STATE OF HAWAII
DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESOURCE MANAGEMENT DIVISION
HONOLULU, HAWAII

May 9, 2022

Board of Agriculture
Honolulu, Hawaii

Subject:	REQUEST FOR CONVERSION OF GENERAL LEASE NO. S-3768; KEVIN JAMES MULKERN AND SUSAN SHAHEEN MULKERN, LESSEE; TMK: (1) 4-1-027:027, LOT 28, WAIMANALO FARM LOTS, KOOLAUPOKO, WAIMANALO, ISLAND OF OAHU, HAWAII
Authority:	Section 166E-4, Hawaii Revised Statutes (HRS), and Sections 4-158-8, Hawaii Administrative Rules (HAR)
Lessee:	Kevin James Mulkern and Susan Shaheen Mulkern
Land Area:	6.836 gross acres
Tax Map Key:	(1) 4-1-027:027 (see Exhibit "A")
Land Status:	Encumbered by Governor's Executive Order No. 4239 to the Department of Agriculture (DOA) for non-agricultural park land purposes dated September 23, 2008
Lease Term:	65 years; December 3, 1963 through December 2, 2028
Annual Rental:	$4,800.00 per year
Character of Use:	Diversified Agriculture

A-2

REMARKS:

General Lease No. S-3768 was awarded by the Board of Land and Natural Resources to Stanley S. Kawabata in 1963. By an unrecorded extension of lease in 1981 and a second extension of lease in 1997, the lease was extended and expires on December 2, 2028. In 2008 the lease was transferred to the DOA via Governor's Executive Order for management purposes. At its meeting held on March 14, 2017, the Board of Agriculture approved the assignment of the lease to Kevin Mulkern and Susan Mulkern. The Mulkern's have been in the nursery and landscaping business since 1975 and plan to develop an orchard of breadfruit and cacao with apiaries interspersed amongst the trees and plants for pollination and honey production on the parcel.

The Lessee has requested a conversion of said lease in accordance with Section 4-158-8, HAR, to a new 35-year lease term, subject to the requirements of the administrative rules which are stated, in pertinent sections as follows:

The department shall:

- Require an appraisal of the parcel in accordance with Section 4-158-21, HAR;
- Impose other lease terms provisions, restrictions, and conditions as provided in this chapter as may be required to protect the State's interest; and
- Require the payment of annual lease rent by appraisal and a premium computed at twenty-five percent (25%) of the annual base rent for each year of the lease equal to the number of years that person occupied the land, but not to exceed seven (7) years

An appraisal was ordered pursuant to Section 4-158-21, HAR, for the purpose of determining the fair market rental for the subject parcel. The new appraised annual rental for this lease shall be set for the first fifteen years of the term versus 1.5% of gross sales, whichever is greater. The appraised annual rental will be applied to the converted lease as of the date of commencement. Additionally, in accordance with the administrative rules, the Lessee will pay a premium equal to 25% of the rental for the new lease for a period of seven (7) years from the commencement of the converted term. The fair market annual rental rate has been determined at $12,570.00 per year.

Further, the Board and Lessee mutually agree to cancel the existing General Lease No. S-3768 by executing a Mutual Cancellation of existing General Lease No. S-3768 subject to execution of the converted general lease documenting the effective date, so that only one lease is in full force and effect. Since the mutual cancellation of lease is agreed upon and executed by the parties and is not due to breach or default by Lessee, Lessee is eligible and qualified for the new general lease, in this respect only. All other eligibility terms must be met to the satisfaction of the Board.

RECOMMENDATION:

That the Board of Agriculture:

1. Approve Lessee's request to convert General Lease No. S-3768 to a new Non-Agricultural Park Lands lease of not more than thirty-five (35) years for its initial lease term subject to the conversion provisions of Chapter 4-158-8, HAR.

2. Approve the Mutual Cancellation of General Lease No. S-3768, subject to the execution of the effective date of the new general lease.

All documents are subject to review and approval as to form by the Department of the Attorney General, and such other terms and conditions as may be prescribed by the Chairperson to best serve the interests of the State.

Respectfully submitted,

BRIAN KAU, P.E.
Administrator and Chief Engineer
Agricultural Resource Management Division

Attachment – Exhibit "A"

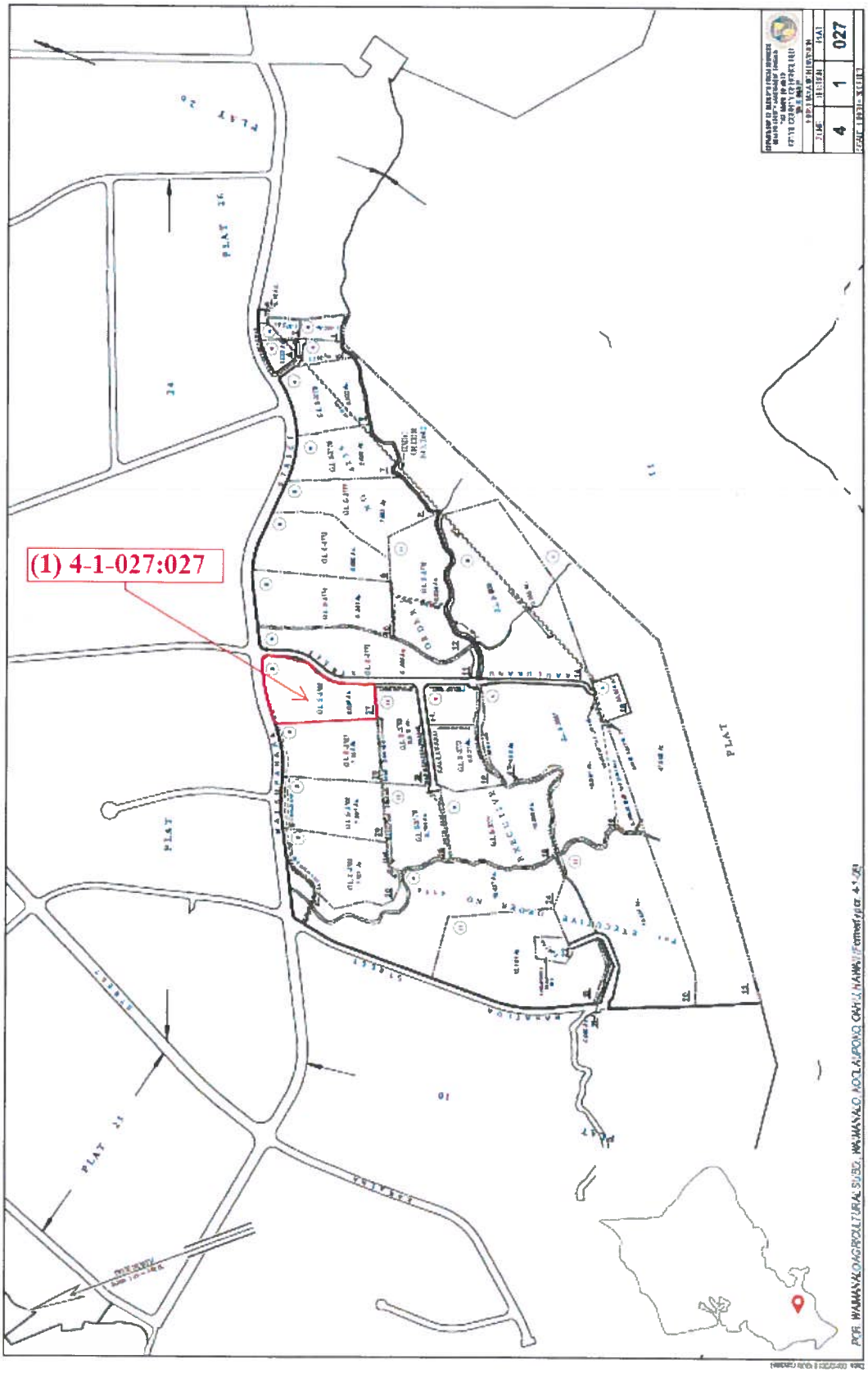
APPROVED FOR SUBMISSION

PHYLLIS SHIMABUKURO-GEISER
Chairperson, Board of Agriculture

A-3

A-4

Exhibit "A"


(1) 4-1-027:027
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PLAT
PCR WAIMANALO AGRICULTURAL SUBD., WAIMANALO, KOOLAUPOKO, OAHU, HAWAII

STATE OF HAWAII
DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESOURCE MANAGEMENT DIVISION
HONOLULU, HAWAII

May 9, 2022

Board of Agriculture
Honolulu, Hawaii

Subject:	REQUEST FOR APPROVAL TO: 1) ADD AND WITHDRAW MEMBERS FOR KLK FARM, LLC, LESSEE; AND 2) EXTEND TERM OF LEASE OF GENERAL LEASE NO. S-6014; TMK: (1) 5-6-006:042; KAHUKU AGRICULTURAL PARK LOT 14, KOOLAULOA, KAHUKU, ISLAND OF OAHU, HAWAII
Authority:	Sections 166-6, 166-7 and 166-9, Hawaii Revised Statutes (HRS), and Sections 4-153-1, 4-153-33(a)(2), (5) and (6)(D), and 4-153-33(b), Hawaii Administrative Rules (HAR)
Lessee:	KLK Farm, LLC
Land Area:	6.196 gross acres
Tax Map Key:	(1) 5-6-006:042 (see Exhibit "A")
Land Status:	Encumbered by Governor's Executive Order No. 3867 to the Department of Agriculture for agricultural park purposes
Annual Rental:	$2,580.00 per year until rental reopening – April 1, 2024
Character of Use:	Diversified Agriculture
Lease Term:	45 years, August 1, 1999 through March 31, 2044
Consideration:	None

A-5

REMARKS:

In 1999, General Lease No. S-6014 was awarded to Jimy S. Inthasone by the Board of Agriculture, and in 2004 the subject lease was assigned to Dana D. Sourinthone and McArt Sourinthone (husband). By mesne assignment, in 2021 the subject lease was assigned to KLK Farm, LLC. The lessee, KLK Farm, LLC is owned by Dana Daody Kiat-A Nan and produces primarily dragon fruit, avocado, lime and lychee which are sold at farmers markets, restaurants, and supermarkets. KLK Farm, LLC requests to add Lisa Lai as a member, and Ms. Kiat-A Nan request termination as a member of KLK Farm, LLC due to physical disabilities and will cease involvement in company operations pursuant to Section 4-153-33(a)(6)(D).

Lisa Lai has been working full-time on Ms. Kiat-A Nan's farm as a manager of day-to-day operations since 2019. Prior to working and farming on the subject farm lot, Ms. Lai owned a 10-acre farm and managed 6 employees. Ms. Lai qualifies as a bona fide farmer with more than two years of fulltime farming experience and meets the eligibility requirements for agricultural parks pursuant to Sections 4-153-1 and 13, HAR. KLK Farm, LLC will continue to qualify as an agricultural corporation with members individually qualified as bona fide farmers.

Subject to the approval of the change of members for KLK Farm, LLC, Ms. Lai requests a 10-year extension of the lease term from April 1, 2044 through March 31, 2054 for a cumulative total of not more than fifty-five years pursuant to Section 4-153-33(a)(2), HAR. The Board of Agriculture may extend the term of the lease to qualify the lease for mortgage lending purposes, pursuant to Section 4-153-33(b), HAR. Consequently, Ms. Lai has been preapproved for a $250,000 loan, subject to Board approval of extension of the lease term. The loan proceeds will be used solely for farming operations and improvements on the demised premises. An appraisal will be ordered by the Lessor for the purpose of determining the fair market annual base rental and additional rents for the subject parcel for the extension period commencing on April 1, 2044, as determined by an independent appraiser.

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RECOMMENDATION:

That the Board of Agriculture: 1) approve the transfer of KLK Farm, LLC members from Dana Daody Kiat-A Nan to Lisa Lai; and 2) approve the extension of General Lease No. S-6014 to expire on March 31, 2054. All documents shall be subject to review and approval as to form by the Department of the Attorney General, and such other terms and conditions as may be prescribed by the Chairperson to best serve the interests of the State.

Respectfully Submitted,

BRIAN KAU, P.E.
Administrator and Chief Engineer
Agricultural Resource Management Division

Attachments – Exhibit "A"

APPROVED FOR SUBMISSION:

PHYLLIS SHIMABUKURO-GEISER
Chairperson, Board of Agriculture

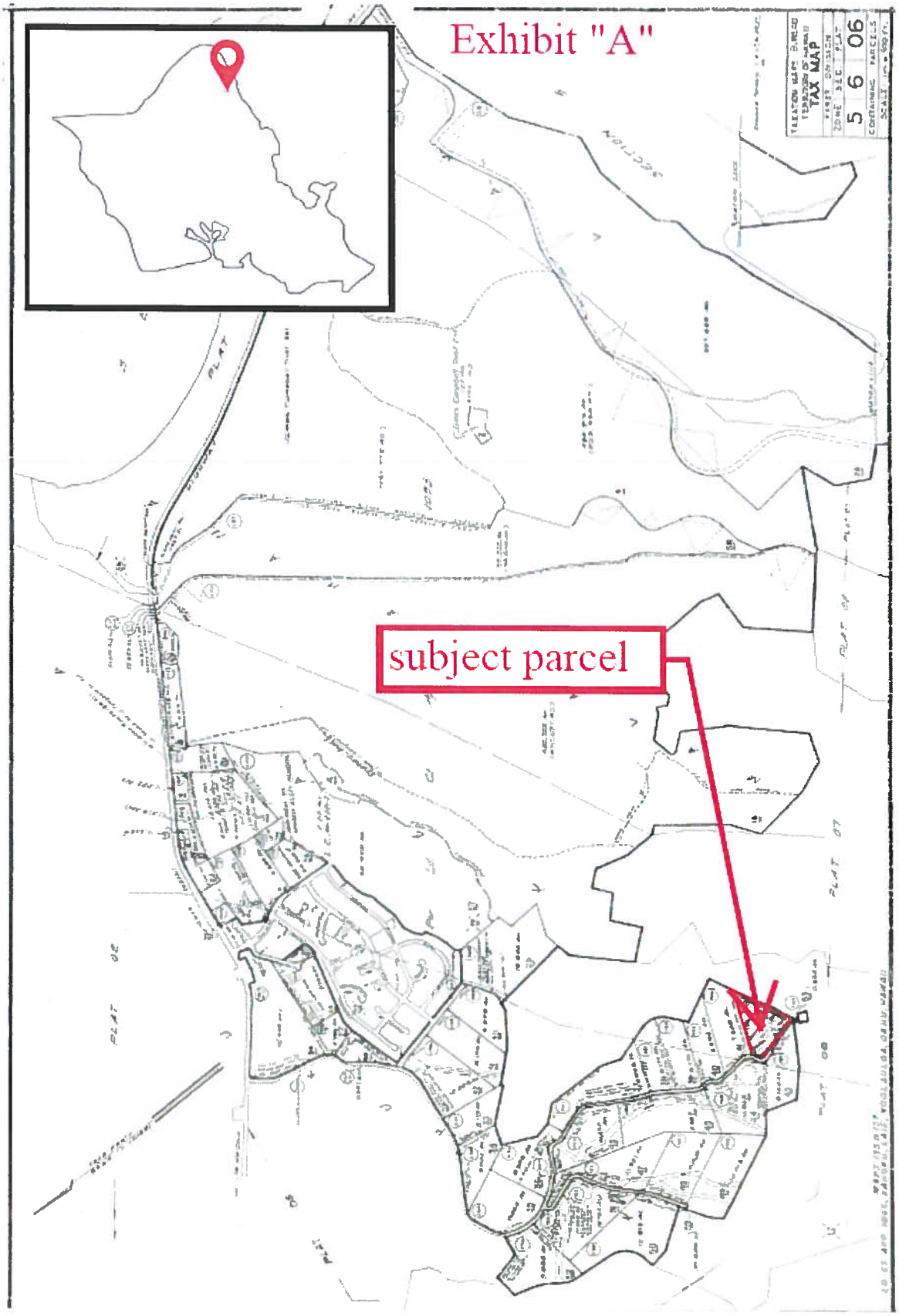
Exhibit "A"
subject parcel

STATE OF HAWAII
DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESOURCE MANGEMENT DIVISION
HONOLULU, HAWAII

May 9, 2022

Board of Agriculture
Honolulu, Hawaii

Subject:	REQUEST FOR APPROVAL TO AWARD LEASES TO VARIOUS AWARDEES AND BACK-UP POSITIONS; TMK NOS. (1) 4-1-027:023 AND (1) 4-1-027:020 & 024, ISLAND OF OAHU, HAWAII
Authority:	Section 166E-8, Hawaii Revised Statutes (HRS), and Section 4-158-22, Hawaii Administrative Rules (HAR)
Tax Map Key:	(1) 4-1-027:023 (Waimanalo, Island of Oahu) (1) 4-1-027:020 & 024 (Waimanalo, Island of Oahu)
Land Area:	(1) 4-1-027:023 15.101 gross acres (1) 4-1-027:020 & 024 38.918 gross acres
Land Status:	Properties set aside to the Department of Agriculture (DOA) by Governor's Executive Order 4534 dated July 21, 2017
Lease Term:	35 years each, commencing upon the completion of pre-requisite requirements and expiration of Right-of-Entry of 6 months
Base Annual Rental:	Various - per qualified applicant bid
Additional Rent:	1.5% of gross proceeds from the sale of commodities produced on the demised premises which exceed the base rental
Character of Use:	Diversified agriculture

A-9

A-10

BACKGROUND:

The Agricultural Resource Management Division received the Waimanalo parcels from the Department of Land and Natural Resources via Governor's Executive Order No. 4534 signed on July 21, 2017 for TMK Nos. (1) 4-1-027:023 and (1) 4-1-027:020 & 024.

In accordance with §166E-8, HRS, and §4-158-22, HAR, a public notice of disposition was published on October 20, 2021 making the subject parcels available for lease. The division received a total of nine (9) applications for the vacant parcels, of which five (5) applicants qualified to submit bid proposals in accordance with the rules. Pursuant to §4-158-1 and 27, HAR, staff has determined that each applicant qualifies as a bona fide farmer with more than two years of years of farming experience and meets eligibility residency requirements of the Non-Agricultural Park Lands Program.

Exhibit "A" attached hereto, lists the applicants, their status and respective bids. Exhibit "B" reflects the locations of the parcels.

In addition to the highest bid proposal, staff identified three backup bid proposals for TMK (1) 4-1-027:023 and one backup bid proposal for TMK (1) 4-1-027:020 & 024.

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RECOMMENDATIONS:

That the Board of Agriculture approve:

1. Issuance of the appropriate Right-of-Entry document to the successful bidders for the lots in accordance with §4-158-22, HAR, and subsequently issue the appropriate general leases subject to the completion of lease pre-requisites.

2. Backup bid proposals per lot as alternatives in the event the highest bidders fail to complete the lease pre-requisites.

All related documents are subject to the review and approval as to form by the Department of the Attorney General, and such other terms and conditions as may be prescribed by the Chairperson to best serve the interests of the State.

Respectfully submitted,

BRIAN KAU, P.E.
Administrator and Chief Engineer
Agricultural Resource Management Division

Attachments: Exhibits "A" and "B"

APPROVED FOR SUBMISSION:

PHYLLIS SHIMABUKURO-GEISER
Chairperson, Board of Agriculture

A-11

A-12

EXHIBIT "A"

VARIOUS NON-AGRICULTURAL PARK LANDS DISPOSITION - 2021

	Waimanalo, Island of Oahu	
Applicant Name	**TMK: (1)4-1-027:023 bid amount**	
Joshua Stamm	$31,600.00	highest bid
Tree Concepts Hawaii LLC	$30,084.00	1st backup
Ahiki Acres, LLC	$30,000.00	2nd backup
Hala Toa Mui Farms, LLC	$21,000.00	3rd backup

	Waimanalo, Island of Oahu	
Applicant Name	**TMK: (1)4-1-027:020 & 024 bid amount**	
Hala Toa Mui Farms, LLC	$31,000.00	highest bid
Joshua Stamm	$30,830.00	1st backup

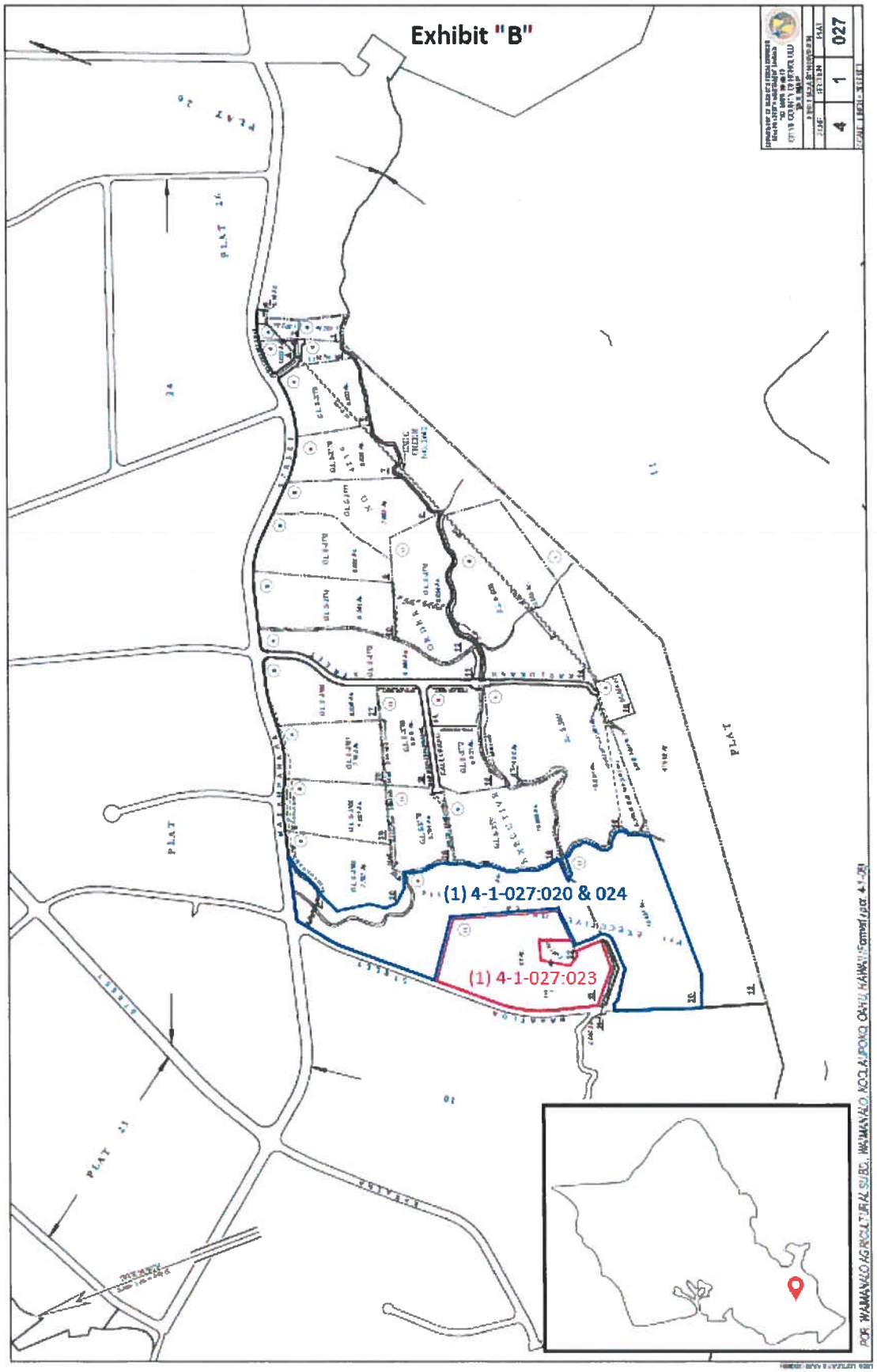
Exhibit "B"
(1) 4-1-027:020 & 024
(1) 4-1-027:023
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PLAT
POR. WAIMANALO AGRICULTURAL SUBD., WAIMANALO, KOOLAUPOKO, OAHU, HAWAII

A-13

STATE OF HAWAII
DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESOURCE MANAGEMENT DIVISION
HONOLULU, HAWAII

May 9, 2022

Board of Agriculture
Honolulu, Hawaii

Subject: REQUEST FOR CONVERSION OF GENERAL LEASE NO. S-4364; PUNA CERTIFIED NURSERY INC, LESSEE; TMK: 3rd DIV/2-4-049:017, LOT 2, PANAEWA FARM LOTS, WAIAKEA, SOUTH HILO, ISLAND OF HAWAII, HAWAII

Authority:	Section 166E-4 Hawaii Revised Statutes, (HRS), and Section 4-158-8, Hawaii Administrative Rules (HAR)
Lessee:	Puna Certified Nursery Inc.
Land Area:	Approximately 12.683 acres
Tax Map Key:	3rdDiv/2-4-049:017 (Exhibit "A")
Lease Term:	55-years, August 17, 1972, through August 16, 2027
Land Status:	Encumbered by Governor's Executive Order No. 4300 to the Department of Agriculture for agricultural purposes, October 14, 2009.
Annual Base Rent:	$4,940/year
Character of Use:	Cultivation, wholesaling, retailing, and packing of nursery products and related purposes.

BACKGROUND:

General Lease No. S-4364 was awarded by the Board of Land and Natural Resources (BLNR) to TSI, LTD, on August 17, 1972. At its meeting held on November 20, 1987, the BLNR consented to the assignment of lease from TSI, LTD to Rainbow Tropicals, Inc. On May 26, 1995, the BLNR approved the assignment of lease from Rainbow Tropicals, Inc. to Gordon C. Heit dba GH Trading Company. On January 22, 1999, the BLNR consented to the assignment of lease from Gordon C. Heit dba GH Trading Company to Puna Certified Nursery Inc (PCN).

General Lease S-4364 was transferred to the Department of Agriculture by Governor's Executive Order No. 4300, dated October 14, 2009.

PCN is an established and thriving business that specializes in the cultivation of dracaenas, palms, and landscaping plants. PCN is utilizing the subject property to cultivate Coconut Palms. They also hold

A-4

A-15

three (3) additional leases with the Department of Agriculture; General Lease No's S-4838, S-4839, and S-4852, all of which are located in the Keahole Agricultural Park.

PCN has requested conversion of the said lease in accordance with Section 4-158-8, HAR, to a new 35-year lease term, subject to the requirements of the administrative rules which are stated, in pertinent sections as follows:

The department shall:

- Require an appraisal of the parcel in accordance with Section 4-158-21, HAR;
- Impose other lease terms, provisions, restrictions, and conditions as provided in this chapter as may be required to protect the State's interest;
- Require the payment of annual lease rent by appraisal and a premium computed at twenty-five percent (25%) of the annual base rent for each year of the lease equal to the number of years that person occupied the land, but not to exceed seven (7) years; and
- Require those qualifying under subsection (a) to meet the bona fide farmer criteria as defined in section 4-158-1, HAR.

An appraisal has been ordered pursuant to Section 4-158-21, HAR, for the purpose of determining the fair market rental for the subject parcel. The new appraised annual rental for this lease shall be set for the first fifteen (15) years of the term versus 1.5% of gross sales, whichever is greater. The appraised annual rental will be applied to the converted lease as of the new date of commencement. Additionally, in accordance with the administrative rules, the Lessee will pay a premium equal to 25% of the rental for the new lease for a period of seven (7) years from the commencement of the converted term. The new fair market annual rental rate has not yet been determined.

Further, the Board and Lessee mutually agree to cancel the existing General Lease No. S-4364 by executing a Mutual Cancellation of existing General Lease No. S-4364 subject to execution of the converted general lease documenting the effective date so that only one lease is in full force and effect. Since the mutual cancellation of the lease is agreed upon and executed by the parties and is not due to breach or default by the Lessee, Lessee is eligible and qualified for the new general lease, in this respect only. All other eligibility terms must be met to the satisfaction of the Board.

RECOMMENDATION:

That the Board of Agriculture:

1. Approve Lessee's request to convert General Lease No. S-4364 to a new Non-Agricultural Park Lands lease of not more than thirty-five (35) years for its initial lease term subject to the conversion provisions of Chapter 4-158-8, HAR with rent reopening's at the expiration of the 15th and 30th years.

2. Approve the Mutual Cancellation of General Lease No. S-4364, subject to the execution of the effective date of the new general lease.

All related documents are subject to the review and approval as to form by the Department of the Attorney General, and such other terms and conditions as may be prescribed by the Chairperson to best serve the interests of the State.

Respectfully submitted,

BRIAN KAU, P.E.
Administrator and Chief Engineer,
Agricultural Resource Management Division

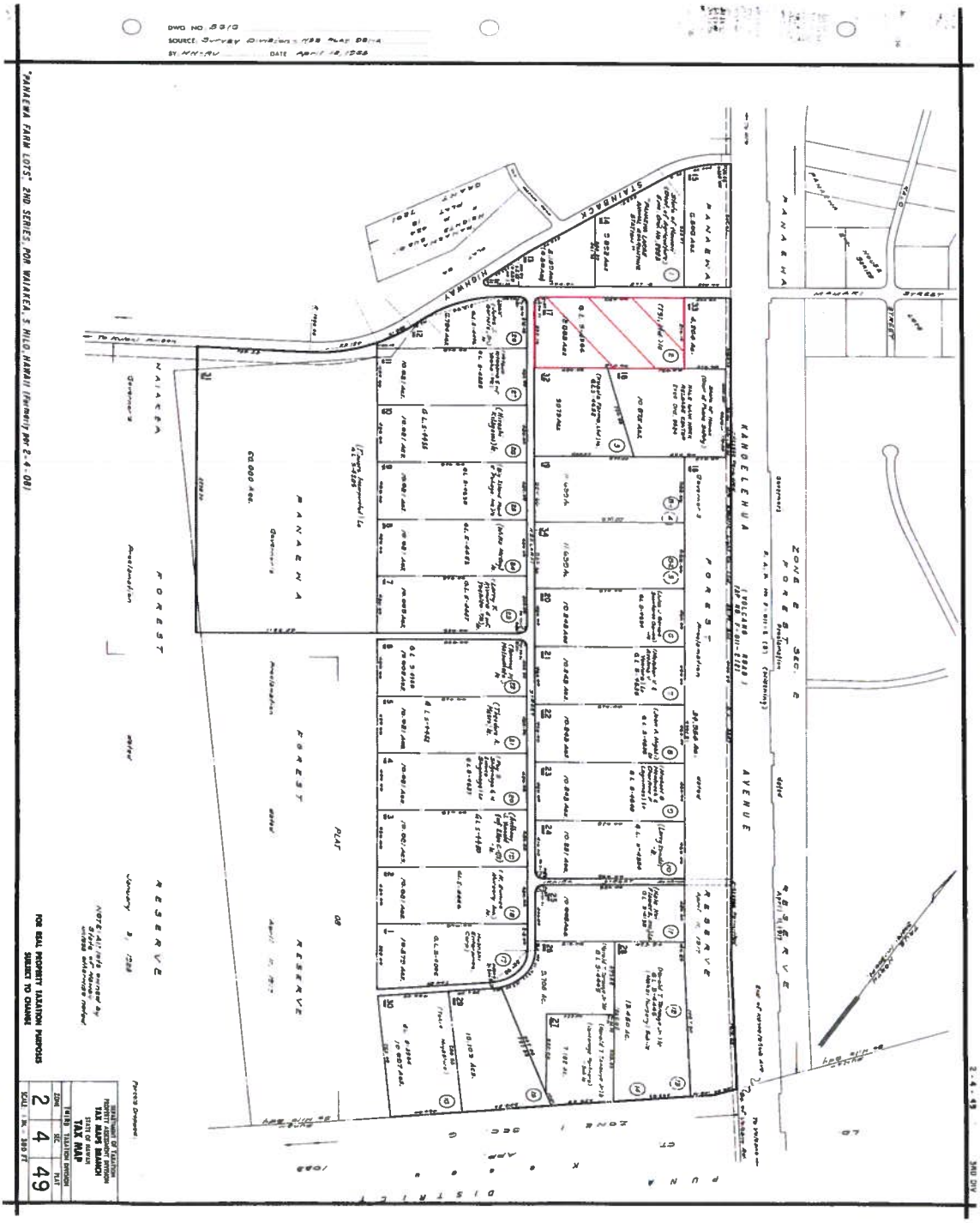
Attachments - Exhibit "A"

APPROVED FOR SUBMISSION:

PHYLLIS SHIMABUKURO-GEISER
Chairperson, Board of Agriculture

A-17

EXHIBIT "A"



STATE OF HAWAII
DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESOURCE MANAGEMENT DIVISION
HONOLULU, HAWAII

May 9, 2022

Board of Agriculture
Honolulu, Hawaii

Subject: REQUEST FOR CONSENT TO ASSIGNMENT OF GENERAL LEASE NO. S-4807; HIKARI NURSERY & LANDSCAPING, INC., LESSEE/ASSIGNOR; DAVID STONE, ASSIGNEE; TMK: 3rd DIV/1-5-116:043, LOT 16, PAHOA AGRICULTURAL PARK, PUNA DISTRICT, ISLAND OF HAWAII, HAWAII

Authority:	Section 166-7 Hawaii Revised Statutes, (HRS), and Section 4-153-33(a)(6)(B), Hawaii Administrative Rules (HAR)
Lessee/Assignor:	Hikari Nursery & Landscaping, Inc.
Assignee:	David Stone
Land Area:	5.335 acres
Tax Map Key:	3rdDiv/1-5-116:043 (Exhibit "A")
Lease Term:	55-years, May 1, 1982, through April 30, 2037
Land Status:	Encumbered by Governor's Executive Order No. 3380, dated February 26, 1988, to the Department of Agriculture for Agricultural Park Purposes
Annual Base Rent:	$880.00 per year (until 5/1/2022 re-opening)
Character of Use:	Diversified Agriculture
Consideration:	$82,000.00

BACKGROUND:

General Lease No. S-4807 was awarded to Eric H. Kama on May 16, 1983, by the Board of Land and Natural Resources (BLNR). On October 25, 1985, BLNR consented to the assignment of lease from Eric H. Kama to Elston T. Takayama and Lew Nakamura. With the approval of the BLNR, General Lease No. S-4807 was assigned from Elston T. Takayama and Lew Nakamura to Hikari Nursery on February 12, 1988. The subject property was transferred to the Department of Agriculture by Governor's Executive Order No. 3380, dated February 26, 1988. At its meeting on February 18, 1993, the Board of Agriculture consented to the assignment of lease from Hikari Nursery to Hikari Nursery and Landscaping, Inc., which is owned and operated by Lew Nakamura and Elston T. Takayama. In 2003, there was a change in Officers of Hikari Nursery and Landscaping, Inc. from Lew Nakamura and Elston T. Takayama

A-18

A-19

to Lew Nakamura and Sheila Nakamura, husband and wife. Hikari Nursery and Landscaping Inc., are currently producing hydroponic cucumbers.

Due to the physical disabilities of the owners, Hikari Nursery and Landscaping, Inc. is requesting the assignment of General Lease S-4807 to David Stone. Pursuant to the terms of General Lease No. S-4807 and Section 4-153-33(a)(6)(B), HAR, an assignment of lease is permitted due to physical disability. The assignment of lease will include a farm dwelling, multiple greenhouse structures, a packing shed with a loading dock, and crops.

David Stone is the Owner and operator of Island Distillers Inc. Since 2014 he has been actively farming on and managing a 2-acre agricultural parcel on Oahu where he grows sugar cane, bananas, and Hawaiian chili peppers. These products are used in the production of distilled beverages under his company, Island Distillers Inc.

Mr. Stone plans to continue and expand the current hydroponic cucumber operation on the subject property. He also plans to cultivate Hawaiian chili peppers, lavender, and vanilla.

Mr. Stone qualifies as a bona fide farmer, with more than two (2) years of full-time farming experience and satisfies the eligibility requirements pursuant to Sections 4-153-1 and 13, HAR.

There is a consideration of $82,000.00 for the assignment of lease. Staff does not recommend an adjustment of the annual rental rate as the consideration amount appears to be consistent with fair market values.

RECOMMENDATION:

That the Board of Agriculture approve the assignment of lease from Hikari Nursery and Landscaping Inc. Lessee/Assignor to David Stone, Assignee, under General Lease S-4807 and the consideration of $82,000.00. All related documents are subject to the review and approval as to form by the Department of the Attorney General, and such other terms and conditions as may be prescribed by the Chairperson to best serve the interests of the State.

Respectfully submitted,

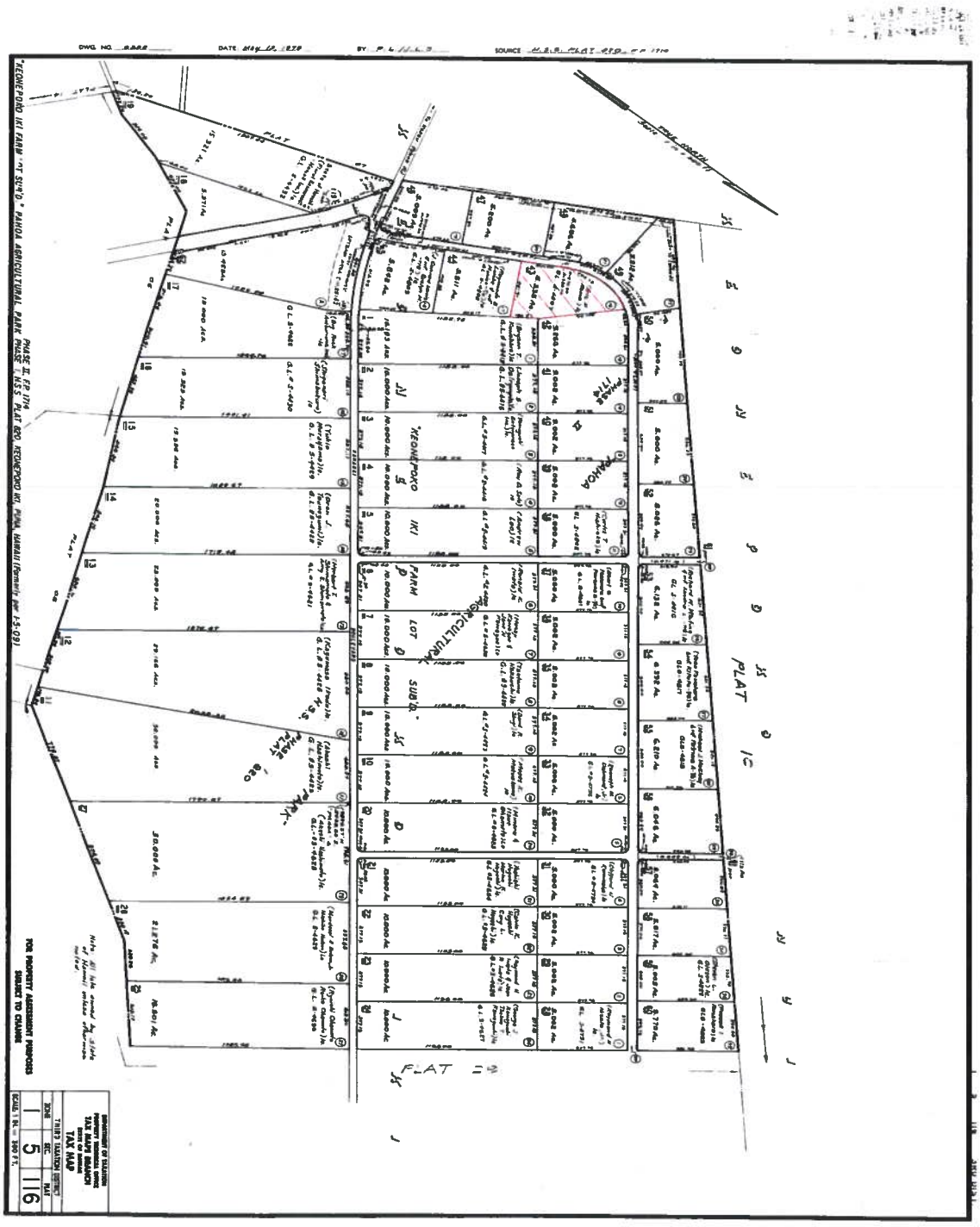
BRIAN KAU, P.E.
Administrator and Chief Engineer,
Agricultural Resource Management Division

Attachments - Exhibit "A"

APPROVED FOR SUBMISSION:

PHYLLIS SHIMABUKURO-GEISER
Chairperson, Board of Agriculture

EXHIBIT "A"



A-20

STATE OF HAWAII
DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESOURCE MANAGEMENT DIVISION
HONOLULU, HAWAII

May 9, 2022

Board of Agriculture
Honolulu, Hawaii

Subject: REQUEST TO APPROVE RENTAL OFFSET, GENERAL LEASE NO. S-4426; JOHN F. GARCIA JR., LESSEE; TMK: 3rd DIV/1-5-116:011, LOT 11, PAHOA AGRICULTURAL PARK, PUNA DISTRICT, ISLAND OF HAWAII, HAWAII

Authority:	Section 166-9 Hawaii Revised Statutes, (HRS), and Section 4-153-24(2), Hawaii Administrative Rules (HAR)
Lessee:	John F. Garcia, Jr.
Land Area:	Approximately 30.000 acres
Tax Map Key:	3rdDiv/1-5-116:011 (Exhibit "A")
Lease Term:	35-years, May 1, 2021 through April 30, 2056
Land Status:	Encumbered by Governor's Executive Order No. 3380, dated February 26, 1988, to the Department of Agriculture for Agricultural Park Purposes
Annual Base Rent:	$2,350.00 per year
Character of Use:	Diversified Agriculture

BACKGROUND:

General Lease S-4426, dated April 19, 2021, was awarded to John F. Garcia, Jr. by the Board of Agriculture on December 15, 2020. The lessee has submitted expense receipts for services, materials and supplies related to the clearing of the subject property readying it for planting of various crops according to his Plan of Utilization and Development. The Lessee requests a rental offset of up to two years of annual lease rents in the amount of $4,700.00 in accordance with 4-153-24(2), HAR, although total expenses exceed this amount.

RECOMMENDATION:

That the Board of Agriculture approve the Lessee's request for rental offsets in the amount of $4,700.00, in accordance with the terms and conditions of General Lease No. S-4426 and Section 4-153-24(2), HAR. All related documents are subject to the review and approval as to form by the Department of the Attorney General, and such other terms and conditions as may be prescribed by the Chairperson to best serve the interests of the State.

Respectfully submitted,

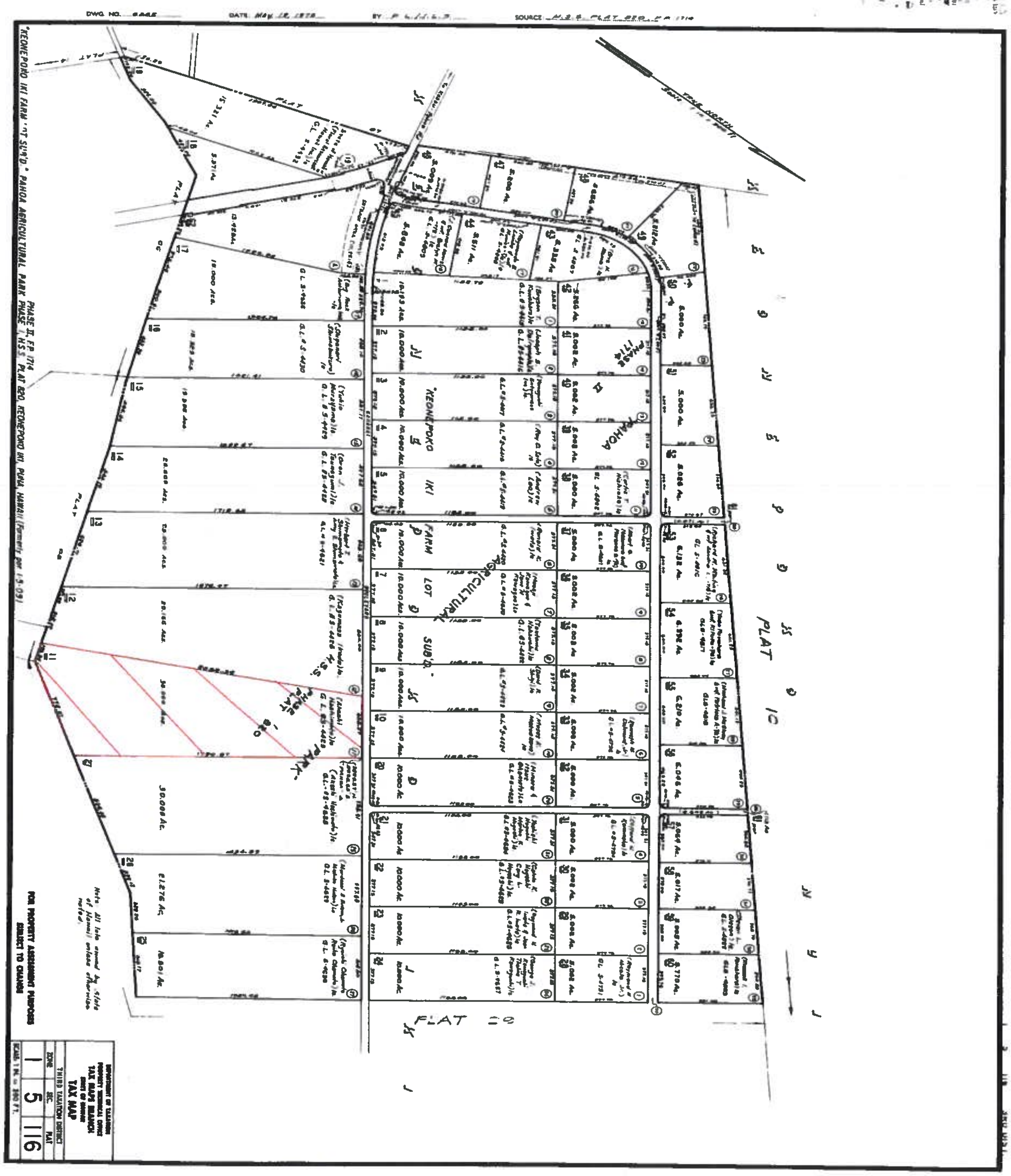
BRIAN KAU, P.E.
Administrator and Chief Engineer,
Agricultural Resource Management Division

Attachments - Exhibit "A"

APPROVED FOR SUBMISSION:

PHYLLIS SHIMABUKURO-GEISER
Chairperson, Board of Agriculture

EXHIBIT "A"



State of Hawaii
Department of Agriculture
Plant Industry Division
Plant Quarantine Branch
Honolulu, Hawaii

May 9, 2022

Board of Agriculture
Honolulu, Hawaii

Subject: Request for Preliminary Review and Approval of the Request from the Hawaii Department of Land and Natural Resources and Hawaii Department of Health to add the Asian Tiger Mosquito, *Aedes albopictus*, Yellow Fever Mosquito, *Aedes aegypti,* and the Southern House Mosquito, *Culex quinquefasciatus*, to the List of Restricted Animals, Part A, by Board Order Pursuant to Hawaii Administrative Rules §4-71-4.2.

I. Background:

In April 2022, the Plant Quarantine Branch (PQB) received permit applications from the Hawaii Department of Health (DOH) and the Hawaii Department of Land and Natural Resources (DLNR) requesting the Hawaii Board of Agriculture (Board) add the Asian Tiger Mosquito, *Aedes albopictus*, Yellow Fever Mosquito, *Aedes aegypti,* and the Southern House Mosquito, *Culex quinquefasciatus*, to the List of Restricted Animals, Part A, by Board Order. The DOH and DLNR requests are included as Attachment A and B respectively.

DLNR is requesting list placement of the Southern House Mosquito, *Culex quinquefasciatus*, for immediate field release applications to suppress mosquito populations in areas where Hawaii fauna are at risk of disease transmission due to the presence of these mosquitoes.

DOH is requesting list placement of all three aforementioned mosquito species for immediate field release applications to suppress mosquito populations in areas where Hawaii residents are at risk of disease transmission due to the presence of these mosquitoes.

II. List Additions By Board Order

Pursuant to Hawaii Revised Statutes (HRS) §150A-6.6, the Board has the authority to adopt administrative rules to make additions to or deletions from the lists required to be maintained under HRS §150A-6.1 through §150A-6.3, which include the List of Restricted Animals, Part A. Changes to the lists can be made without regard to the

notice and public hearing requirements of HRS Chapter 91 provided that there is notice and opportunity for public input regarding additions or deletions to the lists.

Hawaii Administrative Rules (HAR) §4-71-4.2, "Public Input and Notification for Listing," details the specific process that the Board must follow to make a change to the lists maintained by PQB. It requires that, thirty days or more prior to the effective date of the Board order, the Hawaii Department of Agriculture (Department) issue a press release and mail a notice to the Environmental Review Program (formerly the Office of Environmental Quality Control) for publication and to all persons who have made a timely written request of the department for advance notice of the order or the Department's rulemaking proceedings.

The press release is required to have the following information:

a. A statement summarizing the substance of the proposed order which may include examples of the kinds of animals being added to or deleted from the lists required under HRS §150A-6.2;

b. A statement that a copy of the proposed order and the proposed exact changes will be mailed to any interested person who requests a copy upon payment in advance of costs for photocopying, preparing, and mailing the copy;

c. A statement as to where to obtain a copy of the proposed order and the proposed exact changes for inspection, or for pick-up after payment in full of costs for photocopying and preparing; and

d. A statement that the department is soliciting comments regarding the proposed order during the next thirty days, where comments may be forwarded to, and where the proposed order will be discussed.

Pursuant to HAR §4-71-4.2(b), the Department shall consider all oral and written comments and may incorporate those comments in its review of the proposed order by the Advisory Committee on Plants & Animals in a noticed, public meeting.

Pursuant to HAR §4-71-4.2(c), upon approval by the Board at a noticed, public meeting, the order to adopt additions to, or deletions from, the lists of animals shall take effect ten days after the Department gives public notice of the order in a daily or weekly publication of statewide circulation or in separate daily or weekly publications whose combined circulation is statewide.

Due to the urgency of the situations described by DLNR and DOH, PQB will be reviewing both the DOH and DLNR permit applications concurrently with the proposed additions of the three mosquito species to the List of Restricted Animals, Part A via Board Order, provided that the Board preliminarily approves this request. This will allow

the Board to make a final determination on all of the additions to the List of Restricted Animals, Part A via Board Order and permit conditions at a single, future Board meeting.

III. Summary of Proposed Additions to the List of Restricted Animals, Part A

The DOH and DLNR permit applications are requesting the following additions to the List of Restricted Animals (Part A) in Chapter 4-71, HAR:

§4-71-6.5, HAR, List of Restricted Animals (Part A)

Adds Scientific Name: "*Aedes albopictus*" and Common Name "Asian Tiger Mosquito".

Adds Scientific Name: "*Aedes aegypti*" and Common Name "Yellow Fever Mosquito".

Adds Scientific Name: "*Culex quinquefasciatus*" and Common Name "Southern House Mosquito".

Respectfully Submitted,

Becky Azama
Acting Manager, Plant Quarantine Branch

CONCURRED:

Helmuth Rogg, Ph.D.
Administrator, Plant Industry Division

APPROVED FOR SUBMISSION:

Phyllis Shimabukuro-Geiser
Chairperson, Board of Agriculture

B3

https://hdoa.hawaii.gov/wp-content/uploads/2019/08/Plant-and-Non-Domestic-Animal-Quarantine-Non-Domestic-Animal-Import-Rules.pdf

Date: April 6, 2022

To:
Advisory Subcommittee on Entomology

From:
Elizabeth A. Char, MD, Director
Hawaii Department of Health
1250 Punchbowl Street
Honolulu, HI 96813

Gracelda Simmons, Program Manager
Hawaii Department of Health
Vector Control Branch (HDOH VCB)
99-945 Halawa Valley St.
Aiea, HI, 96701

Summary Description of the Requests
In accordance with the provisions of Chapter 150A, Hawaii Revised Statutes, we are requesting to import the following animal commodities:

Commodity	Scientific Name	Quantity
Asian Tiger Mosquitoes (Male Adults)	*Aedes albopictus*	Continued shipments for immediate release.
Yellow Fever Mosquitoes (Male Adults)	*Aedes aegypti*	Continued shipments for immediate release.
Southern House Mosquitoes (Male Adults)	*Culex quinquefasciatus*	Continued shipments for immediate release.

Additionally, we are requesting the listing of *Aedes albopictus*, *Aedes aegypti*, and *Culex quinquefasciatus* mosquito species on the Hawaii Department of Agriculture's (HDOA) List of Restricted Animals Part A given that specific conditions, as outlined and enforced by HDOA, are met at the time of importation. Suggested conditions for importation are included within this application.

Reason for importation:
For immediate field release applications to suppress mosquito populations in areas where Hawaii residents are at risk of disease transmission due to the presence of these mosquitoes.

Shippers:

1) Stephen Dobson, MosquitoMate, Inc.
   2520 Regency Rd.
   Lexington, KY, 40503

2) Verily Life Sciences
   269 E Grand Ave.
   South San Francisco, CA 94080

Importers:

1) Hawaii Department of Health Vector Control Branch - Oahu
   99-945 Halawa Valley St Aiea, HI, 96701, (808) 586-4708

2) Hawaii Department of Health Vector Control Branch - Hilo
   75 Aupuni Street #201, Hilo, HI, 96720, (808) 933-0917

3) Hawaii Department of Health Vector Control Branch - Kona
   79-1015 Haukapila Street Kealakekua, HI, 96750, (808) 322-1507

4) Hawaii Department of Health Vector Control Branch - Maui
   54 South High Street Rm. #301, Wailuku, Maui, HI, 96793, (808) 984-8230

5) Hawaii Department of Health Vector Control Branch - Kauai
   3040 Umi Street, Lihue, HI, 96766, (808) 241-3323

Project:

This is an application for:

-A permit to import three separate male mosquito species: *Aedes albopictus*, *Aedes aegypti*, and *Culex quinquefasciatus*.

-The listing of these mosquito species on the Hawaii Department of Agriculture's (HDOA) List of Restricted Animals Part A given that specific conditions, as outlined and enforced by HDOA, are met at the time of importation. Suggested conditions for importation are included within this application.

As outlined in the suggested conditions for importation, these mosquitoes will either contain the same wild type bacterium (*Wolbachia* spp.) which is already endemic in the three mosquitoes in Hawaii, or will be inoculated with an incompatible bacterium (*Wolbachia* spp.) that is not native to the wild mosquito's current internal fauna. The presence of this different strain of bacteria within the male mosquito's reproductive system will render the imported male mosquitoes unable to successfully mate with wild females found within Hawaii, a process called cytoplasmic incompatibility. Cytoplasmic incompatibility has been used with much success in other parts of the world to reduce mosquito populations and thus reduce the potential of transmission of mosquito vectored diseases. We intend to import male, sexually incompatible mosquitoes for

direct release onto the environment. This process uses cytoplasmic incompatibility to reduce current populations of these species, which are potential vector of human pathogens including Zika virus, dengue virus, chikungunya virus, yellow fever virus, West Nile virus, and lymphatic filariasis. Additionally, these mosquito species are vectors for pathogens to Hawaii's fauna, including pathogens such as avian malaria, avian pox, and dog heartworm. Importing Hawaii lineage mosquitoes which contain the wild type bacterium, will ensure that we can conduct genetic analysis to confirm that the wild *Culex quinquefasciatus* is the wild type originally provided to the collaborators, and that the inoculated mosquitoes are indeed incompatible.

These three species are invasive, disease-spreading mosquitoes that can be found throughout Hawaii. These species were introduced accidentally to Hawaii in either the 1800s or early 1900s. *Aedes albopictus* and *Aedes aegypti* are known vectors of arboviral pathogens such as Zika virus, dengue virus, yellow fever virus, and chikungunya virus. These species are believed to have been the primary vectors during Maui's 2001 dengue virus outbreak, Oahu's 2011 dengue virus outbreak, and Hawaii County's 2015-2016 dengue outbreak, which led to more than 264 cases of the illness. *Culex quinquefasciatus* is also a mosquito species of public health concern as it is known to vector West Nile virus on the US mainland and lymphatic filariasis in other Pacific nations. The species is present on Hawaii, Maui, Molokai, Lanai, Kahoolawe, Oahu, Kauai, and the northwest Hawaiian islands. *Culex quinquefasciatus* can thrive at sea-level to 4800ft in elevation. In Hawaii, these species are also able to transmit pathogens to Hawaii's native forest birds. *Culex quinquefasciatus* is a known vector of avian malaria and *Aedes albopictus* is a vector of avian pox. These diseases have contributed to the extinction of more than half of Hawaii's endemic honeycreepers and continue to pose a risk to the remaining species. Lastly, these mosquito species are known to transmit dog heartworm within pets found throughout Hawaii.

Efforts to suppress these mosquitoes through utilization of traditional vector control methods (*e.g.,* pesticides) are inadequate at a landscape scale, and may be problematic for other non-target state and federally protected invertebrate species including Hawaiian picture-wing flies (*Drosophila* spp.), damselflies (*Megalagrion* spp.), yellow-faced bees (*Hylaeus* spp.) and anchialine pond shrimps (*Vetericaris chaceorum* and *Procaris hawaiana*). Current efforts to control mosquito-vectored disease outbreaks are limited to reducing mosquito breeding site locations and localized applications of various larvicides and adulticides.

On September 6-7, 2016, local, national, and international experts gathered in Hawaii to discuss how to mitigate mosquito-borne diseases. The strategy deemed most favorable in terms of its effectiveness, technical readiness, and safety was *Wolbachia*-based cytoplasmic incompatibility. Cytoplasmic incompatibility results from the presence of a bacterium, *Wolbachia*, in the cells of the mosquito. Many arthropod species, including several native species here in Hawaii, naturally contain strains of *Wolbachia.* Bacteria in the genus *Wolbachia* are a type of arthropod endosymbiont that do not occur in humans or other vertebrates. Approximately 50% of insect species naturally have the bacteria, although many of these insects can survive without *Wolbachia*. Conversely, *Wolbachia*

cannot persist outside of insect cells, as it is an obligate endosymbiont. The largest effect of *Wolbachia* is on mating compatibility between individual insects that carry the bacteria. However, there are secondary effects that are being studied by many labs. These include altered host insect lifespan and reduced vector competence.

In nature, *Wolbachia* are passed from females to their offspring. Different strains of *Wolbachia* have also been introduced into insects in laboratories. If a male mosquito with one type of *Wolbachia* mates with a female mosquito that has a different strain of *Wolbachia* the resulting offspring can be inviable and not develop into mosquito larvae because of a mismatch of cellular signals (loss of the male parental chromosomes) originating from *Wolbachia*. If sufficient numbers, on the order to 10 times the wild population size, of male mosquitoes of a different *Wolbachia* type are released, wild females are more likely to mate with males of a different *Wolbachia* type and are predicted to have far fewer viable offspring. With subsequent releases, this process can significantly suppress the wild population numbers of mosquitoes over the following generations over a geographic area. *Wolbachia* male-based insect control programs have beenhighly successful for reducing local mosquito populations around the world. Results of initial trials in Fresno, California showed decrease of biting *Ae. aegypti* females by 68%, 95%, and 84% during the peak mosquito seasons in 2017, 2018, and 2019 respectively. *Wolbachia* cannot be spread by the released males, because *Wolbachia* are only passed from mother to offspring. It is also worth noting that male mosquitoes do not bite or vector disease.

One way to generate mosquitoes with a different *Wolbachia* type, is by clearing the naturally-occurring *Wolbachia* strain from the mosquitoes using the antibiotic tetracycline. Then *Wolbachia* can be harvested from cells of another insect species (this can be another mosquito or a non-mosquito species) and introduced into the cleared mosquitoes via microinjection. Another method to establish new *Wolbachia* strains is to mate a *Wolbachia*-carrying female insect to males that have been cleared of their naturally-occurring *Wolbachia* via antibiotic treatment. Because *Wolbachia* are maternally inherited (described above), this cross results in all of the offspring inheriting whichever *Wolbachia* strain is contained in the female parent. Incompatible Wolbachia strains can also be naturally present in populations of mosquitoes.

The first shipper listed within this import application, MosquitoMate Inc., holds the US patent, Patent No.: US 7,868,222 B1, for the method of producing an artificial infection in Culicidae species.
(https://patentimages.storage.googleapis.com/55/da/ae/d7cb8b9cb44599/US7868222.pdf)
Additionally, MosquitoMate Inc. offers a commercially available, *Wolbachia* infected male mosquito product for purchase to suppress *Aedes albopictus* mosquito populations via cytoplasmic incompatibility. This product, ZAP Males®, has been reviewed and registered under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). ZAP Males® are a labeled pesticide product with the EPA registration number 89668-4. This product currently has a restriction that only allows for its application in specific states, which does not currently include Hawaii.

(https://www3.epa.gov/pesticides/chem_search/ppls/089668-00004-20171103.pdf)

The second shipper listed is Verily Life Sciences, a CA based company which is in the process of working with a different incompatible *Culex quinquefasciatus.* This company is initiating consultations with the EPA relating to this different *Wolbachia* mosquito and will provide additional information directly to HDOA as needed.

Aedes albopictus, Aedes aegypti, and *Culex quinquefasciatus* mosquito eggs originating from Hawaii stock (aka collected from field sites in Hawaii) have been provided to MosquitoMate and Verily for development and testing of cytoplasmic incompatibility. These mosquitoes have been crossed with female mosquitoes carrying a different *Wolbachia* species as outlined above. These mosquitoes have then been backcrossed with a separate population of mosquitoes originating from Hawaii stock over at least seven generations to ensure Hawaii's wild mosquito genetics are >99% contained within a commercially available product to be applied within Hawaii.

Generations	HI Mosquito Genetics	Crossed MosquitoMate Genetics
0	100.00%	100.00%
1	50.00%	50.00%
2	75.00%	25.00%
3	87.50%	12.50%
4	93.75%	6.25%
5	96.88%	3.13%
6	98.44%	1.56%
7	99.22%	0.78%
8	99.61%	0.39%
9	99.80%	0.20%
10	99.90%	0.10%

On January 17, 2017, the Hawaii Invasive Species Council, an inter-departmental collaboration of the Departments of Land and Natural Resources (DLNR), Agriculture (HDOA), Health (HDOH), Transportation (DOT), Business, Economic Development & Tourism (DBEDT), and the University of Hawaii (UH) passed resolution17-2, specifically pertaining to mosquitoes. Resolution 17-2, entitled, "Supporting Evaluation and Implementation of Technologies For Landscape-Scale Control of Mosquitoes, With a Focus On Mitigating Both Human and Wildlife Health Risks," recognizes that mosquitoes in the State of Hawaii are non-native and an important pest species to control. The resolution supports the implementation of evaluated technologies that are scientifically demonstrated as safe, effective control measures for mosquitoes. (https://dlnr.hawaii.gov/hisc/files/2013/02/HISC-Reso-17-2-signed.pdf).

House Resolution (HR) 297 passed the Hawaii State House in 2019 and further directed "DOA to review the *Aedes aegypti* mosquito with *Wolbachia* bacteria, including *Aedes aegypti* mosquitoes originating from Hawaii stock that could be imported for landscape

scale mosquito control, and render a determination to place it on the appropriate animal import list. Requires DOA, DOH, and DLNR to collaborate on a report to the Legislature with recommendations for appropriate vector control programs." (https://www.capitol.hawaii.gov/session2019/bills/HB297_SD1_.htm)

Additionally, House Resolution (HR) 95 passed the Hawaii State House in 2021 urging DLNR, DOA, DOH and UH to implement a mosquito control program using *Wolbachia* to reduce mosquito population levels throughout the state. (https://www.capitol.hawaii.gov/session2021/bills/HR95_HD1_.htm)

Per Hawaii Revised Statutes §26-13, the Department of Health "shall administer programs designed to protect, preserve, care for, and improve the physical and mental health of the people of the State." Furthermore, Hawaii Administrative Rules (HAR), Title 11, Chapter 26, Subchapter 7 insures that "as a last resort, direct control services may be provided by the Department in special situations due to an imminent vector hazard." The Department is submitting this application to add these three mosquito species to the HDOA List of Restricted Animals Part A to facilitate the importation of these mosquitoes in the event of an imminent vector hazard as outlined within HAR Title 11, Chapter 26. It should be noted that this project has been developed and pursued in close coordination with the Department of Land and Natural Resources. DLNR staff have expertise relating to disease transmission in native wildlife, and their strong support indicates that the use of this approach is not expected to have negative impacts, and in fact is anticipated to benefit rare, threatened and endangered wildlife.

Proposed Required Conditions for Importation via HDOA List of Restricted Animals Part A

Included are proposed conditions, suggested by the HDOH ,Vector Control Branch, that could be required for importation if these three mosquito species are added to the HDOA List of Restricted Animals Part A to ensure any future imports meet safeguards to preserve public health, the environment, and the long-term efficiency of the IIT tool. All of the following suggested requirements would need to be met to obtain importation permitting.

Aedes albopictus, *Aedes aegypti* and *Culex quinquefasciatus*

1. Only mosquitoes originating from a Hawaii stock are allowed for importation.
2. Only mosquitoes containing the same wild-type bacteria as is already present in Hawaii, or a sexually incompatible *Wolbachia* bacteria compared against Hawaii's wild mosquito populations are allowed for importation.
3. Only male mosquitoes are allowed for importation.
4. Only individuals or organizations who have conducted work for EPA registration trials for mosquito biopesticide products and who can provide data on rearing and sorting methodologies are allowed to ship these mosquitoes to Hawaii.
5. Only individuals or organizations listed on the import application are allowed to import/receive these mosquitoes.
6. Only islands with established or incipient wild mosquito populations, as

determined by the Hawaii Department of Health's Vector Control Branch, are allowed to import these mosquitoes.

7. All environmental review processes, including potential Environmental Impact Statements, Environmental Assessments, or other environmental compliance requirements as outlined by State Law and OEQC, must be completed or cited prior to importation.

Specific details for importation

This is an application for:

- A permit to import three separate male, mosquito species: *Aedes albopictus*, *Aedes aegypti*, and *Culex quinquefasciatus*.

- The listing of these mosquito species on the Hawaii Department of Agriculture's (HDOA) List of Restricted Animals Part A given that specific conditions, as outlined and enforced by HDOA, are met at the time of importation. Suggested conditions for importation are included within this application.

Differing *Wolbachia* species will be artificially included within these three mosquito species to facilitate the sexual incompatibility with the wild mosquito species. Within *Aedes albopictus,* the strain of bacterium will be *Wolbachia wPip.* Within *Aedes aegypti,* the strain of bacterium will be *Wolbachia wAlbB.* Within *Culex quinquefasciatus*, the strain of bacterium will be *Wolbachia wAlbA, Wolbachia wAlbB,* or *Wolbachia wPip4.* These *Wolbachia* bacterium are not present within the corresponding species of Hawaii's established mosquito population. The presence of this bacterium will make these males sexually incompatible with the wild, established female mosquitoes. Existing wild-type bacteria strain that may be imported is *wPipV*, which is already found on all of the main Hawaiian islands. Once imported, the male, sexually incompatible males will be released according to EPA and HDOA guidance to suppress the population of the established mosquito populations. Based on the prior use of this technology in California, Florida, and Kentucky, we do not expect releases of these male mosquitoes to have a negative impact on agriculture, the environment, or public health and safety. Existing wild-type bacteria strain that may be imported is *wPipV Culex quinquefasciatus*, and *WAlbA and WAlbB* for *Aedes albopictus* which are already found on all of the main Hawaiian islands.

Persons Responsible

HDOH Director, Elizabeth A. Char, M.D.
1250 Punchbowl Street
Honolulu, Hawaii 96813

Vector Control Manager, Gracelda Simmons
Hawaii Department of Health Vector Control Branch - Oahu
99-945 Halawa Valley St Aiea, HI, 96701, (808) 586-4708

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Alan Takenaka
Hawaii Department of Health Vector Control Branch - Kauai
3040 Umi Street, Lihue, HI, 96766, (808) 241-3323

Locations and Safeguards

All mosquitoes for import will originate from Hawaii biotypes collected from Hawaii. All mosquitoes will be backcrossed for at least 7 generations to ensure >99% Hawaii genetics are contained within the commercially available products to be applied within Hawaii. This backcrossing will also mitigate the risks of infections microorganisms and parasites to the mosquitoes via vertical transmission – thus lowering the risk of the mosquitoes accidentally introducing a new parasite or pathogen. In order for these mosquitoes to acquire and vector a disease, an adult female must blood feed from a disease infected vertebrate, and the pathogen must survive in the mosquito and be injected into another vertebrate during a subsequent blood feeding. As the intended importation of these mosquitoes only includes the importation of male mosquitoes that do not bite or feed on blood, the unintended importation of an acquired pathogen is eliminated. Verification of Hawaii biotypes and *Wolbachia* strains will be conducted on initial shipments of each of the three male mosquito species to verify requirements have been met, in collaboration with University of Hawaii.

These mosquitoes will be imported into Hawaii through the use of commercial cargo flights. Upon reception to Hawaii, the male mosquitoes will be directly released into the laboratory for quality control testing, and into the environment for the purpose of suppressing the wild mosquito populations. These releases will be performed by individuals or organizations certified to apply these mosquito pesticide products to ensure that the product will be applied properly according to the recommended guidelines.

MosquitoMate and Verily will regularly sample release containers by releasing the contents into lab cages and then examining mosquito sex and number. There is an EPA reviewed value of 1 female release per 250,000 males with the MosquitoMate product. A similar value is likely to be estimated for *Culex quinquefasciatus* given that similar automation, engineering and machine learning technology is being applied to sex sorting. However, MosquitoMate and Verily have not previously identified a single female in a release container during the course of the Puerto Rico or Fresno projects. In another example, a published study estimates the probability at less than 1 female per 200 million males (Crawford JE, Clarke DW, Criswell V, Desnoyer M, Cornel D, Deegan B, et al. Efficient production of male Wolbachia-infected *Aedes aegypti* mosquitoes enables large-scale suppression of wild populations. Nat Biotechnol. 2020;38(4):482-92.) To date, PCR monitoring of mosquitoes collected from release field sites have not identified any ZAP infected females.

At least once per year, MosquitoMate and Verily will also conduct longevity and competitiveness studies, comparing the mosquitoes proposed for releases and wild type males. Data from previous trials demonstrate ZAP mosquito longevity and competitiveness to be at least equal to Wild Type males. In addition to Hawaii's import requirements, the shipper and/or receiver will obtain additional permits as required by federal or state agencies.

Wolbachia is an obligate endosymbiont and cannot survive outside of the host invertebrate. *Wolbachia* strains already exist in Hawaii in a range of invertebrates in the wild, including mosquitoes. The presence of Wolbachia endosymbionts is the normal state for 40% to 60% of Arthropods and does not represent an unusual or pathogenic bacterial infection. Wolbachia are not capable of infecting human cells. MosquitoMate and Verily will perform PCR testing on the mosquitoes to confirm the presence of the correct *Wolbachia* bacterium within the shipment lineage to ensure cytoplasmic incompatibility.

The likelihood that introduced strains of *Wolbachia* would become the dominant strains in the environment is highly unlikely. Replacing the dominant *Wolbachia* strain has been done purposefully in the environment for projects that are separate from the approach we are proposing (such as by the World Mosquito Program in Australia and other nations). To clarify, HDOH is NOT proposing a World Mosquito Program type project where the goal is to intentionally force a different dominant *Wolbachia* strain into the wild mosquitoes in the environment and change vector competence of the wild population. However, in these types of programs, they have to release 4 million mixed male AND female mosquitoes in a given location to force a new *Wolbachia* strain to become the dominate strain over an area of 66 km^2. Given the aforementioned EPA reviewed value of 1 female release per 250,000 males with the MosquitoMate product, such an outcome is not expected to occur.

If, somehow population replacement were to occur (despite the estimated 1 female release per 250,000 males) HDOH would cease releases as the released males would

then be able to mate with the wild females with the established *Wolbachia* species. The outcome of this would be that the mosquito species that already exists in Hawaii would continue to exist in the wild, just with a different *Wolbachia* bacteria. We do not anticipate a different *Wolbachia* bacteria having any new or negative effects on human health or the environment.

HDOH approves of this approach being applied at both a very small scale (in remote forest habitat as proposed by DLNR) or at a very large scale (across urban areas and island wide) so long as recommended application guidelines are followed. Compared to conventional chemical pesticides, this approach has zero anticipated non-target impacts on human health or the environment. The scale and scope of applications will likely vary depending on the target mosquito species, proposed area of application, the funding available and mosquito prevalence. As with any pesticide product, if you do not eradicate the species of concern, they will rebound if you stop using the pesticide product. However, we view this as a beneficial aspect of the project as we also know we can stop the process at any time should the Vector Control Branch determine a different approach is preferable to achieve mosquito control objectives.

Data collection will occur during releases using State general funds as well as potential federal funds from partner agencies (CDC or NIH). As the application of the pesticide product is intended to either control or eradicate one of the three target mosquito species, monitoring will include extensive mosquito population surveillance by Vector Control Branch staff and partners following releases to ensure that populations are reduced. HDOH already conducts this type of surveillance monitoring across the state, and would deploy staff and resources as necessary to carry out a pre- and post-monitoring if undertaking a control project for the benefit of public health. Depending on the length of the project, *Wolbachia* genetic monitoring will also occur.

In addition to Hawaii's import requirements, the shipper and/or receiver will obtain additional permits as required by federal or state agencies.

Method of Disposition
Any dead imported mosquitoes will be disposed of as municipal waste.

Abstraction of Organism
Culicidae species are sexually reproducing species. Minimum generation times vary but are approximately three weeks. Mature adults are up to approximately a centimeter in length and can live for a month to a few months. Adult mosquitoes range from 2.0 to 10.0 mm in size with males being smaller than females on average. Mosquito life cycles are well understood for most species, including all those established in Hawaii.

Larvae feed on organic material found in pools of water. Both adult males and females feed on water that contains carbohydrates (water with sap or nectar). Only mature females of certain species seek out and feed on vertebrate blood prior to egg laying. This blood feeding process allows for the transmission of pathogens and parasites.

These species rely on pools of water with organic material for the growth of larvae. Only adult females bite, as they require blood meals from vertebrate hosts to develop their eggs.

Potential Impact to the Environment
These three species are already well established in the wild on all of the main islands in Hawaii. *Aedes albopictus* and *Culex quinquefasciatus* are established statewide and *Aedes aegypti* is well establish on Hawaii's Big Island. An additional three other "biting" non-native mosquito species have also become established: *Ae. japonicus*, *Wyeomyia mitchelli*, and *Ae. vexans*.

Wolbachia are not infectious to humans and are vertically transmitted through the eggs from one generation to another. The *Wolbachia* bacteria are obligate endosymbionts and can only survive inside the insect host's cytoplasm. A mosquito transinfected with a different strain of *Wolbachia* that results in cytoplasmic incompatibility would not be able to successfully reproduce with a wild mosquito due to cytoplasmic incompatibility. Therefore, if individual mosquitoes did become temporarily established, then they will quickly die off over the following generations because of cytoplasmic incompatibility with wild mosquitoes of the same species, with which they would be expected to encounter and mate.

Through the importation we intend to only import male mosquitoes. The sex separation can be performed in a variety of manners including through computer recognition and separation of males and females or through pupal sorting of males and females. However, if both sexes of transinfected mosquito were to be accidently released, they are unlikely to maintain a breeding population of a transinfected mosquito. *Wolbachia* invasions into populations require a critical threshold frequency of infection that needs to be overcome before a novel *Wolbachia* infection can spread into a population. The *Wolbachia* infection rate must exceed 20-45% before it can spread and become established. This is evident in large scale releases such as in Cairns, Australia, where millions of transinfected mosquitoes (both sexes) with *Wolbachia* are released into the environment to control disease transmission, yet they do not easily reach fixation in the wild. If transinfected mosquitoes were to become established, the establishment is likely to be spatially localized due to incompatibility with neighboring mosquito populations.

Potential Impacts of Importation
Pro: Importation of male mosquitoes will allow the implementation of an evaluated technology that has been scientifically demonstrated as a safe and effective control method for mosquitoes on a landscape-scale. These are mosquitoes that are widespread in Hawaii and which have negative impacts to humans, wildlife, and pets. This implementation could be a valuable future resource for mosquito management applications, including eradicating incipient mosquito infestations on neighbor islands, and preventing human disease outbreaks. This would have a wide range of positive effects on human health, the economy, and tourism in Hawaii. Additionally, the application of traditional chemical controls for mosquitoes in both urban and natural

areas is impractical and causes unacceptable non-target impacts, whereas IIT carries no non-target risks to native species, humans or the environment. Furthermore, mosquitoes were first introduced to the Hawaiian Islands in the 1800s, and while they are used opportunistically as prey items, no species native to Hawai‘i are dependent on their presence for survival. The control of mosquito populations in urban or natural area-interfaces would thus cause no negative impacts on Hawaiian species.

Con: It is hard to imagine any negative effects since the species is already established in Hawaii. Importing these organisms will not have any foreseeable beneficial effect to this mosquito species already found in Hawaii. The introduction of, for example, increased genetic variation within the mosquito species will be minimized by crossing the lines to mosquitoes originating from Hawaii.

The presence of unintended accompanying microbiota is minimized by the sterile laboratory rearing conditions used. These mosquitoes have been maintained for many generations in the lab environment and have not had the opportunity to obtain pathogens from the wild from blood feeding. The presence of intended microbiota, the *Wolbachia,* potentially has very positive effects on the societal health, the suppression of human disease vectored by mosquitoes, the environment, via population suppression of mosquitoes that vector avian pathogens, and the economy, through the potential increased tourism and lessened disease burden.

This mosquito species is already well established in Hawaii, as are many different strains of *Wolbachia*. MosquitoMate and Verily have a demonstrated track record of success utilizing sex-sorting methods which are highly effective. In the event that technical difficulties did occur during sex-sorting methods, because of cytoplasmic incompatibility, the escape of female mosquitoes carrying a new *Wolbachia* strain is not expected to be stable over the following generations. Laboratory reared females outcrossing to locally established wild male mosquitoes will result in cytoplasmic incompatibility and the failure of offspring to develop.

There is an extensive body of literature surrounding this mosquito species, its impact upon Hawaii, and Wolbachia-mediated cytoplasmic incompatibility.

Information on *Wolbachia,* with a focus on cytoplasmic incompatibility within mosquitoes:

Atyame, C. M., Cattel, J., Lebon, C., Flores, O., Dehecq, J.-S., Weill, M., Gouagna, L. C. & Tortosa, P. (2015) Wolbachia-based population control strategy targeting*Culex quinquefasciatus* mosquitoes proves efficient under semi-field conditions. *PLoS ONE* 10, e0119288.

Atyame, C. M., Labbé, P., Lebon, C., Weill, M., Moretti, R., Marini, F., Gouagna, L.C., Calvitti, M. & Tortosa, P. (2016) Comparison of irradiation and Wolbachia based approaches for sterile-male strategies

targeting *Aedes albopictus*. *PLoS ONE* 11, e0146834.

Barton, N. H., & Turelli, M. (2011). Spatial waves of advance with bistable dynamics:cytoplasmic and genetic analogues of Allee effects. The American Naturalist, 178(3), E48-E75.

Blagrove, M. S., Arias-Goeta, C., Failloux, A. B., & Sinkins, S. P. (2012). Wolbachia strain wMel induces cytoplasmic incompatibility and blocks dengue transmission in*Aedes albopictus*. *Proceedings of the National Academy of Sciences*, *109*(1), 255-260.

Callaini, G., Dallai, R., & Riparbelli, M. G. (1997). Wolbachia-induced delay of paternal chromatin condensation does not prevent maternal chromosomes from entering anaphase in incompatible crosses of *Drosophila simulans*. *Journal of CellScience*, *110*(2), 271-280.

Dobson, S. L., Marsland, E. J., & Rattanadechakul, W. (2001). Wolbachia-induced cytoplasmic incompatibility in single-and superinfected *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology*, *38*(3), 382-387.

Hamm, C. A., Begun, D. J., Vo, A., Smith, C. C., Saelao, P., Shaver, A. O., ... & Turelli, M. (2014). Wolbachia do not live by reproductive manipulation alone: infection polymorphism in *Drosophila suzukii* and *D. subpulchrella*. Molecular Ecology, 23(19), 4871-4885.

Hoffmann, A. A., Montgomery, B. L., Popovici, J., Iturbe-Ormaetxe, I., Johnson, P.H., Muzzi, F., ... & Cook, H. (2011). Successful establishment of Wolbachia in Aedes populations to suppress dengue transmission. *Nature*, 476(7361), 454.

Hoffmann, A. A., Ross, P. A., and Rašić, G. (2015) Wolbachia strains for disease control: ecological and evolutionary considerations. *Evolutionary Applications*, 8(8),751-768.

Jiggins FM. (2017) The spread of Wolbachia through mosquito populations. *PLoSBiology* 15(6):e2002780.

Laven, H. (1967). Eradication of Culex pipiens fatigans through cytoplasmicincompatibility. *Nature*, *216*(5113), 383-384.

Liao W, Atkinson CT, LaPointe DA, Samuel MD (2017) Mitigating Future Avian Malaria Threats to Hawaiian Forest Birds from Climate Change. *PLoS ONE* 12(1):e0168880.

Mains, J. W., Brelsfoard, C. L., Rose, R. I. & Dobson, S. L. (2016) Female adult Aedes albopictus suppression by Wolbachia-infected male mosquitoes. *ScientificReports* 6, 33846.

Stouthamer, R., Breeuwer, J. A., & Hurst, G. D. (1999). Wolbachia pipientis: microbial manipulator of arthropod reproduction. *Annual Reviews in Microbiology*,*53*(1), 71-102.

Sinkins, S. P., Braig, H. R., & O'Neill, S. L. (1995). Wolbachia superinfections and the expression of cytoplasmic incompatibility. *Proceedings of the Royal Society ofLondon B: Biological Sciences*, *261*(1362), 325-330.

Tram, U., & Sullivan, W. (2002). Role of delayed nuclear envelope breakdown andmitosis in Wolbachia-induced cytoplasmic incompatibility. *Science*, *296*(5570), 1124-1126.

Waltz, E. (2016) US reviews plan to infect mosquitoes with bacteria to stop disease. *Nature* 533, 450-451.

Weinert, L. A., Araujo-Jnr, E. V., Ahmed, M. Z. & Welch, J. J. (2015) The incidenceof bacterial endosymbionts in terrestrial arthropods. *Proc. R. Soc. B* 282, 20150249.

Werren, J. H., Baldo, L., & Clark, M. E. (2008). Wolbachia: master manipulators of invertebrate biology. *Nature Reviews Microbiology*, 6(10), 741.

Zug, R., & Hammerstein, P. (2012). Still a host of hosts for Wolbachia: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. *PLoSONE*, *7*(6), e38544.

***Wolbachia* in Hawaii:**

Atkinson, C. T., W. Watcher-Weatherwax, and D. A. LaPointe. (2016) Genetic diversity of *Wolbachia* endosymbionts in *Culex quinquefasciatus* from Hawaii,Midway Atoll and American Samoa. Technical Report HCSU-074.

Bennett, G. M., Pantoja, N. A., & O'Grady, P. M. (2012). Diversity and phylogeneticrelationships of *Wolbachia* in *Drosophila* and other native Hawaiian insects. *Fly*, *6*(4), 273-283.

Other References:

Anonymous. (2017). To Restore a Mosquito-Free Hawaii. Summary Report of the Workshop to Formulate Strategic Solutions for a "Mosquito-Free Hawaii". Available at: "http://www.cpc-foundation.org/uploads/7/6/2/6/76260637/report_on_mosquito_free_workshop.pdf."

Anonymous. (2020). Three great years of Debug Fresno. Available at: "https://blog.debug.com/2020/01/three-great-years-of-debug-fresno.html"

Dame, D. A., Curtis, C. F., Benedict, M. Q., Robinson, A. S., & Knols, B. G. (2009).Historical applications of induced sterilisation in field populations of mosquitoes. *Malaria Journal,* 8(2), S2.

Johnston, D., *et al*. (2016). Notes from the field: outbreak of locally acquired cases ofdengue fever—Hawaii, 2015. *MMWR. Morbidity and Mortality Weekly Report*, 65(2); 34–35.

Kauffman, E., Payne, A., Franke, M. A., Schmid, M. A., Harris, E., & Kramer, L. D. (2017). Rearing of Culex spp. and Aedes spp. mosquitoes. *Bio-*

Protocol, 7(17).

Mendenhall, I. H., S. A. Tello, L. A. Neira, L. F. Castillo, C. B. Ocampo, and D. M. Wesson. (2012) Host Preference of the Arbovirus Vector Culex Erraticus (Diptera: Culicidae) at Sonso Lake, Cauca Valley Department, Colombia. *Journal of MedicalEntomology* 49 (5): 1092–1102

O'Neill, S. L.; Ryan, P. A.; Turley, A. P.; Wilson, G.; Retzki, K.; Iturbe-Ormaetxe, I.;Dong, Y.; Kenny, N.; Paton, C. J. & Ritchie, S. A. (2018) Scaled deployment of Wolbachia to protect the community from Aedes transmitted arboviruses. *Gates Open Research* 2: 1-18

Richmond JY, McKinney RW, eds. (1999). *Biosafety in Microbiological andBiomedical Laboratories* (4th ed.). ISBN 0-7881-8513-6.

Scott, T. W. (2005). Containment of arthropod disease vectors. *ILAR Journal*, 46(1),53-61.

Takken, W., & Verhulst, N. O. (2013). Host preferences of blood-feeding mosquitoes. *Annual Review of Entomology*, 58, 433-453.

[USFWS] U.S. Fish and Wildlife Service. (2006) Endangered and Threatened Wildlife and Plants; Determination of Status for 12 Species of Picture-Wing Fliesfrom the Hawaiian Islands. *Federal Register* 71 (89): 26835-26852.

Winchester, J. & Kapan, D. (2013). History of *Aedes* Mosquitoes in Hawaii. *Journal of the American Mosquito Control Association* 29(2): 154-163.

Subcommittee on Entomology *Attachment B*
G. Simmons & L. Wells – Hawaii Department of Health
D. Smith & C. King – Hawaii Department of Land and Natural Resources

https://hdoa.hawaii.gov/wp-content/uploads/2019/08/Plant-and-Non-Domestic-Animal-Quarantine-Non-Domestic-Animal-Import-Rules.pdf

Date:4/1/2022

To:
Advisory Subcommittee on Entomology

From:
Suzanne Case
Department of Land and Natural Resources
1151 Punchbowl Street, Honolulu, HI 96813

David G. Smith
Hawaii Department of Land and Natural Resources, Division of Forestry and Wildlife
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Summary Description of the Requests
In accordance with the provisions of Chapter 150A, Hawaii Revised Statutes, we are requesting to import the following animal commodities:

Commodity	**Scientific Name**	**Quantity**
Southern House Mosquitoes (Male Adults)	*Culex quinquefasciatus*	Continued shipments for immediate release.

Additionally, we are requesting the listing of *Culex quinquefasciatus* mosquito species on the Hawaii Department of Agriculture's (HDOA) List of Restricted Animals Part A given that specific conditions, as outlined and enforced by HDOA, are met at the time of importation. Suggested conditions for importation are included within this application.

Reason for importation:
For immediate field release applications to suppress mosquito populations in areas where Hawaii fauna are at risk of disease transmission due to the presence of these mosquitoes.

Shippers:
1) Stephen Dobson, MosquitoMate, Inc.
   2520 Regency Rd., Lexington, KY, 40503

2) Verily Life Sciences
   269 E Grand Ave, South San Francisco, CA 94080

Importers:

1) DLNR Waimano Baseyard – Hawaii Invertebrate Program - Oahu
   2680 Waimano Home Road, Pearl City, HI 96782, (808) 266-7989
2) Kaua'i Branch, Division of Forestry & Wildlife, 3060 Eiwa Street Rm. 306, Lihue, HI 96766. (808) 274-3433
3) O'ahu Branch, Division of Forestry & Wildlife, 2135 Makiki Heights Drive, Honolulu, HI 96822. (808) 973-9778
4) Maui (& Moloka'i) Branch, Division of Forestry & Wildlife, 1955 Main Street, Room 301, Wailuku, HI 96793. (808) 984-8100
5) Hawai'i Branch, Division of Forestry & Wildlife, 19 E. Kawili Street, Hilo, HI 96720. (808) 974-4221

Project:
This is an application for:

- A permit to import male *Culex quinquefasciatus* mosquito species.
- The listing of *Culex quinquefasciatus* mosquito species on the Hawaii Department of Agriculture's (HDOA) List of Restricted Animals Part A given that specific conditions, as outlined and enforced by HDOA, are met at the time of importation. Suggested conditions for importation are included within this application.

As outlined in the suggested conditions for importation, these mosquitoes will either contain the same wild type bacterium (*Wolbachia* spp.) which is already endemic in *Culex quinquefasciatus* mosquitoes in Hawaii, or will be inoculated with an incompatible bacterium (*Wolbachia* spp.) that is not native to the wild mosquito's current internal fauna. The presence of this different strain of bacteria within the male mosquito's reproductive system will render the imported male mosquitoes unable to successfully mate with wild females found within Hawaii, a process called cytoplasmic incompatibility. Cytoplasmic incompatibility has been used with much success in other parts of the world to reduce mosquito populations and thus reduce the potential of transmission of mosquito vectored diseases. We intend to import male, sexually incompatible mosquitoes for direct release onto the environment. This process uses cytoplasmic incompatibility to reduce current populations of this pest mosquito species, which are vectors for pathogens to Hawaii's fauna, including pathogens such as avian malaria, and which can vector West Nile virus, and lymphatic filariasis to humans. Importing Hawaii lineage mosquitoes which contain the wild type bacterium, will ensure that we can conduct genetic analysis to confirm that the wild *Culex quinquefasciatus* is the wild type originally provided to the collaborators, and that the inoculated mosquitoes are indeed incompatible.

Culex quinquefasciatus is an invasive, disease-spreading mosquito that has dispersed across the Hawaiian islands since its accidental introduction in the 1800s. The species is present on Hawaii, Maui, Molokai, Lanai, Kahoolawe, Oahu, Kauai, and the northwest

Hawaiian islands. *Culex quinquefasciatus* can thrive at sea-level to 4800ft in elevation. In Hawaii, the mosquito is able to transmit pathogens to native forest birds. The spread of avian malaria, in particular, has contributed to the extinction of more than half of Hawaii's endemic honeycreepers and continues to pose a risk to the remaining species. *Culex quinquefasciatus* is also known to transmit dog heartworm within pets found throughout Hawaii, and is a concern to human health given its ability to vector West Nile virus on the US mainland and lymphatic filariasis in other Pacific nations.

Efforts to suppress *Culex quinquefasciatus* through utilization of traditional vector control methods (*e.g.,* pesticides) are inadequate at a landscape scale, and may be problematic for other non-target state and federally protected invertebrate species including Hawaiian picture-wing flies (*Drosophila* spp.), damselflies (*Megalagrion* spp.), yellow-faced bees (*Hylaeus* spp.) and anchialine pond shrimps (*Vetericaris chaceorum* and *Procaris hawaiana*). Current efforts to control mosquito-vectored disease outbreaks are limited to reducing mosquito breeding site locations and localized applications of various larvicides and adulticides.

On September 6-7, 2016, local, national, and international experts gathered in Hawaii to discuss how to mitigate mosquito-borne diseases. The strategy deemed most favorable in terms of its effectiveness, technical readiness, and safety was *Wolbachia*-based cytoplasmic incompatibility. Cytoplasmic incompatibility results from the presence of a bacterium, *Wolbachia*, in the cells of the mosquito. Many arthropod species, including several native species here in Hawaii, naturally contain strains of *Wolbachia.* Bacteria in the genus *Wolbachia* are a type of arthropod endosymbiont that do not occur in humans orother vertebrates. Approximately 50% of insect species naturally have the bacteria, although many of these insects can survive without *Wolbachia*. Conversely, *Wolbachia* cannot persist outside of insect cells, as it is an obligate endosymbiont. The largest effect of *Wolbachia* is on mating compatibility between individual insects that carry the bacteria. However, there are secondary effects that are being studied by many labs. These include altered host insect lifespan and reduced vector competence.

In nature, *Wolbachia* are passed from females to their offspring. Different strains of *Wolbachia* have also been introduced into insects in laboratories. If a male mosquito with one type of *Wolbachia* mates with a female mosquito that has a different strain of *Wolbachia* the resulting offspring can be inviable and not develop into mosquito larvae because of a mismatch of cellular signals (loss of the male parental chromosomes) originating from *Wolbachia*. If sufficient numbers, on the order to 10 times the wild population size, of male mosquitoes of a different *Wolbachia* type are released, wild females are more likely to mate with males of a different *Wolbachia* type and are predicted to have far fewer viable offspring. With subsequent releases, this process can significantly suppress the wild population numbers of mosquitoes over the following generations over a geographic area. *Wolbachia* male-based insect control programs have beenhighly successful for reducing local mosquito populations around the world. Results of initial trials in Fresno, California showed decrease of biting *Ae. aegypti* females by 68%, 95%, and 84% during the peak mosquito seasons in 2017, 2018, and

2019 respectively. *Wolbachia* cannot be spread by the released males, because *Wolbachia* are only passed from mother to offspring. It is also worth noting that male mosquitoes do not bite or vector disease.

One way to generate mosquitoes with a different *Wolbachia* type, is by clearing the naturally-occurring *Wolbachia* strain from the mosquitoes using the antibiotic tetracycline. Then *Wolbachia* can be harvested from cells of another insect species (this can be another mosquito or a non-mosquito species) and introduced into the cleared mosquitoes via microinjection. Another method to establish new *Wolbachia* strains is to mate a *Wolbachia*-carrying female insect to males that have been cleared of their naturally-occurring *Wolbachia* via antibiotic treatment. Because *Wolbachia* are maternally inherited (described above), this cross results in all of the offspring inheriting whichever *Wolbachia* strain is contained in the female parent. Incompatible Wolbachia strains can also be naturally present in populations of mosquitoes.

The first shipper listed within this import application, MosquitoMate Inc., holds the US patent, Patent No.: US 7,868,222 B1, for the method of producing an artificial infection in Culicidae species.
(https://patentimages.storage.googleapis.com/55/da/ae/d7cb8b9cb44599/US7868222.pdf)
Additionally, MosquitoMate Inc. offers a commercially available, *Wolbachia* infected male mosquito product for purchase to suppress *Aedes albopictus* mosquito populations via cytoplasmic incompatibility. This product, ZAP Males®, has been reviewed and registered under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). ZAP Males® are a labeled pesticide product with the EPA registration number 89668-4. This product currently has a restriction that only allows for its application in specific states, which does not currently include Hawaii. We reference this, as it is the only registrant in the US currently with a *Wolbachia* mosquito product currently in process of registration with the EPA.
(https://www3.epa.gov/pesticides/chem_search/ppls/089668-00004-20171103.pdf)

The second shipper listed is Verily Life Sciences, a CA based company which is in the process of working with a different incompatible *Culex quinquefasciatus.* This company is initiating consultations with the EPA relating to this different *Wolbachia* mosquito and will provide additional information directly to HDOA as needed.

Culex quinquefasciatus mosquito eggs originating from Hawaii stock (aka collected from field sites in Hawaii) have been provided to MosquitoMate and Verily for development and testing of cytoplasmic incompatibility. These mosquitoes have been crossed with female mosquitoes carrying a different *Wolbachia* species as outlined above. These mosquitoes have then been backcrossed with a separate population of mosquitoes originating from Hawaii stock over at least seven generations to ensure Hawaii's wild mosquito genetics are >99% contained within a commercially available product to be applied within Hawaii.

Generations	HI Mosquito Genetics	Crossed MosquitoMate Genetics
0	100.00%	100.00%
1	50.00%	50.00%
2	75.00%	25.00%
3	87.50%	12.50%
4	93.75%	6.25%
5	96.88%	3.13%
6	98.44%	1.56%
7	99.22%	0.78%
8	99.61%	0.39%
9	99.80%	0.20%
10	99.90%	0.10%

On January 17, 2017, the Hawaii Invasive Species Council, an inter-departmental collaboration of the Departments of Land and Natural Resources (DLNR), Agriculture (HDOA), Health (HDOH), Transportation (DOT), Business, Economic Development & Tourism (DBEDT), and the University of Hawaii (UH) passed resolution17-2, specifically pertaining to mosquitoes. Resolution 17-2, entitled, "Supporting Evaluation and Implementation of Technologies For Landscape-Scale Control of Mosquitoes, With a Focus On Mitigating Both Human and Wildlife Health Risks," recognizes that mosquitoes in the State of Hawaii are non-native and an important pest species to control. The resolution supports the implementation of evaluated technologies that are scientifically demonstrated as safe, effective control measures for mosquitoes. (https://dlnr.hawaii.gov/hisc/files/2013/02/HISC-Reso-17-2-signed.pdf).

House Resolution (HR) 297 passed the Hawaii State House in 2019 and further directed "DOA to review the *Aedes aegypti* mosquito with *Wolbachia* bacteria, including *Aedes aegypti* mosquitoes originating from Hawaii stock that could be imported for landscape scale mosquito control, and render a determination to place it on the appropriate animal import list. Requires DOA, DOH, and DLNR to collaborate on a report to the Legislature with recommendations for appropriate vector control programs." (https://www.capitol.hawaii.gov/session2019/bills/HB297_SD1_.htm)

Additionally, House Resolution (HR) 95 passed the Hawaii State House in 2021 urging DLNR, DOA, DOH and UH to implement a mosquito control program using *Wolbachia* to reduce mosquito population levels throughout the state. (https://www.capitol.hawaii.gov/session2021/bills/HR95_HD1_.htm)

It should be noted that this project has been developed with the full support of, and will be implemented in close coordination with, the Hawaii Department of Health Vector Control Branch. Per Hawaii Revised Statutes §26-13, the Department of Health "shall administer programs designed to protect, preserve, care for, and improve the physical and mental health of the people of the State." DOH has the authority in Hawaii relating to mosquitoes and public health, and their staff have decades of expertise to implement

mosquito surveillance, control and abatement programs.

Proposed Required Conditions for Importation via HDOA List of Restricted Animals Part A

Included are proposed conditions, suggested in collaboration with the HDOH Vector Control Branch, that could be required for importation if *Culex quinquefasciatus* mosquitoes are added to the HDOA List of Restricted Animals Part A to ensure any future imports meet safeguards to preserve public health, the environment, and the long-term efficiency of the IIT tool. All of the following suggested requirements would need to be met to obtain importation permitting.

Culex quinquefasciatus

1. Only mosquitoes originating from a Hawaii stock are allowed for importation.
2. Only mosquitoes containing the same wild-type bacteria as is already present in Hawaii, or a sexually incompatible *Wolbachia* bacteria compared against Hawaii's wild mosquito populations are allowed for importation.
3. Only adult male mosquitoes are allowed for importation.
4. Only individuals or organizations who have conducted work for EPA registration trials for mosquito biopesticide products and who can provide data on rearing and sorting methodologies are allowed to ship these mosquitoes to Hawaii.
5. Only individuals or organizations listed on the import application are allowed to import/receive these mosquitoes.
6. Only islands with established or incipient wild mosquito populations, as determined by the Hawaii Department of Health's Vector Control Branch, are allowed to import these mosquitoes.
7. All environmental review processes, including potential Environmental Impact Statements, Environmental Assessments, or other environmental compliance requirements as outlined by State Law and OEQC, must be completed or cited prior to importation.

Specific details for importation
This is an application for:

- A permit to import male, mosquito species: *Culex quinquefasciatus*.
- The listing of these mosquito species on the Hawaii Department of Agriculture's (HDOA) List of Restricted Animals Part A given that specific conditions, as outlined and enforced by HDOA, are met at the time of importation. Suggested conditions for importation are included within this application.

Within *Culex quinquefasciatus*, the strain of incompatible bacterium will be *Wolbachia wAlbA, Wolbachia wAlbB,* or *Wolbachia wPip4.* These *Wolbachia* bacterium are not present within the corresponding species of Hawaii's established mosquito population. The presence of this bacterium will make these males sexually incompatible with the

wild, established female mosquitoes. Once imported, the male, sexually incompatible males will be released according to EPA and HDOA label directions to suppress the population of the established mosquito populations. Based on the prior use of this technology in California, Florida, and Kentucky, there are no data to suggest releases of these male mosquitoes to have a negative impact on agriculture, the environment, or public health and safety. Existing wild-type bacteria strain that may be imported is wPipV, which is already found on all of the main Hawaiian islands.

Persons Responsible

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DOFAW Administrator, David Smith
DOFAW Entomologist, Cynthia King
Department of Land and Natural Resources – Oahu
1151 Punchbowl Street, Honolulu, HI 96813

DLNR-DOFAW, Hawaii Invertebrate Program Captive Propagation Facility - Oahu
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Hawai'i Branch, Division of Forestry & Wildlife, 19 E. Kawili Street, Hilo, HI 96720. (808) 974-4221

Locations and Safeguards

All mosquitoes for import will originate from Hawaii biotypes collected from Hawaii. All mosquitoes will be backcrossed for at least 7 generations to ensure >99% Hawaii genetics are contained within the commercially available products to be applied within Hawaii. This backcrossing will also mitigate the risks of infections microorganisms and parasites to the mosquitoes via vertical transmission – thus lowering the risk of the mosquitoes accidentally introducing a new parasite or pathogen. In order for these mosquitoes to acquire and vector a disease, an adult female must blood feed from a disease infected vertebrate, and the pathogen must survive in the mosquito and be

injected into another vertebrate during a subsequent blood feeding. As the intended importation of these mosquitoes only includes the importation of male mosquitoes that do not bite or feed on blood, the unintended importation of an acquired pathogen is eliminated. Verification of Hawaii biotypes and *Wolbachia* strains will be conducted on initial shipments of male mosquitoes to verify requirements have been met, in collaboration with University of Hawaii and Department of Health.

These mosquitoes will be imported into Hawaii through the use of commercial cargo flights. Upon reception to Hawaii, the male mosquitoes will be directly released into the laboratory for quality control testing, and into the environment for the purpose of suppressing the wild mosquito populations. These releases will be performed by individuals or organizations certified to apply these mosquito pesticide products to ensures that the product will be applied properly according to the recommended guidelines.

MosquitoMate and Verily will regularly sample release containers by releasing the contents into lab cages and then examining mosquito sex and number. There is an EPA reviewed value of 1 female release per 250,000 males with the MosquitoMate product. A similar value is likely to be estimated for *Culex quinquefasciatus* given that similar automation, engineering and machine learning technology is being applied to sex sorting. MosquitoMate and Verily have not previously identified a female in a single release container during the course of the Puerto Rico or Fresno projects. In another example, a published study estimates the probability at less than 1 female per 200 million males (Crawford JE, Clarke DW, Criswell V, Desnoyer M, Cornel D, Deegan B, et al. Efficient production of male Wolbachia-infected *Aedes aegypti* mosquitoes enables large-scale suppression of wild populations. Nat Biotechnol. 2020;38(4):482-92.) To date, PCR monitoring of mosquitoes collected from release field sites have not identified any ZAP infected females.

At least once per year, MosquitoMate and Verily will also conduct longevity and competitiveness studies, comparing the mosquitoes proposed for releases and wild type males. Data from previous trials demonstrate ZAP mosquito longevity and competitiveness to be at least equal to Wild Type males. In addition to Hawaii's import requirements, the shipper and/or receiver will obtain additional permits as required by federal or state agencies.

Wolbachia is an obligate endosymbiont and cannot survive outside of the host invertebrate. *Wolbachia* strains already exist in Hawaii in a range of invertebrates in the wild, including mosquitoes. The presence of *Wolbachia* endosymbionts is the normal

state for 40% to 60% of Arthropods and does not represent an unusual or pathogenic bacterial infection. *Wolbachia* are not capable of infecting human cells. MosquitoMate and Verily will perform PCR testing on the mosquitoes to confirm the presence of the correct *Wolbachia* bacterium within the shipment lineage to ensure cytoplasmic incompatibility.

The likelihood that introduced strains of *Wolbachia* would become the dominant strains in the environment is highly unlikely. Replacing the dominant *Wolbachia* strain has been done purposefully in the environment for projects that are separate from the approach we are proposing (such as by the World Mosquito Program in Australia and other nations). To clarify, DLNR is NOT proposing a World Mosquito Program type project where the goal is to intentionally force a different dominant *Wolbachia* strain into the wild mosquitoes in the environment and change vector competence of the wild population. However, in these types of programs, they have to release 4 million mixed male AND female mosquitoes in a given location to force a new *Wolbachia* strain to become the dominate strain over an area of 66 km^2. Given the aforementioned EPA reviewed value of 1 female release per 250,000 males with the MosquitoMate product, such an outcome is not expected to occur.

If, somehow population replacement were to occur (despite the estimated 1 female release per 250,000 males) DLNR would cease releases as the released males would then be able to mate with the wild females with the established *Wolbachia* species. The outcome of this would be that the mosquito species that already exists in Hawaii would continue to exist in the wild, just with a different *Wolbachia* bacteria. We do not anticipate a different *Wolbachia* bacteria having any new or negative effects on the environment.

DLNR and DOH feel comfortable utilizing these mosquitoes at a very small scale (in remote forest habitat) or at a very large scale (across urban areas and island wide) so long as recommended application guidelines are followed. The scale and scope of the project will likely vary across time based on the funding available and mosquito prevalence. As with any pesticide product, if you do not eradicate the species of concern, they will rebound if you stop using the pesticide product. However, we view this as a beneficial aspect of the project as we also know we can stop the process at any time. Unfortunately, due to the critical nature of the declines of Hawaiian forest birds, we anticipate mosquito control becoming a long-term management action to be performed (similar to rat control and invasive weed control) annually.

Data collection will occur during releases using the State general funds as well as federal funds from partner agencies (USFWS, USGS, NPS), depending on who is performing the releases. As the application of the pesticide product is intended for the reduction of *Culex quinquefasciatus* mosquito populations, this monitoring will include extensive mosquito population surveillance following releases to ensure that populations are reduced. DLNR is already conducting this type of monitoring in preparation for incompatible mosquito releases. *Wolbachia* genetic monitoring will also occur, likely in partnership with USGS, throughout the release program.

In addition to Hawaii's import requirements, the shipper and/or receiver will obtain additional permits as required by federal or state agencies.

Method of Disposition
Any dead imported mosquitoes will be disposed of as municipal waste.

Abstraction of Organism
Culicidae species are sexually reproducing species. Minimum generation times vary but are approximately three weeks. Mature adults are up to approximately a centimeter in length and can live for a month to a few months. Adult mosquitoes range from 2.0 to 10.0 mm in size with males being smaller than females on average. Mosquito life cycles are well understood for most species, including all those established in Hawaii.

Larvae feed on organic material found in pools of water. Both adult males and females feed on water that contains carbohydrates (water with sap or nectar). Only mature females of certain species seek out and feed on vertebrate blood prior to egg laying. This blood feeding process allows for the transmission of pathogens and parasites.

Culex quinquefasciatus rely on pools of water with organic material for the growth of larvae. Only adult females bite, as they require blood meals from vertebrate hosts to develop their eggs.

Potential Impact to the Environment
Culex quinquefasciatus are already well established in the wild on all of the main islands in Hawaii from sea-level to ~6,000 feet in elevation. and *Culex quinquefasciatus* are established statewide and is well establish on Hawaii's Big Island. An additional five other "biting" non-native mosquito species have also become established: *Ae. albopictus, Ae. aegypti Ae. japonicus*, *Ae. vexans*, and *Wyeomyia mitchelli*.

Wolbachia are not infectious to humans and are vertically transmitted through the eggs

from one generation to another. The *Wolbachia* bacteria are obligate endosymbionts and can only survive inside the insect host's cytoplasm. A mosquito transinfected with a different strain of *Wolbachia* that results in cytoplasmic incompatibility would not be able to successfully reproduce with a wild mosquito due to cytoplasmic incompatibility. Therefore, if individual mosquitoes did become temporarily established, then they will quickly die off over the following generations because of cytoplasmic incompatibility with wild mosquitoes of the same species, with which they would be expected to encounter and mate.

Through the importation we intend to only import male mosquitoes. The sex separation can be performed in a variety of manners including through computer recognition and separation of males and females or through pupal sorting of males and females. However, if both sexes of transinfected mosquito were to be accidently released, they are unlikely to maintain a breeding population of a transinfected mosquito. *Wolbachia* invasions into populations require a critical threshold frequency of infection that needs to be overcome before a novel *Wolbachia* infection can spread into a population. The *Wolbachia* infection rate must exceed 20-45% before it can spread and become established. This is evident in large scale releases such as in Cairns, Australia, where millions of transinfected mosquitoes (both sexes) with *Wolbachia* are released into the environment to control disease transmission, yet they do not easily reach fixation in the wild. If transinfected mosquitoes were to become established, the establishment is likely to be spatially localized due to incompatibility with neighboring mosquito populations.

Potential Impacts of Importation

pro: Importation of male mosquitoes will allow the implementation of an evaluated technology that has been scientifically demonstrated as a safe and effective control method for mosquitoes on a landscape-scale. These are mosquitoes that are widespread in Hawaii and which have negative impacts to humans, wildlife, and pets, and are causing the extinction of native forest birds. Thirty species of main Hawaiian forest birds have become extinct since European contact, and another 11 of the 21 remaining species are federally listed as threatened or endangered. The remaining 21 forest bird species remain at great risk as a result of avian pox and avian malaria. Four honeycreeper species (Akikiki, *Oreomystis bairdi*; Akekee, *Loxops caeruleirostris*; Kiwikiu, *Pseudonestor xanthophrys* and Akohekohe, *Palmeria dolei*) are of particular concern – each are federally endangered, single-island endemics with highly restricted ranges, number fewer than 1,800 individuals, and display recent alarming population declines. DLNR and USFWS have previously attempted to address these declines through bold conservation actions, such as translocations and establishment of captive

populations; however agencies have met with only limited success due to rapidly changing disease-transmission conditions on the landscape. There is an urgent need to develop new conservation tools, including landscape-level mosquito control in order to prevent further extinctions.

The application of traditional chemical controls for mosquitoes in both natural areas is impractical and causes unacceptable non-target impacts, whereas IIT carries no non-target risks to native species, humans or the environment. Furthermore, mosquitoes were first introduced to the Hawaiian Islands in the 1800s, and while they are used opportunistically as prey items, no species native to Hawai‘i are dependent on their presence for survival. The control of mosquito populations in Hawaiian forests would thus cause no negative impacts on Hawaiian species.

Demonstrated application of this approach in Hawaii would have also have a wide range of potential positive effects in that it may facilitate the incompatible insect technique approach being used for human health.

con: It is hard to imagine any negative effects since the species is already established in Hawaii. Importing these organisms will not have any foreseeable beneficial effect to this mosquito species already found in Hawaii. The introduction of, for example, increased genetic variation within the mosquito species will be minimized by crossing the lines to mosquitoes originating from Hawaii.

The presence of unintended accompanying microbiota is minimized by the sterile laboratory rearing conditions used. These mosquitoes have been maintained for many generations in the lab environment and have not had the opportunity to obtain pathogens from the wild from blood feeding. The presence of intended microbiota, the *Wolbachia,* potentially has very positive effects on the societal health, the suppression of human disease vectored by mosquitoes, the environment, via population suppression of mosquitoes that vector avian pathogens, and the economy, through the potential increased tourism and lessened disease burden.

This mosquito species is already well established in Hawaii, as are many different strains of *Wolbachia*. MosquitoMate and Verily have a demonstrated track record of success utilizing sex-sorting methods which are highly effective. In the event that technical difficulties did occur during sex-sorting methods, because of cytoplasmic incompatibility, the escape of female mosquitoes carrying a new *Wolbachia* strain is not expected to be stable over the following generations. Laboratory reared females outcrossing to locally established wild male mosquitoes will result in cytoplasmic

incompatibility and the failure of offspring to develop.

There is an extensive body of literature surrounding this mosquito species, its impact upon Hawaii, and Wolbachia-mediated cytoplasmic incompatibility.

Information on *Wolbachia,* with a focus on cytoplasmic incompatibility within mosquitoes:

Atyame, C. M., Cattel, J., Lebon, C., Flores, O., Dehecq, J.-S., Weill, M., Gouagna, L. C. & Tortosa, P. (2015) Wolbachia-based population control strategy targeting*Culex quinquefasciatus* mosquitoes proves efficient under semi-field conditions. *PLoS ONE* 10, e0119288.

Atyame, C. M., Labbé, P., Lebon, C., Weill, M., Moretti, R., Marini, F., Gouagna, L.C., Calvitti, M. & Tortosa, P. (2016) Comparison of irradiation and Wolbachia based approaches for sterile-male strategies targeting *Aedes albopictus*. *PLoS ONE* 11, e0146834.

Barton, N. H., & Turelli, M. (2011). Spatial waves of advance with bistable dynamics:cytoplasmic and genetic analogues of Allee effects. The American Naturalist, 178(3), E48-E75.

Blagrove, M. S., Arias-Goeta, C., Failloux, A. B., & Sinkins, S. P. (2012). Wolbachia strain wMel induces cytoplasmic incompatibility and blocks dengue transmission in*Aedes albopictus*. *Proceedings of the National Academy of Sciences*, *109*(1), 255-260.

Callaini, G., Dallai, R., & Riparbelli, M. G. (1997). Wolbachia-induced delay of paternal chromatin condensation does not prevent maternal chromosomes from entering anaphase in incompatible crosses of *Drosophila simulans*. *Journal of CellScience*, *110*(2), 271-280.

Dobson, S. L., Marsland, E. J., & Rattanadechakul, W. (2001). Wolbachia-induced cytoplasmic incompatibility in single-and superinfected *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology*, *38*(3), 382-387.

Hamm, C. A., Begun, D. J., Vo, A., Smith, C. C., Saelao, P., Shaver, A. O., ... & Turelli, M. (2014). Wolbachia do not live by reproductive manipulation alone: infection polymorphism in *Drosophila suzukii* and *D. subpulchrella*. Molecular Ecology, 23(19), 4871-4885.

Hoffmann, A. A., Montgomery, B. L., Popovici, J., Iturbe-Ormaetxe, I., Johnson, P.H., Muzzi, F., ... & Cook, H. (2011). Successful establishment of Wolbachia in Aedes populations to suppress dengue transmission. *Nature*, 476(7361), 454.

Hoffmann, A. A., Ross, P. A., and Rašić, G. (2015) Wolbachia strains for disease control: ecological and evolutionary considerations. *Evolutionary*

Applications, 8(8),751-768.
Jiggins FM. (2017) The spread of Wolbachia through mosquito populations. *PLoSBiology* 15(6):e2002780.
Laven, H. (1967). Eradication of Culex pipiens fatigans through cytoplasmicincompatibility. *Nature*, *216*(5113), 383-384.
Liao W, Atkinson CT, LaPointe DA, Samuel MD (2017) Mitigating Future Avian Malaria Threats to Hawaiian Forest Birds from Climate Change. *PLoS ONE* 12(1):e0168880.
Mains, J. W., Brelsfoard, C. L., Rose, R. I. & Dobson, S. L. (2016) Female adult Aedes albopictus suppression by Wolbachia-infected male mosquitoes. *ScientificReports* 6, 33846.
Stouthamer, R., Breeuwer, J. A., & Hurst, G. D. (1999). Wolbachia pipientis: microbial manipulator of arthropod reproduction. *Annual Reviews in Microbiology*,*53*(1), 71-102.
Sinkins, S. P., Braig, H. R., & O'Neill, S. L. (1995). Wolbachia superinfections and the expression of cytoplasmic incompatibility. *Proceedings of the Royal Society ofLondon B: Biological Sciences*, *261*(1362), 325-330.
Tram, U., & Sullivan, W. (2002). Role of delayed nuclear envelope breakdown andmitosis in Wolbachia-induced cytoplasmic incompatibility. *Science*, *296*(5570), 1124-1126.
Waltz, E. (2016) US reviews plan to infect mosquitoes with bacteria to stop disease. *Nature* 533, 450-451.
Weinert, L. A., Araujo-Jnr, E. V., Ahmed, M. Z. & Welch, J. J. (2015) The incidenceof bacterial endosymbionts in terrestrial arthropods. *Proc. R. Soc. B* 282, 20150249.
Werren, J. H., Baldo, L., & Clark, M. E. (2008). Wolbachia: master manipulators of invertebrate biology. *Nature Reviews Microbiology*, 6(10), 741.
Zug, R., & Hammerstein, P. (2012). Still a host of hosts for Wolbachia: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. *PLoSONE*, *7*(6), e38544.

Wolbachia in Hawaii:

Atkinson, C. T., W. Watcher-Weatherwax, and D. A. LaPointe. (2016) Genetic diversity of *Wolbachia* endosymbionts in *Culex quinquefasciatus* from Hawaii,Midway Atoll and American Samoa. Technical Report HCSU-074.
Bennett, G. M., Pantoja, N. A., & O'Grady, P. M. (2012). Diversity and phylogeneticrelationships of *Wolbachia* in *Drosophila* and other native Hawaiian insects. *Fly*, *6*(4), 273-283.

Other References:

Anonymous. (2017). To Restore a Mosquito-Free Hawaii. Summary Report of the Workshop to Formulate Strategic Solutions for a "Mosquito-Free Hawaii". Available at: "http://www.cpc-foundation.org/uploads/7/6/2/6/76260637/report_on_mosquito_free_workshop.pdf."

Anonymous. (2020). Three great years of Debug Fresno. Available at: "https://blog.debug.com/2020/01/three-great-years-of-debug-fresno.html"

Dame, D. A., Curtis, C. F., Benedict, M. Q., Robinson, A. S., & Knols, B. G. (2009).Historical applications of induced sterilisation in field populations of mosquitoes. *Malaria Journal,* 8(2), S2.

Johnston, D., *et al*. (2016). Notes from the field: outbreak of locally acquired cases ofdengue fever—Hawaii, 2015. *MMWR. Morbidity and Mortality Weekly Report*, 65(2); 34–35.

Kauffman, E., Payne, A., Franke, M. A., Schmid, M. A., Harris, E., & Kramer, L. D. (2017). Rearing of Culex spp. and Aedes spp. mosquitoes. *Bio-Protocol*, 7(17).

Mendenhall, I. H., S. A. Tello, L. A. Neira, L. F. Castillo, C. B. Ocampo, and D. M. Wesson. (2012) Host Preference of the Arbovirus Vector Culex Erraticus (Diptera: Culicidae) at Sonso Lake, Cauca Valley Department, Colombia. *Journal of MedicalEntomology* 49 (5): 1092–1102

O'Neill, S. L.; Ryan, P. A.; Turley, A. P.; Wilson, G.; Retzki, K.; Iturbe-Ormaetxe, I.;Dong, Y.; Kenny, N.; Paton, C. J. & Ritchie, S. A. (2018) Scaled deployment of Wolbachia to protect the community from Aedes transmitted arboviruses. *Gates Open Research* 2: 1-18

Richmond JY, McKinney RW, eds. (1999). *Biosafety in Microbiological andBiomedical Laboratories* (4th ed.). ISBN 0-7881-8513-6.

Scott, T. W. (2005). Containment of arthropod disease vectors. *ILAR Journal*, 46(1),53-61.

Takken, W., & Verhulst, N. O. (2013). Host preferences of blood-feeding mosquitoes. *Annual Review of Entomology*, 58, 433-453.

[USFWS] U.S. Fish and Wildlife Service. (2006) Endangered and Threatened Wildlife and Plants; Determination of Status for 12 Species of Picture-Wing Fliesfrom the Hawaiian Islands. *Federal Register* 71 (89): 26835-26852.

Winchester, J. & Kapan, D. (2013). History of *Aedes* Mosquitoes in Hawaii. *Journal of the American Mosquito Control Association* 29(2): 154-163.