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Field comparison of thermal and non-thermal ultra-low-volume applications using water and diesel as solvents for managing dengue vector, *Aedes aegypti*

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Abstract

OBJECTIVES To compare the effectiveness on *Aedes aegypti* (Linneo) (Diptera: Culicidae) of a larvicide-adulticide ULV formulation applied by a thermal or a cold fogger using different solvents.
METHODS We applied, in field conditions, a ULV formulation containing pyriproxyfen and permethrin, using a thermal and a cold fogger and water or diesel as solvent. We determined the effectiveness of these applications on *Ae. aegypti* adults and larvae by different bioassays and measuring Breteau, house and adult indices.
RESULTS When water was used as solvent, the treatments applied with the cold or the thermal foggers were equally effective on adult mortality (close to 90%) and adult emergence inhibition (% EI) (close to 70%). When the thermal fogger was used with water as solvent, the adult mortality outside the houses (85%) was higher, but not significantly different, than with diesel (65%). The contrary happens inside (22% *vs*. 58%), while there were no differences in %EI. Adult and larval indices behaved similarly in all areas, with a slight tendency for the treatments applied using water as solvent to be more effective.
CONCLUSIONS Water-based formulations are equally or more effective than the one applied with diesel as solvent. The use of water as solvent will not only improve the effectiveness of this formulation but also reduce the environmental impact and costs of spraying compared to the use of diesel.

keywords cold fogger, thermal fogger, solvents, mosquito

Introduction

Aedes aegypti (Linnaeus) (Diptera: Culicidae) is the main vector of dengue virus. Rigorous environmental sanitation and source reduction are used as control methods against *Ae. aegypti,* but these methods are neither routinely nor uniformly practiced. During the mid-20th century, the health authorities of American countries, together with the Pan American Health Organization (PAHO), carried out important *Ae. aegypti* eradication campaigns. Eradication was achieved in Argentina in 1965 (Ousset *et al.* 1965). However, by the end of the 1980s, the country was re-infested by the mosquito and since 1997 has suffered a series of small dengue epidemics almost every year until 2009, when a large outbreak occurred involving more than 20 000 cases (Masuh 2008).

When an epidemic is in progress, the goal is to adequately reduce the adult *Ae. aegypti* density as quickly as possible, thereby interrupting transmission of the virus to uninfected hosts; in these cases, ultra-low-volume (ULV) application of various mosquito adulticides is the recommended control method in the Americas (PAHO 1981).

There are two main types of fogging machines: thermal foggers and cold foggers. Thermal fogging is an outdoor space treatment in which the fog is produced by a device that uses heat to break up the insecticide formulation into very small droplets (5–30 μm in diameter) that disperse in the air. When the chemical, usually diluted in oil-based carriers, is heated, it is vaporised in a combustion chamber and expelled to form a dense cloud. Applications should be carried out early in the morning before thermal convection currents lift the fog from the ground (Gratz 1999). In cold foggers, a special device breaks up the formulation into small droplets by mechanical means using a high-pressure pump and a fine nozzle. The cold fogger can dispense formulations and generate the droplets in a precise manner, but its ability to penetrate dense foliage or obstacles is not considered as good as thermal fogging (Barber *et al.* 2007).

When selecting spray equipment and insecticides, factors such as the recommended dose, droplet size, the moving speed of the vehicle, the sprayer cost, etc., are very important. The droplet size of an aerosol insecticide influences its efficiency in killing adult mosquitoes (Mount *et al.* 1975) and depends on several factors, including the particular nature of the solvent used (Mabbett 2003). In Argentina and other Latin-American countries, the use of diesel as a solvent is often recommended, invoking better insecticidal effect, more aerosol dispersion and high visual impact at the time of application. However, only a few studies have compared the effectiveness of insecticides using different solvents and sprayers. Yap *et al.* (2001) compared water and diesel as solvents using a portable thermal fogger for treatments indoors with pyrethroids, while Mount *et al.* (1966) compared thermal and non-thermal aerosols using water or fuel oil as solvent against stable flies (*Stomoxys calcitrans* (Linnaeus) (Diptera: Muscidae)) and *Ae. taeniorhynchus* (Weidemann, Diptera: Culicidae).

In vector mosquito control programs, simultaneous control of adult and larval stages is preferable to reduce the overall vector mosquito population and subsequently decrease or disrupt the transmission of a disease. Outdoor ULV spray tests using a mixture of chemical adulticides with larvicides (Yap *et al.* 1997a,b) have recently been carried out. A new ULV formulation containing permethrin as an adulticide and pyriproxyfen as a larvicide was recently developed in our laboratory and yielded excellent results in an initial field trial (Lucia *et al.* 2008). We also measured the droplet size of this formulation and its effectiveness in semi-field conditions, using different sprayers (cold and thermal foggers) and solvents; we found that the effectiveness on *Ae. aegypti* adults was similar, while on the larvae the water-based formulations were more effective (Harburguer *et al.* 2012).

The objective of this study was to compare, in a field assay, the effectiveness of the larvicide-adulticide ULV formulation applied by a thermal fogger using water or diesel as solvent and as a water-based cold fogger.

Materials and methods

Biological material

We used an insecticide-susceptible CIPEIN strain of *Ae. aegypti* that originated from the Rockefeller strain in Venezuela and has been maintained in our laboratory since 1996. The strain was reared as described in previous reports (Seccacini *et al.* 2006) at the laboratory of the NOA region for Vector Control Program (National Ministry of Health) in Villa Libertad (Misiones, Argentina). Adults 2–3 days old of both sexes and late 3rd or early 4th instar *Ae. aegypti* larvae were used.

Study site

Field tests were conducted in Puerto Libertad. This is a small subtropical city with historical cases of dengue and high *Ae. aegypti* populations. Puerto Libertad has 9000 inhabitants; it is located 50 km south of Puerto Iguazú in the Province of Misiones (Argentina). Almost all the houses in this city are based on two types of construction: wooden walls and sheet metal roofs, and brick walls and tiled roofs. A typical single-storey house has two or three bedrooms, a living room and a kitchen.

Insecticide formulation and equipment

A mixture of permethrin 10% (3-phenoxyphenyl) methyl 3-(2,2-dichloroethenyl)-2,2-) dimethyl cyclopropane carboxylate) and pyriproxyfen 2% (2-(1-methyl-2-(4-phenoxyphenoxy)ethoxy)pyridine) as an emulsifiable concentrate (EC) was formulated by Chemotecnica S.A. (Argentina).

Water or diesel was used as solvent for the EC. When water was used as solvent, 5% of polyethylene glycol 1000 (Química Oeste, S.A., Buenos Aires, Argentina) was added to the mixture as an antievaporant.

A Swingtec Swingfog® SN 101 (Swingtec GmbH, Isny, Germany) thermal fogger was used. The SN 101 is a truck-mounted machine with a net weight of 40 kg, designed to disperse oil and water-based chemicals when the appropriate fogging attachments are used. The cold fogger was a truck-mounted Fog XXI Minor Plus machine (Simpya SRL, La Plata, Argentina), with a net weight of 180 kg, which can be used with oil or water-based chemicals.

Fogging operations and effectiveness assessment

Four areas of about 50 ha, with approximately 200 houses each, were used for ULV treatments. The four areas had similar socioeconomic characteristics and natural barriers such as rivers or large green spaces acting as buffer zones. One of four possible treatments was assigned at random to each area: cold fogger with water as solvent (Area A), thermal fogger with water as solvent (Area B), thermal fogger with diesel as solvent (Area C) and control area or untreated (Area D).

During ULV application, both with the cold and the thermal fogger, the flow was regulated to release 650 cm^3/min; under these conditions, 10 g of permethrin +2 g pyriproxyfen/ha were sprayed. The vehicle speed was approximately 10 km/h and the machine nozzles were pointing towards the dwellings. The spray application route was designed according to WHO protocol (WHO 2003) for ULV treatments starting on the downwind side of the spray area. Applications were carried out during the peak of mosquito flight activity (either before dawn or after sunset) and all windows and doors were open at the time of spraying.

To assess fumigation effectiveness, cylindrical screened sentinel cages built with 18-mesh nylon and 15 cm long ×3 cm in diameter were used, according to WHO protocols with minor modifications (Reiter & Nathan 2006). An hour before treatment, 16 adult mosquitoes (50% of each sex), 1–3 days old and fed on raisins, were transferred to each cage. Ten cages were hung inside 10 randomly selected dwellings (one per house) and 10 more outside, at 3 m from the pulverisation line (corresponding with the edge of the sidewalk) and at 1.5 m from the floor. In addition, ten 250 ml plastic jars (7.5 cm in diameter) containing 15 late 3rd or early 4th instar *Ae. aegypti* larvae in 200 ml tap water were placed inside each house and 10 more outside. This procedure was repeated for all areas, including the control area (Area D).

Both screened cages and plastic jars were taken back to the laboratory after treatment ended and kept at room temperature. Adult mosquitoes in cages were provided hydration and mortality was assessed after 24 h. The 250 ml plastic jars were inspected daily until death or adult emergence of all the individuals to determine the inhibition of adult emergence (EI%). EI% was calculated as shown below and adjusted for larval or pupal mortalities in the corresponding controls according to Mulla *et al.* (1974).

$$EI(\%) = 100 - 100(T/C),$$

where T is the percentage of emergence in treated containers and C is the percentage of emergence in control containers.

Monitoring *Ae. aegypti* populations

Population levels of *Ae. aegypti* larvae were estimated using larval sampling according to PAHO (1994) and expressed as Breteau index (BI) defined as the number of positive containers per 100 inspected houses, and house index (HI) defined as the percentage of houses infested with larvae and/or pupae. The adult index (mean number of *Ae aegypti* adults per house during 10 min of inspection by one person) was also determined. Adults were captured with a manual aspirator (Philips FC 6092 12V, Philips, Amsterdam, Netherlands), placed in drinking cups covered with netting and taken to the laboratory for identification. As adult capture with manual aspirators depends on the skills of the collectors, the same collector was assigned to an area for the entire study period. To control for collection bias, we compared captures in the 2 weeks before the treatments, and if there were no significant differences between areas, we determined that there were no differences in the efficiency of adult capture between the collectors.

Breteau, house and adult indices were determined weekly, starting 2 weeks before treatment and ending once the mosquito population levels returned to pre-treatment values. To assess larvae and adult infestation, 25 houses in each area were randomly selected each week. Entomological studies were carried out for 13 weeks, from 20 March to 12 June 2009.

Statistical analysis

Adult mortality and %EI were corrected with Abbott's formula (Abbott 1925), and compared using one-way analysis of variance (anova); differences between means were compared using Tukey's multiple range test. The accepted level of significance for all comparisons was $P < 0.05$ (Statistica for Windows V7.0; StatSoft Inc, Tulsa, OK, USA). Data of adult mortality and %EI inside or outside the dwellings were analysed individually because we wanted to compare the effectiveness of sprayers and solvents at different locations.

Adult index values were compared using one-way analysis of variance (anova) for each date; differences between means were compared using Tukey's multiple range test. Data from March 23 and April 17 were square root transformed before analyses. The accepted level of significance was $P < 0.05$.

When it was necessary to compare the effectiveness inside and outside the dwellings for the same treatment, we used a Student's *t*-test (Statistica for Windows V7.0; StatSoft Inc, Tulsa, OK, USA).

Results

Treatment effectiveness

Table 1 shows the mortality of caged *Ae. aegypti* adults located inside or outside the houses. The results of the one-way anova analyses inside the dwellings indicate that there were differences between treatments ($F = 3.56$; df = 26; $P = 0.042$). When water was used as solvent indoors, we observed 23% and 45% mortality with the TF and CF, respectively; however, this difference was not statistically significant ($P = 0.22$). When using the TF, however, mortality was significantly higher using diesel (58%) than water (23%) as solvent ($P = 0.034$). As can be seen also in Table 1, there was a significant difference between treatments for adult mortality outside the houses ($F = 4.22$; df = 25; $P = 0.026$). When water was used as solvent, there were no differences in adult mortality between the CF

Table 1 Mortality (% ±95%CI) of caged adults and emergence inhibition (EI% ±95%CI) of *Ae. aegypti* larvae exposed to cold (CF) or thermal (TF) aerosols of permethrin and pyriproxyfen using water or diesel as solvent

	Treatment	Adult mortality (%) (95% CI)	One-way ANOVA *P*-value	Emergence inhibition (%) (95% CI)	One-way ANOVA *P*-value
Inside	CF water (T_1)	45.5 (25.1–65.8)	0.042*	47.1 (32.8–61.2)	0.304
	TF water (T_2)	22.7 (0.00–46.6)		56.6 (48.1–65.2)	
	TF diesel (T_3)	58.4 (37.8–79.0)		52.4 (48.5–56.4)	
Outside	CF water (T_1)	86.1 (76.9–95.2)	0.026*	71.0 (65.7–76.3)	0.034*
	TF water (T_2)	85.4 (73.5–100.0)		62.2 (51.5–72.9)	
	TF diesel (T_3)	64.6 (48.0–81.2)		59.2 (53.9–64.5)	

*At least one treatment is significantly different one-way ANOVA, *P* values for Tukey's multiple range test: Adult mortality: Inside: T1 *vs.* T2 $P = 0.23$, T1 *vs.* T3 $P = 0.59$, T2 *vs.* T3 $P = 0.035$, Outside: T1 *vs.* T2 $P = 0.99$, T1 *vs.* T3 $P = 0.035$, T2 *vs.* T3 $P = 0.063$; EI%: Outside: T1 *vs.* T2 $P = 0.15$, T1 *vs.* T3 $P = 0.034$, T2 *vs.* T3 $P = 0.79$.
All data were corrected for control mortality with Abbott's formula.

(86%) and the TF (85%) ($P = 0.99$). On the other hand, adult mortality was significantly higher with the CF (86%) than with the TF diesel-based application (65%) ($P = 0.035$). The same trend can be seen when comparing the use of water or diesel with the TF, although this result was not statistically different ($P = 0.063$).

The adult emergence inhibition (EI%) inside the houses was not significantly different between any treatments as shown in Table 1 ($F = 1.19$; df = 26; $P = 0.304$). On the other hand, we found significant differences between treatments in EI% when the jars were located outside the houses ($F = 3.79$; df = 26; $P = 0.034$). Adult emergence inhibition when water was used as solvent either with the CF (71%) or the TF (62%) was not statistically different ($P = 0.15$). Also, when the TF was applied, there were no significant differences in the %EI between the use of water (62%) and diesel (59%) as solvents. However, we found that the application using the TF with diesel (59%) was less effective than the one using the CF with water (71%) as solvent ($P = 0.034$).

When we used water as solvent, with both kinds of sprayers, the effectiveness was higher outside than inside the houses (Student's *t*-test, CF adult mortality, $t = -4.12$, df = 18, $P < 0.001$; %EI, $t = -3.57$, df = 18, $P = 0.002$; TF adult mortality, $t = -5.01$, df = 15, $P < 0.001$), except for %EI using the TF (Student's *t*-test, $t = -0.93$, df = 16, $P = 0.36$). However, when the TF was used with diesel as solvent, no differences were found on the effectiveness inside or outside the dwellings, with values between 50 and 60% (Student's *t*-test, adult mortality $t = -0.52$, df = 18, $P = 0.60$; %EI, $t = -2.01$, df = 18, $P = 0.06$).

Entomological evaluation

Entomological surveillance showed that in the four areas assayed, larval and adult index values were very high before the treatment, which is characteristic of this area of Argentina (Masuh *et al.* 2003). Pre-treatment adult index values (Figure 1) were between 3 and 4.5 *Ae. aegypti* adults per house during 10 min of search. No significant differences were observed between the four areas ($F = 2.50$, df = 96, $P = 0.06$; $F = 2.36$, df = 96, $P = 0.17$), indicating that the mosquito population was relatively consistent throughout the region, facilitating the comparison of treatments and ensuring that there were no differences in the efficiency of adult capture between the collectors.

Immediately after treatment, the adult index fell to values below 1.5 in all treated areas. There were significant differences between the areas ($F = 41.02$, df = 96, $P < 0.001$), and all values of the treated areas were significantly lower than the control area. Adult index in Area A (CF water) was significantly lower ($P = 0.012$) than in Area C, where the TF with diesel was used, while there were no significant differences between Area B (TF water) and Areas A and C ($P = 0.26$ and $P = 0.13$). Since the first week after treatment and for seven consecutive weeks, the adult index in all treated areas was significantly lower than the control area, and there were no differences between treatments. After 8 weeks post-treatment (approximately 56 days), the adult index in all areas was not significantly different from the control area ($F = 0.79$, df = 96, $P = 0.50$).

Figure 2 shows the results of the Breteau index (BI). As can be observed, the pre-treatment values of BI were between 110 and 140 for all four areas. BI started to fall almost 2 weeks after treatment in all areas, showing a minimal value of 96, 3 weeks after application. The BI began to rise about 4 weeks after treatment in Area C, maintaining a value close to 120, until week 9 where reached values similar to those in the control area. The increase in this index in Area B was more gradual, starting at week 7 after treatment, but by week 8 had reached

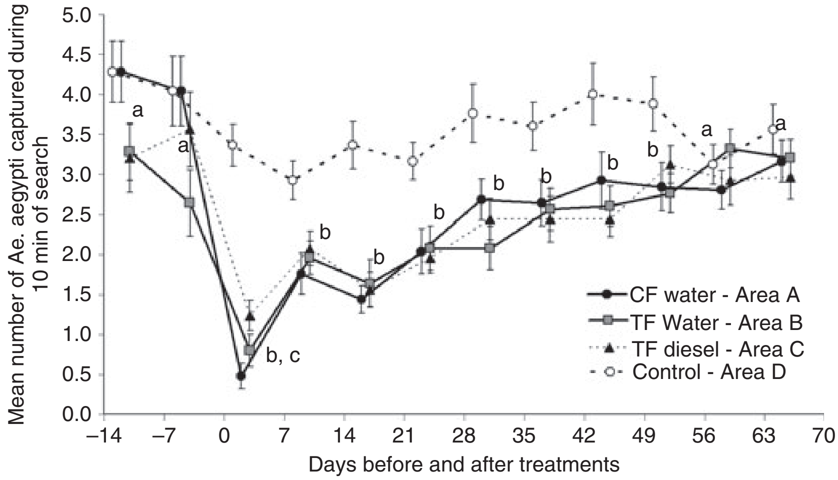

Figure 1 Adult index (mean number of adults captured per house during a 10-min search by one person ±SE) for all treated areas and the control area. Day 0 indicates the day of application. CF: cold fogger, TF: thermal fogger. (a) No significant differences between areas ($P > 0.05$), (b) Adult index in the treated areas is significantly different from the control area ($P < 0.05$), (c) There are significant differences between treatments (in this case, treatment CF water with TF diesel).

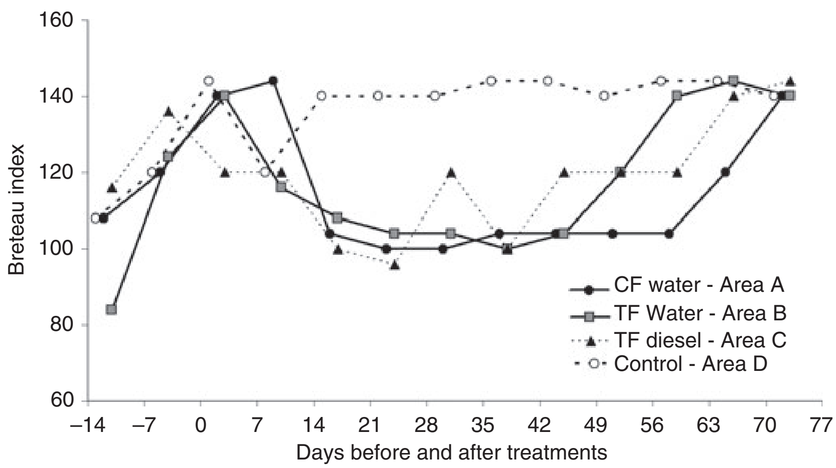

Figure 2 Larval abundance measured as Breteau index (BI) for all treated areas and the control area. Day 0 indicates the day of application. CF, cold fogger; TF, thermal fogger.

similar values to the control area. In Area A, BI behaved in the same way than in Area B.

House index (HI) after treatments behaved similarly to the BI as can be observed in Figure 3, but the decrease was lower and the treated areas reached values similar to those in the control area faster. On week 4 after treatment, HI in Area B reached values around the control area, while in areas A and C took 1 or 2 weeks more.

Discussion

We found that when truck-mounted machines are used, ULV treatment effectiveness inside the dwellings is close to 50% for both adult mortality and %EI, using cold or the thermal foggers, with water or diesel as solvent. These results are in agreement with previous work of Chadee (1985) who found that the mortality rate of caged adult mosquitoes indoors was 30%, applying malathion with a Leco Generator mounted on a truck in St. Joseph, Trinidad. Perich *et al.* (1990, 2000) also obtained similar results: they found that the mortality of caged adults indoors was 20–60%. These results suggest that an insufficient number of insecticide droplets reach inside the dwellings where caged mosquitoes or jars containing larvae were located.

The mortality of caged adults outside the houses using the CF agrees with previous work from our laboratory (Lucia *et al.* 2008) where we found mortality values around 90% with the same formulation at 3 or 6 m from the pulverisation line.

No differences were found between the CF and the TF fogger when water was used as solvent; both were equally effective. Both treatments were also more effective outside than inside the houses. However, when the TF was used with diesel as solvent, the effectiveness was the same inside or outside the dwellings. This could be owing to

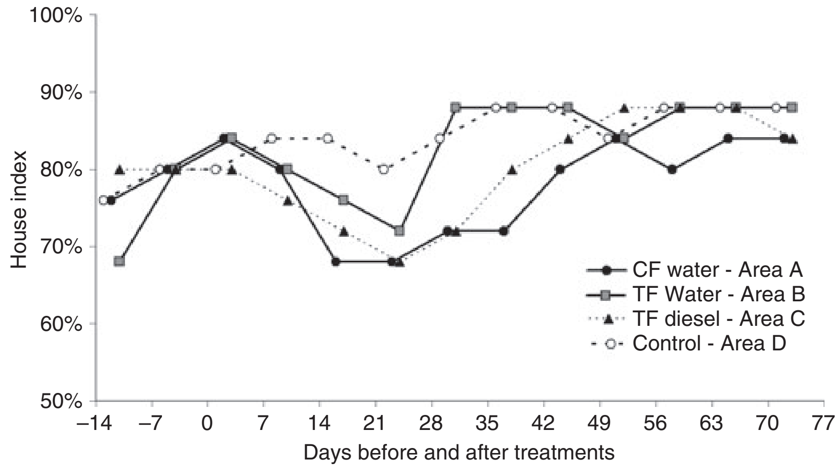

Figure 3 Larval abundance measured as house index (HI) for all treated areas and the control area. Day 0 indicates the day of application. CF, cold fogger; TF, thermal fogger.

differences in droplet size between the two types of solvents (Hoffmann *et al.* 2008; Harburguer *et al.* 2012), as diesel-based formulations have a significantly lower volume median diameter (D_{V50}). As the D_{V50} is low, the droplets remain suspended for longer and at greater distances from the sprayer nozzle, with greater fog dispersal, being more homogenously effective inside and outside.

Yap *et al.* (1997a) compared the effectiveness of a Pesguard® FG 161 formulation using a TF and water or diesel as solvent and found 100% adult mortality at 24 h with both solvents. Nevertheless, the water-based thermal fogging formulation of Pesguard FG 16l seemed to achieve significantly better larvicidal effect than diesel-based spray. We achieved similar results in previous studies from our laboratory performed with the formulation of permethrin and pyriproxyfen, in semi-field conditions (Harburguer *et al.* 2012). However, in this study we found that when we use the TF, the effectiveness on adults outside the houses is higher with water than with diesel, and the contrary happens inside, while there seems to be no difference in %EI. This may be because, while Yap *et al.* (1997b) employed pyrethroids as larvicides, we used the insect growth regulator (IGR), pyriproxyfen, which is effective at lower doses (Seccacini *et al.* 2008) than pyrethroids, so a few droplets containing the IGR reaching the jars with larvae located inside or outside the dwellings suffice to produce the larvicidal effect.

Larval indices (BI and HI) dropped by 30–40 compared to pre-treatment and control values, although this value continued to be very high, is consistent with previous results of our laboratory (Lucia *et al.* 2008). Although these indices behaved similarly in all areas, there was a slight tendency to be more effective when the cold fogger with water as solvent (Area A) was applied. However, as we treated limited areas and not the whole city, the re-infestation with adults from other untreated areas could be an important factor when interpreting changes in these indices.

The efficacy of ULV pesticide application against mosquitoes is based on the premise of an airborne insecticide droplet impinging on a flying mosquito. Therefore, droplet size is a crucial factor modulating the trajectory of aerosols generated by thermal or cold foggers. In previous work from our laboratory in semi-field conditions (Harburguer *et al.* 2012) using the same formulation and portable sprayers, we found significant differences in droplet size between water and oil solvents, although they were all equally effective on *Ae. aegypti* adults. However, this was not the case with the larvicidal effect, where sprays using water as a solvent were significantly more effective than the oil-based formulations.

In this study we assessed, in a field assay, the effectiveness of a larvicide-adulticide ULV formulation applied by a TF using water or diesel as solvent and as water-based cold fogger. From our results, we conclude that the water-based formulation applied with CF or TF is more or equally effective than the one applied with TF in which diesel was used as solvent. Therefore, the use of water as a solvent, both applied with thermal or cold machines, will not only improve the effectiveness of this formulation but also reduce the environmental impact and costs of spraying compared to the use of oil solvents. Further research on the performance of this formulation by sequential applications at the time that indices begin to rise is needed to determine their specific effectiveness on *Ae. aegypti* adults and larvae, especially in situations of dengue epidemics that usually require several sequential applications to rapidly reduce vector density. Cheaper water-based applications with less environmental impact would enhance the feasibility of such sequential cycles.

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