Port of Entry/Exit Pest Monitoring Program
FY22 HISC FUNDS PROJECT REPORT

This annual report includes the following components:
1. Project Overview
2. Report on activities in FY22
3. Economic Analysis by Donna Lee
4. Africanized bee report from UH Bee lab

Should you have any additional questions regarding the implementation of the Port of Entry/Exit Pest Monitoring program, (formerly known as Māmalu Poepoe) in FY22, please do not hesitate to contact Dr. Kaufman at Leyla.V.Kaufman@hawaii.gov.

1. Project Overview

The Port of Entry Pest Monitoring Program (formerly known as Māmalu Poepoe) was originally conceived by representatives from multiple state agencies acting in their capacities as members of the HISC, including the directors or designees from HIDOT, the Department of Health (DOH), the Hawaii Department of Agriculture (HDOA), and the University of Hawaii (UH). These agencies recognized the following areas of shared interests with regard to airport facilities:

- HIDOT seeks to understand the presence and impact of invasive species at airport facilities that may be detrimental to facility operation or user experience,
- DOH seeks to improve its monitoring and research efforts regarding vectors of human diseases at airports, primarily mosquitoes,
- HDOA seeks to improve monitoring and research efforts regarding agricultural pests at airports, namely invasive ants, coconut rhinoceros beetle, and Africanized bees,
- UH seeks to improve research on invasive species distribution and economic impacts, and,
- DLNR is the administrative host of the HISC, which is mandated to provide cabinet-level coordination on invasive species issues.

HISC staff agreed to serve in a coordinating capacity for this project, including management of an interagency project budget and the hiring of a temporary Project Coordinator via a staffing partnership the UH Pacific Cooperative Studies Unit (PCSU) to finalize and implement the Māmalu Poepoe plan.
Utilizing UH PCSU as a project staffing entity has allowed HISC to fund temporary positions and partial full-time equivalences for the pilot project rather than establishing permanent civil service positions. HDOT agreed to fund the program as a 5-year pilot project. The pilot funding ended in March 2022.

2. Report on activities in FY22

- **Interview with project partners**
  HDOT pilot program funds expired in March 2022. During FY 22, the project coordinator and CGAPS staff interviewed project partners about the need to continue with the program. A battery of interview questions was generated for Federal partners (USDA, FWS), State partners (HDOA, HDOH, HDOT, UH), and RCUH project partners (ISCs managers, HAL manager). The responses were in favor for project continuation and for an expansion of efforts to seaports, as well as an expansion of invasive targets.

- **Program expansion**
  Given the overwhelming support from partner agencies, HISC staff worked on securing funds to continue with the program. During FY 22 the project coordinator worked with HISC/DLNR and CGAPS staff to send federal appropriation and Congressional Direct Spending (CDS) requests to the different Congressional offices in Washington DC. HISC staff also briefed local legislators on the program, which resulted in the introduction of a bill. Federal and State requests had budgets for the continuation of efforts at airports and also to expand efforts to seaports and expand the list of invasive targets. The CDS request was approved, and the appropriation went to USDA APHIS. USDA APHIS channeled the funds to DLNR via CAPS financial agreement. The program will expand efforts to the following harbors: Honolulu, Nawiliwili, Kawaihae, and Hilo. The State appropriation went to HDOA.

- **Working Group Meetings**
  Between August 2021 and July 2022, the program coordinator held 1 Questions and Answers meeting with program partners (December 7th 2021) and two Working Group meeting (August 3 2021 and April 21 2022).
  The December Question and Answer meeting was attended by all the representatives from the Invasive Species Committees, Hawaii ant lab, CRB response, UH Bee Lab. The goal for the meeting was to standardize approaches among groups and work out any issues with the NRDS database.

  The working group meeting in August 2021 and April 2022 were attended by 38 participants and 35 representatives from DLNR, DOT, DOA, DOH, UH, CGAPS, CDC, Tripler Medical, USFWS, as well as representatives from the different invasive species committees and the Hawaii Ant Lab. These meetings provided updates on monitoring efforts for mosquitoes, ants, Africanized bees and coconut rhinoceros beetle. It also provided an update on the economic analysis done by UH economist.

4.1 INVASIVE ANTS

The program continues the collaboration with the Hawaii Ant Lab (HAL) and MISC for the ant monitoring. During FY22 the program had two ant surveys at HNL and 3 surveys at ITO, 3 at KOA and 1 at OGG. No new ants to the state were reported at the different facilities. Positives for little fire ant (LFA) were recorded at ITO and KOA airports.

2.2 AFRICANIZED HONEYBEES (AHB)

The University of Hawaii (UH) Bee Lab continues to provide guidance to the program. The UH Bee Lab conducts the DNA analysis and swarm trap processing for swarms intercepted at HNL. Table 1 shows the number of swarm interceptions at airport facilities. All swarms intercepted to data have tested negative to Africanized genes.

Table 1. Number of swarms intercepted at all airports from August 2021 and July 2022

Airport facility	# swarm interceptions	Results of DNA analysis
Lihue	1	-
Honolulu	8	Negative
Kahului	0	-
Hilo	4	Negative
Kona	1	Negative
Total	14	

The program coordinator held 3 action plan meetings for AHB between August 2021 and July 2022). The meetings were held in September 2021, November 2021 and February 2022. In these meetings the group discussed issues like mandatory bee registration, issues regarding the importation of bee semen, buffer zones around airports, trainings for response, outreach materials, among other issues.

2.3 COCONUT RHINOCEROS BEETLE

No beetles were intercepted during FY 2022. Traps are currently being checked every four weeks. The CRB response team has seen increase in CRB detections around HNL. Annual palm surveys were conducted at all airport facilities. During the palm surveys monitoring crews inspect palms for possible CRB damage and count number of palms. Figure 1 shows the clusters of palms at Daniel K Inouye International airport. All palms surveyed were free from CRB damage.

Palm Survey within the AOA of Daniel K. Inouye International Airport
May 31, 2022

Palm Clusters	Survey Result
(#1) 13 coconut palms, 7 fan palms, 5 Manila palms	Possible CRB damage detected on 1 coconut palm
(#2) 3 Manila palms, 7 Macarthur palms, 1 fan palm	No CRB damage detected
(#3) 21 Macarthur palms, 4 Manila palms, 4 coconut palms	No CRB damage detected
(#4) 20 coconut palms	No CRB damage detected
(#5) 10 Manila palms, 2 Macarthur palms	No CRB damage detected
(#6): 18 coconut palms, 6 Manila plams 19 Macarthur palms, 1 date palm	No CRB damage detected

Figure 1. Palm survey inside the AOA at Daniel K Inouye International airport

2.4 MOSQUITOES

No new mosquito species were intercepted during monitoring surveys at airport facilities during this fiscal year. The program continued providing monitoring equipment to offices. The program helped with the response to an interception of a female *Aedes aegypti* (this species is only known to occur in Hawaii island) at Honolulu Harbor in August 2021. The program helped with communications with HDOT partners to gain access to additional harbor sites. The program also provided monitoring traps to help with the response. No other interceptions were recorded.

2.5 BEST MANAGEMENT PRACTICES (BMPS) FOR AIRPORT STAFF AND AIRPORT CONTRACTORS.

The program put together BMPs for all target species. The BMPs outline practices to be followed to maintain and operate airport facilities in a way to minimize the risk of invasive species establishment on airports properties. In FY22 the BMPs were updated to reflect the current CRB interim rule and compliance agreement put in place by HDOA. The document also got a review from HDOT harbors.

Economic rationale for continuing monitoring invasive species at Hawaii airports

Benefits from Monitoring - By specie

Monitoring and rapid response cost - AHB

	Monitoring	Rapid response	Monitoring and response
Oahu	$15,473	$7,056	$22,530
Kauai	$7,075	$4,531	$11,606
Molokai	$0	$0	$0
Maui	$7,527	$4,173	$11,700
Hilo	$19,582	$8,258	$27,840
Kona	$11,203	$5,335	$16,538
All	$60,861	$29,354	$90,214

Benefit from monitoring for AHB at airports

	Monitoring, rapid response, and follow-up*	Terminal Closures* Days per year		
		5	15	20
Cost at 6 airports	$90,214	$2,659,109	$7,977,327	$10,636,436
Benefit-Cost Ratio	Cost	**29:1**	88:1	118:1

*Close one terminal at each airport during swarming

Interpretation of B:C = 29:1

- If an outbreak is likely to occur more than once every 29 years, monitoring to prevent the outbreaks is economically warranted
- If an outbreak is expected to occur only once every 29 years, monitoring to prevent the outbreak, benefits equal the costs
- If an outbreak is expected to occur fewer than once every 29 years, monitoring to prevent the outbreak (at the current level) is not economically warranted.

Benefit from monitoring for LFA at airports

	Monitoring	Mitigation*	Airport Gate Closure*		
			Days per year		
			12	*52*	*260*
Cost at 6 airports	$66,574	$196,210	$713,062	3,089,934	$15,449,672
Benefit-Cost Ratio	Cost	3:1	11:1	46:1	232:1

One gate at each airport is infested and closed for treatment
Mitigation treatment is in place of routine monitoring and response
Gate closure cost compared to monitoring cost

Benefit from monitoring for CRB at airports

	**Monitoring**	**Mitigation**	**Landscape Damage***
		Trapping	*Tree replacement*
Cost at 6 airports	$29,326	$169,688	$539,795
Benefit-Cost Ratio	Cost	6:1	18:1

*13.4% of the palm trees will be damaged irreparably. Overall % based on tree replacement rates of 1% (Kona), 4% (Molokai) and 15% (remaining) airports

Mitigation instead of monitoring and routine response

Landscape damage compared cost of monitoring

Benefit from monitoring for mosquitos at airports

	Monitoring *Cost*	**Response** *Cost*	**Total** *Cost*	Avoided loss in airport net revenues due to active cases of disease for 6-months	
	Per year	*Per incident*	*Per incident One year*	*Net revenues*	*Benefit-cost*
Culex (or all)	$519,137	$190,635	$709,772	$15,318,260	22:1
Anopheles	$519,137	$69,478	$588,615	$15,318,260	26:1
Aedes	$519,137	$11,319	$530,456	$15,318,260	29:1

Progress and Ongoing Needs in Hawaii

Africanized Honeybee Detection and Prevention

Report by E. M. Villalobos - UH Honeybee Project

Historical perspective of swarm trapping in Hawaii

In Hawaii, monitoring for Africanized Honeybees at airports began in 2017 with the use of swarm traps and pheromone lures. Since then, swarm catching procedures were modified in the middle of 2019 when the UH Honeybee Project began assisting with the genetic analysis of the captured swarms. The UH bee team also began recording the amount of honeycomb accumulated and the brood condition of the swarms when they were delivered for screening of their genetic status. Trap occupancy is now checked every 2 weeks and the contents of the trap are examined for bee pests and brood age, which is related to maturity of the colony. The presence of drone bees is recorded, since they represent a possible dispersal route of genetic material linked to the colony being examined.

Swarms are typically composed of young bees and an older queen bee. Once they settle in a swarm trap, the bees quickly begin to build comb. This process is energetically consuming, and the swarm bees carry with them a belly full of honey to begin this process. Honeycomb needs to be created before food storing and egg laying can begin. So, during the first few days the bees will spend most of the time creating and excreting wax, which is then shaped into the cells that form the honeycomb. Once the nest begins to take shape, collection and storage of nectar and pollen can start and the queen is ready to initiate egg laying and produce a new generation of worker bees.

From that point on, the speed of nest development is somewhat variable, depending on the number of worker bees that was part of the swarm, the availability of resources nearby, and the weather. However, the duration of the larval stage of a bee, from egg to pupa, is relatively constant at 6 days. The pupa takes about 12 days to transforms into the adult bee. When our team processes the swarm trap samples, we consider the presence of larvae and capped brood (which is indicative of pupal development) to assess the age of the swarm.

In addition, the appearance of drone cells in the comb structure gives us another indication that the colony has been there for a while. Male bees, or drones, are produced only when a colony is strong enough, which is likely correlated with age of the colony. Thus, swarms do not often produce drones during colony establishment phase. Drones also take longer than worker bees to develop because of their larger body size, 24 days as opposed to 18 by worker bees, so their presence denotes an older colony.

The recommendation to have bi-monthly inspections has had a positive impact in the reduction of colonies with drone brood, thus reducing the potential for dispersal of unwanted genetics via males that could mate with nearby colonies.

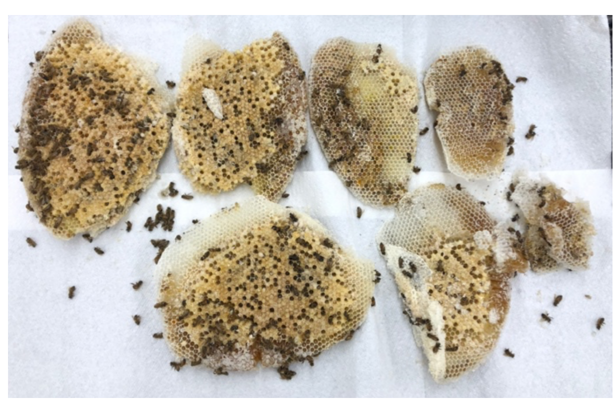
The data revealed that when swarm traps were inspected within 2 to 3 weeks the number of combs was less than swarm traps that had a longer interval between inspections. Table 1 below shows the results of inspection for 3 traps captured at Honolulu airport this year and an average of the past number of combs found in swarm traps when the inspections took place every month.

	Interval Between Inspection (days)	Number of combs (Brood -yes/no)
Oahu Trap 3	11	3 (NO)
Oahu Trap 12	21	4 (NO)
Oahu Trap 2	21	7 (YES)
Older Traps Average (N=7)	30	6 (YES) Range 4 to 7

Table 1 – Shows variation in comb production and brood development by swarms caught at the Honolulu airport in relation to the time interval between inspections.

The images below show Swarm from Trap 12 (2022) (left) and compare it to a swarm from 2019 (right) collected before the change in sampling schedule was implemented. The most important factors to consider are the number of combs produced and the percentage of the comb used in reproduction – evident in the capped brood.

Trap # 2, 2022 - Notice the small size of 2 of the combs



2019 Swarm Trap

Sadly, a collection oversight or a holiday period that interferes with the inspection of a trap can yield a larger population and more brood accumulating in a trap. Consequently, efforts should be made to keep the 2-week interval.

The ideal situation is illustrated here– where the bees are established in the swarm trap and have begun to construct comb but have not produced large amounts of brood and especially no drones.

We would like to discuss the possibility of gathering standardized data on the comb status (number of pieces), rough percentage of the comb with brood, as well, as an estimate of bee population. The population can be easily estimated by using jars with measuring lines that are equivalent to 100 bees or by using measuring scoops for cooking since it is already known that 1 cup is equal to 300 adult bees.

Swarm captures at airports

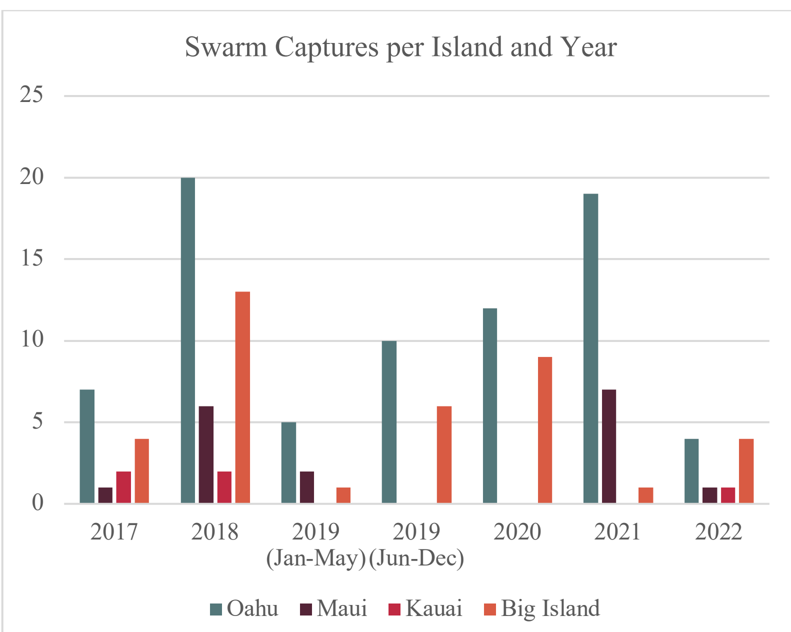


Fig. 1 – *Swarm capture rates/year and site. The year 2019 is divided in 2 since the protocol for inspection frequency changed.*

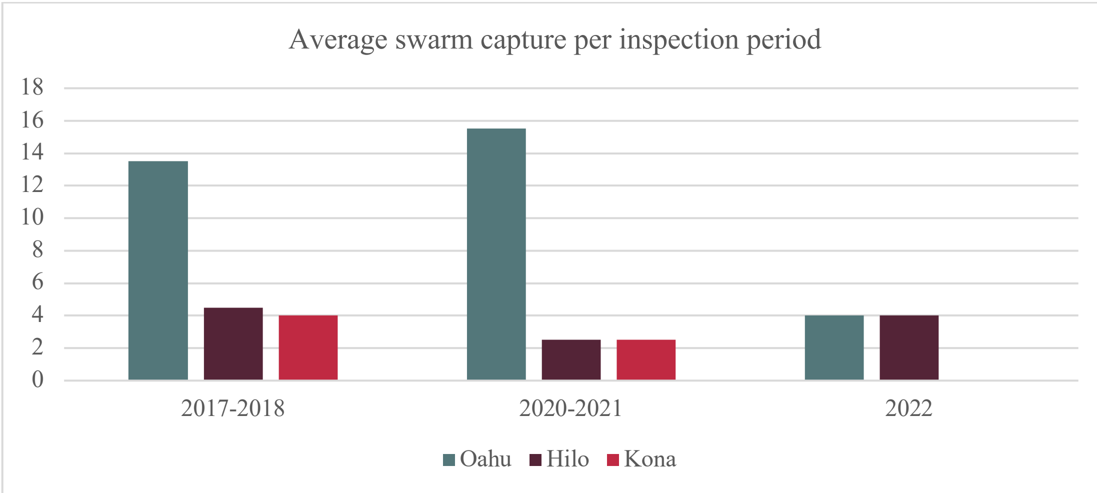


Fig. 2 – *Swarm captures/year by inspection period. During the 2017-2018 swarm trap inspections were every month and the data for 2020-2021, as well as 2022, includes bi-monthly swarm trap inspections. The year 2019 is omitted from this graph since it was the year when inspection changed from once a month to twice a month.*

Possible climatic correlates of swarm capture rates include total precipitation and seasonality of rainfall. The rainfall can affect bees in 2 ways: 1- the bees have less time to forage and grow slower, so feral and managed bees already established on island will

Rainfall in Hawaii is extremely variable depending on location. For example, on Oahu the average rainfall in the 2017-2018 period was close to normal after a previously dry year. In contrast, in Hilo and Kahului the 2018 precipitation was well above average for the state.

Climate studies predict that rainfall levels in Oahu will decline by 30% or more in leeward areas, however, the frequency of random weather events, such as cold fronts that can bring sudden rains, are expected to increase (Longman et al., 2001). Understanding the population dynamics of feral colonies will require longitudinal capture data and weather records. The information collected will provide valuable insight into swarming patterns at each site. If Africanized bees were detected on island, this information, although based on European bees, will most likely be applicable and will help anticipate swarming events based on climate and related food abundance.

In addition to rainfall, plants and bees can be affected by ambient temperature. The apparent decline in swarm capture rates on Oahu, Kona, and Hilo (Fig. 2) suggests a statewide phenomenon. Rainfall in 2022 has been below average for most sites and temperatures in Hawaii continue to rise. It's possible that the low capture rate is related to the decline in abundance and/or health of feral colonies nearby.

Obviously, the objective of the swarm traps is to attract a swarm arriving from outside Hawaii, consequently the low capture rates of what could be local bees should not impact the ongoing monitoring efforts. Invasion events are random, and the frequency of occurrence will not be tied to Hawaii's seasonality. Nevertheless, it would be useful to evaluate the microenvironment in which these traps are located to make sure the dry weather and excessive insulation of the trap are not reducing our capture rates. If a trap is in full sun and the temperature within the trap is too high, this may reduce the appeal of the trap to the scout bees looking for a potential nest site.

One recommendation, based on the current data, is to examine the location of each trap and if possible, use a thermocouple to measure the temperature inside of the nest box and compare it to the ambient at that point. A swarm trap should be a "nest site" that offers shelter from the rain and excessive heat. It would be useful to invest in 2 thermocouples with long leads so that the staff inspecting the traps can collect a reading "in-situ" for each of the traps. Fluke thermocouples are the best for this kind of situation because they are sturdy, portable**, highly accurate**, they can be adapted with a variety of temperature leads and **they give very fast readings** (in Fahrenheit, Kelvin, and in Celsius) which is key for this project. One of the thermocouples could be a travelling tool while the other one stays on Oahu where most of the research is being conducted and we could create heating curves for the traps under different shade cover conditions. However, for this to be successful the team members on the other islands need to learn how to properly use the equipment to avoid getting false readings from direct sunlight exposure or wind effects on the lead, which is very sensitive.

Ensuring that the swarm lure is in good condition, that the trap is not getting excessively wet or too hot are crucial elements to the success of this project.

Bee Genetics and Invasive Subspecies

Import of live bees to Hawaii is illegal, and the only source of official new genetic bee material imported to the islands is in the form of sperm brought by the queen breeders to produce specific types of bee races that the consumers on the mainland US are interested in buying from them.

The Hawaiian archipelago is home to bees of European descent and no Africanized bee has been detected in the past studies (Jara et al., 2014, and Szalanski et al., 2016) nor during the current swarm genetic screening. We are currently focusing a large part of our effort to the screening for Africanized genes in mitochondrial DNA. These tests provide us with information on the genetics of the queen leading the swarm and/or the colony from which the worker bees come from.

The molecular test includes DNA extraction followed by a process involving a restriction enzyme which yields a "positive" or "negative" result with respect to Africanization. In Fig. 3 a positive control from an Africanized bee from Arizona was used, and the local samples show the expected 2 bars that indicate "negative for Africanization".

Fig. 3 – *Results from Restriction Enzyme on cytochrome*

We know from past research that each island seems to have slightly different genetic profiles, all still within the European Honeybee lineage. The subspecies that have been detected in Hawaii include: 1-Lineage C, which includes bees of east European origin such as *Apis mellifera ligustica* and *Apis mellifera carnica;* Lineage M, which includes bees of West Europe descent such as *A. m. mellifera* and *A. m. iberiensis.*

Historically, Lineage C has been dominant (>80% of the bees sampled) on 2 islands: Oahu and the Big Island of Hawaii belong to this lineage (Jara et al. 2014). This in part because of the great concentration of queen breeders on Hawaii island and the past ease through which Oahu beekeepers could obtain bees from these sources. In comparison in Maui, Kauai, and Molokai about 50-60% of the bees belong to Lineage C and roughly 40% belongs to the Lineage M.

However, the access to queens from the Big Island of Hawaii by Oahu beekeepers has greatly decreased. This is in part to new management in some of the queen breeding operations and less interest on their part to ship to small operations on Oahu. There also new regulations to between island bee shipping that would require Hawaii queen breeders to have the boxes inspected for ants before shipping to Oahu. This extra step in the shipping procedure and the relatively small order size from Oahu beekeepers makes selling to Oahu not attractive to these large companies and it has become virtually impossible to get Big Island queens.

Consequently, we are working with the one registered, Department of Agriculture inspected, queen rearing outfit on Oahu to examine their bees from a genetic standpoint. We have obtained DNA from 10 of their breeder colonies and these samples will be added to the ones from wild swarms (25 samples) and local private apiaries (25 samples). The DNA from these bees is now ready for submission to Eurofins, an extremely reputable company in the mainland that is used by other bee researchers, for a more in-depth genetic analysis.

The results from those samples will allow us to examine if the genetics of bees in Hawaii have changed in the past 10 years. Knowing what the allelic composition of the current honeybee population is and how similar it is to the one we sampled 10 years ago will provide valuable information if we were to detect anomalies in the genetics in the future.

The case of Jamaica

Most recently, I had the opportunity to assist the government of Jamaica in their efforts to determine if they had Africanized bees now on the island. The data collected suggests that the Africanized bees are now present in multiple areas in Jamaica and the government now is having to face making changes to the regulations that apply to commercial beekeepers and hobbyist urban beekeepers. That experience has greatly influenced my perception of the threat and potential impact of Africanized bees in Hawaii.

Development of Educational Materials

The program has developed a series of documents and video resources that await final review. One of the most difficult aspects is finding a suitable contact phone number – a line that will take after hours and/or weekend calls as well. We also need to find a suitable website(s) for the materials.

There are also materials that await a few refining touches to become training modules for inspectors and extension agents. For a greater impact these materials need to be finalized with input from the State Apiary Program and hopefully the HDoA.

As we expand into discussions with seaport authorities, we need more than ever to have a way to reach out to the workers, first responders, and the public in general about the threat of Africanization of the honeybee population.