# **INSECT CONTAINMENT FACILITY**

## STANDARD OPERATING PROCEDURE November 2020

Hawaii Department of Agriculture Division of Plant Industry Plant Pest Control Branch Biological Control Section 1428 South King Street Honolulu, Hawaii 96814

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## **INTRODUCTION**

#### Background

The Hawaii Department of Agriculture (HDOA) Insect Containment Facility (ICF), formerly referred to as the HDOA Insect Quarantine Facility (IQF), is under the management and direction of the Biological Control Section of the Plant Pest Control Branch, Plant Industry Division.

## **Mission Statement**

The HDOA-ICF was designed and constructed in 1964 for study of arthropods, primarily insects, and other organisms, imported into Hawaii as potential natural enemies for use in classical biological control of plant pests. Plant pests include phytophagous or plant-feeding insects, mites, snails and slugs, and noxious weeds. HDOA-ICF precludes the escape of organisms brought into the facility as biocontrol agents or hitchhiking contaminants that may have been inadvertently collected and shipped with purposely introduced organisms from overseas or elsewhere.

## Location

The HDOA-ICF is located on the grounds of the Hawaii Department of Agriculture Main Office Complex (HDOA-MOC) at 1428 South King Street, City & County of Honolulu, Island of Oahu (Attachments 1 & 2). The boundaries of the HDOA-MOC are Young Street on the north, Keeaumoku Street on the west, King Street on the south and Pawaa Park on the east. The area is largely a commercial district where small business establishments and residential buildings are located.

## PHYSICAL CONTAINMENT STANDARDS

## SITE DESCRIPTION

## Location

The HDOA-ICF is located in Honolulu equidistant from the major landmarks of the city. It is approximately 1.24 km west of Neal S. Blaisdell Civic Center in Downtown Honolulu and the major tourist resorts in Waikiki on the east; 2.9 km southwest of Honolulu Harbor; 1.6 km northwest of the Punchbowl National Cemetery of the Pacific and approximately 12.9 km southwest of the Honolulu International Airport. The nearest major commercial crop production areas are west of Pearl City, at least 24 km away. The University of Hawaii (UH) Lyon Arboretum is located in Manoa Valley, 5.8 km to the northeast. There are various greenhouses on the UH campus in the lower Manoa valley, 3.7 km from the HDOA. The Foster Botanical Garden is 3.4 km west of the facility.

## **Security Fencing**

The perimeter of the HDOA-MOC is secured with galvanized chain link fence, 6 feet high; with 3 strands of barbed wire that extends a foot above the top of the fence. Besides the front entrance door to the main office, there are two gates by which entrance to and exit from the complex is facilitated. Each of the gates is equipped with a combination lock with a numeric code available only to HDOA-MOC staff. Office hours commence at 6 AM and ends at 5 PM, Mondays through Fridays. The office is closed on Saturdays, Sundays, and holidays.

## **DESCRIPTION OF THE FACILITY**

The HDOA-ICF is an insect containment building designated strictly as a high security facility. The building is a single-story structure with masonry tile walls reinforced with steel bars and concrete and steel roof joists with aluminum roofing. The concrete slab floor has a smooth finish. All interior walls except those in the two vestibules are coated with multiple layers of flat white latex paint. The walls of the vestibules are painted flat black.

The facility consists of seven (7) rooms, namely, two Vestibules, Anteroom, small Storage Room, Main Rearing Room, Screen Room and Back Room (2<sup>nd</sup> rearing room) [Attachment 3]. A moat, 20 cm wide and 10 cm deep, which surrounds the perimeter of the ICF, also extends and runs around the HDOA Insect Propagation Facility, an adjacent building, which is a non-security area. The moat, filled with water, serves as a physical barrier to prevent entry of predators, particularly ants, which could interfere with colonization of biocontrol agents and their hosts (plants or insects).

## **Exterior Door**

The front door is the only entry and main access to the ICF. It is 4.5 cm thick with a vinyl veneer on the exterior surface to resist weathering. The door is fitted with a dead bolt lock and a pneumatic device that shuts all the time. The door is kept locked at all times and can only be opened either by unlatching the lock with a key from the outside or by turning a button from the inside. The door opens inward to the first vestibule.

## Vestibules

Access to the main rearing room is through two vestibules and an anteroom. The first vestibule (V1) is approximately 9 ft long while the second vestibule (V2) is half smaller in size with an inside dimension of 4 by 4 ft. The two doors through V2 are 3.5 cm thick around the perimeters and along the center panel. All the vestibule doors are fitted with felt gaskets to seal any space or clearance between the doors and their jambs. V2 can only accommodate one person at a time.

### Anteroom

The room is painted white and partly illuminated with a black light to trap and capture insects that may have inadvertently escaped from the Main Rearing Room. For emergencies, the anteroom is furnished with a telephone and a fire extinguisher. In addition, the Anteroom serves as a dressing room for workers and authorized visitors who must wear laboratory coats and shower caps prior to entering the Main Rearing Room.

#### Main Rearing Room

This is the largest room within the ICF where rearing and propagation of insects are undertaken. Insects are colonized in rectangular screen cages, small cubical cages or glass jars on counter tops that run the length of the room along the windows. Risk assessment evaluation (non-target studies) of potential natural enemies is likewise done in this room using glass jars, one-gallon jars, or cubical / rectangular cages.

#### Screen Room

The Screen Room is located on the far-right hand corner of the Main Rearing Room. Inside the room, on the left-hand side, is a tightly sealed wooden containment box where consignments of biocontrol agents collected from overseas are opened, processed, and inventoried. The ceiling and the upper halves of the entry side and the right side that face the Main Rearing Room are fitted with an 80-mesh insect screen. The left wall forms the Back Room while the far wall with a glass window makes up the outer wall of the building.

## **Back Room**

Initially, the room was designed as a greenhouse within the ICF but the plan was shelved because of recurring leaks in the glass roof as a result of weathering. Consequently, prior to 1970, the glass was replaced with aluminum as roofing material. Since then, the back room was used as an alternate rearing room for insects that require higher and warmer conditions. The room faces the afternoon sun and there is no AC to cool it except for a fan that is turned on as needed to circulate warm air.

## Windows

All windows are translucent glass reinforced with wire. The panes are held in place with aluminum frames. All crevices are sealed with silicone caulk.

#### Ceiling

The ceiling consists of canec fiberboard painted over with flat white latex paint. All crevices are sealed with white acrylic latex caulk. The single air vent next to the air handler unit of the AC system is securely covered with a double layer of cotton fabric that is finer than 80-mesh screen.

## **Electrical Features**

Gaps or empty spaces around electrical fixtures, service outlets, and equipment, e.g., autoclave, installed through the wall are sealed with acrylic latex caulk to prevent access and escape of insects and other arthropods from the facility. All light fixtures are fixed and sealed to the ceiling.

## **Emergency Exit**

The HDOA-ICF has only one front door through which entry and exit from the building is facilitated. The building, being a highly secured insect containment facility, does not have an emergency exit. In the event of an emergency where escape from the building becomes a priority but the only way through the Anteroom and Vestibules is unsafe or inaccessible, a masonry hammer and a 2-prong sledge hammers, one of which is located on the wall next to the fire extinguisher and another mounted on the opposite side of the wall just to the left of the doorway to the Back Room, are used to breach the windows.

## EQUIPMENT STANDARDS

#### Main Rearing Room

Twin stainless steel sinks with counters, 61 cm x 122 cm, are located to the left of the doorway upon entry into the Main Rearing Room. Off to the right of the doorway is the double door, pass-through autoclave sterilizer for treatment and disposal of all discarded materials. A small portable autoclave which serves as a back-up sterilizer is nearby. Adjacent to it is a laundry washer on casters that can easily be rolled near the sink where it is run to wash laboratory coats and other clothing materials used in insect rearing. A refrigerator and a plant growth chamber are at the far end of the room, just to the left of the Screen Room and doorway to the Back Room. Work counters nearly run the length of the room on the left side. These are made of wood, supported on galvanized pipes, and covered with chemical-resistant white Formica. Formica work counters are also located over the storage cabinets along the right side of the room from where the sterilizers are located to the Screen Room. Eight wooden racks, painted white and on casters, are in the middle of the room where insect holding containers, such as, jars and small aluminum cages of various sizes, glass wares, and other rearing supplies for easy access are kept. Wooden and metal screen cages, 46 x 46 x 61 cm, are routinely used to rear and colonize the insects on the counter tops.

## **Back Room**

White Formica counter tops also line three walls of the Back Room along the windows. Two portable fans are occasionally used in the room to help circulate the air and facilitate exchange with the cooler air in the Main Rearing Room. A hose bib with the length of a garden hose attached to a faucet is used to wash cages and other rearing equipment/tools. Just to the right of the entry doorway are the electrical circuit breakers and panels. A step ladder stationed in the room is used primarily to check and maintain the air handler and air vent systems above the Screen Room.

## Screen Room

The room has a full-size worktable that extends from the wall of the entry door to the main wall of the building. A wooden containment box with a sleeve opening for unpacking shipments is on the worktable along with a lamp. A rack for small cages and jars is on the wall opposite the worktable. A metal tank containing compressed carbon dioxide is securely chained to the wall next to the rack for use in anaesthetizing insects. A variety of supplies and equipment for unpacking shipments and collecting insects is stored on a wooden shelf by the wall above the worktable.

## Vacuum Cleaning System

The HDOA-ICF is not equipped with a centralized vacuum cleaning system. A vacuum cleaner is used to clean the facility. All refuse collected with the vacuum cleaner is emptied into trash containers lined with plastic bags commercially sold for autoclaving.

### **Insect Aspiration System**

A centralized system for insect aspiration was not included when the facility was constructed. Instead, a Kenmore Hepa Filter vacuum cleaner hooked to a modified insect aspirator or a simple aspirator hand-made from rubber tubing and glass pipette are used.

## **OPERATIONAL STANDARDS**

### **Containment Director**

The HDOA-ICF is under the direction of the Quarantine Director (QD) and the Quarantine Officer (QO). The QD, who is the Biocontrol Section Chief, oversees the overall operation of the containment facility. Under the general supervision of the QD, the QO, who is the Insectary Supervisor, oversees the daily operation of the ICF, including access to the facility and the development of quarantine procedures. The QO coordinates the operation of the facility by collaborating with regulatory agencies, cooperators, and project leaders. By working closely with the Plant Pest Control (PPC) Insect Taxonomist, the QO facilitates the determination of natural enemy identification. The QO is responsible for keeping accurate records of all shipments into or out of the facility and for compiling databases of all quarantine activities. The QO ensures that all standards for the HDOA-ICF are being adhered to and that the security of the facility is ensured and maintained at all times.

## **Authorized Personnel**

Access to the HDOA-ICF is highly restricted to prevent escape of organisms from the facility. As indicated on the sign by the main door, only authorized personnel are permitted entry into the ICF. The door always remains locked and secured.

The facility is used primarily by the Insectary Staff to undertake research activities on the biocontrol projects of PPC. Other PPC entomologists and support personnel (technicians) who assist the biocontrol project entomologists are allowed entry into the facility as needed. Researchers from outside entities who undertake joint cooperative biocontrol projects with PPC staff are allowed access into the facility.

The UH project cooperators and other visiting personnel can only enter the ICF during normal HDOA working hours (7:45 AM to 4:30 PM), Mondays through Fridays. Except for authorized personnel and other than normal business hours on week days, there shall be no entry to HDOA-ICF on Saturdays and Sundays. On holidays, non-ICF workers, namely, authorized cooperators, may be granted access only if an Insectary Unit professional staff is present to address facility issues and emergencies. The gates to the HDOA complex are equipped with combination locks for security purposes. Cooperating researchers are not given the combination number to the locks. They shall enter the complex through the Front Office for purposes of identification and registration. Once inside the complex, they can only access the ICF by signing up and borrowing the key to the facility from the Insectary (Insect Propagation) Building.

#### **Project Leaders**

The project leaders are the HDOA insectary entomologists who have been assigned specific projects on classical biological control. Occasionally, researchers from the state, federal or non-government entities whose projects have been approved and accepted by the QD and/or QO as official cooperative projects may likewise be designated as project leaders. However, before the project is approved, the project leader must provide the QD with a brief description of the work including a timetable, identification of the organisms, literature on their biology and behavior, and if available, voucher specimens of the organisms. All cooperating project leaders shall provide the HDOA QD or QO a copy of a current USDA-APHIS-PPQ import permit (PPQ Form 526) for the appropriate organism before any consignment from overseas or elsewhere is brought into the ICF.

The project leader is responsible for providing the required personnel, equipment and supplies for their project. During the course of the work, the Project Leader shall immediately report any discrepancies or any other significant occurrences to the QO. These may include, but are not limited to, diseases, hyperparasitoids and other

contaminants, and any unexplained loss of colonies. The project leader is responsible for providing voucher specimens to the QO for submission to the HDOA Insect Taxonomist. On a routine basis, the Project Leader shall ensure that strict sanitation is practiced in assigned and common work areas. Upon conclusion of the project, the Project Leader is responsible for the proper cleanup of all areas and equipment used and shall restore areas in the facility back to their condition at the start of the project.

## **Facility Visitors**

Entry into the containment facility is restricted to visitors who are directly involved and actively participating in on-going biocontrol projects. Occasionally, service and repair technicians are allowed entry to undertake routine inspection / maintenance work of appliances or electrical fixtures for which they have been contracted or summoned during emergencies. All visitors must abide by the guidelines specified in the protocol and are always accompanied by authorized personnel.

### **User's Agreement**

All authorized users of the ICF who are not staff members of the HDOA Biocontrol Section are initially given an orientation and "in-house training" to acquaint them with the standard operating protocol before they are allowed entry into the facility. In addition, they must sign their names on the HDOA-ICF User's Agreement Form to acknowledge that they have read and understood the ICF standard operating protocol and will abide by its provisions. Any user who does not and can not comply with said protocol will be denied entry to the facility. Cooperating researchers from outside agencies who are authorized to undertake biocontrol projects within the facility have to sign the ICF Log Book to indicate the date and time of entry and exit from the facility on every visit. All users shall plan their schedule in advance to facilitate access to the facility and complete their work within a reasonable period.

## **Visitor Orientation**

A floor plan of the containment facility is attached to familiarize visitors with the general layout of the rooms and other features of the facility (Attachment 3). First-time users are given an orientation session by the QO during their initial visit.

## ACCESSING THE FACILITY

## **Preparation for Entry**

Before entering the facility, users shall leave all outer garments, such as hats, coats, sweaters, jackets, backpacks, in the Insect Propagation Building (Insectary) or, if not possible, in the Plant Pest Control Branch Office area. Research cooperators shall sign-in their names and log in their entry and exit times in the HDOA-ICF Log Book located in the Insectary where they can borrow and return the door key to the ICF.

#### **Entering the Facility**

A diagram is included to show how access to the facility may be facilitated (Attachment 4). The entryway to the ICF consists of two vestibules which require opening and closing three doors. No two doors shall be opened at the same time. Each door has a threshold which one must carefully step over so as not to trip. The deadbolt on the main entry door shall be locked immediately after entry into Vestibule # 1 (V1). Then, by tapping the wall in V1, anyone who might be exiting the facility from the opposite direction is alerted. The person leaving the facility shall yield to the one entering because V1 is much larger than Vestibule #2 (V2), so more time is required to move through V1 than through V2. The person entering shall knock on the second door to alert anyone who might be coming out otherwise he can open the door and access V2 by stepping over another threshold. Once inside the room, the door is closed quickly and the third door that swings inward V2 is open in order to get inside the Anteroom. Again, another threshold is stepped over. Once inside the Anteroom, the door is shut and properly secured. The Anteroom is partly illuminated by a black light that emanates from an insect light trap. Designated as a dressing place, it is in this room where white laboratory coats and shower caps are stored and put on. Only then will access to the Main Rearing Room through another door is allowed. The door is immediately secured and closed behind upon entering the Main Rearing Room.

#### **Preparation for Exit**

When done and before leaving the facility, the worker must ensure that his/her work area is thoroughly cleaned and neatly organized. Prior to exiting, he/she must wash hands and forearms in the sink with a hypochlorite/detergent solution the plastic bucket to make sure that no undesirable organisms are inadvertently taken out of the facility. The worker rinses off the solution under the sink faucet and dries hands with a paper towel. Then, a brush hanging by the wall next to the door is used to brush off the lower part of his/her legs, footwear, and shower cap so as to dislodge unwanted "hitchhikers". Moreover, the worker double checks his/her laboratory coat before access to the Anteroom. He/She closes the door behind then takes off and hangs the coat and shower cap in the coat rack.

Except those in the Vestibules and Anteroom no lights, which are on automatic timer, are turned off inside the facility. Although lights in the Vestibules and Anteroom have manual switches they are always in the off positions and may only be turned on during emergencies.

#### **Exiting the Facility**

The protocol to exit the facility is the same when one enters the building but in reverse. Either way the worker makes sure that every door is secured shut behind before the next door is opened. Again, he/she must exercise caution in stepping over the thresholds so as not to trip. Once inside Vestibule # 1, the door is pulled behind firmly to shut it. While still facing this door, one orients toward the main front door by walking slowly toward it and touching the wall to the left as a guide. At waist level is the door handle. Above it is a turn button for the dead bolt that is twist clockwise to unlock the door. Exit is facilitated by pulling the handle and stepping over the threshold. The entry door is locked immediately with a key to secure the deadbolt in the shut position. In the Insect Propagation Facility, the exit time from the facility is logged in the HDOA-ICF Log Book and access key returned.

## **EMERGENCIES**

## Fires

The HDOA-ICF does not have a fire alarm or a sprinkler system or an emergency exit. However, there is a communication system and fire fighting tools within the facility. The Anteroom and Main Rearing Room has one telephone each. One fire extinguisher is in the Anteroom and a second one is located at the far rear end of the Main Rearing Room by the doorway to the Back Room. Two sledge hammers are mounted above it while another one hangs behind the wall on the left hand side past the doorway to the Back Room. These tools can be used to break the glass windows inside the facility during an emergency. In addition, a garden hose in the Back Room attached to a faucet can also be used in case of a fire.

Should a fire break out in the ICF, trapped workers inside the facility should make every effort to put it out with the fire extinguishers and /or water hose. If unsuccessful and fire is at the rear of the building, workers shall immediately vacate the ICF by exiting through the Anteroom, Vestibules, and the main front door. Use of the phone in the Anteroom should not be attempted. The first person to exit must go directly to the Plant Industry Office to make a 911 emergency call. Then, a warning to all the workers on the premises of the HDOA Main Office complex is sounded off by activating the fire alarm system. A

fire alarm switch is located just inside the main entry doors to the Plant Industry Office at each end of the building. To activate the alarm, follow the instructions on the small, red, alarm unit on the wall by pulling down the switch. Before evacuating to designated assembly areas along bordering streets, check to ensure that all the workers in the adjacent laboratories, particularly, the Insect Propagation Facility (Insectary) and the Plant Pathology Facility have been alerted.

If the fire is at the front end of the building, the telephone in the Anteroom would not be accessible to the workers as they are prevented from exiting the facility through the Anteroom and Vestibules. To facilitate a quick exit, an escape route should be made at the rear of the building, preferably on the south side (King Street) of the Back Room, by breaking a window with the hammers. A metal ladder is in the room and can be used to reach the breached window. Trapped workers should help and assist each other during evacuation. Ensure that all the workers have been accounted for before making a 911 call and activating the fire alarm system in the Plant Industry Office.

**CAUTION**: Do not attempt to extinguish chemical fires.

If a fire breaks out in another building within the HDOA complex, fire alarms will get activated in most of the buildings except the Insectary, ICF, and Plant Pathology Facility. There are no fire alarm systems in these buildings and workers should be alerted during an emergency either by calling them on the telephone or by designated individuals as specified in the HDOA Emergency Preparedness and Response Plan. In the case of the ICF workers, and if safe to do so, they shall immediately vacate the ICF by passing through the anteroom, vestibules and out through the front door. If this is not possible, exit from the facility should be made in the Back Room through a window breached with a sledge hammer as described above. After vacating the facility, workers shall proceed directly to the assembly areas on the south side (King Street) of the HDOA Main Office Complex.

One each of the schematic diagrams for evacuation of facility during emergencies are posted in the Anteroom, back of the door to the Main Rearing Room and behind the wall on the left hand side past the doorway to the Back Room.

## **Medical Emergencies**

A first aid kit is located on the left side of the doorway as one enters the Main Rearing Room. Should there be a medical emergency; a 911 call is made using the telephone in the Main Rearing Room or Anteroom. Concurrently, the supervisory personnel in the Plant Industry office are alerted about the incident. Meanwhile, the general area around the person who needs medical attention is assessed to determine if nothing further will put him at risk. If deemed safe, everything possible should be done to ensure the comfort of the injured person. However, he should not be moved at all unless absolutely necessary. If at all possible, the cause or extent of the problem is likewise determined. If the person attending the injured person is properly trained, first aid or CPR should be administered as appropriate. During the process, the injured person is comforted and kept calm by reassuring him that help is on the way. As soon as emergency medical personnel have assisted the injured person, a report detailing the incident and what transpired thereafter is provided to the appropriate supervisors.

## **Hurricanes and Other Natural Disasters**

All natural disasters, including man-made catastrophes, are covered by the HDOA Emergency Preparedness and Response Plan. The plan was put in place to serve as general guidelines and procedures for employees to follow during an emergency caused by but not limited to earthquakes, hurricanes, tidal waves, lava flows, floods, and fires. The intent is to minimize the threat of the impending disaster and prevent unnecessary loss of human lives and properties.

There are natural disasters that can be tracked down and their occurrence forecasted with precision. During the hurricane season, the Hawaiian Islands occasionally lie in the path of this weather disturbance. More often than not, evacuation of the facility is not immediately issued because the course and arrival of the hurricane can be predicted with accuracy. Nevertheless, various procedures are specified in the emergency plan to deal with an emergency of this nature. Thus, when an official notification to prepare for evacuation is received during normal working hours, all Biocontrol Section personnel shall pitch in to secure all the windows of the ICF. The windows on the outer walls of the building are shielded with sheets of treated plywood (3/4 inch A/C) that have been pre-cut to fit their dimensions. Anchor bolts have been placed on the walls to secure the plywood over the windows with screws. All the pieces of plywood as well as positioning of the anchor bolts on the wall matched perfectly to facilitate ease of installation.

After the plywood pieces have been installed over the windows, all authorized personnel shall access the ICF in order to secure their work areas. Electrical equipment and appliances are turned off and unplugged. Equipment as well as files are covered or stashed inside plastic bags to protect them from potential water and moisture damage. After ensuring that all the rearing cages and containers have been properly sealed, workers shall exit the facility and secure loose objects in outdoor areas surrounding the ICF and adjacent buildings. When done, they shall assist other workers in securing the Insectary and the Plant Pathology buildings. The last facility to be secured is the Plant Industry office.

## SANITATION

## **Cleanup of Work Area**

At the end of the day or after work is completed, the ICF user shall ensure that the work area is clean and free of dust, debris, plant materials, frass, propagation media, and other kinds of refuse. Thrash is discarded into a large receptacle near the sink in the Main Rearing Room. The trash receptacle is lined with a large autoclavable bag. Used items, such as glass wares, plastic vials, plastic cups, jars, and small rearing containers, are soaked overnight in a dishpan by the sink containing 5:100 sodium hypochlorite solution mixed with ca. 5 ml of a dishwashing detergent. These are washed the next day. The larger insect rearing cages are washed in the Back Room with the same cleaning solution and dishwashing detergent. Finally, the user wipes the surface of the workbench or counter with ethyl alcohol (70% or higher concentration) using paper towels.

## Solid Wastes Handling

Solid waste collected in the Screen Room, including those from incoming shipments, shall be discarded into a plastic bag in the wastebasket in the room. When full, the plastic bag is secured by tying both ends then dumping it in an autoclave bag. The bag is sealed and stored under the work table in the Screen Room. The trash is given the highest priority for autoclaving. Meanwhile, trash collected in the Main Rearing Room and Back Room shall be secured in autoclave bags provided in the trash receptacles located near the sink. At the end of the week or as needed, the trash are autoclaved for 30-60 minutes at 122°C (= 250°F) and 15 PSI. Discarded containers or boxes made up of paper materials are autoclaved for 30 minutes. However, soil (potting media), discarded plants and parts including leaves, stems, leaves, and roots and other kinds of wastes are autoclaved for no less than 60 minutes. When autoclaving is completed, treated trash is taken out from the facility. Trash removal is facilitated by opening the rear door of the pass-through (double door) autoclave located outside the building then emptying them into a common dumpster for disposal. Operation of the autoclave is currently handled by a trained Pest Control Technician.

## Soil Handling

Used soil, brought inside the ICF with potted host plants, shall be autoclaved then taken out of the facility through the double-door pass-through autoclave for storage and recycling. Prior to autoclaving, the soil is processed by removing plant stumps and/or roots left in the pot. The soil is emptied from the pot by dumping it through a metal grill that sits on top of a large plastic receptacle. The soil is collected and stored in the receptacle until ready for autoclaving. Wet soil with too much moisture is set aside to dry before it is processed for autoclaving. Soil is laid out in thin layers in stainless steel pans (or cloth sacks) then autoclaved for 60 minutes at  $122^{\circ}C$  (= 250°F). After the treatment, the soil is taken out from the facility as described above.

## **Removal of Objects from the Facility**

Equipment, tools, and other objects shall not be removed from the ICF without prior clearance from the QO. Small objects or tools used routinely in insect rearing, such as scalpel, scissors, dissecting needle, spatula, etc., can be sanitized with a sodium hypochlorite solution or alcohol (70% or higher) before these are taken out of the facility. Complex and sensitive equipment, such as cameras, computers and other special equipment which cannot be treated with liquid sanitizers shall be stored and secured in plastic bags. Bulky objects, such as insect rearing cages, refrigerator, growth chamber, etc., once brought inside, are not allowed to be taken out from the facility.

#### **Trapping Escaped Insects within the Facility**

Occasionally, colonized insects could inadvertently escape from the rearing cages despite efforts by the workers to handle them with care and extra precaution. Black light traps, one in the Anteroom and two in the Main Rearing Room, are employed to catch loose insects and preclude their escape from the facility. The traps are serviced once a week to check and maintain the soap solution in the collection container which kills trapped insects by drowning.

The insects which are reared in the Main Rearing Room and Back Room have already shown some potential as biocontrol agents of various target plant pests. Hence, these insects are considered fairly safe for mass propagation and final evaluation. On the other hand, very little is known about the insects that are still being held inside the Screen Room. In this case, handling of said insects and any other associated organisms must be carefully observed. Thus, in the event that their holding container is accidentally breached, efforts shall be made by the workers to catch and retrieve the insects that have escaped. Under these circumstances, the door in the Screen Room is immediately closed and secured. A search to collect the escapees is initiated. First on the worktable, followed by the window panes and then the light fixtures. After a careful determination and when satisfied that all escapees have been retrieved back, the concealed areas, tight spaces or dark corners in the room that are difficult to access shall be treated thoroughly with a light spray of detergent solution to dispose of any strays that might have been missed during the search.

## Use of Pesticides within the Facility

The use of most pesticides within the facility is not permitted. The only insecticide that is approved for routine use is insecticidal soap. Occasionally, bait which consists of a mixture of sugar, boric acid and water is used to control ants. Detergents and other cleansing solutions such as sodium hypochlorite or alcohol (70% or higher) are used as disinfectants.

#### **General Cleaning of the Facility**

Floors are swept and mopped with a surface disinfectant once a week. More frequent cleaning is left at the discretion of the QO.

## **INCOMING SHIPMENTS**

All incoming consignments that originated from overseas or elsewhere must be taken directly inside the Screen Room of the HDOA-ICF where they are quarantined and processed. In the Screen Room, the shipment is placed inside a sleeved containment box where the OO and/or project leader inspects and opens the container to unpack the contents. Having done so, the shipping container and packing materials are discarded and taken out of the containment box. The discarded materials are emptied into a plastic bag then stashed into another bag, which is autoclavable, before it is stored under the worktable for autoclaving. The contents inside the consignment are usually packaged in a cloth or plastic bag or secured in a paper/plastic carton container. Regardless, before the package is opened, it is thoroughly inspected to check for any organism that might have escaped while the shipment is in transit. If safe to proceed, the security seal of the container is broken and the insects or infested plant materials, as the case maybe, are removed and transferred into a glass jar or a small cage prepared earlier inside the containment box. When the transfer is done, the containment box is double checked within to ensure that no organism has strayed during the transfer. Otherwise, every effort should be made to catch and retrieve back any loose organism. Subsequently, the container holding the newly acquired organisms is taken out of the containment box and placed on the shelf opposite the worktable in the Screen Room. The container is labeled properly and entrusted to the project leader. Once the process is completed, the containment box is cleaned of any waste or refuse that was left behind. The discarded materials are once again secured in an autoclavable bag for subsequent autoclaving.

All appropriate information concerning the consignments including dates of shipment/collection, origin of shipment, sender/recipient, description of specimens/materials, condition of materials when received, etc., are recorded and filed.

## PROPAGATION AND COLONIZATION OF ORGANISMS

Authorized users of the HDOA-ICF shall ensure that all their cages and other insect rearing containers are secured properly to preclude the escape of any organism. Moreover, these containers, which may likewise include jars, vials, tubes, etc., whether they contain dead or living organisms, shall be properly labeled for accurate identification and inventory. In addition, all users shall maintain and upkeep their work areas as well as the facility as a whole at all times. The tools, supplies, and equipment that the workers routinely used shall be taken care of and kept in proper working order. Should there be incidents of cross-contamination of insect cultures, occurrences of disease infections or any unusual event that may put colonies of organisms at risk, the QO must be informed accordingly as soon as possible.

## **RELEASE OF ORGANISMS FROM THE CONTAINMENT FACILITY**

The release of an organism from the HDOA-ICF requires that the colony is disease-free and to the satisfaction of the QO. Once these requirements are satisfied, the release process goes through a protocol as follows:

The applicant shall request an approval from the State of Hawaii Board of Agriculture for the release of the organism from the ICF. The request, which includes a permit application for Restricted Commodities into Hawaii (Form PQ-7) and an Environmental Assessment (EA) report, is submitted to the Board through the HDOA Plant Quarantine Branch (PQB). At the same time, the EA is forwarded to the Office of Environmental Quality Control (OEQC), Hawaii Department of Health, for publication in The *Environmental Notice*, a semi-monthly periodic bulletin published by OEQC. Voucher specimens and authoritative identification of the organism are likewise required. After a review through the Entomology subcommittee and the Committee on Plants and Animals, the Board makes a final decision based on the input made by these advisory committees. If the request to release is approved, a permit is issued by the PQB. The applicant then submits a PPQ Form 526 (Permit to Move Live Plant Pests, Biological Control Agents, Bees, Parasitic Plants or Federal Noxious Weeds) with all the supporting documents to the USDA-APHIS-PPQ Scientific Services in Riverdale, Maryland by logging to their electronic or ePermit system. An in-depth review is initiated by the USDA. If a Finding of No Significant Impact (FONSI) holds, a section 7 Consultation with the U.S. Fish and Wildlife Service (USFWS) follows. If a favorable opinion is received from the USFWS, APHIS-PPQ will issue a news release to the wire services for a 30-day comment period. After which APHIS-PPQ shall determine if a permit to release the organism is justified. A PPQ Form 526 is then issued for the release of the organism from the HDOA containment facility.