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Malathion insecticide resistance in *Aedes aegypti*: laboratory conditions and *in situ* experimental approach through adult entomological surveillance

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Abstract

objective In Brazil, the most common method of controlling outbreaks of arbovirus is by the use of chemical sprays, which kill the insect vector, *Aedes aegypti*. The main objective of this study was to evaluate the resistance of *Ae. aegypti* to the insecticide, malathion, in situ. The location of this study was the municipality of Foz do Iguaçu, in the state of Paraná, Brazil.
methods Ultra-low-volume (ULV) fogging equipment was used, by vehicle, to apply the insecticide in situ, and mosquito populations after treatment were compared with those of control areas. The resistance of strains collected from the municipality was compared to the Rockefeller strain under laboratory conditions.
results We found 220 adult female specimens and 7423 eggs of *Ae. aegypti* in the areas subjected to UBV treatment, whereas 245 adult females and 10 557 eggs were found in the control areas. The UBV treatment area showed no significant difference compared to the control area, for all the indices. Mortality of the Rockefeller colony varied more quickly when there were slight variations in malathion concentration than the Foz do Iguaçu population.

keywords Arboviruses, entomological surveillance, chemical control, ultra-low volume (ULV), Foz do Iguaçu

Sustainable Development Goals (SDGs): SDG 3 (good health and well-being), SDG 15 (life on land), SDG 17 (partnerships for the goals)

Introduction

Arboviruses, associated with zoonoses, circulate widely in tropical and subtropical regions, where there is an abundance of mosquitoes [1]. Among the species of mosquito to transmit arboviruses, *Aedes aegypti* stands out for being highly anthropophilic, for its current territorial expansion [2] and for the ability to transmit arboviruses of relevance to public health, such as dengue (DENV), Zika (ZIKV) and Chikungunya (CHIKV) [3] viruses. The co-circulation of these arboviruses, which cause similar symptoms in humans, represents a great challenge for public health [4].

DENV is the most prevalent human arboviral infection in the world, causing 100 million infections annually and threatening 4 billion people [5,6]. CHIKV received considerable public health attention, following 225 000 cases in Réunion in 2005–06, hundreds of cases in Italy and France, and more than 1 million cases in the Americas [2]. ZIKV has become a global concern, due to its association with Guillain–Barré syndrome, microcephaly and other congenital malformations [7], totalling approximately between 440 000 and 1 300 000 cases in Brazil alone [8].

Historically, the main strategies used in the population management of arbovirus vectors, specifically *Ae. aegypti*, have been the mechanical control of larvae by breeding sites management and application of action insecticides on larvae and adults [9,10]. During outbreaks of arbovirus, the most common response is the use of chemical sprays to kill adult vectors [11,12]. In most cases, spraying is performed through ultra-low-volume (ULV) fogging, aimed mainly at eliminating infected females of *Ae. Aegypti*, capable of transmitting the virus [13]. This integrated approach, using mechanical and chemical control, resulted in a decrease in cases of dengue and yellow fever in the Americas and Asia, between the 1950s and 1990s [14–16]. However, traditional approaches are no longer proving effective [17]. The reasons for this are complex, and they include inadequate implementation of control programmes, lack of human, financial, and infrastructure resources, inadequate public policies, and insecticide resistance (IR) [18].

Chemical control of *Ae. aegypti* in Brazil began in 1985 with the organophosphates temephos and fenitrothion. Between 1987 and 1993, malathion was used in small quantities to replace fenitrothion [19]. Selection pressure by these products on the various phases of the *Ae. aegypti* life cycle resulted in a decrease in efficacy of these insecticides [20]. In 2000, resistance to organophosphates was confirmed [21] and then organophosphates were replaced by pyrethroids. However, in 2002 and 2003, there was already evidence of the resistance of *Ae. aegypti* to these insecticides. The use of pyrethroids was stopped by the Ministry of Health and replaced by malathion in epidemic situations [20]. In 2008, malathion started to be used in some cities of the national territory in areas with resistance to pyrethroid [22], and in Foz do Iguaçu, the substitution of the adulticide occurred only in February 2011 [23].

The incidence of IR has increased rapidly in recent years [24] and is considered the main obstacle to successful control of *Ae. aegypti* [25]. It is a recurring phenomenon in different regions of the world where chemical control is a priority tool in vector control [12]. WHO defines resistance as the ability of an insect population to tolerate a dose of insecticide that under normal conditions would cause their death [20]. Resistance can be thought of as an accelerated evolution in response to selection pressure, which results in the survival of individuals with resistant alleles. The insecticide alone does not produce genetic change, but its continuous use may select resistant individuals [26–28].

Despite intense efforts against the spread of arboviruses in Brazil, all DENV serotypes have advanced and continue to generate outbreaks, with millions of people infected and thousands of deaths [29]. From January to October 2018, 140 893 confirmed cases of DENV infection, 59 584 cases of CHIKV infection and 3308 cases of ZIKV infection were reported, resulting in 163 deaths [30]. Considering that chemical management, using malathion, is the main strategy to combat arboviruses in Brazil, the resistance of *Ae. aegypti* to this insecticide could represent a major challenge to control the transmission of DENV, ZIKV and CHIKV in the country.

This study aimed to evaluate the resistance of the *Ae. aegypti* population in the municipality of Foz do Iguaçu, Paraná, Brazil, to the insecticide malathion, under field conditions and laboratory conditions. We performed vehicle ULV fogging of the insecticide *in situ* and compared strains collected from the field to the Rockefeller strain laboratory conditions. Based on preliminary data from entomological surveillance from the field (unpublished data), long period of Malathion use in Foz do Iguaçu (since 2011) and the resistance to the pyrethroid insecticide acquired previously, it was expected that *Ae. aegypti*, from the municipality of Foz do Iguaçu, would show resistance to malathion, which would be evidenced by (i) a mortality rate of adult mosquitoes below the expected and (ii) higher resistance of the municipality's strains, compared to the Rockefeller strain.

Methodology

Study area

The municipality of Foz do Iguaçu is located in southern Brazil, on the border between Paraguay and Argentina. The climate is classified as mesothermal humid subtropical (Cfa), with hot summers [31]. Average temperatures range from 11 to 33°C, with an average annual precipitation of 1800 mm [32]. It has 258 823 inhabitants living in 87 826 surveyed houses, an urbanisation rate higher than 99%, and a demographic density of 414.58 inhabitants per km^2 [33]. It has a high human development index (0.751, UNDP, 2010. The healthcare system consists of 36 care centres, including four hospitals distributed among the neighbourhoods of the city, with professionals trained to attend, diagnose and notify dengue, zika and chikungunya fevers.[1]

[1]Municipal government of Foz do Iguaçu, Municipal Health Secretariat. Personal communication of Adriana Dias Lourenço Izuka, Director of Primary Health Care – PMFI, on 10 August 2019.

Entomological surveillance procedures

The entomological surveillance procedures adopted by the municipality are based on the use of traps for capturing adult and immature *Ae. aegypti*. These include Adultrap® [34] and ovitraps [35,36]. Adultrap® is evenly distributed throughout the urban territory of the municipality, being present in one in 25 houses, totalling 3476 active traps [37]. These traps are verified periodically by the Zoonoses Control Center of Foz do Iguaçu (CCZ-Foz). Adultrap® is a trap to capture pregnant *Aedes aegypti* females during oviposition, using water as the main attraction. Through three compartments, the trap offers the attraction (water for oviposition); however, the female does not have access to water for oviposition and remains trapped and the trap does not become a breeding ground when exposed for a long time without verification [34,38].

The ovitrap is an oviposition trap and consists of a black container, with a wide mouth and a wooden pallet containing a rough side, placed vertically inside. This container is partially filled with tap water as an attractive for oviposition. Ovipositions are made on straws, which are used to know the abundance of *Aedes* females [35,36,39].

The municipality fully adopts the methods recommended by the Brazilian Ministry of Health to combat arboviruses, according to the National Dengue Control Program and the National Guidelines for the Prevention and Control of Dengue Epidemics recommendations [40,41], which consist of

1 permanent epidemiological surveillance;
2 laboratory surveillance with examinations to confirm cases;
3 integration with primary health care;
4 vector control team (one employee for every 1000 properties);
5 survey of the larval building infestation index according to the national calendar;
6 control of the *Aedes* vector with inspection of properties as a routine service;
7 periodic inspection of strategic points (places that, due to the nature of the establishment, pose a risk of proliferation of the *Aedes* vector);
8 blocking transmission with the use of adulticide through notified cases and applying adulticide in an ultra-low volume (ULV) in areas of intense transmission;
9 social mobilisation actions coordinated and carried out by an education team;
10 active dengue control committee with participation by society representatives;
11 political management of the works.

Adulticide applications by the municipal vector control agency are carried out in a systematic, professional manner and are restricted to the public authorities. The insecticide used is available by the Brazilian Unified Health System. There is no clear evidence of quantitative consumption and ways of using insecticide products by the population. However, the domestic use of these insecticides predominantly includes the chemical pyrethroid group, different from the group evaluated in this work.

Environmental treatment with vehicle ULV

The present study was conducted between November and December, 2017. Eighteen sample blocks (areas) in the municipality, similar to each other in terms of size, number of inhabitants, average income and results in the last LIRAa, were chosen to receive environmental treatment. The size of the sample blocks was defined by georeferencing, using Quantum GIS (QGIS) and PostgreSQL software. Of the 18 sample blocks identified, nine were randomly selected for receiving ULV treatment, while nine were selected for control treatment (Figure 1), as follows:

ULV treatment. Five applications of the adulticide through ULV fogging, applied from a vehicle (vehicle ULV), with an interval of 5 days between each application [41]. The adulticide used was diluted in the following proportions: 67% of a 44% malathion emulsion of oil in water (EW) and 33% water [42]. The two ultra-low-volume adulticide application equipments used were calibrated and certified by technicians from the State Health Department. Both pieces of equipment were used randomly in areas of the two treatments (Control Treatment and Insecticide Treatment).

Equipment 1 – Brand LECO 1800 E – Series 267, regulated with flow rate for application of 210 ml/min, pressure 6.0 pounds, and an engine speed of 2.210 rpm mounted on a Nissan Frontier vehicle. The climatic data recorded were room temperature of 30.1 °C, a relative humidity of 39.0% and wind speed of 5.0 km/h. The technical parameters of droplet sizes fully met the requirements for this activity (median diameter per number of drops – DMN 13.1 μm, median diameter per volume of drops – DMV 16.7 μm, relative amplitude – SPAN 0.7 and the dispersion coefficient – CD 1.4).

Equipment 2 – Brand LECO 120 E – Series 264, regulated with flow rate for application of 210 ml/min, pressure 5.0 pounds and an engine speed of 2250 rpm mounted on a Nissan Frontier vehicle. The climatic data

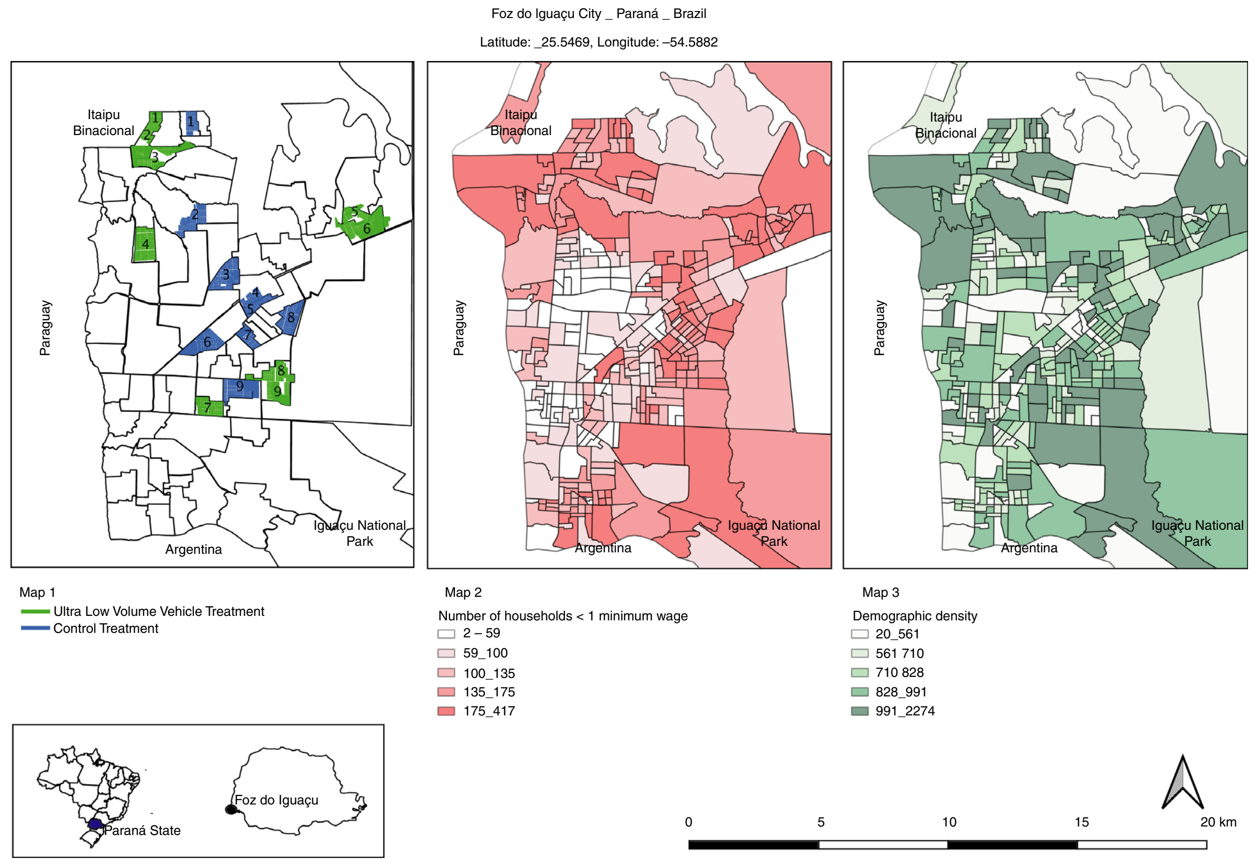

Figure 1 Location maps of the intervention and control areas, social and demographic indicators of the municipality of Foz do Iguaçu, Paraná, Brazil. Map 1: Areas selected for the ULV vehicle (green) and control (blue) treatments; Map 2: Average income earned by domiciled family; and Map 3: Demographic density of the municipality. The spatial divisions represent census tracts of the municipality [33]. [Colour figure can be viewed at wileyonlinelibrary.com]

recorded were room temperature of 28.0 °C, a relative humidity of 52.0% and wind speed of 2.5 km/h. The technical parameters of droplet sizes fully met the requirements for this activity (DMN 12.7 μm, DMV 17.7 μm, SPAN 1.1 and CD 1.4). The travel speed of the vehicles for the application was 10 km/h.

Control areas. No insecticide was applied. According to data from the municipality's Zoonosis Control Center, all activities to combat the vector occurred uniformly in the areas studied, such as (i) health education activities that are divided into lectures, guidelines and mobilisations in November and December of 2017, reaching a total audience of 5844 people; (ii) mechanical elimination of deposits and breeding sites on 6788 deposits in November and 1018 in December; (iii) regular visits by Endemic Control Agents to 15 189 properties in November (month of LIRAa) and 4717 visits in December; (iv) chemical treatment with pyriproxyfen-based larvicide in 44 deposits found in routine surveys in November and 18 deposits in December; and (v) inspections at strategic points (places that, due to the nature of the establishment, offer a risk of proliferation of the *Aedes* vector), totalling 21 visits in November, with 37 deposits eliminated, and 11 visits in December, with 115 deposits eliminated and 1 treated deposit.

The sample blocks were represented by areas equivalent to 1000 residential properties on the land of an average of 300 m^2. There was an Adultrap® trap for every 25 properties. Of the 3476 active Adultrap® installed in the city, 647 were used, which were already present in the study areas, 307 in the insecticide treatment area blocks and 340 in the control area blocks. A hundred and eight (108) ovitraps were used, uniformly arranged between the sample blocks, 54 in the insecticide treatment area blocks and 54 in the control area blocks (six in each block). Adultrap® was checked before the first application of the insecticide, at 24 h after each application, and 15 days after the last application, totalling seven checks, according to Figure 2. The verification of these traps consisted of counting the numbers of adult mosquitoes caught. We also recorded the human population that lives on each property with a trap. The peri-domicile was chosen to install the Adultrap® for presenting the highest yield [36]. The ovitrap was also installed in the peri-domicile.

The captured mosquitoes were sent to the Entomology Laboratory of CCZ-Foz for the identification of female *Ae. aegypti* specimens. Ovitraps were installed in the moment before the first application of adulticide in ULV, remained active for five days in the field when the first reading was made. Subsequently, ovitraps were installed 24 h after the third and fifth adulticide application cycles. These traps also remained active for five days in the field, where the second and third ovitraps readings were made, respectively (Figure 2). The verification consisted of collecting the traps of each sample block.

The ovitraps collected before the first application and after the third application were sent to the Entomology Laboratory at CCZ-Foz, where the identification of *Ae. aegypti* occurred after the hatching. Ovitraps verified after the fifth application was forwarded to the Morphology and Physiology of Culicidae and Chironomidae Laboratory of the Federal University of Paraná (UFPR) for evaluation of malathion resistance, where colonies of the vector *Ae. aegypti* were classified correctly. The eggs adhered to the surface of the Eucatex® palettes of ovitraps were submerged entirely in 700 mL cups containing dechlorinated water (48 h of tap water oxygenation) to stimulate the emergence of the larvae. After 48 h, the larvae were transferred to $35 \times 20 \times 7$ cm trays containing dechlorinated water, where they were fed with fish fry until pupae formed. The immatures were kept in a BOD chamber with a controlled temperature of 26 ± 1 °C and a 12:12 photoperiod.

The pupae were transferred to 50-mL plastic cups and placed in $40 \times 40 \times 40$ cm cages to allow the emergence of adults, who were identified according to Forattini [43]. The adults of *Ae aegypti* were kept in the cages to allow mating; the blood meal was performed twice a week using mice of the Swiss strain (Certification No. 719 of Ethics Animal Experiment Committee – UFPR). The females placed their eggs in filter papers partially submerged in water, which were removed from the cages twice a week. To obtain the F1 generation, these eggs were placed to hatch following the process described

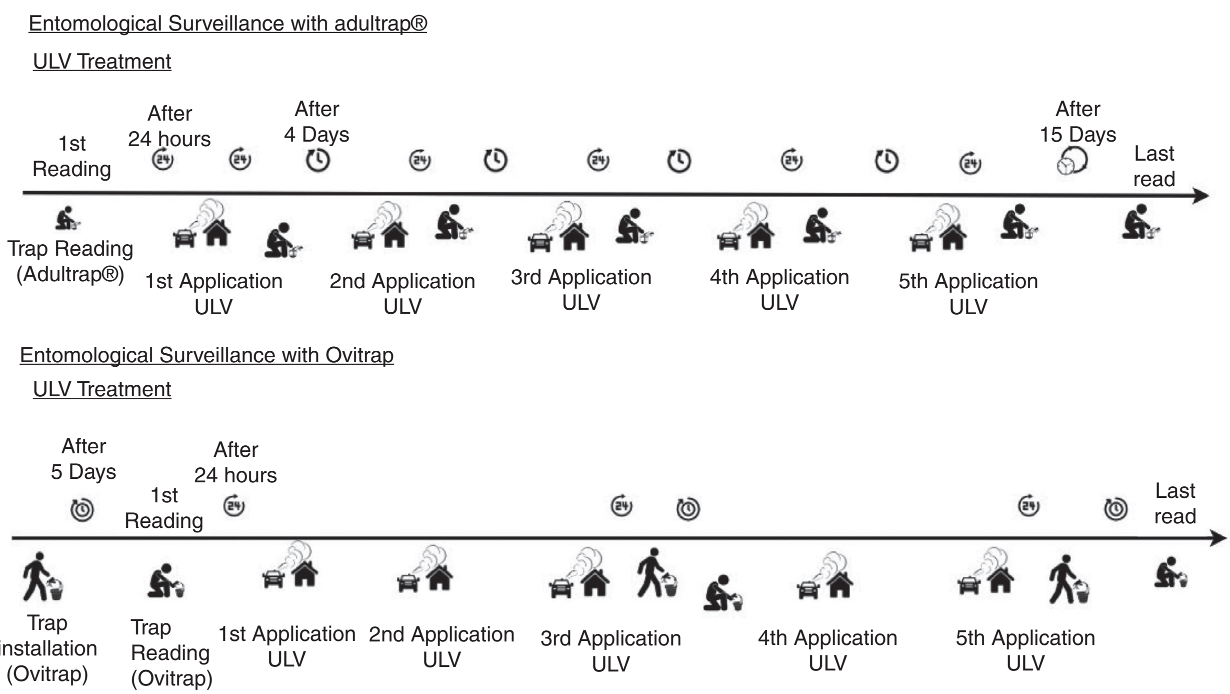

Figure 2 Vehicle ULV environmental treatment and control, entomological surveillance with Adultrap® and ovitrap, performed in the municipality of Foz do Iguaçu, Paraná, Brazil.

above. Likewise, the F2 generation was obtained from the eggs of F1 [43].

Females of the sensitive Rockefeller strain, used as a reference colony, were obtained using the same breeding method described above.

Adult mosquitoes caught in the field in the Adultrap® were taken to the CCZ entomology laboratory where the taxonomic determination was carried out.

Mosquito resistance to malathion laboratory conditions

Insecticide-impregnated papers were prepared according to the standard WHO instructions [44]. Malathion (Pestanal® 98%, Sigma-Aldrich) was used to prepare concentrations ranging from 0.8 to 3% w/v, using olive oil as the carrier. Malathion oil solution was mixed with acetone to ensure its uniform distribution on the qualitative filter paper Whatman® Grade 1 (12 × 12 cm). Impregnated papers were left for 24 h at room temperature (approximately 25°C) to allow complete acetone evaporation and then foil-wrapped and stored at 4 °C until use.

Tarsal contact bioassays were performed using WHO test tubes. Tests were performed using F2 progeny of Foz do Iguaçu or of the Rockefeller strain. Batches of 20–25 non-blood-fed females, 1–3 days old were introduced into an exposure tube wrapped with the impregnated paper and held for 1 h in dark conditions. At the end of the exposure time, females were transferred to a holding tube by gentle blowing, fed with 10% sucrose solution and maintained in a 12:12-h photoperiod. Bioassays were performed at 25 °C on different days. Mortality was determined after 24 h of exposure. Each concentration of insecticide, including control groups, had at least five replicates. The control group consisted of females exposed to papers impregnated with carrier olive oil and acetone, alone.

Data analysis

The number of female *Ae. aegypti* individuals and egg layings was estimated for each sample block, according to the infestation indices generated by the use of traps. Adultrap® was used to generate infestation indices of *Ae. aegypti* at the adult stage:

(a) Percentage of House Infestation of Traps (HIT%) [45]:

$$\mathrm{HIT\%} = \frac{T_{\mathrm{ca}}}{T_V} 100.$$

where T_{CA} = traps that captured *Ae. aegypti*; T_V = total traps verified.

(b) Modified *Stegomyia* Index for Adults (SIA) [36] or Mosquito Index per 1000 Residents (MIR):

$$\mathrm{SIA} = \frac{A_C}{I_{\mathrm{tv}}} 1000.$$

where A_C = total number of *Ae. aegypti* captured; I_{TV} = total residents in the verified houses that have a trap.

(c) Mosquito per House Index (MHI%):

$$\mathrm{MHI\%} = \frac{A_c}{T_{\mathrm{CA}}} 100.$$

where A_C = total number of *Ae. aegypti* captured; T_{CA} = traps that captured *Ae. aegypti.*

The indices of indirect infestation by egg-laying of *Ae. aegypti* were generated using ovitraps:

(d) Percentage of positive traps or Ovitrap Positivity Index (OPI%):

$$\mathrm{OPI\%} = \frac{E_P}{E_V} 100.$$

where E_P = number of positive traps; E_V = number of traps verified.

(e) Average number of eggs per positive trap or Egg Density Index (EDI) [46]:

$$\mathrm{EDI} = \frac{E_T}{E_p}$$

where E_T = total number of eggs; E_P = number of positive traps.

(f) Egg Average Index (EAI):

$$\mathrm{EAI} = \frac{E_T}{E_V}$$

where E_T = total number of eggs; E_V = number of traps verified.

We assessed the effect of environmental treatment (ULV and control treatments) on each index (IPA, IMI and IMH by the Adultrap® method; IPO, IMO and IDO by the ovitrap method) using generalised linear mixed models (GLMM). In these models, fixed effects included experimental time as a categorical factor, with seven levels for the Adultrap® method and three levels for the ovitrap method (each resampling date), as well as interactions with environmental treatment effects. The random-effects structure included both a random intercept, by block (site), and an intercept for each resampling date, nested within block and sub-block (environmental

Table 1 Results of the generalised linear mixed models assessing the effects environmental treatment (ULV and control) on Adultrap (HIT, MHI and SIA) and Ovitrap (OPI, EAI and EDI) indexes during the experiment realised in Foz do Iguaçu, Paraná, Brazil

Index	Variable	Estimate	Std. error	–	*z* value	*P*-value
HIT%	Intercept	−2.48651	0.35756	–	−6.954	***
	Environmental treatment	0.06404	0.49919	–	0.128	0.898
MHI%	Intercept	−1.9610	0.3129	–	−6.267	***
	Environmental treatment	−0.1231	0.4614	–	−0.267	0.79
OPI%	Intercept	−0.4319	0.2120	–	−2.037	*
	Environmental treatment	−0.1559	0.3140	–	−0.496	0.6196

Index	Variable	Estimate	Std. error	df	*t*-value	*P*-value
SIA	Intercept	40.753	7.015	15.995	5.810	***
	Environmental treatment	−4.337	9.879	15.755	−0.439	0.667
EDI	Intercept	93.45	11.47	27.49	8.147	***
	Environmental treatment	−18.48	16.22	27.49	−1.139	0.265
EAI	Intercept	59.717	8.801	26.088	6.785	***
	Environmental treatment	−12.697	12.446	26.088	−1.020	0.317

HIT, House Infestation Trap; MHI, Mosquito per House Index; OPI, Ovitrap Positivity Index; SIA, *Stegomyia* Index for Adults; EDI, Egg Density Index; and EAI, Egg Average Index.
Asterisks represent significance levels: *** < 0.0001, ** < 0.001, * < 0.05.

treatment), [47]. All models were built using the R software package, lme4, assuming a negative binomial distribution for HIT, MHI and OPI data. Significance values for fixed effects were obtained either in the software package, ImerTest, or using the Satterthwaite approximation for degrees of freedom.

The concentration-mortality regressions were estimated using Probit analysis, executed in the R program using the package 'ecotoxicology' [48]. Resistance ratios were estimated by dividing the LC50 value of Foz de Iguaçu by that of the Rockefeller strain, and the LC95 value of Foz de Iguaçu by that of the Rockefeller strain. We performed all statistical analyses in the R statistical environment [49].

Results

Environmental treatment

In total, 220 specimens of adult female *Ae. aegypti* and 7423 eggs were found in the areas that received ULV treatment, and 245 adult female *Ae. aegypti* and 10 557 eggs were found in areas that received the control treatment. The ULV treatment showed no significant difference compared to the control treatment, for all indices used (Table 1, and Figures 3 and 4).

Mosquito resistance to malathion laboratory conditions

Control mortalities, in all experiments, were less than 1%; therefore, no data were adjusted. Tarsal contact bioassays showed that the Foz do Iguaçu strain needed 1.76-fold more malathion than the susceptible Rockefeller strain to reach 50% lethal concentration (LC50) and 1.92-fold at the 95% lethal concentration (LC95) (Table 2). The angular coefficient obtained from females of the Rockefeller strain was 1.18 times higher than that obtained from females of the Foz do Iguaçu population, suggesting that the Rockefeller strain mosquitoes respond more quickly to mortality when there are slight variations in malathion concentration, compared to the Foz do Iguaçu population (Table 2). The confidence intervals of the lethal concentrations evaluated did not overlap, confirming that the treatments are different.

Discussion

Our *in situ* and laboratory condition experiments show that populations of the mosquito *Ae. aegypti*, present in the analysed areas, are resistant to malathion (*sensu* [50]. Resistance can be associated with the continuous use of this organophosphate for the last ten years, throughout Brazil, fostered by public health policies [20].

Laboratory results show that mosquitoes of Foz do Iguaçu are less susceptible to malathion than the Rockefeller strain (control). Notably, despite the lower susceptibility and consequent confirmation of the IR, the mosquitoes of Foz do Iguaçu still showed some susceptibility to this insecticide under laboratory conditions. The *in situ* study demonstrated that the application of malathion through vehicle ULV showed no difference in

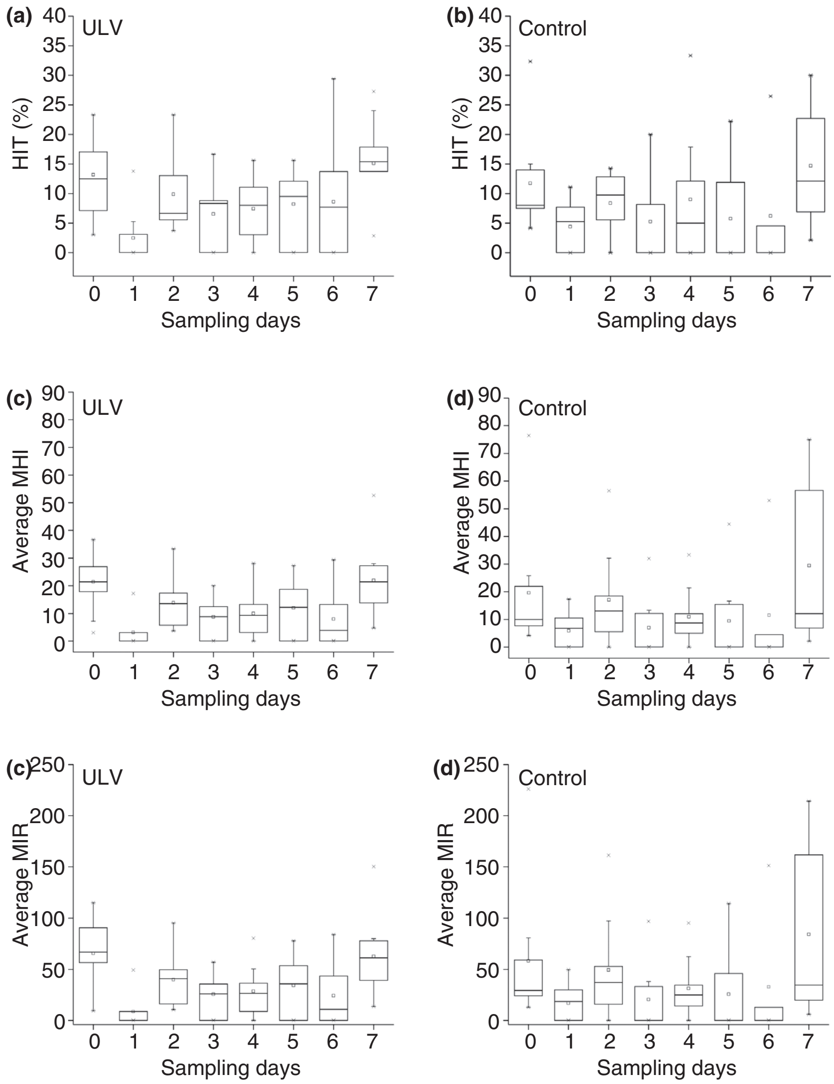

Figure 3 Boxplots of the Adultrap® index values HIT (a and b), MHI (c and d) and MIR (e and f) assessing the effects of environmental treatment (ULV and control), during the seven sampling days.

the mortality of adult vectors, when compared to areas that did not receive chemical treatment. The ineffectiveness of the vehicle ULV methodology can be attributed to a combination of the resistance of the vector, chemical principle used, and ineffectiveness of the 'outdoor' application process.

Despite being one of the main tools for reducing vector density, the massive use of chemical control has reduced the effectiveness of the method [12,51,52]. In Brazil, the application frequency of adulticides, through ULV, should not exceed 5-7 times over a period of 12 months [41]. The application should occur mainly during epidemic periods and in specific situations,however, this advice is not being followed at several locations. Some places have been treated at a frequency above what is recommended by the Brazilian Ministry of Health, with some receiving up to nine applications in a single month [53]. In the municipality of Foz do Iguaçu, some regions received 13 applications of insecticide in 2017, due to the recurrence of disease reported in the region (Zoonoses Control Center, internal records).

In addition to the high frequency of use, the association between the prolonged use of the same insecticide for control of adult insects [54] and the rapid spread of

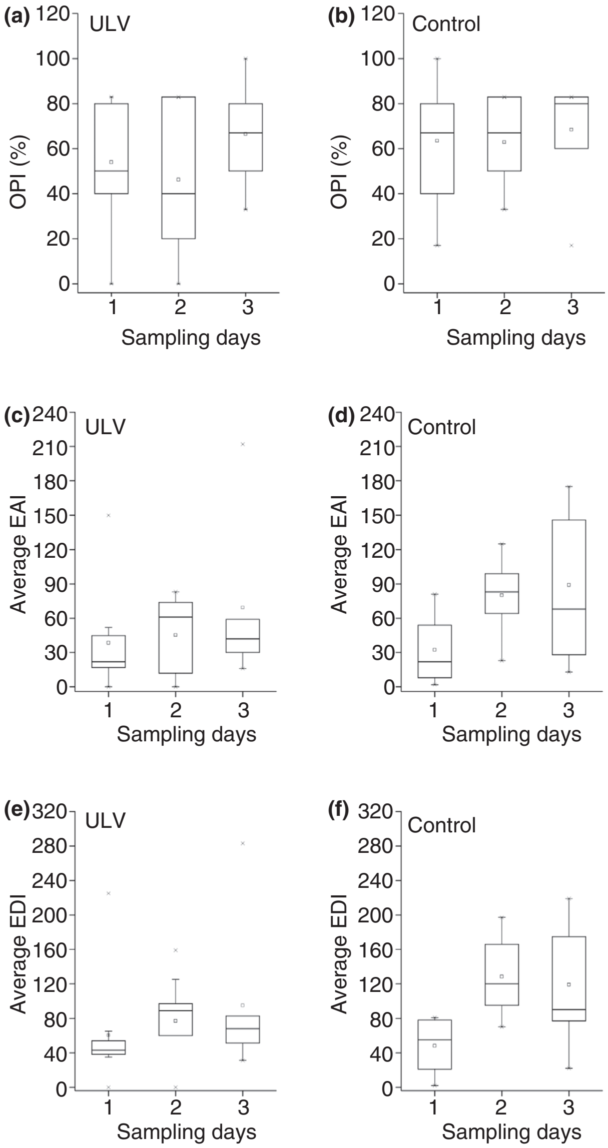

Figure 4 Boxplots of the ovitrap index values OPI (a and b), EAI (c and d) and EDI (e and f), assessing the effects of environmental treatment (ULV and control) during the three sampling days.

potentially resistant mosquitoes [24,27] may also be factors in the development of IR. At the time of this study, malathion is the main substance recommended by the Ministry of Health for the chemical control of adult mosquitoes, throughout Brazil [41]. Thus, the resistance identified in Foz do Iguaçu may also be present in other regions.

The ineffectiveness of the ULV methodology can be partly explained by IR. However, in laboratory conditions experiments, using 100% technical grade malathion, unlike *in situ*, our study showed significant mortality of *Ae. Aegypti,* when compared to the control treatment (without insecticide). ULV fogging was carried out according to Ministry of Health recommendations [41]. The dilution indicated for field conditions is 67% of a 44% malathion EW and 33% water, which may have reduced the effectiveness of the insecticide, compared to laboratory conditions methods. Moreover, spraying and fogging by vehicle ULV often leaves indoor mosquitoes unharmed [55]. Since the mosquito shows endophilic and anthropophilic behaviours, outdoor spraying has limited efficacy against adult *Ae. aegypti* [56].

When there are adverse factors for the application of adulticide, through ULV fogging, there exists a high risk of failure, which reinforces the need for real-time monitoring of the results of this type of procedure. This study has evidenced the viability of entomological surveillance, with Adultrap®, for evaluating the quality of the application of adulticide through ULV fogging, in the field. Adultrap® can remain installed in the field because they are safe and do not become breeding sites [38]. Furthermore, they capture fed females, which are the main targets of spraying with adulticide [41]. This allows immediate analysis and gathering of useful data, after chemical application, as females captured within 24 h post-application were clearly not eliminated by the control measure.

The presence of the tri-border area, the junction of Paraguay, Argentina and Brazil, can affect IR and the potential for new dengue epidemics. International border areas are strategically important for the management of arbovirus transmission, since the countries involved have

Table 2 Concentration–malathion response in virgin females from Foz do Iguaçu and the Rockefeller strain

Population	*N*	Slope (SD)	$LC_{50}(CI_{95})$ µg a.i./cm^2	$LC_{95}(CI_{95})$ µg a.i./cm^2	X^2	DF	*P*-value
Rockefeller	577	8.184 ± 0.64	22.2 (20.5–23.6)	35.2 (32.3–40.0)	3.22	3	0.64
Foz do Iguaçu	960	6.925 ± 0.35	39.2 (36.9–42.1)	67.7 (59.7–80.6)	3.22	4	0.37

CI, confidence interval; LC, lethal concentration; *N*, number of females used in bioassays; SD, standard deviation. µg a.i./cm^2, microgram of active ingredient per square centimetre; X^2, chi-square; DF, degree of freedom.

distinct socio-economic development, legislation, culture and provision of health services [57,58]. The effectiveness of the local health system may be compromised by the flow of people from border populations seeking to enjoy the opportunities they would not find in their country of origin [59]. Such a situation occurs in the tri-border region, where the municipality of Foz do Iguaçu is located, and the three countries offer different opportunities that are sought daily by residents of the three-border cities. The lack of public policies for integrated control, among cross-border countries, affects the responsiveness of local health systems [52,59]. Mobility among populations, promoted by tourism, migration and work-seekers, increases the risk of introducing diseases and increases the difficulty of surveillance [60,61]. This transboundary flow is an important vehicle for the spread of different vectors and the serotypes of dengue, thus increasing the potential for epidemics [62].

Aedes adult stage surveillance traps, among them Adultrap® and ovitrap, rarely produce null indexes, presenting greater sensitivity in detecting *Ae. aegypti* presence, compared to larval research. The positivity indexes of these traps tend to stabilise at high mosquitoes' density [35]. Adultrap® positivity and density indexes agreed with the mosquito seasonality and abundance [35,45]. In field studies, Adultrap® captures were also compared with captures with an aspirator. It was shown that Adultrap® is sensitive to detect *Ae. aegypti* even in low frequency situations. The trap is configured as a potential sensitivity parameter to assess the effectiveness of vector control and its reflexes in reducing the species adults [36,45], as well as in the description of dengue virus transmission levels [35]. The Adultrap® is efficient and reliable for capturing *Ae. aegypti* females in field conditions and their yield was also confirmed, since this trap proved to be as or more efficient for capturing females of pregnant *Aedes* when compared to the aspirator used [38]. Adultrap® also allows monitoring the presence and displacement of *Aedes* [63].

Our field experiment accurately explored the key factors for entomological surveillance, using adult and egg traps. Field surveillance methodologies were used to compare areas with and without insecticide application, allowing us to determine the *in situ* resistance of *Ae. aegypti* to malathion, confirmed by laboratory tests. The relationship between entomological surveillance, in real time, using adult and egg traps in the field (Adultrap® and ovitraps), and the precision of laboratory measurements, is important to decision making in the fight to protect human populations from arboviruses.

The ULV technique has its limitations. Its application, throughout the Brazilian national territory, generates asymmetries in efficiency, caused by resistant or non-resistant populations, and lack of uniformity of application procedures. However, the most important limitation is the use of the product without real-time knowledge of the actual susceptibility of the target population of *Ae. aegypti* to the substance used.

Given the above, we suggest it is important to consider the susceptibility of the vector as a strategic component, a key part of integrated action to reduce vector populations in urban areas. Insecticide reversal tests should precede the new implementation of insecticides. Monitoring with Adultrap® can be considered a strategic tool for evaluating the effectiveness of insecticide application, in real time, that is immediately after the application of the chemical, in the field. These results highlight the importance of studies evaluating the efficacy of methodologies for the chemical control of vectors in field conditions.

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Declaration of interest

None.

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