## Biology and Host Range of *Euselasia chrysippe*

*Euselasia chrysippe* (Lepidoptera: Riodinidae), the golden sombermark butterfly, is proposed for environmental release in Hawaii for biological control of the noxious weed *Miconia calvescens* (Melastomataceae). Native to Central and South America, miconia is considered one of Hawai'i's most invasive plants. With its exceptionally large leaves, it shades and outcompetes other species, effectively forming a monoculture. Uncontrolled growth can overwhelm highly diverse native wet forest ecosystems that are home to critically endangered species and essential to our freshwater resources. Despite major efforts using chemical control, this species continues to proliferate, particularly on Maui and Hawai'i Islands, and long-term management of *M. calvescens* will depend on the use of biocontrol (Ashe 2017). To date only one biocontrol agent has been released against miconia, the leaf spot pathogen *Colletotrichum gloeosporioides*, with only minor impacts in Hawaii (Seixas et al. 2007).

*Figure 1. Euselasia chrysippe adults emerge from pupation*

*Euselasia chrysippe* is a natural herbivore of miconia in the plant's native range in Costa Rica. Among the leaf-feeding natural enemies of miconia, *E. chrysippe* was found the most promising for biocontrol because its larvae feed together in groups, causing more damage to miconia leaves. This gregarious behavior also may improve its defense against natural enemies of lepidopteran species already present in Hawai'i. Extensive testing has shown *E. chrysippe* to be host-specific to miconia and other closely related members of the Melastomataceae family, all of which are non-native weeds in Hawai'i.

Release of *E. chrysippe* is proposed on all islands where miconia has established. Spread of the insect from initial release sites will occur both through natural dispersal and via artificial redistribution by land managers. It is expected that *E. chrysippe* will range statewide in all areas where miconia exists within a few years of release. Feeding by *E. chrysippe* is expected to reduce foliage and suppress vigor of miconia trees, allowing other species to persist and compete, to the long-term benefit of Hawai'i's forests and watersheds. State and federal land management agencies will monitor the effectiveness of the biocontrol.

### Biology of *Euselasia chrysippe*

*Euselasia chrysippe* (Bates 1866) is a butterfly in the family Riodinidae whose native range extends from southern Mexico to Colombia, at elevations from sea level to 1,500m (Nishida 2010). In Costa Rica, it is found on the Caribbean and Pacific slopes in primary and secondary rain forests (Allen 2012; Nishida 2010). Caterpillars and eggs of *E. chrysippe* have been collected only from plants in the family Melastomataceae, specifically several species within the genus *Miconia* and *Conostegia rufescens* (Nishida 2010). Caterpillars feed together in large groups, causing defoliation of otherwise healthy leaves.

*Figure 2. Larval cohort emerged from egg cluster*

Under lab rearing, *E. chrysippe* eggs take approximately one month to hatch, and development of larvae and pupae to emergence of adult butterflies takes roughly another month. Both male and female adults have been shown to live for longer than a month (Nishida 2010). The caterpillars of each cohort develop through six instars, ultimately consuming the

equivalent of one full *M. calvescens* leaf (Johnson 2010). As with all known members of the tribe Euselasiini, *E. chrysippe* caterpillars hatch, feed, rest, molt, and pupate together in sibling cohorts of up to 100 individuals (Allen 2010; Nishida 2010). This gregarious behavior is thought to optimize feeding on tough leaves. In addition, remaining as a large group provides a defense against predation and may contribute to low parasitism rates in their home range (Allen 2010).

*Figure 3. Caterpillars feed gregariously*

**Natural Enemies**: A factor commonly affecting lepidopteran insects introduced for weed biocontrol in Hawai‘i is parasitism by various insects previously introduced accidentally, or purposefully for biocontrol of lepidopteran pests. Reported parasitoids of the genus *Euselasia* include species of Chalcididae, Ichneumonidae, Trichogrammatidae (all in Hymenoptera), and Tachinidae (Diptera) (Nishida 2010). Fortunately, the known parasites of *E. chrysippe* do not occur in Hawaii: one egg parasitoid (*Encarsia* cf. *porteri* (Hymenoptera: Ahelinidae)) and two genera of solitary tachinid parasitoids that attack large larvae and emerge from pupae have been recorded in Costa Rica (Nishida 2010). Species in the subfamily Riodininae do not share the usual parasitoids of Lepidoptera (Hanson et al. 2010), and no members of this family are native or have been introduced to Hawai‘i (Nishida 2002), which further reduces the chance that a specialized parasite of *E. chrysippe* currently exists here.

Generalist predators, however, might significantly impact the immature stages of *E. chrysippe*, which remain exposed on plants throughout their development. In particular, the long development time for eggs means that stage is vulnerable for an extended period. In Costa Rica, *E. chrysippe* eggs were preyed upon by ants, and larvae by hemipteran predators and vespid wasps (Allen 2012).

**Effect on Target Weed**: *Euselasia chrysippe* was selected as a biocontrol for miconia in Hawai‘i because its gregariously feeding larvae can cause substantial damage to leaves. In Costa Rica its eggs and larvae are found on a wide range of sizes of *Miconia* trees, from saplings less than 1m tall to large mature trees. When reared on potted plants, a cohort of 60–80 larvae will consume several hundred square centimeters of leaf tissue – equivalent to the area of one average-sized leaf (Puliafico et al. 2015). Damage is typically distributed across several leaves because larvae move to new feeding areas between meals. Small larvae feed on the under surface of leaves, creating windowing damage, while the later stages feed through the whole leaf lamina. Damage also includes removal of portions of uneaten leaves, presumably to reduce detection by natural enemies (Figure 4).

Although extensive defoliation by *E. chrysippe* is not observed in Costa Rica, its populations are presumed to be limited by natural enemies there. If introduced to Hawai‘i, population growth is expected to be less constrained by enemies, allowing numbers of *E. chrysippe* to increase to levels sufficiently high to cause substantial defoliation. Damage is unlikely to be severe enough to kill miconia trees, but repeated partial defoliations may reduce growth and reproduction of trees and enhance light levels for plants competing with miconia. Future releases of other candidate biocontrol

*Figure 4. Euselasia chrysippe larvae defoliating Miconia calvescens*

agents will aim to impact seed production, population densities, and/or seedling establishment and survival (Johnson 2010).

**Host Range of *Euselasia chrysippe***

Recorded host plants for the genus *Euselasia* include members of the families Euphorbiaceae, Clusiaceae, Myrtaceae, Melastomataceae, Sapotaceae, and Vochysiaceae; however, caterpillars and eggs of *E. chrysippe* have been collected only from Melastomataceae, specifically *Miconia calvescens, M. impetiolaris, M. trinervia, M. elata, M. appendiculata, M. donaena, M. longifolia,* and *Conostegia rufescens* (DeVries 1997; DeVries et al. 1992; Janzen and Hallwachs 2009; Nishida 2010). No-choice host tests conducted by Nishida (2010) found that larvae collected from *M. impetiolaris* would feed on *Conostegia xalapensis* and *M. calvescens* (Melastomataceae) but exhibited no feeding on two *Eucalyptus* spp., *Eugenia truncata*, and *Psidium guajava* (all Myrtaceae), or *Clusia flava* (Clusiaceae).

Host specificity tests with larvae of *E. chrysippe* were conducted from 2012-2014 in laboratories in Hawai‘i, at the Hawai‘i Volcanoes National Park Quarantine Facility, and in Costa Rica, at La Selva Biological Station. Larvae for tests were collected as eggs from several sites in Costa Rica on two of its host plants, *Miconia calvescens* and *Miconia impetiolaris*. An emphasis was placed on testing plants in the order Myrtales, specifically on species within the families Melastomataceae, Myrtaceae, Combretaceae, Lythraceae, and Onagraceae. Relationships within the Melastomataceae were based on Clausing and Renner (2001). In addition, species from more distantly related taxa but with economic, cultural, and/or ecological significance in Hawai‘i were selected based on input from the U.S. Fish and Wildlife Service, consultations with members of the agricultural community, and expert sources on native Hawaiian plants. In total, 73 species of plants from 19 families were examined for suitability as hosts for *E. chrysippe* (Table 1). No-choice tests, with cohorts of 5-10 larvae exposed to leaves of each plant species for 3 days in 90-mm petri dishes, were replicated 4-5 times. Further tests of a subset of melastomes were conducted over longer periods, on potted plants and in petris with leaves replaced every few days, to determine if any are suitable for complete development of *E. chrysippe*.

Results of host specificity studies showed that among the 73 species tested, *E. chrysippe* larvae feed and survive primarily on *Miconia calvescens* and a few close relatives within the tribe Miconieae (Figures 5 and 6). Very low levels of feeding occurred on a few plants in families outside of Melastomataceae, but in all cases, survival of the larvae past the 3-day mark on species in these families was extremely low, and none developed into larger larvae. Among plants occurring in Hawai‘i, only two species other than *M. calvescens* experienced substantial levels of feeding: the melastomes *Clidemia hirta* and *Tetrazygia bicolor*, which have recently been found through genetic analyses to be better placed within the genus *Miconia* (Michelangeli et al. 2020). No Melastomataceae are native to Hawai‘i, and nine of the 15 species naturalized in Hawai‘i have been declared state noxious weeds (Medeiros et al. 1997).

Studies have clearly demonstrated that *E. chrysippe* is host-specific to a narrow subset of Melastomataceae. Results of the host specificity studies are summarized below (Figures 5-7). Laboratory tests are consistent with field observations of *E. chrysippe* in Costa Rica, where eggs and larvae have been collected only from species of *Miconia* and *Conostegia rufescens*, a plant in the same tribe (Nishida 2010). A similar pattern of specificity holds for other species within the genus *Euselasia*. Across numerous studies in various parts of tropical America, *Euselasia* have been found to be narrowly host-specific, with each species specializing within a family of plants (Nishida 2010).

Table 1. Plant species exposed to *Euselasia chrysippe* larvae in no-choice petri tests

| Order<br>Family<br>Tribe | Test Plant Species | Common Name(s) | Native Range* | Present in Hawaii? |
|---|---|---|---|---|
| Myrtales | | | | |
| Melastomataceae | | | | |
| Miconieae | *Clidemia dentata* | | SCA | |
| | *Clidemia discolor* | | SCA | |
| | *Clidemia epiphytica* | | SCA | |
| | *Clidemia hirta* | clidemia, Koster's curse | SCA | yes |
| | *Conostegia subcrustulata* | | SCA | |
| | *Conostegia xalapensis* | | SCA | |
| | *Henriettea turberculosa* | | SCA | |
| | *Leandra granatensis* | | SCA | |
| | *Leandra longicoma* | | SCA | |
| | *Miconia affinis* | | SCA | |
| | *Miconia argentea* | | SCA | |
| | *Miconia barbinervis* | | SCA | |
| | *Miconia calvescens* | miconia | SCA | yes |
| | *Miconia cremadena* | | SCA | |
| | *Miconia elata* | | SCA | |
| | *Miconia gracilis* | | SCA | |
| | *Miconia impetiolaris* | | SCA | |
| | *Miconia longifolia* | | SCA | |
| | *Miconia multispicata* | | SCA | |
| | *Miconia nervosa* | | SCA | |
| | *Miconia prasina* | | SCA | |
| | *Miconia theizans* | | SCA | |
| | *Tetrazygia bicolor* | | NA/SCA | yes |
| Bertolonieae | *Triolena hirsuta* | | SCA | |
| Blakeeae | *Blakea litoralis* | | SCA | |
| | *Topobea maurofernandeziana* | | SCA | |
| Dissochaeteae | *Medinilla cummingii* | | IM | yes |
| | *Medinilla magnifica* | showy medinilla | AU/IM | yes |
| Melastomeae | *Arthrostemma ciliatum* | pinkfringe | SCA | yes |
| | *Dissotis rotundifolia* | pink lady, rockrose | AF | yes |
| | *Heterocentron subtriplinervium* | pearlflower | SCA | yes |
| | *Melastoma sanguineum* | fox-tongued melastome | IM | yes |
| | *Melastoma septemnervium* | Asian melastome | IM | yes |
| | *Pterolepis glomerata* | false meadowbeauty | SCA | yes |
| | *Tibouchina herbacea* | cane tibouchina | SCA | yes |
| | *Tibouchina longifolia* | long leaf glory tree | SCA | yes |
| | *Tibouchina urvilleana* | princess flower, glorybush | SCA | yes |
| Combretaceae | *Terminalia catappa* | false kamani | AU/IM | yes |
| Lythraceae | *Cuphea ignea* | cigar flower | SCA | yes |
| | *Lythrum maritimum* | pukamole | SCA | yes |

* HI =Hawaii, SCA =South & Central America, NA =North America, AU =Australia, AF =Africa, IM =Indomalayan, COS =Cosmopolitan

| Order<br>Family<br>Tribe | Test Plant Species | Common Name(s) | Native Range* | Present in Hawaii? |
|---|---|---|---|---|
| Myrtaceae | *Eucalyptus deglupta* | rainbow eucalyptus | IM | yes |
| | *Eucalyptus globulus* | blue gum | AU | yes |
| | *Eugenia uniflora* | Surinam cherry, pitanga | SCA | yes |
| | *Lophostemon confertus* | brushbox, Brisbane box | AU | yes |
| | *Melaleuca leucadendra* | weeping paperbark | AU/IM | yes |
| | *Metrosideros macropus* | lehua mamo | HI | yes |
| | *Metrosideros polymorpha* | 'ohi'a lehua | HI | yes |
| | *Plinia cauliflora* | jaboticaba | SCA | yes |
| | *Psidium cattleianum* | strawberry guava | SCA | yes |
| | *Psidium friedrichsthalianum* | Costa Rican guava, cas | SCA | yes |
| | *Psidium guajava* | common guava | SCA | yes |
| | *Rhodomyrtus tomentosa* | downy myrtle, rose myrtle | IM | yes |
| | *Syzygium cumini* | Java plum | IM | yes |
| | *Syzygium malaccense* | mountain apple, | AU/IM | yes |
| Onagraceae | *Epilobium ciliatum* | willowherb | NA/SCA/IM | yes |
| | *Fuchsia magellanica* | hardy fuchsia | SCA | yes |
| | *Oenothera laciniata* | cutleaf evening primrose | NA | yes |
| Geraniales | | | | |
| Geraniaceae | *Geranium homeanum* | Australasian geranium | AU | yes |
| Brassicales | | | | |
| Caricaeae | *Carica papaya* | papaya | SCA | yes |
| Malvales | | | | |
| Malvaceae | *Hibiscus rosa-sinensis* | hibiscus | IM | yes |
| | *Theobroma cacao* | cacao | SCA | yes |
| Sapindales | | | | |
| Anacardiaceae | *Mangifera indica* | mango | IM | yes |
| Rutaceae | *Citrus* x *sinensis* | lemon | IM | yes |
| Sapindaceae | *Dodonaea viscosa* | a'ali'i | COS/HI | yes |
| Rosales | | | | |
| Moraceae | *Artocarpus altilis* | ulu, breadfruit | IM | yes |
| Fabales | | | | |
| Fabaceae | *Acacia koa* | koa | HI | yes |
| | *Sophora chrysophylla* | mamane | HI | yes |
| Gentianales | | | | |
| Rubiaceae | *Coffea arabica* | coffee | AF | yes |
| Lamiales | | | | |
| Scrophulariaceae | *Myoporum sandwicense* | naio | HI | yes |
| Proteales | | | | |
| Proteaceae | *Macadamia integrifolia* | macadamia | AU | yes |
| Alismatales | | | | |
| Araceae | *Anthurium* | anthurium | SCA | yes |
| Laurales | | | | |
| Lauraceae | *Persea americana* | avocado | SCA | yes |
| Cyatheales | | | | |
| Dicksoniaceae | *Cibotium glaucum* | hapu'u | HI | yes |

* HI =Hawaii, SCA =South & Central America, NA =North America, AU =Australia, AF =Africa, IM =Indomalayan, COS =Cosmopolitan

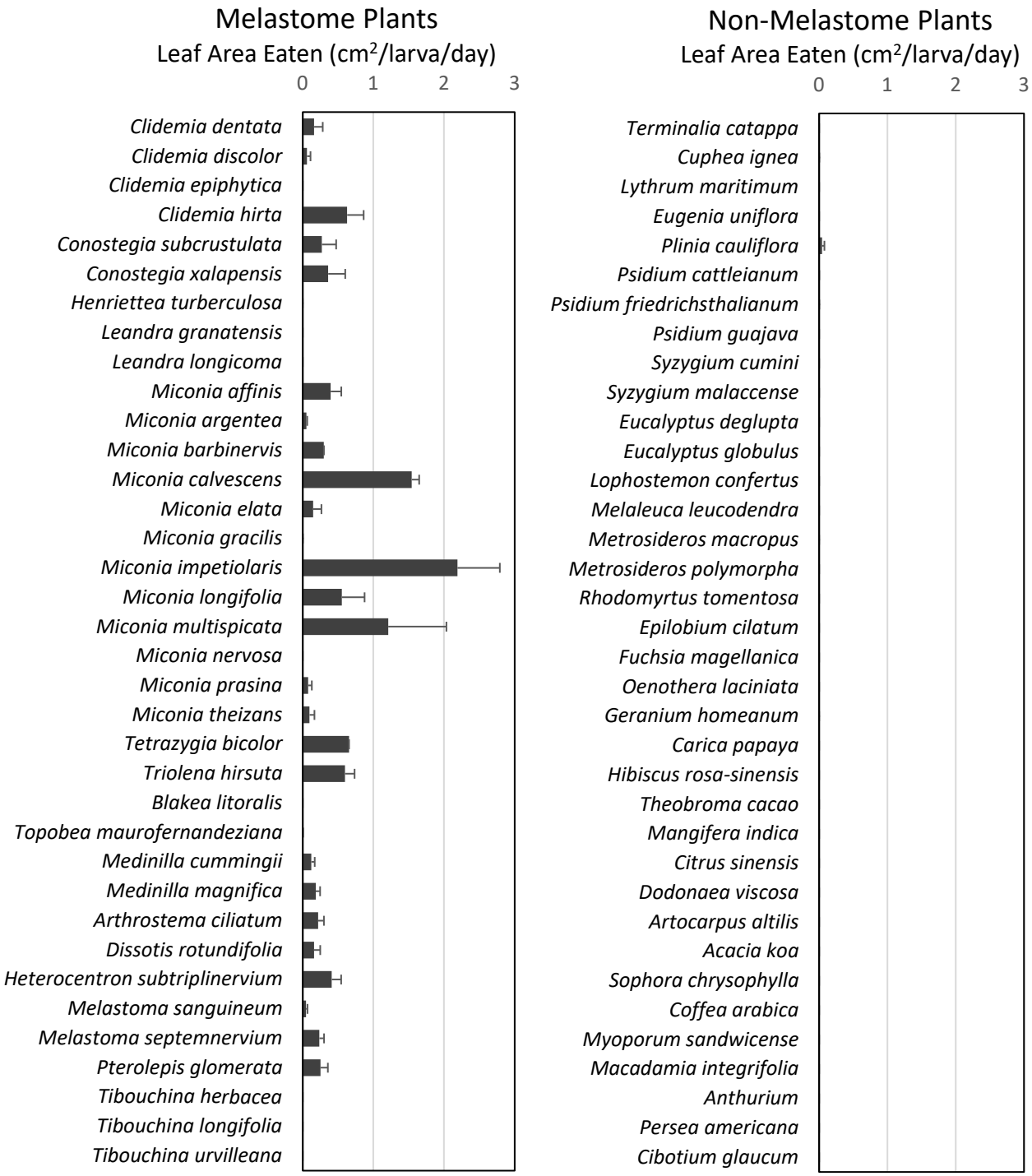


Figure 5. Average feeding damage by mid-sized larvae (instars 3-5) of *Euselasia chrysippe* on plant species in Costa Rica and Hawaii exposed as fresh leaves for 3 days in 90 mm petri dishes in 2012-2014, measured from photos before and after exposure (bar = standard error). Species on left, in the family Melastomataceae, are grouped according to genetic relatedness, and non-melastomes on right are listed in order of genetic distance from Melastomataceae.

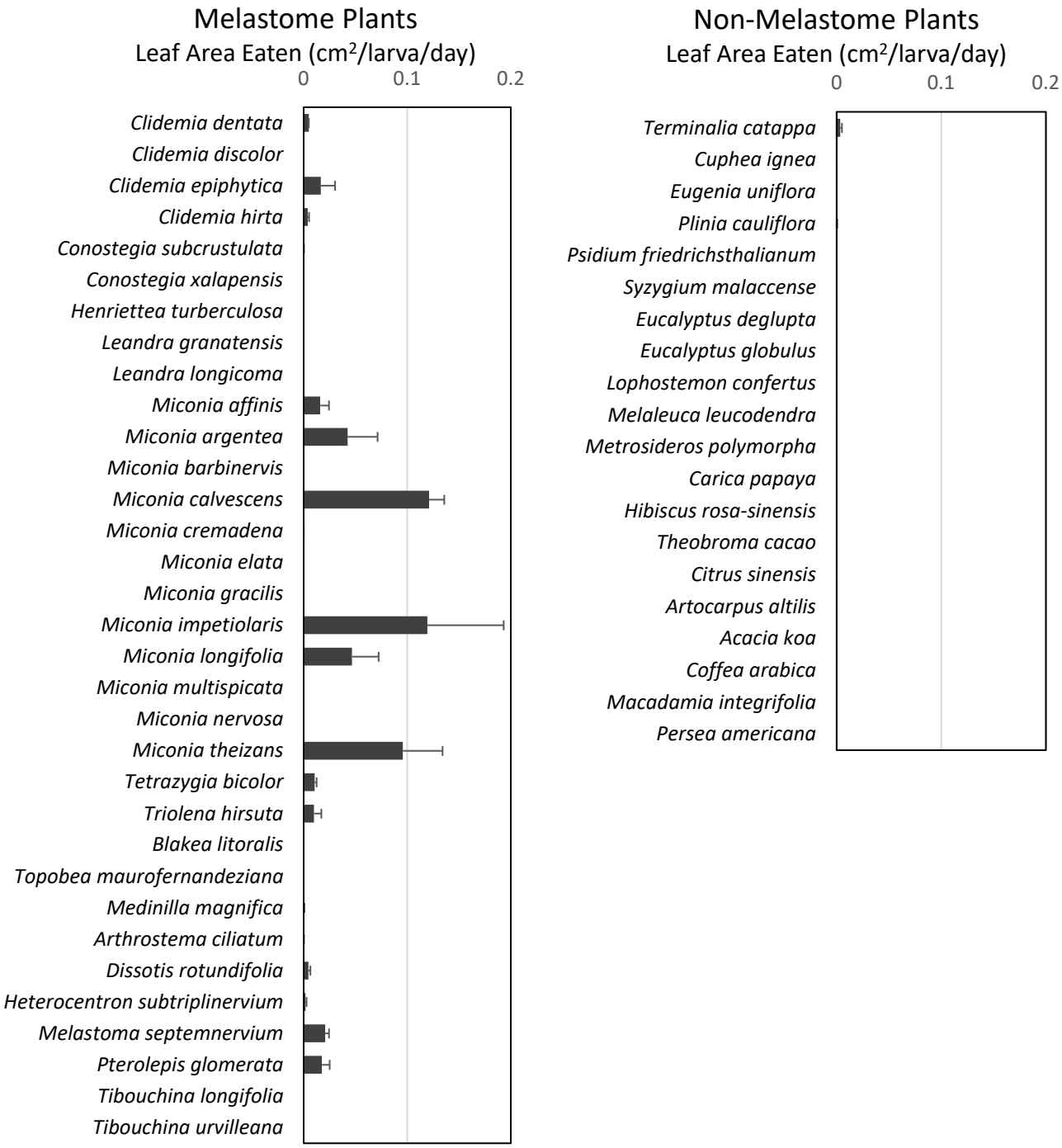


Figure 6. Average feeding damage by small larvae (instars 1-2) of *Euselasia chrysippe* on plant species in Costa Rica and Hawaii exposed as fresh leaves for 3 days in 90 mm petri dishes in 2012-2014, measured from photos before and after testing (bar = standard error). Species in Melastomataceae on left are grouped according to genetic relatedness, and non-melastomes on right are listed in order of genetic distance from Melastomataceae.

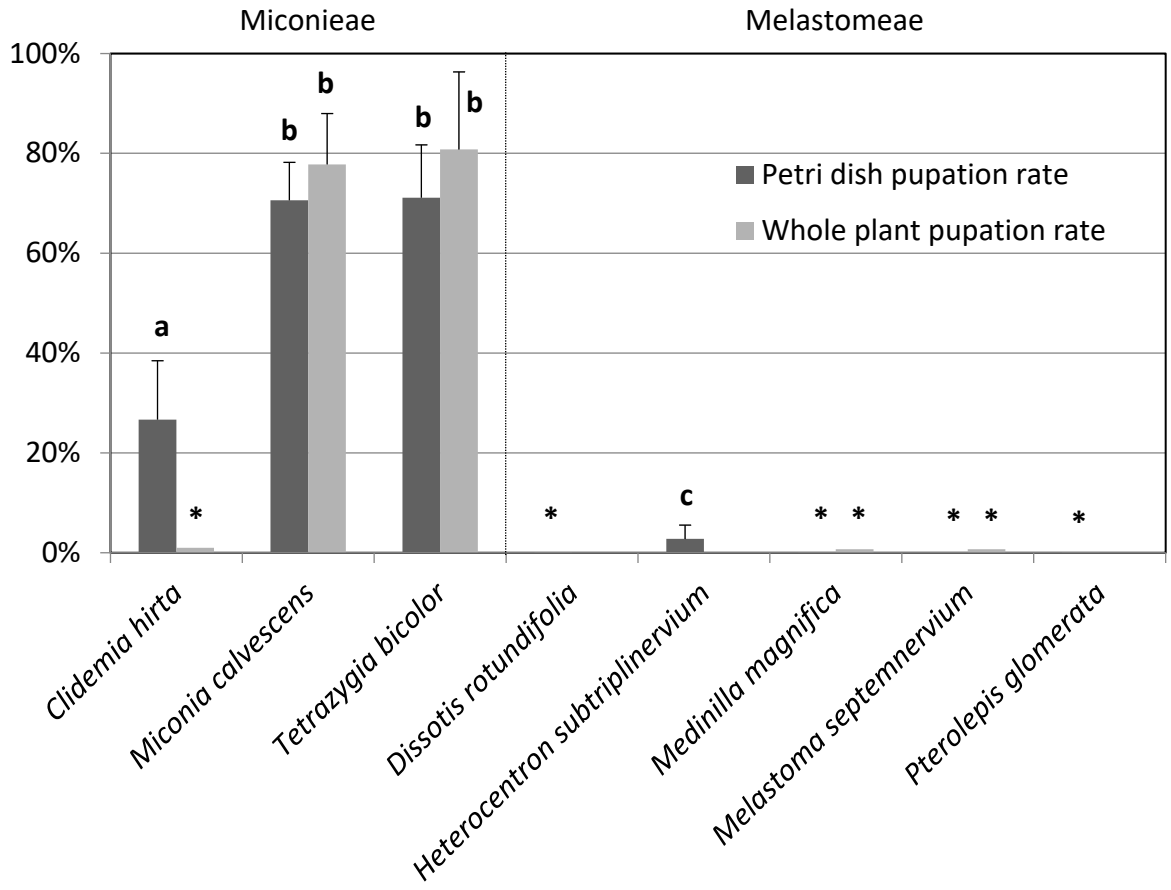


Figure 7. Survival of *E. chrysippe* larvae to pupation (percent average ± standard error) when exposed continuously to leaves in Petri dishes (dark gray) and whole plants (light gray) of test plant species in the tribes Miconieae and Melastomeae (family: Melastomataceae). Results with different letters (a,b,c) are statistically different. Results with an asterisk (*) had negligible survival and were not tested in the statistical model.

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**Process for collection, screening, rearing and field release of *Euselasia chrysippe***

Proposed steps:

1) Egg masses collected from field sites in Costa Rica
2) Larvae reared to adulthood in entomology lab at Univ. of Costa Rica, observing and removing diseased or parasitized individuals at each life stage
3) Adults released in large rearing cage at Univ. of Costa Rica for mating and egg laying
4) Eggs collected at UCR and shipped to Hawaii Volcanoes quarantine
5) Larvae reared to adulthood in containment at Hawaii Volcanoes quarantine, checking for diseased or parasitized individuals at each life stage. In addition to visual screening for natural enemies, we will destructively subsample larvae and adults for genetic analysis to detect potential pathogens.
6) Adults removed from quarantine and released into large rearing cage near Hilo for mating and egg laying
7) Eggs collected from rearing cage and larvae reared to adult in labs or cages at HDOA, Forest Service, or other partner facilities, checking for diseased or parasitized individuals
8) Adults released at field sites of miconia invasion

Protocols:

Immature stages will be inspected twice per week for potential natural enemies. Genetic screening is proposed for pathogens that may be difficult to detect, but are known to affect butterfly larvae, pupae or adults. These include species of fungi, virus and bacteria that commonly infect lepidoptera. If natural enemies are detected at any stage of development, the associated batches of *Euselasia* will be isolated and destroyed if there is no other means of insuring an enemy-free colony. This is mainly relevant for pathogens that might escape visual detection and spread easily, rather than parasitoids that tend to be obvious. Parasitoids include tiny wasps that emerge from eggs, or wasps and flies that emerge from larvae and pupae.

Immatures from a single egg batch (approx. 40-80 individuals) will be reared together as a cohort on a potted miconia sapling in isolation from other egg cohorts. This allows for careful screening of natural enemies and separate handling of males and females from the same cohort to minimize in-breeding. Males from a cohort emerge from pupae one day before females, allowing easy separation and handling of sexes. Duration of life stages are approximately 4 weeks for eggs, 3 weeks for larvae, 2 weeks for pupae, and 4-8 weeks for adults.

Large rearing cages in both Costa Rica and Hawaii will be situated in environments that are expected to lack specialized enemies of *Euselasia*. At the Univ. of Costa Rica, the field cage is situated in a forested area far from natural populations of the insect and its host plants. In Hawaii there are no known specialized natural enemies of *Euselasia*. The Hilo field cage site will be treated to eliminate Little Fire Ant and other potential predators. A few dozen adult male and female butterflies will be introduced to a

cage at a time. Egg masses will be monitored by inspecting undersides of miconia leaves on trees within the cage. Egg masses will be collected by removing all or a portion of each leaf.

The field cage will be constructed of sewn panels of shade cloth suspended by ropes from trees and other structures over several large miconia trees planted in the ground. Edges of shade cloth will be tied together or buried in the ground to close gaps large enough for butterflies to escape. Entry to the cage will be through a shade cloth vestibule to prevent escape by butterflies. The Hilo rearing cage (approx. 30x50x18 ft) is planned for a location with appropriate conditions for *Euselasia* mating and egg laying, on private property away from public access points. The cage will be monitored at least 3 times per week. Although potentially vulnerable to wind or other natural or human-caused damage, the shade cloth cage is expected to be durable for use for 3 years or more. Its main function is to allow rearing of large numbers of egg masses, rather than to prevent possible environmental release of small numbers of butterflies. It can be made available for inspection prior to first use or as needed.

These protocols will allow for screening of 2 generations of *Euselasia chrysippe* prior to environmental release in Hawaii, and one additional generation of screening before field release. All rearing of immatures can be conducted on potted plants in secure environments, where insects are easily inspected for signs of natural enemies. Adult mating and egg laying will occur in rearing cages which can be managed to minimize possible exposure to natural enemies.

# Hawaii Volcanoes National Park Quarantine Facility
# Standard Operating Procedures

## INTRODUCTION

The Hawaii Volcanoes National Park Quarantine Facility is the result of a cooperative agreement among federal and state of Hawaii agencies to implement a biological control program against alien weeds in Hawaii's forests. Participants in the cooperative agreement are: National Park Service; Biological Resources Division of the U.S. Geological Survey; U.S.D.A. Forest Service; Hawaii Department of Land and Natural Resources; Hawaii Department of Agriculture; and University of Hawaii. The Quarantine Facility was completed in March 1984 and certified in November 1984. The original 600 sq. ft. structure was modified in 1996 with addition of 600 sq. ft. of space for a workroom. The entire structure was modified with the addition of a metal roof and skylights in 2003. The facility is located at 3,800 feet elevation in Hawaii Volcanoes National Park, approximately 1 mile southeast of the park headquarters and visitor center.

This facility is designed to contain arthropod agents under evaluation for biological control of noxious forest weeds. Until approved for release by federal and state authorities, agents are considered restricted plant pests. They must be handled with appropriate caution to prevent escape because their establishment could have irreversible negative consequences for the environment. Containment of agents requires a secure, properly maintained building and strict adherence to precautionary protocols by all personnel. These Standard Operating Procedures (SOP) are meant to inform personnel on management policies and procedures to correctly and safely perform duties while working in this specialized structure.

## PHYSICAL CONTAINMENT STANDARDS

### Description of the facility and safeguards

(Refer to attached map and floor plan.)

The Quarantine Facility (Building #338) consists of two anterooms (A and B), the quarantine greenhouse (with walls and skylights made of Lexan polycarbonate), autoclave room, workroom (room 1 of the quarantine addition), temperature cabinet room (room 2), and handling room (room 3). A storage room is located at the external opening of the autoclave.

Entry and exit from the Quarantine Building is through the two anterooms enclosed by three airtight doors. The anterooms are completely dark except for insect light traps formed by windows looking into the Quarantine Greenhouse. There is an Emergency Exit Door in the workroom.

### A. Walls, ceiling, and floors

The Quarantine Facility and adjacent glass greenhouse are surrounded by a water moat to prevent entry of unwanted organisms. Walls are double-walled plywood or Lexan mounted with flexible gaskets. Floors are cement painted grey. Drains in floors are covered with 100-mesh stainless steel screen. Ceilings are sealed wood with insulation. Skylights are sealed, double-pane Lexan polycarbonate. All plumbing and electrical systems located in the walls are sealed with caulking.

**B. Windows**

Double-paned, Lexan windows are present in each room of the quarantine addition. The window in the Autoclave Room is single-paned Lexan, as part of an original design as an emergency exit.

**C. Exterior doors**

The entry and emergency exit doors are steel-plated. The three entry doors through Anterooms A and B are equipped with sensors that indicate via red and green lights when all doors are closed (green) or any door is open (red). The emergency exit is equipped with a sensor that sounds an alarm if the door is opened.

**D. Ventilation**

Air temperature inside quarantine is regulated by two fans that draw outside air through double, 250-mesh stainless steel screens located in the gable over the entrance and exhaust it through double, 250-mesh stainless steel screens located at the opposite end of the Quarantine Greenhouse. Stainless steel screens are protected from clogging dust by standard air filters. Temperature is adjusted by increasing fan rotational speed to increase cooling or decreasing fan speed to allow warming. The fan control box is located near the anteroom entry/exit door.

Hot air generated by temperature cabinets is exhausted from the temperature cabinet room into the adjoining greenhouse by a fan mounted near the ceiling.

**E. Negative pressure**

Negative air pressure within quarantine is maintained by the fans and is indicated by the manometer in the autoclave room. The purpose of negative pressure is to decrease the chance of flying or airborne insects from being sucked out when a door is opened.

**F. Electrical system**

All outlets are on ground-fault interrupt (GFI) circuits. The circuit breaker panel for all electrical sources is located in the Storage Room accessible from outside quarantine. In the event of extended power disruption, National Park staff will deploy a portable emergency generator wired to the power system located in the Storage Room. Use of earplugs is recommended when working in the immediate area while the generator is in operation. The emergency generator will supply all normal quarantine electrical requirements, except for operation of the autoclave.

**G. Communication system**

The Quarantine Facility is supplied with a telephone located in the Workroom (line 808-967-7122 which also serves the Forest Service office in Magma House).

**H. Waste disposal**

Waste materials that can be safely pressure-heated are sterilized in the autoclave before exiting quarantine via the Storage Room. Autoclave doors seal and lock automatically so that only one door can be opened at a time. The external door can only be opened after a sterilizing cycle is completed.

Liquid waste such as dishwashing water is eliminated through the plumbing system that feeds into a closed, covered cesspool. Floor drains covered with stainless steel mesh also are tied to the

cesspool, which serves only the quarantine building.

### I. Fire and chemical safety

Smoke detectors are installed on ceilings of the quarantine greenhouse, workroom, autoclave room, temperature cabinet room, and storage room. Fire extinguishers are hung in the quarantine greenhouse, quarantine workroom, outside Building 342 (plant containment building), outside the HAVO nursery office, and outside the Magma House office.

Emergency exits from quarantine include the main entrance doors and an emergency door in the workroom. An emergency eye-wash and shower are installed in the handling room. A first aid kit is located in the autoclave room. Ear plugs, full-face shields, and gloves are available on site. Chemicals are inventoried and their locations listed within the Chemical Safety Plan, together with safety data sheets (SDS). Inventories and SDS are stored just inside entrances of the office, quarantine, and Building 342.

## OPERATIONAL STANDARDS

### 1. Designation of Quarantine Officer and Quarantine Officer's Supervisor

Quarantine Officer (in training): Nancy Chaney, Natural Resources Specialist
Mailing Address: USDA Forest Service, P.O. Box 236, Volcano, HI 96785
Work tel: 808-967-7122; Fax: 808-967-7158; Cell: 808-333-0433

Quarantine Officer's Supervisor: Dr. Tracy Johnson, Research Entomologist
Work tel: 808-967-7122; Fax: 808-967-7158; Cell: 808-938-7818

### 2. Authorized personnel

Access to the Quarantine Facility is restricted to individuals authorized by the Quarantine Officer. Access is generally limited to individuals involved in biological control research. Visiting guests must be accompanied by the Quarantine Officer or her designee.

### 3. Signs

A. A sign permitting entry to authorized personnel only is posted at the entry to the Quarantine Building.

B. A sign with emergency contact information is posted at the entry to the Quarantine Building.

C. A sign indicating the emergency exit door is posted upon the door.

### 4. Access to the facility

### A. Before entering quarantine

Plan your work in advance so that only required materials and equipment are taken into the quarantine facility. All plants, plant materials, supplies and equipment brought into quarantine shall be free of insects and arthropods. This may be accomplished by visual inspection and removal, treatment with appropriate pesticides, or other approved methods.

**B. Entering the facility**

Wipe off your shoes outside the first entry door. Before entering, check the red and green lights to the left of the entrance door. Enter only if the green light is on. A red light indicates that someone has opened one of the interior doors. In this case you should wait until they have finished entering or exiting. Open only one door at a time after observing that the indicator lights are green.

Upon entry to Anteroom A, place any personal belongings (e.g. hat, coat) on the shelf and/or hooks. Items not needed in quarantine should not be taken further into the facility.

After passing into Anteroom B, put on a laboratory coat. Additional coats are available for guests if needed.

Upon entering the quarantine greenhouse, record your name, the date, time and any materials you have brought with you on the Sign-in Sheet attached to the door.

**C. Exiting the facility**

Wash your hands and take a few moments to visually inspect your clothing for insects. Sign out before exiting and note any items you are removing from the facility. **Materials exiting the facility shall be treated as described under Sanitation** (see below). Remove your laboratory coat inside the quarantine, shake it out, and repeat visual inspection. Check for the green light before opening the door.

Enter Anteroom B and close the door. Hang up laboratory clothing. Brush yourself off starting high and working down. Stamp your feet to dislodge materials on shoes. If at any time during this procedure you observe insects, they should be captured or killed and you should return to quarantine to verify that no others can escape.

If inspection and brushing reveal no insects, proceed to Anteroom A. Shake out personal clothing left in this room before putting it back on. Wipe off your feet on the mat again before exiting.

**5. Sanitation**

**A. Autoclave**

The quarantine autoclave is a Steris double-doored, gravity/laboratory sterilizer with a single-input power supply. The autoclave features microcomputer controls that monitor all cycle phases. It provides both audible and visual notification of progress sterilization.

All items that are small enough and can withstand 120ºC and 20 Atm pressure for 30 minutes should be autoclaved before exiting quarantine. For new or unusually bulky items, place Sterilizer Indicator Strips at the center of the item and check to verify that sterilization was sufficient. Check each load to verify that heat was sufficient to partially melt plastic bag containers.

All soil and plant matter brought into quarantine shall be autoclaved before its removal.

All trash, including residues from wastebaskets and floor sweepings, shall be autoclaved.

Removal of living insects, plants, or plant materials from the quarantine building is prohibited without specific authorization from the Quarantine Officer and regulatory agencies.

**B. Removal of objects from the facility**

Small items (pieces of paper, cameras) that can be thoroughly inspected can be wiped clean and removed.

Items such as plastic pots should be immersed in 5% bleach and held in Anteroom B for several hours, overnight if possible, before being removed.

Lab coats shall be periodically autoclaved and removed by the Quarantine Officer for washing in bleach and hot water. Coats must be shaken clean, and visually inspected inside quarantine.

Equipment that cannot be autoclaved (microscopes, electrical equipment, etc.) shall be decontaminated with alcohol or placed in a plastic bag and fumigated with an appropriate insecticide within Anteroom A. If it is necessary to fumigate an object, this should be done at the end of the day after everyone has left in order to minimize pesticide exposure. Any use of pesticides must be performed in consultation with the Quarantine Officer and follow required safety procedures found in the Chemical Safety Plan.

Larger pieces of equipment (such as refrigerators and temperature cabinets) should be cleaned thoroughly, wrapped in plastic, and fumigated with insecticide for 24 hours. If it is possible to treat all surfaces, spraying with 95% alcohol can be used as an alternative to fumigation.

## 6. Facility maintenance and repairs

Maintenance personnel are authorized for entry only after notifying the Quarantine Officer or the Quarantine Officer's supervisor and receiving a pre-work briefing.

The quarantine facility will be inspected by the Quarantine Officer or Quarantine Officer's supervisor once a month. Items to be checked include: 1) caulking around windows and greenhouse panels; 2) seals around all doors to be sure that latches close properly and that no light can be seen when they are closed; 3) caulking around the autoclave unit, and plumbing and electric lines entering the facility; 4) filters covering air intake and exhaust vents.

A complete inspection of all parts of the facility will be conducted annually. Based on the results of this inspection, maintenance needs will be identified and submitted in writing to the Chief of Resources Management of HAVO.

## 7. Emergencies and contingency plans

Before working in quarantine, familiarize yourself with the location and operation of smoke detectors, fire extinguishers, emergency exits, emergency eye-wash and shower, first aid kit, personal protective equipment and chemicals. Access to emergency equipment must remain unobstructed.

### Earthquakes/Hurricanes

During an earthquake, leave the greenhouse, autoclave room, or temperature cabinet room immediately. Take cover under a metal desk in the workroom or evacuate the building.

Immediately following a minor earthquake (five and lower on the Richter scale) or major storm, the facility should be checked for breaches to quarantine. Areas to be checked include all seals checked in the course of monthly maintenance inspections (see above).

A major earthquake is one in which damage to the physical building could occur and is strong enough to shake loose items off benches. The first and primary goal of the quarantine staff after a major earthquake is human safety.

If quarantine can be entered safely, check all containers with quarantine insects. If breakage has occurred, steps must be taken immediately to prevent insects from escaping. Cracks should be sealed with tape or plastic. Items that have broken or spilled should be sealed in plastic bags.

After verifying that all sleeve cages and other containers are sealed, the entire quarantine facility should be closely inspected to determine if breaks occurred. If breaks are small, quarantine personnel shall repair them immediately. Damage requiring extensive repair will be referred to the National Park Service.

If an approaching major storm poses a threat to the quarantine, all insect colonies shall be packaged in airtight containers and placed inside refrigerators or temperature cabinets with the doors closed and secured.

**Major structural damage**

If it is judged that the physical facility has been damaged to the point that it is no longer operational and may be incapable of containing escaped insects, the following steps shall be taken: 1) If cages have broken open, insects have been freed and escape is possibly occurring, the entire facility will be immediately fumigated. 2) If cages are intact and no insects have escaped, insect colonies shall be packaged in airtight plastic bags, held in the refrigerator or temperature cabinets, and then the quarantine facility fumigated. Later, the colonies shall be packaged and transferred to an alternate quarantine facility for temporary holding.

**Shutdown of quarantine during a park closure due to elevated $SO_2$ levels**

Shut off the quarantine ventilation system at main controls. This will prevent circulation of air in or out of the building. Turn off all appliances except one refrigerator and internal fan. This will serve to reduce heat buildup inside quarantine so that temperature remains moderate and nonfatal to insects. After turning off power to temperature cabinets, leave doors open to allow some air circulation and prevent excessive humidity to insects left inside.

If it is safe to enter the park during a closure (as authorized by park safety officers), quarantine colonies will be inspected and maintained every few days (2-3 times per week if possible). Authorized personnel may minimize exposure to $SO_2$ through use of park-approved respirators and by keeping the time in and out of the park to 1 hour or less. Any access must be controlled with check-in and check-out contacts with both HAVO and Forest Service points of contact. When HAVO officials reopen the park, quarantine will be ventilated and inspected for security and functionality.

**SPECIAL PROCEDURES FOR HANDLING ORGANISMS UNDER PERMIT**

The Hawaii Volcanoes National Park Quarantine Facility is certified for containment of arthropods only. Pathogenic organisms are not tested in this quarantine facility unless they are found in the natural environment in Hawaii.

Only shipments of agents approved for introduction into the quarantine facility by the State of Hawaii and APHIS will be accepted. Approved shipments will be covered by a PPQ Form 526 signed by the appropriate officers for the State of Hawaii and APHIS/PPQ.

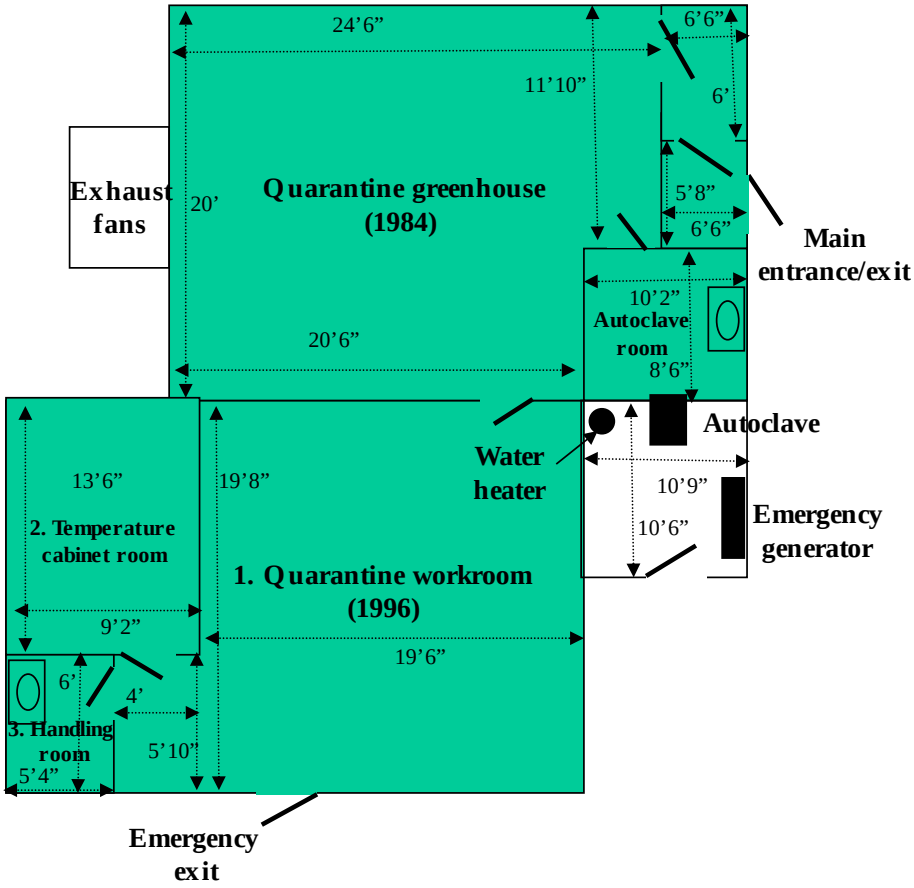
Under no conditions or circumstances will a package be opened before it enters the quarantine facility. State or Federal Inspection personnel may accompany the package to the quarantine facility and be present when it is opened. All arriving shipments shall be unpacked inside the handling room with the door completely closed. Each layer of wrapping as it is encountered will be individually examined for agents. Plant materials and packaging in which insects were received from a foreign country shall be bagged immediately and autoclaved. All instruments and work surfaces shall be sterilized with 95% alcohol.

Live agents shall be removed from the handling room in sealed containers and transferred to sleeve cages in the main quarantine area for rearing and study. Dead or weakened agents shall be checked for pathogens or parasitoids and then preserved or reared for inspection or for voucher specimens. All extraneous species shall be killed and saved.

After receiving and inspecting a shipment, the Hawaii Department of Agriculture and APHIS shall be notified in writing of the shipment, the type and exact number of insects it contained, and their condition. A log will be kept of each shipment received in the quarantine facility and contain all information pertaining to the shipment (i.e., original shipper, dates, number and condition of insects found (both the desired species and extraneous species.

Before transporting live insects to another quarantine facility, approval will be obtained in writing from the State of Hawaii or the APHIS/PPQ. Dead specimens may be removed from the quarantine to be used for voucher or other scientific purposes, but only in consultation with the Quarantine Officer and following appropriate treatment (e.g., alcohol, fumigant, freezing at -29C for >72 h). The release of insects into the field in Hawaii requires state and federal approval. Voucher specimens will be prepared and submitted to both the Hawaii Department of Agriculture and the USDA ARS at Beltsville, Maryland.

## Quarantine Floorplan



United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection & Quarantine
4700 River Road
Riverdale, MD 20737

# Permit to Move Live Plant Pests, Noxious Weeds, and Soil

Importation

**Regulated by 7 CFR 330**

**This permit was generated electronically via the ePermits system**

| | | | |
|---|---|---|---|
| **PERMITTEE NAME:** | Matthew Johnson | **PERMIT NUMBER:** | P526P-20-02009 |
| **ORGANIZATION:** | USDA Forest Service | **APPLICATION NUMBER:** | P526-190826-015 |
| **ADDRESS:** | Hawaii Volcanoes National Park<br>Quarantine Facility<br>Kilauea Research Station, Building 34<br>Volcano, HI 96718 | **FACILITY NUMBER:** | 22 |
| **MAILING ADDRESS:** | P.O. Box 236<br>Volcano, HI 96785 | **HAND CARRY:** | No |
| | | **DATE ISSUED:** | 04/21/2020 |
| **PHONE:** | 808-967-7122 | | |
| **FAX:** | 808-967-7158 | **EXPIRES:** | **04/21/2023** |
| **DESTINATION:** | HI | | |
| **DESIGNATED PORTS:** | HI, Honolulu | | |

Under the conditions specified, this permit authorizes the following:

| Regulated Article | Life Stage(s) | Intended Use | Shipment Origins | Originally Collected | Culture Designation |
|---|---|---|---|---|---|
| Allorhogas clidemiae | Any | Research - Lab | Central America, South America | Originally Collected from Outside the U.S. and Territories | |
| Allorhogas granivorus | Any | Research - Lab | Central America, South America | Originally Collected from Outside the U.S. and Territories | |
| Anthonomus monostigma | Any | Research - Lab | Central America, South America | Originally Collected from Outside the U.S. and Territories | |
| Diclidophlebia lucens | Any | Research - Lab | Central America, South America | Originally Collected from Outside the U.S. and Territories | |
| Euselasia bettina | Any | Research - Lab | Central America, South America | Originally Collected from Outside the U.S. and Territories | |
| Euselasia chrysippe | Any | Research - Lab | Central America, South America | Originally Collected from Outside the U.S. and Territories | |
| Syphraea uberabensis | Any | Research - Lab | Central America, South America | Originally Collected from Outside the U.S. and Territories | |

## SPECIAL INSTRUCTIONS TO INSPECTORS

See permit conditions below

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| | |
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DHS CBP INSPECTORS - SHIPMENT BY BONDED CARRIER
1) Confirm that the carrier of the shipment imported under this USDA PPQ 526 permit is commercially bonded.
2) Confirm that the imported shipment has a valid USDA PPQ Form 599 Red/White label attached to the exterior for routing to a USDA APHIS PPQ Inspection Station or other "Designated Port" as stated on the Permit. A valid label will have the permit number, expiration date, label number, and address of a USDA APHIS PPQ Plant Inspection Station/Designated Port. PLEASE NOTE: In the event of a shipment of bulk container with discrete units, a single PPQ Form 599 Red/White label may be used.
3) Validate the permit in ePermits using the CBP search feature.
4) If a valid PPQ Form 599 Red/White label is not attached to the exterior of the package or the label has been covered or is otherwise not legible, then forward to the nearest USDA APHIS PPQ Plant Inspection Station.
5) If the address on the airway bill does not match the address on the PPQ Form 599 Red/White label then forward the package to the nearest USDA APHIS PPQ Plant Inspection Station/designated port shown on the PPQ Form 599 label. All costs associated with rerouting misaddressed packages will be assumed by the permit holder.

APHIS PPQ INSPECTORS at PIS -High-Risk Invertebrates
Follow the instructions in the Plant Inspection Station Manual for High-Risk Invertebrates Red and White Labeled Packages (must be opened in a sleeved cage; see procedures for handling on page 3-7-39). For questions or concerns, contact the USDA APHIS PPQ Pest Permit Branch in Riverdale, MD, at 301-851-2046, toll free 866-524-5421.

**PERMIT GUIDANCE**

1) Receipt or use of foreign isolates or samples from countries under sanctions requires specific permission from the U.S. Department of Treasury; please refer to https://www.treasury.gov/resource-center/sanctions/Programs/Pages/Programs.aspx
2) This permit does not authorize movement or release into the environment of genetically engineered organisms produced with the regulated organisms described in this permit. Importation, interstate movement, and environmental release of genetically engineered plant pests require a different permit issued under regulations at 7 CFR part 340. Any unauthorized interstate movement or environmental release, including accidental release, of a regulated GE organism would be a violation of those regulations. Additional guidance and contact information for APHIS Biotechnology Regulatory Services, can be found at: https://www.aphis.usda.gov/aphis/ourfocus/biotechnology.
3) If an animal pathogen is identified in your shipment, to ensure appropriate safeguarding, please refer to http://www.aphis.usda.gov/import_export/animals/animal_import/animal_imports_anproducts.shtml
4) If a human pathogen is identified, please refer to the CDC Etiologic Agent Import Permit Program at http://www.cdc.gov/od/eaipp/
5) This permit does not fulfill the requirements of other federal or state regulatory authorities. Please contact the appropriate agencies, such as the U.S. Environmental Protection Agency, the U.S. Fish and



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Wildlife Service, the U.S. Food and Drug Administration, the Centers for Disease Control and Prevention, the APHIS Veterinary Services unit, the APHIS Biotechnology Regulatory Services, or your State's Department of Agriculture to ensure proper permitting.
6) If you are considering renewal of this permit, an application should be submitted at least 90 days prior to the expiration date of this permit to ensure continued coverage. Permits requiring containment facilities may take a longer period of time to process.

## PERMIT CONDITIONS

USDA-APHIS issues this permit to Matthew Johnson, USDA Forest Service, Hawaii Valcanoes National Park, Quarantine Facility, Kilauea Research Station, Volcano, HI 96718. This permit authorizes the importation of any life stages of the various taxa shown under Regulated Article above, collected in/from Central and South American countries, and observed to feed on or be associated with Miconia calvescens,(the target/host plant), to the permit holder Dr. Matthew Johnson, USDA Forest Service, Hawaii Volcanoes National Park, to be received into the USDA APHIS approved containment facility at that address (CF #22).

The imported material may contain various host plant parts of Miconia calvescens, including roots, leaves and stems.

This permit authorizes the possession and rearing of any species imported under this permit for research in the USDA APHIS inspected containment facility (Facility #22) at USDA Forest Service, Hawaii Volcanoes National Park, Kilauea Research Station, Quarantine Facility, Building 34, Volcano, HI 96718, subject to the conditions below.

1. 
- This permit is issued by the United States Department of Agriculture's Animal and Plant Health Inspection Service (APHIS). It conveys APHIS regulations and requirements for the material(s) listed on this permit. It does not reduce or eliminate your legal duty and responsibility to comply with all other applicable Federal and State regulatory requirements.
- The permit number or a copy of the permit must accompany the shipment.
- You must be an individual at least 18 years old, or legal entity such as partnership, corporation, association, or joint venture.
- You are legally responsible for complying with all permit requirements and permit conditions.
- The regulated material and shipping container(s) are subject to inspection by officials of Custom and Border Protection (CBP) and APHIS. CBP or APHIS officials may require the shipment to be treated, seized, re-exported, or destroyed (in part or whole). You will be responsible for expenses.

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- If you violate any applicable laws associated with this permit, you may face substantial civil or criminal penalties. We may cancel all current permits and deny future permit applications.
- Without prior notice and during reasonable hours, authorized Federal and State Regulators must be allowed to inspect the conditions associated with the regulated materials/organisms authorized under this permit.

2. The permit holder must:

- maintain a valid PPQ526 permit so long as the regulated materials/organisms are alive or viable,
- not assign or transfer this permit to other persons without APHIS PPQ authorization,
- maintain an official permanent work assignment, residence, or affiliation at the address on this permit,
- notify the Pest Permit Staff as soon as possible of any change in the permit holder's work assignment, residence, or affiliation,
- notify the Pest Permit Staff of the receipt of unauthorized and/or misdirected shipments of regulated materials/organisms,
- adequately mitigate environmental impacts resulting from unauthorized release of regulated materials/organisms and notify the Pest Permit staff immediately if one occurs,
- notify the Pest Permit Staff if the facility is damaged/destroyed or if you wish to decommission the facility,
- destroy all regulated materials/organisms prior to departure from the organization unless other arrangements are confirmed by the Pest Permit Staff.

Notifications to the Pest Permit Staff must be made via 866-524-5421 or pest.permits@usda.gov within one business day of the event triggering a notification.

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3. All packages for transport must minimally consist of both inner/primary and outer/secondary packages securely sealed so that both are effective barriers to escape or unauthorized dissemination of the listed materials/organisms. The inner/primary package(s) will contain all regulated materials/organisms and must be cushioned and sealed in such a way that it remains sealed during shock, impact, and pressure changes that may occur. The outer/secondary shipping container must be rigid and strong enough to withstand typical shipping conditions (dropping, stacking, impact from other freight, etc.) without opening.
4. After PPQ issues this 526 permit, you will need to request Red/White labels (PPQ Form 599) at least 5 days in advance of your shipping date. If you applied for your permit online using ePermits, you may request the labels using the My Shipments/Labels feature. Otherwise, send your request to Redandwhitelabelrequest@usda.gov. All email requests must come from the permit holder or designee. If requested by the designee, the permit holder must be copied on all requests. Specify the approved port as listed on the permit and the total number of labels needed. You may request additional labels the same way.
   Packages without labels on the exterior may be refused entry.

   Review label instructions at:
   https://www.aphis.usda.gov/aphis/ourfocus/planthealth/import-information/permits/plant-pes ts/or ganisms-shipping-requirements

   You are responsible for instructing your shipper to carefully follow these instructions. You are responsible for each import shipping label issued under this permit.
5. Upon receipt, open the package only in the approved containment facility identified above. Depending on the organism(s) or developmental stage, it may be necessary to open the package inside a cage (glove box or sleeve cage) or use other appropriate means that must prevent the organisms from escaping.
6. After separation of organisms regulated under this permit, along with any necessary host organisms and host plant parts, all other foreign biological material and substrate, including soil, and foreign plant material, if any, must be properly disposed of or destroyed immediately.

   Only authorized/permitted organisms may be retained as live organisms, plus any hosts and plant parts as needed for continued rearing and culture of the regulated organisms until transfer to lab-sourced material. Upon completion of isolations/transfers from imported material (i.e., soil, hosts) these imported materials must likewise be properly disposed of or destroyed immediately, as described above.

   Only secondary containers and packing materials suitable for re-use (such as coolers and icepacks) may be reused, and only after sterilization by autoclave, or with bleach or alcohol, etc., as per protocols established in the SOP's for this facility.



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7. This permit authorizes the importation and possession of live organisms of only those taxa/species listed under "Regulated Article" above, and not authorized under this permit are live cultures of other taxonomic groups from other hosts, or are from other source countries/continents, or received by way of any other permit, except as described below.

   In addition, this permit authorizes continued possession/continued curation of only the live organisms (identified and unidentified) cultured or stored by the permit holder which were imported as authorized on previous permits, of which this is a "renewal". All other such live regulated organisms must be kept under separate USDA APHIS permit, or devitalized.

8. The regulated organisms authorized for import under this permit are to be maintained only in the laboratory area approved for containment at the address indicated under the "Authorizations" above on this permit (CF 22). Any distribution or other removal of live organisms regulated under this permit from the designated area of Containment Facility Forest Service requires a separate prior authorization from APHIS PPQ.

   This permit does not authorize field release, interstate transport, field research, greenhouse work, or any other activities with the regulated organisms authorized for import under this permit outside of the containment facility.

9. All operations must be consistent with information submitted in association with this Containment Facility (CF #22) including the most recent Standard Operating Procedures (SOP's) submitted for the Facility, and any information submitted in association with the inspection of this Containment Facility. This includes, minimally, maintenance of restricted access to unauthorized persons of building and or approved containment areas (key, key card or code), and/or restricted access to unauthorized persons of growth chambers and other equipment (for example by lock) where organisms will be kept, as well as proper/prescribed maintenance of the Autoclave and/or other equipment used to devitalize or sterilize waste.

   The permit holder must insure that all persons working with these regulated organisms
   a) are trained in the importance of approved containment practices;
   b) follow the Standard Operating Procedures (SOP) established for the facility and filed with the USDA APHIS Pest Permit Evaluation Unit at the time of facility inspection; and
   c) are informed of these permit conditions and understand the requirement to adhere to these conditions and the SOP.

   The permit holder shall document such training or familiarization with these permit conditions and the SOP's for the facility, by having copies of both dated and signed/initialed by all persons handing the regulated articles, and have such documentation made available to USDA APHIS upon request.



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10. A separate authorization from USDA APHIS (a new PPQ 526 permit) is required for possession/maintenance of live regulated organisms received under this permit beyond the expiration of this permit. Otherwise, all regulated organisms received under this permit must be devitalized prior to expiration of this permit.

**END OF PERMIT CONDITIONS**

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RESTRICTED ANIMAL LIST (Part A) §4-71-6.5

| SCIENTIFIC NAME | COMMON NAME |
|---|---|
| FAMILY Noctuidae | |
| Antiblemma acclinalis | biocontrol agent, Koster's curse |
| Cucullia verbasci | biocontrol agent, common mullein |
| FAMILY Notodontidae | |
| Cyanotricha necyria | biocontrol agent, banana poka |
| FAMILY Oecophoridae | |
| Agonopterix ulicetella | biocontrol agent, gorse |
| FAMILY Pyralidae | |
| Ephestia kuehniella | moth, Mediterranean flour |
| Galleria mellonella | moth, greater wax |
| Pempelia genistella | biocontrol agent, gorse |
| FAMILY Riodinidae | |
| Euselasia chrysippe | biocontrol agent, Miconia |
| FAMILY Scythrididae | |
| Scythris gallicella | biocontrol agent, gorse |
| FAMILY Sesiidae | |
| Melittia oedipus | biocontrol agent, ivy gourd |
| Pennisetia marginata | biocontrol agent, blackberry |
| FAMILY Tortricidae | |
| Cryptophlebia ombrodelta | moth, litchi fruit |
| [ORDER Orthoptera] | |
| [FAMILY Gryllidae] | |
| [Acheta domesticus] | [cricket, house] |
| ORDER Thysanoptera | |
| FAMILY Thripidae | |
| Scolothrips sexmaculatus | thrips, sixspotted |
| Sericothrips staphylinus | biocontrol agent, gorse |
| CLASS Crustacea | |
| ORDER Decapoda | |
| FAMILY Alpheidae | |
| Athanas (all species in genus) | shrimp, anemone |

**M. TRACY JOHNSON**

Institute of Pacific Islands Forestry
Pacific Southwest Research Station
USDA Forest Service

P.O. Box 236, Volcano, Hawaii 96785
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email: tracy.johnson@usda.gov

## Education

Ph.D., 1995, Entomology, North Carolina State University
Thesis: The role of natural enemies in ecology and evolution of *Heliothis virescens* on transgenic plants.
M.S., 1990, Entomology, North Carolina State University
Thesis: Combined effects of genetically engineered host plant resistance and natural enemies on *Heliothis* populations in tobacco.
A.B., 1984, Biology, University of California - Berkeley

## Work Experience

Research Entomologist, Aug 2000-Present, USDA Forest Service, PSW, Institute of Pacific Islands Forestry
Biological control of weeds in Hawaiian forests, Insect ecology, Post-release monitoring of biocontrol, Non-target impacts of biocontrol, Plant-herbivore-enemy interactions
Junior Researcher, Mar-Aug 2000, Department of Zoology, University of Hawaii – Manoa
Examining population dynamics of the agricultural pest *Nezara viridula* under sublethal biological control by an introduced parasitoid.
Junior Researcher, Dec 1997-Feb 2000, Dept. Entomology, University of Hawaii - Manoa
Quantifying the off-target effects of biological control on the native Hawaiian koa bug, and surveying parasitism of an alien leafhopper invading native forests.
Fulbright Fellow, Oct 1996-Sep 1997, Internatl Centre of Insect Physiology and Ecology, Kenya
Assessing risk of African maize stemborers evolving resistance to transgenic maize expressing toxins of *Bacillus thuringiensis*.
Technician, May 1984 – Dec 1986, Biological Control of Weeds Lab, USDA-ARS, Albany CA
Field studies of native thistles and insects to measure nontarget impact of weevil introduced for biocontrol of weedy thistles; quarantine study of insects shipped from Greece in search for biocontrol agents against thistles.

## Recent Publications

Alfaro-Alpízar MA, Koster SJC, Johnson MT, and Badenes-Pérez FR. 2020. Description, biology, and impact of the fruit-feeding moth, *Mompha luteofascia* sp. n. (Lepidoptera: Momphidae), on *Miconia calvescens* (Melastomataceae) in Costa Rica. *Annals of the Entomological Society of America* 113: 30-39.

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Badenes-Perez, F.R., Castillo, A., and Johnson, M.T. 2014. Damage to *Miconia calvescens* and Seasonal Abundance of *Salbia lotanalis* (Lepidoptera: Crambidae) in Costa Rica. *Environmental Entomology* 43: 877-882.

Hughes, R.F., M.T. Johnson and A. Uowolo. 2013. The invasive alien tree *Falcataria moluccana*: Its impacts and management. Pp 218-223 *in* Wu, Y., T. Johnson, S. Sing, S. Raghu, G. Wheeler, P. Pratt, K. Warner, T. Center, J. Goolsby and R. Reardon (eds), Proceedings of the XIII International Symposium on Biological Control of Weeds.

Conant, P., J.N. Garcia, M.T. Johnson, W.T. Nagamine, C.K. Hirayama, G.P. Markin and R.L. Hill. 2013. Releases of natural enemies in Hawaii since 1980 for classical biological control of weeds. Pp. 230-242 *in* Wu, Y., T. Johnson, S. Sing, S. Raghu, G. Wheeler, P. Pratt, K. Warner, T. Center, J. Goolsby and R. Reardon (eds), Proceedings of the XIII International Symposium on Biological Control of Weeds.

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

November 29, 2022

Board of Agriculture
Honolulu, Hawaii

Subject: Request to Review the Request from Dr. Michael Melzer, University of Hawaii, to Shorten or Exempt the Duration of Quarantine for Coffee Leaf Rust-Resistant Coffee Plants, *Coffea* spp., Subject to Alternative Propagation or Import Procedures, Pursuant to Chapter 4-70-6, Hawaii Administrative Rules.

## I. Background:

The Federal Plant Protection Act currently preempts State and local regulation of plants within foreign commerce to control plant pests. Both coffee leaf rust, *Hemileia vastatrix*, and the coffee berry borer, *Hypothenemus hampei*, are considered regulated pests by the U.S. Department of Agriculture (USDA). Due to the federal preemption, the Department's one-year quarantine period cannot begin until the coffee plants clear the USDA quarantine.

Chapter 4-70, Hawaii Administrative Rules (HAR), Subchapter 4, Introduction of Coffee, regulates the importation of coffee plants and seed for propagation under permit and standard one-year quarantine. The one-year quarantine period is set by Chapter 4-70-6, HAR, Duration of Quarantine, which states "Unless otherwise specified for specific plants in subsequent subchapters, the duration of quarantine shall be one year". However, section 4-70-6, HAR also states that the Board of Agriculture (Board) "may exempt or shorten the period of quarantine under certain conditions of importation or propagation procedure." Dr. Melzer's request (Appendix A) for shortening or exempting the duration of quarantine is brought under this provision of 4-70-6, HAR.

For Board consideration, a request must contain certain substantive items, specifically: (1) the alternative propagation or import procedures that would justify the shortening or exemption of the one-year quarantine; (2) a statement of the petitioner's interest in the subject matter; and (3) a statement of the reasons in support of the proposed shortening or exemption of quarantine. Dr. Melzer's request appears to conform to these prerequisites for Board consideration.

EBI

## II. Alternative Propagation or Import Procedures justifying a Shortening or Exemption of Quarantine

Dr. Melzer proposes to exempt the one-year State of Hawaii quarantine period for coffee seeds imported into Hawaii from Costa Rica under the following:

- Coffee seeds were imported from Costa Rica under USDA Animal and Plant Health Inspection Service (APHIS) permit PCIP-21-00009 (Attachment 1) and Hawaii Department of Agriculture (HDOA) permit 22-03-O-P2052 (Attachment 2). These seeds arrived in Hawaii in July 2021 following inspection by USDA APHIS and HDOA Plant Quarantine.

- Upon arrival to Dr. Melzer's laboratory through October 2021, the coffee seeds were surface sterilized using a diluted sodium hypochlorite (commonly known as bleach) solution, then germinated in aseptic (tissue) culture at the University of Hawaii's Agrosecurity Laboratory, which is directed by Dr. Melzer. Any seeds showing microbial growth or possible contamination prior to surface sterilization were autoclaved.

- A one-year USDA APHIS quarantine period for these seeds began on November 1, 2021, after the last seeds were placed in aseptic (tissue) culture. Following germination and initial growth, seeds were transferred to soil-less medium in a USDA-approved facility. Plants were then required to be grown for at least six months in a soil-less medium to ensure that if there were any pests or diseases, they would have the time to express themselves and be detected by visual inspection.

- Throughout the quarantine period, all plants were routinely inspected by USDA inspectors quarterly and by HDOA inspectors monthly. Additionally, Dr. Melzer or his staff inspected plants daily, except for weekends and holidays.

- Once in soil-less medium, plants were inspected for disease bi-monthly by Dr. John Hu, a University of Hawaii plant pathologist. Approximately 7% of the plants (58/809 plants) were randomly selected and tested by established molecular (PCR-based) assays for *Xylella fastidiosa*, a bacterial pathogen that causes coffee leaf scorch. All plants tested negative with the results included with Dr. Melzer's request in Appendix A. Dr. Melzer noted in his request that there are two systemic diseases of coffee. Besides *X. fastidiosa,*

the other disease, which is a phytoplasma, has only been reported in Colombia and was not tested for.

- There are currently 809 plants and Dr. Melzer is awaiting the final disposition of the plants from USDA.

Dr. Melzer's Standard Operating Procedures (Attachment 3) provides additional details on the importation and quarantine procedures and record-keeping requirements listed above for the coffee plants quarantined in the Agrosecurity Laboratory facility.

## II. Summary of Proposed Shortening and Exemption of Quarantine

Dr. Melzer is requesting an exemption of the State of Hawaii's one-year quarantine of coffee plants should all conditions and requirements of the USDA one-year quarantine be met.

## III. Reasons that the Quarantine should be Shortened or Exempted

Dr. Melzer believes the combination of growth from seed, aseptic (tissue) culture, visual observation by Dr. Melzer and his staff, Federal and State inspection, and strict adherence to standard procedures designed to mitigate risk of pests or diseases over the one-year USDA APHIS quarantine period greatly minimizes the risk of introduction of exotic pests or pathogens. To date, these plants have showed no signs of foreign pests or pathogens, and the randomly sampled plants were all negative for the only known systemic pathogen of coffee that has been reported in Costa Rica, *Xylella fastidiosa*. Therefore, quarantining and observing the plants for another one-year quarantine period for the State of Hawaii will not further reduce the risk of pest introduction.

## IV. Staff Recommendation

Based on the inspection record and current findings of no pests by both USDA APHIS and the PQB throughout the one-year federal quarantine, the PQB recommends that the HDOA one-year quarantine period be exempted by the Board for those coffee plants that satisfactorily complete the USDA APHIS quarantine.

Respectfully Submitted,

BECKY AZAMA
Acting Manager, Plant Quarantine Branch

CONCURRED:

HELMUTH ROGG, Ph.D.
Administrator, Plant Industry Division

APPROVED FOR SUBMISSION:

PHYLLIS SHIMABUKURO-GEISER
Chairperson, Board of Agriculture

College of Tropical Agriculture and Human Resources
**Department of Plant and Environmental Protection Sciences**

UNIVERSITY of HAWAI'I® MĀNOA

## Petition Request for Exemption

Date: September 29, 2022

To: Board of Agriculture

Petitioner: Dr. Michael Melzer, Associate Researcher
University of Hawaii
3190 Maile Way, St. John 315
Honolulu, HI 96822
(808) 956-7887
melzer@hawaii.edu

Through: Hawaii Board of Agriculture
Attn: Phyllis Shimabukuro-Geiser, Chairperson
Hawaii Department of Agriculture

Petition Request:

Petition to exempt approximately 800 coffee leaf rust-resistant coffee plants from State of Hawaii quarantine requirements, allowing their release upon the completion of the USDA-APHIS one-year quarantine and associated permit requirements.

Discussion of Petition Proposal:

Prior to the arrival of coffee leaf rust (*Hemileia vastatrix*) into Hawaii in 2020, my Agrosecurity Laboratory has been involved in efforts to introduce potentially rust-resistant coffee germplasm into the State of Hawaii in a manner that reduces the risk of inadvertent introduction of exotic pests and pathogens. I am the Hawaii representative in the National Clean Plant Network and the National Plant Diagnostic Network, and have an understanding of the risks and invasion pathways involved with importing foreign germplasm for the purpose of propagation. The main risks of coffee germplasm introduction into Hawaii and efforts that have been taken to minimize these risks are as follows:

Viruses: To date, there are no reported systemic viruses of coffee. Coffee ringspot virus (*Dichoravirus coffea*) is the only described virus that naturally infects coffee. It is a non-systemic virus transmitted by *Brevipalpus* spp. mites that causes local lesions on coffee leaves and mites. At present, it has only been reported in Brazil and Costa Rica. Coffee ringspot virus is not transmitted by seed.

Bacteria: There are two systemic bacterial diseases of coffee. Coffee leaf scorch is caused by *Xylella fastidiosa* and symptoms include dieback of the leaf tips and outer perimeter. There are conflicting reports on whether *X. fastidiosa* is seed transmitted in coffee. To mitigate its introduction, visual surveys of plants and molecular diagnostics performed on symptomatic plants (or random plants if no symptoms are present) can be conducted.

Fungi: There are many fungal pathogens and saprophytes of coffee. By germinating coffee seeds in aseptic (tissue) culture, most fungi can be readily detected by contamination of the culture. Obligate biotroph fungi which will not be detected as a contaminant, require a living host to survive. Coffee leaf rust (*Hemileia vastatrix*), would present symptoms on leaf tissue within a one-year quarantine period and be readily observed by inspectors. Although coffee leaf rust is now established in Hawaii, it appears only a single race of the pathogen is present. It is critical to safeguard our coffee industry from invasion by additional races.

In 2018 I initiated efforts to import rust-resistant coffee germplasm from Costa Rica by submitting permit applications to USDA-APHIS and HDOA-PQ and establishing a quarantine facility on the University of Hawaii at Manoa campus for the introduction of such plants. By importing seed and germinating them in aseptic (tissue) culture, we were able to greatly reduce the risk of introducing exotic pests and pathogens. Following inspection by USDA-APHIS and HDOA-PQ, coffee seeds originating from Costa Rica arrived in my laboratory in July 2021.

Following the USDA-APHIS importation permit conditions, all plants were required to be grown outside of aseptic (tissue) culture for at least six months of the quarantine period. This is to allow any pathogens present to develop symptoms on these plants, which would be detected by visual inspection. These visual inspections were conducted as follows during the USDA-APHIS quarantine period:

Agrosecurity Laboratory staff: daily inspection on work days
Plant Pathologist (Dr. John Hu): twice a month once outside of aseptic culture
HDOA-PQ Inspectors: monthly
USDA-APHIS Inspectors: quarterly

No exotic pests or diseases were observed during any of these inspections. Furthermore, 42 coffee plants (or roughly 5% of the total plants in quarantine) were tested for *Xylella fastidiosa* using a PCR-based assay develop at the University of California at Davis. All plants tested negative, with the positive and negative controls used in the assay providing the expected results (results provided at end of petition).

The USDA-APHIS quarantine for these coffee plants started on November 1, 2021 and will terminate on October 31, 2022, should all permit conditions be met. If this petition proposal is not granted, the coffee plants will then have to enter a one-year HDOA quarantine, which was not anticipated at the start of the project. This second quarantine will be detrimental for the following reasons:

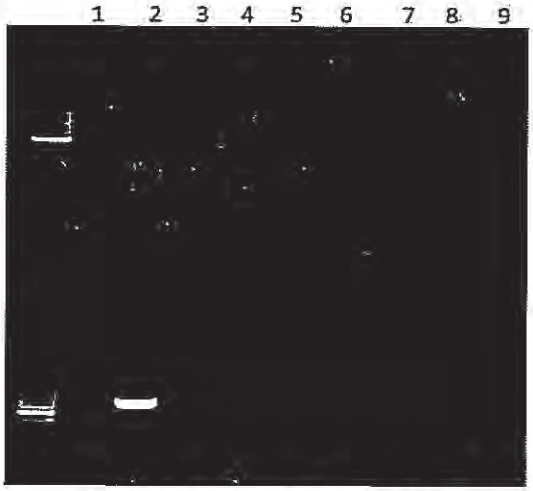
- It will not further safeguard against the introduction of any exotic pests or pathogens and deplete our already limited resources for the project
- This will add another year before we can evaluate the germplasm for its resistance to coffee leaf rust in Hawaii's growing conditions
- Most trees will not likely survive a second year in quarantine due to quarantine facility limitations (at the start of this project we were unaware there would be two consecutive quarantine periods)

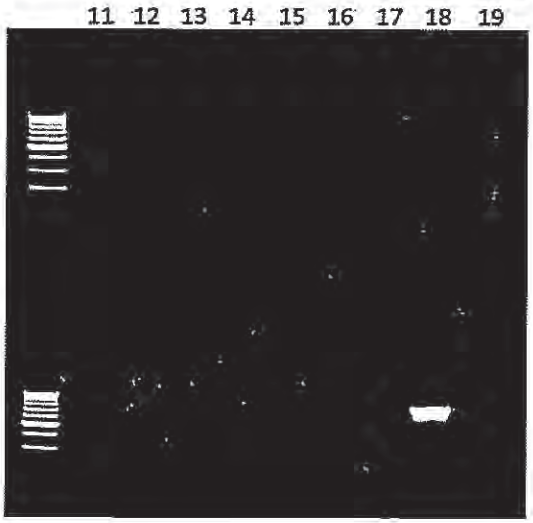
Petitioners Relationship

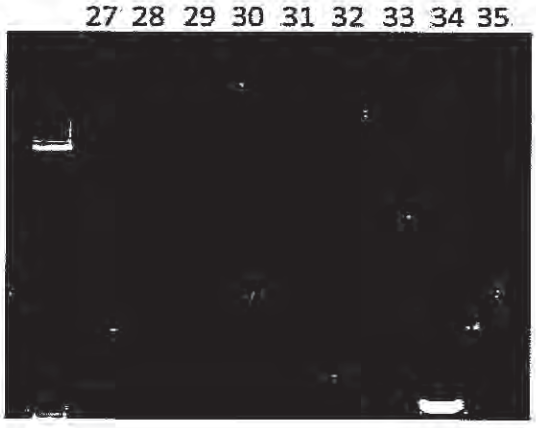
Dr. Michael Melzer is an Associate Researcher in Agrosecurity at the University of Hawaii. His program is focused on the prevention and detection of exotic pests and pathogens of importance to Hawaii and the USA. He has been involved in the National Clean Plant Network since 2013. The NCPN is a USDA-supported network that ensures growers receive high quality crop germplasm from foreign and domestic origins that is free of targeted pathogens. This includes performing therapy on germplasm to eliminate pathogens, maintaining pathogen-free germplasm in foundation block greenhouses, and distributing germplasm to nurseries, growers, and other stakeholders. Dr. Melzer has limited professional interaction and knowledge about Hawaii's coffee industry, but agreed to pursue this germplasm importation due to his knowledge on pest and disease pathways, and to ensure the importation be conducted in a manner that would limit risk to the industry.

Detection of *Xylella Fastidiosa* in Coffee Plants

| Set No. | Sample No. | Plant ID. | Detection of *X. fastidiosa* (YES/NO) | Detection method |
|---|---|---|---|---|
| 1 | 1 | 73 | NO | Conventional PCR |
| | 2 | 650 | NO | Conventional PCR |
| | 3 | 2020 | NO | Conventional PCR |
| | 4 | 2965 | NO | Conventional PCR |
| | 5 | 3034 | NO | Conventional PCR |
| | 6 | 3307 | NO | Conventional PCR |
| | 7 | 3362 | NO | Conventional PCR |
| | 8 | 3453 | NO | Conventional PCR |
| | 9 | 4005 | NO | Conventional PCR |
| | 10 | 4252 | NO | Conventional PCR |
| 2 | 11 | 2044 | NO | Conventional PCR |
| | 12 | 2746 | NO | Conventional PCR |
| | 13 | 190 | NO | Conventional PCR |
| | 14 | 3663 | NO | Conventional PCR |
| | 15 | 3940 | NO | Conventional PCR |
| | 16 | 3884 | NO | Conventional PCR |
| | 17 | 4096 | NO | Conventional PCR |
| | 18 | 4389 | NO | Conventional PCR |
| | 19 | 2422 | NO | Conventional PCR |
| | 20 | 3702 | NO | Conventional PCR |
| | 21 | 3842 | NO | Conventional PCR |
| | 22 | 2828 | NO | Conventional PCR |
| | 23 | 468 | NO | Conventional PCR |
| | 24 | 3979 | NO | Conventional PCR |
| | 25 | 4309 | NO | Conventional PCR |
| | 26 | 4277 | NO | Conventional PCR |
| 3 | 27 | 3920 | NO | Conventional PCR |
| | 28 | 2288 | NO | Conventional PCR |
| | 29 | 2828 | NO | Conventional PCR |
| | 30 | 3055 | NO | Conventional PCR |
| | 31 | 3483 | NO | Conventional PCR |
| | 32 | 3281 | NO | Conventional PCR |
| | 33 | 4309 | NO | Conventional PCR |
| | 34 | 3368 | NO | Conventional PCR |
| | 35 | 3295 | NO | Conventional PCR |



| | | | | |
|---|---|---|---|---|
| 36 | 59 | NO | Conventional PCR | |
| 37 | 2345 | NO | Conventional PCR | |
| 38 | 655 | NO | Conventional PCR | |
| 39 | 3296 | NO | Conventional PCR | |
| 40 | 3952 | NO | Conventional PCR | |
| 41 | 4074 | NO | Conventional PCR | |
| 42 | 715 | NO | Conventional PCR | |
| **Total** | 42 | | | |

**Positive Control amplicon @ 733bp**

United States Department of Agriculture

United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection & Quarantine
4700 River Road
Riverdale, MD 20737

# Controlled Import Permit to Import Restricted or Not Authorized Plant Material

## Regulated by 7 CFR 319.6

**This permit was generated electronically via the ePermits system**

| | | | |
|---|---|---|---|
| **PERMITTEE NAME:** | Dr. Michael Melzer | **PERMIT NUMBER:** | PCIP-21-00009 |
| **ORGANIZATION:** | University of Hawaii | **APPLICATION NUMBER:** | P588-220204-002 |
| **ADDRESS:** | 3190 Maile Way<br>St. John 315<br>Honolulu, HI 96822 | **DATE ISSUED:** | 02/05/2022 |
| | | **EXPIRES:** | 02/05/2023 |
| | | **FACILITY NUMBER:** | 2927 |
| **MAILING ADDRESS:** | 3190 Maile Way<br>St. John 315<br>Honolulu, HI 96822 | **FACILITY ACCOUNT:** | University of Hawaii |
| | | **RESEARCH CENTER:** | |
| | | **FACILITY NAME:** | |
| **PHONE:** | (808) 956-7887 | **FACILITY ADDRESS:** | 3190 Maile Way<br>Honolulu, Hawaii 96822 |
| **ALT. PHONE:** | | | |
| **EMAIL:** | melzer@hawaii.edu | **MAIL ADDRESS:** | 3190 Maile Way, St. John 315<br>Honolulu, Hawaii 96822 |
| **FAX:** | | | |
| **GROWING LOCATION:** | 3190 Maile Way<br>Honolulu, HI 96822 | **FACILITY CONTACT:** | Michael Melzer |
| | | **PHONE:** | 808-956-7887 |
| | | **ALT. PHONE:** | |
| | | **FAX:** | 808-956-2832 |
| | | **EMAIL:** | melzer@hawaii.edu |

**PORTS OF ENTRY:** HI, Honolulu

Under the conditions specified, this permit authorizes the following:

| Article(s) | Countries of Origin | Plant Parts | Grown in U.S. | Intended Use | Total Qty | Qty per Shipment | Number of Shipments |
|---|---|---|---|---|---|---|---|
| Coffea arabica | Costa Rica | Plantlets in vitro, Seed | Yes (Growth Chamber) | Seeds will be germinated and grown in quarantine for 365 days, then propagated for commercial production if healthy after quarantine period. In vitro plantlets will be grown in quarantine as described above | 10000 # seeds and/or cuttings | 10000 # seeds and/or cuttings | 1 |

## SPECIAL INSTRUCTIONS TO INSPECTORS

See permit conditions below

Inspect specifically for coffee berry borer (Hypothenemus hampei) in the seeds as well as other quarantine pests.,

## PERMIT CONDITIONS



| | |
|---|---|
| THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING PPQ HEADQUARTER OFFICIAL VIA EPERMITS. | DATE |
| Nathan Miller | 02/05/2022 |

USDA United States Department of Agriculture

This permit authorizes the importation of the listed articles, under the conditions specified below. A copy of this permit (including all conditions) must accompany all shipments authorized under this permit.

In addition to this permit, certain plants and plant products developed using genetic engineering is regulated under 7 CFR Part 340 and must be imported with a valid APHIS BRS Permit. Please call (301) 851-3935 or email Biotechquery@aphis.usda.gov for more information.

Receipt or use of foreign isolates or samples from countries under sanctions requires specific permission from the U.S. Department of Treasury. See http://www.treasury.gov/resource-center/sanctions/Programs/Pages/Programs.aspx for current country listings.

Any person who violates the Plant Protection Act (PPA) of the United States, individuals or corporations who fail to comply with these conditions and authorizations, or who forge, counterfeit, or deface permits may be criminally or administratively prosecuted and found guilty of a misdemeanor which can result in penalties, a one-year prison term, or both. Additionally, any person violating the PPA may be assessed civil penalties of up to $250,000 per violation or twice the gross gain or gross loss for a violation that results in the person deriving pecuniary gain or a pecuniary loss to another, whichever is greater. In addition, all current permits may be cancelled and future permit applications denied.

This APHIS-issued import permit only covers compliance with APHIS regulations and requirements. Therefore, this APHIS permit for the commodity to be imported does not reduce or eliminate the permittee's legal duty and responsibility to likewise comply with all other Federal and State regulatory requirements applicable to the commodity to be imported.

Some plants may be subject to regulations under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). To determine if a particular species is regulated under Appendix I, II, or III of CITES, please consult the appendices for CITES located at https://ecos.fws.gov

1. This permit authorizes the continued growth and monitoring of the Coffea arabica plants from Costa Rica to Michael Melzer, University of Hawaii in Honolulu, Hawaii for propagation in the approved facility 2927.

2. The person who is issued this PPQ Controlled Import Permit (CIP) and his/her employees or Agents, shall comply with the following permit conditions, which are deemed by the USDA APHIS Administrator to be necessary to prevent the dissemination and establishment of plant pests.

The permit holder must:
(a) be at least 18 years of age, have and maintain an address in the United States that is specified on the permit, and be physically present during normal business hours at that address during any periods when articles are being imported or moved interstate under the permit.
(b) notify the PPQ-CIP unit and local PPQ office if it is necessary to transfer permit ownership, or if there are changes in address or phone number.

Permit Number PCIP-21-00009

| THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING PPQ HEADQUARTER OFFICIAL VIA EPERMITS. | DATE |
|---|---|
| Nathan Miller | 02/05/2022 |

WARNING: Any alteration, forgery or unauthorized use of this Federal Form is subject to civil penalties of up to $250,000 (7 U.S.C.s 7734(b)) or punishable by a fine of not more than $10,000, or imprisonment of not more than 5 years, or both (18 U.S.C.s 1001)

USDA United States Department of Agriculture

(c) keep the CIP permit valid for the duration of possession of any imported plant material that is maintained under his/her possession until released from permit requirements as directed in this permit.
(d) maintain the regulated plant material only at the location specified in the permit, and dispose of the material as described in the permit conditions.
(e) notify the PPQ-CIP unit and local PPQ office, within 5 days, of any signs of potential disease symptoms such as wilting, dying or sudden death, or accidental release or destruction of regulated plant material.
(f) notify the PPQ-CIP unit and the local PPQ office of the receipt of any unauthorized plant material not listed on the permit.
(g) notify the PPQ-CIP unit and local PPQ office, within 5 days, if facilities which contain imported plant material are renovated, decommissioned for any reason or severely damaged by natural disaster.
(h) destroy all regulated plant material prior to the permit holder's departure from the approved facility unless other arrangements are confirmed by the PPQ-CIP unit, or unless a new permit is in place with a responsible party at the facility.
(i) Notification or Contact information for PPQ-CIP unit: Telephone (301) 851-2046 or (877) 770-5990 (Toll-Free Automated System); Fax (301) 734-5786; Email: controlled.import.permits@usda.gov.
(j) Local PPQ office: Honolulu Plant Inspection Station, Telephone (808) 834-3240

3. The plant materials must be selected from apparently disease-free and pest-free sources, free of federal noxious weeds and soil.

4. Inspection:
(a) All regulated plant material is subject to inspection at a PPQ Plant Inspection Station (PIS) at the time of entry to the U.S. If found infected/infested at the PIS, plant material may be destroyed, treated or returned to the exporter at the permittees expense.
(b) Imported plant material may be refused entry if coated or covered with fungicide, insecticide or any other treatments that obscure inspection.

5. Packaging and Shipping:
Packaging:
(a) The shipment must be accompanied by an invoice or packing list indicating its contents, which are clearly labeled, and include the permit number on the bill of lading.
(b) The imported plant material must be packaged in a sturdy closed container to prevent cross contamination and possible escape or introduction of plant pests during shipment.
(c) Imported materials must not be wrapped or otherwise packaged in a manner that impedes or prevents adequate inspection or treatment.
(d) Any packing material used in the consignment of the plant material must meet the requirements of § 7 CFR 319.37-11, and wood packing material used in the consignment must meet the requirements of § 319.40-3(b).

Shipping:
(a) Imported Coffea seeds should be chemically disinfested (e.g. with a 10 percent bleach solution) prior to packaging and shipping. The plant material should only be shipped using clean packaging not previously used.

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USDA United States Department of Agriculture

(b) A green-and-yellow-label is required. Labels will be provided in electronic form (PDF file) to the email address on file and must be printed in color. The green/yellow labels include detailed shipping instructions. Additional labels must be request at least five days in advance through ePermits using the My Shipments/Labels feature. Alternatively, email greenandyellowplantslabelrequest@usda.gov.
(c) Inform the exporter to use a) yellow/green permit label, and b) include a prepaid domestic airway bill in each package that directs the shipment to the final destination after the inspection at PIS. Alternatively, include your preferred courier account number that the PIS may use to generate a domestic airway bill to forward the shipment to the final destination.
(d) When shipping using a bonded carrier such as FEDEX, DHL, etc., attach the green/yellow label to the exterior of the package which will direct the package to the Honolulu PIS, 300 Rodgers Blvd., #58, Honolulu, Hawaii, 96819. DO NOT PLACE ANY OTHER DELIVERY ADDRESS ON THE OUTSIDE OF THE PACKAGE.
(e) Include a copy of the permit inside the package

6. Notification:
(a) Upon arrival of each shipment at the facility/location, the permittee shall notify, by email, the State Plant Health Director (https://www.aphis.usda.gov/planthealth/sphd) and State Plant Regulatory Official (http://nationalplantboard.org/membership/).
(b) The SPHD and the PPQ-Plants for Planting Office (Controlled.Import.Permits@usda.gov) shall be notified within 5 days of any abnormality noticed in the plant material or if the plant material dies. Dead plant material shall be double bagged and either autoclaved or incinerated after being recorded. Please contact the local PPQ office before autoclaving or incinerating.

7. Identity:
(a) Before being used, all imported plant materials must be labeled "Quarantined material", and stored in a locked cabinet or other secure location under the control of the permittee to prevent pilferage and unauthorized use.
(b) From the time of receipt and until the material is fully released by PPQ, the identity of all regulated material must be maintained throughout the permit period by visible labeling. The information on the label must contain the permit number, the genus, species and cultivar, country of origin and date of arrival. Each plant must be tracked and identifiable.

8. Monitoring and Record keeping:
(a) Standard Operational Procedures (SOP) on file must be followed. Any changes to the SOP must be first approved by the permit unit and the Hawaii SPHD office. Please email: controlled.import.permits@usda.gov.
(b)The plants are to be examined periodically by your designated plant pathologist for evidence of plant diseases.
(c) Records must be kept of all pest or diseases associated with the imported plants including abnormalities and death.
(d) Records of any pesticide applications must be maintained.
(e) The facility where the work is performed, and the imported plant materials are grown, are subject to unscheduled inspection by an APHIS representative during regular business hours. At the time of inspection, the importer must make records available for the imported material received under this permit.

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9. Disposal:
(a) After the imported material is removed from the shipping container, the container must be bagged and sterilized by autoclaving, destroyed by double bagging and incineration, or double bagged and sent to a municipal landfill.
(b) All discarded growing media, containers and plant material must be sterilized utilizing methods such as an autoclave, heat application of 121 degrees Celsius (250 F) for minimum of 30 minutes to the core of the treated material, chemical sanitation or incineration prior to disposal into a land fill or municipal sewage. Recommendations for time depending on the type of materials are enclosed below: https://sterilizers.com/autoclave-time-temperature-pressure-chart.html
(c) Unused imported plant materials must be devitalized/destroyed by sterilization using an autoclave or by double bagging and incineration. All related plant waste will be autoclaved or double bagged and incinerated prior to disposal or double bagged and sent to a municipal landfill.

10. Greenhouse/growth chamber requirements:
(a) Access to the imported material must be limited to you, the designated plant pathologist and those you authorize to care for the plants as indicated in the SOP.
(b) A pest management program must be in place to effectively control diseases and pests including arthropods, and insect vectors (e.g. aphids, thrips, and white flies) that potentially could transmit pathogenic organisms.
(c) Records must be kept of all pest or diseases associated with the imported plants that are discovered, as well as any pesticide applications that the plants receive.
(d) A dedicated greenhouse/growth chamber, or a physical barrier such as an insect screening/cage within an approved greenhouse must be used to keep plants isolated and pests from disseminating.
(e) The plant material from individual shipments must be separated physically from any domestic or other imported plants.

11. Quarantine evaluation:

The quarantine period for Coffea arabica is 1 year and may be extended based on plant pests found or pending evaluation as determined by the Permit Unit and the Hawaii SPHD office.

During the quarantine evaluation process:
(a) The seeds may be germinated within individual containers or utilize community containers (germinating sand banks or Magenta boxes) consisting of a pasteurized media. Deflasked plantlets may be grown as described above.
(b) The permittee shall uniquely identify the seed germination containers and maintain a record of the number of seeds planted and the number of plants that emerge per container. The young seedlings initiated in community germination containers should be removed and planted into individual containers (bags) of a previously non-utilized growing media (pasteurized soil and organic material). In vitro plantlets should similarly be removed and planted into individual containers (bags) of a previously non-utilized growing media (pasteurized soil and organic material). The seedlings and deflasked plantlets will be identified and a master record will be maintained which will link the plants and seed lots imported from respective country. The master record shall be maintained while this permit is valid and all information specific to



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the individual plants shall be recorded.
(c) The plants shall be maintained a minimum of 2 meters from any other plant material not regulated under this permit and shall be identified as a growing block with appropriate identification to reflect that the plants are under USDA Quarantine.
(d) Initially the plants shall be monitored and observed at a minimum on a weekly basis for the first 30 days for indications of plant pests and disease. Any indications of plant pests shall be identified and the results recorded within the master record. Notify the local PPQ office if a pest of concern is observed.
(e) After 30 days, the plants shall be routinely monitored for indications of plant pests. Plants identified to have pests shall be removed from the growing facility and destroyed to prevent any cross contamination to other plants within the study. The terminated plant shall have the destruction recorded within the master record and identify the plant pest associated with the plant. Plants that die without indications of a plant pest while under the study shall be evaluated and if possible, the cause of the termination shall be identified and documented in the master record. All growing media, discarded plants and containers associated with the terminated plants shall be sterilized prior to discarding.
(f) Prior to release, results of any diagnostic tests done on the imported material during the quarantine period must be available for review by APHIS.
(g) Some quarantine pests and pathogens include, but are not limited to:
- Arthropods: Hypothenemus hampei (syn. Stephanoderes hampei)
- Fungi: Colletotrichum coffeanum, Colletotrichum kahawae subsp. kawahae, Hemileia vastatrix
- Viruses: Coffee ringspot virus
- Bacteria: Xylella fastidiosa

12. Release:
(a) At the time of release, plants must be mature and have been grown at least 6 months outside of tissue culture, hardened off, and have a fully developed root system.
(b) Prior to release, neither the imported material nor increases may be distributed to any person or location unless approved in advance by the PPQ Controlled Import Permit Unit.
(c) A decision to grant or deny permission to release the plant material will be made at the conclusion of the required PPQ quarantine period. Release will require a written statement from the State Plant Health Director (SPHD) who will consult with the designated plant pathologist and both must inspect the site for compliance and plants for any pests. Request inspection and release by SPHD of your State: https://www.aphis.usda.gov/planthealth/sphd
(d) Alternatively, APHIS PPQ may prescribe additional treatments or measures, if warranted by pest and/or disease findings.

13. Permit validity and renewal:
(a) Controlled import permits are initially issued for one (1) year and may be renewed for up to an additional two (2) years.
(b) As part of the renewal package, the permittee must submit records that include propagation from the imported plant material, testing results, plant abnormalities and death, plant disposal information, and any pest or disease occurrences. Failure to renew to maintain permit validity may result in destruction of all plant material under and associated with the expired permit.

**END OF PERMIT CONDITIONS**

Permit Number PCIP-21-00009

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|---|---|
| Nathan Miller | 02/05/2022 |

ATTACHMENT 2

Permit No.: 22-03-O-P2052

Date: March 22, 2021

State of Hawaii
**DEPARTMENT OF AGRICULTURE**
Plant Quarantine Branch
1849 Auiki Street
Honolulu, Hawaii 96819

# IMPORT PERMIT

(Valid for one shipment within one year)

Permission is hereby granted to introduce the following commodity(s), in accordance with Chapter 4-70. Hawaii Administrative Rules of the Division of Plant Industry, Department of Agriculture, and the conditions listed below. (Each commodity must be inspected by a Plant Quarantine Inspector upon arrival before release.)

| Quantity | Unit | Commodity | Scientific Name |
|---|---|---|---|
| 7000 | each | Green coffee seeds | *Coffea spp.* |
| 500 | each | Tissue-culture coffee plants | *coffea sp.* |
| | | (NO SUBSTITUTIONS ALLOWED) | |

**INSTRUCTION To Shipper:** One copy of permit to accompany shipment to Hawaii.

Object of Importation: Coffee; Seeds and Tissue Culture Plantlets

**Name and Address of Shipper:** Cristal Vitro S. A., Mr. Marco Paez, Apdo, Postal 1084-2050 San Jose,

**Phone:**

**Name and Address of Importer:** University of Hawaii at Manoa, Mike Melzer, 3190 Maile Way St. John #315

Honolulu, HI 96822 **Phone:** 808-956-7887

CHIEF PLANT INSPECTOR

Phyllis Mimabukuro-Reiser

CHAIRPERSON, BOARD OF AGRICULTURE

FOR OFFICIAL USE ONLY

STATION _____ ARRIVAL DATE _____ FLIGHT/SHIP _____

WAYBILL NO. _____ INSPECTION DATE/TIME _____ INSPECTOR _____

REMARK _____

ATTACHMENT 2
Permit No.: 22-03-O-P2052
Date: March 22, 2021

## PLANT QUARANTINE BRANCH
## Permit Conditions

**Condition**

COFFEE TC andSeeds for Propagation conditions - final- Mike Melzer-UH-CTAHR-PEPS-P2052

# Permit Conditions for the Introduction of Propagative Coffee Plant Material and Seeds Requiring One Year Quarantine

Permit no. **22-03-O-P2052**

1. The restricted article(s), **Tissue cultured Coffee plants, seeds or other propagative parts, *Coffea (all species in genus)*, shall be used for propagation**, provided that the restricted article(s) complete a one-year quarantine in a Plant Quarantine Branch (PQB) approved quarantine facility under the supervision of the PQB. The restricted article(s) shall not be released from quarantine unless approved by the PQB chief in writing.

2. Importation of the restricted article(s) into the State from any location within the continent of Africa is prohibited due to the presence of coffee berry disease.

3. The restricted article(s) shall be quarantined at the approved site, **St. John Plant Science Laboratory, University of Hawaii, 3190 Maile Way, Honolulul, Hawaii 96822. Agrosecurity Laboratory Room 315 and Plant Tissue Culture Room 004 (D&E)**, a site inspected and approved by the PQB prior to importation. A site inspection and approval by the PQB chief is required prior to the movement of the restricted article(s) to another site.

4. The permittee, **Dr. Mike Melzer**, shall be responsible and accountable for the restricted article(s) imported, from the time of their arrival to their final disposition.

5. The restricted article(s) shall be maintained by the responsible person, **Dr. Mike Melzer, 3190 Maile Way, St. John Rm. 315, Honolulu, Hawaii 96822**, or by trained or certified personnel designated by the permittee.

6. The restricted article(s) may be imported in the follow manner:

   a. Restricted article(s) imported via cargo, passenger baggage, hand carried package or other similar means, shall be immediately presented to a PQB inspector upon arrival at the port of entry for inspection.

   b. All parcels containing the restricted article(s) imported via the United States Postal Services (USPS), United Parcel Service (UPS), Federal Express (FedEx) or other similar transportation means, shall be addressed to:

Hawaii Department of Agriculture
Plant Quarantine Branch
1849 Auiki Street
Honolulu, Hawaii 96819
Office Phone: 808-832-0566

7. Each shipment of the restricted article(s) shall be accompanied with the following documents:

   a. An original phytosanitary certificate issued by a government official certifying the shipment's origin and pre-entry treatments that the restricted article(s) have been subjected to;

   b. A copy of the valid PQB permit for the restricted article(s); and

   c. An invoice, packing list, or other similar PQB-approved document listing the scientific and common names of the restricted article(s), the quantity of the restricted article(s), the shipper, and the permittee for the restricted article(s).

8. The restricted article(s) shall be subjected to a PQB approved broad-spectrum insecticide and broad-spectrum fungicide prior to importation, to mitigate the risk of insect, disease and/or other pest infestation and subsequent introduction into the State.

9. At least four sides of each parcel containing the restricted article(s) shall be clearly labeled in plain view with "Live Plants" and "This Parcel May be Opened and Delayed for Agriculture Inspection", in 1/2" minimum sized font.

10. The restricted article(s) shall be subjected to the following upon entry into the quarantine facility:

    a. The restricted article(s) shall be dipped in Malathion solution per label prior to planting; and

    b. The restricted article(s) and media shall be drenched with a mixture of Ridomil and Tilt per label after planting

11. The restricted article(s) shall be subjected to post-entry inspections as deemed necessary by the PQB for the detection of any insect, disease, and/or other pest.

12. If a potentially detrimental insect, disease, or other pest is detected in or on the restricted article(s) upon inspection, the PQB chief may order the chemical

treatment or the destruction of any or all of the restricted article(s) imported under permit, including any associated planting materials.

13. The approved site, restricted article(s), and records pertaining to the restricted article(s) under permit may be subject to post-entry inspection by the PQB. The permittee shall make the site, restricted article(s), and records pertaining to the restricted article(s) available for inspection during normal business hours upon request by the PQB inspector.

14. During PQB inspections at the approved site, representative samples of the restricted article(s) or any other article directly related to the restricted article(s) may be taken for further examination to determine the presence of, and/or the identity of, any insects, diseases and/or other pests.

15. The permittee shall adhere to the use, facility, equipment, procedures, and safeguards described in the permit application as approved by the PQB Chief.

16. The permittee shall have a biosecurity manual available for review and approval by the PQB, at the time of the initial site inspection and any subsequent post-entry inspection(s), which identifies the practices and procedures to be adhered to by the permittee, to minimize or eliminate the risk of theft, or accidental release of the restricted article(s), including the risk of introduction and spread of insects, diseases, or other pests associated with the restricted article(s) into the environment. The permittee shall adhere to all practices and procedures as stated in this biosecurity manual.

17. The permittee shall immediately notify the PQB chief in writing under the following circumstances:

    a. Any theft, accidental release, disease outbreaks, pest emergence and/or mass mortalities involving the restricted article(s) under this permit.

    b. Any changes to the approved sites, facilities or containers used to quarantine the restricted article(s).

    c. If a shipment of the restricted article(s) is delivered to the permittee without PQB inspection and approval for entry into quarantine at the permittee's approved site. If a shipment of the restricted article(s) is delivered to the permittee without PQB inspection and approval for entry into quarantine, the permittee shall immediately transfer custody of the entire shipment, including the shipping container, to the PQB.

    d. The permittee will no longer import or possess the restricted article(s) authorized under this permit. In that event, the permit will be cancelled.

18. The permittee is responsible for all costs, charges, or expenses incident to the inspection or treatment of the restricted article(s) as provided in Act 173, Session Laws of Hawaii 2010, section 13, including, if applicable, charges for overtime wages, fixed charges for personnel services, and meals.

19. It is the responsibility of the permittee to comply with any applicable requirements of municipal, state, or federal law pertaining to the restricted article(s).

20. The permittee shall submit to the PQB chief a copy of all valid licenses, permits, certificates or other similar documents required by other agencies for the restricted article(s). The permittee shall immediately notify the PQB chief in writing when any of the required documents are suspended, revoked, or terminated. This permit issued by the PQB chief may be cancelled upon revocation, suspension, or termination of any license, permit, certificate or similar documents the permittee is required to have for the restricted article(s).

21. Any violation of the permit conditions may result in citation, cancellation of the permit, and enforcement of any or all of the penalties set forth in HRS §150A-14.

22. A cancelled permit is invalid and upon written notification from the PQB chief, all restricted article(s) listed on the permit shall not be shipped or moved interisland. In the event of permit cancellation, any restricted article(s) imported may be moved, seized, treated, quarantined, or otherwise disposed of, at the discretion of the PQB chief. Any expense or loss in connection therewith shall be borne by the permittee.

23. The permittee shall agree in advance to defend and indemnify the State of Hawaii, its officers, agents, and employees for any and all claims against the State of Hawaii, its officers, agents, or employees that may arise from or be attributable to any of the restricted article(s) that are introduced under this permit. This permit condition shall not apply to a permittee that is a federal or State of Hawaii entity or employee, provided that the State or federal employee is a permittee in the employee's official capacity.

College of Tropical Agriculture and Human Resources
**Department of Plant and Environmental Protection Sciences**

UNIVERSITY of HAWAI'I® MĀNOA

# STANDARD OPERATING PROCEDURES

## for Receiving Plant Material for Propagation: Coffee

Agrosecurity Laboratory

USDA Containment Facility #2927

August 2022

Michael J. Melzer, Assistant Researcher in Agricultural Security

3190 Maile Way, St. John 315
Honolulu, HI 96822

(808) 956-7887
(808) 436-9169 (cell)
(808) 956-2832 (fax)
melzer@hawaii.edu
www.agrosecurityhawaii.com

CONTENTS

# I. INTRODUCTION

## 1. Background information

Coffee has been grown for about 200 years in Hawaii. It was Hawaii's primary agricultural crop in the early 1800's and remains one of Hawaii's most important agricultural commodities with current annual production values of approximately $60 million. At present, there are over 800 coffee farms on 7,200 acres statewide, most of which are located in the South Kona district of the island of Hawaii. The largest coffee plantation is located on the south shore on the island of Kauai. As one of Hawaii's most visible and valuable commodities, ensuring Hawaii remains free of important pests and pathogens is critical in the maintenance and expansion of this industry. The recent introduction and spread of coffee berry borer and coffee rust are examples of how exotic pests and pathogens can place an entire industry in peril.

Coffee rust, caused by the fungi *Hemileia vastatrix* and *H. coffeicola*, is perhaps the most economically important disease of coffee, causing over $1 billion in damage in South and Central America, and the Caribbean. Coffee rust is present in most coffee growing regions around the world, and has finally reached Hawaii's shores. Recently, severe and well-publicized outbreaks of coffee rust have occurred in South and Central America which are decimating harvests. The fungus infects leaves and causes them to drop prematurely, decreasing the plant's ability to photosynthesize, weakening it and reducing bean yield the following year. In parts of the world where the rust was newly introduced, early efforts to eradicate the disease by rouging trees in a buffer zone surrounding the infection area, met with early success. However, because rust spores are spread by wind, the disease eventually reemerged, infecting trees downwind and the eradication efforts failed. So far, no successful eradication programs have ever been implemented once the rust has been found in a new location. It appears only one race of coffee rust has established in Hawaii, which will aid in successful management. However, it is vital that additional coffee rust races be prevented from entering Hawaii.

Another important disease of coffee in South America, particularly in Brazil, is coffee leaf scorch. Coffee leaf scorch is caused by the fastidious bacterium *Xylella fastidiosa*. *X. fastidiosa* causes disease in several crops including grapevine (Pierce's disease) and citrus (citrus variegated chlorosis) and is transmitted by sharpshooter insects. The glassy-winged sharpshooter (*Homalodisca vitripennis*), a vector of *X. fastidiosa*, is a recent introduction to Hawaii and potentially capable of spreading coffee leaf scorch through Hawaii's coffee farms. The *X. fastidiosa* strain that infects coffee is also capable of infecting citrus, thereby posing a threat to two of Hawaii's agricultural crops.

Coffee berry disease, caused by the fungus *Colletotrichum kahawae* subsp. *kahawae*, is another important disease that is currently found only in Africa. Unlike the more superficial anthracnose on coffee fruit caused by other *Colletotrichum* spp., *C. kahawae* subsp. *kahawae* does impact the

coffee bean and has a greater threat on production. A closely related pathogen, *C. kahawae* subsp. *ciggaro*, has been reported on tree tomato in Colombia.

This document has been developed by Principal Investigator of the University of Hawaii's Agrosecurity Laboratory (UH-AL). It describes in detail the objectives, activities, and day-to-day operation and safeguarding procedures for personnel to follow when handing and imported coffee material while in the UH-AL. It also includes contingency plans to manage emergencies. Copies of this document, upon U.S. Department of Agriculture Animal and Plant Health Inspection Service Plant Protection and Quarantine (USDA-APHIS-PPQ), will be distributed to the Hawaii Department of Agriculture (HDOA) and all personnel working on imported coffee plants for information and reference.

## 2. Major objectives and activities

### *2.1 Major objectives*

The major objectives at the UH-AL that specifically relate to coffee importation are 1) the maintenance of imported coffee material in both tissue and soilless media culture in a manner that minimizes the risk of the spread of exotic pests and pathogens that may be harmful to Hawaii's agricultural and natural ecosystems (quarantine); and 2) the visual and diagnostic surveillance of imported coffee material while under quarantine for the presence of targeted pests and pathogens (diagnostics). The quarantine period required by HDOA is 1 year.

### *2.2 Major activities*

The major activities at the UH-AL that specifically relate to this SOP are listed below:

- Secure storage of coffee propagules (seed and tissue culture explants)
- Aseptic tissue culture of coffee seed/seedlings
- Soilless culture of coffee seedlings (post aseptic tissue culture)
- Visual surveillance for exotic pests and diseases on seedlings during culture
- Serological and molecular diagnostics for exotic pathogens

## 3. Sites

This document covers the two sites where work will be performed as part of receiving coffee material for propagation:

i. UH-AL (St. John 315): tissue culture manipulation, diagnostics
ii. Tissue culture rooms (St. John 004): growth and observation of plants in tissue culture and soil-less medium

**4. Physical location and administration**

UH-AL: Located on the 3rd floor, Room 315, of St. John Plant Sciences Laboratory (St. John), a six-story building that consists of laboratories, offices, conference rooms, and classrooms. The UH-AL is administered by the Department of Plant and Environmental Protection Sciences, within the College of Tropical Agriculture and Human Resources.

Tissue culture rooms: Located on the ground (0) floor, Room 004, of St. John. The tissue culture rooms are administered by the Department of Plant and Environmental Protection Sciences and Department of Tropical Plant and Soil Sciences, within the College of Tropical Agriculture and Human Resources

## II. PHYSICAL AND CONTAINMENT STANDARDS

**1. Description of the sites**

St. John, which harbors the UH-AL and Tissue culture rooms, is a six-story concrete structure reinforced with steel bars and beams on the grounds of the UH-Manoa campus in Manoa valley, Honolulu. The building is bounded by Mid Pacific Institute to the north, Maile Way to the south, the Agricultural Engineering Institute to the west, and East-West Road to the east.

St. John is located approximately 5km from downtown Honolulu, 6km from Honolulu Harbor, and 15km from Honolulu International Airport, which represent the major ports of entry on the island of Oahu. The UH-Manoa campus is adjacent to the H-1 Interstate Highway, Honolulu's major freeway artery. The UH-Manoa campus is approximately 32km southeast of the closest commercial coffee plantation on the island of Oahu.

**2. Security**

The UH-AL and Tissue culture rooms are located on the UH-Manoa campus which has guard stations at entry points and houses the Department of Public Safety (http://www.manoa.hawaii.edu/dps/), a security service that polices the campus 24 hours a day, 7 days a week, 365 days a year. St. John, which houses the UH-AL, and Tissue culture rooms, has five entry doors that are locked between 5pm and 7am as well as weekends and state holidays.

**3. Demarcation and description of the sites**

UH-AL: A multi-purpose laboratory, with six rooms all under the supervision of the Principal Investigator. The walls of the UH-ACL are cement and drywall, the floating ceiling is composed of acoustic tile or concrete, and the floor is a ceramic tile on concrete. The walls and ceiling of the main laboratory are painted in an off-white latex paint, and the floor tile is in a yellow mosaic. The two entry doors into the UH-AL are wood and are closed and locked when the lab is unoccupied.

Tissue culture rooms: Consists of an ante room (004) that leads into six individual tissue culture rooms (004A-F). For this SOP, 004D and 004E will be utilized. The ante room is accessed by a wooden door that is closed and locked at all times when not occupied. The individual tissue culture rooms are repurposed cold storage units with heavy metal doors and concrete floors, walls, and ceilings. The walls and ceiling are painted a pale yellow color and the floor is unfinished. Access to the individual tissue culture rooms is restricted by a padlock on the entry doors.

## III. EQUIPMENT STANDARDS

### 1. Benches, tables, and other furniture

UH-AL: Equipped with five fixed laboratory benches located in the middle and perimeter of the main laboratory. Chairs with an impermeable covering are used at these tables. Wooden cabinet systems with glass doors provide storage.

Tissue culture rooms: Equipped with mobile wire racks and small tables.

### 2. Waste disposal

UH-AL: Has trash bins lined with black garbage bags for non-hazardous and non-biological wastes. This waste is collected daily by custodial staff. The red bins with closing lids are lined with red autoclave bags are used to store biological wastes. When full, this biological waste is autoclaved prior to disposal by laboratory staff trained on autoclave use. Two Steris Amsco Renaissance (models 3023 and 16VS) autoclaves are located in a room (St. John 311) adjacent to the UH-AL. These autoclaves are regularly serviced and are evaluated using a commercial integrator test on a monthly basis. Standard conditions are 121 $^{o}$C at 15 psi for at least 20 min, with duration dependent on volume and density of the material to be devitalized.

Tissue culture rooms: No waste disposal at this location. Waste will be collected in red autoclave bags and brought to UH-AL for proper disposal.

### 3. Liquid waste sterilization

Liquid waste containing biological materials are either autoclaved in glass containers as described above and disposed down the sink, or are treated with bleach (approximately 0.5% final concentration of sodium hypochlorite) for 30 min and disposed down the sink. All non-biological liquid waste is disposed of by certified personnel following UH Environmental Health and Safety Office (EHSO) guidelines.

### 4. Biosafety cabinet

The UH-AL has a Baker SterilGARD biosafety cabinet that is annually certified. This biosafety cabinet is used to perform any manipulations on foreign plant material or materials with the potential of harboring exotic pests or pathogens.

## IV. OPERATIONAL STANDARDS

### 1. Containment Director

All work conducted at the UH-AL is under the direction of the Principal Investigator/Containment Director:

Name: Michael Melzer
Address: 3190 Maile Way, St. John 315, Honolulu HI, 96822
Telephone: (808) 956-7887
Email: melzer@hawaii.edu

### 2. Responsibilities of the Containment Director

The UH-AL-Containment Director (UH-AL-CD) is responsible for the physical and operation integrity of all work conducted at the UH-AL and other locations where the project is undertaken. He is responsible for all foreign plant specimens that enter, are retained, and leave the locations. The UH-AL-CD is also responsible for ensuring any staff working with the plant specimens are properly trained in their procedures, all permit conditions, EHSO and Biosafety requirements, and are compliant with this SOP. The UH-AL-CD is responsible for keeping accurate records for regulatory agencies, cooperators, and any other relevant stakeholders. The UH-AL-CD is responsible for maintaining the cleanliness of the facility, and reports all SOP infractions and non-compliant equipment to USDA-APHIS-PPQ.

### 3. Plant Pathologist

A Plant Pathologist of record will be responsible for bi-monthly visual inspections of all regulated plants upon their transfer to soilless media. In the event that the plant pathologist cannot conduct the weekly inspection, a substitute plant pathologist designated as qualified by the Plant Pathologist will conduct the inspection. Data to be recorded will include i) date and time of inspection; ii) the number of dead plants or those displaying symptoms of a disease of regulatory importance; iii) and detailed photographs of the affected plants. This information will be recorded as hard and electronic copies (see attachment 10 for hard copy example) and available to regulatory authorities upon request.

### 4. Signage

The entrance to the UH-AL provides signage on biosafety and contact information. The Tissue culture rooms are identified with the sign “Authorized Personnel Only”. In addition, a reminder “This door must always be kept closed”. The names and contact information of the UH-AL-CD is also posted on the entrance doors to these facilities.

**5. Accessing the facilities**

Only authorized personnel are allowed entry. Visitors, collaborators, and maintenance personnel are allowed entry only when escorted by authorized personnel and must be briefed on the procedures and risks associated with any materials that may be contained in the facility. A log book will be maintained in the location where the coffee is grown to document visitors and activities conducted by authorized personnel.

a) The UH-AL-CD and/or authorized personnel are required to inform visitors to the activities in the UH-AL and Tissue culture rooms when active manipulation of plant material associated with the permit is undertaken.

b) Visitors and authorized personnel entering the UH-AL and Tissue culture rooms are required to wear personal protective equipment (PPE) as determined by the EHSO. This typically includes a lab coat and safety glasses. Authorized users have keys or passcodes required to open entry doors.

c) Visitors and authorized personnel are to return any PPE upon leaving any of the facilities.

**6. Sanitation**

a) Miscellaneous articles and equipment used in plant tissue culture will be sanitized by thorough flaming of the item using a Bunsen burner or heated glass beads when possible. Items that cannot be sanitized in this manner will be autoclaved as described in **III 2. Waste disposal**. Surfaces in the UH-AL will be decontaminated with either a freshly made 10% bleach solution (~0.5% sodium hypochlorite) followed by 70% ethanol, or with the use of a commercial disinfectant.

b) Equipment, tools, and miscellaneous items are generally not to be removed from any of the facilities. In the event that an item needs to be removed, separate procedures are to be followed, depending if there has been any evidence of an exotic pest or pathogen present on the permitted plant material.

*When there is no evidence of exotic pests or pathogens*

i) No active manipulation of the permitted plant material shall occur at the time of object removal.

ii) The UH-AL-CD will be informed of object removal.

iii) If the object is uncomplicated with no potential hiding places, it can be removed with a thorough visual inspection for exotic pest species. More complex items should be disassembled and thoroughly inspected to ensure no exotic pests are present.

*When there is evidence of exotic pests or pathogens*

i) No active manipulation of the permitted plant material shall occur at the time of object removal.

ii) The UH-AL-CD will be informed and participate in object removal.

iii) USDA-APHIS-PPQ will be contacted for guidance. Simple items must be thoroughly inspected and wiped down with a disinfectant such as 10% bleach solution (0.5% sodium hypochlorite) followed by a 70% ethanol solution. If possible, more complex items must be double-bagged, removed from the location and either incubated at 50$^{o}$C or -20$^{o}$C for at least 24 hours prior to unpacking. Alternatively, the equipment can be fumigated within the bag with an appropriate pesticide prior to unpacking.

c) Maintenance within any of the facilities outside the scope of authorized personnel will be conducted by University of Hawaii's Office of Planning and Facilities. Any maintenance needs will be brought to the attention of the UH-AL-CD, who will submit an electronic maintenance request through departmental support staff. It will be noted in the request that there is a need for the maintenance worker(s) to consult with the UH-AL-CD prior to any entry or maintenance activity. The UH-AL has tools, ladder, and other equipment to facility many repairs. The use of these tools, which will remain in the UH-AL once repairs are completed, are preferred over the entry and removal of outside tools.

d) In the event of fire: The UH-AL is equipped with a fire extinguisher, fire alarm, and emergency exit, but there is no sprinkler system. In the event of a fire within the facility, the following steps are to be followed.

i) Personnel should immediately try to put out the fire with the fire extinguisher if possible. If the fire cannot be extinguished in a safe manner, all personnel should immediately exit the facility. The first person to exit should operate the closest fire alarm.

ii) If personnel cannot safely exit the facility, perhaps due to a fire in the vestibule or doorway that cannot be extinguished, an emergency call to campus security x66911 should be placed. The occupants should remain low to the floor to reduce smoke inhalation.

iii) No personnel are to return to the UH-AL until the fire department officials declare the area safe.

In the event of a medical emergency: A first aid kit is present next to the doorway of the main entrance to the UH-AL and can be used for minor cuts, burns or abrasions. An emergency eyewash station is present in the UH-AL sink area for removing contaminants from eyes. Another emergency eyewash station and body shower are located in the hallway. For chemical exposures, consult the MSDS sheet for rinse times, or rinse for at least 15min. For more serious medical emergencies, call x66911 for an ambulance. For less urgent situations, proceed to the University Health Services building at 1710 East-West road. All medical emergencies must be reported to the Containment Director when appropriate. The Containment Director is responsible for reporting serious incidents to the University of Hawaii Environmental Health and Safety Office using the "Biological Laboratory Incident Report" form attached to this SOP.

Utility Outage: In the event of a major utility outage, notify the PEPS Department Secretary and the CTAHR Office of Planning and Management Systems.

**7. Cleaning and de-infesting the facility**

If an exotic pest or pathogen capable of surviving or remaining viable outside of the plant host is identified on the permitted plant materials, the materials must be sanitized as described above and the facilities cleaned and de-infested. Work surfaces in the UH-AL are to be sanitized with either a freshly made 10% bleach solution (~0.5% sodium hypochlorite) followed by 70% ethanol, or with the use of a commercial disinfectant. Walls, floors, and benches in the Tissue culture rooms will be sprayed with a freshly made 10% bleach solution (~0.5% sodium hypochlorite) using a backpack sprayer and appropriate PPE. After 30 min, a second application will occur. After 30 min, the surfaces will be sprayed with a water. Additional water applications will occur as needed.

**8. Opening and handling packages from foreign sources**

Valid copies of the appropriate USDA-APHIS-PPQ 588, HDOA PQ-7 and University of Hawaii BSP-2 must accompany all packages. If the package does not have evidence of USDA-APHIS inspection, the Honolulu Plant Inspection Station will be immediately notified by phone for guidance (808) 834-3240. Date, tracking number, and time of arrival will be entered onto a hard copy of the USDA-APHIS-PPQ 588 permit and filed into the "Permits" binder in St. John 315A. All unpackaging will take place in a certified biosafety cabinet located in St. John 315. The packaging will be photo-documented at all steps of the unpackaging process to ensure containment integrity. All packaging materials will be placed in autoclave bags for devitalization. Plant materials will be stored in double-containment in a dedicated, labeled, and locked storage container or incubator at appropriate temperature (typically 4$^{o}$C up to room temperature). At no point will samples come in contact with, or be inoculated onto, living plants, cell cultures, etc.; At no point will there be any attempt to regenerate or allow replication of any plant pest or pathogen present in the plant material.

**9. Culturing of Regulated Plant Materials**

**Coffee Seeds**

1. Upon arrival, and in a biosafety cabinet, each volume of coffee seeds will be incubated in 10 volumes of deionized water containing at least 0.5% v/v sodium hypochlorite and 0.5-1.0% Tween 20. The seeds will be gently shaken for 15 min.
2. The solution will be drained and the seeds will be washed twice with sterile deionized water, 5 min per wash.
3. The seeds will be placed in 4" tissue culture vials (1 seed/vial) or magenta boxes (up to 4 seeds/box) containing MS-based coffee germination medium. Vials will be capped, sealed with parafilm or cloth surgical tape, and given a unique identification code associated with

the plant. An electronic spreadsheet will be used to monitor the progression of each seed/vial through the tissue culture process.

4. Seeds will be germinated in the Tissue culture rooms with monthly medium transfers (or as needed), and transferred to larger vessels if needed. All tissue culture manipulations will occur either in the biosafety cabinet or laminar flow hood located in UH-AL.
5. After sufficient foliar and root growth (perhaps 2-6 months), the seedlings will be transferred to sterilized soil-less medium in 1.5 x 8" grow cones. This transfer will occur in the UH-AL and plants will be placed in large, sealed bags when transported back to the Tissue culture rooms. Grow cones will be placed in racks (98 or 200 plants/rack) and maintained using sterile irrigation water. Any excess irrigation water will be collected and devitalized by autoclaving.
6. Plants will be visually examined by the Plant Pathologist (or their designee) on a bi-monthly basis for the presence of disease symptoms (see attachment 11).

***In vitro* (tissue culture) Plantlets or Explants**

1. As above for "Coffee Seeds", starting at step 5. Plantlets are inspected upon arrival for any contaminants/microbial growth on the media. Those with such contamination are devitalized by autoclaving. Those without contamination are maintained in tissue culture until of sufficient size to transfer to soil-less medium.

**10. Pest monitoring and control**

To prevent the establishment of arthropod pests, yellow sticky cards will be placed wherever coffee plants are grown. These traps and the presence of pests on foliage will be visually monitored by authorized personnel for the presence of potential pathogen vectors or herbivorous arthropods. The presence of such vectors or arthropods will result in the application of an appropriate insecticide or acaricide following label instructions.

**11. PPQ Regulatory Requirements**

To be provided by USDA-APHIS-PPQ

## V. DIAGNOSTICS FOR EXOTIC COFFEE PATHOGENS

### 1. *Xylella fastidiosa*, coffee ringspot virus, and *Colletotrichum* spp.

Any plants growing in tissue culture or soilless media that are displaying symptoms of coffee leaf scorch caused by *Xylella fastidiosa*, will samples will be tested for this pathogen. If no symptomatic plants are observed, we will randomly select and test 5% of the coffee seedlings in culture. From these samples, DNA will be extracted using either a commercial kit or other standard DNA extraction procedure (such as protocols utilizing Trizol® or CTAB). The DNA will undergo

conventional and/or real-time PCR assays for the presence of X. fastidiosa. The permit holder has DNA of *X. fastidiosa* that will serve as a positive control for the assays.

Samples from tissue culture plants suspected of having coffee ringspot will have RNA extracted using either a commercial kit or other standard DNA extraction procedure (such as protocols utilizing Trizol® or CTAB). An RT-PCR assay using primers targeting the Brazilian isolate of Coffee ringspot virus will be used. PCR reactions will be visualized by agarose gel electrophoresis and any PCR products close to the size of the expected amplicon will undergo sequencing for further identification.

Any plants testing positive for these pathogens or display symptoms of coffee leaf rust or infection by *Colletotrichum* species (specifically *C. kahawae* subsp. *kahawae*) will be immediately devitalized by autoclaving. The permit holder will then alert relevant state and federal regulatory agencies for further guidance.

## VII. ATTACHMENTS

1. Geographic location of the UH-AL in relation to the major airport (HNL) and seaport (HH) of Oahu

2. St. John Plant Science Laboratory Situation Map on the UH Manoa campus

3. UH-AL Floor Plan

4. UH-AL Entry Doors and Biosafety Cabinet

5. Plant Tissue culture rooms Floor Plan and Entry Door

6. Tissue culture rooms 004D and 004E

7. Tissue culture and soil-less media growth systems

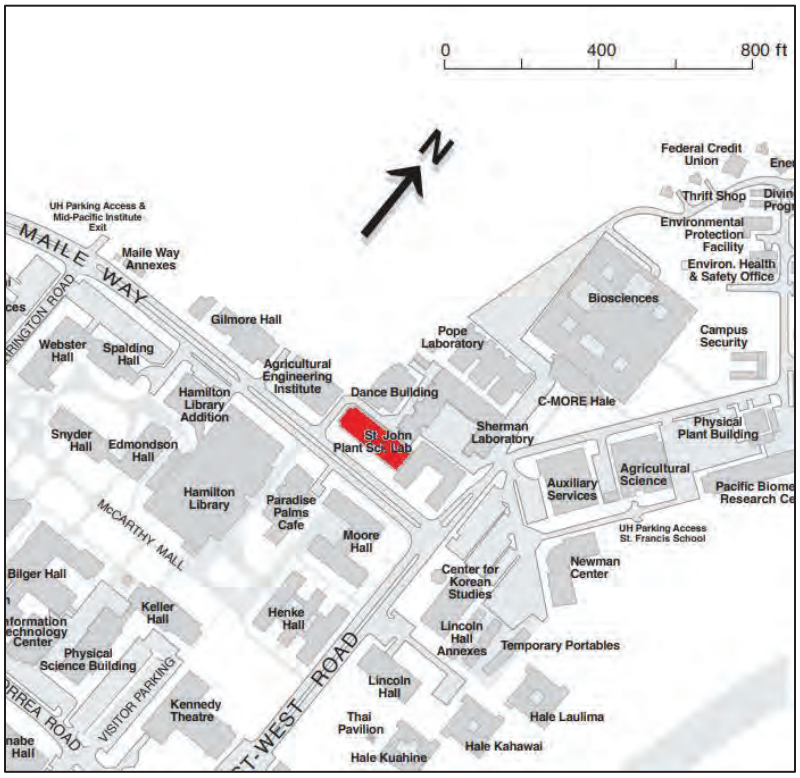
8. Biological Incident Report

9. Visual Inspection Report

10. Staff Acknowledgment Template for Approved SOPs

**Attachment 1.** Geographic location of the UH-AL in relation to the major airport (HNL) and seaport (HH) of Oahu

**Attachment 2.** St. John Plant Science Laboratory Situation Map on the UH Manoa campus




**Address:**

St. John Plant Science Laboratory
University of Hawaii
3190 Maile Way
Honolulu, HI 96822

Agrosecurity Laboratory
Room 315

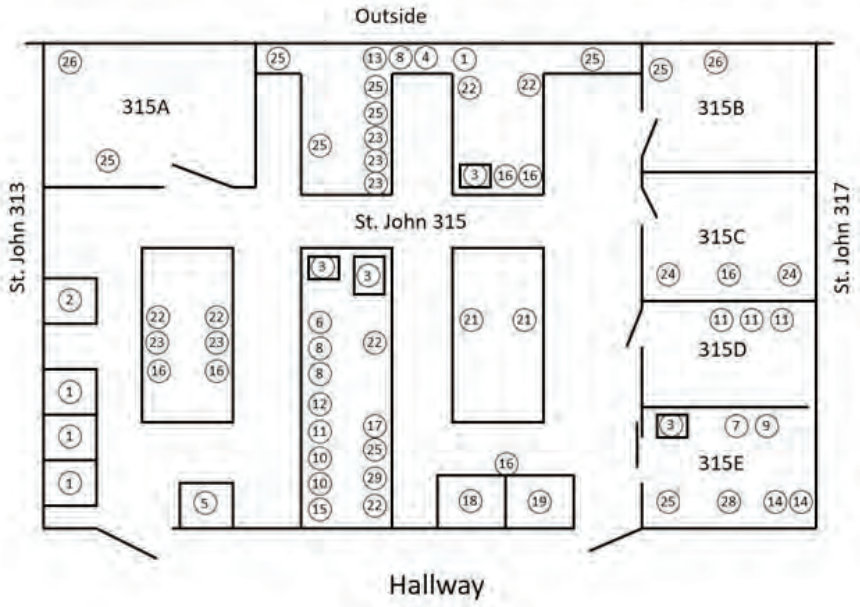
Plant Tissue Culture
Room 004 (D&E)

**GPS:**
21.301693, -157.815370

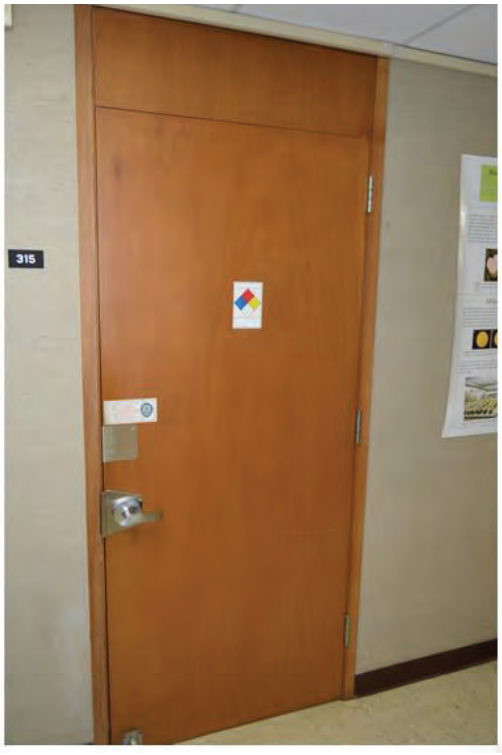
**Attachment 3.** UH-AL Floor Plan

# Agrosecurity Laboratory
## St. John Room 315 Floor Plan & Equipment List

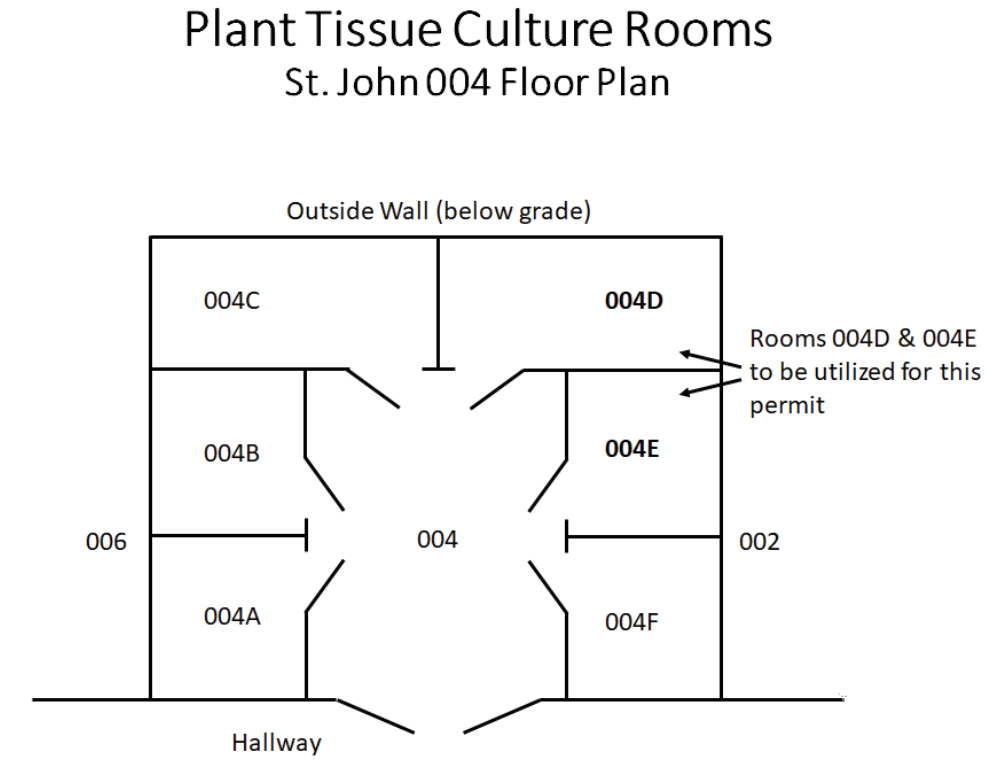
1. Refrigerator/Freezer
2. Floor Centrifuge
3. Sink
4. Plate washer
5. Fume Hood
6. Water Bath
7. Electrophoresis Equipment
8. Incubator/Shaker
9. Microwave
10. Analytical Balance
11. Stir Plate
12. pH Meter
13. qPCR Thermocycler Station
14. Tissue Disruptor
15. Tabletop Incubator
16. Vortexer
17. Spectrophotometer
18. Laminar Flow Hood
19. Biological Safety Cabinet
20. Drying oven
21. Microscope
22. Microcentrifuge
23. Thermal cycler
24. PCR reaction assembly hood
25. Computer
26. Printer
27. -80°C freezer
28. Gel documentation system
29. Nanodrop

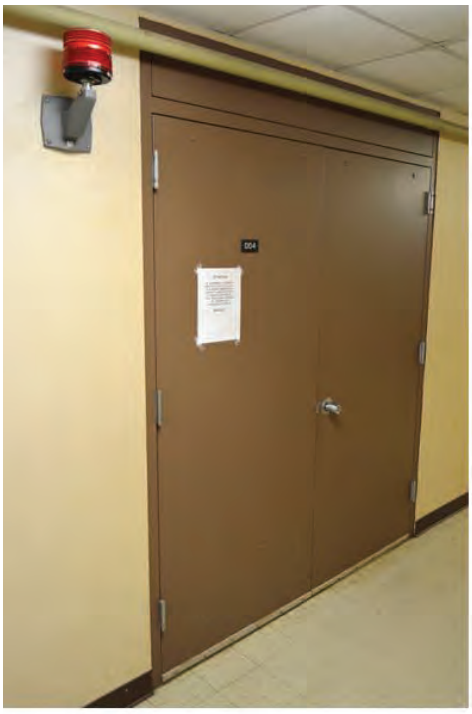


**Attachment 4.** UH-AL Entry Doors and Biosafety Cabinet


**Attachment 5.** Tissue culture rooms Floor Plan and Entry Door




Although St. John 004 is locked at all times, the alarm system is for climate control of the rooms, not security.

**Attachment 6.** Tissue culture rooms 004D and 004E

## Plant Tissue Culture Rooms
### St. John 004D


St. John 004D is locked at all times and has restricted entry


HEPA Filters (with blue pre-filters) installed over exhaust vent on Feb 11 2020

# Plant Tissue Culture Rooms
## St. John 004E


St. John 004E is locked at all times and has restricted entry


HEPA Filters (with blue pre-filters) installed over exhaust vent on Feb 11 2020

**Attachment 7.** Tissue culture and soil-less media growth systems

Short and tall tissue culture tubes in a standard rack (36 tubes/rack)

Ray Leach Cone-tainers (aka grow cones) (1.5 x 8”) in rack (98 or 200 cones/rack depending on model).

**Attachment 8.** Biological Incident Report

## BIOLOGICAL LABORATORY INCIDENT REPORT

University of Hawaii's Biological Laboratory Incident Report should be completed as soon as possible after a lab accident involving an employee or student's injury or "near miss."

Lab accidents, incidents or near misses do happen. Reporting the incident within 24 hours after the incident is required to help prevent recurrence, prevent others from potentially being injured and because facts can be easily forgotten as time passes. Facts gathered within the first 24 hours are still fresh in your memory. Over time, facts or what we think are the facts may be lost or changed or altered by opinions of others as we speak about the incident. "Facts" may also change to protect a coworker.

**Notification.**
Any lab accident resulting in personal injury or illness:

- Call 911 if necessary
- Contact UH Animal Welfare and Biosafety Program, Research Compliance Director, Mr. Norman Magno at 956-6446, 956-9061 or nmagno@hawaii.edu
- Contact the appropriate school/college Human Resources
- The Supervisor should also contact the appropriate school/college HR and Risk Management.

**Completing the Biological Laboratory Biological Laboratory Incident Report form**

**Section 1.** To be completed by the person reporting the incident.
The person must document

1. The name of the person completing the form
2. The name of the supervisor notified, including the date and time of notification
3. A detailed description of the incident.

This section must be completed within 24 hours of the incident and submitted to supervisor

**Section 2.** To be completed by the Supervisor.
Within 24 hours of receiving the Incident Response Form or notification of the incident, the supervisor must document his/her assessment of the incident and corrective actions to mitigate and prevent future recurrences.
The supervisor must also notify the Lab Director, UH IBC , and the Animal Welfare & Biosafety Program Office (AWBP) at 956-9061..

**Evaluation**

A review of the incident by the Principal Investigator, Lab Director, AWBP Office and the Supervisor to determine the effectiveness of the corrective actions taken must be completed and documented within 5 days of notification. If necessary, follow-up must be done in a timely manner.

# BIOLOGICAL LABORATORY INCIDENT REPORT

TO BE COMPLETED BY PERSON INVOLVED IN THE INCIDENT. Submit completed form to your supervisor within 24 hours of the incident.

| | | | |
|---|---|---|---|
| COLLEGE/SCHOOL/DEPARTMENT | | | |
| LOCATION | | DATE OF OCCURANCE | TIME AM PM |
| PERSONAL INJURY YES NO | | WORK RELATED INJURY YES NO | |
| PROPERTY DAMAGE YES NO | | MEDICAL ATTENTION/TREATMENT YES NO | |
| REPORTED BY | | DATE REPORTED | TIME AM PM |
| PERSON NOTIFIED | | DATE REPORTED | TIME AM PM |

**DESCRIPTION OF INCIDENT.** Describe in detail what took place. Include the date and time of the incident, the location the incident occurred, list all witnesses and others who were involved. (What personal protective equipment was used? What equipment was being used?)

**ASSESSMENT.** Explain the possible cause of the incident (Why did this occur?); What factors were involved? List any pertinent information.

**PREVENTIVE MEASURES.** Describe the measures that will be taken or will be taken to prevent reoccurrence.

| Reviewed by: | Print Name and Sign | | |
|---|---|---|---|
| Supervisor: | | Date: | |
| Principal Investigator: | | Date: | |
| Lab Director | | Date: | |

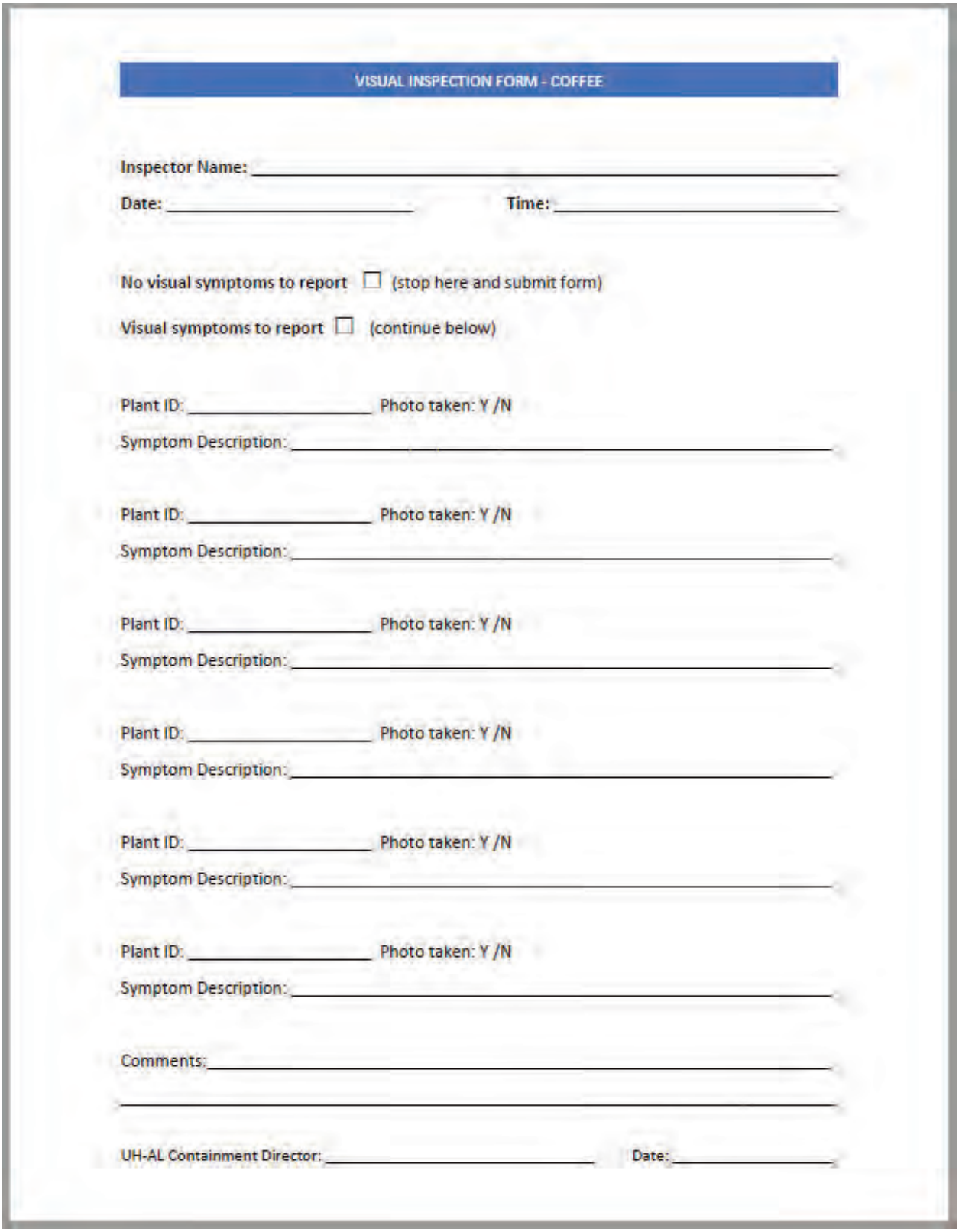
## Lab Biological Laboratory Incident Report
## Supervisor's Assessment

**TO BE COMPLETED BY THE SUPERVISOR AND SUBMIT TO AWBP OFFICE WITHIN 24 HOURS OF NOTIFICATION OF THE INCIDENT.**

*The Supervisor should also contact the appropriate school/college HR and Risk Management if the events resulted in work related injury/ illness.*

| |
|---|
| **ASSESSMENT OF THE PROBLEM:** Explain the plausible cause of the incident (Why did this occur?); What factors were involved? Was there sufficient training or should more be required? What PPE was provided? What PPE was used? What PPE should have been used? What environmental factors (building, noise, vapors, lighting) were involved? What corrective action(s) have been taken? List the preventive measures proposed and any other pertinent information. |
| **PREVENTIVE MEASURES**. Describe the measures that will be taken or will be taken to prevent reoccurrence |
| Supervisor submitting Report: _____ Date: _____ |
| Reviewed by: _____ Date: _____ |
| Reviewed by: _____ Date: _____ |
| Reviewed by: _____ Date: _____ |
| Reviewed by: _____ Date: _____ |

**Attachment 9.** Visual inspection report form



VISUAL INSPECTION FORM - COFFEE

Inspector Name: _____

Date: _____ Time: _____

No visual symptoms to report ☐ (stop here and submit form)

Visual symptoms to report ☐ (continue below)

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Comments: _____

_____

UH-AL Containment Director: _____ Date: _____

**Attachment 10.** Staff Acknowledgment for Approved SOPs

**STAFF ACKNOWLEDGMENT**

I have read and understand the SOP's and agree to adhere to the requirements listed

| Name | Date | Signature |
|---|---|---|
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# VISUAL INSPECTION FORM - COFFEE

**Inspector Name:** John Hy

**Date:** 03/30/2022 **Time:** 1:42 pm

**No visual symptoms to report** ☑ (stop here and submit form)

**Visual symptoms to report** ☐ (continue below)

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Comments: _____

_____

UH-AL Containment Director: _____ Date: 03/30/2022

## VISUAL INSPECTION FORM - COFFEE

**Inspector Name:** John Fry

**Date:** 04/21/2022 **Time:** 4:00pm

**No visual symptoms to report** ☑ (stop here and submit form)

**Visual symptoms to report** ☐ (continue below)

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Comments: _____

_____

UH-AL Containment Director: _____ Date: 04/21/2022

## VISUAL INSPECTION FORM - COFFEE

**Inspector Name:** John Hu

**Date:** 05-02-2022 **Time:** 11:00 A

**No visual symptoms to report** ☑ (stop here and submit form)

**Visual symptoms to report** ☐ (continue below)

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Comments: _____

_____

UH-AL Containment Director: _____ Date: 05-02-2022

## VISUAL INSPECTION FORM - COFFEE

**Inspector Name:** John Fry

**Date:** 05/10/2022 **Time:** 1:55 pm

**No visual symptoms to report** ☑ (stop here and submit form)

**Visual symptoms to report** ☐ (continue below)

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Comments: _____

_____

UH-AL Containment Director: John Fry Date: 05/10/2022

# VISUAL INSPECTION FORM - COFFEE

**Inspector Name:** Dr. John Hu

**Date:** 6/27/22 **Time:** 3:57 PM

**No visual symptoms to report** ☑ (stop here and submit form)

**Visual symptoms to report** ☐ (continue below)

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Comments: _____

_____

UH-AL Containment Director: _____ Date: 6/27/22

# VISUAL INSPECTION FORM - COFFEE

**Inspector Name:** Dr. John Hu

**Date:** 07/07/2022 **Time:** 11:26 AM

**No visual symptoms to report** ☒ (stop here and submit form)

**Visual symptoms to report** ☐ (continue below)

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Comments: _____

_____

UH-AL Containment Director: _____ Date: 7/7/2022

## VISUAL INSPECTION FORM - COFFEE

**Inspector Name:** Dr. John Hu

**Date:** 7/26/2022 **Time:** 11:01 AM

**No visual symptoms to report** ☒ (stop here and submit form)

**Visual symptoms to report** ☐ (continue below)

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Comments: _____

_____

UH-AL Containment Director: _____ Date: _____

# VISUAL INSPECTION FORM - COFFEE

**Inspector Name:** Dr. John Hui

**Date:** 8/4/2022 **Time:** 12:30 PM

**No visual symptoms to report** ☒ (stop here and submit form)

**Visual symptoms to report** ☐ (continue below)

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Comments: _____

_____

UH-AL Containment Director: _____ Date: 8/4/22

## VISUAL INSPECTION FORM - COFFEE

**Inspector Name:** Dr. John Hu

**Date:** 8/24/2022 **Time:** 12:00 PM

**No visual symptoms to report** ☒ (stop here and submit form)

**Visual symptoms to report** ☐ (continue below)

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Comments: _____

_____

UH-AL Containment Director: _____ Date: 08/24/2022

# VISUAL INSPECTION FORM - COFFEE

**Inspector Name:** Dr. John Hu

**Date:** 9/7/2022 **Time:** 1420

**No visual symptoms to report** ☑ (stop here and submit form)

**Visual symptoms to report** ☐ (continue below)

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Comments: _____

_____

UH-AL Containment Director: _____ Date: 9/7/2022

# VISUAL INSPECTION FORM - COFFEE

**Inspector Name:** Xupeng Wang

**Date:** 9/30/22 **Time:** 14:45

**No visual symptoms to report** ☑ (stop here and submit form)

**Visual symptoms to report** ☐ (continue below)

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Comments: _____

_____

UH-AL Containment Director: Xupeng Wang Date: 9/30/22

**Attachment 9.** Visual inspection report form

VISUAL INSPECTION FORM - COFFEE

Inspector Name: Dr John Hu

Date: 10/11/22 Time: ~~10/12~~ 10:25 AM

No visual symptoms to report ☒ (stop here and submit form)

Visual symptoms to report ☐ (continue below)

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Comments: _____

_____

UH-AL Containment Director: _____ Date: 10:25 A.M.

**Attachment 9.** Visual inspection report form

**VISUAL INSPECTION FORM – COFFEE**

Inspector Name: Dr John Hu

Date: 10/19/2022 Time: 11:49 A

No visual symptoms to report ☑ (stop here and submit form)

Visual symptoms to report ☐ (continue below)

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Plant ID: _____ Photo taken: Y /N

Symptom Description: _____

Comments: _____

_____

UH-AL Containment Director: _____ Date: 10/19/22

| Date | Visitor (✓) | Name | Purpose |
|---|---|---|---|
| 7\|31\|21 | | Asoka de Silva | Set up coffee seeds in tissue culture. |
| 8\|2\|21 | | Asoka de Silva | Check coffee seeds |
| 8\|3\|21 | | Asoka, Tomie, Allie | check plants |
| 8\|10\|21 | | Tomie, Allie | Check plants |
| 8\|23\|21 | | Asoka de Silva | Checked remaining coffee seed tubes |
| 8\|24\|21 | | Asoka de Silva | Added 99 more coffee seed tubes |
| 8\|26\|21 | | Asoka de Silva | Added 102 more coffee seed tubes |
| 8\|27\|21 | | Asoka de Silva | Added 107 more coffee seed tubes |
| 8\|30\|21 | | Asoka de Silva | Checked tubes, eliminated contaminated coffee seed tubes. |
| 8\|31\|21 | | Asoka de Silva | Autoclaved all contaminated coffee seed tubes |
| 9\|7\|21 | | Asoka de Silva | Check plants |
| 9\|8\|21 | ✓ | Tedie Lancaster | inspection CHDOA |
| 9\|8\|21 | ✓ | EDWARD O'CALLAGHAN | " |
| 9\|9\|21 | | Asoka de Silva | Checked plants. |
| 9\|10\|21 | | Asoka de Silva | Checked plants, 214 seeds added |
| 9\|13\|21 | | Asoka de Silva | Check plants |
| 9\|14\|21 | | Asoka de Silva | Check Plants |
| 9\|15\|21 | | Asoka de Silva | Check plants |
| 9\|16\|21 | | Asoka de Silva | Check plants |
| | | | Also added 284 coffee seed tubes Batch #7 |
| 9\|17\|21 | | Asoka de Silva | Check plants |
| 9\|20\|21 | | Asoka de Silva | Check plants |
| 9\|21\|21 | | Asoka de Silva | Check plants |
| 9\|22\|21 | | Asoka de Silva | Check plants |
| 9\|23\|21 | | Asoka de Silva | Check plants |
| 9\|23\|21 | | Asoka de Silva | Added 216 coffee seed tubes |
| 9\|24\|21 | | Asoka de Silva | Check plants |
| 9\|27\|21 | | Asoka de Silva | Check plants |
| 9\|28\|21 | | Asoka de Silva | Check plants |
| 9\|29\|21 | | Asoka de Silva | Check plants |
| 9\|30\|21 | | Asoka de Silva | Check plants 396 seeds added |
| 10\|1\|21 | | Asoka de Silva | Check plants |
| 10\|4\|21 | | Asoka de Silva | Check plants 396 seeds added |
| 10\|5\|21 | | Asoka de Silva | Check plants |

ATTACHMENT 3

| Date | Visitor (✓) | Name | Purpose |
|---|---|---|---|
| 10/6/21 | | Asoka de Silva | Check plants |
| 10/7/21 | | Asoka de Silva | Check plants |
| 10/8/21 | ✓ | Techie Lancaster | inspection |
| 10/8/21 | ✓ | Jarett Law | Insp. |
| 10/8/21 | ✓ | Dexter Sugimoto | Insp Post Entry |
| 10/8/21 | | Asoka de Silva | Inspection & check plants |
| 10/11/21 | | Asoka de Silva | Check plants |
| 10/12/21 | | Asoka de Silva | Check plants |
| 10/13/21 | | Asoka de Silva | Check plants 396 seeds added |
| 10/14/21 | | Asoka de Silva | Check plants |
| 10/15/21 | | Asoka de Silva | Check plants 396 ~~36~~ seeds added |
| 10/18/21 | | Asoka de Silva | Check plants |
| 10/19/21 | | Asoka de Silva | Checked plants |
| 10/20/21 | | Asoka de Silva | Checked plants |
| 10/21/21 | | Asoka de Silva | Check plants |
| 10/22/21 | | Asoka de Silva | Check plants; Added 396 seeds |
| 10/23/21 | | Asoka de Silva | Ch Added 396 seeds |
| 10/25/21 | | Asoka de Silva | Added 396 seeds |
| 10/25/21 | | Asoka de Silva | Check plants |
| 10/26/21 | | Asoka de Silva | Check plants, Remove contamin. fo AC |
| 10/27/21 | | Asoka de Silva | Check plants, Removed contam. for AC |
| 10/27/21 | | Ferrell Daste | visitor |
| 10/28/21 | | Asoka de Silva | Check plants, Removed contam for AC |
| 10/29/21 | | Asoka de Silva | Check plants; added 396 ~~36~~ seeds |
| 10/30/21 | | Asoka de Silva | Check plants, added 441 ~~396~~ seeds |
| 11/1/21 | | Asoka de Silva | Checked plants, Removed contaminated seeds |
| 11/2/21 | | Asoka de Silva | Checked plants, Removed contaminated seeds |
| 11/3/21 | | Asoka de Silva | Checked plants |
| 11/4/21 | | Asoka de Silva | Checked plants; removed contaminated seeds (AC) |
| 11/5/21 | | Asoka de Silva | Checked plants; removed contaminated seeds (AC) |
| 11/6/21 | | Asoka de Silva | Checked plants, Removed & autoclave seeds. |
| 11/8/21 | | Asoka de Silva | Checked plants; Removed & autoclaved contam-seeds |
| 11/9/21 | | Asoka de Silva | Checked plants; Removed & AC contamin. seeds |
| 11/10/21 | | Asoka de Silva | Checked plants. |
| 11/12/21 | | Asoka de Silva | Checked plants |
| 11/15/21 | | Asoka de Silva | Checked plants, Removed & AC contam. seeds |

ATTACHMENT 3

| Date | Visitor (✓) | Name | Purpose |
|---|---|---|---|
| 11/16/21 | | Asoka de Silva | Checked plants, Removed and AC contam. seeds |
| 11/17/21 | ✓ | Dexter Sugimoto | Check on Post Entry plants |
| 11/17/21 | | Asoka de Silva | Check plants |
| 11/18/21 | | Asoka de Silva | Checked plants |
| 11/19/21 | | Asoka de Silva | Checked plants |
| 11/22/21 | | Asoka de Silva | Checked plants |
| 11/23/21 | | Asoka de Silva | Checked plants |
| 11/24/21 | | Asoka de Silva | Checked plants |
| 11/26/21 | | Asoka de Silva | Checked plants |
| 11/29/21 | | Asoka de Silva | Checked plants |
| 11/30/21 | | Asoka de Silva | Checked plants |
| 12/1/21 | | Asoka de Silva | Checked plants |
| 12/2/21 | | Asoka de Silva | Checked Plants |
| 12/3/21 | | Asoka de Silva | Checked plants |
| 12/6/21 | | Asoka de Silva | Checked plants |
| 12/7/21 | | Asoka de Silva | Checked plants |
| 12/8/21 | | Asoka de Silva | Checked plants |
| 12/9/21 | | Asoka de Silva | Checked plants |
| 12/10/21 | | Asoka de Silva | Checked plants |
| 12/13/21 | | Asoka de Silva | Checked plants |
| 12/14/21 | | Asoka de Silva | Checked plants |
| 12/15/21 | | Ben Tottori | Checked Plants |
| 12/16/21 | | Cade Kane | Checked plants, removed contaminated seeds |
| 12/17/21 | | Ben Tottori | Checked Plants/removed contaminated seeds |
| 12/20/21 | | Ben Tottori | Checked Plants/removed contaminated seeds |
| 12/21/21 | | →BIAS Lehmann | Inspection |
| 12/21/21 | | Dexter Sugimoto | Inspection Post Entry |
| 12/21/21 | | Cade Kane | Checked plants |
| 12/22/21 | | Ben Tottori | checked plants |
| 12/23/21 | | Cade Kane | Checked plants |
| 12/27/21 | | Ben Tottori | Checked plants/removed contaminated seeds |
| 12/28/21 | | Cade Kane | Checked plants |
| 12/29/21 | | Ben Tottori | checked Plants |
| 12/30/21 | | Cade Kane | checked plants |
| 1/3/22 | | Ben Tottori | checked plants/removed contaminated seeds |
| 1/4/22 | | Cade Kane | Checked plants |

ATTACHMENT 3

| Date | Visitor (✓) | Name | Purpose |
|---|---|---|---|
| 1\|5\|22 | | Ben Tottori | cheched plants |
| 1/6/22 | | Cade Kane | Checked plants |
| 1/7/22 | | Ben Tottori | checked plants |
| 1/10/22 | | Ben Tottori | checked plants |
| 1\|11\|22 | | Asoka de Silva | Checked plants |
| 1\|12\|22 | | Asoka de Silva | Checked plants |
| 1\|13\|22 | | Asoka de silva | Checked plants |
| 1\|14\|22 | | Asoka de silva | Checked plants |
| 1\|18\|22 | | Asoka de Silva | Checked plants |
| 1\|19\|22 | | Asoka de Silva | Checked plants |
| 1\|20\|22 | | Asoka de Silva | Checked plants |
| 1\|21\|22 | | Asoka de Silva | Checked plants |
| 1\|24\|22 | | Asoka de Silva | checked plants |
| 1\|25\|22 | | Asoka de Silva | Checked plants |
| 1/25/22 | | Monika Panfylio | inspect facility/permit conditions |
| 1/25/22 | | Eliki Siga | Inspect facility compliance |
| 1\|26\|22 | | Asoka de Silva | Checked plants |
| 1/28/22 | | Ben Tottori | checked plants/removed contaminated plants |
| 1\|31\|22 | | Asoka de Silva | Checked Plants |
| 2\|1\|22 | | Asoka de Silva | Checked Plants |
| 2\|2\|22 | | Asoka de Silva | Checked plants |
| 2\|3\|22 | | Asoka de Silva | Checked plants |
| 2\|4\|22 | | Asoka de Silva | Checked plants |
| 2\|7\|22 | | Asoka de Silva | Checked plants |
| 2\|8\|22 | | Asoka de Silva | Checked plants |
| 2\|9\|22 | | Asoka de Silva | Checked plants |
| 2\|10\|22 | | Asoka de Silva | Checked plants |
| 2\|11\|22 | | Asoka de Silva | Checked plants |
| 2\|14\|22 | | Asoka de Silva | Checked plants |
| 2\|15\|22 | | Asoka de Silva | Checked plants |
| 2\|16\|22 | | Asoka de Silva | Checked plants |
| 2\|17\|22 | | Asoka de Silva | Checked plants |
| 2\|22\|22 | | Asoka de Silva | Checked plants |
| 2\|23\|22 | | Asoka de Silva | Checked plants |
| 2\|24\|22 | | Asoka de Silva | Checked plants |
| 2\|25\|22 | | Asoka de Silva | Checked plants |

| Date | Visitor (✓) | Name | Purpose |
|---|---|---|---|
| 2/28/22 | | Asoka de Silva | Check plants |
| 3/1/22 | | Asoka de Silva | Check plants |
| 3/2/22 | | Asoka de Silva | Check plants |
| 3/3/22 | | Asoka de Silva | Check plants |
| 3/4/22 | | Asoka de Silva | Check plants |
| 3/4/22 | | TOBIAS LEHMANN | Inspection |
| 3/7/22 | | Ben Tottori | Check Plants |
| 3/9/22 | | Ben Tottori | Check Plants |
| 3/9/22 | | Asoka de Silva | Check plants |
| 3/10/22 | | Asoka de Silva | Check plants |
| 3/11/22 | | Ben Tottori | Check Plants |
| 3/14/22 | | Ben Tottori | Check Plants |
| 3/15/22 | | Asoka de Silva | Check plants |
| 3/16/22 | | Ben Tottori | Check Plants |
| 3/17/22 | | Asoka de Silva | Check plants |
| 3/18/22 | | Asoka de Silva | Check plants |
| 3/21/22 | | Ben Tottori | Check plants |
| 3/22/22 | | Asoka de Silva | Check plants |
| 3/23/22 | | Asoka de Silva | Check plants |
| 3/24/22 | | Asoka de Silva | Check plants |
| 3/28/22 | | Asoka de Silva | Check plants |
| 3/29/22 | | Asoka de Silva | Check plants |
| 3/30/22 | | John Hu | inspect plants |
| 3/30/22 | | Asoka de Silva | Check plants. |
| 3/31/22 | | Asoka de Silva | Check plants |
| 4/1/22 | | Asoka de Silva | Check Plants |
| 4/1/22 | | Rex Haraguchi | Post Entry Inspection |
| 4/1/22 | | MIKE MELZER | " " |
| 4/4/22 | | Ben Tottori | Check plants |
| 4/4/22 | | Cade Kane | added labels to racks |
| 4/5/22 | | Asoka de Silva | Check plants |
| 4/6/22 | | Asoka de Silva | Check plants |
| 4/7/22 | | Asoka de Silva | Check plants |
| 4/8/22 | | Asoka de Silva | Check plants |
| 4/11/22 | | Ben Tottori | Check plants |
| 4/13/22 | | Ben Tottori | Check plants |

| Date | Visitor (✓) | Name | Purpose |
|---|---|---|---|
| 4/14/22 | | Asoka de Silva | Check plants |
| 4/18/22 | | Asoka de Silva | Check plants |
| 4/19/22 | | Asoka de Silva | Check plants |
| 4/20/20 | | Asoka de Silva | Check plants |
| 4/21/20 | | Asoka de Silva | Check plants |
| 4/21/2022 | | John Ith | check plants |
| 4/22/22 | | Asoka de Silva | Check plants |
| 4/25/22 | | Asoka de Silva | Check plants |
| 4/26/22 | | Asoka de Silva | Check plants |
| 4/27/22 | | Ben Tottori | check plants |
| 4/28/22 | | Asoka de Silva | Check plants |
| 4/29/22 | | Asoka de Silva | Check plants |
| 5/2/22 | | Asoka de Silva | Check plants |
| 5/2/2022 | | John Ith | check plants |
| 5/2/22 | | Asoka de Silva | Check plants |
| 5-3-22 | | Mathew Cao | Inspection & Check plants |
| 5-3-22 | | Wendy Sueno | Inspection (USDA) |
| 5/4/22 | | Asoka | Check plants |
| 5/5/22 | | Asoka | Check plants |
| 5/6/22 | | Asoka | Check plants |
| 5/6/22 | | Tiffany Ho | State Ag Insp. |
| 5/9/22 | | Asoka | Check plants |
| 5/10/2022 | | John Ith | Check plants |
| 5/10/22 | | Asoka | Check plants |
| 5/13/22 | | Ben | check plants |
| 5/16/22 | | Ben | Check Plants |
| 5/18/22 | | Ben | check plants |
| 5/20/22 | | Ben | check plants |
| 5/23/22 | | Ben | Check plants |
| 5/25/22 | | Ben | Check Plants |
| 5/27/22 | | Ben | check plants |
| 5/31/22 | | Asoka | Check plants |
| 6/1/22 | | Asoka | Check plants |
| 6/2/22 | | Asoka | Check plants |
| 6/3/22 | | Asoka | Check plants |
| 6/6/22 | | Ben | Check Plants |
| 6/7/22 | | M.JM. | CHECK PLANTS |

| Date | Visitor (✓) | Name | Purpose |
|---|---|---|---|
| 6/8/22 | | Misty McElyea | HDOA inspection |
| 6/8/22 | | Ben Tottori | Check Plants |
| 6/13/22 | | Ben Tottori | check Plants |
| 6/14/22 | | Asoka de Silva | Check plants |
| 6/15/22 | | Asoka de Silva | Check plants |
| 6/16/22 | | Asoka de Silva | Check plants |
| 6/17/22 | | Asoka de Silva | Check plants |
| 6/20/22 | | Asoka de Silva | Check plants |
| 6/21/22 | | Asoka de Silva | check plants |
| 6/22/22 | | Asoka de Silva | Check plants |
| 6/23/22 | | Asoka de Silva | check plants |
| 6/24/22 | | Asoka de Silva | Check plants |
| 06/27/22 | | John Irby | check plants |
| 06/27/22 | | Asoka de Silva | Check plants |
| 6/28/22 | | Asoka de Silva | Check plants |
| 6/29/22 | | Asoka de Silva | Check Plants |
| 6/30/22 | | Asoka de Silva | Check Plants |
| 7/1/22 | | Asoka de Silva | Check Plants |
| 7/5/22 | | Asoka de Silva | Check plants |
| 7/6/22 | | Asoka de Silva | check plants |
| 7/7/22 | | John Irby | check plants |
| 7/7/22 | | Asoka de Silva | Check plants |
| 7/8/22 | | Ben Tottori | Check plants |
| 7/11/22 | | Shelby Chino | HDOA inspection |
| 7/11/22 | | Asoka | Check plants |
| 7/12/22 | | Asoka | Check plants |
| 7/13/22 | | Asoka | Check plants |
| 7/14/22 | | Asoka | Check plants |
| 7/15/22 | | Asoka | |
| 7/15/22 | | Ben Tottori | Check plants |
| 7/18/22 | | Ben Tottori | Check Plants |
| 7/19/22 | | Asoka | Check plants |
| 7/20/22 | | Asoka | Check plants |
| 7/21/22 | | Asoka | Check plants |
| 7/22/22 | | Asoka | Check plants |
| 7/25/22 | | Asoka | Check plants |

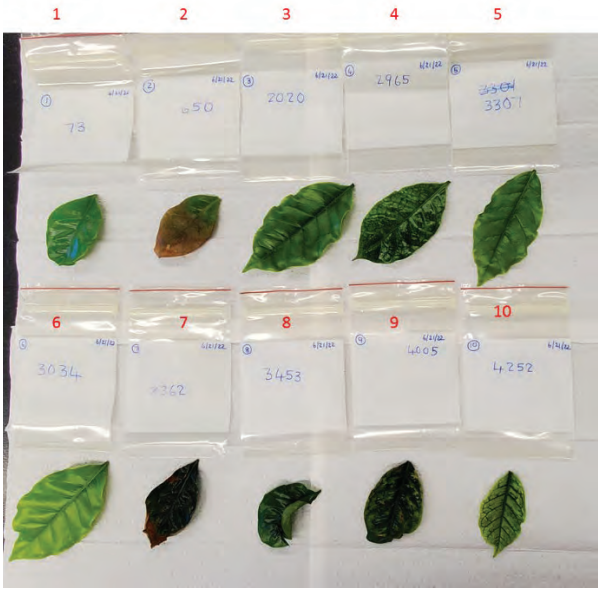
| Date | Visitor (✓) | Name | Purpose |
|---|---|---|---|
| 7/26/22 | | Asoka de Silva | Check plants |
| 7/26/22 | | John Ith | Check plants |
| 7/27/22 | | Asoka de Silva | Check plants |
| 7/28/22 | | Asoka de Silva | Check plants |
| 7/29/22 | | Asoka de Silva | Check plants |
| 8/1/22 | | Asoka de Silva | Check plants |
| 8/2/22 | | Asoka de Silva | Check plants |
| 8/3/22 | | Asoka de Silva | Check plants |
| 8/4/22 | | John Ith | Check plants |
| 8/4/22 | | Asoka de Silva | Check plants |
| 8/5/22 | | Asoka de Silva | Check plants |
| 8/8/22 | | Asoka de Silva | Check plants |
| 8/9/22 | | Asoka de Silva | Check plants |
| 8/10/22 | | Asoka de Silva | Check plants |
| 8/11/22 | | Asoka de Silva | Check plants |
| 8/12/22 | | Asoka de Silva | Check plants |
| 8/15/22 | | Asoka de Silva | Check plants |
| 8/16/22 | | Asoka de Silva | Check plants |
| 8/17/22 | | Asoka de Silva | Check plants |
| 8/18/22 | | Asoka de Silva | Check plants |
| 8/23/22 | | Misty McElyea | site inspection |
| 8/23/22 | | Asoka de Silva | Check plants |
| 8/24/22 | | John Ith | Check plants |
| 8/24/22 | | Asoka de Silva | Check plants |
| 8/25/22 | | Asoka de Silva | Check plants |
| 8/26/22 | | Asoka de Silva | Check plants |
| 8/29/22 | | Asoka de Silva | Check plants |
| 8/30/22 | | Asoka de Silva | Check plants |
| 8/31/22 | | Asoka de Silva | Check plants |
| 9/1/22 | | Asoka de Silva | Check plants |
| 9/2/22 | | Asoka de Silva | Check plants |
| 9/6/22 | | Asoka de Silva | Check plants |
| 9/7/22 | | John Ith | Check plants |
| 9/7/22 | | Asoka de Silva | Check plants |
| 9/8/22 | | Asoka de Silva | Check plants |
| 9/9/22 | | Asoka de Silva | Check plants |

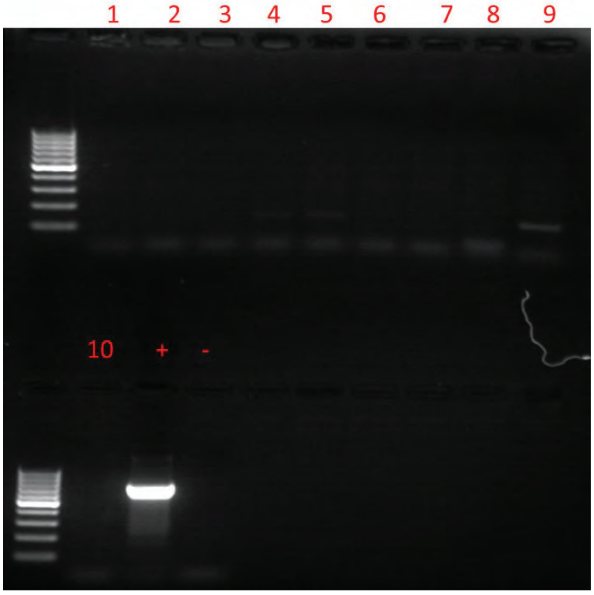
| Date | Visitor (✓) | Name | Purpose |
|---|---|---|---|
| 9/9/22 | ✓ | Wendy Sheno | Check plants, inspection |
| ✓ | ✓ | Matthew Gee | ✓ |
| 9/12/22 | | Asoka de Silva | Check plants |
| 9/13/22 | | Asoka de Silva | Check plants |
| 9/14/22 | | Asoka de Silva | Check plants |
| 9/15/22 | | Asoka de Silva | Check plants |
| 9/16/22 | | Asoka de Silva | Check plants |
| 9/19/22 | | Asoka de Silva | Check plants |
| 9/20/22 | | Asoka de Silva | Check plants |
| 9/21/22 | | Asoka de Silva | Check plants |
| 9/22/22 | | Asoka de Silva | Check plants |
| 9/23/22 | | Asoka de Silva | Check plants |
| 9/23/22 | | Shelby Chiang | Inspection |
| 9/26/22 | | Jonathan Kam | Inspection |
| 9/27/22 | | Asoka de Silva | Check plants |
| 9/28/22 | | Asoka de Silva | Check plants |
| 9/29/22 | | Asoka de Silva | Check plants |
| 9/30/22 | | Asoka de Silva | Check plants |
| 9/30/22 | | Ferrell Daste | Visitor |
| 9/30/22 | | Asoka de Silva | Check plants |
| 10/3/22 | | Xupeng Wang | check plants |
| 10/4/22 | | Asoka de Silva | Check plants |
| 10/5/22 | | Asoka de Silva | Check plants |
| 10/6/22 | | Asoka de Silva | Check plants |
| 10/7/22 | | Asoka de Silva | Check plants |
| 10/10/22 | | Asoka de Silva | Check plants |
| 10/11/22 | | Asoka de Silva | Check plants |
| 10/11/22 | | John Ith | Check plants |
| 10/12/22 | | Asoka de Silva | Check plants |
| 10/13/22 | | Asoka de Silva | Check plants |
| 10/14/22 | | Asoka de Silva | Check plants |
| 10/17/22 | | Asoka de Silva | Check plants |
| 10/18/22 | | Asoka de Silva | Check plants |
| 10/19/22 | | Asoka de Silva | Check plants |
| 10/19/22 | | John Ith | Check plants |

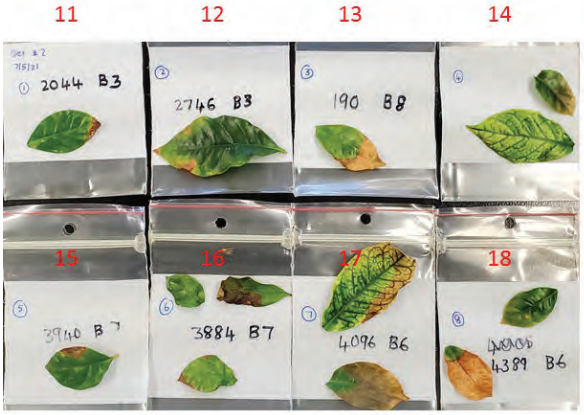
ATTACHMENT 3

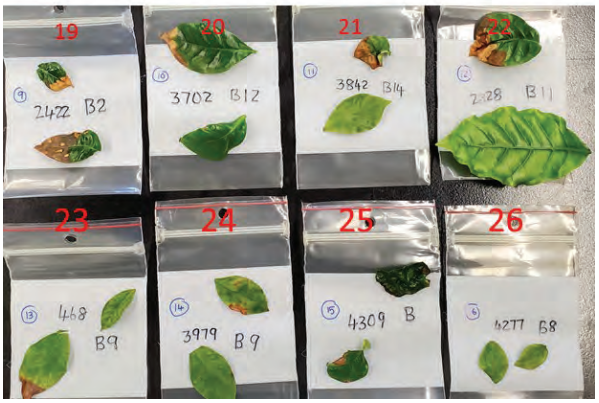
| Date | Visitor (✓) | Name | Purpose |
|---|---|---|---|
| 10/20/22 | | Asoka de Silva | Check plants |
| 10/21/22 | | Asoka de Silva | Check plants |
| 10/21/22 | ✓ | Wendy Sheno | USDA inspection |
| 10/21/22 | ✓ | Mary Beust | USDA inspection |
| 10/24/22 | | Asoka de Silva | Check plants |
| 10/25/22 | | Asoka de Silva | Check plants |
| 10/26/22 | | Asoka de Silva | Check plants |
| 10/27/22 | | Asoka de Silva | Check plants |
| 10/28/22 | | Asoka de Silva | Check plants |
| 10/31/22 | | Asoka de Silva | Check plants |
| 11/1/22 | | Siming Liu | Inspection USDA |

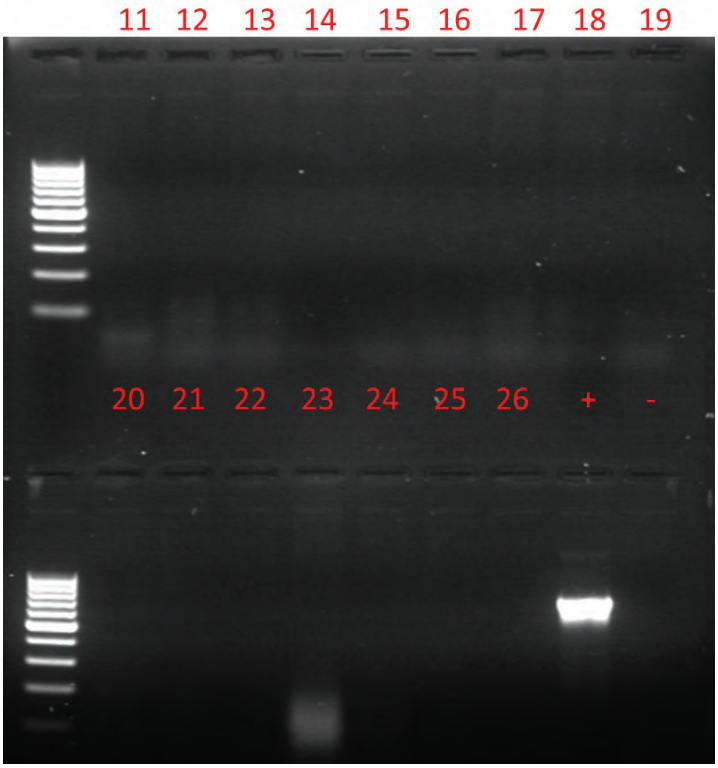
# PCR detection of Xylella fastidiosa (raw data)

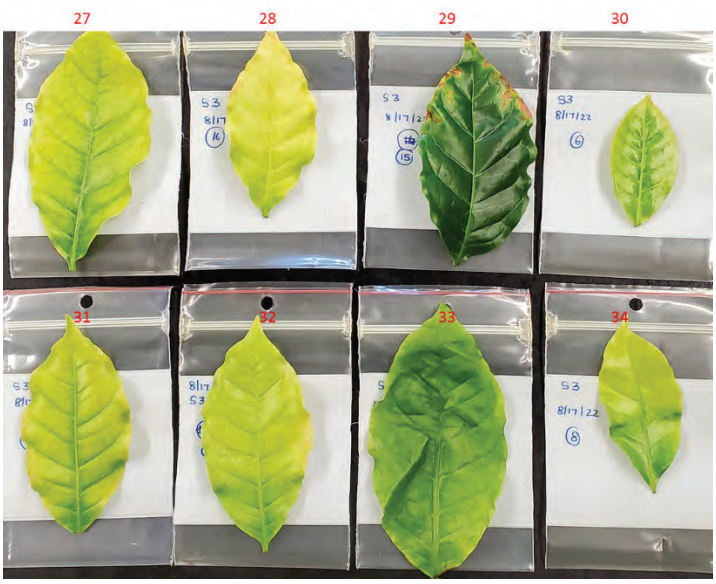
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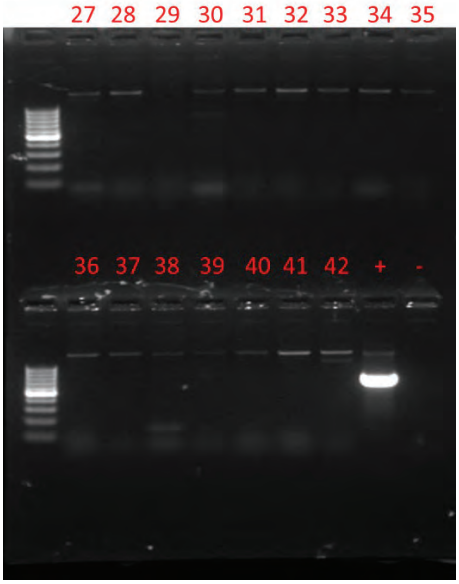
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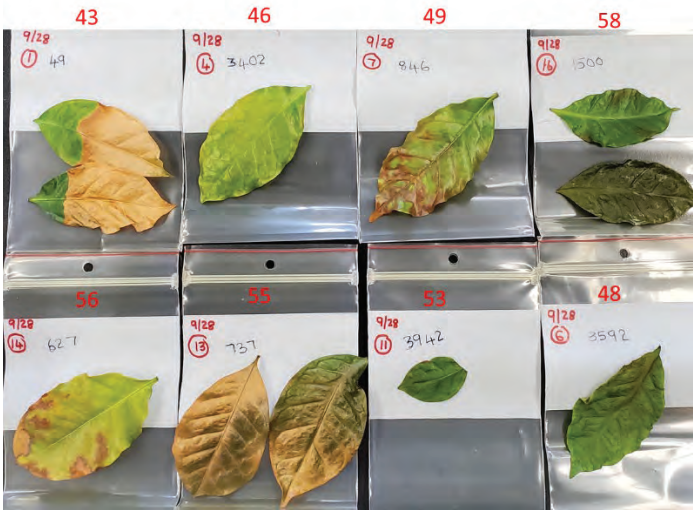
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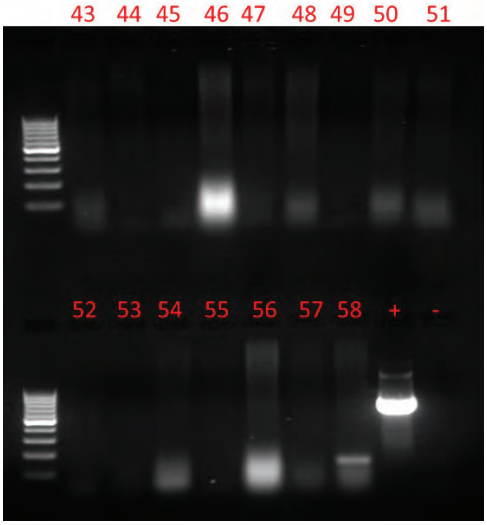
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State of Hawaii
Department of Agriculture
Plant Industry Division
Plant Quarantine Branch
Honolulu, Hawaii

November 29, 2022

Board of Agriculture
Honolulu, Hawaii

Subject: Request to Review the Request from David Smith, Department of Land and Natural Resources, to Designate the Kahului Airport on Maui and the Lihue Airport on Kauai as Additional Ports of Entry, Pursuant to Chapter 150A-5(10), Hawaii Revised Statutes, for the importation of the Southern House Mosquito, *Culex quinquefasciatus* Inoculated With *Wolbachia* Bacteria, for Mosquito Suppression.

## I. Background:

At its June 28, 2022, meeting, pursuant to Hawaii Administrative Rules §4-71-4.2, the Board of Agriculture (Board) approved a Board Order placing the southern house mosquito, *Culex quinquefasciatus*, on the List of Restricted Animals, Part A (RA List). The Board also approved the importation and approved permit conditions for *C. quinquefasciatus,* provided the Board Order was approved by the Governor.

On September 6, 2022, Governor Ige approved the Board Order to place *C. quinquefasciatus* on the RA List. The Plant Quarantine Branch subsequently issued permit 23-10-O-E1023 to the Department of Land and Natural Resources (DLNR) on October 19, 2022. Permit condition 11 states: "The restricted article(s) shall be imported only through the port of Honolulu, except as designated by the Board. Entry into Hawaii through another port is prohibited unless designated by the Board."

On November 14, 2022, DLNR submitted a request to the Board to designate Kahului Airport on Maui and Lihue Airport on Kauai as approved ports of entry for the importation of *C. quinquefasciatus* under permit 23-10-O-E1023 (see Appendix A). This request is brought before the Board pursuant to Hawaii Revised Statutes §150A-5(10), Ports of entry, which states: "None of the articles mentioned in this section shall be allowed entry into the State except through the airports and seaports in the State designated and approved by the board." The port of Honolulu is currently the only full port of entry.

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## II. Summary of Reasons for Additional Ports of Entry

DLNR's provides the following reasons for additional ports of entry beyond Honolulu:

- Optimal mosquito fitness provides for the best chance of suppressing existing *Culex* populations. Transit times of less than 24 hours from production site to release site are ideal and direct shipments to Maui and Kauai are the best way to achieve this.

- Additional ports of entry allow for added transportation flexibility.

## III. Staff Recommendation

The Plant Quarantine Branch (PQB) proposes the following recommendations:

Based on the information contained in the November 14, 2022 request, and subsequent communication with DLNR, the Plant Quarantine Branch (PQB) recommends allowing the initial "test shipments" to Lihue and Kahului airports in December 2022, as they could likely be accommodated by existing staff as they would likely be limited in size and frequency. PQB understands that the information garnered from these initial shipments is necessary for DLNR to be able to assess actual shipping requirements.

The PQB recommends the approval of Kahului Airport as an approved port of entry for the duration of this project as there is likely adequate on-island staff to accommodate the addition of a single shipment once a week.

The PQB cannot recommend adding Lihue Airport as a port of entry in perpetuity at this time because the PQB cannot adequately assess if existing levels of staffing could accommodate the increased inspectional volumes in a timely manner without knowledge of shipment sizes, times, and specific modes of transportation. DLNR has indicated in communications subsequent to their November 14, 2022, written request that this type of shipping information is not yet available and only empty test shipments have occurred via air cargo. This specific information is particularly important with regards to Lihue Airport because there are only two inspectors on-island to cover all inspections.

Respectfully Submitted,

BECKY AZAMA
Acting Manager, Plant Quarantine Branch

CONCURRED:

HELMUTH ROGG, Ph.D.
Administrator, Plant Industry Division

APPROVED FOR SUBMISSION:

PHYLLIS SHIMABUKURO-GEISER
Chairperson, Board of Agriculture

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DAVID Y. IGE
GOVERNOR OF HAWAII

SUZANNE D. CASE
CHAIRPERSON
BOARD OF LAND AND NATURAL RESOURCES
COMMISSION ON WATER RESOURCE MANAGEMENT

ROBERT K. MASUDA
FIRST DEPUTY

M. KALEO MANUEL
DEPUTY DIRECTOR - WATER

AQUATIC RESOURCES
BOATING AND OCEAN RECREATION
BUREAU OF CONVEYANCES
COMMISSION ON WATER RESOURCE MANAGEMENT
CONSERVATION AND COASTAL LANDS
CONSERVATION AND RESOURCES ENFORCEMENT
ENGINEERING
FORESTRY AND WILDLIFE
HISTORIC PRESERVATION
KAHOOLAWE ISLAND RESERVE COMMISSION
LAND
STATE PARKS

**STATE OF HAWAII**
**DEPARTMENT OF LAND AND NATURAL RESOURCES**

POST OFFICE BOX 621
HONOLULU, HAWAII 96809

November 14, 2022

TO: Phyllis Shimabukuro-Geiser
Chairperson, Board of Agriculture

FROM: David G. Smith
Administrator, Division of Forestry and Wildlife

SUBJECT: Import Permit 23-10-O-E1023: Requesting Board approval to designate Kahului Airport and Lihue Airport as permitted ports of entry for the import of southern house mosquitoes (*Culex quinquefasciatus*) into Hawaii

## I. BACKGROUND

On June 28, 2022, the State of Hawaii Board of Agriculture approved restricted article(s), Hawaiian biotype southern house mosquito, *Culex quinquefasciatus*, inoculated with *Wolbachia* bacteria to be imported into Hawaii for field-release in area-wide mosquito suppression programs for the benefit of critically endangered forest birds.

Import Permit 23-10-O-E1023 outlines the Department of Agriculture, Plant Quarantine Branch permit conditions for shipment, import, and release. Condition 11 states: "The restricted article(s) shall be imported only through the port of Honolulu, except as designated by the Board." Phase 1 implementation of the Department of Land and Natural Resources (DLNR) area-wide mosquito suppression program includes the release of male mosquitoes on Maui and Kauai. Permit 23-10-O-E1023, as issued, allows for shipment to, holding, and release of the restricted article(s) on Maui and Kauai, following initial entry into the port of Honolulu and HDOA inspection.

For *Culex* incompatible (*Wolbachia*) males to have the best chance of suppressing invasive wild *Culex* populations, the incompatible males must arrive at the release point(s) with optimal health and fitness. Based on previous mosquito control projects where incompatible male mosquitoes were packed and shipped, the total time in transit held in the cold chain is a critical aspect of fitness at the destination - minimizing transit time increases male viability, longevity, and mating competitiveness. Transit time of <24hr from production site-to-release site is essential to preserve incompatible male fitness, moreover shortened transit times provide more flexibility for

the timing of male mosquito releases. For releases on Maui and Kauai, it is therefore important to have incompatible male mosquitoes sent directly to each island to minimize transit times.

With our partners, DLNR is currently exploring all transport options (including commercial and cargo carriers), as both transit time and economics must be considered. A combination of transit approaches may be required to mitigate risks if, for instance, commercial flights are canceled. DLNR aims to start test shipments as soon as December 2022, followed by weekly mosquito shipments throughout 2023, with the hope of extension through 2024. However, shipments will not commence until DLNR obtains HDOA approval following the inspection of holding facilities.

If this request to add Kahului Airport and Lihue Airport is granted, it will improve the likelihood that this initial incompatible male intervention can suppress wild southern house mosquito populations in critically endangered forest bird habitats for the benefit of these irreplaceable biocultural resources.

II. REQUEST

Pertaining to Import Permit 23-10-O-E1023, DLNR respectfully requests that two additional ports of entry be designated by the Board: Kahului Airport and Lihue Airport.

Respectfully submitted,

DAVID G. SMITH, Administrator
Division of Forestry and Wildlife