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Raquel Ramos Date

The Effect of Volatile Pyrethroid Insecticides on Pyrethroid-Resistant *Aedes aegypti* in Mérida, Mexico

By

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Global Environmental Health

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The Effect of Volatile Pyrethroid Insecticides on Pyrethroid-Resistant *Aedes aegypti* in Mérida, Mexico

By

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2019

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Abstract

The Effect of Volatile Pyrethroid Insecticides on Pyrethroid-Resistant *Aedes aegypti* in Mérida, Mexico

By Raquel Ramos

Aedes aegypti is the principal vector transmitting dengue, chikungunya, and Zika viruses in the Americas. They live in close association with humans, primarily in urban areas. Due to the low cost and low mammalian toxicity, pyrethroid insecticides for vector control have been widespread in order to decrease the spread of *Aedes* born viruses. There have been significant barriers with the use of common protective measures and an increase in insecticide resistance. One of the main resistance mechanisms is knockdown resistance which occurs by reducing the sensitivity of sodium channels to pyrethroids. Advancements in insecticides, specifically polyfluorinated pyrethroids have been made, but more research on insecticide resistant mosquito control is needed. This study was conducted to measure the effectiveness of volatile pyrethroid metofluthrin emanators on field and lab resistant *Ae. Aegypti* mosquitoes (New Orleans, Cienega de Flores, Itzincab, and Juan Pablo), with mutations in Mérida, Mexico. A total of 1,099 mosquitoes were used and three different levels emanators (0,1,4). Houses and apartments were the unit of treatment and the endpoint of focus was mosquito survival after treatment. Experiment One: statistical difference among resistant and susceptible strains, F (1, 147) =78.62, p = <0.0001. Experiment Two: statistically significant interaction between the effects of strain and treatment level on survival, F (2, 322) = 10.55 p = <0.0001. Experiment Three: statistically significant interaction between the effects of genotype and treatment level on survival was confirmed (F (8, 457) = 3.73 p = 0.0003). Survival increased with mutation. 40% of mosquitoes with genotype WT, WT survived versus 72.55% of mosquitoes with genotype Mut, Mut. Our experimental study deliver important proof effectiveness of emanators is limited in populations with high levels of resistance and mutation. Survival increased with mutation. Mosquitoes with one or two mutations had higher percentage of survival versus mosquitoes without mutation. Probability of survival had a similar result. The estimated probability of survival for WT, WT is 0.31 (0.24-0.39) versus 0.71 (0.64-0.77) for Mut, Mut. We acknowledge the need to implement strategies that involve understanding knockdown resistance frequencies in monitoring and management programmes in complex urban areas.

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Introduction

I. Pyrethroids and Resistance

Due to the low cost and low mammalian toxicity, pyrethroid insecticides for vector control have been widespread. Pyrethroid insecticides are a large class of synthetic analogues of natural Pyrethrins from the flower extracts of Chrysanthemum spp. They assist in the control of nearly all arthropod pests [1]. Based on their chemistry and effects, pyrethroids can be classified into two groups: Type I and Type II [2]. Pyrethroids mainly affect insects' peripheral and central nervous systems by binding to a target site in the voltage -gated sodium channel (*Vgsc*) or *Vssc* within the nerve membrane [1, 2]. These channels are responsible for the initiation and propagation of action potentials in excitable cells. Prolonged opening of sodium channels and lack of an α-cyano group at the phenoxybenzyl alcohol position results in the repetitive firing of the neuron (Type I) [1]. Prolonged opening of sodium channels and an α-cyano-3-phenoxybenzyl alcohol moiety results in depolarization of the membrane, which leads to conductance blockage (Type II) of the nervous system [1]. Type I displays low potency, knockdown, and high repellence. Type II pyrethroids are much more potent with acute lethal effects [1].

The increased use of insecticides has led to pyrethroid resistance, leading to treatment failure [3]. Numerous point mutations in sodium channels are known in pyrethroid-resistant insect species. Many of these mutations have been confirmed to reduce the sensitivity of channels expressed in pyrethroids [1].Mutations in the voltage-sensitive sodium channel gene (*Vssc*), which are the primary target of pyrethroid insecticides, have been recognized in Aedes aegypti and linked to pyrethroid resistance [1]. One of the main resistance mechanisms is knockdown resistance (*kdr)* [1, 2]. *Kdr* occurs by reducing the sensitivity of sodium channels to pyrethroids due to reducing binding of the insecticides at the target site and can cause resistance

since pyrethroids target sodium channels [2]. About 25 sodium channel mutations are associated with *kdr* or *kdr*-like pyrethroid resistance in various pest species [3]. The frequency of *kdr* mutations has been increasing rapidly worldwide. In Mexico, the frequency of *kdr* in *Aedes aegypti* has increased from < 0.1% in 1996–2000 to 88.3% in 2007–2009 [3].

II. *Aedes aegypti* Problems and Control

Aedes aegypti is the principal vector transmitting dengue, chikungunya, and Zika viruses in the Americas. They live in close association with humans, primarily in urban areas. They live and breed in and around houses and develop in various peridomestic water-holding containers; this makes them very difficult to control [3]. Females have proven to be a major nuisance because they most commonly feed and rest indoors and may not even venture outside if there are available larval development sites indoors [4] . Since there is no widely licensed vaccine or specific treatment for any of these diseases, vector control has become the principal strategy for preventing transmission [2]. Insecticides have been the most important component to vector control and management of mosquito-associated diseases [5, 6]. Still, *Aedes*-borne viruses (ABV) are becoming more of a global public health concern due to increased geographical spread and insecticide resistance [6, 7]. All ABVs are transmitted primarily by the tropical yellow fever mosquito *Aedes aegypti*, and to a lesser extent by *A*. *albopictus*, the Asian tiger mosquito [8]. There have been significant barriers with the use of common protective measures such as long-lasting insecticidal nets (LLNs) and indoor residual spraying (IRS): time needed to spray, gaining entrance, community compliance, and the development of insecticide resistance in mosquitoes [5, 6, 9]. More research on how insecticide resistant mosquitoes can be controlled is needed, but there have been advancements in insecticides, specifically polyfluorinated pyrethroids [10].

III. Emanators

Metofluthrin and transfluthrin (polyfluorinated pyrethroids) are less polar insecticides with lower melting points, are slightly volatile, and release vapor in the air. At low doses, airborne pyrethroids can repel, deter, inhibit feeding, and reduce the fecundity of mosquitoes as opposed to higher doses of insecticides that induce mortality via direct toxicity[5, 9]. These insecticides consistently kill mosquitoes at lower concentrations than monofluorinated pyrethroids, such as flumetrhin [11]. Volatile insecticides, which contain synthetic pyrethroids, are available in different forms. Emanators are spatial repellents and release low concentrations of volatile insecticides from a point source consist of fibers woven together that are treated with pyrethroids [9, 12]. The primary aim of emanators is to create a vector-free space and reduce long-term mosquito survival by disrupting host-seeking and blood-feeding behaviour [12].

Currently, the World Health Organization's Vector Control Advisory Group includes volatile insecticides under the "spatial repellent" product class [5, 9]. Emanators incorporating metofluthrin and transfluthrin have shown to have high efficacy for reducing mosquito bite exposure [5, 9]. However, these exploratory studies did not test efficacy on different strains of pyrethroid-resistant mosquitoes with known mutations that cause pyrethroid resistance. It is unclear whether the known mutations cause resistance alone or cause resistance in combination with other alleles [13]. Understanding resistance mechanisms is critical for the effective management of pyrethroid resistance and usage of pyrethroid insecticides [1]. Past research has evaluated the entomological impact of volatile pyrethroids on urban *Ae. Aegypti* and found that they have a continued impact on mosquito contact indoors, and efficacy increases by using a fan to increase airflow and locating the pyrethroid in the center of the room [5, 14]. Studies conducted in Puerto Rico, Trinidad, the Dominican Republic, and Cuba highlighted the need for

finding alternative strategies to control *Aedes* mosquitoes due to reduced sensitivity to repellents because of resistance and *kdr* mutations [15-17]. The advantages of emanators [10] make them a promising control strategy compared to other interventions but the current knowledge of how they can overcome genetic resistance and control mosquitoes with mutations remains unclear [10].

IV. Our Study

To assess the full protective potential of passive emanators containing the volatile pyrethroid metofluthrin (10% active ingredient by weight), this study was conducted to measure their effectiveness on field and lab resistant *Ae. Aegypti* mosquitoes (New Orleans (NO), Cienega de Flores (CdF), Itzincab (ITZ), and Juan Pablo (JP)), with mutations in Mérida, Mexico. A total of 1,099 mosquitoes were used and three different levels emanators (0,1,4). Our study used houses and apartments as the unit of treatment and the endpoint of focus was indoor *Ae. Aegypti* survival after 24h exposure of treatment.

Materials and Methods

Ethics statement:

We did not require Institutional Review Board approval because this was an experimental study where mosquitoes were released into uninhabited houses and apartments rented on long-term contracts.

Study area:

The study was conducted in the city of Mérida in the Yucatán peninsula of southern Mexico. Mérida (20°58′ N/ 89°37′ W) is 10 m above sea level and covers an area of 858.41 km^2 with a population of ~ 900,000. The Yucatan peninsula is dominated by limestone, is flat and low, and has subtropical climate. Average maximum temperatures in Mérida range from

29°C in December to 34°C in July; precipitation typically occurs from May to October, with a peak from June to September [5]. Dengue and other ABVs are endemic throughout the Yucatán peninsula, with peak dengue transmission typically occurring between July and November. Peak time for mosquito abundance in Mérida is usually July to October [4, 5].

Metofluthrin emanators:

Devices that release volatile insecticides from a point source (here termed emanators) consist of fibers woven together that have been treated with volatile pyrethroids. The emanators consists of a methacrylate polymer net impregnated with 10% w/w (ca. 0.217 g) of the synthetic, volatile pyrethroid metofluthrin (Sumitomo Chemical Company Ltd. Chuo-ku, Tokyo, Japan) [5, 18]. Various iterations of this formulation are currently registered in Australia, Singapore, Malaysia and Thailand where they are sold for indoor mosquito bite prevention. The net is contained within a 95 mm x 160 mm plastic holder designed to be hung or placed in rooms with gentle air circulation to encourage volatilization [5, 9, 18]. Laboratory and semi-field experiments have shown emanators to be highly effective against pyrethroid-susceptible *Ae*. *aegypti* indoors, remaining effective for 3 weeks after deployment [5].

Experimental design

To understand the entomological impact of metofluthrin emanators on the survival of *Ae*. *aegypti* with mutations, we implemented three different randomized experiments with individual apartments and houses as the units of treatment and analysis. Experiment One took place on May 16, 2017; Experiment Two took place on May 10 and 13, 2017; and Experiment Three occurred on June 28, 2017. A total of 1,099 mosquitoes were used and a total of 1,053 were collected. All mosquitoes used were females. Survival was measured by how many mosquitoes lived at the end of 24 h exposure to the emanator(s). Landing was measured at 30

minutes, 60 minutes, and 24 hours. The mosquitoes were not allowed to feed and landing from legs and knees were counted for 5 minutes (2 minutes for Experiment 3). There were four different landing sites. Feeding data was collected in Experiment 1 and consisted of counting landing/feeding on forearms for 5 minutes. Landing and feeding data was was analysed separately.

Experiment 1: Preparatory study

Experiment One was a preparatory trial that allowed us to understand survival of three strains (CdF: lab susceptible, ITZ: field resistant, JP: field resistant) in rooms with the different treatment levels (0 emanators, 1 emanator, 4 emanators). 149 mosquitoes were released and collected. The emanator(s) were allowed to emanate for 1 hour before the mosquitoes were introduced. The mosquitoes were initially kept in separate rooms in 28C. They were placed in single cups (n=10 mosquitoes) in a test room where they were allowed to acclimate for 10 minutes. The mosquitoes were then allowed to land/feed on a forearm for 5 minutes. After this they were returned to a separate room. Survival of mosquitoes was measured after 24 h.

Experiment 2: Preliminary analysis in experiment houses

Three treatments (0 emanators, 1 emanator, and 4 emanators) and two strains of mosquitoes: ITZ (Field Resistant) and JP (Field Resistant) were used in houses. 350 mosquitoes were released and 328 mosquitoes were collected. The size of houses was approximately 120-140m^3. Each house contained two bedrooms, one bathroom, and one or two main/living rooms. The temperature ranged from 27-34 C and the humidity ranged from 28-60%. Inside the experimental houses, the furniture was all standard, as described by Dunbar et al [5]. The bedrooms contained one bed (made of white PVC and black cloth), one small table (made of black plastic), six hung clothes (three white shirts and three colored shirts). The main/living

rooms contained two tables (made of black plastic) and four chairs (two white and two dark colored). The windows were opened but, along with the drains, they were screened from the inside and outside. And all the doors and furniture were sealed. 175 mosquitoes were used for each strain (ITZ and JP), 50 mosquitoes were released within each house, and they were allowed to acclimate for 30 minutes. The emanators were then added. Survival was measured after 24 h and all collected mosquitoes (live and dead) were stored in RNAlater to preserve the mosquitoes for subsequent analysis.The experiment was replicated twice, once for ITZ and once for JP.

Experiment 3: Evaluation of the impact of strains and mechanisms of resistance

Two treatments (0, and 1 emanator) and and four strains of mosquitos: New Orleans (Lab Susceptible), Cienega de Flores (Field Susceptible), Itzincab (Field Resistant), and Juan Pablo (Field Resistant) were used in eight houses. Experiment Three was replicated in three blocks (each block testing all four strains and the strains were assigned to 0 and 1 emanators). 25 mosquitoes were released within each house (1-8) for each block (1-3) resulting in 600 total mosquitoes. The size of houses was approximately 120-140m^3. Each house contained two bedrooms, one bathroom, and one or two main/living rooms. The temperature ranged from 29-34 C and the humidity ranged from 62-80%. Inside the experimental houses, the furniture was all standard. The bedrooms contained one bed (made of white PVC and black cloth), one small table (made of black plastic), six hung clothes (three white shirts and three colored shirts). The main/living rooms contained two tables (made of black plastic) and four chairs (two white and two dark colored). The inside and outside of windows were closed and the drains were screened. All the doors and furniture were sealed with screens. Additions to the houses from Experiment 2 were buckets of water with cloth for moisture, oscillating fans on low (four per house) and ant baits at each entrance. During the treatment group, the mosquitoes were allowed to acclimate for

30 minutes then the emanator was added. Survival was measured after 24 h and the live and dead mosquitoes were collected separately and stored in RNAlater. Landing and feeding data were also collected. Landing from feet to knees was counted and personnel was kept consistent within each room and house. This data was analysed separately.

Detection of *kdr* alleles

Detection of *kdr* alleles was conducted following the procedure in Devine et al [5]. Genomic DNA extraction from field-caught mosquitoes was performed using Extracta DNA Prep for PCR–Tissue (QuantaBio, Beverly, MA). Individual whole mosquitoes were added to 25 µL of extraction reagent. Samples were incubated at 95°C for 20 min. Once cooled to room temperature, 25 µL of stabilization buffer was added to the samples, which were kept at -20°C until use. Allele-specific PCR methods were used to detect *kdr* mutations with known function. Genotypes were characterized using a CFX-96 RT-PCR system (BioRad, Hercules, CA) under specific cycling and melt curve conditions. Primers used were adopted from Saavedra-Rodriguez et al [19] and Yanola et al [20]. PCR reagents and conditions were based on Deming et al [21] and Saavedra-Rodriguez et al [19].

Statistical Analysis

Data from Experiment 1, Experiment 2, and Experiment 3 from the Merida sites were analysed for differences in survival. Differences among allele, strain, and treatment frequencies were analysed using contingency tables with significance tested through the chi-square statistic. Logistic regression and generalized linear mixed models were performed to compare the variation of resistance in mutations, strains, and treatments. All analyses were performed in the SAS® software (SAS Institute. 2011). To determine the level of statistical significance between strains and genotypes for each treatment, generalized linear mixed models (GLM) with Odds ratios

(with 95% confidence intervals) were calculated to determine whether mutations in combination in an individual were more likely to be associated with resistance than mutations occurring singly. An odds ratio of 1 indicates that there is no relationship between resistance and the genotype under investigation. If 95% confidence intervals of the odds ratio do not span the value "1" then this suggests that the genotype is associated with resistance. Predicted probability of survival and 95% confidence intervals were also calculated in order to further understand survival of mosquitoes based on genotype and strain. To determine the level of statistical significance between treatment and control arms for each entomological indicator, generalized linear mixed models (GLMM)

Single Mutation and Mutation in Combination Analysis:

Research has shown that 1023 and 1565 alleles are directly implicated in pyrethroid resistance [2]. There were nine possible genotype combinations with five of them containing a mutation on one or both of the 1023 and 1565 alleles (**Table 3**) We tested whether mutations influenced resistance to the emanators by genotyping surviving and dead mosquitoes.

Results

Experiment One: Preparatory Study

Among the149 total mosquitoes collected (**Table 1**), survival was consistent with strain. The resistant strains (ITZ and JP) had a higher percent of survival versus the susceptible strain (CdF) (**Figure 1**). A two-way ANOVA was conducted that examined the effect of strain and treatment level on survival. There was a not statistically significant interaction between the effects of strain and treatment level on survival, F (4, 148) =1.98, p = .10. There was also no statistical difference between treatment levels, F (2, 146) =2.04 , p =.13. There was a statistical difference among resistant and susceptible strains, F (1, 147) =78.62, p = <0.0001. Simple main effects analysis showed that resistant strain mosquitoes had significantly higher survival rates in

rooms with 0 emanators (p = <.0001), 1 emanator (p = <.0001), and 4 emanators (p = <.0001). The odds of surviving 4 emanators was 0.67 (0.26-1.51) times higher than surviving 0 emanators and 0.37 (0.14-0.99) times higher than surviving 1 emanator.

Experiment 2: Preliminary Analysis in Experimental Houses

Experiment Two was conducted as a preliminary analysis of survival of field resistant strains in experimental houses. This experiment helped us to understand the best methods for Experiment Three. 350 mosquitoes were released and 328 were collected (**Table 2).** Since both strains used were field resistant, no significant difference in survival was expected between the strain and survival was expected to decrease with addition of emanators (**Figure 2)**. A two-way ANOVA was conducted that examined the effect of strains and treatment level on survival. There was a statistically significant interaction between the effects of strain and treatment level on survival, F (2, 322) = 10.55 p = <0.0001.

Experiment Three: Evaluation of the Impact of Strains and Mechanisms of Resistance

Of the 576 mosquitoes collected after Experiment Three (**Table 1**), the genotypes of 475 were sequenced. 293 mosquitoes were a resistant strain (ITZ or JP) and 274 had either one or two mutations (**Table 3**). A two-way ANOVA was conducted that examined the effect of genotypes and treatment level on survival. There was a statistically significant interaction between the effects of genotype and treatment level on survival, F (8, 457) = 3.73 p = 0.0003. Survival increased with mutation. 40% of mosquitoes with genotype WT,WT survived versus 72.55% of mosquitoes with genotype Mut,Mut (**Table 4**). Probability of survival had a similar result. The estimated probability of survival for WT,WT is 0.31 (0.24-0.39) versus 0.71 (0.64-0.77) for Mut,Mut (**Table 5**). Mutation on both 1023 and 1565 was found frequently and had the highest percentage of mosquitoes survive (102 total mosquitoes with 15.58% surviving), but did

not have the highest odds of surviving. Compared to WT,WT, Mut, Mut had an odds ratio of 4.08 whereas mosquitoes with Mut on 1023 allele and Het on 1565 allele had an odds ratio of 5.36 (**Table 4**). Both these genotypes of mosquitoes had significant p values (<0.05).

Discussion

This experimental study evaluated the entomological impact of metofluthrin emanators on field and lab resistant *Ae*. *Aegypti* with mutations on the 1023 and 1565 alleles. Our study builds on evidence that *kdr* or *kdr*-like mutations reduced channel sensitivity to both type I and type II pyrethroids [22, 23]. These mutations would result in a population with cross-resistance to pyrethroids once population resistance to one pyrethroid has is developed. Our research shows that this resistance can be caused due to homozygous or heterozygous mutation.

Historically, insecticides have been an essential component to managing mosquito-associated diseases, especially in the absence of vaccines or drugs [5, 6]. There are six classes of insecticides recommended for use against adult mosquitoes: organochlorines, organophosphates, carbamates, pyrethroids, pyrroles, and phenyl pyrazoles [6]. Pyrethroids are used globally as an effective strategy to control insect vectors of human diseases, such as malaria and Dengue fever. Long-lasting insecticidal nets (LLNs) and indoor residual spraying (IRS) have been two of the most commonly used measures for protection against ABVs [6, 9]. Massive spraying of insecticides has greatly limited ABVs, but there have been significant barriers leading to outbreaks. Effectiveness is challenged by the amount of time it takes to spray interiors, the difficulty of gaining entrance, and community compliance. The idea of using vaporized chemicals to create bite-free protected areas has become more widely studied [9, 24, 25]. Polyfluorinated synthetic pyrethroids, transfluthrin, and metofluthrin are popular molecules of focus. Both have high vapor pressures and are suitable for formulating devices that facilitate

volatilization at room temperature [24]. Recent research has shown that the most effective pyrethroid insecticides are applied to solid surfaces where mosquitoes could have greater exposure, such as house walls and ceilings[5, 9]. Metofluthrin and transfluthrin (fluorinated pyrethroids) are less polar insecticides with lower melting points, are slightly volatile, and can release vapor in the air. At low doses, airborne pyrethroids can repel, deter, inhibit feeding, and reduce the fecundity of mosquitoes as opposed to higher doses of insecticides that induce mortality via direct toxicity [5, 9]. Devices that release chemicals passively, at room temperature, are portable, low cost, and suitable for deployment in resource-poor environments. Transfluthrin-treated plastics[26], metofluthrin treated paper concertinas [27, 28] and metofluthrin-impregnated polyethylene mesh [24] are all examples of this type of device.

The increased development of resistance in mosquitoes to commonly used insecticides contributes to the outbreaks of these diseases [5, 6]. Spreading mosquito resistance to pyrethroids has become a serious global challenge for effective insecticide-based vector control operations [29, 30]. Numerous studies have shown that multiple, complex resistance mechanisms are likely responsible for insecticide resistance [6] , and despite early optimism that its fast toxic action would not produce resistance, pyrethroid resistance has become a dilemma. Resistance to insecticides among several mosquitoes, such as *Anopheles gambiae* and *Culex pipiens,* emerged over 25 years ago [31]. However, pyrethroid resistance and cross-resistance to other insecticides have become critical issues leading to the reemergence of insect vector-borne diseases globally. Increased insecticide residence in mosquitoes has been identified in more than six countries, affecting all major vector species and all classes of insecticides and negatively impacting current control measures [6, 32]. Resistance to pyrethroids is mediated by a range of mechanisms that involve point mutations in specific genes or up-regulation of metabolic enzymatic pathways [33].

An effective form of resistance, knockdown resistance (*kdr*), is caused by mutations in the sodium channels, which results in a lower sodium channel and neuronal sensitivity to pyrethroids [34-36]. This type of resistance mechanism has been documented globally in all major arthropod pests and disease vectors, including *Aedes* mosquitoes. There has been limited research and understanding of the impact of conventional pyrethroid resistance mechanisms on the efficacy of metofluthrin and transfluthrin [37, 38].

Our experiments expanded on the research on the efficacy of metofluthrin and transfluthrin on resistant mosquitoes. Analysis of survival showed that survival was higher in resistant strains versus susceptible strains. Genotype combinations were diverse in the control and treatment arms, and most of the live mosquitoes collected had either one or two mutations. This strongly suggests that the *kdr* mutations present in mosquitoes surviving the emanators were subject to selection pressure, as opposed to studies that have reported the opposite [5]. Interventions that permit significant mosquito survival or that prevent mosquitoes taking full blood meals may increase the proportion of potentially infectious mosquitoes in the population or encourage multiple, partial blood feed [5]. Sometimes, spatial repellents can be presented as less vulnerable to the development of resistance than more conventional insecticides [25], disruption of natural behaviour can affect survival and exert significant selection pressure that facilitates adaptive mutations [5, 39]. Although the relationship in Experiment 3 is not directly linear, there appears to be a relationship between survival and genotype. Research has shown that mutations on the 1565 and 1023 are strongly associated with pyrethroid resistance pyrethroids [40-42]. Specifically, our results show that having one mutation on either the 1565 or 1023 alleles or mutation on both alleles increases probability of survival.

We also acknowledge the limitations within our experiments. Since the NO strain is supposed to be lab susceptible, evidence suggests there was contamination because it was found to have mutations. The experiment should also be repeated with mosquitoes from the potential study area and treatment levels should be changes to 0 emanators, 1 emanators, and 2 emanators per room. The use of 4 emanators in room cause eye irritation and was therefore discontinued in Experiment 3.

Conclusion:

The high levels of resistance and their association with high frequencies of *kdr* mutations (1023 and 1565) obtained through artificial selection, suggest an important role of these mutations in conferring resistance to metofluthrin emanators. Metofluthrin emanators could help to control *Aedes Aegypti* mosquitoes if they can be deployed for epidemiologically significant periods at a high coverage in populations with limited resistance and mutations [10, 11]. Our experimental study deliver important proof that in populations with high levels of resistance and mutation, the effectiveness of these emanators is limited. Survival increased with mutation. Mosquitoes with one or two mutations had higher percentage of survival versus mosquitoes without mutation. Probability of survival had a similar result. The estimated probability of survival for WT,WT is 0.31 (0.24-0.39) versus 0.71 (0.64-0.77) for Mut,Mut. We acknowledge the need to implement strategies that involve the monitoring of *kdr* frequencies in insecticide resistance monitoring and management programmes. This will require elements of community development and ownership in complex urban areas.

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Tables & Figures:

	Experiment One	***Experiment Two***	***Experiment Three***
# Mosquitoes released	**149**	**350**	**600**
# Mosquitoes collected	**149**	**328**	**576**
# Treatments used	**3**	**3**	**3**
# Strains used	**3**	**2**	**4**

Table 1: Number of mosquitoes released and collected, number of treatments used, and number of strains used for each experiment

	0 Emanators	***1 Emanator***	***4 Emanators***
CdF (susceptible)	18 (33.33%)	13 (13.51%)	6 (0%)
ITZ (resistant)	20 (100%)	20 (100%)	16 (62%)
JP (resistant)	20 (85%)	19 (94.74%)	17 (88.24%)

Table 2: Total number of mosquitoes released in Experiment 1 by strain in each treatment and percent that survived

	0 Emanators	***1 Emanator***	***4 Emanators***
ITZ (resistant)	48 (16.67%)	76 (60.53%)	57 (24.56%)
JP (resistant)	36 (72.22%)	50 (56.00%)	61 (54.10%)

Table 3: Total number of mosquitoes released in Experiment 2 by strain in each treatment

	0 Emanators	*1 Emanator*
NO (susceptible)	47 (100%)	46 (0%)
JP (resistant)	51 (82.61%)	48 (46.30%)
ITZ (resistant)	73 (53.70%)	75 (46.30%)
CdF (susceptible)	51 (82.61%)	51 (82.61%)

Table 4: Total number of mosquitoes released in Experiment 2 by strain in each treatment

	0 EMANATORS					*1 EMANATOR*				
STRAIN GENOTYPE	***NO***	***JP***	***ITZ***	***CdF***	***Total***	***NO***	***JP***	***ITZ***	***CdF***	***Total***
WT,WT	16	7	1	5	28	19	6	2	4	31
WT,HET	2	7	5	12	26	1	5	5	1	12
HET,WT	3	5	2	3	13	4	1	1	3	9
HET,WT	3	5	2	3	13	4	1	1	3	9
HET,HET	10	9	16	8	43	7	10	11	5	33
HET,MUT	1	19	10	5	35	4	21	9	1	35
MUT,HET	3	0	7	0	10	7	2	5	12	26
WT,MUT	11	9	6	2	28	3	10	2	0	15
MUT,WT	0	1	0	11	12	0	0	0	11	11
MUT,MUT	1	15	27	2	45	1	19	33	4	57

Table 5: Total number of mosquitoes released in Experiment 3 by strain and genotype for 0 treatments and 1 treatment

1023, 1565	***Total Number***	***Dead***	***Alive***	***Odds of Surviving (95% Confidence Intervals) compared to WT, WT***
0: WT, WT	65	39 (60%)	26 (40%)	----
1: WT, Het	38	30 (78.95%)	8 (36.36%)	0.405 (0.161-1.022)
2: Het, WT	22	14 (63.64%)	8 (36.36%)	0.841 (0.309 – 2.291)
3: Het, Het	76	39 (51.32%)	37 (48.68%)	1.421 (0.726– 2.780)
4: Mut, WT	23	11 (47.83%)	12 (52.17%)	1.573 (0.608 – 4.066)
5: WT, Mut	43	18 (41.86%)	25 (58.14%)	1.573 (0.608 – 4.066)
6: Het, Mut	70	35 (50%)	35 (50%)	1.527 (0.775 – 3.007)
7: Mut, Het	36	8 (22.22%)	28 (77.78%)	5.361 (2.115-13.591)
8: Mut, Mut	102	28 (27.45%)	74 (72.55%)	4.080 (2.117 - 7.863)

Table 6: Total number of mosquitoes, percentage dead, percentage alive, and odds of surviving compared to WT, WT for each genotype combination (Experiment 3)

1023, 1565	*Estimated Probability*	*95% Confidence Intervals*
0: WT, WT	0.314	(0.2434-0.395)
1: WT, Het	0.3606	(0.2951-0.4317)
2: Het, WT	0.4096	(0.3511-0.47076)
3: Het, Het	0.4605	(0.465 -0.5129)
4: Mut, WT	0.5122	(0.4649 - 0.55928)
5: WT, Mut	0.56367	(0.51589 - 0.610305)
6: Het, Mut	0.613797	(0.56093-0.664102)
7: Mut, Het	0.66162	(0.601168 - 0.717215)
8: Mut, Mut	0.70635	(0.63785 -0.766630)

Table 7: Estimated probability of survival and 95% confidence intervals for each genotype of mosquito in Experiment 3 (correlates with **Figure 1**)

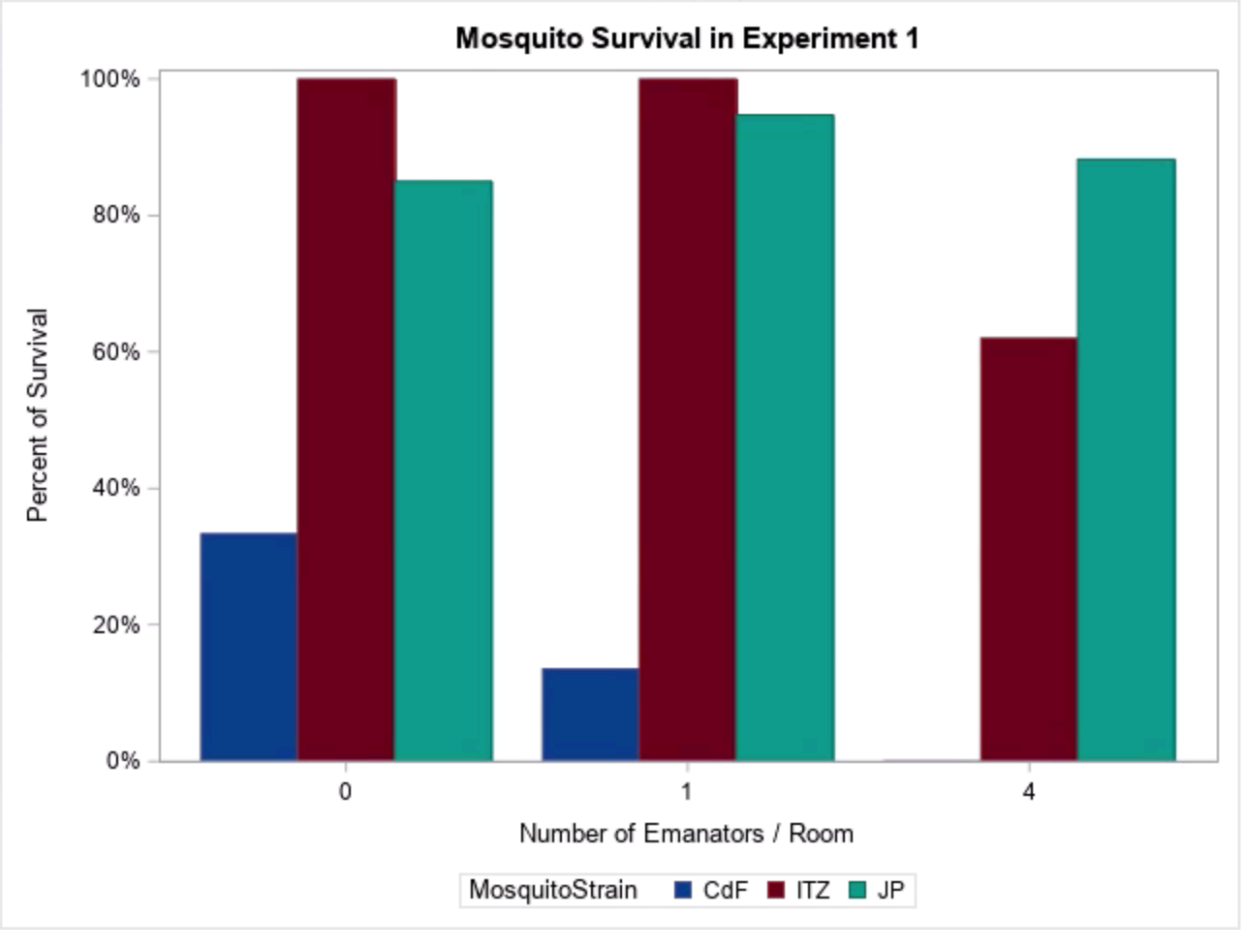

Figure 1: Percentage of survival by strain for each number of emanators in Experiment

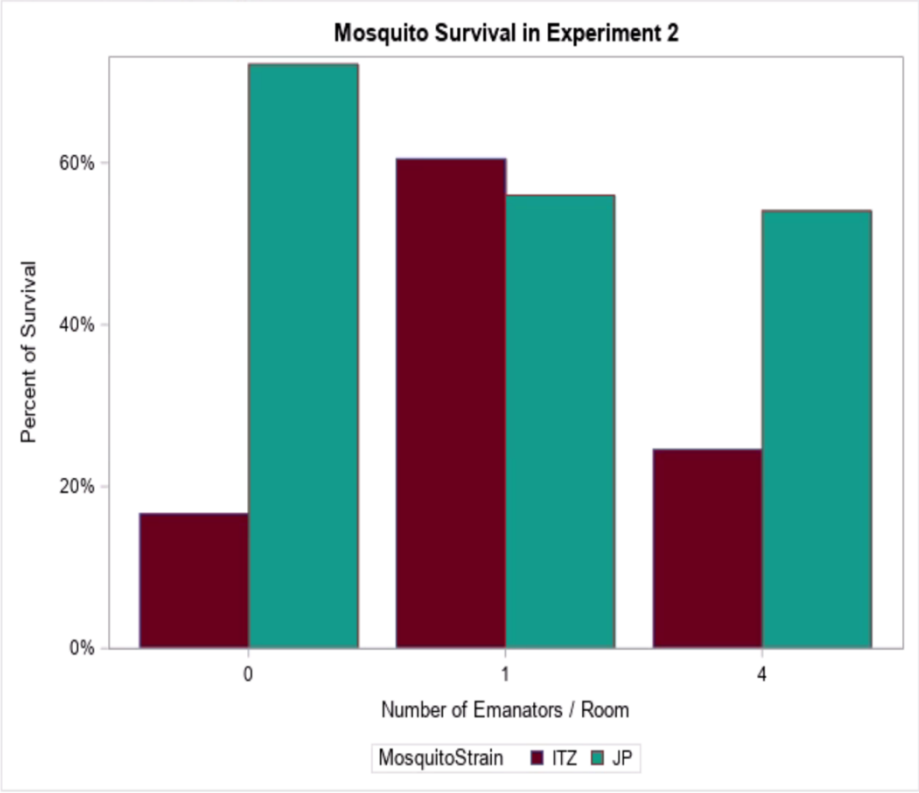

Figure 2: Percentage of survival by strain for each number of emanators in Experiment 2

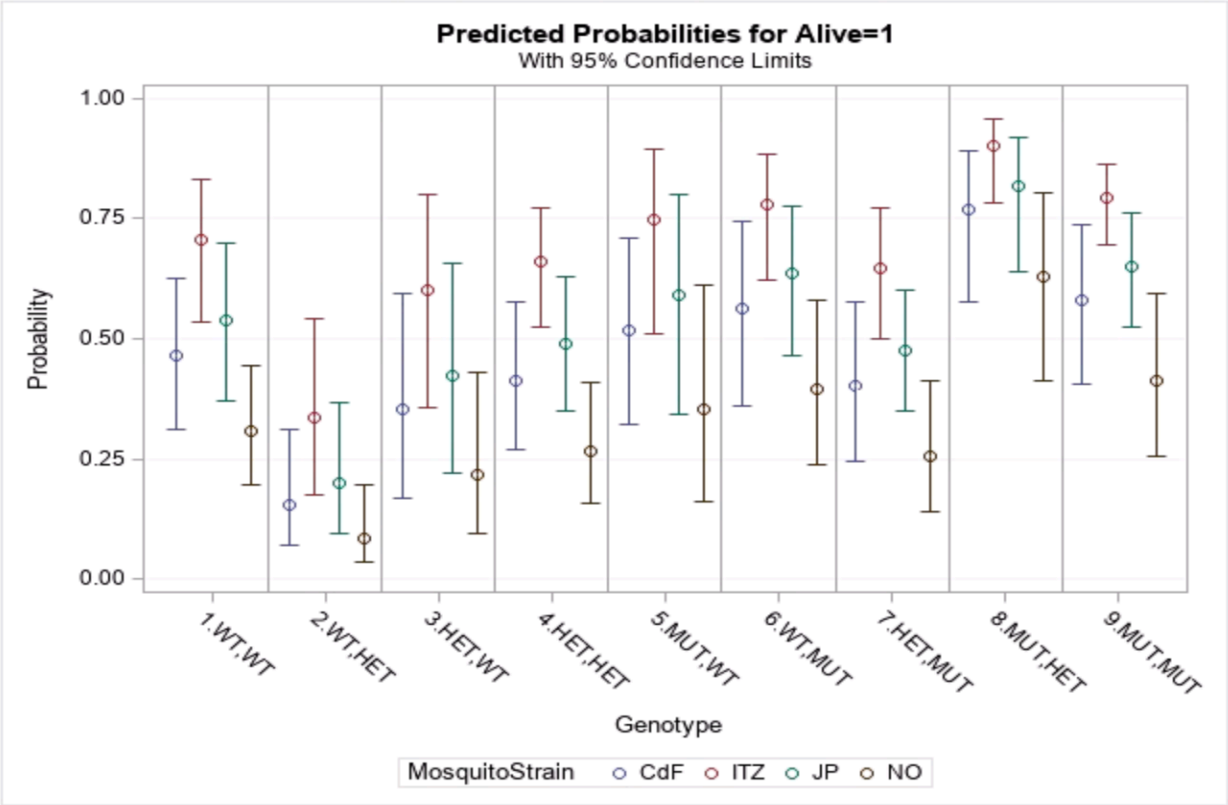

Figure 3: Predicted probability of survival (with 95% confidence intervals) of each genotype for the four strain