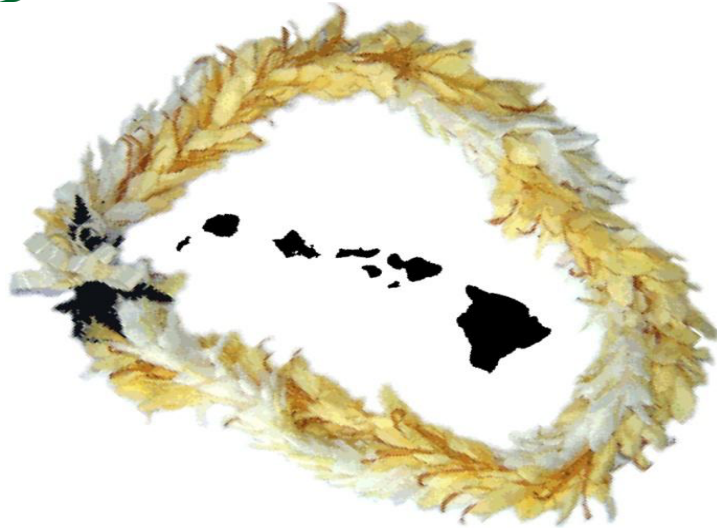




# Interagency Points of Entry, Points of Exit Monitoring Plan

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## ***Māmalu Poepoe***

*Strategic Biosecurity for Hawai'i*

**2014 - 2022**

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# Table of Contents

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I. INTRODUCTION	6
Project Scope and Description:	6
History	7
Target Species	7
Current Situation	7
Additional Priorities and Gaps	9
II. GOALS	10
III. OUTCOMES & STRATEGIES	10
Outcomes:	10
Strategies:	11
IV. PARTNER ROLES & RESOURCES	13
V. BUDGET	14
VI. Citations	15
VII. Appendices	16
I. Monitoring protocol for Africanized honeybees.	16
Site selection:	16
Set up:	16
Checking:	16
Removal:	16
Swarm trap processing	17
Protocol for collecting honey bees to sample for genetic testing:	18
Safety Protocol	19
If Stung:	19
II. Monitoring Protocol for Coconut Rhinoceros Beetle (CRB)	20

Panel Trap	20
Barrel Trap	21
Trap Servicing	22
Visual Surveys	23
III. Standardized surveillance and monitoring procedure for Mamalu Poepoe airport ant surveys	24
Purpose	24
Scope	24
Work instructions	24
Overview	24
Planning the survey	25
Bait preparation	25
Conducting the survey	26
Keeping records	26
File Naming Conventions	29
Data capture and management procedure for mamalu Poepoe Invasive Ant Surveillance	31
Downloading and storage of data	31
IV. SOP for Mosquitoes Standard Operational Procedures Purpose	40
1. Purpose	40
2. Introduction - Hawaii's Ports of Entry	40
3. Mosquito Surveillance Procedures	40
3.1 Ovitrap Operation	41
3.1.1 Ovitrap Mosquito Rearing	41
3.1.2 Additional Larval Surveillance	42
3.2 Biogents Gravid Aedes Trap (BG-GAT) Operation	43
3.3 Biogents Sentinel (BGS) Trap Operation	44
3.4 Gravid Mosquito Trap Operation	45
3.5 CDC Light Trap Operation	46
3.6 New Jersey Light Trap Operation	47
3.7 Mosquito Magnet Trap Operation	48
4. Mosquito Identification by Developmental Stage	49
4.1 Eggs	49

4.2 Larvae	50
4.3 Pupae	51
4.4 Adults	51
5. Handling of Mosquito Specimens Following Invasive Collection & Notification Chain	52
6. Current Mosquito Surveillance Schedule at Each Port of Entry	54
7. Inventory of Surveillance Tools at Each Port of Entry	56
8. Port of Entry GIS and Spatial Data	56
9. Mosquito Surveillance Data Management and Action Thresholds	57
10. Integrated Mosquito Management	58
10.1 Larval Control – Source Reduction	58
10.2 Larval Control – Larviciding	58
10.3 Adult Control – Harborage Reduction	59
10.4 Adult Control – Space / Ultra Low Volume (ULV) Spraying	59
10.5 Adult Control – Residual / Barrier Spraying	60
10.6 Personal Protective Equipment (PPE)	60
10.6.1 PPE – Gloves	61
10.6.2 PPE – Eye Protection	61
10.6.4 PPE – Respiratory Protection	62
10.6.5 PPE – Maintaining Clothing and Personal Protective Equipment	62
10.7 Pesticide Resistance Management	63
References	64
V. CO2 cylinders and propane tanks facts and safety plan	65
Facts and safety plan for the use of CO2 cylinders for mosquito traps	65
Facts	65
Safety plan tanks	65
Facts and safety plan for the use of propane tank for mosquito trap	66
Facts	66
Safety plan for propane tanks:	67
Resources used	68

# 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*<sup>1</sup>. 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 focuses 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 (Hasty, personal communi. 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.

Invasive ants can cause severe damage to agriculture, while some species of ants can also bite and sting people and cause severe pain and serious skin reactions to people and animals. Little fire ant (LFA) is currently widespread in Hawaii island and present at Hilo International Airport (ITO), while other islands are under prevention, containment, or eradication efforts. High populations of LFA in previous years prompted complaints from ITO workers (workers being stung at checking counters for example). An economic study estimated that current control costs for LFA on Hawaii island alone nearly averages \$200 million a year. Continuous monitoring and control of this species as part of the existing monitoring project has significantly reduced populations of LFA at ITO. The red imported fire ant (RIFA) is not currently present in the State. This ant species is highly invasive and extremely aggressive (Gutrich et al. 2007), posing a serious threat to human health. RIFA is estimated to cause a potential \$200 million a year in damages to tourism and agricultural sectors should it establish in Hawaii.

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 Tropilaelaps 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 ports 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 ports 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"> <li>• Coordination support</li> <li>• Funding support</li> <li>• Planning support</li> </ul>
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"> <li>• Vector staff monitoring at airports</li> <li>• Entomology expertise</li> <li>• Mosquito control equipment</li> </ul>
Department of Transportation	Lead for Airports and Harbors	<ul style="list-style-type: none"> <li>• Funding</li> <li>• Travel for project staff</li> <li>• Staff support (access, landscaping adjustments)</li> </ul>
Department of Agriculture	Agricultural and Environmental biosecurity	<ul style="list-style-type: none"> <li>• Expertise (entomologists, monitoring protocols, response)</li> <li>• Space on each Island</li> </ul>
University of Hawai'i College of Tropical Agriculture and Human Resources	Research and Outreach (Extension)	<ul style="list-style-type: none"> <li>• Expertise (entomologists, research)</li> <li>• Grad Students</li> <li>• Lab Space at Extension on each island</li> </ul>
Island Invasive Species Committees	Early Detection, Control/ Eradication of Incipient Species, Outreach	<ul style="list-style-type: none"> <li>• Infrastructure to host monitoring staff</li> <li>• Response capacity</li> <li>• Flexibility</li> </ul>
Pacific Cooperative Studies Unit, Research Corporation of Hawai'i	Project hosting and fiscal sponsor support	<ul style="list-style-type: none"> <li>• Mechanism to administratively house project and staff</li> </ul>
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"> <li>• Expertise (entomologist, monitoring protocols, procedure)</li> <li>• Supplemental monitoring at their locations</li> <li>• Staff for response</li> </ul>

## 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
<b>Total</b>	<b>\$ 32,577</b>

Year 2	
Salaries and Fringe	
d. Program Coordinator	\$ 280,000
e. 6-10 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
<b>Total</b>	<b>\$ 333,000</b>

Years 3-5	
Salaries and Fringe	
f. Program Coordinator	\$340,000
g. 6-12 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
<b>Total</b>	<b>\$378,141</b>

# 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

## I. Monitoring protocol for Africanized honeybees.

***This is an already existing protocol developed by the HDOA Apiary staff.***

Swarm traps are used to monitor high-risk areas. Swarm traps are to be placed with-in a one-mile radius around ports or areas of concern for introduction of new pests and diseases. 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.

### Site selection:

- Placed at eye level, not above the head (as high as is comfortable for maintenance)
- Not placed near area with high public activity
- ~1 mile radius around ports or airports, ~ ¼ mile spacing between traps
- Attached to a fence or tree with zip ties

### Set up:

- Entrance hole facing open area (non-restricted flight path in and out)
- Fresh lure (Mannlake lures last 3 months, we also have 1-year lures)
- Trap label clearly visible

### Checking:

- Visual check first for bee activity from a distance of 5-10 feet
- If no bees are present, walk up and lightly tap trap, log info
- If bees are present, remove and replace trap (see below)
- Replace lure every 3-6 months

### Removal:

- Wear a bee suit and gloves, work together (2 or more)



- Bag trap in black plastic trash bag, zip tie close, label trap (see next slide)
- Replace old trap with new trap & lure. Attach to original location. You can use the bee catchers to collect the stragglers.
- As soon as possible, freeze trap for 48 hrs

Swarm traps should be checked every 4 weeks between November and February and every two weeks between March and October (swarm season). 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:

- Wear protective gear
- Prepare replacement trap (with lid, zip ties, label, and lure)
- 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
- Label trap (Name and Location of Trap, Date, Collectors, Temperament upon retrieval, any other out-of-the-ordinary observations)
- BIISC will drop off trap to HDOA Hilo office (cheryl/maria/Stacey). OISC will drop off trap to UH. All other ISCs will place trap in freezer as soon as possible (freeze for 48-72 hours before processing). KISC and MISC will also follow steps for swarm trap processing.

## Swarm trap processing

To sample a swarm (after it is dead):

Take pictures of inside trap and throughout processing. You can upload pictures directly in the NRDS app.

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.

#### Look for Brood Diseases

- Look for common brood diseases such as; American and European Foulbrood, Chalkbrood, stonebrood, sac brood
  - If foul brood is suspected collect samples separately (Apiary staff on Big Island or Dr. Ethel Villalobos at UH Manoa) will use test kits for preliminary determining presence of disease.

#### Protocol for collecting honey bees to sample for genetic testing:

It is particularly important to sample any bees suspected to be Africanized, such as those caught in swarm traps in the Biosecurity risk zones.

Label should include the following:

(WHERE): trap location/number

(WHO): who collected the swarm

(DATE): date of collection

(COMMENTS): are the bees aggressive? Is the trap heavy?

#### 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:

Ethel Villalobos  
3050 Maile Way Room 310  
Honolulu, HI 96822

---

#### Genetic variation bee sample specimen

Collector:                      Date:                      Island:  
Location description/trap number:  
Swarm trap number

Comments:

---

## Safety Protocol

Always work together, this is a team effort!

Assume Africanization, any swarm encountered could potentially be aggressive. Move slow and deliberately, be prepared.

No persons with a known bee allergy should do this work. Prepare by having an epi-pen and Benadryl with you at all times. (epi-pens must be acquired through a prescription from your doctor).

Drink plenty of water while you are out in hot, humid conditions. Take breaks to hydrate!

### If Stung:

Stay calm, walk away, ASAP scrape stinger along surface of skin to remove with finger nail, or credit card edge

Do not pinch stinger to remove, this releases more venom into your skin

Wash site with soap as soon as you are able (masks pheromone, eliminates bacteria at sting site)

Ice site if needed, take a Benadryl if localized symptoms persist and hinder normal activity (do not drive after consuming Benadryl)

Monitor reaction

if reaction moves from a localized reaction near sting site (normal), to affecting the whole body this is considered a bee allergy and needs to be taken very seriously.

**\*If throat constriction occurs, use epi pen immediately. Do not drive, seek emergency assistance immediately \***

## II. Monitoring Protocol for Coconut Rhinoceros Beetle (CRB)

Selection of trapping location and trap type has been done in coordination with the CRB response team. The CRB response team will provide trapping materials and will provide training to monitoring staff.

Besides monitoring traps, monitoring staff will help record potential breeding sites at airports. If potential breeding sites are identified, the location (GPS coordinates), dimensions, and a “suitability score” will be recorded. The suitability score is a 0-5 scale with zero or one points given for 5 categories.

**Moisture:** 1 point if the material is at least as moist as cake. Dry material gets 0 points.

**Particle size:** 1 point if over 50% of the material has a particle size less than 1”.

**Palm composition:** 1 point if over 20% is composed of palm material.

**Decomposition:** 1 point if the material is decomposing.

**Presence of other larvae:** 1 point if there are larvae of other beetles present.

Monitoring staff will also help identify palm locations around the airports. Palm surveys (to detect signs of CRB feeding damage) will be done once a year.

**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 by 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 for field deployment. The trap is non-lethal and designed to be suspended from trees, fences 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 aggregation lure that attracts 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. If hanging from a hook, select a location with sufficient clearance around the trap to prevent collision with tree branches or other obstructions that might damage or entangle the trap. When attaching the trap body to a fence or pole with zip ties, ensure that the trap is vertical so that beetles impacting the vanes will fall into the funnel and cup below.
3. Select a location that will allow ease of access for servicing and maintenance by program personnel.
4. Avoid placing traps that require a pole to access within twenty (20) feet of power-lines or overhead wires.
5. If placed in trees, select those with limbs which are sturdy/strong enough to support the weight of the trap even if the cup fills with water if the drain hole becomes plugged.
6. 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.
7. 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 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. Enter results in the NRDS app
  - 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. Clear debris from the collection cup and ensure that the drain hole is clear.
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.).
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 completely decomposed
  - c. Possible presence of CRB

### Servicing Data

The information for each servicing is recorded directly onto the NRDS App which contains the following information:

1. Status of the trap
2. Result – indicate if CRB was found in trap during the servicing
3. Adults Count – if CRB adults was collected in the trap, list the total count
4. Lure Change – indicate if the lure was changed during the servicing
5. 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. Palm surveys are done once a year. 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. Teams will walk and/or drive the accessible areas with palms
4. The following data will be collected and entered onto the datasheet:
  - a. Date – calendar date of the survey
  - b. Initials – initials of survey personnel
  - c. Total Healthy Palms – total number of palms trees not exhibiting CRB damage
  - d. Total CRB Damaged Palms – total number of palm trees exhibiting CRB damage
  - e. Notes – list any facts or details related to the visual survey

### III. Standardized surveillance and monitoring procedure for Mamalu Poepoe airport ant surveys

#### Purpose

The purpose of this document is to outline the procedures for conducting surveillance and monitoring for exotic ants at airports for the Mamalu Poepoe project

#### Scope

This standard operating procedure provides a standardised surveillance method for use at Hawaiian points of entry that targets the detection of invasive ant species.

#### Work instructions

##### Overview

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 45-60 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) for the site. 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 (i.e. SPAM) 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
- Spare batteries
- Hi-Viz vests
- Supermarket bags or other large bags
- First aid kit
- pens
- Permanent markers

#### 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. Also, plan to do the survey during when rain is not expected.

Surveys may be conducted in teams of 2 or as individuals. Each surveyor should be able to place and collect around 200-400 sample vials in a day at a single site/sub-site. If working in teams, one person can collect and track GPS data while the other collects and labels sample vials, or one person can deploy and collect sugar baits while the other deploys and collects protein baits. When preparing baits, use the number of samples from previous surveys to estimate the number of samples needed and prepare the sample vials the day before. 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. It's best to make only enough baited vials 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 color lids 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. Alternatively, you can puree the peanut butter and luncheon meat together to form a paste. Protein based bait should be prepared by placing a small, thin smear of peanut butter or paste blend on the inside of each bait vial (do not coat the inside of the vial). In addition, a small cube of luncheon meat is to be placed inside each vial if not blended with the peanut butter.

Use a vial with a different color lid for sugar baits. The sugar baits contain a small, thin smear of light colored jelly or jam on the inside of each vial (do not coat the inside of the vial). The jam should be light colored and not contain seeds, lumps or rinds.

### Conducting the survey

The aim of the survey is to thoroughly sample the ants at the site. This is done by placing bait vials at 10m intervals in a grid pattern over the entire area, alternating between protein baits and sugar baits. It is not important to have the grids at exactly this spacing as long as the spacing is approximately correct. Sections that are all concrete or bitumen do not need to be thoroughly sampled because few ants nest in these locations. However, ants will nest within cracks in the concrete, especially if there is a small amount of vegetation. Common ant habitats are listed in Table 1 and it is important that these are all sampled.

Bait vials should be collected 45-60 minutes after placement. As a guide, surveyors 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 45-60 minutes. GPS waypoints should be taken during pick up for every sample, even if no ants are present. Every sample for a given survey round must be assigned a UNIQUE 3 digit waypoint ID (ex, 001, 056, 348, etc...). Any waypoint ID that has the potential for being confused with another should be underlined to indicate the correct order (ex, 001 vs. 100, 006 vs. 900). Make sure handwriting is neat, clearly legible, and numbers are written in a way to avoid confusion. All vials with ants must be capped upon collecting and labelled with the corresponding waypoint ID.

Surveillance should not occur during or immediately 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 re-survey the rain-affected section.

Bait vials should be placed in the shade where possible. Vials should be positioned with the entrance 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 assigned a GPS waypoint ID and noted/recorded as a 'random, no-bait collection'.

### Keeping records

If multiple surveys are being conducted within a short timeframe and samples are being stored for processing, a field data sheet must be filled out and kept with the samples in order to

differentiate samples from different surveys. Field data sheets must also be filled out for a single survey if samples are collected by multiple people and have overlap in sample number labelling. If this is the case, the sample numbering will have to be adjusted in the final databases (mapping/GIS and excel) in order to have all samples with a UNIQUE identification number. Please refer to table 2 for site code for each airport. In some cases, a 'sub-site' should also be listed for easy future referencing.

Below is an example of the form:

## Survey Sample Field Data Sheet

GPS number(s)	date		
	start time		
	end time		
Site Code	Sub-site		
Surveyor(s)			
	first vial		last vial
vial numbers			
SIGNED	team member 1..... team member 2.....		
Survey notes			

### Keeping records (cont...)

Global positioning systems (GPS) allow streamlined data collection. During the survey, mark unique, 3 digit waypoints for every sample placed and record the waypoint number on the vial. After the day's survey, download the waypoints onto a computer using Mapsource or Garmin Basecamp. Combine data from all GPS units into a single file. Once ALL data from a given survey round for an airport has been compiled in the proper format, save the file as a .gpx and a .txt (tab delimited text ) file. The final (complete) .gpx and .kml must then be uploaded to the Mamalu Poepoe Google Drive in the appropriate folders for the site. The data in the .txt file will be added to the database using Google Sheets as described below.

### File Naming Conventions

In order to ensure consistent data storage, files are to be named as follows:

Filename = [caps] + [port code] + [date]

Site codes can be found in Table 2, and the date convention to be used is YYYYMM. For example, the files produced after a survey at Hilo airport conducted between 10 and 12 March 2009 would be:

***Mp\_ITO\_200903.txt*** is a text file containing waypoint details for each sample. This file will be used by to enter species information.

***Mp\_ITO\_200903.gpx*** is a GPS readable file containing waypoint, route and date-time details for the survey. This file can be loaded back onto a GPS should any sample require further investigation.

***Mp\_ITO\_200903.kml*** is a file that can be read by Google Earth showing each sample waypoint and tracks data. This can be used to produce a map of the survey site showing where each sample was placed. This file can also be converted into shape files for use with ArcGIS mapping software.

Table 1. A 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.

Table 2. Site codes for Hawaiian airport and sea port codes.

Island	Port	Site Code	Frequency of surveys
Hawaii	Hilo Airport	ITO	Quarterly
Hawaii	Kona Airport	KOA	Quarterly
Oahu	Honolulu airport	HNL	Twice a year
Molokai	Hoolehua airport	MKK	once a year
Maui	Kahului airport	OGG	Twice a year
Kauai	Kauai Island Airport	LIH	Once a year

### Data capture and management procedure for mamalu Poepoe Invasive Ant Surveillance

This procedure describes how surveillance data from Mamalu Poepoe surveillance activities are to be downloaded, manipulated and stored for future use.

The location of sample vials placed on ports are recorded with a Global Positioning System (GPS). Where two GPS units are used on the same port survey, Each unit should have a realistic set waypoint range. For example: GPS unit 1 may have a waypoint range of 001-200 while GPS unit 2 has a range of 201-400. The combined waypoints from unit 1 and unit 2 will each have a unique number and geo-location.

When setting the GPS units,

### Downloading and storage of data

There are a number of programs that can be used to download and store data from GPS units including freeware programs Garmin Basecamp<sup>1</sup>, DNRGarmin<sup>2</sup> and Garmin Mapsource<sup>3</sup> (no longer supported by Garmin, but old software still works), and GPS Babel<sup>4</sup>. A free online file conversion utility is also available at if you do not have Google Earth Plus installed.

The following instructions apply when working with **Garmin Mapsource**:

1. connect the GPS to the computer but do not turn on power
2. start the Mapsource program
3. click the icon at the top of a gps unit with an arrow pointing away from it (*"receive from device"*) a popup window will appear showing the GPS unit and allowing a choice of waypoints, tracks and routes to be downloaded. [figure 1].

<sup>1</sup> Go to <https://www.garmin.com/en-US/shop/downloads/basecamp>

<sup>2</sup> Go to <http://www.dnr.state.mn.us/mis/gis/tools/arcview/extensions/DNRGarmin/DNRGarmin.html>

<sup>3</sup> Go to [http://www.gpsvisualizer.com/convert\\_input](http://www.gpsvisualizer.com/convert_input)

<sup>4</sup>Go to <https://www.gpsbabel.org/>

4. if your GPS does not appear in the “device” box, click “*find device*”. If the device can not be found, check the cables and make sure the GPS is turned on.
5. choose to download waypoints, tracks, and routes.
6. the program will download these and show the waypoints in the left hand section of the screen.
7. disconnect but do not erase the data from the GPS unit, yet.
8. repeat steps 1-7 with additional GPS units used in the survey. Mapsource will not allow duplicate waypoints so it is important that all samples have a unique waypoint ID in order to combine data.
9. once all data has been combined, save the GPS file by clicking on “File” and choosing “save as”. Give the file an appropriate name and select “GPS eXchange Format (\*.GPX)” in the “file type” entry. The file name should comply with the format outlined in the previous section
10. save the file again (with the same name) but this time as a **tab-delimited text file**, [see figure 3]
11. open the txt file by right clicking the file, choosing open-with, and selecting Microsoft Excel as the program [figure 4]. The waypoint data will now open in Excel and allow you to modify columns to suit your needs [figure 5].
12. Log into the MP Google Drive and open the appropriate database in Google Sheets. If no database has been set up, you can start a new Google Sheets file.
13. Add a new tab to the database and name it according to the file naming conventions
14. copy the entire worksheet from Excel and paste into the new database worksheet (new tab in Google Sheets [figure 6]. All changes will automatically save to the Drive
15. Open the .gpx file in Google Earth. Select your file from the “Temporary Places” list. Right click and select “save place as”.
16. Make sure to name the file appropriately and select .kml in the “file type” entry field.
17. Double check that all files are complete and data is saved correctly by re-opening each file and scanning the contents.
18. Once confirmed that all the files are complete and data is correct, you may now clear the data from the GPS units.
19. Upload the completed files onto the Mamalu Poepoe Google Drive making sure they are saved into the correct files.
20. **Do not delete the working files from your desktop until you receive confirmation that the files on the Drive are accessible and complete.**



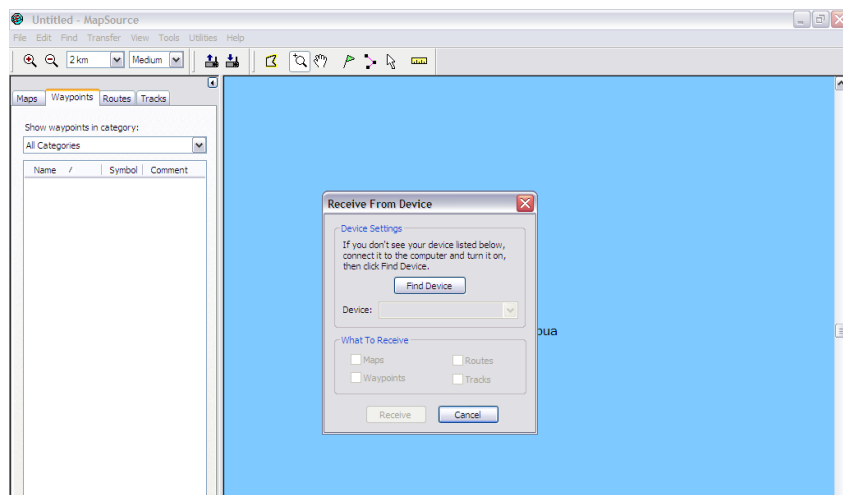


Figure 1. retrieve screen in Garmin Mapsource

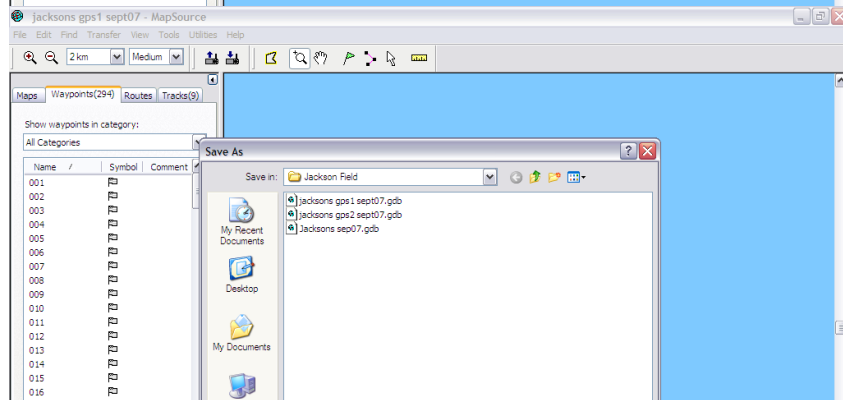


Figure 2 save-as screen for saving to a “GPX” file

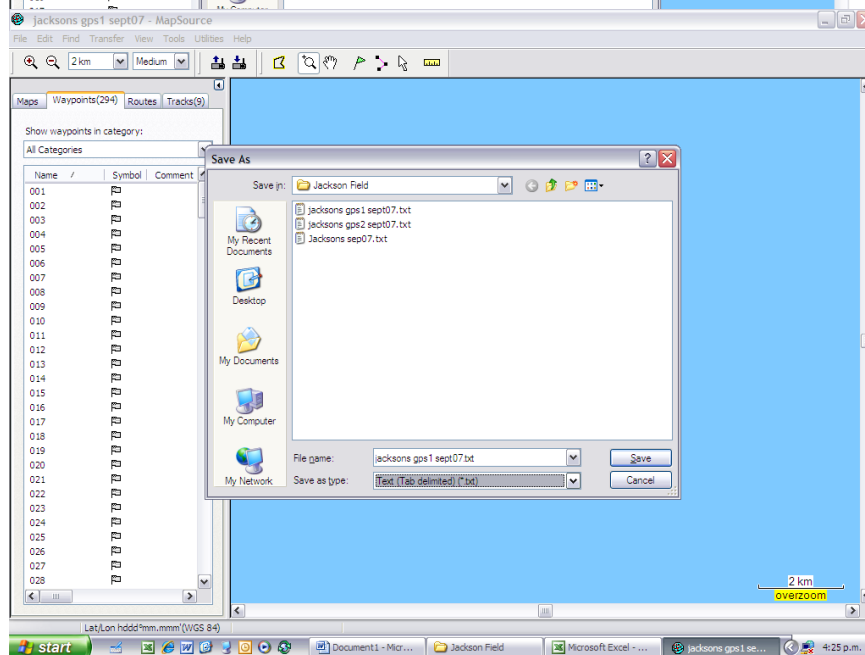


Figure 3. saving as a tab-delimited text file

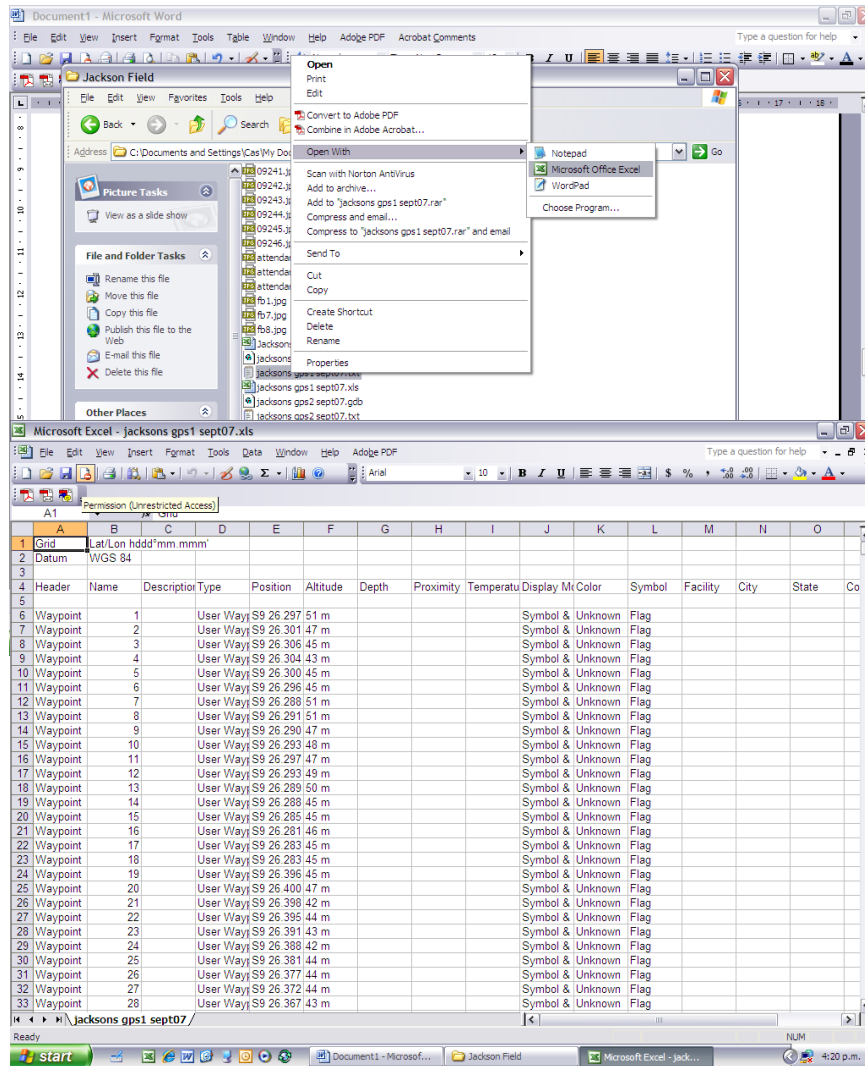


Figure 4. open the txt file with Microsoft Excel

Figure 5. txt file open in Excel

Databases - Google Drive 20170914\_MP\_LIH.xlsx

doc.google.com/spreadsheets/d/17EEEdDN1HimnG9plowK2sCedcG14dU11KXP-YC3h8/edit#gid=1669722016

20170914\_MP\_LIH.xlsx

File Edit View Insert Format Data Tools Add-ons Help All changes saved in Drive

Comments Share

Grid

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Grid	Lat/Lon hddd?mm.mmm'												
2	Datum	WGS 84												
3	QA/QC:	MM												
4														
5	Header	Name	GPS ID	Bait	ID		ID By:	Type	Position	Altitude	Depth	Proximity	Temperat	Display M Co
6	Waypoint	001	KISC7	NIL				User Wayy	N21 58.57	226 ft				Symbol & Ur
7	Waypoint	002	KISC7	NIL				User Wayy	N21 58.57	225 ft				Symbol & Ur
8	Waypoint	003	KISC7	pb/s	Paratrechina longicornis		AW	User Wayy	N21 58.57	227 ft				Symbol & Ur
9	Waypoint	004	KISC7	NIL				User Wayy	N21 58.57	228 ft				Symbol & Ur
10	Waypoint	005	KISC7	pb/s	Paratrechina longicornis		AW	User Wayy	N21 58.58	230 ft				Symbol & Ur
11	Waypoint	006	KISC7	NIL				User Wayy	N21 58.58	225 ft				Symbol & Ur
12	Waypoint	007	KISC7	pb/s	Brachymyrmex obscuria		AW	User Wayy	N21 58.58	228 ft				Symbol & Ur
13	Waypoint	008	KISC7	NIL				User Wayy	N21 58.58	226 ft				Symbol & Ur
14	Waypoint	009	KISC7	pb/s	Paratrechina longicornis		AW	User Wayy	N21 58.59	226 ft				Symbol & Ur
15	Waypoint	010	KISC7	NIL				User Wayy	N21 58.59	225 ft				Symbol & Ur
16	Waypoint	011	KISC7	NIL				User Wayy	N21 58.59	225 ft				Symbol & Ur
17	Waypoint	012	KISC7	NIL				User Wayy	N21 58.59	227 ft				Symbol & Ur
18	Waypoint	013	KISC7	NIL				User Wayy	N21 58.59	228 ft				Symbol & Ur
19	Waypoint	014	KISC7	Jelly	Paratrechina longicornis	Brachymyrmex obscuria	AW	User Wayy	N21 58.59	227 ft				Symbol & Ur
20	Waypoint	015	KISC7	NIL				User Wayy	N21 58.59	229 ft				Symbol & Ur
21	Waypoint	016	KISC7	NIL				User Wayy	N21 58.59	227 ft				Symbol & Ur
22	Waypoint	017	KISC7	pb/s	Cardiocondyla emeryi	Pheidole megacephala	AW	User Wayy	N21 58.59	227 ft				Symbol & Ur
23	Waypoint	018	KISC7	NIL				User Wayy	N21 58.59	229 ft				Symbol & Ur
24	Waypoint	019	KISC7	NIL				User Wayy	N21 58.59	228 ft				Symbol & Ur
25	Waypoint	020	KISC7	NIL				User Wayy	N21 58.60	228 ft				Symbol & Ur
26	Waypoint	021	KISC7	NIL				User Wayy	N21 58.60	229 ft				Symbol & Ur
27	Waypoint	022	KISC7	Jelly	Plagiolepis alluaudi		AW	User Wayy	N21 58.61	228 ft				Symbol & Ur
28	Waypoint	023	KISC7	pb/s	Pheidole megacephala		AW	User Wayy	N21 58.61	230 ft				Symbol & Ur
29	Waypoint	024	KISC7	NIL				User Wayy	N21 58.61	230 ft				Symbol & Ur
30	Waypoint	025	KISC7	pb/s	Paratrechina longicornis		AW	User Wayy	N21 58.61	231 ft				Symbol & Ur
31	Waypoint	026	KISC7	NIL				User Wayy	N21 58.61	230 ft				Symbol & Ur
32	Waypoint	027	KISC7	pb/s	Paratrechina longicornis		AW	User Wayy	N21 58.61	230 ft				Symbol & Ur

+ MP\_LIH\_201703 MP\_LIH\_201709

Figure 6. Copy the worksheet from Excel and paste into the new tab of the Google Sheets database.

The following instructions apply when using **Garmin Basecamp**:

1. start the Basecamp program
2. connect the GPS to the computer but do not turn on power
3. the gps unit should appear in the “Devices” list with a file named “internal storage” [figure 7]. This file contains all of the data within the connected GPS unit. If your GPS does not appear in the “device” list, unplug the device and try connecting to a different port.
4. drag and drop the file “internal storage” into “My Collection” in the Library list [figure 8].
5. Rename “internal Storage” following the File Naming Convention described above.
6. disconnect but do not erase the data from the GPS unit, yet.
7. repeat steps 1-3 with additional GPS units used in the survey.
8. Drag and drop “internal storage” for each GPS into the file for the survey you are working on under “My Collection” [figure 9]. Basecamp will not allow duplicate waypoints in a file so it is important that all samples have a unique waypoint ID in order to combine data. If duplicate waypoints are entered, Basecamp will automatically add a ‘0’ before the waypoint ID of the duplicate. This makes the data messy and difficult to reference.
9. once all data has been combined, highlight the file you want to save (if multiple items in “My Collection”). Save the GPS file by clicking on “File” and choosing “Export”. Make sure the file name is correct and select “**GPS eXchange Format (\*.GPX)**” in the file type entry. The file name should comply with the format outlined in the previous section
10. export the file again (with the same name) but this time as a **tab-delimited text (.txt)** file
11. Export the file a third time as a “**KML 2.2 Document, v2 (.kml)**” file. This can be done in Basecamp without opening Google Earth.
12. Double check that all files are complete and data is saved correctly by re-opening each file and scanning the contents.
13. Once confirmed that all the files are complete and data is correct, you may now clear the data from the GPS units.
14. Upload the completed .gpx and .kml files onto the Mamalu Poepoe Google Drive making sure they are saved into the correct files.
15. **To save the .txt file in the database on the Drive**, open the appropriate database in Google Sheets
16. Add a new tab to the database and name it according to the file naming conventions
17. Open the .txt file using Microsoft Excel by right clicking the file, select “open with”, then selecting “Excel”
18. copy the entire worksheet from Excel and paste into the new database worksheet (new tab in Google Sheets). All changes to the database will automatically save to the Drive

**Do not delete the working files from your desktop until you receive confirmation that the files on the Drive are accessible and complete.**

Like Google Earth, Garmin Basecamp will retain items in the “My Collection” list for future use. You can delete lists from “My Collection” when you are finished, or save for future use or as a back-up. Always double check and make sure you are working with and adding data to the correct list when multiple lists are present.

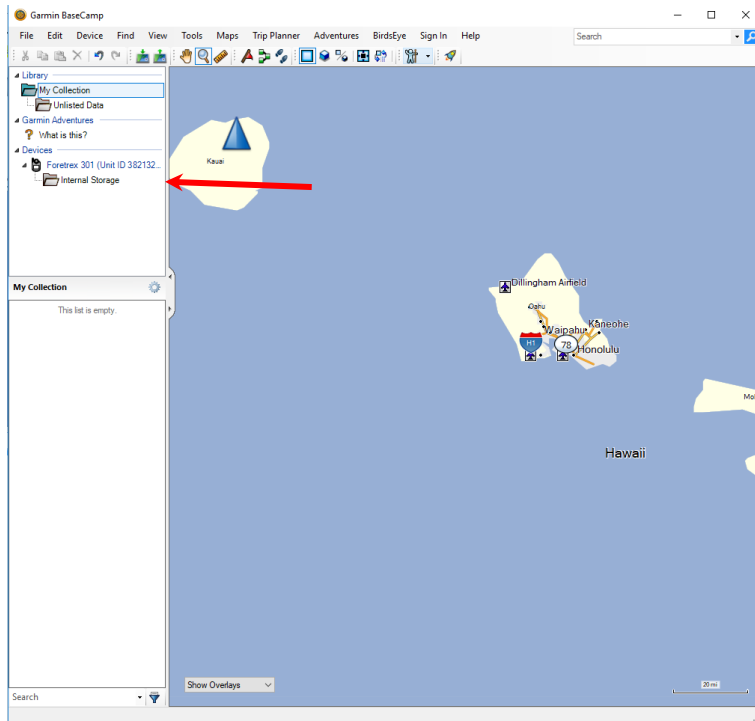


Figure 7: Device will appear in the “Devices” list.

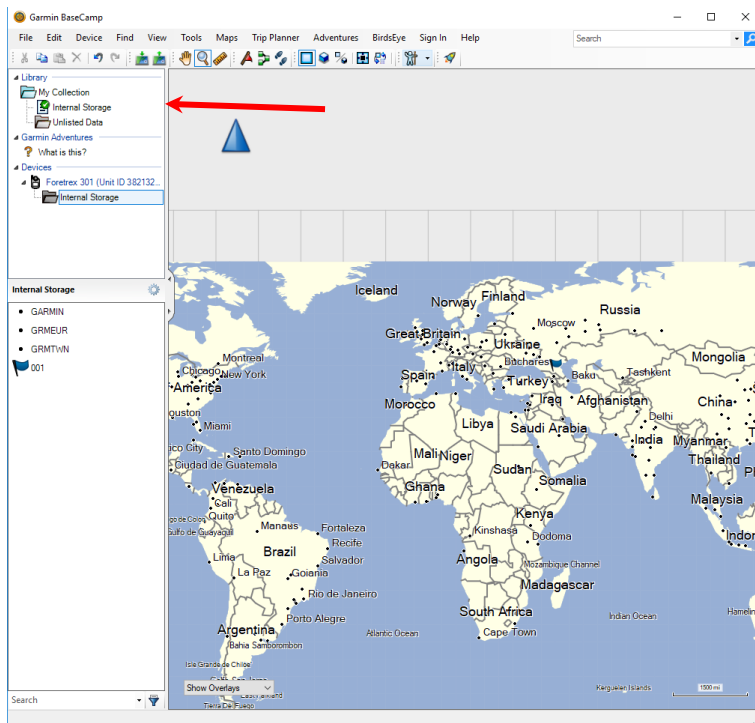


Fig 8: Drag and drop the “internal Storage” folder into “My Collection” and rename using the proper file naming convention.

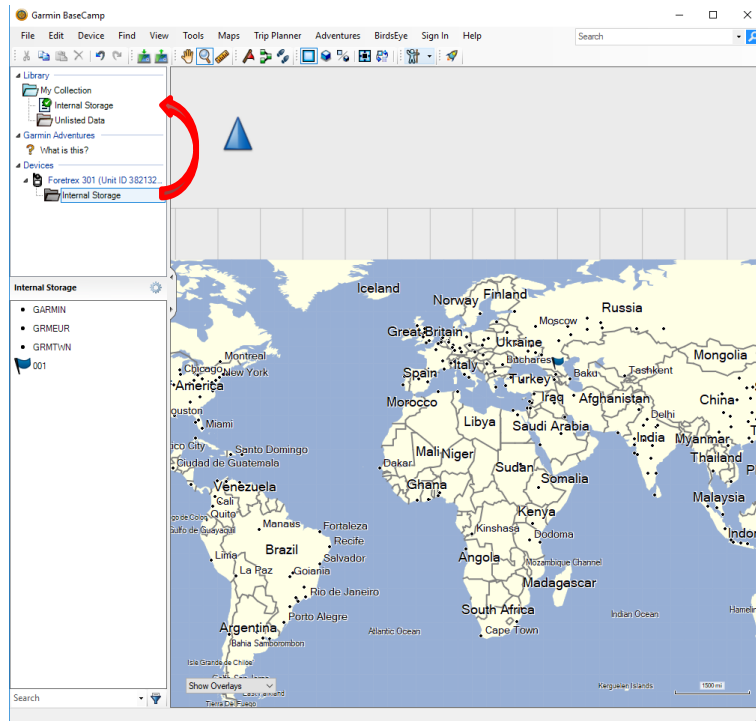


Fig 9: Drag and drop “internal storage” from additional GPS units into the new working file under “My Collections”.

## Specimen Identification

Only qualified persons (such as entomologists and those who underwent ant identification training) may identify specimens collected during MP surveys. All species ID's must be confirmed by a qualified individual via voucher specimen submission.

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 numerous 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. Each identifier will submit THEIR OWN voucher specimen collection individually. For example: if 2 people are working on specimen identification, there will be 2 sets of voucher specimens sent to HAL.

## Data Entry

All species ID's will be entered into the Google Sheets database for each site. Each complete survey for the site will have its own tab in the database. Once the GIS data has been copied and pasted into the database, extra columns will need to be added for “GPS ID”, “Bait Type”,

“Species ID” (2 columns), and “ID By”. These columns will immediately follow the “waypoint” column. Samples with no ants will have NIL recorded as the species ID and those with ants will have the complete scientific name (*Genus species*) recorded as the species ID. Enter the identifier’s initials in the “ID By” column.

At the top of the worksheet, an entry should be added for “Quality Assured/Quality Control (QA/QC) BY” where the person who confirmed the initial ID’s enters their initials.

## IV. SOP for Mosquitoes Standard Operational Procedures

### Purpose

#### 1. Purpose

This document describes activities for routine mosquito monitoring and control at airport facilities, guidelines to counteract population increases of established mosquito species, and response strategies for interceptions of new species at airport facilities in Hawaii. This document describes optimal use of resources, planning, monitoring, and decision-making.

#### 2. Introduction - Hawaii's Ports of Entry

Hawaii's geographic isolation provides natural protection from invasive species. However, increased movement of people and goods has facilitated the arrival of invasive species into Hawaii. Ports of entry (POE) into Hawaii include commercial, noncommercial, and military seaports and airports.

Hawaii has seaports and airports on every main island. These ports are international or domestic (arrivals only from the US mainland or interisland). Once invasive species establish on one island, they easily make their way to neighboring islands through interisland trade and travel.

Surveillance and control of vectors at ports of entry prevent invasive species from establishing in local environments. These activities also prevent the exportation of local vector species to other destinations. Ports of entry can provide suitable habitats for the establishment of various mosquito species as they provide ample resting and breeding site locations. Specifically, some airport facilities in Hawaii have forested areas inside or in the vicinity of the facilities that can provide both resting and breeding sites. Other facilities have additional breeding sites nearby (resorts with water ponds, open sewer facilities, etc.). Table 1 provides examples of breeding and resting sites that can be found at ports.

#### 3. Mosquito Surveillance Procedures

The Hawaii Department of Health utilizes various mosquito monitoring techniques and traps to collect mosquitoes at ports of entry. The following sections describe traps and the operational procedures for their use by the Hawaii Department of Health at ports of entry into Hawaii to monitor for invasive mosquito species. Operations manuals for these traps can be found in Appendix 2. If CO2 canisters or propane tanks are being used along with a mosquito trap, additional safety is provided in Appendix 6.



### 3.1 Ovitrap Operation

Ovitrap (Figure 1) are small metal, glass or plastic containers, usually dark in color, containing water and a substrate (e.g., wood, seed germination paper, cloth, plant gel) where female mosquitoes lay their eggs. Ovitrap detect the presence of egg-laying females which preferentially lay eggs in artificial containers, such as *Ae. aegypti*, *Ae. albopictus*, and other *Aedes* spp. Adequate sampling requires regular (weekly) trapping at fixed sites, which represent habitat types present in the community. Ovitrap should not be deployed in the field for more than one week at a time because they could become larval sites that contribute to adult mosquito proliferation.



Figure 1. Ovitrap

**PROS:** inexpensive, easily deployed, and easily overlooked by pedestrians. A small number of ovitrap is usually enough to determine vector presence; fewer than 100 ovitrap can reliably estimate abundance in a large urban neighborhood. Typically, one ovitrap is placed per city block. Lastly, ovitrap data is easy to analyze and is usually expressed as the percentage of positive ovitrap (ovitrap with eggs). The mean number of eggs per ovitrap can be used to estimate adult mosquito abundance.

**CONS:** ovitrap data may require caution as ovitrap compete with naturally occurring larval habitats and the estimates from oviposition surveys may not accurately reflect the abundance of gravid females under some conditions. Some degree of training in microscopy may be needed for accurate egg counting especially when there is debris on the oviposition surfaces. The collected eggs need to be hatched and reared out in the laboratory and the larvae or adults identified to species, which requires trained personnel. If traps are not serviced at a timely manner, they can be a breeding site.

1. Label collection sticks with date and location information and place them in the desired trap location.
2. Fill ovitrap 2/3 full of new grass infused water. Leave trap in desired location for a maximum of one week.
3. Collect the stick from the ovitrap and inspect the ovitrap water for mosquito eggs and larvae. Collect all the eggs and larvae from the ovitrap and bring them to a lab for identification.
4. Dry collected eggs and collection sticks for 10 days, then count the number of eggs under a microscope. Record live and dead eggs for a total number of eggs per stick.
5. After recording the number of eggs from each collection stick, begin the mosquito rearing process.

#### 3.1.1 Ovitrap Mosquito Rearing

1. Place a collection stick with deposited mosquito eggs in a breeding container and fill with water.
2. Add 2-3 pinches of fish food as a nutrient source for hatched mosquito larvae.

3. When the larvae reach 3rd and 4th stage instars, remove the stick and pour out half of breeding water from the breeding container. Be sure to keep all larvae within the container
4. Attach the top section of the breeding container with the flight funnel to the rearing container. (If additional mosquito food is required, add a fish food at this step)
5. Upon emergence, the adult mosquitoes will fly up the funnel and into the top compartment of the container. When all the adult mosquitoes are dead in the container, count and record the number of adult females and males per breeding jar.
6. Wash and dry the materials as appropriate between uses.

### 3.1.2 Additional Larval Surveillance

In addition to the use of ovitraps to collect mosquito eggs and larvae, additional larval surveys should be regularly completed at known breeding sites throughout and surrounding the airport facility. This larval surveillance should include the documentation and mapping of permanent standing water sources (e.g. storm drains, bromeliad plants), the sampling of these permanent standing water sources to collect immature mosquitoes that may be found at the POE, and the removal of temporary breeding sites (e.g. construction materials, debris, uncovered driving barriers) throughout the facility, if possible. Additionally, inspectors should be on the lookout for changes (such as replacing landscape plants that harbor mosquito larvae – such as bromeliads) to the airport facilities that may promote mosquito breeding.

Table 1 is a World Health Organization (WHO) listing of potential mosquito breeding and harborage sites where larval or adult mosquitoes may be collected for identification at ports of entry.

Table 1: Potential Breeding and Resting Places of Mosquitoes at Ports, Airports, Ground Crossings and in the Periphery Areas (WHO, 2016)	
<b>Breeding sites</b>	<b>Resting Places</b>
Ponds	Human dwellings/restrooms
Puddles	Sheds
Ditches	Indoor hanging objects
Surface drains	Crevices
Grassy and marshy land	Bushes/vegetations - wild and in gardens
Pits and depressions	Underneath furniture
Scrap water and water-containing depressions in sheeting	Curtains
Containers of different varieties and shapes	Underneath or on the inside of tanks
Water chambers	Cargo boxes /holds
Hydrants	Work stations
Tires including fenders	Walls of buildings and underneath roofs
Ground and overhead water tanks	Open luggage/cargo boxes or containers
Septic tanks	Cartons and containers
Terraces/roof tops	Scrapped crafts, vehicles, vessels, etc.
Curing waters in construction/development sites	Tire dumps
Wells	Abandoned structures and buildings
Iron or loader buckets	Tree holes

### 3.2 Biogents Gravid Aedes Trap (BG-GAT) Operation

**DESCRIPTION:** Gravid traps take advantage of female mosquito oviposition behavior. Biogents Gravid Aedes Traps (BG-GAT traps) are enclosed containers of water (sometimes with an attractant infusion) that mimic typical artificial container oviposition sites. Once female mosquitoes enter a trap, they encounter a killing agent (insecticide and/or an adhesive). Most traps also include a barrier to prevent trapped females from dropping eggs into the water. BG-GAT traps (Figure 2.) can also be treated on its inner surface with insecticide or oil to kill captured mosquitoes on contact.

**TARGET:** Gravid container-breeding female mosquitoes; *Aedes aegypti* and *Ae. albopictus*.

**PROS:** Target-specific for container-breeding *Aedes* spp. Traps are low maintenance as collection intervals can be extended to one week or more. A high-density trap deployment can also be used as a population reduction strategy.

**CONS:** Traps may support larval development if not inspected regularly. Larger traps are highly visible and water infusions may smell offensive to nearby individuals.

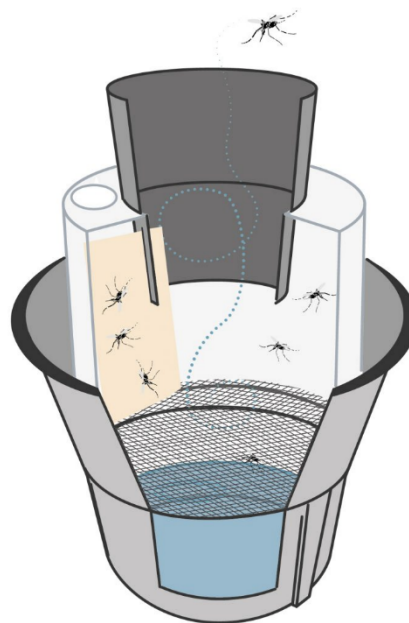


Figure 2. GAT Trap

1. Fill the bucket with new grass infused water up to the drainage holes (when you don't have grass infused water, add a very small hand full of hay or grass as an attractant). Replace grass infused attractant once every two weeks.
2. Put the catch bag over the open end of the transparent chamber. Tighten the top part of the catch bag by pulling the cord. Secure the catch bag with the mounting ring. (After strong or frequent rain, the catch bag might hang into the water due to its own weight making the identification of the captured mosquito difficult. If you cannot avoid placing the trap where it is subject to rain, it is recommended fixing the catch bag in place with the mounting ring.).
3. Place the transparent chamber on the water filled trap bucket.
4. Insert the black funnel in the chamber.
5. Place a residual insecticide sprayed cloth on the catch bag within the trap. Replace this cloth at least once a month or as environmental condition dictate due to exposure to a water sprinkler, heavy rainfall, or physical damages.
6. Collect all specimens in the catch bag and bring them back to a lab for further identification.

**PS:** Place BG-GATs in shaded, humid, and wind protected locations.

### 3.3 Biogents Sentinel (BGS) Trap Operation

**DESCRIPTION:** The Biogents-Sentinel (BGS – Figure 3) trap is a lightweight pop-up cylinder with an opening on its top surface. A funnel is fitted into the opening and a small electric fan pulls mosquitoes into a collection bag. Traps are usually equipped with odorant lures available from Biogents. CO<sub>2</sub> canisters or dry ice can also be used in conjunction with, or as an alternative to, Biogents lures. Newer versions of the trap feature a weighted flap that closes to prevent mosquitoes escaping if the fan fails.

**TARGET:** Primarily *Aedes aegypti* and *Ae. albopictus*, however other species are also attracted to the trap. Target species will change depending on attractants used as CO<sub>2</sub> will broaden the diversity of the mosquito collection.

**PROS:** Sensitive for human-seeking mosquitoes, especially *Aedes* spp. Traps have high portability with low visibility. Specimens do not pass through a fan and are often in better condition than other fan trap collections.

**CONS:** Requires battery maintenance or connection to a power outlet, meaning frequent collection may be required depending on power source. Predators and ants may damage or remove specimens from the traps. May need to shelter the traps to prevent rain damage. Some evidence suggests that certain populations of *Ae. aegypti* may be repelled by the 1-octen-3-ol bait found in the BG lure.

1. Open the BGS trap carrying bag and remove the components.
2. Unhook the eyelet on the carrying handle and open the trap body.
3. Attach the funnel net (optional) and then the catch bag over the protruding ring of the intake funnel. Tighten the top of the catch bag.
4. Insert the intake funnel with the attached funnel net and catch bag into the opening on top of the cover.
5. Unwrap the plastic label from the BG-Lure cartridge.
6. Pop out the white BG disc from the hole in the cover when using the BG-Lure cartridge.
7. Insert the desired attractant into the trap. When using the BG-Lure cartridge it can be placed into the hole on the cover.
8. Connect the battery to the battery cable. Alternatively, you can connect the power transformer. Connect the open ends of the battery cable with the ventilator cable. Please pay attention to always connect with the arrows in one line.
9. When the power is turned on, the shutter will automatically tilt open. When the power is shut off or if the battery fails, the shutter automatically closes which ensures that the caught mosquitoes cannot escape.



(c) Biogents AG

Figure 3. BGS Trap

10. Alternatively, you can place the battery within the body of the trap. For this purpose, open the trap by unhooking the clips of the cover and remove the cover.
11. Place the battery into the fixed straps located on the bottom of the body and tighten the strap.
12. Connect the battery cable to the battery and guide the other end of the cable out of the opening on the side of the trap.
13. Connect the open ends of the battery cable with the ventilator cable. The trap is now running and the shutter of the intake funnel will tilt open. You can easily disconnect and connect the cables to switch the trap off or on without the need of opening the trap body.
14. Collect all specimens in the catch bag and bring them back to a lab for further identification

### 3.4 Gravid Mosquito Trap Operation

**DESCRIPTION:** Gravid traps (Figure 4) take advantage of female mosquito oviposition behavior. Unlike BG-GAT traps, gravid traps are open containers of water (sometimes with an attractant infusion) that mimic typical potential open oviposition sites. Instead of coming into contact with an insecticide, a structure (either a toolbox or a tower container) containing a fan, a capture chamber, and a battery power sources is placed over the open source of water. As mosquitoes approach the water source to oviposit, they are sucked into the holding chamber and held there for collection.



*Figure 4. Gravid Trap*

**TARGET:** Trap target dependent on the infusion used. Grass infused water typically used to attract *Aedes* mosquitoes. Infusions using animal waste or other wastewater sources typically used to attract *Culex* mosquitoes.

**PROS:** Very effective for capturing gravid female mosquitoes, particularly in dry and arid areas with limited alternatives for oviposition.

**CONS:** Handling of waste water and the development of infusions can be of concern. Traps need to be checked regularly as traps are dependent upon battery sources power and maintenance. May serve as a potential breeding source if traps are not regularly maintained.

1. Place the black collecting duct with the large "O" ring around it into the hole in the bottom of the case. The pick-up tube should be placed so that the longer end is outside the toolbox.
2. Place the chamber's mesh sleeve so that it goes on the outside of pick-up tube.
3. Install full charged 6V battery.
4. Place the toolbox case on top of the black tray so that the feet rest on the outside edges of the tray.



5. Fill the black tray with 2-3 days grass infused water until the water is approximately one inch from the mouth of the intake duct.
6. Turn on switch and close lid to toolbox. Lid must be completely closed for trap to operate properly.
7. Collect sample every 24 hours or before the power source of the trap expires.

### 3.5 CDC Light Trap Operation

**DESCRIPTION:** CDC light traps (Figure 5) are typically used for collection of mosquitoes for arbovirus surveillance. Baited traps are suspended from a tree or other vertical structure as a bait or CO<sub>2</sub> is supplied as an attractant. CO<sub>2</sub> is typically supplied by a cooler of dry ice that releases CO<sub>2</sub> above or in the general proximity to the trap. A small electric fan pulls mosquitoes into a collection net or container. Bait variations include using CO<sub>2</sub> provided by gas cylinders or by yeast fermentation vats, but gas cylinders are prone to theft and yeast fermentation often produces inconsistent rates of CO<sub>2</sub>. Non-target insects or other species may also be attracted to yeast fermentation mixtures. These traps can be used with or without a light as an additional attractant. A BG lure may also be used with the CO<sub>2</sub> trap to enhance attractiveness for *Aedes* spp.

**TARGET:** Host-seeking adult female mosquitoes. Nocturnal mosquitoes or those active right at dusk or dawn are particularly attracted, which typically includes *Culex*, certain *Aedes*, and *Anopheles* mosquito species. CO<sub>2</sub> can be used to attract diurnal mosquitoes as well.

**PROS:** Broad range of target species. The trap height can be adjusted to attract different invasive species.

**CONS:** May exhibit collection bias related to host-seeking differences in local vector ecology. The trap requires regular battery and CO<sub>2</sub> replacement. Specimens may be damaged as they pass through the fan towards to collection net. CDC light traps may capture large numbers of non-target insects, especially if using lights. The trap may not be attractive to blood-fed females.

1. Identify a site location where the trap can be securely hung and that is near potential mosquito habitats. If possible, traps should be hung ~30 feet from the nearest building and away from other sources of artificial light.
2. Hang the top of the trap, the protective shield, ~5 to 6 feet above the ground using a light trap stand or by attaching it to an existing structure. Traps can also be hung from CO<sub>2</sub> canisters or containers containing dry ice to increase their attractiveness.
3. Attach the light and fan electrical components to the underside of the protective shield.



Figure 5. CDC Light Trap

4. *Attached the collection net or container to the underside of the electrical components so that mosquitoes attracted to the light and/or CO<sub>2</sub> will be captured.*
5. *Connect the trap to the appropriate power sources, including a battery or an electrical socket.*
6. *Ensure that the trap light bulb, fan, and potentially the CO<sub>2</sub> source are working properly.*
7. *Collect sample every 24 hours or before the power source of the trap expires.*

### 3.6 New Jersey Light Trap Operation

**DESCRIPTION:** Similar to the CDC light trap, the New Jersey light trap (Figure 6.) is a trap uses a light source as an attractant for nocturnal mosquito species. Mosquitoes are attracted to the light, before being sucked into a collection net or chamber by a small electrical fan. Unlike the CDC light traps, New Jersey light traps are designed to be left within the environment at all times with a metal protective covering and topper that protects the electrical components and the collected mosquitoes from environmental conditions. Additionally, New Jersey light traps are powered through a plug to an electrical outlet that provides consistent power to the trap, allowing it to collect mosquitoes over the course of multiple weeks without maintenance. CO<sub>2</sub> can also potentially be used to increase the traps effectiveness with diurnal species, but this is not typical.

**TARGET:** Host-seeking adult female mosquitoes. Nocturnal mosquitoes or those active right at dusk or dawn are particularly attracted, which typically includes *Culex*, certain *Aedes*, and *Anopheles* mosquito species.

**PROS:** Broad range of target species. The trap height can be adjusted to attract different invasive species. Relatively low maintenance as traps do not need to be checked daily as they are plugged into a wall outlet, not a battery.

**CONS:** Requires being plugged into a wall or having access to AC power, which limits trap placement and means they may not be set up in the ideal mosquito habitat. Mosquito samples must pass through the fan, potentially removing scales and other mosquito identifiers. May capture large numbers of non-target insects within the traps.

1. *Identify a site location where the trap can be permanently hung including sites that have permanent and solid structures, that have ready access to a power outlet, and that are near potential mosquito habitats. If possible, traps should be hung ~30 feet from the nearest building and away from other sources of artificial light.*
2. *Hang trap with installed light bulb at the identified location using proper supportive materials such as chains and hooks supported by wood studs or metal*



Figure 6. New Jersey Light Trap

- hangers. Traps should be mounted 5 to 6 feet above ground.*
3. *Insert, attach, and tighten collection container or net within the trap's metal cylinder below the traps fan.*
  4. *Connect trap to power source and ensure that both the light bulb and fan are operational. Change bulb and perform maintenance upon unplugged traps as needed.*
  5. *Collect samples at least once a week to ensure sample integrity and that they will still be able to be identified.*

### 3.7 Mosquito Magnet Trap Operation

DESCRIPTION: Mosquito Magnet traps (Figure 7) attract blood seeking mosquitoes through the conversion of propane into CO<sub>2</sub> to mimic a potential host's breathing behavior. Mosquitoes that are attracted to the omitted CO<sub>2</sub> are then sucked into a chamber by a fan located within the trap. An additional octenol attractant is used within the trap to provide greater attractiveness at shorter ranges. Mosquito samples caught within the trap usually die within 24 hours from dehydration. The propane tank and the additional octenol attractant should be replaced every three weeks for optimal performance. The electrical components of the trap are powered by an internal rechargeable battery that has the potential to last an entire mosquito season, or ~6 months. Insect containers should be checked weekly to prevent sample degradation and to ensure samples are identified as soon as possible.



*Figure 7. Mosquito Magnet Trap*

TARGET: Host-seeking adult females.

PROS: Relatively low maintenance trap as they do not need to be checked daily due to the consistent CO<sub>2</sub> production from the propane tanks. Minimal battery usage meaning the battery only needs to be changed once per mosquito season.

CONS: Additional safety considerations need to be taken while handling propane tanks. Nontarget insects may be attracted to the CO<sub>2</sub> produced and the collection may be impacted by predators that enter the trap to feed upon captured insects.

Additional safety information regarding the usage of propane tanks can be found in the separately provided appendix materials.

1. *Place base upside down on a flat surface, unfold U-shaped support leg until it locks in place.*
2. *Turn base and leg assembly right-side-up and place support pole into cavity on base.*
3. *Place trap power head onto pole. Press down firmly with two hands to secure. Assembled trap should have the front of power head/trumpet placed farthest from the wheels in the same direction as base U-shaped legs.*



4. *The battery is not fully charged at the factory; charge the battery before the first use. Under proper usage, one charge lasts an entire mosquito season.*
5. *Place trap in an upwind, shaded location that is near groundcover or potential harborage sites.*
6. *Place propane tank onto the molded cradle.*
7. *Make sure propane tank is fully closed. Attach propane regulator hose to propane tank. Secure tightly by hand.*
8. *Turn propane on by slowly turning valve counter-clockwise. Take care not to turn too quickly to avoid engagement of safety valve.*
9. *Insert additional octenol attractant as needed inside the plume tube.*
10. *To start the trap, press and hold the ON/OFF button for three seconds until the orange LED illuminates. RUN will show on the display. WARM and the indicator arrows will also flash on the display. The LED (indicator above ON/OFF button) will be orange.*
11. *The fan will ramp from low speed to high speed after approximately 3 minutes. The display will flash WARM and the LED will be orange for approximately 15-25 minutes while the trap is warming up. After the trap reaches operating temperature, the LED will be green and WARM will disappear from the display.*
12. *To turn off the trap, press the ON/OFF button. RUN will show on the display. COOL and the indicator arrows will flash on the display. The LED will be green. After a two-minute cool down, the fan will stop, the LED will turn off and OFF will show on the display.*

## 4. Mosquito Identification by Developmental Stage

Following mosquito collection, the mosquito specimens need to be identified to species to determine their invasive status. This section highlights key background information related to identifying mosquitoes at their various life stages. Complete mosquito identification keys for various mosquito species of concern can be found in Appendix 3.

### 4.1 Eggs

Mosquito eggs (Figure 8) can be identified to genus by the manner in which they are laid (singularly or sticking together to form rafts that float on the water's surface), by their general shape, their size, and by the structure of their outer chorion (egg shell). Some mosquito eggs can also be identified to species. However, identification keys exist for only a few species.

Egg identification to species requires laboratory equipment. For identifying or sorting eggs before they hatch to larvae, a classic microscope or binocular can be used (100x).



Figure 8. Examples of Mosquito Eggs

Left – *Culex* egg raft

Top Right – *Aedes* eggs on a substrate

Bottom Right – *Anopheles* eggs with floats

*Culex* and *Culiseta* eggs are deposited one hundred or so at a time and are held together by surface tension to form structures called rafts.

Eggs of *Aedes* spp., along with some other genera, are deposited individually and appear to be very similar in shape and structure to the naked eye. However, microscopic examination shows very distinctive patterns on the chorion (eggshell) that can be used for identification. *Aedes* spp. lay eggs on dry surfaces that will be submerged in water following a rain event.

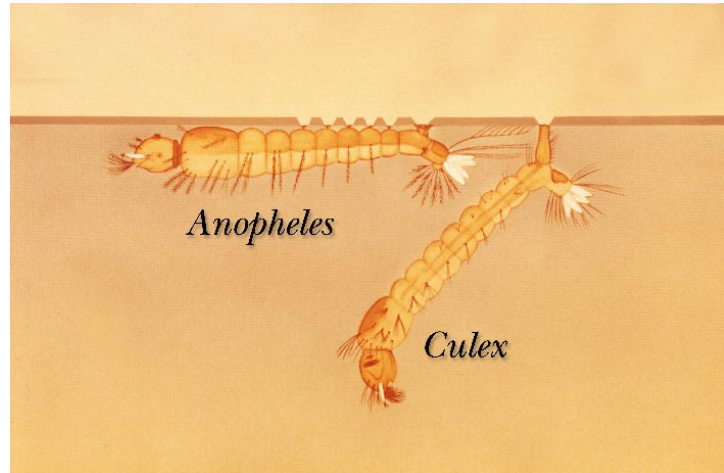
Eggs of *Anopheline* spp. are also deposited individually, but on the water surface. To keep them afloat, these eggs have structures on their sides called floats.

## 4.2 Larvae

Mosquito larvae (Figure 9) are reared from collected eggs or are directly collected from larval breeding sites, such as through netting or dipping. In all cases, the collected material should be inspected in a white plastic tray (1 liter) filled with clear water from the collection site. The

juvenile larvae are transferred with a plastic pipette into a fully labelled container filled with water for subsequent rearing to L4 or adults. For long-term storage they can be kept in airtight tubes filled with 70% ethanol.

Larvae can be identified by their morphology at almost all instars (L1 to L4). However, descriptions and identification keys are usually based on L4 instars. Identification can usually be performed under a binocular microscope, but some characters may need higher magnifications requiring inspection of slide-mounted samples under a more powerful microscope. Specimens can also be identified through molecular tools.



*Figure 9. Mosquito Larvae*

*Anopheles (no siphon) and Culex (with siphon)*

*Anopheline* and *Culicine* larvae can be distinguished in the field through their different resting positions in the water or through the presence/absence of a siphon.

#### 4.3 Pupae

Pupae can be collected and stored in the same way as the larvae. However, it is recommended to keep them alive after collection in water from the collection site to be reared to the adult stage in a laboratory. Pupae often drown during transport, but survival rates can be increased if there are regular stops to allow pupae the opportunity to breathe.

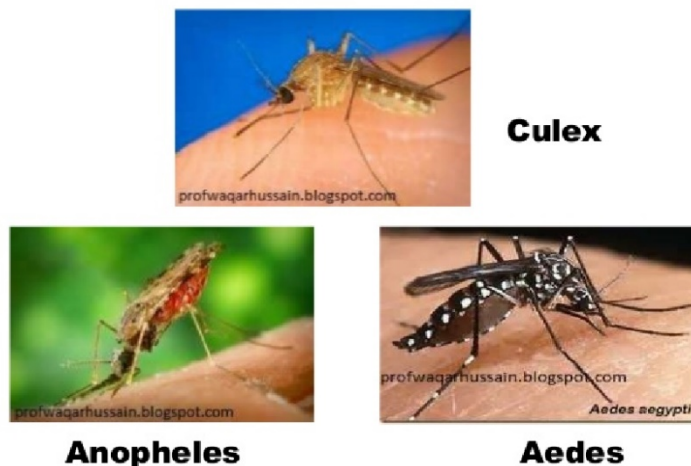
The identification of pupae is possible to genus level, but identification to species level is uncommon as there are few distinct morphological characteristics. Therefore, few pupal identification keys or complete descriptions of pupae to species exist. It is therefore recommended to collect them live and to rear them to adults for identification. Well-developed male pupae can be dissected and the genitalia used to identify them in the same way as for adult males. Specimens can also be identified through molecular tools.

#### 4.4 Adults

Mosquito adults (Figure 10) can be caught through various trap types, through aspiration from resting sites (indoors in shelters, outdoors from the vegetation, etc.) or hosts (animal or human), and by netting amongst vegetation. However, routine surveillance of mosquitoes is more effectively performed with traps. Adults can be stored both dry or frozen. For long-term

preservation or identification, adults must be pinned, labelled, and stored in an insect collection box.

Adult specimens must also be sorted by gender. Adult females can be identified to species level by morphologic differences using a binocular microscope (preferably above 50x). The main diagnostic features are coloration patterns on the legs, abdomen, and thorax, which are produced by patches of colored scales. Fresh specimens may be identified by colorations, but these fade in older specimens as scales are easily dislodged. Therefore, identification using coloration may be unreliable. Specimens can also be identified through molecular tools.



*Figure 10. Adult Mosquitoes by Selected Genus*

In addition to the mosquito identification keys provided in Appendix 3, the following key developed by Darsie and Ward (2005) may be useful.

## 5. Handling of Mosquito Specimens Following Invasive Collection & Notification Chain

Specimens will be collected from the relevant traps throughout the airport facilities by relevant Vector Control staff (workers, inspectors, entomologist). Specimens are euthanized prior to processing (freezing at -20C or below, exposure to ethyl acetate, etc.). Once specimens are dead, they are sorted by species and gender, and the results will be entered into the HawaiiSurv online database.

Recovery of an unknown or unusual adult mosquito caught near a POE will be forwarded to and inspected by a Vector Control Branch entomologist for identification.

If a new species is identified, the notification flow chart (Figure 11) provides the protocol on how the sample should be transferred and how external partners should be notified of the alien identification. These partners include the Department of Transportation, the Department of Land and Natural Resources, the Center of Disease Control (CDC), the Department of Defense, and other relevant partners as determined by the Department of Health.

If an abnormal mosquito specimen is collected from an individual or agency outside of the Department of Health, this individual is to complete the Abnormal Mosquito Identification Form (Appendix 6) before submitting the specimen to a Hawaii Department of Health entomologist for secondary identification.

## Notification Chain Following Invasive Mosquito Species Identification

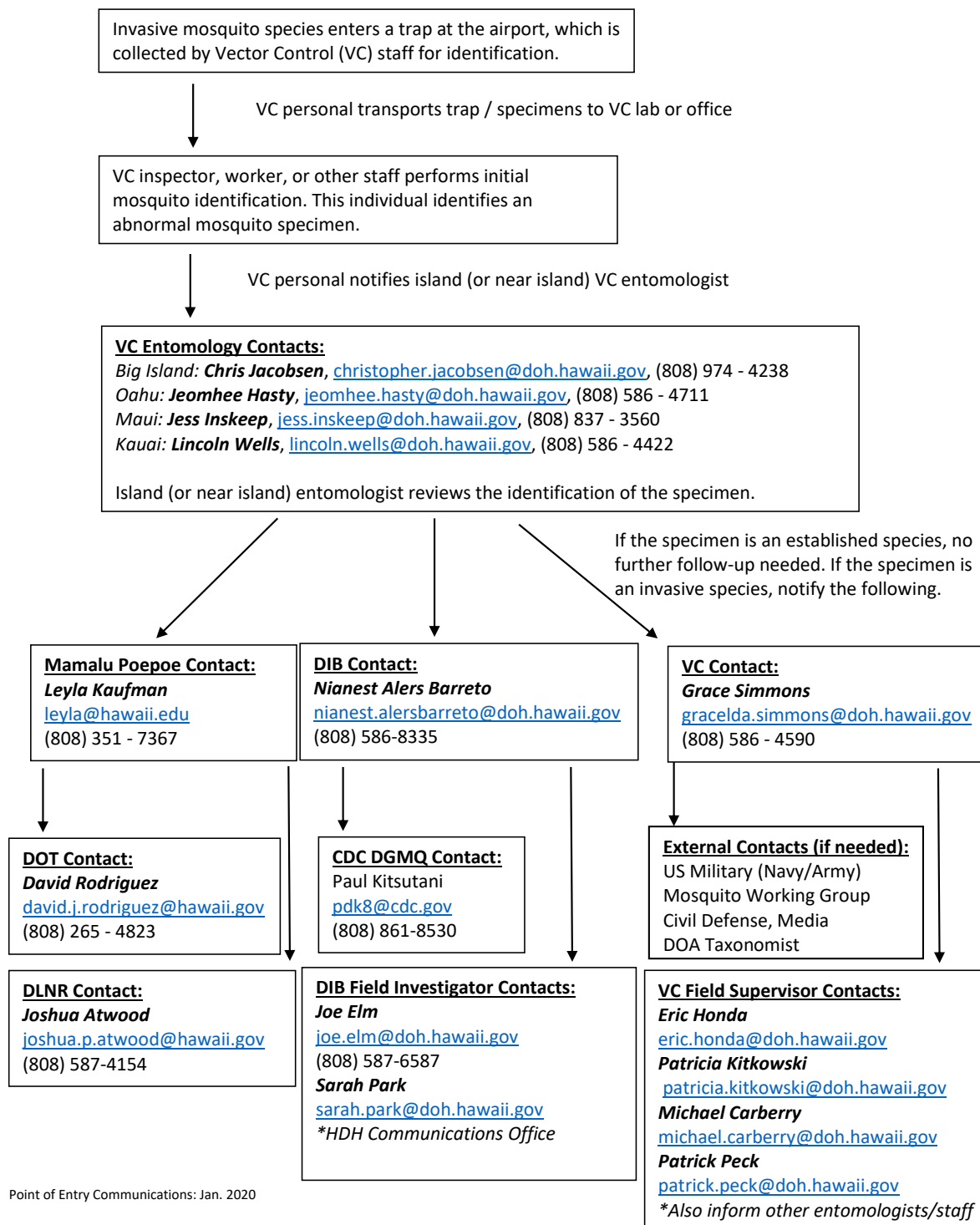


Figure 11. Notification Chain Following Invasive Mosquito Species Identification

## 6. Current Mosquito Surveillance Schedule at Each Port of Entry

The mosquito surveillance and standard collection routine of the HDOH VCB at the various airport facilities is described in Table 2. Following the identification of an invasive species, the Hawaii Department of Health will alter this schedule to increase both the scope and intensity of the surveillance efforts. This can include increasing trap density up to the total number of traps available on each island (POE, Section 7), increasing the use of mosquito attractants such as CO2 from gas canisters or dry ice, and surveying beyond the airport facility to determine the extent of the incursion. These determinations will be made by the Hawaii Department of Health based on the biology of the introduced species and the environment surrounding that specific airport.

Table 2. Mosquito surveillance schedule and details b-y airport

		<b>Ovitrap</b>	<b>GAT traps</b>	<b>BG traps</b>	<b>CDC light</b>	<b>NJ light</b>	<b>Gravid</b>	<b>Magnet trap</b>
Lihue Airport	Frequency of service	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	-
	Total # traps serviced	35	14	8	5	2	7	-
	Number of locations	7	7	8	5	2	7	-
	Lure /attractant	Tap Water	Tap Water	BGLure	UV Black Light	Light	Tap Water	-
Honolulu Airport	Frequency of service	Weekly	Weekly	Monthly	-	Weekly	Bi-weekly	-
	Total # traps serviced	55	10	4	-	1	6	-
	Number of locations	11	10	4	-	1	6	-
	Lure /attractant	Grass Infusion	Grass Infusion	CO2; Dry Ice	-	UV Black Light	Grass Infusion	-
Molokai Airport	Frequency of service	-	-	-	-	-	-	-
	Total # traps serviced	-	-	-	-	-	-	-
	Number of locations	-	-	-	-	-	-	-
	Lure /attractant	-	-	-	-	-	-	-
Kahului Airport	Frequency of service	Weekly	Weekly	Mon. to Wed.	Mon. to Wed.	-	Weekly	-

	Total # traps serviced	41	5	6	4	-	3	-
	Number of locations	15	5	6	4	-	3	-
	Lure /attractant	Water	Water	BG Lure	UV Light	-	Water	-
Hilo Airport	Frequency of service	Mondays	-	Mon & Fri.	Mon & Fri.	None	Mon & Fri.	Thur.
	Total # traps serviced	20	-	9	3	-	1	4
	Number of locations	20	-	9	3	-	1	4
	Lure /attractant	Grass infusion	-	Varies: BG-Lure, UV light, C02, grass infusion	BG-lure and C02	-	Grass infusion	Magnet Lures Lurex and Octenol
Kona Airport	Frequency of service	Every other month	-	Mon & Fri	Mon & Fri	None	Mon & Fri	Mondays
	Total # traps serviced	25	-	2	1	-	1	2
	Number of locations	25	-	2	1	-	1	2
	Lure/ attractant	Grass infusion	-	BG-Lure and BG-lure with light	BG Lure	-	Grass infusion	Mosquito Magnet lures

## 7. Inventory of Surveillance Tools at Each Port of Entry

Following the identification of an invasive mosquito species at a POE increased surveillance may be appropriate to determine the extent of the incursion. While the quantity and diversity of traps deployed may be influenced by the invasive mosquito species identified and the location of the identification, the VCB staff will account for the total number of traps available on each island that may be used for invasive mosquito collection.

As of **10/01/2019** VCB staff have access to the number of traps outlined below in Table 3.

Table 3. Trap and battery availability by island

Island	Site	Ovitrap s	GAT Trap s	BG Trap s	CDC Light Trap s	NJ Light Traps	Gravi d Traps	Magne t Traps	12V Bat.	6V Bat.
Big Island	Hilo	750	25	35	18	22	8	4	45	50
Big Island	Kona	11 glass 232 plastic	24	11	1	1	1	2	41 good	7
Kauai	Lihue	92	26	43	10	17	19	0	37 (14 New, 23 Used)	41 (26 New, 15 Used)
Maui	Kahului	41	7	16	4	0	3	0	15	0
Oahu	Honolulu	500+	25	50		25 21 – Need Repairs	36	0	44	22

## 8. Port of Entry GIS and Spatial Data

The evaluation of geographic information system (GIS) data is a key attribute within mosquito monitoring plans. GIS and spatial data can assist with the visualization of current mosquito surveillance efforts, assist with the identifications of gaps in current monitoring, and help identify regions that may require additional attention due to the biological or geographic attributes of the region. As a part of monitoring and surveying for invasive mosquito species at Hawaii's POE,



spatial data will be collected and entered into relevant data management systems and may be provided to relevant surveillance partners as determined appropriate by the HDOH.

The spatial data at the POEs related to mosquito monitoring can include but is not limited to current mosquito trap and larval monitoring locations, potential mosquito harborage sites, known artificial standing water locations, and data related to the locations of natural water sources, such as ditches, wetlands, streams, lakes, etc. Additionally, spatial monitoring of weather data, such as current and annual averages of precipitation, wind, and moisture zones, can be included to help directly daily monitoring efforts as well as provide insights related to long term POE surveillance planning. This information can be documented for the POE facilities and regions near the airport (e.g., a 0.5 km buffer zone surrounding the facilities).

HDOH VCB Branch GIS data related to POE monitoring is currently managed through an ArcGIS online dashboard that stores and manages relevant spatial data at the POE. This dashboard can be accessed at:

<http://histategis.maps.arcgis.com/apps/opsdashboard/index.html#/4361e309553846808f67e8ecb7f22d2a>

In addition to the information currently being collected and entered into the ArcGIS POE Operations Dashboard, additional GIS information, including links to the State of Hawaii's GIS Program, to NOAA data, and to additional datasets can be found within the separately provided Appendix 6.

## 9. Mosquito Surveillance Data Management and Action Thresholds

Following the identification and sexing of the mosquito samples collected within the traps at Hawaii's POEs, the corresponding mosquito surveillance data will be entered into UC Davis' HawaiiSurv online database. Entry into HawaiiSurv allows for the evaluation of mosquito population trends at POEs. These trends may indicate biological changes within the mosquito populations, environmental changes that modulate mosquito development within the facility environment that may impact the mosquito lifecycle. This data will be made available to the Mamalu Poepoe Program Coordinator as needed for reporting purposes to program funders or relevant partners or partner agencies. Data entry into HawaiiSurv also facilitates the reporting of Hawaii's mosquito surveillance data to federal partners at the CDC.

Mosquito surveillance data will also be used to develop relevant action thresholds for established mosquito species. These action thresholds will be species and site specific and will suggest control procedures or monitoring adjustments. The identification of one invasive species specimen may be enough to institute a chemical pesticide control program and increased monitoring and surveillance at the POEs. Significant increases in previously established mosquito species of concern may also require the implementation of control measures. Analysis of trends in trap interceptions for species already established within Hawaii can be used to identify these increases in established mosquito populations, which may be caused by changes to the airport environment. Action thresholds and analysis of mosquito surveillance data will be a key outcome of the monitoring efforts in addition to the monitoring for invasive mosquito species. The development of action thresholds requires multiple years of data to determine seasonal trends in established populations.

## 10. Integrated Mosquito Management

Following the identification of an invasive mosquito species or a significant increase in the population of an established mosquito species, control measures may be instituted in an attempt to eradicate or suppress the mosquito populations. This section outlines the mosquito control techniques utilized by the Hawaii Department of Health and provides safety information to ensure that these materials are being implemented correctly and in accordance with the law. Larvicide and adulticide application information and labels are also included in the separately provided Appendices 4 and 5.

### 10.1 Larval Control – Source Reduction

Source reduction eliminates larval habitats available to mosquitoes and it is one of the most effective strategies to control mosquitoes. Source reduction can be either permanent or temporary. Permanent source reduction strategies can include sanitation and cleanup of areas with breeding containers or drainage or prevention of standing water occupying low lying areas. Screening can also permanently control larvae within tanks and barrels. While not ideal, temporary source reduction can be effective if conducted every 7-10 days. Temporary reduction often entails cleaning, flushing, or dumping/exchanging water from containers or areas that cannot be permanently eliminated (e.g. flower vases & small water features). Larvaciding may be a recommended companion practice in such areas to extend the interval between reduction activities. Recommended strategies to document and accomplish source reduction at ports are listed below:

- Quarterly, thoroughly survey port areas and identify and remove all small or temporary breeding sources possible.
- Monthly inspect wastewater treatment facilities of the port.
- Provide recommendations to port authorities for strategies to implement that are beyond our scope of services.
- Identify, document, and map areas for which permanent source reduction is not possible (i.e. equipment that holds water, storm drains, bromeliad plants, etc.). Develop action plans for such areas. Maps should be updated annually.
- Monthly look for changes to the port areas that might create new breeding sources. Construction activities and barricades can often support breeding of mosquitoes.
- Be alert for novel breeding locations in hardscape urban areas.

### 10.2 Larval Control – Larviciding

Larviciding is the process of chemically controlling mosquitoes when they are in the larval or pupal form. Larvicides are often recommended for areas in which permanent source reduction cannot be accomplished. Controlling mosquitoes when they are in the water is an effective approach because the mosquito is somewhat constrained and known breeding sites can be recorded and routinely monitored. There are numerous products available for abatement practices. It is important to become familiar with the longevity, mode of action, target stages and signs of efficacy of the various products utilized by the program. Additionally, when possible,

treatment efficacy should be verified by follow-up surveys of the treated locations. As with other types of pesticides, development of a rotation strategy among the classes of available larvacides is recommended to prevent resistance. Application of larvacides requires strict adherence to the product labeling to ensure personal and environmental safety and control of the breeding source. Larvicide labels (separately provided in Appendix 4) should be reviewed prior to each use of a product. **Labeling supersedes this SOP.**

### 10.3 Adult Control – Harborage Reduction

As with larvae, environmental modification can reduce adult populations. Activities should focus on eliminating harborage within port areas. Recommendations for ports should include elimination of overgrown areas by regular mowing ground cover, pruning trees and shrubs to reduce areas of heavy shade, increasing air flow, and landscaping with plants that have growth habits that do not favor mosquito breeding or refuge.

### 10.4 Adult Control – Space / Ultra Low Volume (ULV) Spraying

Aside from arbovirus presence (suspected or confirmed) within an area, adulticiding is rarely employed by the HDOH VCB. However, there may be instances when adult mosquito populations need to be controlled within POEs. Space spraying relies on tiny droplets of pesticides suspended in the air contacting adult mosquitoes directly. This approach uses minute quantities of pesticide which do not persist beyond period of droplet suspension. Adulticide labels (Appendix 5) should be reviewed prior to each use of a product. **Labeling supersedes this SOP.**

Application success relies on multiple factors. First, adult mosquitoes should be active during the time of application. For this reason, it is important to identify the target species and its behavior so that pesticide applications can be scheduled at an appropriate time coinciding with their activity. Second, environmental factors must favor droplet longevity and that droplets will be carried on wind currents near ground level. This requires moderate to high humidity levels, low wind speeds, and temperature inversions. Inversion conditions are found when cooler air is near the ground with warmer air above it. Lingering morning mist is a good example of inversion conditions. Below are a few conditions that favor the development of an inversion:

- 1) Radiation from surface objects into a cloudless or near cloudless sky with 25% or less cloud cover.
- 2) Light and variable winds with minimal mixing of the lower atmosphere (especially 0 to 3 mph). Remain cautious with winds of 4 to 6 mph.
- 3) Time of day; begins in the mid to late afternoon especially 3-5 hours before sunset and intensifies throughout the night until dawn and then dissipates approximately 2-3 hours after sunrise.

Finally, droplets must be of the appropriate size to impinge on mosquitoes. Droplet size requirements are specified on pesticide labels and may be modified by spray devices. Space

spraying is effective in open areas as well as places of light to moderate vegetation. Infested areas of dense vegetation may benefit from high volume residual spraying rather than space sprays. Also, it is important to note space sprays may not reach areas where wind currents do not flow (e.g. recessed areas beneath stairwells). These areas should be identified and directly sprayed when a space spraying strategy is employed. Labelling will provide guidance on minimum required PPE for the application. **Labeling supersedes this SOP.**

## 10.5 Adult Control – Residual / Barrier Spraying

Residual or barrier spraying differs from space spraying in application and effect. Most importantly spray droplets are deposited on surfaces and remain lethal to adult mosquitos for days or weeks following application. While space and ULV applications use as little as 8 oz/acre, residual spraying is most effective with 40 or more gallons/acre applied using a coarse spray to surfaces. Residual sprays should specifically target habitat, harborage and resting surfaces of mosquitoes (i.e. dense vegetation, undersides of leaves, humid recessed areas). Within port areas, rock and concrete surfaces may serve as resting surfaces. At such sites pesticides should be formulated for application to such materials (i.e. microencapsulated or polymer formulations). Selection of such products ensures prolonged activity and can limit potential of pesticide movement through rain or other water sources. As with space sprays, applications can be in response to public complaints or importation of infested materials. Residual sprays can also be applied as a preventative strategy in high risk or special areas. As with all pesticides, labeling must be followed and supersedes any recommendations of this SOP. Labeling will provide guidance on minimum required personal protective equipment for the application. It's important to note that regular use of residual sprays can increase potential of resistance development as multiple generations are exposed to the treatment and the exposure dosage decreases over time. While rotating between pesticides with differing modes of action could manage resistance, few chemical classes are available for mosquito control. There is some evidence that rotation among types of pyrethroids can delay resistance. Recently some mosquito control products have begun including pyrethroids and neonicotinoids (e.g. Temprid FX; cyfluthrin and imidacloprid). Such a mixture offers advantage over a single mode of action. These products should be selected if resistance of the target population is suspected such as when mosquitoes have been imported from an area with high incidence of pyrethroid resistance (e.g. Florida). Another resistance management strategy is to simultaneously larvicide extensively within the treatment areas to potentially expose the population to a second mode of action and target a different life stage.

## 10.6 Personal Protective Equipment (PPE)

For work that does not involve pesticides, Occupational Safety and Health Administration (OSHA) regulations may apply, but more likely the employee's assessment will inform the type of PPE required to carry out the tasks. PPE comprises the clothing and devices worn to protect one's body. Basic protective work clothing consists of: long-sleeved shirts, long pants, closed toe shoes, and socks. In addition to basic protective work clothing, waterproof gloves are recommended for source reduction activities since breeding sources and containers may have

environmental pathogens (i.e. leptospirosis) or other hazards. Gloves for source reduction should have sufficient thickness to prevent contact with sharp materials encountered during source reduction activities. Protective eye wear is also recommended to prevent possible eye injury due to inadvertent splashing of containers during source reduction activities.

A pesticide label lists the PPE that an applicator, handler, and early-entry worker must wear. Wearing anything less is illegal and dangerous. All pesticide handlers (e.g., applicators, mixers and loaders, and flaggers) are responsible for following the pesticide label, including wearing PPE. Wearing PPE can lower the chances of injury by reducing the potential for dermal, respiratory, ocular, and oral exposure.

#### 10.6.1 PPE – Gloves

Pesticide handlers get by far the most exposure from pesticides on their hands and forearms. Research has shown that workers mixing pesticides received 85% of the total exposure on their hands and 13% on their forearms. The same study showed that wearing protective gloves reduced exposure by 99%. Protective gloves are essential to protect your skin. Pesticide labels often require waterproof gloves or one of the following glove types: nitrile rubber, butyl rubber, neoprene rubber, barrier laminate, and Viton®.

Each glove type varies significantly in how well it protects from the different solvents in formulated products. Read each label to determine which glove type is appropriate. Pesticide labels require either waterproof gloves for solid or water-based formulations or chemical resistant gloves for non-water solvents (e.g., alcohols, ketones, and petroleum distillates) used in different formulations.

For liquid products that use a solvent other than water, EPA requires the label to specify the glove type needed for adequate protection. Read the label carefully to ensure you have the correct protective glove material. As a rule, thicker gloves (of the same material under identical conditions) provide longer breakthrough times. A pesticide label's specification of glove type is generally based upon a thickness of 14 mils, except for polyethylene and barrier laminate gloves. **Use the 14 mils thickness as a rule of thumb when selecting glove materials that appear on the pesticide label.**

Reusable gloves should be rinsed thoroughly during each break and at the end of the workday. Absorbed pesticides will continue to enter the material if not removed. Gloves should be properly maintained and rinsed and discarded when beginning to show wear. Check gloves for integrity before each use.

#### 10.6.2 PPE – Eye Protection

Eyes readily absorb pesticides. When a label says to wear protective eyewear, you may use goggles, face shields safety glasses with shields at the front, brow, and temple, or a full-face respirator. Use personal preference from OSHA evaluated eyewear to select eyewear that protects you for the task. Eyewear made of impact-resistant material, such as polycarbonate, can protect from flying objects, such as granular pesticides. However, safety glasses will not adequately protect your eyes from pesticide splashes.

#### 10.6.4 PPE – Respiratory Protection

Some pesticides may expose the applicator to toxic gases, vapors, and/or particulates (solids or liquids). A respirator is a safety device that protects you from inhaling contaminated air. The pesticide label states whether you must use a respirator and if so, which type. The respirator type is based on the pesticide formulation, application method, and environment where the application is made.

Particulate filters remove dusts, aerosols, or sprays suspended in the air. Particulate filters DO NOT remove gases or vapors. EPA regulations require that you replace particulate filters according to respirator manufacturer recommendations or pesticide labeling (whichever is more frequent). If there are no other use directions, dispose of particulate filters after eight hours of cumulative use.

Chemical cartridges or canisters use sorbents to remove contaminant specific gases and vapors, but they do not remove particles. The most typical chemical cartridge or canister specified by the label for pesticide applications is an organic vapor removing (OV) cartridge or canister. Always use the type of chemical cartridge or canister purifying element required by the pesticide label. Keep purifying elements sealed until ready to use. Although it is not a requirement, some respirator manufacturers stamp the expiration date of purifying elements on the outside of the product package. Do not use a purifying element after the expiration date, even if it was never opened. The service life of a chemical cartridge or canister depends on the type and concentration of pesticide, the user's breathing rate, and humidity. Chemical cartridge respirators, when selected appropriately, are essentially 100% efficient until the gas or vapor breaks through. Any taste, smell, or irritation indicates that breakthrough of the pesticide has occurred. Cartridges should be changed immediately whenever you detect breakthrough in the mask. Once used, an organic vapor cartridge must be disposed of.. Any pesticides trapped by the sorbent in the cartridge may desorb over time, and can be inhaled if used again. Always dispose of chemical cartridges at the end of a workday unless the manufacturer directs otherwise.

Before using any respirator, receive medical evaluation to ensure wearing a respirator will not endanger your health. Next, read and understand the manufacturer's instructions and NIOSH approval certificate that accompany the respirator and its components. For full protection, conduct a fit test before wearing a tight-fitting particulate-filtering face piece, half mask, or full-face mask. When wearing a tight-fitting respirator, nothing must interfere with the seal between the surface of the mask and your face, including facial hair and eyewear.

#### 10.6.5 PPE – Maintaining Clothing and Personal Protective Equipment

At the end of each workday, wash all work clothes and PPE. Some items, such as clothes and coveralls, can be washed using electric washer and dryer appliances. Other items, such as gloves, protective suits, goggles, aprons, boots, and eyewear, require hand washing. Wear protective gloves when handling contaminated items. Rinse and discard disposable items.

Dispose of any non-reusable or contaminated item carefully to prevent cross-contamination or contamination of others who might handle the discarded item. Dispose of heavily contaminated items as household hazardous waste.

## 10.7 Pesticide Resistance Management

Development of insecticide resistance among mosquito populations regularly exposed to pesticide applications is a real concern; resistance has been documented in major vectors of human disease and many US jurisdictions have documented levels of resistance among mosquito populations. Preventing this phenomenon and preserving the functionality of available pesticides is a core aim of an integrated mosquito management program. Successful resistance management depends on reducing the selection pressure that a particular mode of action or chemistry exerts on a population.

There are several strategies that can reduce the risk of pesticide resistance developing, beginning with reducing the volume or frequency of pesticide applications. Frequency of chemical intervention is an important driver of resistance by applying constant selection pressure on a population and allowing resistant individuals to dominate within the population. While resistant individuals may survive exposure to pesticides, there is often a fitness cost (i.e. reduced robustness, longevity or offspring) associated with acquisition of the resistant characteristics, which is a disadvantage in the absence of the insecticide. This phenomenon has been found in both the lab and field.

When possible, non-pesticide focused control strategies should also be used against mosquitoes in addition to reliance on pesticide intervention. When pesticide applications are needed regularly within a port area, pesticides should be rotated after 1 to 2 applications to a product with a differing mode of action. This strategy prevents subsequent generations of mosquitoes from exposure to the same mode of action, which reduces the likelihood of selecting resistant characteristics. Mixing pesticides with differing modes of action is another approach that may be beneficial. In all instances care should be taken to apply products at the recommended dose based on label specifications. Areas should be monitored following treatment to ensure efficacy of the application by monitoring the mosquito population size. In the case of treatment failure, the cause should be investigated to determine if it was from environmental conditions, applicator error, or pesticide resistance.

If insecticide resistance is suspected within a population of mosquitoes, the CDC bottle bioassay can be performed. This testing method determines if insecticide resistance has developed by exposing adults from the select population of mosquitoes to an insecticide. The adult mosquitoes within the bottle can be observed over the course of two hours to determine the rate at which they are killed or “knocked down”. If mosquitoes are surviving beyond the exposure time expected to kill or knock down that specific mosquito species, resistance to that particular pesticide may have developed.

More information on the bottle bioassay testing technique, including the rates at which mosquito species should be killed or knocked down by specific pesticides, can be found on the following CDC website: <https://www.cdc.gov/zika/vector/insecticide-resistance.html>

## References

World Health Organization [WHO]. 2016. Vector Surveillance and Control at Ports, Airports and Ground Crossings.

Darsie, R.F. and Ward, R.A. 2005. Identification and geographical distribution of the mosquitoes of North America, North of Mexico. University of Florida Press.



## V. CO2 cylinders and propane tanks facts and safety plan

### Facts and safety plan for the use of CO2 cylinders for mosquito traps

#### Facts

Carbon dioxide (CO<sub>2</sub>) 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 CO<sub>2</sub> 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 CO<sub>2</sub> tanks to lure mosquitoes to traps. CO<sub>2</sub> will be provided at a flow of 250 - 400 ml/min. Flow rate will be controlled with an adjustable valve. Humans emit 250 ml CO<sub>2</sub> per minute (exhale). The amount of CO<sub>2</sub> released for trapping is therefore for equivalent to one person or two people exhaling CO<sub>2</sub>. 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 tanks

The CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> cylinders at airport facilities: CO<sub>2</sub> cylinders will be used to lure mosquitoes to traps. Cylinders will use a CO<sub>2</sub> regulation kit that will ensure a constant release rate of 250-400 ml/min. The plume of CO<sub>2</sub> produced mimics human exhalation and thus makes traps more efficient at trapping mosquitoes. The use of CO<sub>2</sub> 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

### Facts

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 for propane tanks:

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 place 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](https://www.ccohs.ca/oshanswers/chemicals/chem_profiles/carbon_dioxide.html)

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