

DAVID Y. IGE  
Governor

JOSH GREEN  
Lt. Governor



PHYLLIS SHIMABUKURO-GEISER  
Chairperson, Board of Agriculture

**DEPT. COMM. NO. 217**  
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December 26, 2019

The Honorable Ronald D. Kouchi,  
President and Member of the Senate  
Thirtieth State Legislature  
State Capitol, Room 409  
Honolulu, HI 96813

The Honorable Scott K. Saiki,  
Speaker and Member of the House of  
Representatives  
Thirtieth State Legislature  
State Capitol, Room 431  
Honolulu, HI 96813

Dear President Kouchi, Speaker Saiki, and Members of the Legislature:

For your information and consideration, I am transmitting a copy of the Report on the Importation and Use of *Aedes Aegypti* with *Wolbachia* Bacteria for Landscape Scale Control of Mosquitos in a Vector Control Program as required by Act 106, SLH 2019. In accordance with Section 93-16, Hawaii Revised Statutes, I am also informing you that the report may be viewed electronically at <http://hdoa.hawaii.gov/>.

Sincerely,

A handwritten signature in cursive script that reads "Phyllis Shimabukuro-Geiser".

Phyllis Shimabukuro-Geiser, Chairperson  
Board of Agriculture

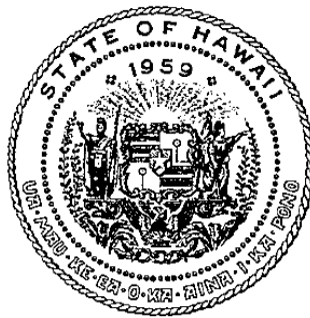
Enclosures



**REPORT TO THE THIRTIETH LEGISLATURE  
2020 REGULAR SESSION  
STATE OF HAWAII**

**REPORT ON THE IMPORTATION AND USE OF AEDES AEGYPTI WITH WOLBACHIA BACTERIA  
FOR LANDSCAPE SCALE CONTROL OF MOSQUITOS IN A VECTOR CONTROL PROGRAM**

**ACT 106, SLH 2019**



**PREPARED BY:**

**HAWAII DEPARTMENT OF AGRICULTURE  
HAWAII DEPARTMENT OF HEALTH  
HAWAII DEPARTMENT OF LAND AND NATURAL RESOURCES**

**DECEMBER 2019**

## Background

Act 106, SLH 2019 (House Bill 297 HD1 SD1) requested a report from the Hawaii Department of Agriculture (DOA), Hawaii Department of Health (DOH) and Hawaii Department of Land and Natural Resources (DLNR) on the importation and use of the *Aedes aegypti* mosquito with *Wolbachia* bacteria for use in a vector control program to control mosquitos on a landscape scale to the Legislature no later than twenty (20) days prior to the convening of the Regular Session of 2020. Importation of *A. aegypti* falls under DOA rules and regulations, and DLNR and DOH would be the primary agencies undertaking the project from a mosquito control standpoint. HB 297 listed ten (4) items of interest, which are shown below along with a response for each.

## Responses

- (1) Findings and recommendations regarding the importation and release of the *Aedes aegypti* mosquito with *Wolbachia* bacteria, including *Aedes aegypti* mosquitoes originating from Hawaii stock that could be imported for landscape scale mosquito control:

In addition to the list placement process listed below in (2), there also exists a mechanism to import an unlisted organism without list placement. Unlisted organisms are considered to be prohibited until list placement after Board review. However, importation of an unlisted organism can occur by special permit on a case-by-case basis for research that has been determined to not be detrimental to the environment, subject to Board review. This process does shorten the time requirements to do the initial research, however, in order to release the animals into the environment, rulemaking is required for list placement and new conditions for release would need to be established by the Board.

- (2) Determination for the placement of the *Aedes aegypti* mosquito with *Wolbachia* bacteria on the appropriate animal import list pursuant to section 150A-6.2, Hawaii Revised Statutes, and the status of the import list placement process:

The Hawaii Board of Agriculture (Board) has not yet conducted a formal review of *Aedes aegypti* mosquitos with *Wolbachia* bacteria. Procedurally, the Department does not place organisms on lists without an intent to initiate importation as it could be viewed as potentially providing preferential treatment for an applicant that may not want to face public scrutiny through the rulemaking process. Additionally, placement is normally requested from a non-departmental entity. However, pursuant to Hawaii Administrative Rules 4-71-6.5(b), which defines permitted introductions of non-domestic animals into the State, covers *A. aegypti* list placement based on the organism and proposed use. By definition, there is only

one list that would be appropriate to place *A. aegypti*, the List of Restricted Animals (Part A).

Currently, the Department maintains four lists of non-domestic animals: List of Conditionally Approved Animals (CA List); List of Restricted Animals, Part B (RB List); List of Restricted Animals, Part A (RA List), and List of Prohibited Animals (Prohibited List). These lists are generally structured by placing animals and their likely uses against their potential risk to agriculture, the environment, or public health and safety, with animals on the CA List generally being the lowest risk and animals on the Prohibited List being so potentially devastating that no importation can be allowed.

Placement of an animal on the CA list would allow for individual possession, businesses, or institutions. Essentially anyone in the State can import these animals. Common examples would be live seafood for consumption or guinea pigs for pets. Placement on the RB List disallows individual possession but allows for commercial uses such as aquaculture. Common examples would be various tilapia species for aquaculture production. Placement on the RA List is more restrictive by allowing importation only for research by universities or government agencies or exhibition in a municipal zoo or aquarium. Common examples would be Tigers at the Honolulu Zoo or biocontrol agents for potential release. Placement on the Prohibited List would prevent any person from importing any of these animals. A common example is Piranha.

Based on these criteria, placement on the CA List or RB list would allow *A. aegypti* mosquitos with *Wolbachia* bacteria as a pet or to be used for commercial purposes. Placement on the Prohibited List would preclude any possibility of research in the State. Knowing the risk that any mosquito species can pose to human/animal health, placement on the RA List would limit the research to only Government agencies or universities, subject to Board approved permit conditions, which would likely be extremely restrictive. Placement on any other list would be inappropriate for the requested need to conduct research of the possibility of using *A. aegypti* mosquitos with *Wolbachia* bacteria.

Once a request/information is received from DOH, the review process can begin.

- (3) Recommendations, plans, timeline, and implementation costs for a mosquito control program using the *Aedes aegypti* mosquito with *Wolbachia* bacteria, if permitted, and, if not permitted, recommendations for other alternative vector control means:

## **Recommendations:**

Overall, the DOA, DOH and the DLNR support further research and work towards the implementation of mosquito control programs utilizing *Wolbachia* infected mosquitoes. The following sections provide additional context related to the risk of mosquito-borne disease introductions to Hawai'i, the current status of *A. aegypti* establishment within Hawai'i, the impacts mosquitoes are having on our native bird populations, and on *Wolbachia* infected mosquitoes and their potential use in a vector control program.

## **Mosquito-Borne Diseases and *Aedes aegypti***

The introduction and transmission of mosquito-borne diseases poses a significant health threat to Hawai'i. Individuals originating from countries or regions where these mosquito-borne diseases are endemic may import these pathogens into the state. Among Hawai'i residents, the DOH's Disease Investigations Branch has identified 26 confirmed imported cases of Zika virus infection, 14 confirmed imported cases of chikungunya virus infection, and 61 confirmed imported cases of dengue virus infection since 2015. These imported cases are in addition to the 238 confirmed locally acquired cases of dengue in Hawai'i residents that occurred during the 2015-2016 Hawai'i Island outbreak. These cases of mosquito-borne disease have the potential to transmit their infections to others within Hawai'i through the bite of mosquito species that are established within the state.

One mosquito species that can transmit these pathogens is *Aedes aegypti*, the yellow fever mosquito. This species is a non-native, invasive species that was introduced to Hawai'i within the past 200 years. The presence of this invasive mosquito species poses a continued threat to residents and visitors in Hawai'i as it can transmit mosquito-borne diseases of concern, it has a strong preference for feeding upon humans, and its preferred habitat is within or near human dwellings. Within Hawai'i, the current known distribution of the *A. aegypti* mosquitoes is limited to Hawai'i Island, though individual *A. aegypti* mosquitoes have been intercepted at airports in Honolulu and on Maui. Control of these mosquitoes is directed by the DOH's Vector Control Branch. Control activities include the application of chemical adulticides and larvicides, the reduction of mosquito habitats, and the distribution of educational materials to the public related to how they can control mosquitoes and prevent mosquito bites.

In addition to their potential impacts on human health, mosquitoes within Hawai'i also significantly impact the health of our animals. The Hawaiian honeycreepers represent a spectacular biological radiation of birds, yet, they are among the most highly imperiled group of birds globally. Introduced diseases transmitted by non-native mosquitoes are responsible for much of the decline in Hawai'i's forest birds. Avian pox was introduced to the Hawaiian Islands in the late 1800s, and avian malaria was introduced in the early 1900s. With no prior exposure or natural immunity, native forest birds are highly susceptible to

these non-native pathogens. Avian malaria, which is transmitted by the southern house mosquito (*Culex quinquefasciatus*), is the most significant mortality factor for the majority of Hawaiian forest birds. Over the past 30 years, nine more species of forest birds have gone missing and are most likely extinct. The remaining native birds live at high elevations where there is still some healthy forest habitat with few mosquitoes. However, rising temperatures and drought are enabling mosquitoes and the associated pathogens to expand their distributions into these higher elevations. Recent research on Kaua'i demonstrates that the population of nearly all native bird species there have sharply declined, and some species are projected to disappear within the next decade. Reducing the impacts of mosquito-borne diseases, like avian malaria, could help prevent that from occurring. Lastly, in addition to their impacts on our native bird species, mosquitoes also transmit heartworm disease to pets within Hawai'i. These heartworms can infect both dogs and cats leading to severe illness or even to the death of the animal. While heartworm treatments are available if the animal receives the proper diagnosis, infection prevention is the best defense against heartworm disease.

### ***Wolbachia* Infected Mosquitoes**

*Wolbachia* is a genus of bacteria that is found in an estimated 50% of all insects, including many that are found within Hawai'i. These bacteria are passed on from mother to offspring within insects. *Wolbachia* are intracellular bacteria, meaning they live within the cells of the insects. Because of this high level of specificity, they cannot survive outside of insect cells and are considered harmless to humans and other vertebrates. While *Wolbachia* are naturally passed from mother to offspring in many insect species, these bacteria can also be artificially introduced to insect species of interest, such as *A. aegypti* mosquitoes, to produce a desired outcome. Within the context of a vector control program, these mosquitoes with artificial *Wolbachia* infections can be used in two separate ways that hinge upon similar concepts. Within both types of programs, this artificial introduction is a non-GMO technique as the genetic material of the mosquitoes and of the *Wolbachia* bacteria are not being altered.

#### **a. *Wolbachia* to Reduce the Mosquito's Ability to Transmit Pathogens**

Within the first type of *Wolbachia* related vector control program, *Wolbachia* are artificially introduced to a mosquito population to reduce that mosquito's ability to transmit pathogens, such as Zika, dengue, and chikungunya. As noted within Act 106, this is an approach that is currently being implemented by the World Mosquito Program. Within this type of program, mosquitoes are collected from a site, artificially infected with the *Wolbachia* bacteria, reared to a large population size, and then their lineage is re-released back into the environment. The overall mission of this type of

program is to have the mosquito population remain within the environment and to have all mosquitoes infected with the *Wolbachia* bacteria to reduce their ability to transmit the pathogens of concern. Because this type of program relies on the bacteria being passed on from one generation of mosquito to the next within the wild, female mosquitoes containing the *Wolbachia* are released alongside males. While these female mosquitoes have a reduced ability to transmit pathogens, they do bite and require a blood meal, which may be considered a public nuisance. However, in communities where such programs have already been initiated, the public has generally supported the short-term increase in mosquito populations as a trade-off for reducing the risk of contracting infectious mosquito-borne diseases in the long-term.

**b. *Wolbachia* to Reduce Mosquito Populations**

The second type of *Wolbachia* related vector control program aims to reduce the total numbers of mosquitoes within the environment. This type of program relies on the mass release of male mosquitoes that contain *Wolbachia*. These male mosquitoes will be unable to produce offspring with wild females that either do not contain *Wolbachia* or that contain a different type of *Wolbachia*, which will suppress the overall mosquito population. Similar sterile insect programs have been implemented to control screwworms on the continental US and to control fruit flies within Hawaii, however they typically use radiation rather than *Wolbachia* to sterilize the males. Within this type of program, mosquitoes are collected from a site, artificially infected with the *Wolbachia* bacteria, reared to a large population size, and then the males of this lineage are re-released back into the environment. Males do not bite or require blood, meaning they do not transmit pathogens and are not considered a public nuisance.

In the United States, the process of artificially introducing *Wolbachia* to mosquito species is patented (Patent Number US 7,868,222 B1) by the University of Kentucky Research Foundation with Dr. Stephen L. Dobson noted as the inventor. As outlined in the abstract of the patent documentation, this approach of artificially introducing *Wolbachia* into mosquito species can be used to control mosquito reproduction or to limit the mosquito-borne pathogens:

“A method is provided for producing an artificial infection in a Culicidae (mosquito) species. The mosquitoes include species within the Subfamilies Culicinae and Anophelinae, and the species include *A. albopictus*, *A. aegypti* and *A. polynesiensis* infected with a *Wolbachia* infection. The infection may be a strain of *Wolbachia* which does not normally or naturally infect the selected mosquito species. The artificially infected *Aedes* mosquito can be introduced into a mosquito population to control the reproduction capability of the population by introducing an incompatible *Wolbachia* infection. The present method can be used as a novel means to limit mosquito-borne pathogens and thus control or prevent mosquito-borne diseases such as dengue, lymphatic filariasis, etc.”

Under this patent, MosquitoMate has the exclusive license to produce and distribute mosquito control technologies utilizing Dr. Dobson's method of artificially infecting mosquito populations with the *Wolbachia* bacteria. MosquitoMate is currently in the development of mosquito population suppression products for the control of the *A. aegypti* mosquito. This product corresponds to the second type of *Wolbachia* related vector control program as outlined above. To date, MosquitoMate has successfully completed trials of their product, have received EPA registration for a similar biopesticide product (Zap Mosquitoes) using *Wolbachia* for the control of *A. albopictus* mosquitoes, and are planning on undergoing an EPA review under the Pesticide Registration Improvement Extension Act (PRIA 4) towards registering their *A. aegypti* product in November 2020.

The use of *Wolbachia* infected mosquitoes to reduce overall mosquito populations has some significant advantages over traditional mosquito control techniques. For instance, a control program utilizing *Wolbachia* infected mosquitoes could be implemented at a landscape scale. These *Wolbachia* infected mosquitoes can be released at a scale that would allow for the suppression of mosquitoes across an entire community or region. While chemical pesticides controls could also be regularly implemented at this scale, relevant concerns exist regarding where they can be applied and their potential impacts on human health. Another significant benefit of *Wolbachia* programs is that they are implemented using mosquito-specific techniques that do not significantly impact other native or beneficial species. Hawaii is known as the endangered species capitol of the world, and the presence of incredibly rare and declining native species across the entire state may limit the potential for area-wide applications of chemical pesticides. The last major benefit of implementing this type of *Wolbachia* based vector control program is that it is self-limiting, meaning the *Wolbachia* from the mosquitoes will not persistently remain within the environment following the death of the sterile males. This ensures that the control program can be stopped at any time by ending the releases of the sterile males.

#### **Plans:**

The DLNR and the DOH have begun the process of developing lineages of mosquitoes, originating from Hawai'i, to be infected with *Wolbachia* for their use in potential programs to reduce mosquito populations through the release of sterile male mosquitoes. At this time, there are no specific plans to implement the other type of *Wolbachia* based program that requires the release of both male and female mosquitoes and that reduces the mosquitoes' ability to transmit pathogens as outlined within Act 106. However, as the legal and regulatory framework surrounding this technology evolves, implementation of such a program may also be possible.



In November 2016, DLNR Division of Forestry and Wildlife (DOFAW) directed \$50,000.00 to the University of Hawai'i at Manoa and University of Hawai'i at Hilo to initiate development of a *Wolbachia* infected *C. quinquefasciatus* mosquito. UH researchers collected mosquitoes from local *C. quinquefasciatus* populations on Oahu and Hawai'i Island and attempted to infect laboratory mosquito populations with *Wolbachia* bacteria. State funding concluded in December 2017, and the researchers were not successful in accomplishing the project objectives over that period. In the interim, DLNR DOFAW secured an additional \$50,000.00 in matching funds from the U.S. Fish and Wildlife Service (USFWS) to continue the project. Federal funds were administered by DLNR DOFAW and passed through to UH researchers from August 2018 through June 2019. Despite the additional funding, the project still did not result in the development of a *Wolbachia* infected *C. quinquefasciatus* mosquito, due to the complexity of methodologies and technical specialization required for such an undertaking. In addition to these efforts, several federal agencies, including the USFWS and the National Parks Service, are currently participating in and supporting the development of *Wolbachia* infected *C. quinquefasciatus* mosquitoes originating from Hawai'i for the preservation of native forest birds. This work includes collaborating with external university partners towards the development of these mosquitoes.

The DOH has also recently taken steps towards the development of *Wolbachia* infected Hawaiian mosquitoes. In August 2019, the HDOH Vector Control Branch was awarded \$61,989 from the Hawai'i Invasive Species Council (HISC) for the development and maintenance of a lineage of *Ae. aegypti* and *Ae. albopictus* mosquitoes originating from Hawai'i that will be artificially infected with the *Wolbachia* bacterium. Collection and submission of mosquito specimens from Hawai'i to the MosquitoMate laboratory is expected to occur beginning in 2020.

#### **Timeline:**

The timeline for the implementation of such a *Wolbachia* based *A. aegypti* population reduction program relies on a variety of factors. The following is a best estimation for the earliest a *Wolbachia* based program could be implemented within Hawai'i for the control of *A. aegypti* mosquitoes.

Using the funds provided through HISC, the DOH intends to collect and submit both *A. aegypti* and *A. albopictus* mosquito specimens originating from Hawai'i to the MosquitoMate laboratory beginning in early 2020. There, these lineages of Hawaiian mosquitoes will be infected with *Wolbachia* and reared at the MosquitoMate laboratory by the end of 2020. In November 2020, MosquitoMate will undergo their EPA review under the Pesticide Registration Improvement Extension Act (PRIA 4) for their *A. aegypti* product. Following the receipt of their EPA biopesticide registration for the *A. aegypti* product, MosquitoMate will undergo two separate HDOA review processes. The first review process

will be to register their *A. aegypti* product as a biopesticide within Hawai'i. The second process will be to allow for the importation of this biopesticide product into Hawai'i as outlined in Sections 1 and 2 of this report. It is expected that these review processes will take approximately one year to complete. Therefore, the earliest potential start date for the development of a mosquito rearing facility within the state for the production of *Wolbachia* infected *A. aegypti* mosquitoes is early 2022. The development of the facility, the importation of the *Wolbachia* infected mosquitoes from MosquitoMate's laboratory, the establishment of a colony of *Wolbachia* infected mosquitoes at the rearing facility, and the mass rearing of these mosquitoes will likely take an additional six months to a year of production time before releases could begin.

### **Implementation Costs:**

Cost estimates for the implementation of a *Wolbachia* based mosquito control program arise from our current understanding of the *A. aegypti* mosquito distribution and density on Hawai'i Island, the nature of Hawai'i Island's rugged terrain and lack of road access in certain areas, and estimates for the development of a rearing facility on island to produce the mass quantities of *Wolbachia* infected, sterile male mosquitoes to be released into the environment. Cost estimates are also guided by previous *Wolbachia* based release programs that have been implemented in both Florida and California using MosquitoMate's technology. Based on our best understanding of these factors, it is estimated that the first-year cost for the development of such a project would be ~\$2.5 million, which would include the cost of developing the mosquito rearing facility and the associated costs regarding the first year of *A. aegypti* mosquito releases.

The cost estimate of ~\$2.5 million includes a one-time investment of ~\$450,000 to develop the rearing facility and to purchase the mass mosquito rearing equipment. In addition to the control of *A. aegypti*, the facility and the equipment could also be used to rear other *Wolbachia* infected mosquito species, such as *C. quinquefasciatus* and *Ae. albopictus*, that are present statewide and that are of public health and ecological concern. The annual cost for the development of the mosquitoes to treat the regions infested with *A. aegypti* is ~\$1,750,000. This estimate includes the cost of mass producing the mosquitoes for release, of the associated supplies required for this mass production, and of the distribution and release of the mosquitoes throughout the infested areas of Hawai'i Island. Lastly, an annual expenditure of ~\$300,000 is estimated for the personnel costs associated with running the rearing facility and the distribution of the mosquitoes across the island.

This cost estimate does not include certain associated costs, such as the significant level of public outreach and community engagement that would be required to ensure understanding of and support for the project.

### **Vector Control Alternatives to *Wolbachia*:**

Mosquito control measures are usually implemented and evaluated in a comprehensive manner under a broad integrated pest management (IPM) plan. These IPM plans can include and promote the use of chemical, biological, and cultural control methods that can reduce adult mosquito populations, larval mosquito populations, and the environments in which these mosquitoes breed and reside. Current mosquito control efforts are targeted at specific areas of concerns, such as points of entry into the state, near the properties of confirmed imported cases of disease, etc. These ongoing efforts are not considered to be landscapes scale controls or with the aim of eradicating a specific mosquito species. However, along with these outlined *Wolbachia* programs, additional programs or technologies could be implemented to control mosquitoes at the landscape scale.

While not completely analogous to the current *A. aegypti* situation on Hawai'i Island, *A. aegypti* eradication successfully occurred during the 1940s on Oahu. Guided by the Territorial Board of Health and the Malaria Control in War Areas program (the precursor organization to the U.S. Centers for Disease Control and Preventions), over 1,500 mosquito control personnel across the island of Oahu conducted mosquito control activities. These activities included door to door inspections of every property on Oahu, removal of any rubbish or other water holding containers that served as mosquito breeding locations, and broad applications of chemical pesticide treatments, including aerial treatments and treatments around individual homes. These large scale, collaborative control measures occurred from 1943-1946 followed by three years of control measures directed solely by the Territorial Board of Health. By 1949, the *A. aegypti* mosquito was no longer found on Oahu.

With regards to new technologies that could be implemented for landscape scale mosquito control, the DOH, in collaboration with the University of California, Davis, is currently evaluating the potential use of a new mosquito kill trap, the In2Care trap, on Hawai'i Island. This type of trap includes active ingredients that are coated onto an adult mosquito that flies into and out of the trap. These active ingredients both kill the adult mosquitoes over the course of a few days and allows the mosquito to distribute a larvicide to additional breeding sites where they land to lay their eggs. For this type of trap to be effective on a landscape scale, many more traps would need to be deployed and maintained over a significant period. Additional concerns related to the trap, such as potential toxicity to fish and other aquatic insects, does place a limitation on the number of traps that can be distributed over any given area.

Lastly, new control techniques involving genetically modified mosquitoes are on the horizon of scientific and regulatory possibility, though there are currently no specific plans to utilize

this technology in Hawai'i. These types of programs are commonly referred to as RIDL programs, release of insects carrying a dominant lethal gene. These types of programs implement a variety of different gene modification strategies in attempts to reduce mosquito populations. Trials of these type of programs are currently being implemented in multiple locations, including Brazil, the Cayman Islands, and the Florida Keys.

**References:**

1. <https://bioone.org/journals/journal-of-the-american-mosquito-control-association/volume-29/issue-2/12-6292R.1/History-of-Aedes-Mosquitoes-in-Hawaii/10.2987/12-6292R.1.short>
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  12. <https://www.oxitec.com/our-technology>
  13. <http://hawaii.gov/hdoa/labels/10833.1.pdf>
- (4) Any other relevant findings, recommendations, and any necessary proposed legislation:

None at this time as the Statutes and Rules already have the existing processes to achieve the goal of Landscape Level Control of *A. aegypti*.