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Major Article

Impact of insecticide resistance on the field control of *Aedes aegypti* in the State of São Paulo

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ABSTRACT

Introduction: The need to control dengue transmission by means of insecticides has led to the development of resistance to most of the products used worldwide against mosquitoes. In the State of São Paulo, the *Superintendência de Controle de Endemias* (SUCEN) has annually monitored the susceptibility of *Aedes aegypti* to insecticides since 1996; since 1999, surveys were conducted in collaboration with the National Network of Laboratories (MoReNAa Network) and were coordinated by the Ministry of Health. In this study, in addition to the biological characterization of insecticide resistance in the laboratory, the impact of resistance on field control was evaluated for vector populations that showed resistance in laboratory assays. **Methods:** Field efficacy tests with larvicides and adulticides were performed over a 13-year period, using World Health Organization protocols. **Results:** Data from the field tests showed a reduction in the residual effect of temephos on populations with a resistance ratio of 3. For adults, field control was less effective in populations characterized as resistant in laboratory qualitative assays, and this was confirmed using qualitative assays and field evaluation. **Conclusions:** Our results indicated that management of resistance development needs to be adopted when insect populations show reduced susceptibility. The use of insecticides is a self-limiting tool that needs to be applied cautiously, and dengue control requires more sustainable strategies.

Keywords: *Aedes aegypti* control. Field control. Insecticide resistance.

INTRODUCTION

Currently, dengue fever can only be controlled by using vector control, since vaccines are not yet available[1,2]. Among the methods used for vector control, chemical control with insecticides is an important tool that interrupts transmission of the disease, and its effectiveness depends not only on operational requirements, but also on susceptibility of the insects to insecticides.

Many studies in Brazil have reported development of resistance in *Aedes aegypti* to most insecticides used[3-8]. In São Paulo, since 1996, resistance of different populations of *A. aegypti* has been monitored yearly[9]. Analysis of the evolution of vector resistance to insecticides in the State of São Paulo, Brazil, as well as the response of populations to the doses used in the field, might contribute to understanding of the process of resistance development, allow assessment of the impact of resistance in control operations, and provide support for the choice of management strategies for more effective vector control.

In this study, we analyzed the field response of *A. aegypti* populations that showed some degree of resistance in laboratory assays and assessed the management strategies implemented.

METHODS

After the susceptibility of larvae and adult mosquitoes was characterized using methods recommended by the World Health Organization (WHO)[10-15], the impact of resistant populations was evaluated by routine chemical treatments using standardized protocols. Classification of the susceptibility of *A. aegypti* populations followed the criteria for the interpretation of diagnostic doses using bioassays recommended by the World Health Organization[15], where a mortality rate of > 98% characterizes a population as *susceptible*; < 80% as *resistant*; and between 80 and 98% as *reduced susceptibility*. Resistance was also characterized in qualitative assays for both larvae and adults using dose-response assays and estimation of the resistance ratio (RR). For each population, 3 assays were conducted, and the results were expressed as the average of the 3 assays. The response was considered satisfactory when the mortality rate of *A. aegypti* larvae was 80% higher than that in the Rockefeller strain, a susceptible reference strain that was used for monitoring biological responses of *A. aegypti* field populations.

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In order to assess the impact of resistance, the effectiveness of insecticide treatment in simulated field conditions was evaluated in routine laboratory experiments using commercial products and application techniques typically used in control programs. These included focal application of larvicides (temephos), space spray, and residual treatments for adult control. For the latter, the products evaluated were organophosphates (fenitrothion, malathion, and pirimiphosmethyl); pyrethroids (cypermethrin, deltamethrin, and permethrin); and carbamate (bendiocarb).

Evaluation of field control of larvae

The efficacy of focal treatment was evaluated by analyzing the data from tests of residual effect of larvicides according to the methodology recommended by Andrighetti et al.[16] and the WHO[17]. Over the period of evaluation (2004-2010), the residual effect of temephos was assayed 11 times for 7 *A. aegypti* populations that presented different RRs at 95% lethal concentration (RR_{95}; Araçatuba, Marília, Presidente Prudente, São José do Rio Preto, São Sebastião, Santos, and Sorocaba). In each field evaluation, *A. aegypti* larvae (F1 or F2) were exposed to treatments, paired with larvae of the Rockefeller strain. The biological response, expressed as the percentage of mortality of treated larvae over time, of *A. aegypti* larvae was compared to the percentage of mortality observed in the larvae of the Rockefeller strain, and the results were expressed as the percentage of response compared to that in the Rockefeller strain.

Evaluation of field control of adults

Evaluation of ultralow volume treatment efficacy: for evaluation of spatial treatment, bioassays were conducted using sentinel cages[18-20] during 2000-2001 (cypermethrin and malathion), 2002 (permethrin), 2007 (deltamethrin), 2008–2009 (malathion), and 2011-2012 (deltamethrin). In the first two years of the tests, several populations were assayed (Barretos, Campinas, Marília, Ribeirão Preto, Santos, and São José do Rio Preto). Subsequently, the tests were performed using populations that showed different levels of resistance to pyrethroids in laboratory experiments, namely, populations from Marília, which showed diminished susceptibility, and those from Araçatuba and Santos, which showed high and very high resistance, respectively.

Evaluation of residual treatment: application of insecticides on the surfaces was evaluated by conducting cell wall assays[21,22]; these experiments were performed using *A. aegypti* populations from Marília and Santos in 2002–2003. The tests were performed using 3 groups of insecticides: pyrethroids (cypermethrin and deltamethrin), organophosphate (fenitrothion), and carbamate (bendiocarb). In all these assays, the Rockefeller strain was used as a control for the field populations. Furthermore, among the organophosphates tested, the field response to fenitrothion was compared with that to pirimiphosmethyl in 2 populations, in 2004, namely, Marília and Araçatuba. A new evaluation of the residual activity of insecticides was performed in 2011, using 3 groups of insecticides, the organophosphate fenitrothion, the pyrethroid deltamethrin, and the carbamate bendiocarb. Three different tests were performed using doses of 2.3 (standard deviation: 0.5), 34.7 (7.5), and 378.3 (22.5), respectively, for fenitrothion, deltamethrin, and bendiocarb. The assay results for populations from Araçatuba, Marília, and Santos were again compared with those for the Rockefeller strain.

Data analysis

The National Network for Monitoring Resistance to Insecticides in *A. aegypti* (MoReNAa) has used a resistance ratio ≥ 3 at the level of 95% lethal concentration as a criterion indicating the need for replacement of larvicide[23]. For field efficacy tests, satisfactory control rates are thought to have been achieved when the mortality rates exceeded 80%[17,21,22,24]. The criterion for interpretation also included comparison of the response to that of the susceptible reference strain (Rockefeller): response <70% of that of the sensitive strain suggests that the evaluated product needs to be replaced[25].

Population response to field treatment was compared using analysis of variance (ANOVA; Kruskal-Wallis) by first converting the mortality data to arcsin values[17].

RESULTS

Evaluation of field control of larvae

The results were grouped according to the RR_{95} obtained in the laboratory experiment. Group I consisted of 4 populations that presented RR_{95} of < 3.0; group II comprised 12 populations presenting with RR_{95} between 3 and 5; and group III were 4 populations with RR_{95} of ≥ 5. The percentage mortality in each group and replicate was pooled according to the RR_{95} groups. The average mortality, relative to that observed in the Rockefeller strain (standard deviation), for groups I, II, and III, was 85.7 (12.0); 67.5 (7.4), and 59.2 (13.2), respectively. The difference among the group means were significant (ANOVA, $p = 0.0003$). Data are shown in **Figure 1**.

Evaluation of field control of adults

Evaluation of ultralow volume treatment efficacy: results from tests performed in 2000/2001 with cypermethrin and malathion are shown in **Figure 2**. Populations of Barretos, Ribeirão Preto, Santos, and São José do Rio Preto, which were classified as *resistant* to cypermethrin in laboratory assays[13], presented a mortality level below 80% in field treatments with this product, while populations from Marília and Campinas, which were classified as having *decreased susceptibility* presented mortality above 80%. For malathion, all field populations were classified as susceptible in laboratory assays and presented mortality rates above 90% in field treatments.

Sentinel cage assays with another pyrethroid product (permethrin) were performed in 2002 for populations from Marília (*decreased susceptibility* for cypermethrin and *resistant* to permethrin in laboratory assays) and from Santos (*resistant* to both cypermethrin and permethrin in laboratory assays). The use of permethrin did not facilitate satisfactory control of the Santos population (mean mortality: 43.3%; standard deviation: 26.4), whereas the Marília population could be controlled at a level

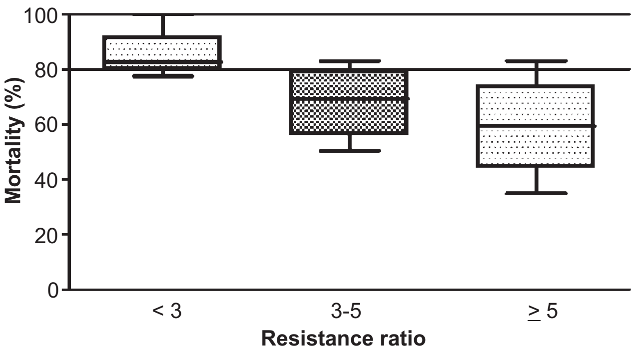

FIGURE 1 - Mean mortality of *Aedes aegypti* populations treated with temephos, with levels of resistance expressed as resistance ratio 95.

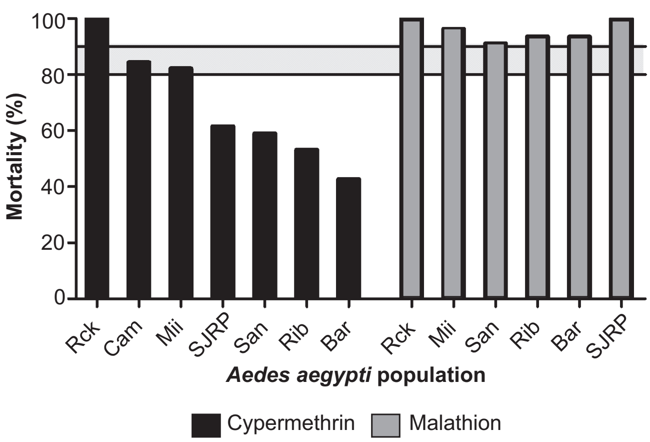

FIGURE 2 - Mean mortality of *Aedes aegypti* populations exposed in sentinel cages to ultralow volume treatment with cypermethrin (dose: 0.6g a.i./house) and malathion (dose: 200g a.i./ha). Tests were performed during 2000-2001. Rck: Rockefeller; **Cam:** Campinas; **Mii:** Marília; **SJRP:** São José do Rio Preto; **San:** Santos; **Rib:** Ribeirão Preto; **Bar:** Barretos.

similar to that possible using cypermethrin (mean mortality: 85.5%; standard deviation: 14.3).

The results of *A. aegypti* populations exposed to ultralow volume (ULV) treatment with malathion (an organophosphate) in sentinel cages are shown in **Figure 2**; a high level of field control was obtained for all the populations. This result was expected, since these populations were susceptible to malathion under laboratory conditions.

In 2007, a new evaluation was performed using the pyrethroid deltamethrin. In this evaluation, Araçatuba (*resistant*) and Marília (*decreased susceptibility*) populations were exposed to ULV treatment. The objective of this new evaluation was to determine whether the response was different under field conditions. The level of control was below the acceptable level for both the populations (23.8% [16.3] and 51.1% [33.7], mean mortality for Araçatuba and Marília populations, respectively).

In 2008-2009, new field evaluations of the impact of treatment with malathion on ULV were performed, in order to compare the results with those obtained in 2001. Tests with sentinel cages were performed using the populations from Marília and Barretos. Although the results were not significantly different, and the mean mortality of both the populations was still under the level of acceptable control, the mortality rates were slightly lower than those observed in 2001.

During 2011 and 2012, a new series of sentinel cage tests were performed with deltamethrin, using the 3 populations from São Paulo that showed different levels of resistance to pyrethroids (cypermethrin and deltamethrin): Araçatuba, Marília, and Santos. The resistance status of the populations was measured and compared using qualitative (diagnostic dose) and quantitative (dose-response) assays, as per the standard protocol (WHO); the results are presented in **Table 1**. The mean mortality observed in the 3 field evaluations performed using the 3 mosquito populations was 22.7 (23.7) for the Santos population, 39.7 (26.6) for the Marília population, and 28.2 (29.5) for the Aracatuba population, whereas for Rockefeller, the susceptible strain, the mean mortality was 83.9 (19.2).

The average mortality of all field populations was significantly lower than that of the Rockefeller strain ($p < 0.0001$). All the paired comparisons were significantly different between the populations ($p < 0.001$). The mean mortality in the field showed a good correlation to that obtained using diagnostic tests (coefficient of correlation [Pearson test] = 0.93) and a weak correlation with the resistance ratio (coefficient of correlation [Pearson test] = 0.51).

TABLE 1 - Susceptibility of adult *Aedes aegypti* to deltamethrin according to population and type of assay.

	Type of assay	
Population	DD/status*	RR LC_{95}
Araçatuba (SP)	64.3/R	72.0
Marília (SP)	73.5/R	40.9
Santos (SP)	49.6/R	95.0

***DD**: diagnostic dose, 18mg of active ingredient (a.i.)/m^2*; **R:** resistant; **RR:** resistance ratio; **LC_{95}**: lethal concentration 95%.

Evaluation of residual treatment

Data of the residual effect of experiments involving surface treatments with the 3 groups of insecticides: pyrethroids (cypermethrin and deltamethrin); carbamate (bendiocarb); and organophosphate (fenitrothion) are shown in **Figure 3**. We demonstrated that, although the mosquito population from Marília presented diminished susceptibility to cypermethrin, all the products showed an acceptable level of control. On the other hand, the population from Santos, which was resistant to pyrethroids in laboratory assays, was not effectively controlled

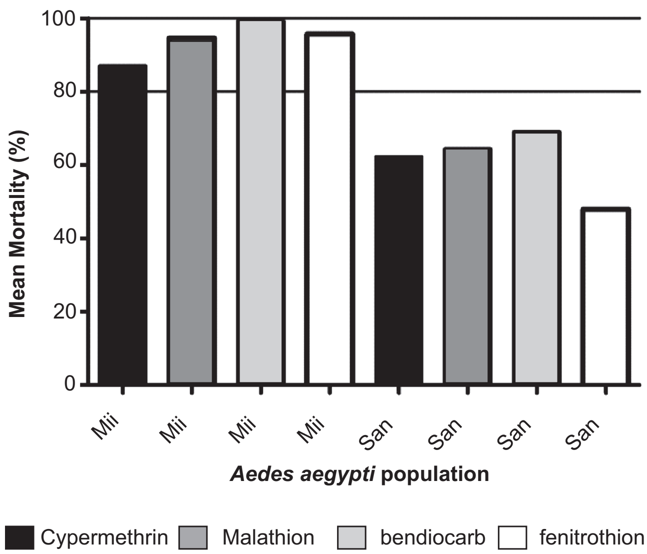

FIGURE 3 - Mean mortality of *Aedes aegypti* populations exposed to surface treatment with four insecticides. Tests were performed during 2002-2003. Mii: Marília**; San:** Santos.

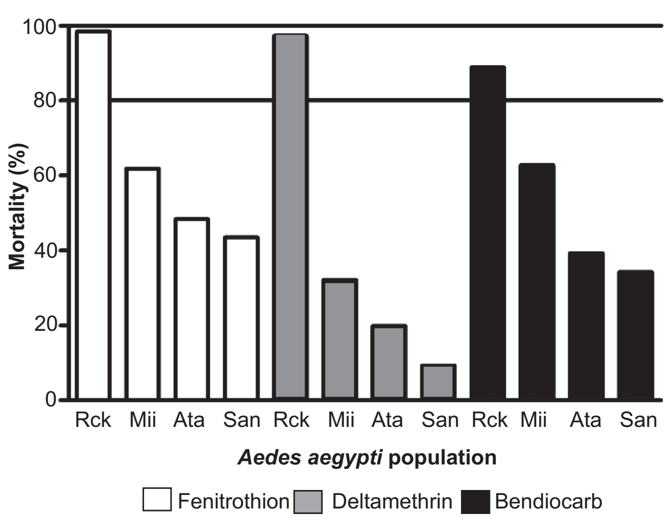

FIGURE 4 - Mean mortality observed in *Aedes aegypti* populations exposed to surface treatment with deltamethrin, bendiocarb, and fenitrothion. Tests were performed in 2011. Rck: Rockefeller; **Mii:** Marília; **Ata:** Araçatuba; **San:** Santos.

by any group of insecticides. The results of the field response to fenitrothion compared with that to pirimiphosmethyl indicated that, for the Marília population, both the products were effective: mean mortality of 83.6 (15.2) and 89.9 (10.9), respectively, for fenitrothion and pirimiphosmethyl. However, for the Araçatuba population, better control was observed with fenitrothion, with a mean mortality of 80% (standard deviation: 27.1), than with pirimiphosmethyl (mean: 42.5%; standard deviation: 49.8).

The results from the tests performed in 2011 indicated that all 3 products were effective at the tested doses for the Rockefeller strain. For all 3 field populations, no product provided acceptable levels of control (mean mortality: > 80%), as shown in **Figure 4.**

DISCUSSION

Field control of larvae

Resistance to temephos may cause a reduction in the duration of its residual effect. This impact has been observed in the Caribbean region[26], in Brazil[27] and, more recently, in Colômbia[28].

The residual effects are known to vary according to the type of breeding site used for the tests. Studies performed in 2001 in Manaus[29] showed variation in the duration of the effect of temephos, depending on the kind of container used, as well as the commercial formulation available. According to MoReNAa, a larvicide needs to be substituted when it provides field control of less than 70% of what was observed for a susceptible reference strain (Rockefeller strain in our study) and management is recommended when the field population shows an RR of > 3[25]. Results from efficacy tests in São Paulo showed the suitability of this criterion, since, at this level of resistance, field failure is possible because of the diminished residual effect of the larvicide treatment.

According to this criterion, the population from Santos was the first to show a lack of efficacy under field conditions, and temephos needed to be substituted by a *Bacillus thuringiensis var. israelensis*-based larvicide in 2001[9]. Efficacy tests conducted between 2005 and 2008 showed a residual effect of the larvicide in all populations tested and identified the need for replacing the larvicide; the replacement was required on a medium term in Araçatuba, Barretos, Ribeirão Preto, São José do Rio Preto, and Sorocaba regions and on the long term in the regions of Bauru, Marília, and Presidente Prudente. Despite the urgent need for adequate management, the results showed that the use of temephos needed to be replaced throughout the entire State of São Paulo. In 2010, the larvicide temephos was replaced by diflubenzuron (an insect-grown regulator) in the Cities of São Paulo capital, Santos, Sorocaba, Ribeirão Preto, and Presidente Prudente. Temephos was still used until 2013 in the Araçatuba, Campinas, Taubaté, and Marília regions.

Taken together, data from the semi-field assays showed that, for the populations that showed an RR to temephos > 5, field failure was a considerable evident. For those populations with an RR 3-5, the average control was also below the level accepted by the Brazilian Monitoring Scheme[23]. Finally, populations with an RR of up to 3 showed acceptable control under field conditions. This correspondence between the results of laboratory and field tests of larvicides improves the efficacy of the monitoring results and permits an estimation of the success of field control, suggesting that the results may facilitate management strategies.

Adult control

The control level for adults was below the acceptance criteria or below 70% for all populations that showed a resistant status in laboratory experiments when exposed to ULV levels of pyrethroids in the field and in residual treatments since 2000. In Martinique, similar results were obtained for ULV treatment for the pyrethroid-resistant *A. aegypti* populations [30].

The results showed that the São Paulo Dengue Control should manage resistance to pyrethroids by substituting cypermethrin with malathion in ULV treatments and with fenitrothion for residual treatments. Between 2001 and 2003, cypermethrin was gradually replaced throughout the entire state[9].

Apparently, resistance to pyrethroids in São Paulo remained unchanged for about 7 years after the discontinuation of their use, and the effectiveness data from 2007 to 2009 indicated that use of malathion is still recommended.

After 10 years of the replacement of cypermethrin with fenitrothion, the field response to residual treatment, evaluated in 2011, showed that the tested populations of *A. aegypti* (Araçatuba, Marília, and Santos) did not show acceptable levels of control by means of fenitrothion or to the 2 other compounds: deltamethrin and bendiocarb. These results indicated that the available products were ineffective in controlling mosquitoes over a 15-day period, which is the duration of visiting and treatment at strategic sites in the Dengue Control Program[31,32]. Management procedures such as rotation[33] and mosaics[34] should be considered in order to avoid development of resistance and field control failure.

There was clear correspondence between laboratory and field responses to insecticides for adult control, as indeed also for larvicides. The diagnostic dose test, although unable to discriminate the levels of resistance, corresponded to field performance: all mosquito populations characterized as resistant presented a field response below the levels stipulated as acceptable (> 80% mortality or > 70% of the response of the susceptible reference strain). Populations characterized in the laboratory as having reduced susceptibility showed field responses within acceptable levels. The correlation between tests with qualitative assays (**Table 1**) suggested that a simpler test using only one diagnostic dose could be used as a guide for monitoring resistance, and would permit inference of the success of field control. This is an important tool, since the resistance of *A. aegypti* to insecticides has been noted worldwide[35,36], and because chemical control involves additional drawbacks, such as the difficulties of application in urban areas[37] and the inefficiency of equipment available for field control[38].

A decreased response in field control of mosquito populations was found for populations showing an RR of 3 at the lethal concentration of 95% for the larvicide temephos. For the adults, there was lack of effectiveness in the field for all populations of *A. aegypti* characterized as resistant to pyrethroids in the laboratory. A series of longitudinal tests indicated that the laboratory results permitted inference of field performance.

The management strategy involving the substitution of insecticides in areas with populations showing resistance[9] and restriction of chemical control in the entire state, introduced since 2001[39], has not been adequate for reversing the resistance detected or preventing the development of resistance in other regions. Importantly, the actual strategy of using insecticides in São Paulo (group of organophosphates for larvae and adult insects) for a long time; as also used in the past in other states of Brazil, apparently may have contributed to the high levels of resistance to the larvicides.

The São Paulo Superintendency of Endemic Disease Control strategy of assessing the impact of insecticide resistance on vector control is an important tool for guaranteeing the quality of field operations[38]. Data presented in this study allowed measurement of the operational impact of resistance; this may be used as a reference for monitoring programs and as an alert that insecticide resistance is reducing the efficacy of management strategies for controlling dengue transmission.

CONFLICT OF INTEREST

Some data from this study were included in a PhD thesis, entitled *Resistance mechanisms of Aedes aegypti L. (*Diptera: Culicidae*) to insecticides* presented to UNESP for obtaining a doctoral degree in general and applied biology.

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