EID MÉDITERRANÉE
OPÉRATEUR PUBLIC EN ZONES HUMIDES
Démoustication

STUDY REPORT

Study on the effectiveness of the Zikaseal® device against the proliferation of *Aedes albopictus* in storm drains

December 2024

Direction technique (DT)

Evaluation of the effectiveness of the Zikaseal® in reducing the risk of tiger mosquito (*Aedes albopictus*) egg-laying and proliferation in storm drains

Summary

1. GENERAL INFORMATION

1.1 Généralités

Demandeur	Université de Copenhague, Danemark
Installations d'expérimentation	EID Méditerranée - Direction technique Pôle Laboratoire et expérimentation Pôle lutte préventive moustique-tigre et santé publique 165, avenue Paul Rimbaud F-34184 Montpellier Cedex 04 - France

1.2 Personnel de l'expérimentation

Coordination	N. LE DOEUFF-LE ROY, F. JEAN
Rédaction	N. LE DOEUFF-LE ROY
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1.3 Calendrier de l'expérimentation

Début de l'expérimentation	05/09/2024
Date de remise du rapport final	10/02/2025

1.4 Distribution et archivage

Distribution des rapports Demandeur : 1 ; EID Méditerranée : 1

Archivage : L'EID Méditerranée archivera les données suivantes pendant au moins dix ans : protocole d'essai, rapports d'essai et final, et données brutes. Toutes les données sur support informatique produites sont conservées indéfiniment.

1.5 Certification

L'EID Méditerranée bénéficie de la triple certification QSE (Qualité Sécurité et Environnement) selon les normes ISO 9001, ISO 14001 et l'OHSAS 18001. Les tests en laboratoire et les essais sur le terrain sont conduits en respectant les exigences de ces normes. Les appareils et matériels de mesurage (pesée, volumétrie, thermo-hygrométrie) sont soumis à une vérification métrologique régulière.

2. OBJECTIFS DE L'ÉTUDE

The *Aedes albopictus* mosquito (Skuse, 1894) has been present in mainland France since 2004. Since then, it has been expanding its range every year. In 2024, the species was considered to be established in 78 departments of mainland France (Source: Santé publique France).

Because of the nuisance it causes and its ability to transmit arboviruses such as dengue fever, chikungunya and Zika to humans, the pest control of this species, which is based in particular on the elimination of breeding sites, represents a public health issue.

Depending on their physical characteristics and the context, storm drains in the public domain can be highly productive of adult mosquitoes (mostly *Aedes* and *Culex genus*). Management of these drains by the organisations responsible requires regular larvicide treatments that are costly in terms of manpower. Researching, finding and testing suitable, operational and sustainable autonomous systems that would limit larval production in these breeding grounds at lower cost is an important added value in the fight against mosquitoes on a regional scale.

Against this backdrop, the Department of Public Health at the University of Copenhagen asked EID Méditerranée to carry out a study to assess the effectiveness of an adaptable system to prevent mosquitoes, including the tiger mosquito *Aedes albopictus*, from laying eggs and proliferating in storm drains.

The aim of this open-field study on the assessment of the risk of proliferation with the Zikaseal® is to provide an entomological expert opinion on the capacity of this innovative device to eliminate access to residual stagnant water in certain storm drains, which is responsible for the proliferation of urban mosquitoes on the public domain. This is the purpose of this report.

The Zikaseal® prototype is intended to provide a low-cost, sustainable operational solution to the risk of larval production in storm drains by preventing mosquitoes from gaining access to them. The overall aim of this study is to evaluate the potential effectiveness of such a device in the field under experimental conditions, using a device simulating a larval breeding ground of the same size.

This study aims to respond to these two hypotheses:

1. The use of the Zikaseal® system, under experimental conditions, prevents any adult mosquitoes from emerging in the lower part of the device
2. Using the Zikaseal® system under experimental conditions prevents mosquitoes from gaining access to the stagnant water in the lower section.

3. MATERIELS ET MÉTHODES

3.1 Biological material

The mosquitoes used come from the population of Aedes albopictus (Diptera; Culicidae) reared in the insectarium of the EID Méditerranée laboratory since 2021. Their rearing conditions are 27 +/- 1 °C and RH % 70 +/-10 % with a photoperiod of 16 h day / 8 h night.

Under these conditions, the eggs are laid on blotting paper and then hatched in a separate tank. The larvae are then reared to reach a sufficient level of development corresponding to the L3 to L4 larval stage.

The larvae are then reared to the adult stage. The adult mosquitoes are then collected using a mouth aspirator, trying to collect mainly female mosquitoes. The tests were carried out with adult females fed with a 10% honey solution. The blood meal required by the female mosquitoes for ovogenesis was provided on Hemotek®, using capsules containing sheep blood. The blood-fed females are kept for 3 days to encourage oviposition retention. After this period, between 25 and 30 mosquitoes are introduced into each aquarium.

3.2 Trial n°1: Protocol

3.2.1 Set-up design

The devices are made of a BG-GAT® trap bucket (Biogents®) for the lower part, to which a wooden board with a 20 cm diameter opening is attached, thus ensuring separation from the upper part. The upper part consists of a transparent chamber (upper part of the BG-GAT®), covered with mosquito netting so that no mosquitoes can enter or leave the experimental device. The zikaseal system is positioned between the lower and upper parts, at the opening on the board.

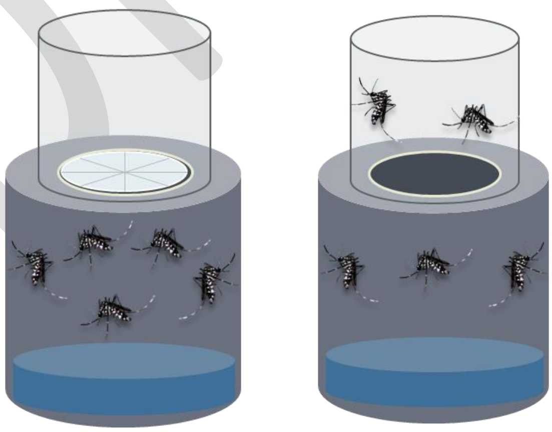

Figure 1: Diagram of experimental set-up for trial 1

The sticky cards are placed in the upper part, above the Zikaseal system for set-up 1. These sticky cards are suspended vertically to capture mosquitoes in flight.

To ensure that no mosquitoes can escape, the overflows of the traps are sealed with gaffer tape. The mosquito nets are held in place by elastic bands.

Figure 2: Photograph of the experimental set-up before reading the results

The experiment was carried out on 4 set-ups, divided into 2 modalities, 3 equipped with the Zikaseal® system and 1 considered as a control to check the mosquito rate survival inside the set-ups.

3.2.2 Study design

This experiment was deployed on the EID-Méditerranée site to facilitate the transport of mosquitoes and ensure regular monitoring of the devices, in particular to track the survival of individuals placed inside the devices. All the devices and the mosquitoes placed inside them are then subjected to the same conditions (weather, temperature, humidity). To monitor the experimental conditions, a Testo® data logger was placed in one of the devices. This tool enables us to collect daily temperature variations and air humidity levels throughout the day and for the duration of the experiment.

Following the failure of the initial protocol (see 3.3.4 Problems encountered; Annex n°1), it was decided to replace the larvae with adult mosquitoes. This test was carried out on the basis of 3 replicas equipped with Zikaseal '**Zik1.1; Zik1.2; Zik1.3**' and a single control '**C1.1**'. The purpose of this control was to monitor the survival of the mosquitoes throughout the experiment.

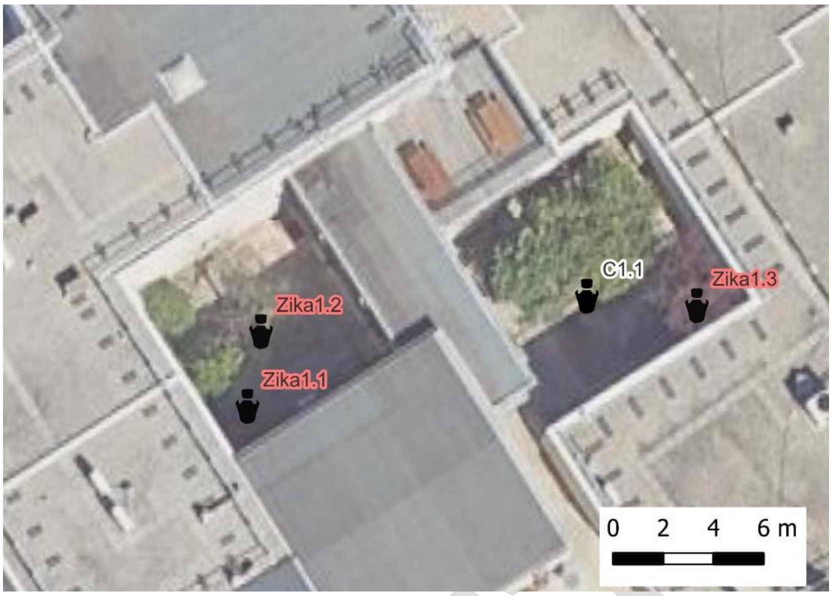

Figure 3: Location of outdoor set-ups on the EID-Méditerranée site

Adult mosquitoes (from EID-méditerranée insectariums) were introduced directly into the devices using a mouth aspirator. In the devices with Zikaseal®, the mosquitoes were placed in the lower part (Figure 1).

3.2.3 Data collection and analysis

After 15 days, the sticky cards in the upper sections are collected, making sure that no flying mosquitoes are still present. The card is placed in a plastic bag, along with the date of collection and the device number.

In Set-up 1 equipped with Zikaseal®, the remaining mosquitoes that had not managed to pass through the Zikaseal® were recovered and counted. As the devices were subject to weather conditions and rain, individuals that had died during the experiment were sometimes recovered in poor condition. The devices were therefore meticulously inspected in order to recover a number of post-experiment individuals (N at Day +15) as close as possible to the number of mosquitoes placed at the start of the test (N at Day +0), in order to ensure that the devices were impermeable. These results give us the rate of mosquitoes able to pass through the Zikaseal®.

3.2.3 Problems encountered.

Experiment 1 was to be carried out with L3-L4 stage larvae in the Zikaseal® and Control set-ups. However, during experiment 1 (set-up 1 and 2), we observed very high larval and pupal mortality and very few individuals were therefore able to reach the adult stage, despite the use of a strain from a 'local' population, dechlorinated water and clean containers. The main hypothesis is that this failure seems to be linked to external conditions. The weather and climate in September were relatively cool and damp for the end of summer. The data logger placed during the trial shows temperatures that vary greatly during the day, with temperatures dropping during the nights. Over the period of the test, 76% of the data recorded was below 20°C (air temperature), with an average of 18.4°C ± 2.6. As the water takes longer to warm up, it is highly likely that the larvae lived in water that was always below 20°C. However, below 20°C, the survival rate of the larvae drops, with higher mortality for the pupal stage (60% mortality at 20°C at constant temperature) (Rozilawati et al. 2016).

The mortality observed, and therefore the failure of this experiment, can probably be explained by the cool temperatures (and daily fluctuations, added to the fact that these laboratory larvae come from successive generations accustomed to optimal conditions (T° = 27°C).

3.3 Trial n°2: Protocol

3.3.1 Set-up design

The conception of the set-ups is the same as for trial n°1, with the exception that there is no mosquito net on the top of the device. The aim of this device is to show that mosquitoes coming from outside cannot gain access to the lower part, which is protected by Zikaseal® technology. Here too, the overflows will be covered with adhesive (gaffer tape) so that no mosquitoes can get through and skew the results of this test.

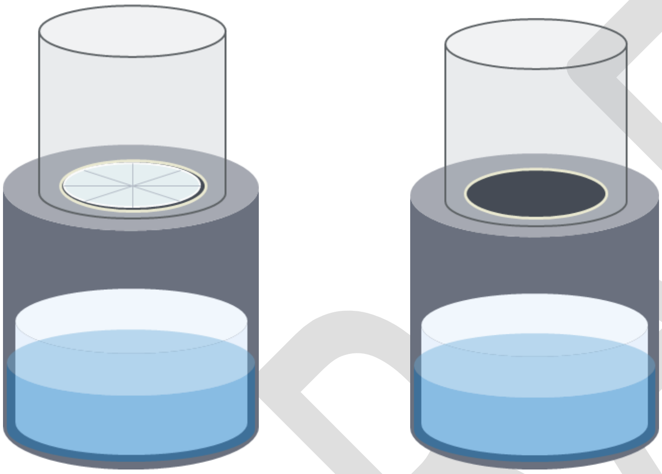

Figure 5a: Diagram of experimental set-up for trial 2

Figure 5b: Photograph of the egg collection tray in the experimental set-ups

The bucket serving as the base of the set-up will be filled with water, to imitate a stagnant water point such as a storm drain. A strip of egg-laying paper is laid out in a circle in the device, taking care to immerse half the egg-laying paper and press it against the sides.

3.3.2 Study design

The experiment is being conducted on 6 systems divided into 2 modalities with 3 Controls « **C2.1 ; C2.2. C2.3** » and 3 with the Zikaseal® « **Zik2.1 ; Zik2.2 ; Zik2.3** »

Ces 6 dispositifs sont disposés à différents endroits par pairs, chacune composée d'un réplica Zikaseal® et d'un Témoin. Pour cette expérimentation, 2 paires ont été placé dans des jardins privatifs (sur ls vile de St-Clément-de-Rivière et Clapiers) et la troisième est situé sur le site de l'EID-Méditerranée. Dans chacun des lieux sélectionnés, la présence d'*Aedes albopictus* est avérée. Afin de limiter la concurrence entre les dispositifs, une distance d'une dizaine de mètre au minimum est respectée entre les deux modalités d'un même pair.

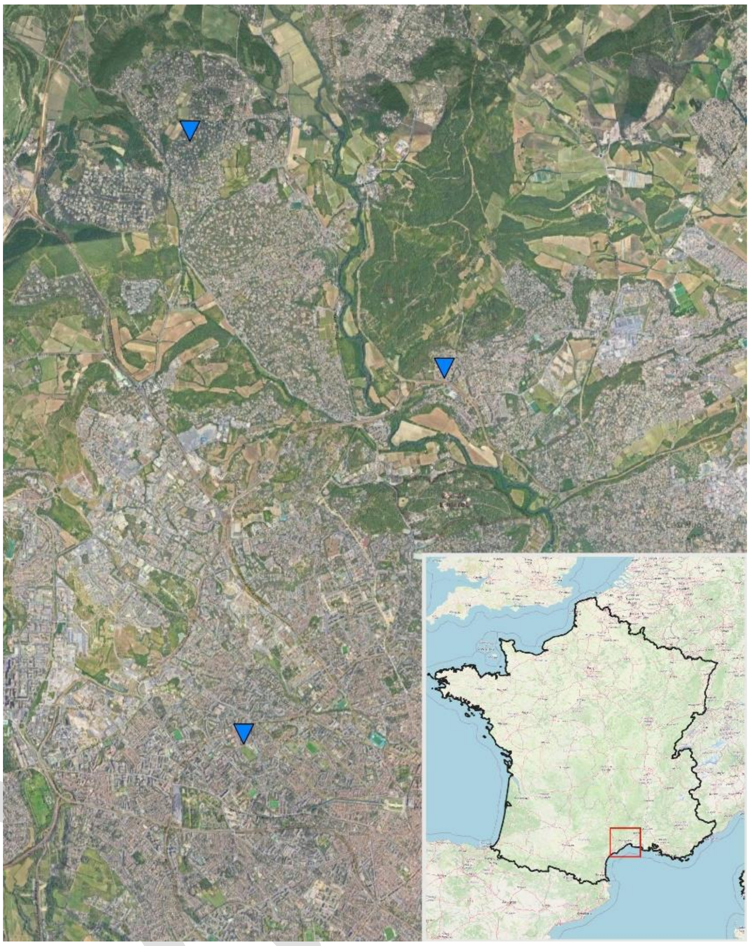

Figure 6: Localisation experimental set-up for trial 2

The set-ups fitted with Zikaseal® were always installed in what we considered to be the most favourable locations, i.e. in the vegetation and away from direct sunlight.

3.3.3 Data collection and analysis

Every week, the devices placed on the EID-Méditerranée site are inspected. Egg-laying papers are recorded after 2 to 3 weeks in the field, to ensure that a sufficient number of eggs have been laid.

Date de Pose	Collecte n°1	Collecte n°2
5 Septembre	27 Septembre	28 Octobre

The egg-laying papers are collected and placed individually in a plastic bag marked with the date, place of collection and replica number. They are then dried separately in the EID-Méditerranée laboratory.

Then, the eggs are identified and counted under a binocular magnifying glass

4. RESULTS

4.1 Results from trial n°1

modality	Day 0	Day end	adult inoculated	Mosquitoes collected on sticky card	Mosquitoes under Zikaseal®		Missing mosquitoes
					Dead	Alive	
Z1.1	30/09/2024	10/10/2024	25-30	1	15	8	[3-7]
Z1.2	30/09/2024	10/10/2024	25-30	0	24	1	[0-5]
Z1.3	30/09/2024	10/10/2024	25-30	0	20	5	[0-5]

On all the set-ups, at least 80% of the mosquitoes were found [81%-97%], whether they were trapped on the sticky card (in the upper part) or were recovered in the lower part, under the Zikaseal®. On each device, some individuals were still alive and had not managed to pass through the Zikaseal®, even 10 days after the beginning of the experiment. However, the majority of the mosquitoes placed [60% to 95%] were found dead.

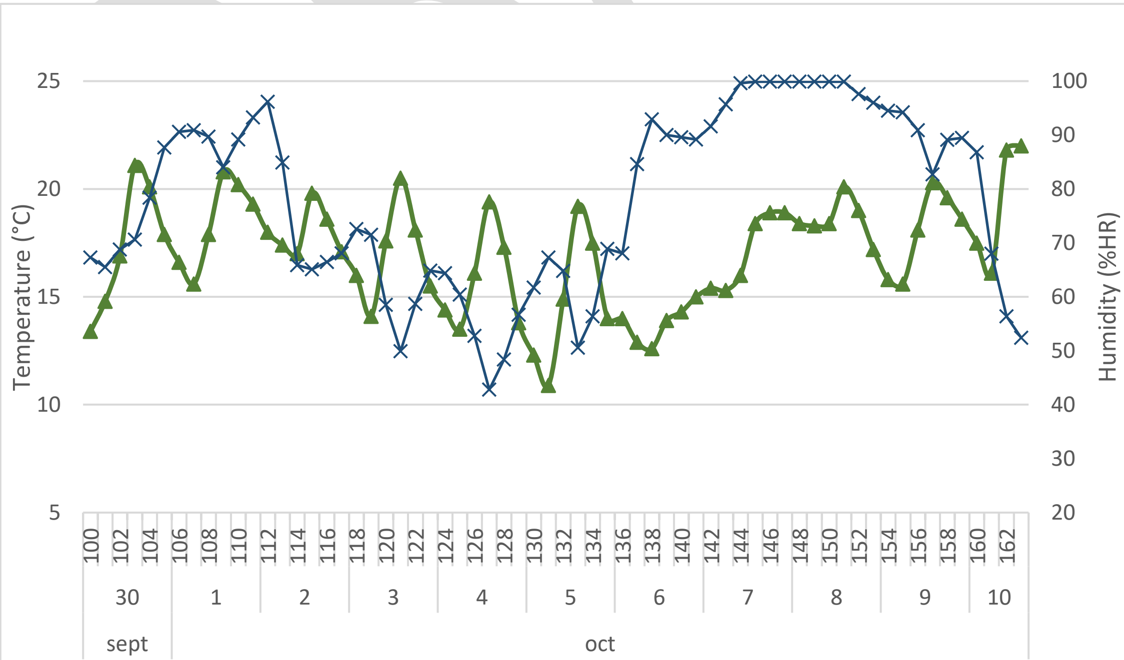

Figure 7: Temperature and humidity in the air during the experimentation (trial n°1)

4.2 Result from trial n°2

The data collected were implemented in R software (version 4.4.1) to obtain these graphical results and statistical analyses, and are presented in Figure 8. The results are very clear, since after 2 months of installation in the field, no eggs were laid on the oviposition supports collected in the 'ZIKASEAL' devices. Furthermore, no larvae were observed in the water at the bottom of the device. Conversely, in the 'CONTROL' devices, several hundred eggs were counted on the oviposition papers (Figure 7), which proves that the tiger mosquito was still active at the time of this test.

In this test, the devices simulating rainwater drains protected by Zikaseal® were never colonised by *Aedes albopictus*, nor by any other species. The Kruskal-Wallis test on the data collected confirmed the results observed.

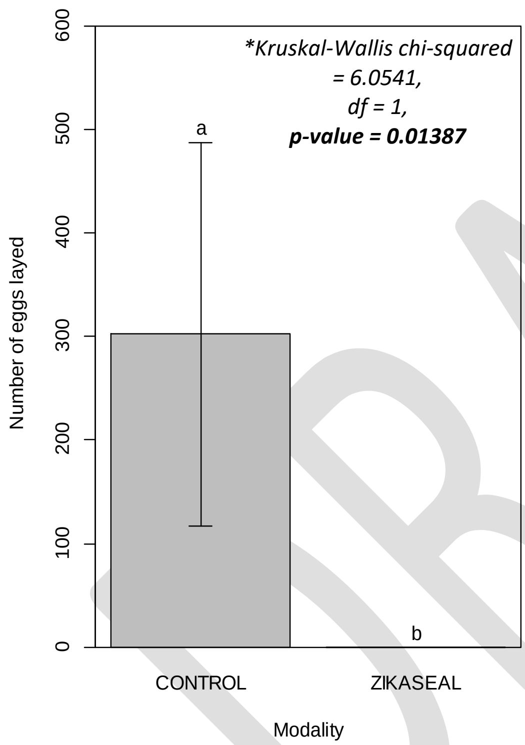

Figure 9: Graph of the number of eggs laid on "CONTROL" and "ZIKASEAL" set-up (Collect n°1, 27/09/2024)

Figure 9: Aedes albopictus eggs collected on the CONTROL C2.2

5. DISCUSSION

The results of experiment 1 show that mosquitoes trapped under the zikaseal® cannot pass through the prototype and reach the upper part of the experimental device. Only one mosquito was found on one of the sticky cards of the device. The fact that this was the only mosquito present in the upper part (of the entire test) raises the question of its ability to access the upper part. In fact, the spaces in the zikaseal device are all smaller than 1.0 mm (insert maximum size observed). The most likely hypothesis is that this mosquito got into the upper part when checking the devices during the experiment. In any case, even if a mosquito did manage to get through the Zikaseal®, this small proportion, between 1% and 2%, does not call into question the barrier effect provided by this device.

Experiment No. 2 confirms the results of Experiment No. 1. After several weeks in the field, the devices fitted with Zikaseal® were never colonised by the *Aedes albopictus* species, since no eggs or larvae were observed when the test was read (Figure 8), confirming the effectiveness of the device. In addition, as the system acts as a physical barrier, it is possible that its installation limits the diffusion of chemical signals used by mosquitoes in search of breeding sites.

These two complementary experiments show that Zikaseal®, as designed, prevents mosquitoes from gaining access to the water (lower part) or emerging adults from leaving the larval nest.

The contribution of storm drains to total mosquito production is difficult to estimate and can vary greatly depending on the type of drains, the climate and the topology of the area. However, they may be responsible for a large proportion of local mosquito production (Arana-Guardia et al. 2014). In a study carried out in north-east Italy, Carrieri et al. showed that rainwater drains are in some places the most uniformly distributed larval habitat and can therefore have a significant effect on mosquito abundance (Carrieri et al. 2011). Also, according to data collected via the *Mosquito alert* project (CREAF, CEAB-CSIC, et ICREA 2017), 42% of the 14,214 drains inventoried contain water, highlighting the quantity and share represented by these breeding grounds linked to rainwater networks. In addition, these breeding grounds are permanent, meaning that they are likely to produce mosquitoes continuously (during the period of activity of the mosquito species). The productivity of these breeding sites shows the importance of integrating the management of storm drains into strategies for controlling mosquito vectors and, more generally, for vector control. (Paploski et al. 2016 ; Ocampo et al. 2014).

In most studies on the management of stormwater networks, the control methods and strategies used are based on regular insecticide treatments. (Anderson et al. 2011; Flacio et al. 2015). A method consisting of filling in the part retaining water has recently been evaluated in Barcelona on around forty storm drains, the configuration of which has been modified so that there is no longer any water retention (Treskova et al. 2024). However, this technique cannot be used everywhere, depending on the configuration of the gullies, the cost of intervention and local regulations. Apart from this, there are few innovative, long-term alternatives for controlling the proliferation of mosquitoes in storm drains. Zikaseal® technology can be an additional tool in the fight against urban breeding sites. This system can be used in urban environments as part of an integrated control strategy. The system does not require any modification to the structure of the gullies, and therefore does not change the way they operate. What's more, its design, based on a 3D scan of the drains, will make it easier to install by adapting to the various profiles of storm drains.

This study shows the effectiveness of the Zikaseal® system if it were installed on a storm drain. Considering that an equipped downspout would have a low or zero productivity (according to the results obtained in the 2 trials), the installation of Zikaseal® on a downspout network would make it possible to eliminate a large quantity of larvae breeding sites. However, this study is subject to several limitations. Firstly, our results do not consider the system's ability to remain 'watertight' by ensuring that the device closes properly. And secondly, they do not allow us to determine whether or not the installation of the system disturbs the drain's absorption capacity, which could pose a problem in the event of heavy rain and possibly increase the risk of flooding. Even so, this permanent solution can reduce the operational costs associated with larvicide treatments over the long term. Given the diversity of storm drains shapes and sizes, sometimes even within the same town, the adaptability of

Zikaseal® to different storm drain configurations will be an important criterion for this solution to be adopted by municipalities.

6. CONCLUSION

The aim of the study was to assess the risk of *Aedes albopictus* laying eggs and proliferating in facilities fitted with Zikaseal® devices. The results show that the Zikaseal® system considerably reduces the risk of proliferation of a drain, by acting as a physical barrier preventing mosquitoes from gaining access to the water. This study could be supplemented by field observations of Zikaseal® devices installed on rainwater drains known to be mosquito-producing.

However, it is important to underline that the effectiveness of the device in a real-life situation depends on its ability to remain in a completely closed position outside periods of heavy rain and to retain its properties over time. The devices must not have any manufacturing defects (holes, malfunctions) that could impair the effectiveness of Zikaseal®. In addition, the installation of Zikaseal® on the inlets of gullies must be rigorously carried out to ensure that there are no gaps that would allow mosquitoes to pass through. The structure on which Zikaseal® is installed must also prevent any mosquitoes from getting through.

As the device is mobile and can open and close according to the pressure exerted on it, we recommend a visual check every season, or at least after a heavy rainfall episode. The aim is to ensure that the Zikaseal® is still in place and that no objects or waste are interfering with its effectiveness

It should be noted that the study was carried out on the *Aedes albopictus* species. The results and conclusions of this study on the risk of proliferation are therefore valid for this species. Considering that the other urban species present in Europe, in particular *Culex pipiens*, are generally larger than the tiger mosquito, we can conclude that the zikaseal system will be effective against other species that may be found in storm drains.

BIBLIOGRAPHIE

Anderson, John F., Francis J. Ferrandino, Douglas W. Dingman, Andrew J. Main, Theodore G. Andreadis, et James J. Becnel. 2011. « Control of Mosquitoes in Catch Basins in Connecticut With Bacillus thuringiensis israelensis, Bacillus sphaericus, and Spinosad ». *Journal of the American Mosquito Control Association* 27 (1): 45‑55. https://doi.org/10.2987/10-6079.1.

Arana-Guardia, Roger, Carlos M. Baak-Baak, María Alba Loroño-Pino, Carlos Machain-Williams, Barry J. Beaty, Lars Eisen, et Julián E. García-Rejón. 2014. « Stormwater drains and catch basins as sources for production of *Aedes aegypti* and *Culex quinquefasciatus* ». *Acta Tropica* 134 (juin):33‑42. https://doi.org/10.1016/j.actatropica.2014.01.011.

Carrieri, M., P. Angelini, C. Venturelli, B. Maccagnani, et R. Bellini. 2011. « *Aedes albopictus* (Diptera: Culicidae) Population Size Survey in the 2007 Chikungunya Outbreak Area in Italy. I. Characterization of Breeding Sites and Evaluation of Sampling Methodologies ». *Journal of Medical Entomology* 48 (6): 1214‑25. https://doi.org/10.1603/ME10230.

CREAF, CEAB-CSIC, et ICREA. 2017. « Mosquito Alert ». https://www.mosquitoalert.com/.

Flacio, Eleonora, Lukas Engeler, Mauro Tonolla, Peter Lüthy, et Nicola Patocchi. 2015. « Strategies of a Thirteen Year Surveillance Programme on *Aedes albopictus* (Stegomyia Albopicta) in Southern Switzerland ». *Parasites & Vectors* 8 (1): 208. https://doi.org/10.1186/s13071-015-0793-6.

Rozilawati, Harun, Saranum Mohd Masri, K. Tanaselvi, Zairi Jaal, Nazni Wasi Ahmad, et Lee Hanlim. 2016. « Effect of temperature on the immature development of *Aedes albopictus* skuse ». *Southeast Asian Journal of Tropical Medicine and Public Health* 47 (janvier):731‑46.

Treskova, Marina, Tomás Montalvo, Joacim Rocklöv, Charles Hatfield, Frederic Bartumeus, Shouro Dasgupta, João Encarnação, et al. 2024. « The effectiveness of mosquito-proof storm drains on adult mosquito and mosquito larvae abundance: protocol of the IDAlErt storm drAin randomiSed controlled trial (IDEAS) in Barcelona, Spain ». Research Square. https://doi.org/10.21203/rs.3.rs-3870309/v1.

Annex

Annex 1: Tableau de données brutes

insert tab data

Annex 2 : Protocole initial de l'Expérimentation 1 :

This trial thus comprises 2 modalities of 3 replicas each: set-up 1, equipped with Zikaseal and set-up 2, being the controls (thus without the Zikaseal® device) which will be named respectively Zikaseal with ' Zik1 ; Zik2 ; Zik3 " and Control " C1 ; C2 ; C3 '.

Once the device is assembled (t0), 50 larvae (L3 - L4 stage) are added to the stagnant water tank (5cm of water rested for 24H) of each replica with larval food as soon as the test is launched (20 adapted larval food granules). Food will be added as required to maintain good larval development, limit cannibalism and promote survival.