistical analysis. For determination of intrinsic activity of each larvicide, three replicates with larvae from different rearing batches were made at different times and the results were pooled for analysis ($n = 300$ per dose). In case of mortality in the control treatment $> 5\%$, larval mortality was corrected by using the formula of Abbott.³⁴ Results were analyzed by using log-probit method of Finney³⁵ and probit software of Raymond and others.³⁶ This software estimates the slope of regression lines and 50% and 90% lethal concentrations (LC_{50} and LC_{90}) with 95% confidence intervals (CIs). The Bora-Bora and Vauclin strains were considered as having different susceptibilities to a given insecticide when the ratio between their $LC_{50/95}$ had CIs excluding the value of 1 (significant at $P < 0.05$).

Regarding the simulated field trial, emergence inhibition rates (% EI) and 95% CIs were calculated for the average of the 3 replicates per insecticide according to the formula $\% EI = ((C - T)/C) \times 100$, where C is the emergence in the control and T is the emergence in the treated container at the same time period. For each formulation, curves are presented until the % EI decreased to $< 80\%$, which corresponds to the threshold generally considered for reapplication of the treatment.²⁹

For large-scale field trials, the RD of *Ae. aegypti* larvae and pupae for the five insecticide treatments and their SEs over time were estimated on the logit scale by using a mixed model analysis of variance in a split-plot design (see below). The RD was estimated by using the formula of Mulla:³⁰ $RD = 100 - (C1/T1) \times (T2/C2) \times 100$, where C1 is the average number of larvae in control containers prior to treatment (D0) calculated separately for each location, and C2 is the average number of larvae in control containers at each location and day of sampling. The log (number of larvae and pupae + 1) per container was

used to calculate average values prior to back-transformation. T1 is the number of larvae and pupae on D0 in each container to be treated with insecticides, and T2 is the number of larvae and pupae in each treated container for each day of sampling. Estimates of RD with negative values were set to zero. Analysis of RD was conducted on a logit scale ($\log[RD/(1 - RD)]$). To do so, values of RD were transformed by the equation $0.005 + 0.99 \times (RD/100)$ to avoid values of either 0 or 1.

Analysis of variance used to analyze the data involved a split-plot design in which the three localities were treated as whole-plots (random, nominal) and the effect of insecticide treatments (fixed, nominal) was tested against the interaction between treatments and localities (random, nominal). The model also estimated variation among containers within each locality and treatment (random, nominal). The sub-plot level of the model involved the effects of day of sampling and the interaction between day of sampling and treatments. These fixed nominal effects were tested against the residual error variation. The restricted maximum likelihood method in JMP[®] version 7.1 (SAS Institute, Cary, NC) was used to fit the model.

Containers that disappeared during the experiment or that were emptied completely (e.g., because of domestic use) were considered missing data. Estimates of RD are shown until populations recovered to $> 20\%$ of their initial size.

RESULTS

Insecticide resistance status of *Ae. aegypti* in Martinique. Results of larval bioassays on the Bora and Vauclin strains are shown in Table 1. For each strain and each insecticide, the dose-mortality relationships were fitted by regression ($P > 0.05$). With the susceptible Bora strain, pyriproxyfen, diflubenzuron, and temephos showed the highest insecticidal activity, followed by *Bti* and spinosad. The Vauclin strain showed strong resistance to temephos ($RR_{50} = 44$ and $RR_{95} = 175$). The slope of the regression line for temephos was significantly lower in the Vauclin strain (2.1) than in the Bora strain (8.1), indicating a heterogeneous response of mosquitoes to this insecticide. A slight but significant reduction of pyriproxyfen and diflubenzuron insecticidal activity was observed in the Vauclin strain ($RR_{50} = 2.2$, $RR_{95} = 1.9$) than in the susceptible Bora strain ($RR_{50} = 1.8$). Conversely, the Vauclin strain was fully susceptible to *Bti* ($RR_{50} = 1$) and spinosad ($RR_{50} = 0.6$).

TABLE 1
Resistance status of *Aedes aegypti* (Bora and Vauclin strains) against the five larvicides tested, Martinique*

| Larvicide | Strain | Slope (SE) | LC ₅₀ (95% CI) (µg/L) | LC ₉₅ (95% CI) (µg/L) | RR ₅₀ (95% CI) | RR ₉₅ (95% CI) |
|---------------|---------|------------|----------------------------------|----------------------------------|---------------------------|---------------------------|
| Temephos | Bora | 8.5 (0.4) | 3.7 (3.6–3.8) | 5.7 (5.5–6) | – | – |
| | Vauclin | 2.1 (0.1) | 160 (150–180) | 1,000 (870–1,180) | 44 (40–48) | 175 (150–205) |
| <i>Bti</i> | Bora | 5.4 (0.5) | 120 (110–130) | 250 (220–280) | – | – |
| | Vauclin | 4.9 (0.6) | 130 (120–140) | 270 (220–330) | 1 (0.8–1.3) | 1.1 (0.7–1.7) |
| Pyriproxyfen | Bora | 3.5 (0.2) | 0.11 (0.10–0.11) | 0.32 (0.29–0.36) | – | – |
| | Vauclin | 3.9 (0.5) | 0.24 (0.21–0.27) | 0.64 (0.48–0.84) | 2.2 (1.8–2.6) | 1.9 (1.3–3.0) |
| Spinosad | Bora | 3.9 (0.6) | 350 (310–400) | 920 (640–1,340) | – | – |
| | Vauclin | 4.2 (0.2) | 220 (210–230) | 550 (510–600) | 0.6 (0.5–0.8) | 0.6 (0.5–0.8) |
| Diflubenzuron | Bora | 5.5 (1.2) | 1.7 (1.4–2.1) | 3.4 (2.4–4.9) | – | – |
| | Vauclin | 6 (1.1) | 3.1. (2.8–3.5) | 5.9 (4.7–7.5) | 1.8 (1.1–3) | 1.7 (0.6–4.5) |

*LC₅₀ = 50% lethal concentration; CI = confidence interval; RR₅₀ = 50% resistant ratio; *Bti* = *Bacillus thuringiensis* var *israeliensis*. Resistant ratios = LC₅₀ of vauclin strain/LC₅₀ of Bora strain; RRs in **bold** are significantly different from 1 ($P < 0.05$).

Residual activity of formulated insecticides in simulated field trial. During the trial, outdoor temperatures recorded daily ranged from 19°C to 35°C. The temperatures recorded in the containers ranged from 24 to 28°C and the pH was 7.7 ± 0.22 . Limited variation in temperature and pH was recorded between treatments throughout the trial, suggesting that the environmental conditions did not have an impact on the efficacy of the different formulated insecticides.

Data from the semi-field trial and the corresponding statistical analysis are shown in Figure 2 and Table 2, respectively. During the trial, the emergence inhibition rate of the control group was generally low (< 10%) except in cohorts 1, 5, and 11 in which it was > 20%. Analysis of variance showed that there was a significant effect of the treatments over time (effect TD, Table 2). The results showed that the EI for the temephos and *Bti* decreased to < 80% after 140 days and 200 days, respectively. With temephos, the EI varied between 90% and 100% until 130 days, and the efficacy of *Bti* remain maximal (100%) until 110 days. The unusually long persistence of *Bti* WG in drums (EI > 80% until 190 days) is unusual.

The residual activities of spinosad and pyriproxyfen were dosage-dependent. The EI of pyriproxyfen GR decreased to < 80% after 160 and 260 days (0.02 mg/L and 0.05 mg/L, respectively). Pyriproxyfen GR (0.05 mg/L) showed the longest residual activity in comparison to all other insecticides tested ($P < 0.05$). Spinosad GR showed far lower residual activity than pyriproxyfen GR, i.e., 110 days and 150 days were

required to go below the cut-off point (EI₈₀) at concentrations of 0.1 mg/L and 0.5 mg/L, respectively. No increase in residual activity was noted for the spinosad DT formulation compared with spinosad GR, despite similar application rates (0.67 mg/L versus 0.5 mg/L).

Large-scale field trials. The field trial was conducted during February 2008–August 2008. During this period, the average temperature per month was 27°C and ranged from 21°C in February to 33°C in August at the three study sites. The average precipitation over the trial was 60 mm per month, with a maximum of 91 mm in August and a minimum of 32 mm in March. The rainiest day was in April (32 mm). The pH recorded in the containers remained constant and close to neutrality in each locality (range = 6.4–8.2), and temperatures within drums ranged from 25°C to 33°C throughout the trial.

The efficacy of the different formulated products was expressed in term of RD of *Ae. aegypti* over time (Figure 3). Analysis of variance (Table 2) showed that there was a strong and significant difference of RD among treatments over time (effect DT; $P < 0.001$). These results were not influenced by random variation among the different localities (effect L, coefficient of variance < 1%) or by an interaction between localities and treatments (effect LT coefficient of variance < 3%). However, the treatment effect was strongly significant ($P = 0.008$).

In the control containers, there was a time-dependant decrease of mosquito density that reached a minimum level of 37% of the original abundance 98 days post-treatment (D98)

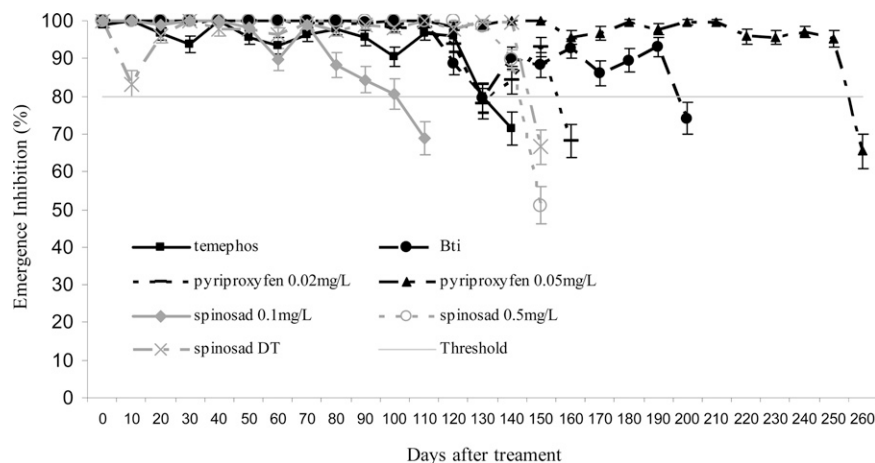


FIGURE 2. Emergence inhibition rates and their 95% confidence intervals in simulated field trials for the larvicide formulations tested against *Aedes aegypti*, Martinique.

TABLE 2

Split-plot repeated measures analysis of variance for treatment effects on *Aedes aegypti* in semi-field trials and field experiments, Martinique*

| Source | No. | DFnum | DFden | F | P | CV |
|--|----------|---------|-----------|---------------|--------------|-----------|
| Emergence inhibition in semi-field assay | | | | | | |
| Treatment (T) | 4†/3‡ | 3†/2‡ | 8†/6‡ | 2.64†/6.47‡ | 0.12†/0.032‡ | — |
| Day (D) | 29†/18‡ | 28†/17‡ | 224†/102‡ | 29.73†/25.17‡ | < 0.001†‡ | — |
| TD | 116†/54‡ | 84†/34‡ | 224†/102‡ | 2.09†/2.79‡ | < 0.001†‡ | — |
| Container (C) treatment | 12†/9‡ | — | — | — | — | 48%†/10%‡ |
| Relative density | | | | | | |
| T | 5 | 4 | 6.806 | 8.75 | 0.008 | — |
| D | 22 | 21 | 792.9 | 17.36 | < 0.001 | — |
| DT | 110 | 84 | 791.8 | 2.04 | < 0.001 | — |
| Locality (L) | 3 | — | — | — | — | < 1% |
| LT | 15 | — | — | — | — | 2.3% |
| C (LT) | 62 | — | — | — | — | 24.9% |
| Emergence inhibition in field experiment | | | | | | |
| T | 5 | 4 | 6.99 | 7.55 | 0.011 | — |
| D | 20 | 19 | 824.5 | 15.31 | < 0.001 | — |
| DT | 80 | 76 | 822.7 | 1.44 | 0.009 | — |
| L | 3 | — | — | — | — | < 1% |
| LT | 15 | — | — | — | — | < 1% |
| C (LT) | 62 | — | — | — | — | 13.97 |

*No. = number of parameters; DFnum = degrees of freedom numerator; DFden = degrees of freedom denominator; CV = coefficient of variance.

† = first experiment.

‡ = second experiment.

(Figure 3). With *Bti* tablets, the RD decreased drastically from 100% to 1% (95% CI = 0.4–4%) at D2, but rapidly increased again to 22% (95% CI = 7–49%) at D28. This finding indicates a fast-killing effect but a low residual activity of this insecticide in plastic containers. The RD with diflubenzuron decreased to 8% one week after the treatment but increased beyond the threshold of 20% RD twice at D77 (34% RD) and D98. We suspect the first increase in RD was caused by natural fluctuation rather than a real loss of efficacy. The residual activity of spinosad was 100% until 98 days post-treatment and was comparable to that of diflubenzuron.

The relative density of *Ae. aegypti* exposed to pyriproxyfen is shown in Figure 4. Because the RD of mosquitoes strongly fluctuated over the time, it was difficult to estimate its residual efficacy by using this criteria. This finding is most likely explained by the specific mode of action of pyriproxyfen that acts only at the pupal stage. Thus, the data indicated the natural fluctuation of larval density in the containers.

The emergence inhibition rates of the different formulations against fourth instar larvae and pupae of *Ae. aegypti* collected in the different containers and tested in the labo-

ratory are shown in Figure 5. Statistical analysis (Table 2) showed that the EIs were not correlated with the study site effect (effect L coefficient of variance < 1%). However, the treatment effect was significant ($P = 0.011$). Of the variance explained, 13% could be attributed to the effect of random variation among containers (Table 2). The EI rates for *Bti* and pyriproxyfen were > 95% two days after the treatment, but rapidly decreased to 1.7% (SE = 0.6–5%) and 7.9% (SE = 3–21%) 28 days post-treatment. This period of 28 days corresponded to the duration required to obtain relative densities beyond 20% for *Bti* under field conditions (Figure 4). The EI with spinosad was strong (SE = 85–100%) and lasted for 98 days, before decreasing to 37% (SE = 10–76%) after 105 days (Figure 5). The EI for diflubenzuron decreased to < 80% after 126 days (21%, SE = 5–59%).

DISCUSSION

This study tested the field efficacy of conventionally used and alternative larvicides for the control of *Ae. aegypti*, the main dengue vector in Martinique. The simulated field trial

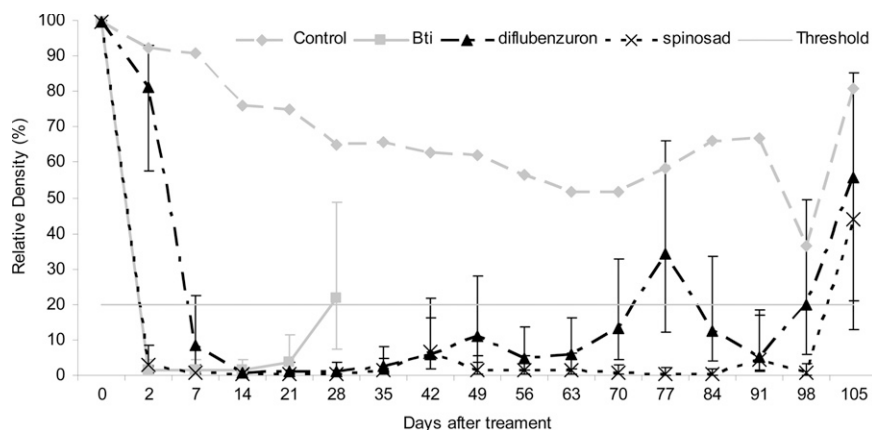


FIGURE 3. Relative densities and standard errors of *Aedes aegypti* in breeding habitats after treatments with *Bacillus thuringiensis* var *israeliensis* (*Bti*), diflubenzuron, and spinosad, Martinique.

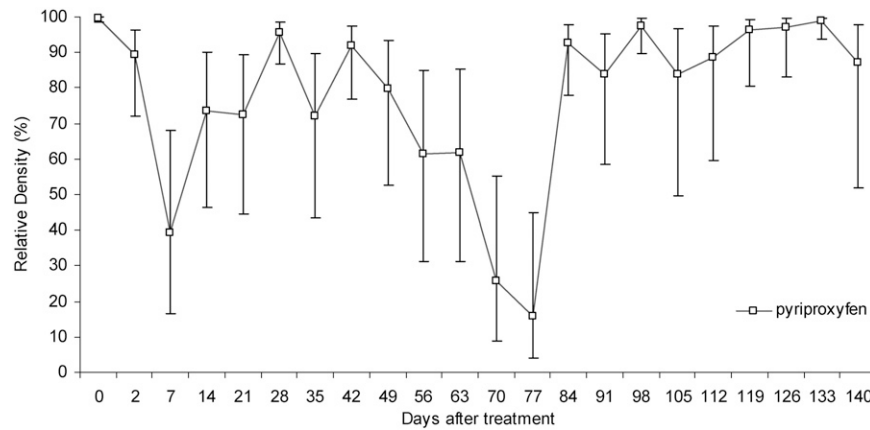


FIGURE 4. Relative densities and standard errors of *Aedes aegypti* in breeding habitats after treatments with pyriproxyfen, Martinique.

conducted at Fort de France showed 28 weeks and 20 weeks residual activity (EI > 80%) for *Bti* and temephos, respectively, against the local Vauclin population. In a similar study conducted in Malaysia on *Ae. aegypti* and *Ae. albopictus*, the efficacy of temephos and *Bti* was much lower (efficacy = 7–9 weeks and 11 weeks, respectively).¹⁵ The rather long residual activity of *Bti* and temephos in this study could be questioned because it is known that *Bti* toxins sediment rapidly from the water surface³⁷ and because of the high level of temephos resistance in the Vauclin population. Regarding temephos, this finding may be explained by an absence of degradation by ultraviolet light and by the fact that the dosage (1 mg/L) applied in drums corresponded to a concentration that killed 95% of the resistant mosquitoes in laboratory conditions (Table 1). Regarding *Bti*, the high dose used (5 mg/L) and the absence of organic content may have extended the duration of activity, as reported.¹⁷

In the same conditions, spinosad GR at a concentration of 0.1 mg/L showed the lowest residual activity (EI > 80% for 14 weeks). A similar performance of spinosad (16 weeks) was observed against *Ae. aegypti* in semi-field conditions.³³ Spinosad GR and DT applied at a concentration of 0.5 mg/L showed longer and comparable residual activity (20 weeks), but the DT formulation is easier to use in the field because the insecticide immediately sank to the bottom of the container,

unlike the granules, which floated on the surface of the water for several days.

The long efficacy of pyriproxyfen (EI > 80% for almost 9 months) has been reported,^{38,39} although its residual activity was much longer in our simulated conditions than in previous conditions. However, the performance of larvicides in well-controlled conditions has already been pointed out by several authors^{17,40} and should be interpreted with caution because of the absence of direct exposure to rain, sunlight, and organic matter could lead to an over-estimation of the residual activity of an insecticide. Simulated-field experiments represent a useful method for screening new insecticides and/or to select dosages for field application, but they cannot be used to predict the performance of formulated products under real conditions.

As expected, a lower residual activity was observed for all insecticides during the field trial compared with the simulated-field trial. *Bti* DT and pyriproxyfen GR lost their efficacy after 4 weeks (RD > 20% and EI < 80%), and diflubenzuron DT and spinosad DT provided much longer residual activity (14 weeks). As reported,¹⁷ the poor efficacy of *Bti* (1 tablet/50 L, equivalent a concentration of 5 mg/L) may render the control of *Ae. aegypti* populations in Martinique difficult in preventing dengue. No data could be generated for temephos because it was banned in 2009 from the insecticides available

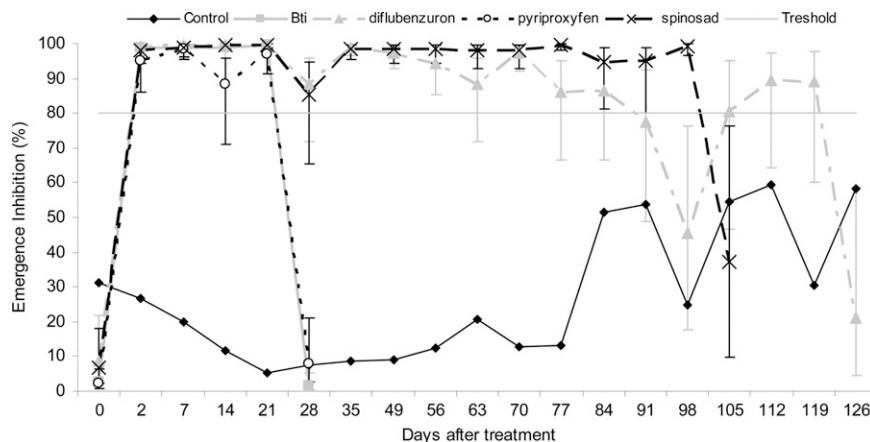


FIGURE 5. Emergence inhibition rates and standard errors of the fourth instar (L4) larvae and pupae collected in the breeding habitats treated against *Aedes aegypti*, Martinique.

for use in the European Community. However, a previous study conducted in Martinique showed that 18% of the containers treated with the organophosphate were positive for *Ae. aegypti* 28 days post-treatment (Yp-Tcha MM and Etienne M, unpublished data). Organophosphate resistance is widespread in dengue vectors in the Caribbean and probably explains the poor performance of temephos against wild *Ae. aegypti* mosquitoes.

The short residual activity of pyriproxyfen contrasts with that obtained in the simulated field trial. Sihuincha and others⁴¹ demonstrated longer performance (i.e., mortality rate > 80% for 5 months) against *Ae. aegypti* in water tanks treated with the same dosages in Peru. Similar to *Bti*, pyriproxyfen may have degraded because of environmental conditions. As previously observed by Vythilingham and others,³⁹ temperatures in the containers periodically increased to as high as 33°C and this increase might have contributed to reduced residual activity of pyriproxyfen. The low residual activity of pyriproxyfen GR in Martinique and its specific mode of action on pupae may render its future use and evaluation at an operational scale difficult.

In contrast, spinosad DT (0.5 mg/L) showed good residual efficacy and emergence inhibition rates against *Ae. aegypti* (15 weeks). The fast killing effect of spinosad DT (> 97% reduction of mosquitoes 2 days post-treatment) and a long-residual effect may be attributed to the specific composition of the DT tablet, which is made of two specific layers: an outer layer consisting of an effervescent system that provides fast release of the AI, and a second layer formulated to dissolve the insecticide gradually over time into the water.³³ Similar to spinosad, diflubenzuron remained effective in drums for up to 15 weeks. This finding is consistent with the results of Thavara and others,²⁰ who demonstrated that diflubenzuron can provide effective control of *Aedes* mosquitoes for 3–4 months in the field. The long residual activity of spinosad and diflubenzuron and their favorable environmental profile¹ make them very attractive alternatives for dengue vector control in water tanks and water storage containers in the tropics.

During the field trial, a time-dependent reduction in the number of larvae and pupae was observed in control containers. This result is probably explained by the simultaneous presence of treated and control containers in the same localities in which the effect of insecticide treatments led to a global reduction in the numbers of mosquitoes emerging and ovipositing in the local *Ae. aegypti* population. This finding clearly suggests an impact of the treatments at a community level. The absence of locality-dependent effects on the effect of insecticide treatments (coefficient of variance < 1%) may be explained by the fact that the three localities were 4 km from each other and experienced similar environmental conditions. Whatever the treatment or locality, 25% of the variance in relative density that could be explained was caused by variation among containers. Variation of temperature in the larval habitats over time could be responsible for some of this change. However, a more likely explanation is that each container experienced its own particular environmental variation, e.g., rainfall, habitat use, sunlight exposure, organic content. This variation at the level of individual containers could influence the presence or absence of larvae beyond the presence of an insecticide. This explanation indicates the necessity of having replicate containers in field trials to avoid accurate estimates of mosquito density being obscured by random variation among individual containers.

Finally, our study showed that the field population of mosquitoes from Vauclin was highly resistant to temephos, tolerant to pyriproxyfen and diflubenzuron, but fully susceptible to *Bti* and spinosad. The absence of *Bti* resistance even after 12 years of use for larval control is encouraging if it is used for regularly treated larval habitats (< 4 weeks). As suggested, this finding is probably caused by the complex structure of the multiple *Bti* toxins, which may slow down the acquisition of resistance in natural populations.⁴² Similarly, the absence of spinosad resistance in the Vauclin population is promising and may be explained by the fact that this pesticide has never been used for mosquito control in the Caribbean. Moreover, because of its unique mode of action on post-synaptic receptors and the simultaneous action of two neurotoxins, one might expect a lower risk for the appearance of resistance compared with classical synthetic insecticides.^{43,44} Unfortunately, decreased susceptibility to pyriproxyfen and diflubenzuron was observed in the Vauclin population (significant resistant ratios).

Previous authors have reported similar findings in mosquito populations exhibiting high levels of resistance to temephos.^{45,46} In Martinique, the organophosphate temephos has been used since the 1970s but has recently been abandoned because of European Biocide legislation. Previous studies showed that temephos resistance is widespread in the Caribbean and South America, and this resistance is strongly associated with increased activity of glutathione S-transferases, α -esterases and β -esterases, and, to a lesser degree, mixed function oxidases.^{9,47} Because diflubenzuron and pyriproxyfen have never been used in public health programs in Martinique, it is possible that the cross-tolerance of mosquito larvae to IGR has arisen through the extensive use of temephos for vector control. This suggestion may have operational consequences for dengue vector control and the future use of IGRs if these products may contribute to selection for existing resistance mechanisms in natural populations of *Ae. aegypti*.

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