Aerial release of *Aedes aegypti* male mosquitoes using an unmanned aerial vehicle: a novel control strategy

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Abstract

Objective. To development of a methodology for the chilling, handling, transport, and release of male *Aedes aegypti* mosquitoes, reared in insectary conditions to release in the field with unmanned vehicles to compete sexually with wild males in the field. **Materials and methods.** A population of *Ae. aegypti* from different areas in Tapachula, Chiapas, was used. Laboratory tests were conducted: Effect of temperature and cooling time on the knockdown, recovery of males, and copulatory success. **Results.** The chilling temperature of 3 ± 1°C for 30 min, was used as a knockdown temperature before handling, packing, transportation, and aerial release. The males subjected to the entire process, including the semi-field aerial release test, showed normal sexual behavior activity, obtaining 100% of females inseminated. **Conclusion.** These results present the feasibility of applying a new control methodology using unmanned aerial vehicle (UAV) as support for the sterile insect release technique (SIT), use of *Wolbachia* or both, in male *Ae. aegypti*, for the design of strategies to control their populations.

Keywords: *Aedes aegypti*; unmanned aerial vehicles; sterile insect technique

Valdez-Delgado KM, Ríos-Delgado JC, Nettel-Cruz JA, Angulo-Kladt R, Villarreal-Treviño C. Liberación aérea de mosquitos *Aedes aegypti* machos utilizando un vehículo aéreo no tripulado: estrategia novedosa de control. Salud Publica Mex. 2023;65:387-393.
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Resumen

Objetivo. Desarrollar una metodología para el enfriamiento, manejo y transporte de mosquitos *Aedes aegypti* machos, criados en condiciones de insectario, para su liberación en el campo con vehículos no tripulados. **Material y métodos.** Se utilizó una población de *Ae. aegypti* de diferentes zonas de Tapachula, Chiapas. Se realizaron pruebas de laboratorio para determinar el efecto de la temperatura y tiempo de enfriamiento en el derribo, recuperación de machos y éxito copulatorio. **Resultados.** Se utilizó la temperatura de enfriamiento de 3 ± 1°C durante 30 min como temperatura de derribo para el manejo, empaque, transporte y liberación de machos *Ae. aegypti*. Los machos sometidos a todo el proceso, incluida la prueba de liberación aérea de semi-campo, mostraron una actividad de comportamiento sexual normal, obteniendo 100% de hembras inseminadas. **Conclusión.** Estos resultados presentan la factibilidad de aplicar una nueva metodología de control utilizando un vehículo aéreo no tripulado como apoyo a la técnica de liberación de insectos estériles (SIT), uso de *Wolbachia* o ambos, con machos de *Ae. aegypti*, para diseñar estrategias de control de sus poblaciones.

Palabras clave: *Aedes aegypti*; vehículos aéreos no tripulados; técnica de insectos estériles

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In Mexico, *Aedes (Stegomyia) aegypti* is widely distributed from the northern part to the south of the country.[1] It was recently reported at an altitude of 2 250 masl in Mexico City.[2] It is a highly anthropophilic and anthropophagic mosquito linked to dengue virus transmission.[3] In southern Mexico, an outbreak of Zika and chikungunya occurred in 2014, with *Ae. aegypti*.[4,5] Despite the enormous efforts to control this species of mosquitoes using chemical products to kill larvae,[6] and adults,[7] the populations of these vectors continue to be a serious problem. The problem of arbovirus transmission between susceptible human populations continues to be a priority in health systems. One of the reasons why chemical control has not been effective is because the vectors are highly resistant to insecticides used in public health,[8,9] and the lack of vaccines against these arboviruses. Another determining factor is that the adult females remain in hidden places of houses during the aerial fumigation.

There are alternative control methods such as biological control using other insects, *e.g.*, *Toxorhynchites* larva for larval biological control,[10] crustaceans as predators of eggs,[11] and entomopathogenic fungi for adults.[12] Recently, experiments have been carried out with irradiated sterile males and males inoculated with *Wolbachia* sp. bacteria.[13] Advances in modernizing vector control and monitoring tools and developing new ones offer an excellent opportunity to strengthen vector control.[14] Also, in its global vector control response 2017–2030, the World Health Organization (WHO) noted the urgent need to develop and integrate innovative mosquito control methods, including the sterile insect technic (SIT), particularly against *Aedes* sp. vectors.

The release procedures and the intrinsic biological phenomena of the mosquito males are vital knowledge to apply the sterile male technique, which is necessary to manage and release them effectively to compete against wild males to copulate with wild females. The development of an effective aerial release strategy is required to ensure cost-effective releases of sterile male mosquitoes, especially when large areas of ground need to be covered. The various parts of the release process, step by step, need to be analyzed in order to carry out an effective male mosquito release technique; from the cold knockdown of males, handling, packaging, transportation to the release area, use of the unmanned aerial vehicle (UAV), and the impact of all these steps on the sexual competitiveness of males to ensure competition for field females. These procedures can be applied to male *Ae. aegypti* using the SIT, or infected with *Wolbachia* sp., to be released with UAV technology as an alternative strategy for dengue vector control in endemic countries.

Materials and methods

Mass breeding of *Aedes aegypti*

For all the experiments, *Ae. aegypti* mosquitoes (Tapachula strain) from different areas of Tapachula city, Chiapas were used. This population of mosquitoes has been established for one year (12 generations) under controlled conditions: temperature 24 ± 1°C, relative humidity 45 ± 8%, 12:12 hr light: dark. The larvae were reared according to standardized techniques.[15,16] Pupae were sexed using a glass plate separator (John W. Hock Co., Gainesville, FL, USA). All the experiments were carried out in the *Centro Regional de Investigación en Salud Pública* (CRISP) insectary in Tapachula, Chiapas, Mexico, from January to December 2018.

Effect of temperature on the knockdown and recovery of males

A hundred virgin males from 2 to 4 days of age were placed in wooden cages (10 x 10 x 10 cm), with three repetitions, under seven temperature treatments: -4, -2, 0, 2, 4, 6, and 8°C for 30 min to determine the knockdown temperature. The flight activity recovery time of 50 and 100% of the cooled males was recorded at a temperature of 30ºC.

Effect of cooling time and recovery of males

To determine the effect of chilling of male *Ae. aegypti* at the time of knockdown and recovery. One hundred virgin males from 2 to 4 days of age were placed in wooden cages (10 x 10 x 10 cm) with three repetitions. These males were placed in a refrigerator at 3ºC for 15, 30, 45 min, and 1, 24, 48, and 72 hr with a 1 min ramp to reach the indicated temperature inside the container. The recovery time of the flight activity of 50 and 100% of the males at environmental temperature (30°C) was recorded.

Effect of cooling temperature on copulatory success

The effect of low temperatures applied to *Ae. aegypti* males and its impact on the insemination of females was determined. For this experiment, 100 virgin males aged 2 to 4 days were used and placed in wooden cages covered with mesh (10 x 10 x 10 cm). The males were subjected to temperatures of 0, 2, 3, 4, 6, and 8ºC for 30 min, applying a ramp of 1 min, since the cage was introduced to the refrigerator, to reach the test temperature inside the cage.

Control adults were maintained at 25 ± 1ºC. Once the cold exposure time was over, the males were transferred to the aluminum cage (30 x 30 x 30 cm) together with 100 virgin females from 2 to 4 days of age, at 30ºC, waiting a few minutes for all the males to recover from the cold. The females do not get cold. From that moment, they remain in copulation, taking a random sample of 30 females from each treatment at times of 1, 24, and 48 hr for spermathecae analysis.[16]

Effect of cooling time on copulatory success

The effect of chilling time on copulatory success was determined in 100 virgin males from 3 to 5 days of age, subjected to different chilling times at 3°C. The males were placed in the refrigerator for 15, 30, 45 min and 1, 72 hr. Male mosquitoes were placed in 10x10x10 cm wooden cages covered with mosquito netting. When these mosquitoes were introduced to the refrigerator, they were given a 1 min ramp to reach the temperature indicated inside the cage. The control consisted of males subjected to the same manipulation but without applying cold. After 48 hr of coexistence, a random sample of 30 females were taken to extract the three spermathecae, recording the number of positives to sperm.[16] They were provided with cotton soaked in a 10% sucrose solution in all the treatments.

Effect of handling, cooling, packing, transport, UAV release, and recapture on copulatory success at greenhouse semi-field test

As a final test, 15 000 virgin male mosquitoes, 2 to 4 days old, were used to determine the time at each step and the effect of the cooling, packing, transporting, UAV release, and recapture process on sexual success. A Rio Florido Field Experiment's greenhouse was selected 3.5 x 25 x 10 m high, long, and wide, respectively), where a UAV release test under semifield conditions was carried out.

Results

Effect of knockdown temperature on male flight recovery time

When applying different cold temperatures, it was found that the temperature of 8ºC did not knock down the males; it only immobilized them, and when they were exposed to room temperature (30ºC), they began to fly immediately. For the rest of the test temperatures, when the knockdown temperature decreased, the recovery time of the males increased. From 6 to 0ºC, the 50% mean recovery time (± standard deviation, SD) ranged from 1'13" (± 0.23) to 2'68" (± 0.23), respectively. Regarding the 100% mean recovery time (± SD) presented a range of 2'88" (± 1.25) to 7'07" (± 0.79), respectively. By decreasing the temperature from 0ºC to -4ºC, the mean recovery time (±SD) increased significantly with ranges from 9'92" (± 5.29) to 10'35" (± 3.45), for 50% and 13'06" (± 5.04) and 12'77" (± 4.43) for 100%, respectively (table I).

Effect of cooling time on male recovery time

The temperature of 3 ± 1ºC was chosen as optimal for applying to males because it provides the recovery time required for handling in the laboratory and release in the field. However, it is important to notice that when the cooling time was longer, the recovery time was also longer. Specifically, in the first hour of cold application, after 1 hr, the recovery time remains constant. When applying 15' of chilling, the 100% mean recovery time (± standard deviation, SD) was 0.46" (± 0.04). When the cold time was increased to 30' and 45', the mean recovery time (± SD) was 4'24" (± 0.61) and 5'26" (± 1.28), respectively. Subsequently, when placing males for more extended periods of 1, 24, 48, and 72 hr, the mean recovery time (± SD) remained with slight variation between these treatments, with a time duration of 9'35" (± 0.05), 8'67" (± 0.55), 8'36" (± 0.05) and 9'05" (± 1.01), respectively (table II).

Table I

THE EFFECT OF TEMPERATURE ON THE KNOCKDOWN AND FLIGHT RECOVERY TIME OF MALE *AEDES AEGYPTI*, UNDER INSECTARY CONDITIONS. TAPACHULA, CHIAPAS, MEXICO

*Temperature (°C)**	*50% recovery time (min) (±SD)*	*100% recovery time (min) (±SD)*
8	No knockout	No knockout
6	1'13" (0.23)	2'88" (1.25)
4	2'04" (0.45)	3'10" (1.11)
2	2'34" (0.77)	5'14" (1.34)
0	2'68" (0.23)	7'07" (0.79)
-2	9'92" (5.29)	13'06" (5.04)
-4	10'35" (3.45)	12'77" (4.43)

* The males were placed under the temperature referred by 30 min.

Effect of different cooling times on copulatory success

In the present study, we detected that males were negatively affected in their sexual activity after cooling. At 1 hr of cohabiting at the 8ºC treatment, males copulating with females were observed; however, when analyzing the spermathecae, the insemination rate was 53.3%. As the temperature decreased, the percentage of inseminated females gradually decreased to 50, 43.3, and 30% at 6, 4, and 3ºC, respectively. When applying 2ºC to males, the temperature impact was more significant, with only 10% of females inseminated. At 0ºC, no inseminated females were observed. When analyzing the spermathecae of females cohabiting with males for 24 hr, a significant increase in the insemination rate above 70% was observed at temperatures from 2 to 8ºC; at 0ºC, 100% negative spermathecae remained. After 48 hr, all treatments presented 100% of females inseminated, including the temperature of 0ºC. In the control test, 100% of the females were inseminated after one hour of cohabiting males and females (table III).

Table II

Effect of different cooling times on the average recovery time of males of *Aedes aegypti* at 3ºC, in insectary conditions. Tapachula, Chiapas, Mexico

*Cooling time**	*50% recovery time min (±SD)*	*100% recovery time min (±SD)*
15'	0.26" (0.01)	0.46" (0.04)
30'	2'32" (0.01)	4'24" (0.61)
45'	2'52" (0.81)	5'26" (1.28)
1 hr	3'61" (0.45)	9'35" (0.05)
24 hr	3'46" (0.41)	8'67" (0.55)
48 hr	3'11" (0.02)	8'36" (0.05)
72 hr	3'40" (0.01)	9'05" (1.01)

*Three replicates of 100 males per cage.

Table III

The effect of cooling temperatures by 30 min on the copulatory success of *Aedes aegypti* males at three times of female copulation under insectary conditions. Tapachula, Chiapas, Mexico

Temperature (ºC)	*Positive spermathecae (%)* (n=30)*		
	1 hr	*24 hr*	*48 hr*
8	16 (53.3)	28 (93.3)	30 (100)
6	15 (50.0)	24 (80.0)	30 (100)
4	13 (43.3)	30 (100)	30 (100)
3	9 (30.0)	28 (93.3)	30 (100)
2	3 (10.0)	21 (70.0)	30 (100)
0	0 (0)	0 (0)	30 (100)
Control‡	30 (100)	-	-

* Includes females with at least one positive spermathecae.
‡ Unchilled males

Impact of cooling time on the sexual success of *Aedes aegypti* males

Under insectary conditions and according to the control where males without cold application were utilized, 90% of the females of *Ae. aegypti* have two inseminated spermathecae and a small percentage (10%), with three. In general, the application of cold hours did not affect this pattern, although there were variations in the number of positive spermathecae. The treatment with the highest number of females with three positive spermathecae was 45' cold, which produced positivity in females of 66.6% with two spermathecae and 33.4% with three. In the group of males that were kept for 72 hr at 3º±1 C, no adverse effects were found on their sexual activity since 96% of the females were inseminated with two spermathecae and 3.4% with three spermathecae. The males that were subjected to the entire process of cooling, handling, packaging, transport, introduction, and release with a drone and recapture under semi-field conditions presented 83.3% of females with two positive spermathecae and 16.6% with three, after cohabiting with virgin females for 48hr (table IV).

Table IV

Effect of different cooling times on the sexual success of males *Aedes aegypti* copulating with females for 48 hr, at 3 ± 1ºC, under insectary conditions. Tapachula, Chiapas, Mexico

Experimentation area	*Cooling time*	*Positive spermathecae (%) (n=30)*		
		1	*2*	*3*
Laboratory	Control*	0	27 (90)	3 (10)
	15'	0	27 (90)	3 (10)
	30'	0	23 (76.6)	7 (23.3)
	45'	0	20 (66.6)	10 (33.4)
	60'	0	25 (83.3)	5 (16.6)
	72 hr	0	29 (96.6)	1 (3.4)
Semi-field	30'‡	0	25 (83.3)	5 (16.6)

* Unchilled males.
‡ Males subjected to chilling, translated, drone release, and recapture process.

Timing of each step and impact of cooling, packing, transportation, UAV deposition, release, and recapture on male copulatory success

After the semifield test, the males subjected to the entire process showed normal sexual behavior activity, obtaining 100% of females inseminated with 83.3 and 16.6% of females with two and three positive spermathecae, respectively (table IV, figure 1). This process guarantees the male mosquito quality, improves releases without damage, and reaches successful copulation. Even if the transport of mosquitoes takes more time than the test in this study, our experiments show that mosquitoes can recover and copule after long cooling periods.

Discussion

The main objective of this work was to use new tools such as a UAV to release males of *Ae. aegypti* in endemic areas of arboviruses such as Zika, dengue, and chikungunya. The results are the basis for applying new tools using the sterile insect technique (SIT) or males infected by the endosymbiotic bacterium *Wolbachia* sp. through induced incompatibility.[17] In both cases, the goal is to prevent these arboviruses transmission by suppressing *Ae. aegypti* natural populations or by blocking transmission through *Wolbachia*.[18] These strategies require the release of a large quantity and excellent quality of insectary-reared male mosquitoes to reduce the native population quickly and efficiently. The males to be released must meet the requirements for using mosquitoes with favorable characteristics, especially in areas of difficult access.[19]

In this study, we initially applied different temperatures from 8°C to -4°C to determine the time when the males recovered from the knockdown. After this, different cooling times were applied from 15 min to 72 hr. When analyzing the cooling time and the impact on the activity of the males, although the cooling and the recovery time were longer during the first hour, from 1 to 72 hr, the recovery time did not change. This may be because the males entered a state of quiescence, with interruption or a decrease in metabolic activity to survive by using their energy reserves.[20] This shows the high capacity of *Ae. aegypti* to tolerate extreme cold and heat conditions. Based on the results and according to the distance between the CRISP and the UAV release area, it was decided to use a temperature of 3 ± 1°C for 30 min. This selected temperature and time were considered optimal for manipulating males in the laboratory and the field. The study recommended that temperature and cooling time is species-specific for a given transfer time under a tropical zone's temperature and relative humidity, such as Tapachula, Chiapas. Therefore, these

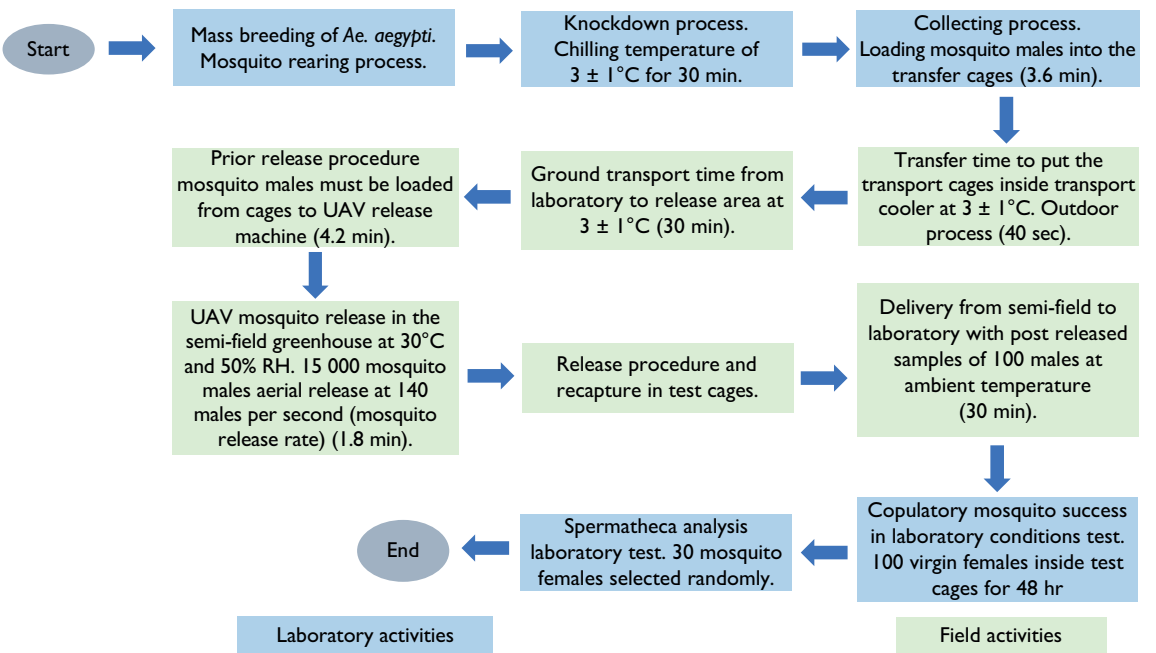

FIGURE 1. SUMMARY DIAGRAM PROCEDURE TO DETERMINE THE EFFECT OF HANDLING, COOLING, PACKAGING, TRANSPORT AND RELEASE OF THE MALES USING UNMANNED AERIAL VEHICLE, TO EVALUATE THE COPULATORY SUCCESS IN THE LABORATORY, IN TAPACHULA, CHIAPAS, MEXICO. JANUARY TO DECEMBER 2018

results should not be considered a rule but a guide to applying in other geographical areas with *Ae. aegypti* or other *Culicidae* species.[21,22]

An important finding of the present study was that cold temperature had an adverse effect on the sexual ability of males. However, it only delays the total insemination of the spermathecae because this negative effect was temporary, as it was shown by finding that all spermathecae from females between 24 to 48 hr were inseminated. When analyzing different temperatures and the insemination rate, it was found that even at the temperature of 8ºC, which did not knock down the males, it reduced the sexual efficiency by 53.3%. This can be explained because insects are poikilothermic, which means that their body temperature depends on the temperature of the surrounding environment.[23] The cold temperature probably affected the introduction of the aedeagus into the vagina for sperm deposition because male and female copulation activity was observed, but there was no insemination. This should be considered in a release model of sterile males or with *Wolbachia* sp., where males require at least 24 hr to recover, and once this period has passed, their sexual behavior will be normal, even after 72 hr of cooling (table IV). Another study with *An. arabiensis* showed that cold induces stress in males, affecting sexual activity.[23] In other species of *Anopheles* sp., it was observed that stress due to changes in environmental temperature does not always cause negative effects on sexual activity, but instead activates mechanisms that induce copulation; it is the same case as *An. pseudopunctipennis* and *An. darlingi*, where high temperatures during the day (30 ± 1°C) followed by low temperatures at dusk (25 ± 1°C) caused stress followed by comfort, which activated the copulation system in both sexes.[15,24] Although these reports did not use extreme temperatures to knock down males, the principle is the same: stress followed by comfort induces copulation.[16] Some insects under different stress conditions induce the synthesis of heat shock proteins (HSP), which contribute to the survival under extreme conditions of heat, cold, starvation, and anoxia. In the present work, males with the cold application probably survived via HSP synthesis.[25,26]

Conclusions

The use of unmanned aerial vehicles to release *Ae. aegypti* male mosquito is a novel strategy that can be applied for mosquito control programs as the use of males infected with *Wolbachia*, sterile insect techniques (SIT) or both, to control this important vector of Zika, dengue, and chikungunya virus.

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