



Interagency Points of Entry, Points of Exit Monitoring Plan



Māmalu Poepoe

Strategic Biosecurity for Hawai‘i

2014 - 2019

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I. INTRODUCTION

Project Scope and Description:

Māmalu Poepoe connotes “Sphere of Protection” and is a program funded by the Hawaii department of transportation (HDOT) for a five year period. Funds are to be used to increase surveillance of target species at airport facilities statewide.

Human transportation activities are a major pathway for the introduction of invasive species across the globe and require comprehensive management strategies to prevent the risks of their introduction and establishment. Risk assessments, interdiction, inspections, and monitoring high-risk areas are all critically important. This plan focuses on establishing (1) routine monitoring for a specific set of high risk incipient insect pests at major points of entry in the Hawai‘ian Islands where they are likely to first appear and (2) planning for adequate response capacity upon detection of a pest. The target pests selected are ones that are not easily detected or often missed through inspections due to the many pathways through which they may enter, especially, their ability to hitchhike undetected on planes, boats, people, and cargo. For the purpose of this plan, the definition of point of entry is adapted from WordNet 3.1 as *a port in the United States where the entry and exit of people and merchandise occurs*¹. Early detection and rapid response to a pest occurrence at these points is important to both prevent further establishment on the given island itself, but also to prevent the exit and further interisland spread of the pest.

The first phase of this effort will focus on 6 airports within the state: Honolulu International, Hilo International, Kona International, Kahului, Moloka‘i, and Lihue. Honolulu International is a primary concern due to the volume of people and incoming traffic from the most diverse number of locations. However, there is also concern about the lack of knowledge of what may occur or be arriving at other major points of entry in Hawai‘i that are not being monitored. Following the implementation of the first phase and building upon a successful model for monitoring, the working group will work to reach out and include additional points of entry (i.e. harbors, private shipping ports etc.). This plan outlines a 5-year interagency strategy to implement statewide points of entry monitoring and strengthen response capacities.

This document was developed by an interagency working group facilitated by the Hawai‘i Invasive Species Council (HISC). The plan supports HISC directive 2 in Chapter 194-2 of the statute, which established the HISC to advise, consult, and coordinate interagency efforts related to invasive species. It also supports state priorities in both prevention and response and control.

History

Invasive species surveillance at ports of entry is a valuable tool that helps track presence and status of already introduced invasive species, as well as offers the opportunity for early detection of potential invaders. Early detection followed by rapid response can prevent the establishment of harmful species. In 2000-2001, Hawai'i Department of Agriculture (HDOA) conducted a 100% inspection of all incoming cargo at Kahului airport. Results from these inspections showed that an average of one new insect species was intercepted each day (HDOA, 2002). Proactive measures to prevent the establishment of new invasive species can save millions of dollars to the state and the nation. Pimentel et al. (2000) estimated that biological invasions cost roughly \$137 billion annually in the US alone. Many invasive species in Hawai'i arrive first to one island and they subsequently invade other islands due to the interisland movement of people and goods. Increased airport surveillance for key pests is therefore imperative to prevent the continued spread of invasive species.

Target Species

Target species were selected collaboratively by the working group with DOH and DOA as the primary leads. The focus of this project is primarily high-risk insect pests that pose significant human health, economic, and environmental concern. Criteria for selection of target species was based on these key factors:

- High priority pest due to threats posed by introduction and spread.
- A species that is easily missed through current inspections/monitoring regimes due to the characteristics of its mobility and/or is not currently being monitored for.
- Ability to set up a feasible and time efficient monitoring program for the group of species selected given available funding and resources.

1. Mosquitoes (*Aedes sp.*, *Culex sp.*, *Anopheles sp.*)
Lead: DOH
2. Ants (*Solenopsis invicta*, *Wasmannia auropunctata*)
Lead: DOA
3. Coconut rhinoceros beetle (*Oryctes rhinoceros*)
Lead: DOA
4. Africanized honeybee (*Apis mellifera scutellata*)
Lead: DOA

Current Situation

Due to recent economic crises resulting in less funding to these priority detection areas, there is little to no monitoring for invasive species at major points of entry across the state beyond selected foreign, interstate and interisland cargo inspections. Cuts to the Hawai'i Department of Health (DOH) Vector

Control Branch in 2009 resulted in decreased capacity for mosquito surveillance increasing Hawai'i's vulnerability to the introduction and spread of mosquito-borne illnesses, which poses serious threats to human and ecological health. Though many of these positions were later restored in 2016 and 2017, the gap in monitoring and control had demonstrable impacts in the State's ability to mitigate invasive species. Since January 2012, *Aedes aegypti* (an efficient dengue fever vector mosquito) has been detected 7 independent times at Daniel K. Inouye International Airport (formerly, Honolulu International Airport), with the most recent detection occurring in August 2014. As a result, DOH has been working closely with the Hawai'i Department of Transportation (DOT) to assess the airport grounds and implement changes to reduce suitable mosquito habitat and has also approached HISC about increasing interagency coordination to proactively fill a gap in Hawai'i's biosecurity by collaboratively this more thorough statewide points of entry monitoring program.

Additionally, it has recently been discovered that Hilo International has infestations of Little Fire Ant throughout its grounds. The economic consequences of invasive ants establishing throughout the state could be staggering. It has recently been estimated that the economic impact of Little Fire Ants on the island of Hawai'i alone could reach \$170,000,000, making it imperative that it not be allowed to spread further among the islands. The cost of Red Imported Fire Ants establishing in the state is estimated at \$200,000,000 within 10 years of its introduction. In response to the Little Fire Ant issues at Hilo International, DOT Airports is working with DOA to assess and begin treating known infestations. They are also actively participating in this collaborative effort as a key component to assess and establish routine monitoring for ants at the other airports as well.

The coconut Rhinoceros beetle (CRB) was detected in Hawai'i on December 23, 2013 at the Joint Base Pearl Harbor Hickam military facility during routine trap checks (HDOA, 2014). This invasive pest is currently restricted to O'ahu and is still under eradication efforts. Coconut trees are an important component of urban forestry and are widely recognized as a symbol for the state. The loss of coconut palm trees due to CRB could affect tourism, which is Hawai'i's primary industry. The CRB response team on O'ahu monitors traps at several sites at Honolulu International Airport (HIA). These sites are located outside the secured areas of the facility. A few traps are located inside the secured areas and are serviced by the US Department of Agriculture's Animal and Plant Health Inspection Service (USDA APHIS) staff. We are currently evaluating the need for increasing trapping sites inside secured areas at HIA as well as at airports in neighboring islands, where this invasive pest has not yet been detected.

Africanized honeybees (AHB) are a major threat to the state of Hawai'i. This single, unwanted species of honeybee could endanger Hawai'i's beekeeping industry and Hawai'i's \$14 billion tourism industry as these bees would become a public health hazard (HDOA, Apiary Program). Besides monitoring for Africanized honeybees swarm traps could also help detect the incursion of other unwanted species such as *Apis cerana*, and invasive parasites such as Varroa mite and Tropiaelaps mites. Varroa mite is currently restricted to O'ahu and Hawai'i islands. The HDOA Apiary program currently keeps traps around seaports and airports at all major islands. Trap sites at airports have previously only included non-secure areas, as no current agencies have the capacity to monitor traps inside secured areas at

airports. The program coordinator and apiary staff are currently identifying swarm trap locations at all main airports.

Additional Priorities and Gaps

The working group recognizes that this project is a positive and meaningful step in the direction of increased biosecurity for Hawai'i but also that there are still gaps that require further attention. For example, one major area of concern for DOH is the movement of rodents (a primary vector for human and wildlife disease) around and between points of entry. A testing program of rodents at these areas would allow for increased early detection and response to health hazards. There are additional high-risk vertebrate species such as snakes and frogs that have been directly linked to spread via hitchhiking through human transportation. The establishment of snakes in the state would be ecologically disastrous for Hawai'i. Coqui frogs have prompted emergency management strategies on the Big Island, been eradicated from Kaua'i, are being managed on Maui and O'ahu and are not currently known on Molokai. Mitigating the interisland spread of coqui is a very high statewide priority as well. Lastly, there are also plant species of concern that are either currently established and need to be surveyed and treated at a point of entry to prevent further spread (i.e. fountain grass at Honolulu Int'l) or also may first show up at these major hubs of transportation activities.

While not all of the known gaps in Hawai'i's biosecurity at points of entry can be addressed in the scope of this one project, through this interagency partnership we will explore value added opportunities to further mitigate the spread of these and other potential high-risk species at points of entry. This project provides the potential opportunities to leverage resources and partnerships to achieve not only the primary goals within its scope, but to also help partnering programs achieve goals related to Hawai'i's overall well-being.

II. GOALS

1. Foster cooperation, coordination and communication among partner agencies regarding invasive species surveillance at airport facilities in Hawaii.
2. Improve the state's capability to prevent invasive species introductions through systematic monitoring efforts
3. Increase the security of Hawaii's people, natural resources, food supply and economy through an interagency monitoring program of incipient pests at major airports over the next five years.

III. OUTCOMES & STRATEGIES

Outcomes:

Short Term (Yr. 1):

- 1A.** Increased interagency collaboration
- 1B.** Development of monitoring protocols for target species
- 1C.** Increased capacity to communicate and respond to target species detections
- 1D.** Increased knowledge of target species occurrence/ introductions at six main airports

Mid Term (Yr. 2-5):

- 2A.** Increased knowledge of changes in target species occurrence at airports over time
- 2B.** Major airports are regularly and consistently monitored through partnerships
- 2C.** Response protocols in place for new detections
- 2D.** Studies completed on trap efficacy for mosquitoes and economic benefits of monitoring

Long Term (Yr. 5-on):

- 3A.** Risk for target species occurrence at points of entry is better understood
- 3B.** Clear response protocols & resources for implementation in place
- 4C.** Reduced risk of establishment of target species due to effective surveillance efforts.
- 3C.** Partners have a clear understanding of the costs and benefits of an airports monitoring program and are prepared to make a decision on whether to maintain monitoring efforts past the five-year pilot program

Strategies:

- i. Prioritize list of target species in the current plan
Leads: Coordinator, Project Working Group
- ii. Prioritize airport facilities in the current plan
Leads: Coordinator, Project Working Group
- iii. Determine current lead agencies' surveillance capacity at different airport facilities (for species listed in the plan)
Leads: Coordinator, lead agencies
- iv. Working with partners, conduct a gap analysis of existing surveillance efforts and identify need for expansion at different airports
Leads: Coordinator, lead agencies
- v. Assess and recommend appropriate landscape modifications at points of entry
Leads: DOT, with input from Coordinator
- vi. Work with partners to identify and allocate resources
Leads: Coordinator, in partnership with DOT, DOH, DOA, UH CTAHR, ISCs
- vii. Implement and complete baseline survey for ants at airports
Leads: DOA and Hawaii Ant Lab
- viii. Compile information on all current monitoring occurring and response resources available at points of entry and identify additional needs and gaps
Leads: Coordinator
- ix. Develop appropriate target species & monitoring protocols
Leads: DOA, DOH, Coordinator
- x. Designate/hire/provide FTE for monitoring staff
Leads: ISCs, DOH, DOA, with input from Coordinator
- xi. Establish UH graduate student project for data collection and analysis
Leads: UH CTAHR
- xii. Train staff in monitoring protocols
Leads: Coordinator, ISCs, DOA, DOH

- xiii. Implement monitoring at 6 main airports
Leads: Coordinator, Project Staff, ISCs
- xiv. Develop clear detection communication protocols
Leads: Coordinator
- xv. Establish UH project to provide economic analysis for cost of monitoring vs. cost of inaction
Leads: UH CTAHR
- xvi. Partner with DOT, non-profits, and private business to expand monitoring to additional points of entry
Leads: CGAPS, Project Working Group
- xvii. Develop ICS based response plans for target species, starting with mosquitoes
Leads: Coordinator, DOH, DOA
- xviii. Implement table top response exercise based on ICS response plan for mosquitoes
Leads: Coordinator
- xix. Identify funds for response capacity
Leads: Coordinator, Project Working Group

IV. PARTNER ROLES & RESOURCES

Agency	Role	Resources
Hawai'i Invasive Species Council	Interagency and partner coordination, cabinet-level direction and decision making	<ul style="list-style-type: none"> ● Coordination support ● Funding support ● Planning support
Coordinating Group on Alien Pests	Interagency communication and outreach	Coordination and outreach support
Department of Health	Human and Environmental Health	<ul style="list-style-type: none"> ● Vector staff monitoring at airports ● Entomology expertise ● Mosquito control equipment
Department of Transportation	Lead for Airports and Harbors	<ul style="list-style-type: none"> ● Funding ● Travel for project staff ● Staff support (access, landscaping adjustments)
Department of Agriculture	Agricultural and Environmental biosecurity	<ul style="list-style-type: none"> ● Expertise (entomologists, monitoring protocols, response) ● Space on each Island
University of Hawai'i College of Tropical Agriculture and Human Resources	Research and Outreach (Extension)	<ul style="list-style-type: none"> ● Expertise (entomologists, research) ● Grad Students ● Lab Space at Extension on each island
Island Invasive Species Committees	Early Detection, Control/ Eradication of Incipient Species, Outreach	<ul style="list-style-type: none"> ● Infrastructure to host monitoring staff ● Response capacity ● Flexibility
Pacific Cooperative Studies Unit, Research Corporation of Hawai'i	Project hosting and fiscal sponsor support	<ul style="list-style-type: none"> ● Mechanism to administratively house project and staff
Counties	Local Environmental and Human Health	
Military (JBPH-HI) / Army	Responsible for environmental impact of activities (Major vector due to amount of movement of people and equipment)	<ul style="list-style-type: none"> ● Expertise (entomologist, monitoring protocols, procedure) ● Supplemental monitoring at their locations ● Staff for response

V. BUDGET

DOT Airports Division is allocating \$300,000 yearly for a period of 5 years, beginning in the fourth quarter of FY15 and extending through the third quarter of FY20.

Year 1 (Fourth Quarter of FY15 Only)	
Salaries and Fringe	
a. Program Coordinator	\$ 22,832
b. 5-6 Monitoring Staff (partial FTEs)	
c. UH Grad Student, Economic Analysis	
Travel (training & meetings) <i>30 interisland trips</i>	\$ 3,090
Materials & Maintenance	\$ 1,100
DOH Equipment Maintenance for Mosquito Response	\$ 5,000
Complete Baseline Survey: Ants	\$ 0
Indirect costs	\$ 555
Total	\$ 32,577

Year 2	
Salaries and Fringe	
d. Program Coordinator	\$ 280,000
e. 4-6 Monitoring Staff	
a. UH Grad Student, Data Analysis	
b. UH Grad Student, Economic Analysis	
Travel (training & meetings) <i>30 interisland trips</i>	\$ 16,000
Materials & Maintenance	\$ 32,000
Indirect costs	\$ 1,000
Total	\$ 333,000

Years 3-5	
Salaries and Fringe	
f. Program Coordinator	\$340,000
g. 6-8 Monitoring Staff	
c. UH Grad Student, Data Analysis	
a. UH Grad Student for Economic Analysis	
Travel (training & meetings) <i>__ interisland trips</i>	\$ 16,000
Materials & Maintenance	\$ 10,000
Contingency response funds	\$11,141
Indirect costs	\$ 1000
Total	\$378,141

VI. Citations

HDOA. 2002. Kahului Airport Pest Risk Assessment. Department of Agriculture, Plant Quarantine Branch, Honolulu. 41pp

HDOA. 2014. Coconut Rhinoceros Beetle *Oryctes rhinoceros* (Linnaeus) (Coleoptera: Scarabaeidae). New Pest Advisory. No. 14-01.

Pimentel, D., Lach, L., Zuniga, R., Morrison, D., 2000. Environmental and economic costs associated with non-indigenous species in the United States. *BioScience* 50 (1), 53–65.

Princeton University "About WordNet." WordNet. Princeton University. 2010.
<<http://wordnet.princeton.edu>>

VII. Appendices

Monitoring protocol for Africanized honeybees.

This is an already existing protocol developed by the HDOA Apiary staff. Communication protocol needs to be developed for all airports.

Monitoring records (dates of swarm trap placement or location changes, dates checked, dates lures are changed, bees caught and inspection results) will be made available to Noelani Waters, Apiary Program (noelani.waters@Hawaii.gov).

Collect 300 bees in 70% ethanol from any swarms caught (details below).

Swarm traps are used to monitor high-risk areas. Swarm traps are to be placed in a one-mile radius for swarm trap placement has been created around each of these locations. (within 1 mile of ports) for new pests and disease. Swarm traps are designed to intercept colonies that are looking for a home. Their attractiveness is based on their location, microclimate, size, and proximity to competing habitat.

Honeybee swarms consist of a queen and several thousand worker bees. They form a cluster (often on a tree or post) and send out scouts to search for appropriate cavities to move into. These scouts are more likely to find the swarm trap if it has an attractive odor in it, such as bee pheromones or familiar nest smells from previous inhabitants.

Swarm traps should be:

- Placed in a large tree or bush if possible, the profile where a scout bee would search
- As high as is comfortable for maintenance
- Minimizing visibility and exposure to passers-by
- Equipped with a lid to provide weather protection
- Anchored securely to prevent any swaying or movement
- Lid should be oriented parallel to the ground (not sideways)
- Pheromone lure tube pushed into the trap through small hole

Swarm traps should be checked every 4-6 weeks if possible. If no bees are caught, log date each trap was checked, lures changed, and any comments about its function or state on attached log form. If bees are observed coming and going, it could be a colony or scouts. If it is a colony, the swarm must be removed and sampled.

When removing a swarm trap:

- Take GPS point
- Wear protective gear
- Have two people in field if possible

- Cut zip ties to clear colony for bagging
- Bag colony quickly in large heavy plastic trash bag
- Seal bag with duct tape or zip ties
- Place in freezer when possible, but if not available leave in car in hot sun
- If immediate colony death is necessary, poke hole in bag and penetrate cap with ball point pen housing, using like straw to spray ether (starter fluid) inside swarm trap
- Do not open bag until there is no buzzing inside

To sample a swarm (after it is dead):

Look for small hive beetle

- Small hive beetle adults are very attracted to swarm traps. Observe the bucket for adults and larvae (they prefer the periphery or any hiding places)

Look for Varroa and Tropilaelaps mites

- Collect adult bees (1 cup at a time) and shake vigorously in alcohol bee shaker. This will dislodge mites, which go through screen and sink to bottom of alcohol. Sample at least 4 cups of bees.
- Using uncapping fork, pull pupae out of sealed cells and observe for mites. Drone brood (larger cells) should always be sampled when available, otherwise sealed worker brood will do. Sample as much of the brood as possible.
- Observe combs and brood closely for Tropilaelaps mites, see photo.

Take a sample for Genetic Testing (see details and sample information required, attached):

- Collect about 300 fresh bees in a clean, unused vial with alcohol- at least 70% ethanol. If fewer bees are collected, the test may still be possible but try for 300. They should be freshly dead and not desiccated.
- Samples will be processed by Hilo Apiary staff with required data.

Take note of anything that looks unusual or unhealthy, take photo and samples, store in freezer until Apiary staff can be consulted.

Protocol for collecting honey bees to sample for genetic testing:

Note: Non-Apiary staff send bees and associated data from each sample using form on last page.

The goal of this project is to survey the honey bee populations throughout the state to sample genetic variation. It is particularly important to sample any bees suspected to be Africanized, such as those caught in swarm traps in the Biosecurity risk zones. Other bees, managed or feral, can also be sampled from any location. In an apiary, separate samples may be collected from 3-5 colonies.

Data to collect includes: location description, GPS coordinates, colony type (feral, swarm trap, or managed), beekeeper, if known, Queen source, if known, and any other comments (e.g. if the bees were abandoned, aggressive, or suspicious).

Procedure:

1. Collect about 50 bees in a clean, unused vial with alcohol- at least 70% ethanol. If fewer bees are collected, the test may still be possible but try for 50. If they are dead, they should be freshly dead and not desiccated. (Note: stings may also be collected in the event of a stinging incident in which bees are unavailable.)
2. Record the required information using the form below, using pencil or alcohol-proof ink.
3. Place label with unique identifier inside vial, using pencil on paper, to ensure identity is not lost in shipping. Make sure that the label information is alcohol-proof (pencil is best).
4. Make sure the vial is closed tightly, and mail the sample with a copy of the completed data form to:

Apiary Program
Hawai'i Dept of Agriculture
16 E. Lanikaula St.
Hilo, HI 96720

Genetic variation bee sample specimen

Number written on label inside vial: _____

Collector: _____ Date: _____ Island: _____

Location description:

GPS coordinates:

Feral, swarm trap or managed?

Temperament of colony:

Beekeeper, if known:

Queen source, if known:

Comments:

Monitoring Protocol for Coconut Rhinoceros Beetle (CRB)

Selection of trapping location and trap type will be done in coordination with the CRB response team on O‘ahu. The CRB response team will provide trapping materials and will provide training to monitoring staff. Program coordinator will line up help to monitor traps in secured areas. New CRB trapping locations will be selected inside the secured areas of airport facilities.

Besides monitoring traps, monitoring staff will help record existing mulch piles at airports. If mulch piles are identified additional information will be recorded such as type of material, chipped or unchipped, how long the pile has existed in its current location, whether it is permanent or not, internal temperature pile maintains, whether it maintains moisture throughout the year, etc.

Monitoring staff will also help identify palm concentration locations around the airports. Identifying what species of palm are in these concentrations and how many of each. They will also survey all palms on Airport properties every 6 months for signs of CRB feeding damage.

Text for this portion of the plan has been taken from the Standard Operational Procedures (SOP) – Field Activities- Coconut Rhinoceros Beetle. Refer to SOP for complete description

The delimiting survey (trapping) is used as a tool to monitor for both the presence AND absence of the beetle in a determined area over a designated time period. This activity is initiated once a positive detection for CRB has occurred and/or there is a high risk of a future discovery of the beetle in a previously beetle-free area. Currently, two (2) versions of monitoring traps are employed to conduct delimiting surveys in the Hawai‘i response: Panel and Barrel.

Panel Trap

Panel Traps used on the Hawai‘i Response are manufactured and supplied by a national recognized pest management company. Traps are shipped as individual components and assembly is required before deployment in field. The trap is non-lethal and designed to be suspended in trees or from buildings. It is constructed of durable light-weight plastic and designed to endure prolonged exposure to outdoor weather conditions. It uses a chemical aggregate lure and a custom designed ultra-violet (UV) light as attractants for both sexes of the adult beetle. Its dimensions are forty-eight inches by sixteen inches and predominantly black in color.

Panel Trap Placement:

Prior to any trap being deployed in the field, permission and/or authorization for the placement will be obtained before proceeding. This shall be the primary requirement for any trap placement.

1. Suspend at a height that will prevent tampering/access by unauthorized individuals or interfere with the general public's path of travel (recommend the bottom of the collection cup of trap be approximately 8' – 12' above the ground).
2. Select a location with sufficient clearance around the trap to allow for free movement in the wind.
3. Select a location that will allow ease of access for servicing and maintenance by program personnel.
4. Position the trap such that the solar cell in the hood section is exposed to direct or partial sunlight for 2 – 3 hours daily to ensure adequate charging of the batteries.
5. Avoid placing trap within twenty (20) feet of power-lines or overhead wires.
6. If placed in trees, select those with limbs which are sturdy/strong enough to support the weight of the trap and resist the swaying action the trap in the wind.
7. If trap is placed in the canopy of a tree and not readily visible, attach flagging tape to the trunk or in a conspicuous location. This will assist with locating the trap for servicing and maintenance or should it need to be rehung/replaced.
8. Avoid placing traps on or within 50 yards of palm trees.

Barrel Trap

The Barrel Trap is a modified trapping method designed to attract CRB through the use of a chemical lure and a ultra-violet light similar to the Panel Trap. It has the added attractant property of using actual mulch material, the preferred breeding material for the beetle. Barrel Traps are much larger than Panel Traps and are usually deployed in areas near/around mulch piles or where no suitable location for a suspended Panel Trap can be found. Because they are placed on the ground and can be tampered with, controlling access to the trap location is essential and a primary consideration in its use.

Barrel Trap Placement:

1. Deploy the trap on a hard, level surface with a vegetation-free radius of three (3) feet.
2. Select a location which cannot be easily accessed by unauthorized individuals.
3. Ensure trap is in plain view, to assist with trap being serviced and maintained and avoid damage by vehicular traffic.
4. Prevent trap being moved or toppled by strong wind by securing to a near-by stationary object or placing heavy weights in the barrel section with the mulch material.
5. Avoid placing traps within 50 yards of palm trees.

Trap Deployment Data

For each trap deployed in the field a "Trapping Card" is generated by the field staff responsible for its physical placement. All pertinent information of the trap location will be recorded and entered into a mapping platform program such as ArcGIS, where data will be compiled, analyzed and shared for the creation of maps.

The following deployment information will be collected and entered into a “Trapping Card” immediately after placement into the field:

1. Location of the response (State, County, Island, etc.)
2. Latitude coordinates – obtained from GPS unit or equivalent
3. Longitude coordinates – obtained from GPS unit or equivalent
4. Address – street address of or nearest to the trap location, if no address is available then a brief description of the immediate area surrounding the trap
5. Trap Type – check box for type of trap deployed (BR = barrel, PT = panel)
6. Trap Deploy – check box only if this trap is a new deployment
7. Initial – initials of staff personnel deploying the trap
8. Date – calendar date of initial trap deployment
8. Grid – number of the one-mile² grid the trap is deployed within, the number is generated and obtained from the Mapping/Data section of the program
9. Sub-grid – number of the 1/8-mile² grid the trap is deployed within, the number is generated and obtained from the Mapping/Data section of the program
10. Site Diagram – a map, hand-drawn or printed, is placed into this space showing the immediate area where trap is located with trap location, roadways, prominent landmarks and other notable features.

When entering information into the “Trapping Card”, accuracy is essential. To increase the functionality and usefulness of the card, the following should be considered when filling the required fields:

- When recording the latitude and longitude coordinates for the trap location, place the GPS unit or equivalent as close to the trap as possible
- If no physical address is available, describe prominent landmarks or structures in the area surrounding the trap
- Note the building or type of tree the trap is located on or near
- When drawing the map, orient directional North to the top edge of the “Trapping Card”
- Clearly mark the location of the trap in the site diagram section of the card.

The completed “Trapping Card” is returned to a data collection group for confirmation and processing. A unique identification “Trap Number” will be generated and assigned to each trap based upon the information submitted regarding the trap.

For ease of tracking trap activities, only grid square and sub-grid numbers will be used when generating Trap Numbers. This number will be printed onto labels and attached to the trap at the location site in the field corresponding the data collected on the “Trapping Card” and will be used for future reference for the particular trap.

Trap Servicing

Deployed traps are serviced and maintained at different time intervals, depending on the location of the trap in relation to the positive breeding site. The servicing and maintenance are

essential to monitor for beetle captures and ensure the trap is functioning properly. Traps deployed within the “buffer zone” are serviced at the schedule of two services per week. Traps placed at airports facilities will be monitored once a month. The servicing intervals may be adjusted by the response managers as they see appropriate.

Servicing and Maintenance Procedures

1. Check for presence/absence of beetles in the collection containers of the trap
 - a. Note results on the trap card
 - b. If beetles captured, place into a thick plastic container or Nalgene bottle (the beetles have been recorded chewing through thin plastic and fiberglass screens)
2. Verify the solar powered UV light is functioning properly (see trap assembly section for instructions)
3. Check viability of the chemical lure
 - a. The manufacturer recommends an effective field period of 42 – 45 days after activation
 - b. Areas with higher than normal (>90 degrees Fahrenheit) daily temperatures may reduce the effective field period
4. Check and ensure the physical details of the trap (location coordinates, type of tree, trap numbers, etc.) correspond to the information listed on the Trapping Card
5. Ensure other aspects of the trap, including proper assembly and deployment procedures are followed
6. Barrel Traps – Inspect mulch material in trap for:
 - a. Adequate moisture
 - b. Material has not completed decomposed
 - c. Possible presence of CRB

Servicing Data

The information for each servicing is recorded directly onto the Trapping Card which was created when the trap was first placed into the field. Trapping Cards are returned to the data collection group for processing after each servicing. The data is compiled into reports and maps and used to update the response managers on the progress of trapping activity.

The following servicing information will be collected and entered onto a “Trapping Card” (see Sample 2 below):

1. Date – calendar date of trap servicing
2. Initials – initials of staff personnel servicing the trap
3. Result – indicate if CRB was found in trap during the servicing
4. Adults Count – if CRB adults was collected in the trap, list the total count
5. Lure Change – indicate if the lure was changed during the servicing
6. Comment – list any notable actions which occurred or were required during servicing

Visual Surveys

Visual Surveys are an essential tool to assist with determining the extent of predation on palm trees within a designated area by the beetle and to track the extent of damage over time. The surveys also serve to locate and track potential breeding sites for CRB. Data collected during the surveys are:

- Total number of palm trees exhibiting signs of CRB damage
- Total number of palm trees not exhibiting signs of CRB damage
- Locations of potential breeding sites for CRB

Procedures

1. Survey personnel will be trained to recognize the characteristics of CRB damage to palm trees prior to conducting visual surveys
2. Survey personnel will be divided into 2-person teams
3. Each team will be assigned and provided with a map of a grid square(=1 square mile) by the data collection group
4. Each grid square will be divided into four half-mile square quadrants
5. Teams will walk and/or drive the accessible areas of each quadrant
6. A “Tree Count” card (see Appendix B) will be used by each team to record data of the grid square
7. The following data will be collected and entered onto the “Tree Count” card (see Sample 3 below):
 - a. Date – calendar date of the survey
 - b. Initials – initials of survey personnel
 - c. Quad Complete – note if survey of the quadrant was completed
 - d. Total Healthy Palms – total number of palms trees not exhibiting CRB damage
 - e. Total CRB Damaged Palms – total number of palm trees exhibiting CRB damage
 - f. Notes – list any facts or details related to the visual survey

When the “Tree Count” card is completed, it is returned to the data collection group for verification and processing. The card will be cataloged and filed and will be referred to in the future for follow-up action by the response managers.

Invasive Ant Surveys

Introduction

There are over 12,000 species of ants currently described. Hawai'i has no native ant representatives; however, about 57 ant species are currently known to be established in the islands. Hawai'i's role as a hub for tourism, trade, and military transport contributes to its continuing assault by foreign species (Messing and Wright, 2006). In average 20 new arthropod species invade the Islands each year (Beardsley 1979); some of them get established and become a real problem. Invasive ant species can pose a significant threat to human health, biodiversity, agriculture and other economic sectors. Active surveillance provides the opportunity for early detection and rapid response against invasive species (Hulme 2006). Only when invasions are caught early will the chance of eradication remain high (NISC 2008). Early detection and rapid response success stories include the successful eradication of the Red Imported Fire Ant (RIFA) in New Zealand (MAF, 2005), as part of the New Zealand National Invasive Ant Surveillance Programme (NIAS).

The Mamalu Poepoe program intends to increase invasive ant surveillance at main airport facilities in Hawai'i. The main objectives are to detect the incursion of new ant species into the state and to monitor already present exotic ant species (monitor changes in composition, distribution, etc.). This will be achieved by conducting bi-annual surveys at identified high risk sites at airport facilities.

In order to increase chances of early detection high priority/risk sites need to be identified. Priority/risk sites may include:

1. Green areas (including planters and potted plants) inside commuter, interisland, and overseas terminals
2. Green areas (including planters and potted plants) immediately outside all terminals
3. Air cargo areas
4. Baggage sorting and storage areas

Newly landed invasive ants will be in search for suitable habitats for nest construction. Many sites at airports facilities feature large areas of flat concrete or asphalt pads that are constantly disturbed by vehicle traffic etc., and are not suitable habitats for nest construction (NIAS, 2014). Ants will therefore seek undisturbed sites and habitat around the margins of concrete or asphalt pads where there are cracks or gaps in the surface and some vegetation and detritus has gathered (e.g. light poles, fence-lines, gutters, drains, buildings) (NIAS, 2014). These habitats tend to be of low quality and poorly resourced (e.g. little food), which inhibits colony growth and promotes constant foraging activity. Offering attractive food sources (baits) in a resource-poor environment, nearby risk sites increases chances of locating and capturing foraging exotic ant species (Peacock et al., 2015).

Little Fire Ant (LFA)

The Little Fire Ant (LFA), *Wasmannia auropunctata*, is an aggressive pest native to Central and South America (Starr et al., 2008). LFA are a devastating and costly invasive species currently moving and established throughout the State of Hawai'i. They cause painful stings, are linked to blindness in pets, and significantly threaten local food security by making farmlands less productive and unworkable (HISC, 2015). LFA are transported to new sites in potted plants or on plant materials, green waste, and rubbish (CTAHR, 2010). They are serious pests that infest yards and agricultural fields, and they will move into houses and other structures. It was first detected in Hawai'i on the Big Island in 1999. Delimiting surveys at the time confirmed that this species was well established and widespread in the Puna and Hilo Districts. This species has also been detected on the islands of Kaua'i, O'ahu and Maui. Population on those islands are currently being contained and controlled. The Hawai'i Department of Agriculture (HDOA) regularly checks nursery shipments by baiting, to avoid the accidental spread of this invasive species to neighboring islands.

The Red Imported Fire Ant (RIFA)

The Red Imported Fire Ant (RIFA), *Solenopsis invicta*, is recognized as one of the most serious pests worldwide. It poses a significant threat to biodiversity, human health, and the horticultural and agricultural industries. *This species is considered* highly invasive because of its high reproductive capacity, large colony size, ability to exploit human disturbances, wide food range and ability to sting. Its stinging ability allows it to subdue prey and even repel larger vertebrate competitors from resources (ISSG, 2014). The fire ants are very aggressive and will attack anything that disturbs their nest. They are named after the painful burning sensation, similar to a bee or wasp sting, that is left by their venom. They damage crops by feeding directly on them, and the mounds they build interfere with farming and can turn recreational spaces into no-go areas. This species is not currently present in Hawai'i, and is regarded as a high priority for early detection, rapid response and eradication by the Hawai'i Department of Agriculture (HDOA).

Ant survey protocol

The Hawai'i Ant Lab (HAL) staff will assist with ant surveys. Surveys will be done following existing protocols developed by HAL (Vanderwoude and Mann, 2011, Hawai'i Ant Group, 2017). Two baseline surveys will be conducted in a calendar year at Honolulu International, Kona International, Hilo International and Kahului airports. Molokai and Lihue airports will one have one survey a year. O'ahu HAL team will assist with surveys at Honolulu International Airport and Molokai airport, while HAL staff on Big Island will assist with surveys at all other airport facilities. The program will assist in obtaining AOA access when necessary.

Surveys will target all invasive ant species. Surveys will be done following specifications described in Table 1.

Survey protocol:

All Mamalu Poepoe surveys will be conducted following the HAL CAPS Standard Operating Procedures (Hawai'i Ant Group 2017).

Surveillance for ants is accomplished by placing vials baited with attractive food items in a grid pattern over the entire area to be surveyed, and collecting the vials after 30-60 minutes exposure. While the baits are in the field, any ants foraging nearby will be attracted to the baits and these can be sealed inside the vials and identified in the laboratory. Procedures that specifically target Little Fire Ants or Red Imported Fire ants are different, and the procedures for delimiting, monitoring and general surveillance are also slightly different. This procedure only covers general surveillance.

Equipment and supplies needed:

Bait materials

- A sufficient number of “Biolab” 60cc bait vials or similar. It can be helpful to have two different coloured lids so different bait types can be distinguished in the field. Light coloured lids are preferred as it makes lab sorting more efficient.
- 1 jar of “creamy” peanut butter per 500 baits
- 1 tin firm luncheon meat per 500 baits
- 1 jar of jam or jelly (clear, without lumps or seeds)

Bait preparation

- Paper or plastic plates
- 2 dessert spoons for mixing
- A sharp knife

Survey equipment and supplies

- Map of survey area
- Sample summary sheets
- GPS if available
- Spare batteries
- Hi-Viz vests
- Supermarket bags or other bags
- First aid kit
- pens
- Permanent marker vivid pens

Planning the survey:

When planning the survey, work out the area you want to cover and obtain a map or aerial image of the site. Google Earth is a good source of maps but most ports have port plans which can also be used. Contact site management at least a day before the survey to make sure you have permission to enter

and arrange any port passes etc that might be needed. In the case of an airport or sea port, try to pick a time when no planes are expected or ships are being loaded/unloaded. Also, plan to do the survey during clear weather when rain is not expected.

Each team should be made up of three people and one team should be able to place and collect around 200-400 bottles in a day. When planning the survey organise staff in lots of three (3, 6, or 9 people) one person to record complete sample sheet and/or mark waypoints, one person to deploy sugar baits and one person to deploy protein baits. When preparing baits, make up around 100 per person working in the survey. If you know the size of the area you have to cover and the survey type, you can also use these rates to work out how many days you will need to complete the survey. Keep a record of the number of baits placed and the total person-hours for each site for planning future surveys.

Bait preparation

Different ants are attracted to different food types so a mixture of bait types is used. Its best to make only enough baits for a days work. This way the baits will be fresh and attractive to ants (ants are not as interested in old baits). If possible, make them up the day before and store them in a refrigerator overnight. This way you can make an early start before temperatures get too hot.

You will make two types of bait: a protein bait and a sugar bait. When you lay them out in the field, these will be placed alternately – a protein bait, then a sugar bait, then a protein bait etc. Use vials with different colors for each bait type. That way you will know which is which. Keep these in separate bags.

Protein baits contain a smear of peanut butter and a small cube of luncheon meat. Protein based bait should be prepared by smearing a line of peanut butter (half the size of a pea) on the inner side of each bait container on the side corresponding with the back of the label¹. In addition, a small cube of luncheon meat is to be placed inside each vial.

Use a vial with a different colour lid for sugar baits. The sugar baits contain a smear of light coloured jelly or jam on the inner side of each bait container on the side corresponding with the back of the label. The jam should be light coloured and not contain seeds, lumps or rinds. A little water can be mixed with the jam to make it easier to spread

Conducting the survey

The aim of the survey is to thoroughly sample the ants at the site. This is done by placing baits in a grid pattern over the entire area, placing protein baits and sugar baits alternately. The spacing between baits is different for each survey type and these are listed in Table 1 and 2. It is not important to have the grids at **exactly** these spacings as long as the spacings are approximately correct. Sections that are all concrete or bitumen do not need to be sampled because few ants nest in these locations. Common ant habitats are listed in Table 3 and it is important that these are all sampled.

Bait vials should be collected 30-60 minutes after placement. It takes much less time to retrieve vials than it does to deploy them. As a guide, teams should place vials for one hour, then stop and retrieve the vials they have deployed in the order they were deployed. This way, the vials placed at the

beginning will have been out for 60 minutes and the ones deployed last will have been exposed for about 30-45 minutes depending on ant species.

Surveillance should not occur during or after rain when the ground surface is still wet, or on windy days. Also no rain is to occur between placement of bait traps and their retrieval. If rain is imminent, it is a good idea to stop deploying baits and retrieve the ones already out. If this is not possible, collect the baits one hour after the rain has stopped. If not many ants are at the baits, it might be necessary to resurvey the rain-affected section.

Bait vials should be placed in the shade where possible. As a hint place your vials away from prevailing wind and angle the entrance slightly to the ground. This helps prevent vials filling with water if you encounter a sudden down pour.

Any unusual ants (that look different to common established species) sighted while conducting surveillance should also be collected even if not they are not found going into a sample vial. Such ant samples should be recorded as a random, no-bait collection.

Data collection and storage:

All survey samples will have a corresponding GPS point. All data will be stored online on the MP Google Drive. Each airport site will have their own folder on Google Drive which will contain a data file (to store the Excel files with all gps data and ant ID's) and a mapping file (to store gps data in .gpx and/or .kmz file format for use with MapSource or Google Earth). Downloading and saving of the GPS data will follow the procedures outlined in the CAPS SOP however, file names will indicate MP for Mamalu Poepoe instead of CAPS. In addition to saving the data as a gpx file through MapSource all data will also be saved as a kmz file through Google Earth.

Ant ID's, recording and quality control:

All ants will be identified to the species level and recorded in the gps database (excel file) for that survey. No ant counts will be collected since population densities are outside the scope of the project. Samples with no ants present will be recorded as NIL for the species ID in order to verify that no ants were present and that it was not merely a missing sample. Bait type (peanut butter and spam vs. jelly) must also be recorded for each sample.

Voucher specimens for each species will be saved for every survey and mailed to HAL in Hilo for quality control identification verification. Voucher samples will consist of several ants of the same species from multiple samples placed in a single vial and labeled with the following information:

Species ID

Date Collected

Airport Site

Name of Identifier

Each species will have its own voucher sample (DO NOT MIX SPECIES). The purpose of the voucher samples are to verify ID's and ensure consistency among all identifiers working on this project. If any mistakes in ID's are made they can quickly be resolved through quality control measures such as voucher samples.

Reporting and data compilation:

HAL will only be responsible for collecting storing data on the MP Google Drive. HAL will not be responsible for report preparation.

Table 1. Survey specifications for Red Imported Fire Ant (RIFA) and other exotic ants

Specifications/survey type	Detection survey	Delimitation survey	Post treatment monitoring
Methods	Bait vials	Bait vials	Bait vials
Baits/food lures	Sugar and protein alternated	Sugar and protein alternated	Sugar and protein alternated
Vial spacing	100/ha, 1 vial every 10m	200/ha, 1 vial every 7m	400/ha, 1 vial every 5m
Time in the field	30-60 min	30-60 min	30-60 min
Survey frequency	Every six months	Immediately, if results negative follow up every six months for 2 years. If results positive, treat and monitor out to delimiting boundary	Six months for 2 years beyond last detection.
Buffer zone	50m beyond site boundary	2km from known nest(s)	200m beyond treated areas.

Source: Vanderwoude and Mann (2011).

Table 2. List of common ant habitats

1. Tree trunks (visual inspection and bait at base if appropriate).
2. Flowers and trunks of trees.
3. Shrubs and poles.
4. Building edges and foundations.
5. Concrete slab edges.
6. Cracked concrete.
7. Disturbed sites.
8. Drains and culverts.
9. Electrical generators and fittings.
10. Exposed rocks.
11. Fence palings.

12. Grass areas.
13. Verges.
14. Hot water pipes and heaters.
15. Isolated weeds.
16. Logs.
17. Loose gravel.
18. Low vegetation (including grass).
19. Plant pot bases.
20. Road margins.
21. Rubbish piles.
22. Shiny surfaces.
23. Soil.
24. Tree crotches and hollows.
25. Vertical surfaces.
26. Weed and plant re-growth.
27. Wooden structures.
28. Underneath stones or concrete rubble.

Source: Vanderwoude and Mann (2011).

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Draft plan for mosquito adult trap efficacy tests and testing infusion waters for ovitraps and gravid traps

Introduction

According to The American Mosquito Association, there are over 3,000 species of mosquitoes worldwide. Mosquitoes may be of public health relevance either when they occur in high densities and cause a nuisance or when they transmit diseases (Petric et al., 2014). The most common and dangerous species of mosquitoes fall into the *Culex*, *Anopheles* and *Aedes* genera. There are no mosquitoes native to Hawai'i and so far only six species of biting or blood feeding mosquitoes have become established. *Culex quinquefasciatus*, the southern house mosquito, is the most ubiquitous mosquito in Hawai'i and it is a known vector of avian malaria, avian pox, human and canine filariasis, and West Nile virus (WNV). *Aedes albopictus*, the Asian tiger mosquito, is a known vector of dengue fever, chikungunya, dog heartworm and a minor vector of avian malaria. *Aedes vexans* is a vector of dog heartworm and can be a competent bridge vector for WNV. *Aedes aegypti*, the yellow fever mosquito, is a vector of yellow fever, chikungunya, dengue fever, zika virus, and other diseases. *Aedes japonicus japonicus*, the Asian bush mosquito, is not considered a major vector of human diseases but has been implicated in cases of La Crosse encephalitis, Japanese encephalitis, WNV and has been found to be a competent vector of several other encephalitis viruses under laboratory conditions. *Wyeomyia mitchellii*, the bromeliad or pineapple lily mosquito, is not known to vector any human diseases.

The Mamalu Poepoe program aims at enhancing surveillance of invasive mosquito species already established in Hawai'i, as well as other mosquitoes that could potentially become established. Airport facilities to be included in the program are: 1) Lihue Airport, 2) Honolulu International Airport, 3) Molokai Airport, 4) Kahului Airport, 5) Hilo International Airport and 5) Kona International Airport. Human activity, especially the global movement of trade goods and increased movement of humans, has dramatically facilitated the importation of invasive mosquitoes and their associated vector-borne pathogens (European Centre for Disease Prevention and Control, 2012; Vaux and Medlock, 2015). The Hawai'ian Islands are particularly vulnerable to accidental introduction of nonnative species, such as mosquitos due to the high volume of military and commercial air traffic. From a biosecurity standpoint, mosquito surveillance at ports of entry is imperative to prevent the introduction and establishment of new species of mosquitoes and associated vector-borne diseases. Surveillance of invasive mosquito species not yet known to be present or established in an area can be triggered by risk of introduction depending on certain circumstances such as: commercial trade of goods associated with transport of invasive mosquito species (eg. used tires, plant material) or air traffic originating in areas known to have invasive mosquitoes and/or mosquito-borne endemic/epidemic areas.

Even though it is not feasible to locally test the efficacy of trapping devices for mosquitoes not currently present in Hawai'i, the incorporation of traps/lures known to work well in other areas can be valuable early detection tools. In those cases surveillance measures need to be customized and proportionally tailored to the level of identified risk, based on the level of trade and travel with regions that are already

colonized by invasive mosquito species (European Centre for Disease Prevention and Control, 2012). Surveillance of already established mosquito species is also very important to detect population outbreaks, outbreaks of vector-borne diseases and to assess effectiveness of control efforts.

The aim of this program is to develop economically-viable surveillance strategies for invasive mosquito species already present in Hawai'i as well as for species at risk of introduction. By testing the efficacy of different trapping devices we can select the most appropriate strategies for mosquito surveillance at different airport facilities in Hawai'i. An economic analysis can help identify the best trap or trap combinations that can provide reliable information.

Mosquito surveillance studies

Mosquito species differ in their choices of breeding habitats, flight ranges, feeding behavior, and activity rhythms. Those factors are critical in selecting the best surveillance methodologies (World Health Organization, 2016). There are several trapping devices commercially available that use a variety of cues for mosquito attraction such as olfactory cues, visual cues, carbon dioxide, heat, etc., and they can influence the efficacies for different genera or species (Lühken et al. 2014). It is often advisable to use several types of traps at a single trap site to collect a representative sample of the species active at that location. The use of more than one trap type and using as many as economically feasible should increase the chance of detecting invasive mosquitoes (California Department of Public Health, 2016).

Testing lures

Several semiochemicals have been identified as mosquito lures including CO₂, lactic acid, octenol, butenol and others (Anderson et al. 2012). Lures can significantly increase the efficacy of mosquito traps. For this section of the study we will test the efficacy of the following lures: 1) CO₂, 2) BG-lure 3) BG-lure + CO₂ and 4) trap only (no lure - control)

4x4 Latin square experiments will be carried out at Hilo International Airport, with 4 trapping locations. At each trap location, individual traps will be placed ~ 20m away from each other. Lures will be tested using BG 1 -sentinel traps. BG-1 traps will be covered with black fabric to increase attractiveness. Traps will be rotated to the next location after 24 hours.

The best and most cost effective lure or lure combination will be used in to test the efficacy of different adult traps.

Testing the efficacy of different adult traps:

Several studies report significant differences in adult mosquito catches between trapping methods. The selection of the most efficient trapping method(s) is crucial to effectively monitor activity of mosquito species already present but also to ensure incursions of exotic mosquitoes are identified at an early stage, allowing for successful control and eradication efforts (Vaux and Medlock, 2015). Trap type choice depends on the target species, the environmental conditions at the selected trap sites, and the availability of resources (dry ice, power supply, trap cost, cost of maintenance, etc.).

Adult traps to include in the efficacy study at Hilo airport will include the following:

- CDC miniature light trap (incandescent light) – literature shows that this trap can work well for *A. vexans*, *Culex* spp. and *Anopheles* spp. Often used with CO₂ to increase catches. One of the disadvantages of these traps is that it attracts other insects. Lures such as Lurex, BG-Lure and Octenol can be added to increase catches.
- CDC blacklight – similar to the CDC miniature light trap, but uses a UV blacklight lamp. This type of trap is known to reduce the collection of non-targets.
- BG sentinel trap – with BG-Lure and/or CO₂ – number one trap used worldwide, tends to have higher capture rates of mosquitoes (male and female) and wide variety of species. These traps use AC power or batteries. Some disadvantages of this trap: expensive, bulky, easy to vandalize. Previous studies have shown that these traps attract mainly unfed females and males (California Department of Public Health, 2016).
- GAT trap (Gravid *Aedes* trap) – attract gravid females. Low cost trap that uses visual and olfactory cues to attract gravid females. Females are captured after they are knocked down by a killing agent. Studies done in the past show that canola oil can be used as efficient killing agent and as an alternative to low-dose pesticides.
- CDC gravid traps – attract gravid females. Preferred trap to capture potentially infectious females as they attract egg-laying females (which have already taken a blood meal and thus may be infected). Traps will be run with clean water as well as the common hay infusion water. Clean water will be used to attract *Aedes* mosquitoes, whereas the hay infusion water will be used to lure *Culex* mosquitoes.

Field trials to compare the performance of different traps at selected sites at airport facilities using a Latin square design scheme. At each site traps will be positioned 20-30 m from each other. Traps will be randomly rotated to the next position to reduce point specific differences.

Some traps may have limited success if adult population is small or patchy in the environment. After the most efficient and cost effective traps are identified a separate study can be designed to test the most efficient trap densities to increase the likelihood of catches.

GAT traps and CDC gravid traps will be compared separately as these traps are known to take more time to effectively trap mosquitoes.

Mosquito magnet trap (Executive model - does not need AC power) – utilizes propane tank to generate power to run a fan to catch mosquitoes and to generate carbon dioxide as an attractant. Chemical attractants such as Lurex (L-lactic acid) and Octenol (1-octen-3-ol) can be added to increase trapping efficacy. This trap is commonly used in Europe for surveillance of invasive mosquito species.

Mosquito magnet traps are to be used within the AOA to avoid vandalism or loss of traps. A total of three traps will be placed inside the AOA at each airport facility.

Test different infusions to use as attractant for ovitraps and gravid traps

An array of chemicals and environmental factors are known to influence mosquito oviposition response (Bentley and Day 1989). Organic infusions of fermented plant material or animal waste are commonly

used to attract gravid females to ovitraps and gravid traps (Service 1993). Moreover, it has been documented that different infusion waters can attract different species of mosquitoes depending on the season (Burkett and Mullen, 2008; McPhatter et al., 2009). Grass infusion waters have proved to be effective in trapping *Culex* females, but have been relatively ineffective in collecting *Aedes* females. We will attempt to test several infusion materials to target mosquitoes other than *Culex* spp. The aim for this portion of the study is to test different infusion materials to attract *A. albopictus* and/or *A. aegypti*. Infusions to be tested will include locally available plant materials such as: koa leaves, pili grass, native sedges (*Carex* spp.), and bamboo leaves. Material will be tested at multiple times throughout the year to account for seasonal variabilities.

There are no standard methods for producing infusion water, nevertheless previous studies have shown that fermentation period, biomass of organic material per volume of water, and methods of fermentation can have significant effects on the attraction to gravid females (Burkett-Cadena and Mullen, 2008, Ponnusammy et al, 2010). A small pilot study can be conducted to test the most effective fermentation period and fermentation process for infusion water production.

Test different size ovitrap containers and/or density of ovitraps

Previous studies have shown that container size can significantly influence the number of eggs laid by container-breeding *Aedes* species (Davis, et al., 2016). A study can be conducted to test if this holds true under Hawai'i's conditions. The study conducted by Davis et al. (2016) also showed that the presence of flowering plants nearby ovitraps had a significant effect on the number of eggs oviposited. The study can also assess if the presence of selected flowering plants nearby ovitraps could attract more gravid females to the traps.

The California Department of Public Health (2016) highlights that detection success of container breeding *Aedes* species with ovitraps may be dependent on the number of ovitraps deployed per area. A separate study can test the number of traps per area that may effectively detect *A. albopictus* and *A. aegypti*. By using an adequate density of traps per area we could also improve the chances of early detection of other invasive container-breeding *Aedes* species.

Study and monitoring sites will be selected in collaboration with DOH staff. Maps with tentative sites will be made available to airport manager and to fire department chief to approve the use of monitoring equipment at those sites.

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CO2 cylinders and propane tanks facts and safety plan

Facts and safety plan for the use of CO2 tanks

Carbon dioxide (CO₂) is a colorless, odorless, non-flammable gas. In terms of worker safety, Occupational Safety and Health Administration (OSHA) has set a permissible exposure limit (PEL) for CO₂ of 5,000 parts per million (ppm) over an 8-hour work day, which is equivalent to 0.5% by volume of air.

For the purpose of this project we will use 5 lb CO₂ tanks to lure mosquitoes to traps. CO₂ will be provided at a flow of 250 - 400 ml/min. Flow rate will be controlled with an adjustable valve. Humans emit 250 ml CO₂ per minute (exhale). The amount of CO₂ released for trapping is therefore for equivalent to one person or two people exhaling CO₂. This rate is therefore not a concern.

Cylinders will be legibly marked, for the purpose of identifying the gas content. Such marking shall be by means of stenciling, stamping, or labeling, and shall not be readily removable. Whenever practical, the marking shall be located on the shoulder of the cylinder.

While in use the cylinders will be placed and secured in an upright position. When not in use, cylinders will be stored in a ventilated storage facility and secured upright at all times.

What are the stability and reactivity hazards of carbon dioxide?

- **Chemical Stability:** Normally stable.
- **Conditions to Avoid:** High temperatures. Temperatures above 52.0 °C (125.6 °F)
- **Incompatible Materials:** Increased risk of fire and explosion on contact with: metal powder or dusts. Not corrosive to metals.
- **Hazardous Decomposition Products:** None known.
- **Possibility of Hazardous Reactions:** None known.

Safety plan:

The CO₂ cylinders will be used in shaded areas and exposed to ambient temperature that does not exceed 100°F.

What are fire hazards and extinguishing media for carbon dioxide?

- **Flammable Properties:** Does not burn.
- **Suitable Extinguishing Media:** Not combustible. Use extinguishing agent suitable for surrounding fire.
- **Specific Hazards Arising from the Chemical:** Can displace oxygen in the air, causing suffocation. Gas may accumulate in hazardous amounts in low-lying areas especially inside confined spaces, resulting in a health hazard. Closed containers may rupture violently when heated releasing contents. In a fire, the following hazardous materials may be generated: very toxic carbon monoxide, carbon dioxide.

Safety plan: 5 lb CO₂ cylinder tanks will be used in ventilated areas and will not be exposed to high temperatures to become a hazard.

What handling and storage practices should be used when working with carbon dioxide?

Handling: Prevent accidental contact with incompatible chemicals. Use the pressure regulator appropriate for cylinder pressure and contents. Secure cylinder in an upright position. Protect cylinders from damage. Use a suitable hand truck to move cylinders; do not drag, roll, slide, or drop.

Storage: Store in an area that is: cool, dry, well-ventilated, out of direct sunlight and away from heat and ignition sources, temperature-controlled, secure and separate from work areas, on the ground floor or preferably, if storing in large volumes, in an isolated, detached building. Always secure (e.g. chain) cylinders in an upright position to a wall, rack or other solid structure.

Safety plan: a pressure regulator will be used when operating the cylinders. Cylinders will be secured upright at all times

Justification for the use CO₂ cylinders at airport facilities: CO₂ cylinders will be used to lure mosquitoes to traps. Cylinders will use a CO₂ regulation kit that will ensure a constant release rate of 250-400 ml/min. The plume of CO₂ produced mimics human exhalation and thus makes traps more efficient at trapping mosquitoes. The use of CO₂ will improve monitoring and surveillance efforts for already established mosquitoes as well as other mosquitoes that could potentially get established.

Facts and safety plan for the use of propane tank for mosquito trap

Propane or liquid propane gas (LPG) is a colorless odorless flammable gas used in cooking, heating and power generation. The odor of propane is from an added odorant. Commercially available "propane" fuel, or LPG, is not pure. Propane gas is denser than air and when release will have a tendency to sink. Propane is flammable when mixed with air (oxygen) and can be ignited by many sources, including open flames, smoking materials, electrical sparks, and static electricity.

Effective April 1, 2002 Washington State adopted a requirement that all propane cylinders with a capacity of less than 40 pounds must have an Overfill Protection Device (OPD). An OPD is a safety feature that helps prevent small propane cylinders from being overfilled. An overfilled cylinder doesn't have enough space left if the liquid expands when exposed to warmer temperatures.

What are the stability and reactivity hazards of propane?

- Chemical Stability: Normally stable.
- Conditions to Avoid: Open flames, sparks, static discharge, heat and other ignition sources.
- Incompatible Materials: Increased risk of fire and explosion on contact with: oxidizing agents (e.g. peroxides), halogens (e.g. chlorine). Not corrosive to: aluminum alloys, carbon steel.
- Hazardous Decomposition Products: None known.
- Possibility of Hazardous Reactions: None known.

Safety plan:

The mosquito magnet trap will be used at a distance of at least 20 feet from any building and away from any source of flammable vapors from liquids such as gasoline, solvents, etc.

What handling and storage practices should be used when working with propane?

Handling: Eliminate heat and ignition sources such as sparks, open flames, hot surfaces and static discharge. Post "No Smoking" signs. Only use where there is adequate ventilation. Immediately report leaks, spills or failures of the safety equipment (e.g. ventilation system). In the event of a spill or leak, exit the area immediately. Never work on pressurized system. Use piping and equipment designed for high pressures and cold temperatures. Isolate and purge all equipment, piping or vessels prior to maintenance or repairs.

Storage: Store in an area that is cool, well-ventilated, out of direct sunlight and away from heat and ignition sources. An approved, fire-resistant area. Separate from incompatible materials. (e.g., oxygen, chlorine gases) On the ground floor or preferably, in an isolated, detached building. Clear of combustible and flammable materials (e.g. old rags, cardboard). Electrically bond and ground containers. Ground clips must contact bare metal. Always secure (e.g. chain) cylinders in an upright position to a wall, rack or other solid structure. Avoid bulk storage indoors. Inside of buildings, cylinders shall be stored in a well-protected, well-ventilated, dry location, at least 20 (6.1 m) feet from highly combustible materials such as oil or excelsior. Cylinders should be stored in definitely assigned places away from elevators, stairs, or gangways. Assigned storage spaces shall be located where cylinders will not be knocked over or damaged by passing or falling objects, or subject to tampering by unauthorized persons. Cylinders shall not be kept in unventilated enclosures such as lockers and cupboards.

Safety plan: Propane tank will be used following recommendations for Mosquito Magnet trap. It will be used away from heat, or ignition sources, in well ventilated areas. The tank, when in use will always be placed outdoors. When not in use, the propane tank will not be stored at airport facilities.

Transportation of small cylinders

- Always transport and store a cylinder in a secure and upright position so it will not fall, shift, or roll.
- Always close the cylinder valve and, if required, seal with a plug, even if the cylinder is empty. Ask your propane retailer if a plug is required.
- Never keep a filled cylinder inside a hot vehicle or transport it inside a closed trunk.
- Always place the cylinder in a well-ventilated area of the vehicle.
- Always proceed directly to your destination and immediately remove the cylinder from your vehicle.
- The law places limits on the number of cylinders and the amount of propane that can be transported in closed-bodied vehicles such as passenger cars and vans. Ask your propane retailer for more information on state and local codes that apply to you.

Safety plan: propane tank will be transported following the transportation requirements described above.

Justification for the use of propane tank at airport facilities: We request the use of propane tank (20lb) to run Mosquito Magnet traps. These traps have 15 patents, and are known to work well. All models of Mosquito Magnet are provided with well thought-out propane consumption regulation and control systems.

Mosquito magnet trap will be placed away from any ignition source and in well ventilated areas. Traps will be placed at least 20 feet away from any buildings
A product manual is attached to this document.

Resources used

<https://access.ewu.edu/Documents/HRRR/ehs/Procedures/PROPANE%20AND%20PROPANE%20TANK%20%20SAFETY.pdf>

<http://www.nfpa.org/public-education/by-topic/safety-in-the-home/gasoline-and-propane/propane-safety>

https://www.ccohs.ca/oshanswers/chemicals/chem_profiles/carbon_dioxide.html

<http://www.cornelius-usa.com/stellent/groups/web/@guest/documents/manual/002811.pdf>