Larval Emergence from *Aedes aegypti* (Diptera: Culicidae) Eggs Exposed to Hot Air[1]

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Abstract *Aedes aegypti* (L.) (Diptera: Culicidae) represents a severe threat to human well-being and health due to the arthropod-borne viruses (arboviruses) it transmits. Its control is implemented mainly through massive applications of insecticides directed to the larval and adult stages. To develop an additional method for combating this vector, eggs (7–15 d old) were exposed in groups of 20 to a stream of hot air at temperatures between 32 ± 2°C and 147 ± 2°C for 5 s. The cumulative percentage of emerged larvae at 24 h and 48 h posttreatment was recorded as a measure of response to the hot air treatment. In the untreated control, which was exposed to room temperature (26 ± 2°C), the cumulative emergence of larvae at 48 h was 99.2 ± 1.7%. The cumulative percentage of larval emergence at 48 h ranged from 97.2% at 87 ± 2°C to 67.7% at 147 ± 2.4°C. The biological efficacy of this proposed hot air treatment was, thus, not acceptable. The natural biological attributes of the *Ae. aegypti* eggs in withstanding heat and desiccation appear to have protected them against the various levels of temperature tested.

Key Words hot air treatment, yellow fever mosquito, heat tolerance

The yellow fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae), has adapted to live and thrive in urban and periurban settlements in tropical and subtropical areas (Powell and Tabachnik 2013). Females require vertebrate blood for egg development, and the acquisition of the bloodmeal provides an avenue for disease transmission (Hansen et al. 2014, Gonzales and Hansen 2016). This vector causes discomfort or death due to the transmission of arthropod-borne viral (arboviral) diseases such as classic dengue, hemorrhagic dengue, Zika, yellow fever, Mayaro fever, and chikungunya (Mayer et al. 2016). Dengue has received particular attention from international organizations and governments of affected countries. Globally, an estimated 3.9 billion persons are at risk of being infected with this disease (WHO 2022). Annually, 390 million people contract it and 96 million people experience severe symptoms (WHO 2022). In 2021, in the Region of the Central American Isthmus and Mexico, 111,227 cases of dengue were detected, of which

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1,399 were severe and 54 people died (PAHO 2021). The problem with *Ae. aegypti* is further amplified by considering the remaining diseases that are transmitted by this mosquito. The adverse effects of these diseases on the people and economies of the affected regions are sufficient reasons to consider this vector the "most dangerous animal for humans" (Powell 2016). The main drivers for the geographic expansion of this invasive mosquito species are globalization and changes in the environment, including climate change (Wesula Lwande et al. 2020), and consequently, its impact is expected to increase.

The countries affected by this vector initiate organized actions against mosquito species shortly before the rainy seasons begin. These actions consist of destroying or disabling containers that can collect rainwater, which are required for both oviposition and larval development (WHO 2022). Once the rains begin, insecticides are used against larvae and adults. Of the active ingredients applied against larvae, some have marginal activity against pupae, for example pyriproxyfen (Darriet and Corbel 2006, Fiaz et al. 2019). Adults are combated with insecticides indoors and outdoors (CENAPRECE 2020). Impregnation of fabrics is also used as protection from mosquitoes entering houses or places where people sleep (Faulde et al. 2012). In some countries, housing units are treated with insecticide-containing paints (Mosqueira et al. 2010, 2015, Schiøler et al. 2016, Poda et al. 2018).

The fight against this pest has several weak links. The use of insecticides to reduce the density of larvae and adults may have adverse consequences for human health and the environment and also can lead to the development of insect resistance to the insecticides (Chino-Cantor et al. 2014, López et al. 2014, López-Solís et al. 2020). However, no actions are taken anywhere in the world for the direct control of *Ae. aegypti* eggs (WHO 2022), perhaps because no chemical substances have been identified to possess acceptable ovicidal properties.

Additionally, it is easier to combat larvae and adults with insecticides during the rainy season. Outside this period in which both larvae and adults appear, reducing the density of the eggs that this pest leaves scattered throughout habitats is not considered essential, which may be a significant flaw in its integrated management. Consequently, studies are needed to identify methods, which are economically feasible and respectful of the environment and human health, to allow for effective control of the eggs of this vector. Therefore, the objective of this study was to determine the impact of a stream of hot air at a variety of temperatures on the emergence of larvae from exposed eggs of *Ae. aegypti*.

Materials and Methods

Insects. We used the New Orleans strain of *Ae. aegypti* obtained from a colony maintained at the Universidad Autónoma de Nuevo León, Mexico. Colony maintenance followed the methods of the World Health Organization (WHO 2005) at 26 ± 2°C, 70 ± 5% relative humidity (RH), and a photoperiod of 12:12 h (light: darkness).

Hot air treatment. A hot air gun (model 1800 W STXH2000-B3; Stanley®, Towson, MD), with an airflow of 300–500 L/min, was used to create the stream of

hot air. The temperature was regulated by modifying the speed of the airflow, adjusting the temperature regulator of the gun, and varying the distance between the hot air emission point and the ovitrap. The hot air temperature was measured with a mercury thermometer (Taylor®, Oakbrook, IL) with –20°C to 150°C and divisions of 1°C.

Bioassay. The experimental units for this study consisted of ovitraps containing 20 eggs (7–15 d old) each. The ovitraps were placed in 5-cm-diameter petri dishes containing 5 mL of agar (Bacto® Agar; Becton, Dickinson and Company, Mexico City, Mexico). The hot air gun was calibrated to the desired temperatures before exposing the eggs. The petri dish with the ovitrap was then exposed for 5 s to a current of hot air at a speed of 3.7 ± 1 m/s at a specific temperature. Twenty-four temperature levels were evaluated ranging from 32 ± 2°C to 147 ± 2°C (Table 1). Thirty repetitions with 20 eggs each were performed for each temperature. Each repetition included an untreated control.

Subsequently, the ovitrap treated with hot air was placed in a 150-ml container containing 100 ml of a solution of water with sugar (24 g/L of water). Before the water was used to hatch the eggs, it was heated to the boiling point after which the heat source was removed and the water was allowed to cool at room temperature. Once the water reached 30°C, sugar was added at the indicated proportion. The mixture of water-sugar increases the hatching percentage, as suggested by Quispe-Pretel et al. (2015). The experimental units were kept under controlled environmental conditions inside a bioclimatic chamber (model TFFU2065FWA; Thermo Scientific, Waltham, MA) at 26 ± 2°C, 70 ± 5°C RH, and a photoperiod of 12:12 h (light: darkness). After 24 h, the percentage of emerged larvae was recorded in each container. At 48 h after exposure, the accumulated number of hatched larvae was recorded, and the emerged larvae were examined to determine any visible adverse effects of the treatment.

Statistical analysis. Before statistical analysis, the data were transformed using the arcsine of the square root of the percentage of emerged larvae/100. Larval emergence rather than egg mortality was measured and analyzed. These transformed data were subjected to analysis of variance, and treatment means were compared using Tukey's honestly significant difference (HSD) at $P = 0.05$ (SAS Institute 2008).

Results

There were significant statistical differences among treatments at both 24 h ($F = 4.67$; df = 24, 725; $P \leq 0.001$) and 48 h ($F = 5.22$; df = 24, 725; $P \leq 0.001$). In the untreated control, a mean ± SE of 97.0 ± 3.7% of the larvae emerged at 24 h with an accumulated larval emergence of 99.2 ± 1.7% at 48 h (Table 1). In terms of the cumulative percentage of larval emergence, temperatures of 137, 142, and 147°C were the only temperature treatments that statistically differed from that of the control (Tukey's HSD, $P = 0.05$) with larval emergences of 80.0 ± 2.0%, 75.0 ± 2.0%, and 67.7 ± 2.4%, respectively. Larval emergence in all other temperature treatments tested was not statistically different from that of the control (Tukey's HSD, $P = 0.05$). This same pattern of response was observed at 24 h after

Table 1. Mean ± SE percentage of larval emergence from eggs of *Ae. aegypti* exposed to a stream of hot air (3.7 ± 1 m/s) at different temperatures.

	% Emergence	
Temperature (°C)	**24 h**	**48 h**
32 ± 2	80.2 ± 2.0 bcde	89.3 ± 1.8 abc
37 ± 2	80.5 ± 2.3 bcde	90.8 ± 1.3 abc
42 ± 2	86.2 ± 2.4 abcd	90.5 ± 2.6 abc
47 ± 2	87.5 ± 2.0 abcd	92.0 ± 2.4 ab
52 ± 2	93.2 ± 1.3 abcd	95.7 ± 1.2 ab
57 ± 2	91.0 ± 2.0 abcd	92.3 ± 2.1 abc
62 ± 2	91.7 ± 1.2 abcd	93.2 ± 1.4 ab
67 ± 2	90.5 ± 1.49 abcd	96.0 ± 1.4 a
72 ± 2	93.3 ± 1.7 abcd	95.7 ± 2.2 a
77 ± 2	93.3 ± 1.4 abc	95.7 ± 1.4 a
82 ± 2	95.3 ± 1.4 abc	96.8 ± 1.4 a
87 ± 2	95.8 ± 1.2 abc	97.2 ± 1.2 a
92 ± 2	87.8 ± 2.3 abcd	90.7 ± 2.4 ab
97 ± 2	90.7 ± 2.2 abc	91.80 ± 2.0 ab
102 ± 2	86.8 ± 2.0 abcd	89.2 ± 2.0 abc
107 ± 2	90.7 ± 2.1 abcd	92.7 ± 1.9 ab
112 ± 2	84.5 ± 2.7 abcde	86.2 ± 2.9 abc
117 ± 2	92.7 ± 1.7 abc	94.2 ± 1.8 ab
122 ± 2	87.8 ± 3.2 abcd	88.3 ± 3.3 abc
127 ± 2	93.0 ± 1.9 abc	93.8 ± 2.0 ab
132 ± 2	85.0 ± 2.3 abcde	87.5 ± 2.1 abc
137 ± 2	78.7 ± 2.0 cde	80.0 ± 2.0 bcd
142 ± 2	74.2 ± 1.9 de	75.0 ± 2.0 cd
147 ± 2	66.8 ± 2.2 e	67.7 ± 2.4 d
Untreated control	97.0 ± 3.7 a	99.2 ± 1.7 a

Percentage means within each postexposure time and followed by the same lowercase letter are not significantly different (Tukey's HSD, $P = 0.05$). For each level of temperature tested, 600 eggs were exposed (30 replications of 20 eggs each).

exposure (Table 1). No visible abnormalities were observed in the larvae that emergence following any of the temperature treatments.

Discussion

In our preliminary testing, we experienced a high variability (e.g., 2 to 87%) in the percentage of larval emergence in the untreated controls at 12 and 48 h after placement of the ovitraps in the containers with water. We subsequently adopted the methods of Quispe-Pretel et al. (2015) of placing the ovitraps into sugar water as described above. Using that method, we observed a high level of emergence that remained consistent with low variability.

Overall, we obtained a high level of larval emergence in the untreated controls, with a level of 97.0 $\pm$ 3.7% at 24 h and 99.2 $\pm$ 1.7% cumulative emergence at 48 h (Table 1). Larval emergence from eggs treated with temperatures ranging from 32°C to 132°C did not differ statistically from that of the untreated control (Tukey's HSD, $P = 0.05$). Only the treatments with 137°C, 142°C, and 147°C yielded larval emergence levels that were statistically lower than that of the untreated control (Tukey's HSD, $P = 0.05$), with the lowest level of emergence only at 66.8 $\pm$ 2.2% (147°C, at 24 h)

We, therefore, concluded that treatment of *Ae. aegypti* eggs with streams of hot air did not provide an acceptable level of biological efficacy (e.g., $\geq$90%) to merit field studies.

The conditions to which the eggs of this vector were subjected are lethal for the same biological stage of other species of insects. For example, Hernández-Rivera et al. (2022), using a similar method, found a positive correlation between temperature and egg mortality in *Tetranychus urticae* Koch (Acari: Tetranychidae), reporting 100% egg mortality at 65 $\pm$ 1.5°C.

It is inferred that there are two approaches to increase the biological efficiency of hot air. The first would be to increase the temperature. However, working with temperatures above 147 $\pm$ 2°C would represent a risk for the applicator. The second approach would be to increase the time of exposure to temperature. Others found this approach to improve efficacy. Smith et al. (1988) used heat exposures of 2, 15, and 120 min, while Macfie (1920) used 5 min. Davis (1932) increased exposure time from 24–48 h to 7 d. Although feasible under laboratory conditions, both of these approaches are impractical in the field and could have deleterious impacts to the habitat.

Our results could be due to the impermeable characteristics of the *Ae. aegypti* egg chorion (Mundim-Pombo et al. 2021) that provides support and protection for the egg (Suman et al. 2011). We could not rule out the possibility that specific chorion characteristics have evolved to yield a consistent phenotype that is highly resistant to the selective forces of the environment, providing an effective defense against stressors, as suggested by Brittany et al. (2016).

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