

Dynamics of the control of *Aedes (Stegomyia) aegypti* Linnaeus (Diptera, Culicidae) by *Bacillus thuringiensis* var *israelensis*, related with temperature, density and concentration of insecticide¹

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ABSTRACT. Dynamics of the control of *Aedes (Stegomyia) aegypti* Linnaeus (Diptera, Culicidae) by *Bacillus thuringiensis* var *israelensis*, related with temperature, density and concentration of insecticide. The dynamics of the control of *Aedes (Stegomyia) aegypti* Linnaeus, (Diptera, Culicidae) by *Bacillus thuringiensis* var *israelensis* has been related with the temperature, density and concentration of the insecticide. A mathematical model for biological control of *Aedes aegypti* with *Bacillus thuringiensis* var *israelensis* (Bti) was constructed by using data from the literature regarding the biology of the vector. The life cycle was described by differential equations. Lethal concentrations (LC50 and LC95) of Bti were determined in the laboratory under different experimental conditions. Temperature, colony, larvae density and bioinsecticide concentration presented marked differences in the analysis of the whole set of variables; although when analyzed individually, only the temperature and concentration showed changes. The simulations indicated an inverse relationship between temperature and mosquito population, nonetheless, faster growth of populations is reached at higher temperatures. As conclusion, the model suggests the use of integrated control strategies for immature and adult mosquitoes in order to achieve a reduction of *Aedes aegypti*.

KEYWORDS. Biological control; environmental variable; Mathematical model.

RESUMO. Dinâmica do controle de *Aedes (Stegomyia) aegypti* Linnaeus, (Diptera, Culicidae) por *Bacillus thuringiensis* var *israelensis*, relacionada com a temperatura, densidade e concentração do inseticida. Foi elaborado um modelo matemático do controle biológico de *Aedes aegypti* com foco em *Bacillus thuringiensis* var *israelensis* (Bti). Na construção do modelo foram utilizados dados da literatura sobre a biologia do vetor, no qual o ciclo de vida foi descrito através de equações diferenciais. As concentrações letais (CL50 e CL95) do Bti foram determinadas no laboratório sob diferentes condições experimentais. As variáveis temperatura, colônia, densidade de larvas e concentração do bioinseticida acusaram diferenças significativas quando analisadas no modelo geral, porém quando analisadas individualmente, apenas a temperatura e concentração apresentaram diferenças. As simulações do modelo indicam que a temperatura afeta inversamente a produção de indivíduos e que os pontos máximos de produção de mosquitos são atingidos mais rápido a temperaturas maiores. Concluímos, com a simulação do modelo, que estratégias integradas de controle de imaturos e adultos devem ser utilizadas para atingir redução expressiva da população de *Aedes aegypti*.

PALAVRAS-CHAVE. Controle biológico; Modelo matemático; variáveis ambientais.

Dengue and dengue hemorrhagic fevers are diseases which etiologic agents are transmitted by the mosquito vector *Aedes aegypti* Linnaeus, 1762. This species is also capable of transmitting the yellow fever virus. The vector as well as the disease has become an economic and public health problem. Affected countries invest a significant part of the gross domestic product in the vector-disease control. Despite these efforts, the number of cases in Latin America oscillates year by year and in some cases, epidemics appear (Gubler 2002).

Currently, the main strategies directed at controlling the vector for dengue in Brazil involve chemical organophosphate (OP) and pyrethroid (P) products. In some cases, the population of mosquitoes changes its susceptibility, developing resistance (Macoris *et al.* 1995; Macoris *et al.* 2003, Lima *et al.* 2003, Braga *et al.* 2004; Duque *et al.* 2004). The resistance to insecticides can be reduced by rotation with non-chemical products based upon *Bacillus thuringiensis* var

israelensis (Bti), which have demonstrated efficiency for almost thirty years and safety in the environment (Goldber & Margalit 1977; Kroeger *et al.* 1995; Almalraj *et al.* 2000; Batra *et al.* 2000; Russell *et al.* 2003).

In the control of mosquitoes with Bti, it is crucial to take into account data for the following variables: temperature, larvae density, origin of populations and concentrations. Previous work has shown that such variables can modify the effectiveness of control efforts on the species of interest (Becker *et al.* 1992, Katbeh-Bader *et al.* 1999). In specific cases, the mathematical models allow us to analyze all these variables within a specific time interval. This can be done for laboratory or real conditions, predicting the dynamics of populations and helping us to take new directions of control.

Earlier studies based upon mathematical models presented predictions of the action of insecticides on dengue incidence (Newton & Reiter 1992; Esteva & Vargas 1998, 1999) including

environmental factors and the life cycle of the mosquito when dengue is present (Focks *et al.* 1993a,b; Focks *et al.* 1995) or the effect of copepods on the control (Duque *et al.* 2004). Nonetheless, they are designed to study the disease, and in some cases without the inclusion of vector biology. For this reason, the present study aims to understand the dynamics of *Ae. aegypti* control with *Bacillus thuringiensis* var *israelensis* H-14, by the use of a mathematical model simulated with environmental variables and with biological data on the species.

MATERIAL AND METHODS

The methodology of this study is divided in three parts: first, the construction of the mathematical model; second, bioassays with Bti to determinate LC_{50} and LC_{95} ; third, mathematical model simulation with biological data obtained from the literature.

First part: the model was established using differential equations adjusted to the *Ae. aegypti* biology. Data from the literature allowed determining the rates for birth, increase and survival of each daily biological stage of the species. The control of Bti and treatment alternative is continuous during the period of incidence of the mosquito. Parameters are defined as follows; θ : daily oviposition rate; μ : unhatched eggs rate; α : development rate from O to L_1 ; σ_1 : L_1 mortality rate; π : development rate from L_1 to L_2 ; σ_2 : L_2 mortality rate; γ : development rate from L_2 to L_3 ; σ_3 : L_3 mortality rate; ρ : mortality rate of L_3 by Bti action; ψ : development rate from L_3 to L_4 ; σ_4 : mortality rate of L_3 ; λ : development rate from L_4 to P; τ : mortality rate of pupae; Ω : development rate from Pupae to Adult; ε : daily mortality rate of adults; C: daily adult emergence rate; B: amplitude modulation of function oscillations; b: frequency of oscillations; t: simulation time (Table I). The starting values for simulations are: O = 125, L_1 = 25, L_2 = 24, L_3 = 23, L_4 = 22, P = 22 and A = 29.

Second part: reared larvae from laboratory were subjected to susceptibility tests with Bti using the liquid product Vectobac-AS (Sumitomo Chemical) 1200 international toxic units (ITU) per milligram. Different Bti concentrations from 0.01 ppm and 0.50 ppm were tested to determine the mortality limits from 1% and 99%. Five concentrations were ultimately selected for the essays: 0.03 ppm, 0.06 ppm, 0.12 ppm, 0.18 ppm and 0.25 ppm. The bioassays followed procedures established by WHO (1981).

Larvae were assayed for two different density values: 20 or 30 individuals from late third instar and/or earlier fourth at temperatures of 20, 25 or 30 °C and five replicates for each test. The larvae were kept in 300 ml polystyrene vessels (cups) with 150 ml of "Ouro Fino" brand mineral water. In addition to the control assay, five tests were carried out for each larvae density and temperature. During the experiments food was not offered to the larvae, and cups were kept in climatized chambers with 80% ($\pm 10\%$) relative humidity.

For the third part of the methodology, mortality was evaluated 24 hours after the addition of the product. The LC_{50}

and LC_{95} values were estimated by the Probit GW-Basic analysis (Finney 1981). Statistical calculation of density, temperature and concentration was obtained through variance analysis (ANOVA), where the dependent variable was the mortality by Bti action. The results from the Homogeneity of Variance test (Levene test) were analyzed at significance levels of 5% ($\alpha=0.05$), the analyses were carried out using the *Statistical Package for the Social Sciences* (SPSS) v10.0.

RESULTS

A model is presented with seven differential equations (e1...e7) formed by the following variables: O: number of eggs laid by females from variable A; L_1 : number of first instar larvae; L_2 : number of second instar larvae; L_3 : number of third instar larvae; L_4 : number of fourth instar larvae; P: number of pupae; A: number of adults (Figure 1); $CA/2\theta \text{ Sen}^2$ = this function determines the fluctuation of adults originated from each female oviposition. It is divided by two according to the sexual male/female ratio, which tends to be 1:1, *i.e.*, from the total of adults, only one half produce eggs.

Differential equations system:

$$\frac{dO}{dt} = \frac{CA}{2} \theta + B \text{Sen}^2 (bt) - \mu O - \alpha O \quad \text{e.1}$$

$$\frac{dL_1}{dt} = \alpha O - \sigma_1 L_1 - \pi L_1 \quad \text{e.2}$$

$$\frac{dL_2}{dt} = \pi L_1 - \sigma_2 L_2 - \gamma L_2 \quad \text{e.3}$$

$$\frac{dL_3}{dt} = \gamma L_2 - \sigma_3 L_3 - \psi L_3 - \rho L_3 \quad \text{e.4}$$

$$\frac{dL_4}{dt} = \psi L_3 - \sigma_4 L_4 - \lambda L_4 \quad \text{e.5}$$

$$\frac{dp}{dt} = \lambda L_4 - \tau P - \Omega P \quad \text{e.6}$$

$$\frac{dA}{dt} = \Omega P - \varepsilon A \quad \text{e.7}$$

Bioassays with *Bacillus thuringiensis* H-14: The temperature, concentration and density interaction of two *Ae. aegypti* colonies evaluated (Londrina and Rockefeller) indicates the existence of an inversely proportional pattern of concentration versus temperature, *i.e.* at higher temperatures, a smaller bacillus concentration will be needed to obtain a satisfactory effect (Tables II - V).

The "Levene" test (homogeneity of variances) showed a normal distribution ($\alpha=0.05$, $p < 0.111$). The analysis of variance (ANOVA) only revealed significant differences when all the variables were analyzed together. The values obtained were:

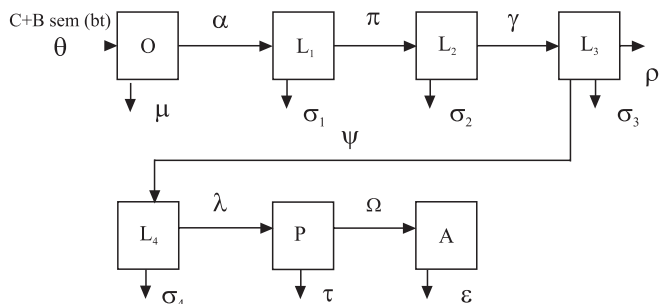


Fig. 1. Flow diagram of the model.

Rockefeller or Londrina colonies ($\alpha = 0.05$, $f = 12.709$, $p < 0.001$), Temperatures ($\alpha = 0.05$, $f = 32.552$, $p < 0.0001$), Concentration ($\alpha = 0.05$, $f = 39.920$, $p < 0.0001$) and Density 20 or 30 ($\alpha = 0.05$, $f = 4.798$, $p < 0.034$) when compared to the Mortality dependent variable.

When the variables were analyzed alone, they did not present relevant differences related to colony ($\alpha = 0.05$, $f = 2.636$, $p < 0.110$) and density ($\alpha = 0.05$, $f = 2.456$, $p < 0.123$). This indicates that results are strongly influenced by the temperature. The Tukey HSD (*Honestly Significant Difference*) test showed differences for the temperature ($p < 0.05$) and concentration variables. There are differences for 20°C when compared to data for 25 and 30°C. The concentrations that did not present differences are 0.12 ppm / 0.18 ppm and 0.18 ppm / 0.25 ppm.

Simulations: The simulations carried out present population increase of immature and adult *Ae. aegypti* in different scenarios depending on the efficiency of the control applied with Bti (ρ), at 20, 25 or 30°C during a 12 month period. In this dynamics, four hypothetical possibilities were evaluated for the Bti efficiency based on different treatments: a) $\rho = 0$, this value indicates the control (without Bti); b) $\rho = 0.5$, represents 50% of larval mortality; c) $\rho = 1$, 100% of larval mortality; and d) $\rho = 1$, 100% of larval mortality in addition to a treatment for adults of 0.5 (50% of mortality) as suggestion for an integrated control (Figures 2, 3 and 4).

The simulation at 20°C shows a population increase with stabilization after 8 months (Figure 2). When the efficiency (ρ) of 50% is simulated the population decreases, although this effect is not enough because the objective is to reach a higher number of controlled individuals (Figure 2b). By increasing

Table I. Values of the rates in percentages (%) used in the simulation of the model for *Aedes aegypti*.

Parameter	20 °C	25°C	30°C
θ	61.1 ^e	78.9 ^e	78.9 ^e
μ	29.61 ^f	29.61 ^f	29.61 ^f
α	70.39 ^f	70.39 ^f	70.39 ^f
σ_1	0 ^e	(24°C)4 ^e	0 ^e
σ_2	0 ^e , 3.8 ^b , 7.5 ^a	1 ^c , 3.8 ^b , 7.5 ^a	1 ^c , 3.8 ^b , 7.5 ^a
π	100 ^f	100 ^f	100 ^f
γ	100 ^c , 96.2 ^b , 95.5 ^a	99 ^c , 96.2 ^b , 95.5 ^a	99 ^c , 96.2 ^b , 95.5 ^a
ρ	0, 50, 100	0, 50, 100	0, 50, 100
σ_3	1.9 ^c , 3.8 ^b , 2.8 ^a	2.1 ^c , 3.8 ^b , 2.8 ^a	5 ^c , 3.8 ^b , 2.8 ^a
ψ	98.1 ^c , 96.2 ^b , 97.2 ^a	97.9 ^c , 96.2 ^b , 97.2 ^a	95 ^c , 96.2 ^b , 97.2 ^a
σ_4	6 ^c , 3.3 ^b , 10 ^a	3.2 ^c , 3.3 ^b , 10 ^a	6.3 ^c , 3.3 ^b , 10 ^a
λ	94 ^c , 96.7 ^b , 90 ^a	96.8 ^c , 96.7 ^b , 90 ^a	97.5 ^c , 96.7 ^b , 90 ^a
ι	3.19 ^c , 15 ^b , 10 ^a	15.9 ^c , 15 ^b , 10 ^a	1 ^c , 15 ^b , 10 ^a
Ω	91.80 ^d , 97 ^a	62.58 ^d , 97 ^a	66.34 ^d , 97 ^a
ϵ	4.56 ^f	4.56 ^f	4.56 ^f
C	0.50 ^e	0.50 ^e	0.50 ^e
B	8	8	8
b	2	2	2
T	24	24	24

For some rates, there is only a single value. When there are more because of the different papers, the simulation was always conducted with the lower value.

Hien (1975)^a, Bar-Zeev (1957)^b, Bar-Zeev (1958)^c, Rueda *et al* (1990)^d, Costero *et al* (1998)^e, Crovello & Hacker (1972)^f.

the efficiency up to 100% the number of larvae diminishes but the reduction is still insufficient (Figure 2c).

The information generated by the model indicates that effective control strategies focused on larvae and adults must be applied concomitantly with cultural actions. The lack of persistence of this method evaluated with environmental and cultural characteristics leads to continuous appearance of breedings and then a renewal of new adult generations. This factor makes the control actions difficult.

By including control of adults we observe a higher efficiency for reducing the population (Figure 2d). In order to make the mosquito control more effective, according to the model, adult treatment is necessary (Figures 3 and 4) in addition to the biological treatment applied to the larvae (L3). We also observe in the simulation that high temperatures influence inversely the production of individuals, therefore, the highest peaks of mosquito population are reached faster at low temperatures. This fact must be applied in the new strategies for mosquito control (Figures 2 to 4).

Table II. Lethal concentrations at the 50% and 95% levels for *Bacillus thuringiensis* H-14, confidence interval (CI), x^2 , for the temperatures 20, 25 or 30°C, density 20 larvae per replicate of *Aedes aegypti* colony Rockefeller.

Temperature	LC _{50ppm}	[]	CI		LC _{95ppm}	[]	CI		x^2
20°C	0.3062	3.825 °C to 20 °C	0.0271199	0.374682	0.6364	3.625 °C to 20 °C	0.484466	1.042998	2.9288
25 °C	0.0797	4.430 °C to 20 °C	0.073252	0.086802	0.1732	4.230 °C to 20 °C	0.151675	0.206291	0.1147
30 °C	0.0692	0.130 °C to 25 °C	0.061251	0.72693	0.1507	1.130 °C to 25 °C	0.133177	0.177028	3.1183

[]:Proportional increase in concentration depending on temperature: lower temperatures / higher temperatures

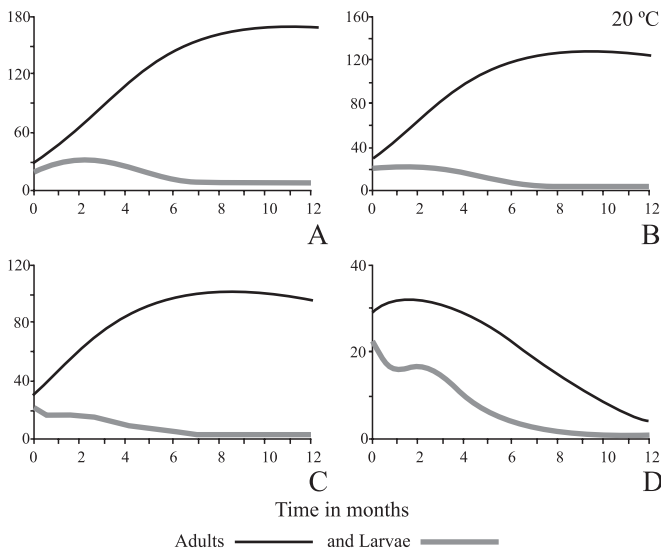


Fig. 2. Scenarios for the control of *Aedes aegypti* using parameters from table 1, simulated 20°C. A = number of adults is represented by the fine line and L3 = number of larvae of third instar is represented by thickest line. Varying the effectiveness of the Bti (ρ), a; $\rho = 0$, b; $\rho = 0.5$, c; $\rho = 1$ and d; $\rho = 1$ including alternative treatment with 50% of adult mortality.

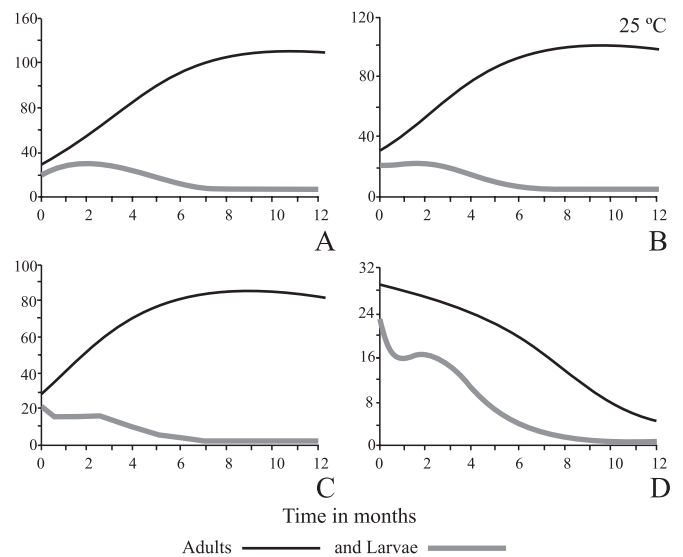


Fig. 3. Scenarios for the control of *Aedes aegypti* using parameters from table 1, simulated 25°C. A = number of adults is represented by the fine line and L3 = number of larvae of third instar is represented by the thickest line. Varying the effectiveness of the Bti (ρ), a; $\rho = 0$, b; $\rho = 0.5$, c; $\rho = 1$ and d; $\rho = 1$ including alternative treatment with 50% adult mortality.

DISCUSSION

This study meets these expectations quite closely and it reaches our objective by describing the dynamics of the biological control of *Ae. aegypti*. The focus on the construction of models related to mosquitoes is directed to the analysis of diseases. Only a small number of studies focus on vector control. In expecting to represent the action of every single variable and explore them with differential equations, a different methodology was followed than in earlier works. Our model was directed at the larval stage as consequence of the work of Newton & Reiter (1992) which reveals that the impact of insecticides at ultra-low volume (ULV) for the adults is minimal and only delays a dengue epidemic.

The inclusion of function *Sin* allows the equations system to provide useful information for vector control programs. The model can assume periods with a higher production of mosquitoes (rain, temperature and humidity) that could be compared with the index used to evaluate the amount of mosquitoes and their relationship with the disease.

In the experiments related to the determination of lethal concentrations (LC_{50} and LC_{95}), it was observed that the Bti

dose varies depending on the temperature. In relation to the larvae density and colony variables, the concentrations of the product did not influence the lethal concentrations.

In the model we observe that part of the changes taking place in the population density are influenced by the temperature, particularly at values below the thermal limit of the species. For low temperatures, higher concentrations of entomopathogenic bacteria (Bti) are required in order to get the same effect produced at high temperatures.

This fact has also been demonstrated by Becker *et al.* (1992) with *Bacillus thuringiensis* H-14 versus *Aedes vexans*, *Aedes aegypti* and *Culex pipiens*, and Katbeh-Bader *et al.* (1999) by using *Bacillus thuringiensis* H-14 versus *Culiseta longiareolata* (Macquart). According to Ramoska & Pacey (1979), an explanation for this fact is that high temperatures increase the metabolic activity of mosquitoes, and therefore the bacillus ingestion rates. Thus, there is a direct influence on the mortality at high temperatures because of the high concentrations of the pathogen into the digestive system (Ramoska & Hopkins 1981). This is relevant and attention must be paid because the handbooks used by different

Table III. Lethal concentrations at the 50% and 95% level of *Bacillus thuringiensis* H-14, confidence interval (CI), x^2 , at the temperatures 20, 25 and 30°C, density 30 larvae per replicate of *Aedes aegypti* colony Rockefeller.

Temperature	CL _{50ppm}	[]	CI		CL _{95ppm}	[]	CI		x^2
20°C	0.2588	2.425 °C to 20 °C	0.237996	0.292949	0.5947	1.625 °C to 20 °C	0.470474	0.877733	2.9288
25 °C	0.1066	4.530 °C to 20 °C	0.098446	0.115572	0.3684	3.330 °C to 20 °C	0.316975	0.442830	0.1147
30 °C	0.0570	1.830 °C to 25 °C	0.051962	0.062076	0.1797	2.230 °C to 25 °C	0.158516	0.209766	3.1183

[]:Proportional increase in concentration depending on temperature: lower temperatures / higher temperatures

Table IV. Lethal concentrations at the 50% and 95% level of *Bacillus thuringiensis* H-14, confidence interval (CI), x^2 , at the temperatures 20, 25 and 30°C, density 20 larvae for replicate of *Aedes aegypti* colony Londrina.

Temperature	LC _{50ppm}	[]	CI	LC _{95ppm}	[]	CI	x^2
20°C	0.0919	1.325 °C to 20 °C	0.085028-0.098806	0.1879	125 °C to 20 °C	0.168222-0.217406	6.368
25 °C	0.0657	2.930 °C to 20 °C	0.059670-0.072042	0.1799	2.230 °C to 20 °C	0.156613-0.214053	3.0495
30 °C	0.0307	2.130 °C to 25 °C	0.0254843-0.034829	0.0847	2.130 °C to 25 °C	0.071168-0.111868	2.2112

[]:Proportional increase in concentration depending on temperature: lower temperatures / higher temperatures

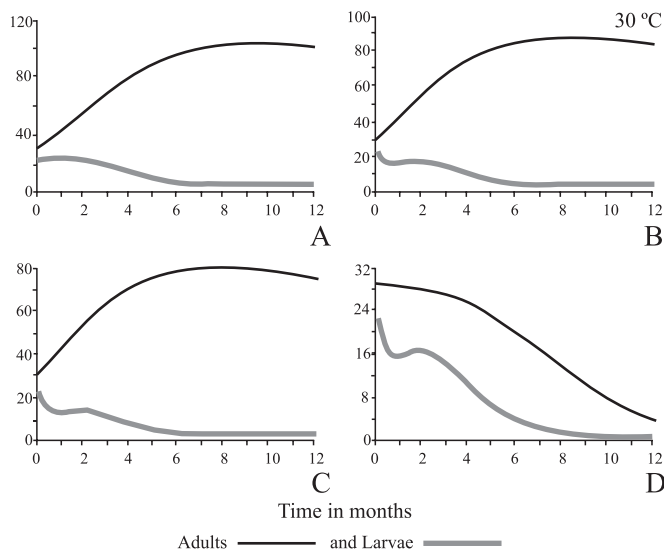


Fig. 4. Scenarios for the control of *Aedes aegypti* using parameters from table 1, simulation for 30°C. A = number of adults is represented by the fine line and L3 number of larvae of third instar is represented by the thickest line. Varying the effectiveness of the Bti (ρ), a; $\rho = 0$, b; $\rho = 0.5$, c; $\rho = 1$ and d; $\rho = 1$ including alternative treatment with 50 % adult mortality.

mosquito control sanitary agencies do not strictly consider this parameter.

The density provided little information, perhaps as consequence of the small amplitude of densities used in the experiment. Nevertheless, the density presented a significant difference when it was analyzed within the whole set of variables (temperature, colony and concentration). According to Becker *et al.* (1992), the density interferes in the efficiency of lethal concentration, indicating that in places with a high larvae density, the control efficiency may reduce with the standard concentrations.

The Rockefeller and Londrina colonies presented different susceptibilities as to lethal concentrations. By comparing both colonies, Londrina is less susceptible to Bti as expected in the first instance, since the reference colony should be more susceptible. Thiéry *et al.* (1999) have already observed differences between *Ae. aegypti* populations and the ‘bora-bora’ reference colony which was created over twenty years ago in the laboratory with more susceptibility than the former colony collected in French Guiana. The colonies with more stabilization time in laboratory tend to be more affected by the different products used to date.

The Londrina colony presented more susceptibility, probably because it was subjected to natural conditions of its original place and then it was placed under the stress of the controlled temperature, humidity and photo-phase of the room where the experiments were conducted.

When we compare the results of the present simulation with the experimental data from a similar study such as the model of Newton & Reiter (1992), it is confirmed that the introduction of isolated agents does not considerably reduce the population of mosquitoes. Our model, formed by different scenarios of control where Bti is applied, indicates the need to add different strategies and methods in order to obtain more efficiency for the control of young forms of *Ae aegypti*.

As previously mentioned, the model uses experimental data which tend to elevate the laboratory rates compared to the values found in field. Nonetheless, the rates applied to the model simulation can be modified to approach the natural conditions required according to the experience and convenience of researchers when they design the scenarios for the model. We point out the importance of the continuous evaluation that must follow the methods and control strategies.

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Table V. Lethal concentrations at the 50% and 95% levels of *Bacillus thuringiensis* H-14, confidence interval (CI), x^2 , at the temperatures 20, 25 and 30°C, density 30 larvae for feedback from the *Aedes aegypti* colony Londrina.

<i>Aedes aegypti</i> Londrina	LC _{50ppm}	[]	IC	CL _{95ppm}	[]	CI	x^2
20°C	0.1562	1.325 °C to 20 °C	0.147600-0.165341	0.3335	125 °C to 20 °C	0.299725-0.382922	0.2090
25 °C	0.0661	2.930 °C to 20 °C	0.061198-0.071059	0.1707	2.230 °C to 20 °C	0.152792-0.195571	2.2562
30 °C	0.0600	2.130 °C to 25 °C	0.05390-0.066109	0.2429	2.130 °C to 25 °C	0.208677-0.293724	1.9278

[]:Proportional increase in concentration depending on temperature: lower temperatures / higher temperatures

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