

**MASS-REARING *LILIOCERIS CHENI* GRESSITT  
AND KIMOTO,  
AIR POTATO BEETLE (APB):  
STANDARD OPERATING PROCEDURES**

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# PREFACE

*Lilioceris cheni* Gressitt and Kimoto (Coleoptera: Chrysomelidae) is currently being mass-reared by the Florida Department of Agriculture and Consumer Services Division of Plant Industry (FDACS-DPI) at a facility in Gainesville for biological control releases to assist in management of infestations of air potato (*Dioscorea bulbifera* L.) throughout six states including Florida. This Standard Operating Procedures (SOP) manual is designed for employee training and for transferring current rearing technologies to other organizations that plan to mass-rear *Lilioceris cheni* or similar herbivorous beetles.

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## SECTION I. INTRODUCTION

### A) INTRODUCTION

The air potato vine (*Dioscorea bulbifera* L., Dioscoreaceae) has a wide native range including much of Asia, tropical Africa and northern Australia (Burkill 1960; Coursey 1967; Tindall 1993). Introduction into the United States occurred in Alabama during the late 1700s and southern Florida in the early 1900s (Morton 1976). Since then, it has spread throughout Florida, as well as Alabama, Georgia, Hawaii, Louisiana, Mississippi, South Carolina and Texas. There are additional reports in several other states though the survivability of the vine in those areas is unknown. The vine grows rapidly, climbing tree canopies and crowding out native plant species. It can persist in poor soil types and generates large numbers of bulbils, from which new vines sprout. It is considered one of the most aggressive weeds ever introduced into Florida. It forms dense blankets which smother native trees and understory plant species. In central and southern Florida, it is considered one of the more common natural area weeds, and the Early Detection and Distribution Mapping System (EDDmapS) shows vine infestations in all 67 Florida counties. Most natural and disturbed habitats are susceptible to air potato infestation, including floodplain forests, marshes, scrub forest, sinkholes, tropical and subtropical hammocks, waterways and urban lots (Schultz 1993). Air potato is listed as a noxious weed by the Florida Department of Agriculture and Consumer Services and by the Florida Exotic Pest Plant Council as a Category I invasive plant: “species which are altering native plant communities by displacing native species, changing community structures or ecological functions, or hybridizing with natives”.

Most air potato management methods have proven temporary and ineffective (Wheeler et al. 2007). Chemical control is costly and requires repeated basal and foliar sprays over several years. Damage or death to non-target plants often occurs during these treatments. Additionally, new vines often continue to sprout from underground tubers when herbicide treatments cease. Mechanical control of air potato is labor-intensive and time-consuming. Temporary removal is possible when vines and bulbils are hand-collected and destroyed. Eradication, however, requires the removal of underground tubers which can be difficult to access and completely remove.

Biological control was determined to be the most promising management strategy for this invasive plant after the discovery of the air potato beetle (APB) (*Lilioceris cheni*: author (Chrysomelidae) (Wheeler et al. 2007). Laboratory production of tens of thousands of beetles is more cost-effective than mechanical and chemical control methods. Beetles representing two different biotypes were collected from locations in Nepal and China. Host-specificity testing was performed on 41 non-target species, and *L. cheni* was found to be highly host-specific. Release and establishment of the beetle in Florida have resulted in a massive reduction in vine biomass, bulbil production and spread of the vine. This program can be considered one of the major successes in the field of biological control (Rayamajhi et al. 2019).

Releases in previously targeted and newly identified locations across the southern states are warranted to ensure the widespread establishment of beetles and overall reduction of air potato vine throughout the infested regions. We continue working with our collaborators across the region to identify areas where *D. bulbifera* is a major nuisance and work towards releasing *L. cheni* at those sites. Individuals

of *L. cheni* are currently established and reproducing in the field in Alabama, Florida, Georgia, Louisiana and Mississippi.

Although air potato beetle appears well-established and widespread in Florida and have made an impact on vine biomass in many release areas (some showing significant vine and bulbil reduction), vines continue to regrow from underground tubers each spring (Rayamajhi et al. 2019). Infestations in the southern half of the state tend to display less beetle impact and produce more propagules than infestations in the northern half; applying earlier releases to these regions may further improve the efficacy of the biological control agent on those infestations. Examining the effect of several years of heavy beetle feeding at any given location is warranted to determine if beetles can induce enough stress on vines to cause mortality in some tubers.

## **B) LIFE CYCLE OVERVIEW**

### **i) Air Potato**

In spring, air potato vines sprout from underground tubers and bulbils that were dropped the previous growing season. The vines cannot support their own weight and will climb vegetation to reach sunlight. Vines grow 1.5-11.8 centimeters a day and up to 23 meters total (Rayamajhi et al 2016). This characteristic enables the plant to cover native vegetation quickly, thereby limiting the availability of light to other flora. Even vines which have been fed upon by beetles will continue to grow for the entire growing season, another characteristic that makes it a successful invader. As temperatures drop, the vine will begin to allocate resources to reproduction, generating bulbils, and begin leaf senescence. Flowers and seed production are very rare in Florida. Bulbils, which can weigh up to 1 kilogram, will drop to the ground where they will sprout new vines the following season (Rayamajhi et al. 2016). The underground tubers will also overwinter and produce new vines in the spring.

### **ii) Air Potato Beetle (Figure 1)**

The life cycle of the air potato beetle is holometabolous and spans 28 days, allowing for rapid production. Adult beetles bite the leaf veins resulting in cupping of plant leaves. Eggs are most often laid in the cupped leaves. This is an important indicator used to assess oviposition rates in the mass-rearing program. It has been reported that female adults can lay more than 1,200 eggs in their lifetime (Manrique et al. 2017). The pale white eggs will become yellowish if fertilized and larvae will eclose in approximately four days. First instar larvae are also yellowish but become grey to red with black legs as they progress through the four larval instars. The larval stage lasts in total approximately eight days. After this time, larvae will drop off the plant and cluster in the soil to pupate. They secrete a substance that solidifies into a hard puparium. The pupal stage lasts approximately 14 days. Adults emerge and require approximately 10 days before mating begins and the cycle continues. Adults are assumed to live over five months (Pemberton and Witkus 2010). They have also been observed to go several weeks without food. Immediately after emergence adult insects must have a food source or they will die within two to three days. Insects which are provided a food source immediately postemergence can survive with minimal dietary resources, especially those that overwinter as adults. Larvae will not survive without leaf material (pers. obs. Kraus).

Field observations suggest that beetles have successfully overwintered in Florida for several years (Rayamajhi et al. 2019). When beetles emerge from diapause (in areas where diapause occurs) there is a short period before feeding upon vines begins, likely due to post-diapausal physiological changes. Adults kept outdoors during the mass rearing process will also experience this phase of reduced feeding behavior. After adults begin feeding on vines they will begin to mate and lay eggs in areas with sufficient air potato tissue. Beetles will completely defoliate plants if allowed to do so. It is vital, therefore, to monitor oviposition and beetle density in rearing rooms so as not to allow vines to be completely defoliated. Much is still unknown about beetle overwintering and diapause. The critical threshold for temperature is unknown, and the impact of a complex of viruses has not been studied. In addition to mass rearing beetles, there is a need for experimental field- and laboratory-based research to answer these questions.

## SECTION II. FACILITIES AND MAINTENANCE

### A) FACILITIES

It is a common practice in the mass rearing of insects to separate the host from the insect being reared. Rearing the air potato beetle is unique in this respect. In the initial phase, while vines are growing, it is important to maintain separation; however, after a certain amount of vine growth (approximately six weeks), the beetles can be released onto vines and monitored. To accomplish this, a minimum of two fully isolated rooms, preferably buildings, is recommended. Large operations may be modular, requiring multiple rooms or buildings for each of the two components to back up production and provide for testing new materials, equipment or procedures. Construction using resistant materials such as cinderblock and incorporating flush surfaces to allow easy cleaning and limiting potential insect hiding locations or pests and pathogens is required. At FDACS-DPI, the operation begins small with two rooms for beetle isolation and two temperature-controlled greenhouses for plant tissue. As the season progresses, a greenhouse and an outdoor screenhouse are planted and infested with the beetle. When outdoor temperatures permit, large-scale outdoor rearing commences.

| Minimum Facility Requirements             |                                                                                                                                                                                                               |
|-------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Room                                      | Purpose                                                                                                                                                                                                       |
| Greenhouse                                | All season production. Specifics are discussed in Section II.                                                                                                                                                 |
| APB Rearing Room                          | APB oviposition on air potato leaves and development of larvae to be collected during the fourth instar and finish rearing in pupation bins. Discussed in Section III.                                        |
| Screen House                              | Main season production only. Specifics are discussed in Section II.                                                                                                                                           |
| Component                                 | Considerations                                                                                                                                                                                                |
| Heating, Ventilation and Air Conditioning | The HVAC system of the facility requires separate zones for each room to properly maintain the required temperatures. Proper air circulation is maintained within each room to limit temperature and humidity |

|                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                          | <p>gradients. Sufficient air exchange and filtration is required to provide a healthy environment for the employees and the organisms being reared; OSHA regulations state four to 12 air changes per hour for laboratory settings. Buildings must maintain a positive air pressure differential from the exterior to prevent the ingress of pests and pathogens.</p> <p>Buildings with a central hallway work best, with all rooms maintaining a positive air pressure differential from the hallway. For operations that cannot be separated by a hallway, the HVAC systems are designed such that the dirty rooms, those most likely to contaminate remaining rooms, have a negative pressure differential compared to cleaner rooms.</p> |
| Lighting                                 | Supplemental very high output (VHO) fluorescent lighting (48" 4-lamp fixture with T5-VHO 95-watt 6500°K fluorescent lamps, which supply 7200 initial lumens) is hung 7-10 feet above beetles. A 14L:10D photoperiod, controlled by automatic timers, is most efficient.                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Entryways                                | Entryways into each room are to be fully sealed when closed to limit pest and pathogen ingress. Doors are at least four feet wide and thresholds level with the floor to be easily traversed with utility carts.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Monitoring Systems                       | Temperature and humidity are monitored continuously and checked frequently in all rooms and environmental chambers. Battery back-up or independently powered units are recommended for electronic monitoring equipment.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Additional Recommended Components</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Back-up Generator                        | Power outages can be lethal to the insects and plants, mainly due to temperature or humidity extremes; poor development also occurs during a prolonged power outage. An ideal back-up system will be of sufficient size to power all essential equipment, but the exact power rating will depend on the individual facility. An automatic transfer switch is used to start generators in the event of a power outage. Temperatures must be maintained within acceptable ranges.                                                                                                                                                                                                                                                              |
| Alarm Systems                            | When a power outage occurs, or temperatures exceed acceptable levels, an alarm system alerts personnel via cell phone and audible alarm to facilitate rapid remediation. Alarms can be tied into available monitoring systems to indicate the locations where problems are occurring. Battery back-up or independently powered alarm equipment is required.                                                                                                                                                                                                                                                                                                                                                                                  |
| Environmental Chamber                    | Use to store unsprouted bulbils for later planting.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |

## **B) EQUIPMENT AND SUPPLIES**

- **Field Cages (Figure 2)**
- **Humidifiers**
- **Dehumidifier**
- **RO (Reverse Osmosis) water system**

- Pesticide sprayer
- Hose with spray-nozzle attachment
- Automatic timer for lights
- T5VHO lighting fixtures (48 in.)
- Log books or data logger
- Nursery containers (10 gallon) (Figure 3)
- Potting mix
- Slow-release complete fertilizer
- Micronutrient fertilizer
- Bone meal
- Horticultural oil
- Insecticidal soap
- Pyrethrum micro-fogger
- 6' & 8' bamboo stakes (Figure 4)
- Garden clippers
- Tray or cardboard box
- Container for bulbils
- Latex/nitrile gloves
- 4L storage tubs with screened ventilation holes in lid (Figure 5)
- 12L clear storage bins
- Self-adhesive label tape
- Medium-grade vermiculite
- Square net with extendable pole
- Tri-fold paper towels
- Plastic spray bottles
- Dish soap
- 70% isopropyl alcohol
- Moistened wicks (Figure 6)

### **SECTION III. AIR POTATO CARE**

The health of the host plant is critical to the rearing process. A healthy, rapidly growing plant will produce more foliage and therefore more APB. Proper fertilization, watering and pest management procedures must be followed.

#### **A) PLANT PROPAGATION AND USE**

- i) **Plant Source:** Bulbils are generally collected from air potato infestations throughout the state; many are obtained through cooperation with local governments and environmental groups. Bulbils are also produced in greenhouses on vines utilized for foliage production. Some bulbils will produce tubers that can be utilized in subsequent growing cycles.

- ii) **Propagule Storage:** Store unsprouted bulbils for up to one season at 10° C and <40% RH. Plan for a minimum of six weeks for a suitable percentage of bulbils to begin sprouting after being removed from storage.

iii) **Propagation Methods**

- (1) **Bulbils:** Bulbils are aerial storage organs formed on the vines and are the most common source for propagating air potato vines. A minimum of two months is required for sufficient growth to accumulate to be suitable for air potato beetle rearing.
- (a) Spread bulbils out in a tray or cardboard box in a warm area (27±2° C).
  - (b) Once bulbils begin to sprout, place them into a 10-gallon container filled with potting mix and press the bulbils firmly into the media.
  - (c) Water newly planted bulbils regularly.
  - (d) The bulbils will start rooting within one to two weeks.
- (2) **Tubers:** Tubers are underground storage organs which form annually and will sprout in the spring. Best utilized for main-season rearing, tubers are typically slower to sprout than bulbils but provide a recurring source of propagative material.
- (a) Harvest tubers every two years after the air potato vine has senesced.
  - (b) Repot tubers 2 inches below the fresh soil surface in a clean container.
  - (c) Fertilize repotted tubers twice annually and water regularly.
  - (d) First-season tubers should be left in the soil. Sprouting bulbils can be rooted in the same containers.
- iv) **Growing Conditions:** Grow plants in greenhouses, screen houses, field cage and indoors with supplemental grow lights. Monitor and manipulate plants to prevent intertwining of vines.
- (1) **Environmental conditions:** Logged by a digital data logger.
- (a) **Temperature:** 27±2° C
  - (b) **Relative humidity:** 65% (60 – 80% acceptable)
  - (c) **Lighting:** 14L:10D (6:00AM to 8:00PM) (Indoors only)
- (2) **Containers and soil:** Pot plants in the 10-gallon container in which they will grow for two years.
- (3) **Cage maintenance:** All cages are inspected regularly for tears; dead plant material is removed from inside the cage and debris are removed off the top of cages as applicable.

## B) PLANT MAINTENANCE

- i) **Water:** Assess plants regularly for proper soil moisture; water when the top inch of the potting mix is dry. Generally, this equates to watering 2-3 times per week; particularly dry periods may require daily irrigation.
- ii) **Fertilization:**
  - (1) Every three months, fertilize plants with approximately 1 Tbsp. of slow-release fertilizer per 3 gallons of the plant container.
  - (2) Once yearly, 1 tsp. each of a micronutrient blend and bone meal should be applied per 3 gallons of the plant container.
- iii) **Indoor Rearing:**
  - (1) If plants will be transferred later, ensure the vines do not become intertwined by compressing the growth on the bamboo stakes and training vines to grow on themselves.
  - (2) Remove dead and heavily damaged leaves from plants in beetle production as needed.
  - (3) When the *D. bulbifera* vines have senesced, remove bamboo stakes. Cut off all dead foliage, inspect vines, collect any bulbils and discard the dead vines.
- iv) **Outdoor Rearing:** If air potato vines are being grown uncontained outdoors, take extra care to ensure they do not become established.
  - (1) Keep vines away from trees and other naturally growing vines (e.g. *Smilax* or muscadine grape) that they can climb on to reach the tree canopy.
  - (2) Pull sprouts from the ground immediately when noticed, ensuring the entire tuber/bulbil is removed.
  - (3) Carefully collect any bulbils that form on these vines. All bulbils, even the smallest ones, must be collected.

## C) PEST MANAGEMENT

- i) **Key Pests:** Mites, thrips and mealybugs are the most common pests of air potato. In the bulbil and leaf production facilities and the small-scale production plant growing facility, air potato beetles can also become pests.
- ii) **Scouting:** Inspect all growing facilities for pests daily.
  - (1) **Mites and thrips:** Visually inspect plants, especially new growth, for signs of damage. Collect damaged material and inspect under a microscope.
  - (2) **Mealybugs:** When scouting for these pests, pay attention to the regions where petioles attach to the leaves, vines attach to growing bulbils and vines sprout from soil surface.
  - (3) **Air potato beetles:** Air potato beetles are a pest in production areas designated beetle-free. If beetles are accidentally introduced, remove beetles by hand and monitor for any

new damage or signs of larvae over the next week. If caught early, accidental beetle introductions can be easily controlled.

**iii) Treatment Procedures:** Before applying any insecticide, required Personal Protective Equipment, as outlined in the respective SDS, must be worn.

- (1) NOTE:** Pesticide applications, except for soap and oil treatments, must be discussed with the production manager prior to any application being made. The production manager must approve all pesticide applications and ensure adherence to any applicable OHSA regulations. Pesticide application cannot be conducted without explicit approval from the production manager.
- (2)** If a facility scheduled to have APB released for rearing has an infestation of mites or thrips and the plants are at the proper stage for beetle release, beetles can be released. Beetles will often out-compete mites and thrips. The decision to release beetles for rearing or treatment of mites is based on production needs and the severity of the infestation; consult with a supervisor in such cases prior to any applications being made.
- (3) Soap and oil method:** Combine 2 oz. of horticultural oil with 3 oz. of insecticidal soap per gallon of solution. Reverse osmosis (RO) or distilled water should be used when available. Spray on both upper and lower surfaces of all affected foliage.
  - (a)** Soap and oil may be used if plants are small and not severely intertwined. (Refer to 4a for large intertwined vines).
  - (b)** For widespread infestations, it may be necessary to treat all the plants in the greenhouse with soap and oil. It is up to the production manager to determine the threshold at which it is necessary to treat plants with broad spectrum insecticide. This will halt colony growth and is one of the most difficult decisions to make in beetle production. This threshold will differ depending on the characteristics and abilities of each rearing facility to manage pests. For example, depending on available personnel, mechanical control of pests may be possible in one facility but not another.
- (4) Insecticide treatment:** For all insecticide applications, clear and prominent signage is posted at all entryways into the treated area. Date of application, no-entry period and the insecticide formulation used must be clearly indicated.
  - (a)** Once plants are moderately large and intertwined, soap and oil applications are not effective. If treatment is required, fog the facility with a pyrethrin insecticide. Guidelines set forth on the pesticide label must be followed for all pesticide applications. If possible, apply insecticides on Fridays when greenhouses will not be entered until the following Monday. Plants cannot be used for rearing APB for 2-3 weeks if a pyrethrin insecticide is applied. Before bringing pyrethrin-treated plants back into the production cycle, test a small batch of plants for residue by adding beetles and assessing survival rate. Soap-and-oil-treated plants generally may be used within two days without the need for residue testing.

## D) PATHOGEN MANAGEMENT

Both *Pythium* sp. and *Rhizoctonia* sp. have been detected in some plants. These pathogens infect both the roots and portions of the stem just above the soil line. Infected plants will show signs of wilting and may have necrotic regions at the crown and on roots. In severely affected plants, the vascular tissue at the crown will be completely rotted and/or hollow. Plants displaying these symptoms must be removed from the growing facility and autoclaved before disposal.

## E) PLANT CARE CHECKLIST

|           |                                                                                                                                                        |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| Daily     |                                                                                                                                                        |
|           | Scout for pests                                                                                                                                        |
|           | Check environmental conditions                                                                                                                         |
|           | Inspect soil moisture                                                                                                                                  |
|           | Manage vines for small-scale rearing                                                                                                                   |
|           | Sweep floors and ensure proper drainage                                                                                                                |
| Monthly   |                                                                                                                                                        |
|           | Download data                                                                                                                                          |
| Quarterly |                                                                                                                                                        |
|           | Fertilize all plants with a slow-release fertilizer at a rate of ½ tsp. per gallon of the plant container.                                             |
| Annually  |                                                                                                                                                        |
|           | Fertilize all plants with a micronutrient blend at a rate of 1 tsp. per 3 gallons of the plant container. To be done in late winter/early spring.      |
|           | Fertilize all plants with bone meal at a rate of 1 tsp. per 3 gallons of the plant container. To be done in late summer/early fall.                    |
| As Needed |                                                                                                                                                        |
|           | Remove dead and heavily damaged leaves.                                                                                                                |
|           | Decide on required treatments based on pest species and severity noted during scouting as outlined in SOP.                                             |
|           | Discuss planned pesticide applications with the production manager prior to application. Soap and oil applications are excluded from this requirement. |
|           | Document any treatments to be made in respective Room Logbook(s).                                                                                      |
|           | Place appropriate signage and markers as necessary.                                                                                                    |
|           | Prepare treatment; only the required quantity should be prepared.                                                                                      |
|           | Apply prepared treatment only to the areas that need treatment and have been properly marked as such.                                                  |
|           | Clean PPE, application equipment and self to prevent contamination.                                                                                    |

## SECTION IV. AIR POTATO BEETLE REARING

### A) ENVIRONMENTAL CONDITIONS

Maintain these environmental conditions for the entire APB rearing period. Data is logged on a digital data logger.

- i) **Temperature:**  $27 \pm 2^{\circ} \text{C}$
- ii) **Relative Humidity:**  $65 \pm 10\%$
- iii) **Lighting:** 14L:10D (6:00AM to 8:00PM)

- (1) Check cage lighting system timers for proper operation daily.
- (2) At the end of the workday, turn off all other overhead lights. This is easy to overlook when the plant lighting system is turned on.

### B) PREDATOR MANAGEMENT

- i) **Vertebrates:** Frogs, lizards and occasionally birds will feed upon adult and larval air potato beetles. Capture and remove them when found within indoor/greenhouse rearing spaces.
- ii) **Arthropods:** Major pests that can disrupt beetle rearing activities include carpenter ants, fire ants and various wasps.

- (1) **Ants:** Attempt to locate carpenter and fire ant colonies.
  - (a) If a colony is located inside a plant container, remove the entire container.
    - (i) Using a small shovel, remove all air potato vines from the container. Wash all roots thoroughly to remove remaining ants.
    - (ii) Discard the remaining soil and thoroughly rinse the container.
    - (iii) Fill the pot with fresh soil, replant the vines and place them back into production.
  - (b) If the colony cannot be located or is not found within a container, treat with commercially available fire ant bait according to the directions given on the label.
- (2) **Wasps:** Use nets to capture wasps within indoor/greenhouse rearing facilities. Scout the area for wasp nests, which may be found inside dense air potato foliage or other semi-protected areas within the rearing facility.
  - (a) Treat nests with appropriate pesticide spray or capture all adults with an aerial net. Take care to not spray more foliage than necessary.
  - (b) With gloved hands, remove any foliage that was contaminated with pesticide residues.

## C) PRODUCTION

### i) Production Scales:

- (1) **Small-scale production:** Rear beetles in small rooms or field cages. Indoor small-scale production is used primarily to start building the beetle colony early in the year before the greenhouse is ready for beetle release. Small-scale rearing uses plants grown in 10-gallon containers in a beetle-free facility. Plant tissue can be collected from these plants to feed beetles in containers.
- (2) **Large-scale production:** Large-scale rearing is done in a greenhouse or screen house. Beetles are released onto plants grown in place in 10-gallon containers.
- (3) **Outdoor beetle production:** Rear beetles by growing potted air potato vines outdoors. This population is slow to start, but generally offers high yields with lower inputs than the conventional indoor rearing. Extra care must be taken to ensure air potato vine is properly contained and controlled.

### ii) Production Tasks:

#### (1) Setup:

- (a) Set up plant containers with bulbils and/or tubers based on the given propagation methods [see Section II, A, iii].
- (b) Release approximately five beetles per 10-gallon container in the production area; actual release numbers may need to be adjusted based on plant condition and timing.

#### (2) Daily maintenance:

- (a) Check for oviposition and larvae daily; a lack of oviposition may indicate pests, plant health issues or an insufficient number of adult beetles. If no pests are found, and there are no plant health issues, more adult beetles should be released.
- (b) Evaluate adult beetle populations, paying attention to oviposition levels. If oviposition is high, beetles may need to be removed in anticipation of larvae consuming large amounts of leaf tissue. Vines must be given the chance to recover and grow new leaf tissue. If oviposition is low, more adult beetles may need to be released.
- (c) Collect fourth-instar larvae and adult beetles as necessary [see Section III, D].

## D) COLLECTION

### i) Larvae:

- (1) Larvae will begin to reach the proper age for collection roughly 10 days after oviposition, a few days before pupation occurs.

- (2) Look for larvae on the underside of foliage damaged by feeding and on nearby petioles and stems.
- (3) Clip leaves containing larvae at the petiole and place entire leaf into a 4L bin with a ventilated lid. Minimizing disturbance of larvae increases survival rates.
  - (a) When collecting larvae in the greenhouses, check the concrete floor for larvae that have been disturbed and dropped during the collection process. If not collected, these larvae will desiccate and die.
- (4) Transfer the stack of leaves, glossy side up, to a 12L pupation bin filled halfway with moistened vermiculite. The larvae should mostly be on the undersides of the leaves facing the vermiculite. No more than 100 larvae are recommended per container. Vermiculite should be sterilized prior to use.
- (5) If the 12L container lid fits without compressing the leaves, latch the lid; if the leaf stack extends above the edges of the container, place another 12L container upside down over the stack to retain humidity and keep larvae from escaping.
- (6) Write the date collected, number of larvae and biotype on a label and attach to top of lid (or overturned 12L container). If biotype is mixed, or they were found in outdoor rearing areas, indicate as such. Larvae that are close to pupating may drop from the foliage when disturbed.
- (7) Place the fourth-instar larvae in pupation bins [Section III, E, 4].

## ii) Adult Beetles:

- (1) Adult beetles are best collected by placing a container or cupped hand below them and gently picking them off the plant.
- (2) Adults generally drop from plants when disturbed and may play dead.
- (3) Collect beetles into a 4L tub with one of the circular vents removed or collect into a plastic test tube and transfer to a 4L container.
- (4) **Note:** Adults may congregate in the peak of the greenhouse or screen house, especially late in the season. Collect these at least three times per week by using a net to “sweep” them from the ceiling.

## E) CARE

- i) **Larval Care:** APB larvae feed on air potato leaves for approximately one week, then drop to the ground to pupate within the substrate for approximately 16 days. If suitable substrate is not available for pupation, such as in a greenhouse or screen house, significant larval mortality will occur due to desiccation. To avoid these losses, collect fourth-instar larvae and maintain in pupation bins until they emerge as adult beetles.

**(1) Preparing vermiculite:**

**(a)** Autoclave

**(b)** Larval rearing bin setup:

**(i)** Take a small, one-liter watering can and fill with RO water past the 1L mark, about 48 ounces.

**(ii)** Pour half of the watering can (roughly 24 ounces) into the bottom of a 12L bin.

**(iii)** Wearing a paper face mask to avoid inhaling small particles, fill the 12L bin halfway full of autoclaved vermiculite.

**(iv)** Gradually pour the rest of the RO water from the can into the bin and mix with a metal scoop until the vermiculite is moist, but not clumpy.

**(2) Maintaining fourth-instar larvae:**

**(a)** Keep all 12L bins inside at  $27\pm 2^{\circ}$  C and under T5VHO 14:10 lights.

**(b)** Check 12L larvae bins every day and replace leaves if they are slimy and rotting. If a leaf is still in good condition replacement may not be necessary.

**(i)** If rotting leaves are found, first clip a stack of fresh leaves and place next to work area with the undersides upturned.

**(ii)** Take the old stack of leaves from the bin and carefully sort through it for unpupated larvae. Gloves are highly recommended for this activity.

**(iii)** Gently remove larvae from the old leaves and transfer them to the undersides of the leaves in the fresh stack. The amount that can go on a leaf depends on the size of the leaf, but 3-5 larvae is generally recommended.

**(c)** Note any mortalities on the bin label.

**NOTE:** When transferring larvae, touch the slimy abdomen as little as possible and try to utilize their natural defense mechanism, dropping when disturbed, to your advantage by positioning them over the fresh leaves and getting what larvae you can to drop by gently brushing from the cut leaf into the container. The rest of the larvae may be gently plucked or pried off.

**(3) Maintaining early-instar larvae:** Although maintaining early-instar larvae in tubs is generally avoided, they must sometimes be collected late in the season to avoid death due to frost or a lack of healthy leaf material. Overwintering beetle populations may still produce eggs, which will hatch and need maintaining.

**(a)** Keep early-instar larvae in a 4L ventilated tub on fresh leaves. Check daily for rotting leaves and replace as needed using the same technique as with the fourth-instar larvae [Section III, E, 2].

**(b)** Keep temperature at  $27\pm 2^{\circ}$  C and use T5VHO 14:10 lights.

**(4) Pupating larvae:**

- (a) Once all larvae in the 14L bins have dropped from the leaves into the vermiculite to pupate, remove the leaves and note the date of final pupation on the bin label to accurately estimate adult emergence dates.
- (b) Move the bin to pupation shelves and let it rest undisturbed for 12 days.
- (c) After 12 days, place one or two fresh leaves in the bin, underside down, to provide food for the emerging beetles.
- (d) Check the bin for emerging beetles daily. Note emergence dates and number of beetles on the bin label.
- (e) Move emerged beetles out of bins and ship, overwinter or start populations in beetle-rearing areas.
- (f) Once all beetles have emerged, or after three days with no emergence, discard vermiculite and wash container with soap and water.
- (g) Transfer bin label to pupation log book.

**ii) Adult Beetle Care:** Adult beetles are stored in a 4L Rubbermaid container with ventilated lid. They may be kept short-term while waiting to be shipped, or long-term through the winter.

**(1) Setup:**

- (a) Place 50-100 adult beetles in a container. If beetles are newly emerged, or will be stored more than one week, do not place more than 50 per container.
- (b) Label container lid with date and number of beetles. If more beetles are added, amend the label to include subsequent dates and amounts.
- (c) Place 2-5 air potato leaves in each container or include a section of artificial diet [see Section III, E, iii]. For hydration, lightly mist the leaves or container with water, or provide a soaked wick.

**(2) Environmental conditions:**

- (a) **Temperature:**  $27 \pm 2^{\circ} \text{C}$
- (b) **Relative humidity:**  $65 \pm 10\%$
- (c) **Lighting:** 14L:10D

**(3) Daily Maintenance:**

- (a) Gently transfer beetles to a new 4L container with fresh food.
- (b) Empty and thoroughly clean the used container by washing with soap and water and stacking to dry overnight. Wipe container lid clean with 70% isopropyl alcohol, wipe dry or air dry and replace on new tub.

### **iii) Artificial Diet**

#### **(1) Ingredients:**

- (a)** 35 g Colorado potato beetle diet mix
- (b)** 3.3 g agar
- (c)** 1.25 mL KOH
- (d)** 170 mL water
- (e)** 3.5 g fresh air potato leaf

#### **(2) Instructions:**

- (a)** Follow instructions included with Colorado potato beetle diet.
- (b)** Immediately pour diet mix in long, roughly 1-inch-wide, strips across the wax paper.
- (c)** Let the diet cool and harden for 20 minutes and then cut the wax paper into 1-inch square sections and freeze in a clean, sealed tub.

#### **(3) Guidelines for providing diet to adult beetles:**

- (a)** Do not feed diet to newly eclosed beetles or larvae.
- (b)** If only feeding diet, or only diet and bulbils, be sure to also include a tri-fold paper towel and a source of water such as a moistened wick.
- (c)** Change the diet every other day when temperatures are cool and every day in warm conditions.

## **F) OVERWINTERING**

Due to the life cycle of plants in the field and limited plant availability, it is not feasible to maintain a large breeding population throughout the winter. For that reason, beetles are kept in cold storage, either in an environmental chamber or outdoors. Beetles seem to fare best outdoors on a shaded patio.

### **i) Preparing Containers**

- (1)** Keep no more than 50 beetles in a 4L tub with ventilated lid.
- (2)** On the lid label, note beetle biotype, date and number of beetles added.
- (3)** Feed beetles 2-3 lightly misted, fresh leaves; small bulbils (2 cm across or smaller) or diet. If not misting container or leaves, provide an alternate water source such as a soaked wick. Bulbils should only be used as a supplementary food source.
- (4)** If the tubs are kept outdoors and temperatures will be below 10° C, add a dry tri-folded paper towel for shelter.

ii) **Maintenance**

- (1) Refresh food and containers as needed. Beetles will eat more and produce more frass in warm temperatures and less when it is cool. Diet, leaves and containers may not need to be changed as often in cold weather.
- (2) Transfer beetles to new tubs as needed and wash dirty tubs.

iii) **Overwintering in Environmental Chamber**

(1) Environmental conditions:

- (a) Temperature: 17° C day and 14° C night
- (b) Relative Humidity: 65% +/- 10%
- (c) Light Schedule: 10.5L:13.5D

- (2) Four to six weeks prior to release into rearing facility, remove tubs from chamber and hold at room temperature (27° C); clean as needed.

iv) **Overwintering Outdoors**

- (1) Beetles kept outside all winter will resume reproductive activity sooner than beetles kept in an environmental chamber and can be used for small-scale rearing as soon as reproductive activity is noticed.
- (2) Store beetles in a safe area, away from sun and rain.
- (3) Move beetles indoors if the temperature will drop below 1° C.
- (4) Monitor containers often as temperature increases will elicit an increase in beetle activity, requiring more frequent food replenishment and cleaning.

## SECTION V. SHIPPING PROCEDURES

### A) PACKING SHIPMENT

- i) Place 50-100 beetles in a deli container supplied with 8 to 10 leaves. This provides sufficient food for two days and reduces impacts during shipping.
- ii) Place an ice pack in an insulated shipping container.
- iii) Place bubble wrap immediately around the ice pack to separate it from the container(s) of beetles.
- iv) Include a prepaid merchandise return label in each box for the recipient to return the shipping material free of charge.
- v) Include release instructions/FAQ information sheet (see next page) in each box for the recipient to release beetles effectively and confidently.

## SECTION VI. Air Potato Beetle Release Instructions/FAQ

**Release Instructions: Do not separate the beetles out of their deli cups, KEEP THEM ALL TOGETHER**

To release the beetles simply empty out the plastic container of beetles and air potato leaves near your air potato vine. You do not need to place them on the vine, they will disperse themselves. We recommend releasing all the beetles in one spot so that they can mate, increase their population size and thus increase the damage done to the air potato vines.

Please note that the air potato beetles will disperse themselves, as they can fly up to 10 miles from their release point!

***Be patient, it takes some time for the beetles to become effective***

Please keep in mind it will take a few weeks for your beetles to get settled in. Once settled, they will lay eggs which will hatch in 2-4 days, then the larvae (babies) will feed for about a week. When the larvae are ready to pupate (metamorphose), they will move to the soil. In about two weeks they will emerge from the soil as adult beetles. About a week after emergence the adult beetles will start laying eggs and the cycle will continue once again. *The entire life cycle from egg to adult takes about a month and the beetles live on average 6 months to one year.*

Beetle damage will be noticed first, then larvae damage. Once the larvae begin eating you should start seeing a *drastic* increase in damage to the air potato vines.

***The air potato beetles will usually come back each year***

When the air potato vines senesce around October the air potato beetles will enter diapause in order to overwinter. During diapause, they will sleep for a long period of time in the leaf litter, until they re-emerge in the spring, about a month after the air potato vines start growing again. Re-emergence is variable and is based on fall/winter's weather conditions.

### ***But, they will not eliminate your vine***

While the beetles will feed on older leaves and occasionally the bulbils (air potatoes), they prefer to eat the new growth. This hinders its growth significantly, prevents it from climbing high into the canopy and prevents the vine from choking other plants. Most importantly, the damage the beetles cause reduces the vine's resources, meaning the damage the beetles cause reduces the number of new bulbils (air potatoes) produced and thus limits the spread of the vine. If you are interested in eradicating air potato vine from your yard, it is recommended you also manually remove the vine's means of propagation and energy storage—the aerial bulbils and underground tubers.

## **SECTION VII. REFERENCES**

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## Appendix 1



**Figure 1:** *Lilioceris cheni* eggs, larvae and adults. Photos by Eric Rohrig, FDACS-DPI



**Figure 2:** Field cage. Photo by Emily Kraus, FDACS-DPI



**Figure 1: 10-gallon container. Photo by Emily Kraus, FDACS-DPI**

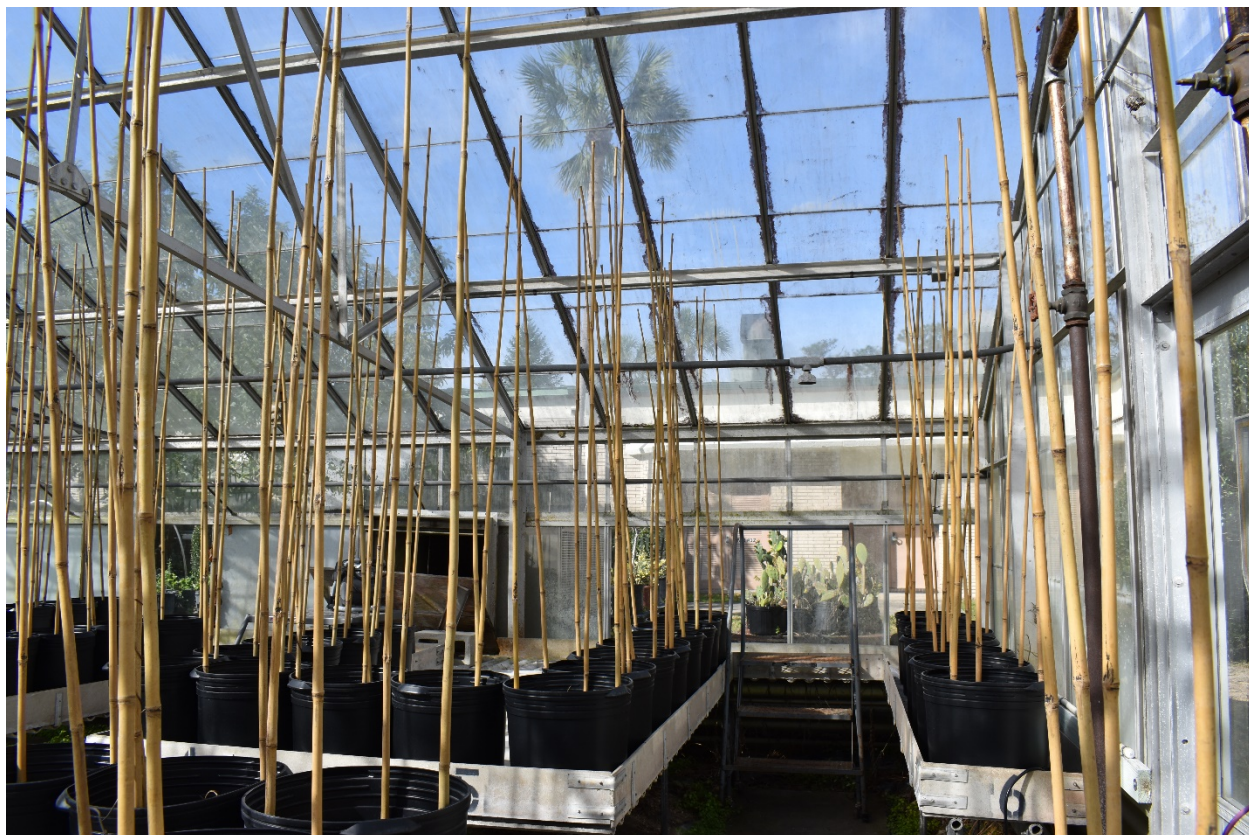


**Figure 2: Bamboo poles in 10-gallon containers. Photo by Emily Kraus, FDACS-DPI**



**Figure 3: 4L storage tub with screened ventilation in lid. Photo by Emily Kraus, FDACS-DPI**



**Figure 4: Cotton wicks. Photo by Emily Kraus, FDACS-DPI**