

MASS-REARING *TAMARIXIA RADIATA* STANDARD OPERATING PROCEDURES

DIVISION OF PLANT INDUSTRY

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PREFACE

Tamarixia radiata Waterson (Hymenoptera: Eulophidae) is currently being mass-reared by the Florida Department of Agriculture and Consumer Services Division of Plant Industry (FDACS-DPI) at facilities in Gainesville and Dundee for augmentative biological control releases to assist in management of Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), populations throughout the state of 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 *T. radiata* or similar parasitoids.

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SECTION I. FACILITIES AND EQUIPMENT

a) FACILITIES

There are a few specific facility requirements for successfully mass rearing *Tamarixia radiata* due to the tritrophic nature of the system. Physical separation of host plant production, ACP rearing and *T. radiata* must be maintained to avoid cross contamination which could lead to system failure. Small operations should plan on a minimum of three fully isolated rooms, or preferably buildings. Large operations may be modular, requiring multiple rooms or buildings for each of the three components to backup 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 for pests and pathogens is required. The overall design should consider the required traffic patterns and ensure that movements through rooms susceptible to contamination are restricted to the minimum required for production.

Minimum Facility Requirements	
Room	Purpose
Greenhouse	Produce healthy Orange Jasmine plants in a controlled, pest-free environment. Specifics are discussed in SECTION II.
ACP Rearing Room	Psyllid oviposition onto Orange Jasmine, growth of nymphs to required life-stage, and production of additional psyllid adults to maintain the colony. Specifics are discussed in SECTION III.
<i>Tamarixia radiata</i> Rearing Room	<i>Tamarixia radiata</i> oviposition onto psyllid nymphs, rearing <i>T. radiata</i> to the adult stage, and sorting collections for both product shipments and colony maintenance. Specifics are discussed in SECTION IV.
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 gradients. Sufficient air-exchange and filtration is required to provide a healthy environment for the employees and the organisms being reared. OSHA regulations state 4 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. 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 when compared to cleaner rooms.

Dehumidifiers	Standalone dehumidifiers rated at a minimum of 45 pints per day are used within the greenhouses and rearing rooms. Dehumidifiers are set to the lower RH value given for the RH range of each room.
Lighting	Supplemental very high output (VHO) fluorescent lighting is installed 11 in. above the top of rearing cages with a 14L:10D photoperiod (48" 4-lamp fixture with T5-VHO 95 watt 6500°K fluorescent lamps, which supply 7200 initial lumens).
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.
Additional Recommended Components	
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. The <i>Tamarixia</i> mass-rearing facility at Dundee utilizes a 36 KW generator to back-up ventilation and cooling systems in both the greenhouse and psyllid rearing rooms, and to power the water pump; the <i>T. radiata</i> rearing rooms are protected with additional insulation.
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.

b) EQUIPMENT

- i) **Cage** – The screen cages for rearing ACP and *T. radiata* are custom made of pine wood painted white. Joints are sealed with silicone caulk to eliminate crevices.

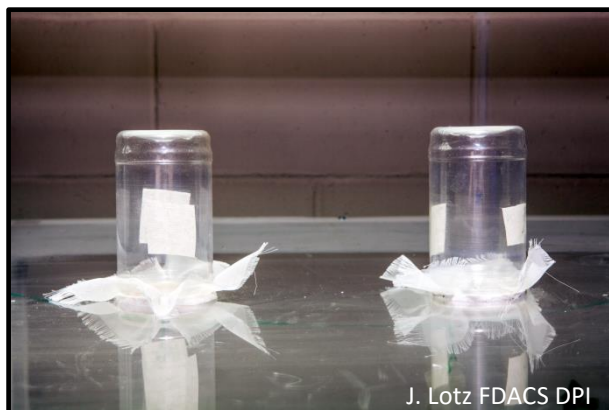


Figure 1b: Top of rearing cage showing collection jars inserted.



Figure 1a: Front view of rearing cage.

Cage Dimensions	Large Cage 31 in. W x 36 in. H x 36 in. D	Small Cage 24 in. W x 25 in. H x 24 in. D
Specifications given are for large cages; measurements must be adjusted for small cages which are otherwise built in a similar fashion.		
Front & Door	The frame for door is placed at bottom center, remainder with 243 μ m mesh screening fabric (SEFAR #: PA-68GG-243). The door is made of $\frac{1}{4}$ in. thick pine plywood or clear acrylic, 15 $\frac{3}{8}$ in. W x 25 in. H. A 6 in. diameter PVC tube is inserted into the center of the door, flush on the inner side and protruding 2 in. outward. A 12 in. long cloth sleeve of 243 μ m mesh is glued or clamped to the PVC tube and tied loosely at the other end.	
Top	The top is a clear $\frac{1}{4}$ in. thick acrylic panel. Two 2 $\frac{1}{2}$ in. diameter holes are cut out from the top and are located 9 in. on either side of the center point halfway back from the front of the cage. A 2 in. hole is made in the center of two collection jar lids and a piece of 1 mm mesh pass-through fabric screening is glued to each lid to cover the hole. The 1 mm mesh screening excludes ACP from entering the collection jar. The adapted collection jar lids are glued into each of the 2 $\frac{1}{2}$ in. cutouts made at the top of the cage.	
Sides & Back	Enclosed with the 243 μ m mesh.	
Bottom	Solid $\frac{1}{2}$ in. pine plywood painted with a durable white paint.	
Trays	Fiberglass or hard plastic trays measuring 16 in. W x 30 $\frac{1}{2}$ in. L are positioned on top of two 30 in. long $\frac{3}{4}$ in. PVC tubing which are placed roughly a quarter of the width away from each side of the cage and oriented front to rear. This protects the cage bottom from water and further limits ants any access to the plants.	

- (1) **Hood** – A black cloth hood designed to fit loosely over a *T. radiata* rearing cage is used during wasp collection periods so that the only light entering into the cage is through the collection jars. The size of the cloth is at least 2 in. larger than each cage dimension to allow for easy placement and removal. Cutouts are made so that the hood fits around the base of each collection jar. Additionally, a slit is cut the entire height of the hood front to allow access to the cage with the cover in place. A black utility trash bag with cutouts for the collection jars is placed on top of the cloth hood to further block light transmission.



Figure 2: Cage prepared for collection, covered with hood.

- (2) **Restricting mesh** – A loose section of 243 μm mesh screening is placed between the mesh pass-through screening and the collection jar to prevent insects from entering the jar outside of collection periods.
- (3) **Cleaning** – Once all insects and plants have been removed, the cage is cleaned thoroughly as follows to limit the occurrence of pests or pathogens:
- (a) All plant material and loose soil is removed and discarded.
 - (b) All surfaces of the cage and plant trays are wiped with a solution of a detergent and water, and finally with water only.
 - (c) If the above cleaning is inadequate, the cage and trays are wiped with 75% isopropyl alcohol. **CAUTION:** Do not use alcohol on the acrylic surfaces because it will cause cloudiness and possibly cracking.
- (4) **Cage maintenance** – All cages are inspected regularly for cracks in the acrylic panels; tears in screening; and sealing at the doors, joints, and collection jars. Back-up cages are required when the primary cages are being repaired.

ii)**Aspirator** – Aspirators are used to collect and sort both *T. radiata* and ACP adults without injuring the insects. One aspirator is needed for each rearing room, psyllid oviposition room, and sorting hood.



Figure 3: Aspirator assembly with attached collection vial.

Vacuum pump	Gast DOA-P704-AA vacuum pump
Nalgene tubing from the vacuum pump to the aspirator	A 4 in. piece of $\frac{5}{16}$ in. diameter tubing is attached to the vacuum inlet port. Additionally, a 2 in. piece of $\frac{3}{16}$ in. diameter tubing is fitted inside the $\frac{5}{16}$ in. diameter tubing to serve as a reducer for connecting the $\frac{1}{8}$ in. diameter main tubing. This $\frac{1}{8}$ in. tubing is inserted into the $\frac{3}{16}$ in. diameter tubing attached to the aspirator vacuum port. The overall length of the $\frac{1}{8}$ in. tubing is room-dependent, and is based on the distance from the furthest cage to the vacuum pump. The length of the tubing must enable easy maneuverability with only minimal slack.
Aspirator stopper	Two $\frac{1}{8}$ in. diameter holes each placed in line $\frac{3}{8}$ in. off center are bored through a 1 $\frac{1}{4}$ in. tapered rubber stopper. A 3 in. piece of $\frac{1}{8}$ in. diameter copper tubing bent 45 degrees is fitted into each hole, one being the vacuum port and the other the intake port. On the inner portion, the vacuum port is covered with a small piece of fabric mesh screening, with openings less than 243 μ m, to exclude insects and other materials. The vacuum port is fitted with a 2 in. section of $\frac{3}{16}$ in. tubing into which the main $\frac{1}{8}$ in. vacuum tubing from the vacuum pump is inserted. The intake port is fitted with a 2 in. section of $\frac{3}{16}$ in. tubing into which a 2 in. section of $\frac{1}{8}$ in. tubing is inserted. The $\frac{1}{8}$ -in. intake tubing is tapered 45 degrees on the outside end.

(1) General aspiration procedure

- (a) Set the aspirator to -6.5 bars for ACP or -5 bars for *T. radiata*. Greater suction can cause injury to the insects.
- (b) Fit rubber stopper of aspirator to a collection vial; make sure there are no leaks around the stopper.
- (c) Place intake tube over adults at a slight angle.
- (d) Aspirate adult into vial

(2) Potential aspiration problems

- (a) **Adults fly away during collection.** Approaching the adults from the abdomen may minimize the escape response.
- (b) **Many adults crushed during collection.** Improper aspiration angles or moving too quickly can cause adults to be crushed. Experience and practice are required to not injure the insects during aspiration.
- (c) **Adults are not readily aspirated.** The aspirator may be clogged. Clean the screen at the point where the aspiration line enters the stopper. Also, verify that the line is not kinked. Placing the line over your shoulder may limit the potential for stepping on the line.
- (d) **Adults seem to get stuck at the inlet.** This may be due to a buildup of honeydew at the inlet. Clean the inlet after each collection.

iii) **Sorting Hood** – The sorting hood is designed to allow a technician to quickly count and sort *T. radiata* or ACP while preventing contamination, and also to ensure the required number of insects is contained within each collection vial. A light panel is operated in the collection hood to attract the insects; and the sorting hood is covered with a black cloth to exclude external light. The hood is placed on a firm surface at a comfortable height for a seated technician.

Construction	Made of ½ in. laminated pine plywood
Front Section	Front box is 38 in. W x 26 ½ in. H x 12 ½ in. D and connects to the rear light source at a 45-degree angle with a 16 in L panel which tapers on the upper edge. Light switch located at front of box.
Work Surface	Bottom panel has an arc-shaped cutout to comfortably fit personnel when the black cloth is down; 14 in. D from arc apex to light source.
Front Panel	38 in. W x 26 ½ in. H with 29 in. cutout at the center; upper edge of cutout arc-shaped with apex 2 in. from top of hood.
Light Housing	23 ½ in. W x 23 ½ in. H x 9 ½ in. D with two 21 in. T5 fluorescent bulbs. Frosted diffuser panel with pine quarter trim covering inner face of light housing.

(1) Sorting collections: After collecting the insects, regardless of the method, the collections must be sorted to ensure that only *T. radiata* or ACP adults are in the vials. Pest insects encountered should be killed. Sorting is done within a hood to contain the insects. Sorting is performed as follows:



Figure 4: Lighted sorting hood with aspirator and collection vial.

- (a)** Place collection jars and sufficient collection vials to process the specimens inside the sorting hood.
 - (b)** Turn on the light panel. Fit the rubber stopper of the aspirator to a collection vial, making sure there are no leaks.
 - (c)** Pull the black cloth down to create a complete enclosure, ensuring that the light panel is the strongest light source.
 - (d)** Tap the base of a collection jar lightly on the work surface of the collection hood so that insects fall to the bottom. Unscrew the lid, and place the jar on the work surface open end down. Remove a few insects at a time from the collection jar by lightly tapping it on the work surface and aspirating the insects into a collection vial. Be sure to aspirate insects from the walls of the hood and the light panel as well.
 - (e)** After 150 adults have been collected in a vial, tap the bottom of the vial on a hard surface to bring the adults away from the vial opening. Disconnect the vial from the aspirator and quickly cap it.
 - (f)** Connect a new vial to the aspirator and continue collecting adults until none remain.
 - (g)** Store vials of adults in an environmental chamber [see Error! Reference source not found.] until they are shipped or used for subsequent rearing cycles.
- (2) Cleaning:** Spray the inside surfaces of the sorting hood with 75% isopropyl alcohol and wipe down with a clean paper towel. Allow at least 3 minutes before subsequent use.

SORTING PROCEDURE

Sorting Collections [see (1)]	
	Label collection vials with the species, ACP or <i>Tamarixia</i> , and date of collection. For <i>Tamarixia</i> collections also label the vials with the strain to be collected.
	Affix a ¼ in. x 3 in. strip of honey-soaked paper towel into each collection vial.
	Place the collection jars and prepared collection vials into the sorting hood.
	Turn on the back light panel of the sorting hood and lower the black cloth behind you.
	Uncap a collection vial and place the rubber stopper of the aspirator into the opening. Ensure the stopper fits snugly.
	Turn on the aspirator; verify proper operation and check for leaks.
	Uncap a collection jar or unsorted collection vial and place open end down against the work surface of the collection hood. Lift and move the collection jar/vial to another location to allow a few insects to exit at a time.
	Aspirate all target insects and ensure pests are not aspirated. Kill any non-target insect and discard.
	Replace the collection vial with a new collection vial when 150 insects have been collected. Be sure to cap the full vial.
	Repeat the above three steps until all insects have been sorted.
	Remove the collection jars and vials from the sorting hood.
	Clean the inside of the sorting hood with 75% isopropyl alcohol and wipe down with a paper towel.
	Turn off the back light.
	Store labeled and sorted collection vials in the environmental chamber.
	Allow a minimum of 3 minutes before sorting subsequent collections.

SECTION II. ORANGE JASMINE (*Murraya paniculata* (L.) Jack) CARE

The health of the host plant is critical in the rearing process. A healthy rapidly growing plant will produce more flush and, by virtue of this, more psyllid nymphs for parasitization. Proper fertilization, watering, pest management, and pruning must be followed.

a) EQUIPMENT AND SUPPLIES

EQUIPMENT	SUPPLIES
1) Dehumidifier	1) Log books or data logger
2) RO (Reverse Osmosis) water system	2) Nursery containers (1 gal.)
3) Pesticide sprayer	3) Potting mix (A well-draining potting mix, e.g. Fafard 52)
4) Pruning shears	4) Slow-release complete fertilizer (e.g. Osmocote Pro Fusion 19-6-9)
5) Hose with spray-nozzle attachment	5) Micronutrient fertilizer (e.g. Micromax Premium)
6) Automatic timer for lights	6) Micronutrient spray (e.g. Cal-Mag plus)
7) T5VHO lighting fixtures (48 in.)	7) Horticultural oil
	8) Insecticidal soap
	9) Liquid rooting hormone (e.g. Dip'n Grow)
	10) Rooting medium (e.g. Fafard 3B)

b) PLANT PROPAGAION

i) **Source of Plants:** Both cuttings and seeds are collected from ornamental plantings in Central and South Florida.

ii) Propagation Methods:

(1) **Cuttings** - Orange Jasmine can be propagated via cuttings. It takes from 6 months to a year before the plants are suitable for use in the rearing system.

(a) Cut shoots 4-6 in. long from a healthy plant. Select shoots that have healthy flush at the tip and are hardened at the cut end. Cut the shoot at 45° or more; straight cuts root poorly.

(b) Dilute rooting hormone as indicated by manufacturer.

(c) Dip the cut end of each shoot into the diluted rooting hormone and place with others into a tray filled with rooting medium.

(d) Keep rooting shoots in a covered tray to maintain high humidity levels or, when utilizing an automated system, mist the foliage hourly until the cuttings take root. Ensure the medium always stays moist. If the leaves die then the cutting likely will die also.

- (e) Place the tray in a shaded area devoid of overhead lighting. The space under a greenhouse bench will suffice.
- (f) After rooting occurs, about a month, transfer each cutting to an individual 1-gal. container filled with potting mix and fertilize with a slow-release fertilizer. Slowly introduce plants to higher light levels while maintaining a high humidity environment.
- (g) Keep the soil moist for a 2-3 more weeks until the plants are well established, after which place the plants under normal plant care operations. The plants will need an additional 5-10 months depending on growth-rate to be suitable for use in the rearing operations.

(2) **Seed** - Orange Jasmine also can be grown from seed, and it takes a year before the plants are suitable for use in the rearing system.

- (a) Plant 2-3 seeds in the center of a 4" container containing potting mix at a depth of ½ in. and water containers regularly to maintain proper soil moisture.
- (b) At one month, thin to one seedling per container. Identify the most vigorous seedling and remove the remaining seedlings from the container. Fertilize with a slow-release fertilizer at a rate of 1/4 tsp. per container.
- (c) At 4-6 months, transplant to 1-gal. containers and fertilize with 1 tsp. of the slow-release fertilizer. At this point the plants may be placed under normal plant care operations. The plants will need an additional 6-8 months depending on growth-rate to be suitable for use in the rearing operations.

iii) **Growing Conditions:** Plants are grown inside greenhouses and systematically pruned to produce maximum new flush about 2 weeks before use in the rearing system. If plants are grown outdoors then they must be trimmed, cleaned [see c)] and inspected for pests [see d)] before being transferred to the greenhouse.

(1) **Environmental conditions:**

- (a) **Temperature** - $28 \pm 4^{\circ} \text{C}$
- (b) **Relative humidity** - 60%
(50 – 80% acceptable)
- (c) **Lighting** - 10L:14D
(7:30AM to 5:30PM).



Figure 5: *Murraya paniculata* grown in a greenhouse.

(2) **Containers and soil:** Initially, plants are potted in 1-gal. containers filled with the potting mix. Plants too large for 1-gal. containers are transplanted into 2-gal. containers or discarded. Fewer 2-gal. containers will fit in the rearing cages.

(3) Fertilizer: Fertilize plants with 1 tsp of the slow-release fertilizer for each gallon of the plant container, i.e. 1 tsp - 1 gal. container, 2 tsp - 2 gal. container. Due to the rigorous scouting for pests which requires the plant to be removed from its container, using a formulation that will cake together, such as Osmocote Pro Fusion, limits the loss of fertilizer. Once yearly, 1 tsp of the micronutrient blend should be applied for each gallon of the plant container.

(a) Occasionally a curling of new flush occurs that the psyllids avoid. This can be corrected using a micronutrient spray such as Cal-Mag plus.

(4) Liming: An annual application of 3/4 tsp of dolomitic lime is applied to 1 gal. pots to correct acidification which occurs from the degradation of organic matter in the potting media blend.

(5) Water: Plants are assessed regularly for proper soil moisture; when the top in. of the potting mix becomes dry it is watered. Generally, this equates to watering once or twice per week.

c) PLANT MAINTENANCE

i) Pruning: Cutting off all growing tips is extremely important to both prevent pest infestations in the greenhouses and stimulate the plants to flush. Prior to moving plants into greenhouses, they are trimmed short enough to fit inside rearing cages, including the anticipated growth from new flush. Plants are trimmed to 24 in. high or less for cages used in the FDACS-DPI rearing system. Additionally, 2 weeks before plants are used in the rearing cycle they are pruned to induce flush. Twice as many plants as needed are pruned to allow for sufficient plant selection.

ii) Cleaning: Plants are cleaned at several points in the rearing process to limit contamination or unintentional releases of either ACP or *T. radiata*.

(1) Place the plant in a designated sink.

(2) Using a commercial-grade spray valve, spray both the upper and lower surfaces of all foliage with water and use a sponge to remove any honeydew, waxes, oils, or other contaminants from the leaf surfaces.

(3) Rinse plant a second time with the spray valve and then shake off excess moisture and set the plant aside.

d) PEST MANAGEMENT

i) Key pests: Ants, aphids, psyllids, and whiteflies are encountered most often but scales and mites are occasional pests. Specific pests include the Asian citrus psyllid, *Diaphorina citri*; cowpea aphid, *Aphis craccivora* Koch; whitefly, *Bemisia tabaci* (Gennadius); brown soft scale,

Coccus hesperidum L.; citrus snow scale, *Unaspis citri* (Comstock); broad mite, *Polyphagotarsonemus latus* (Banks); citrus red mite, *Panonychus citri* (McGregor); twospotted spider mite, *Tetranychus urticae* Koch; sixspotted mite, *Eotetranychus sexmaculatus* (Riley); ghost ant, *Tapinoma melanocephalum* (Fabricius); tawny crazy ant, *Nylanderia fulva* (Mayr); and red imported fire ant, *Solenopsis invicta* Buren. Photographs to aid in identification can be found in **Appendix B**.

ii) **Scouting:** All plants are inspected individually for pests at least once weekly.

(1) **Aphids, psyllids, whiteflies, scales, and mites:** To scout for these pests, visually inspect yellow sticky traps and the plants, especially the new growth, for adults or immatures.

(a) General procedures:

(i) Remove and discard infested shoots.

(ii) Inspect all nearby plants.

(iii) Treat plants with an insecticide if necessary.

(b) Insecticide treatment: Before applying any insecticide required Personal Protective Equipment, as outlined in the respective MSDS, must be worn. 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 are clearly indicated. To make a soap and oil mixture, combine 2 oz. of horticultural oil with 3 oz. of insecticidal soap per gallon of solution; use RO or distilled water if available. Both upper and lower leaf surfaces are sprayed completely with the soap and oil mixture.

(i) **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 OSHA regulations. Without explicit approval from the production manager the pesticide application will not be conducted.

(ii) If fewer than five adjacent plants are infested, spot-treat with soap and oil. For spot treatments, yellow tape is used to delineate the perimeter of the treated area.

(iii) For widespread infestations, it may be necessary to treat all of the plants in the greenhouse with soap and oil. A general threshold of pest abundance and distribution is used to gauge the severity of an infestation depending on pest species. Considering psyllids for example, if more than ~5% of plants in a greenhouse are found to be infested, treating the entire greenhouse may be warranted.

- (iv) If the infestation is catastrophic, for example >30% plants infested, the entire greenhouse may require an application of soap and oil or fogging with a pyrethrin insecticide, such as Pyrethrum TR. Guidelines set forth on the pesticide label must be followed for all pesticide applications. If possible, insecticides are applied on Fridays when greenhouses will not be entered until the following Monday. Clear and prominent signage is posted at all entryways into the treated area. Date of application, no-entry period, and the insecticide formulation used are clearly indicated. Plants in the greenhouse will not be available for ACP rearing for 2-3 weeks if a pyrethrin insecticide is applied. Before bringing pyrethrin-treated plants back into the production cycle, a small batch of plants is tested to verify ACP will survive on the plants. Soap and oil treated plants generally may be used within 2 days without the need for testing.
 - (c) Additional procedures for aphids: Infested plants are treated, labeled as having been infested with aphids, moved to a designated area, and not used for a minimum of two months.
 - (d) Procedures for citrus snow scale: Due to the potential for heavy infestations, limited number of occurrences, and difficulty eliminating this scale, all infested plants are bagged and discarded.
- (2) Ants:** To scout for ants, remove the plant from the container and inspect the potting mix.
- (a) Scouting procedures for ants:
 - (i) Plants are inspected over a clean solid surface, generally a utility cart.
 - (ii) To remove a plant from a container, the hand is placed flat against the soil surface with the trunk of the plant in-between the index finger and thumb.
 - (iii) Tilt the plant, remove the root ball from the container, and inspect both the root ball and inside the container. After inspection, loose soil or fertilizer that falls onto the clean surface is collected and placed back into the container.
 - (b) If ants are found:
 - (i) Over a designated container, outside of the greenhouses, remove all potting mix from the container and shake as much as possible from the roots.



Figure 6: Inspecting a container for ant infestation.

(ii) Rinse the roots under running water.

(iii) Replant the plant in clean potting mix.

iii) **Preventive Measures:** Limit the movement of pests into the rearing facilities and cages. This minimizes pest outbreaks and the need for corrective action.

- (1) **Plant movement:** Movement of plants between buildings or rooms occurs at several points in the rearing system, and provides opportunities for pests to be introduced. Every time plants are moved they are thoroughly inspected for pests. If pests are found, the plants are trimmed, treated, or discarded based on pest species and severity of the infestation.
- (2) **Staff movement:** The general work flow is designed to limit cross contamination. The general work-flow is greenhouse, psyllid room, *Tamarixia* room, and last sorting and shipping. At times, it is necessary to move counter to the general work-flow and in these situations both a thorough shake-down of clothing and a 15-minute minimum wait-period must be performed. Designated lab coats for each room will help to further limit cross contamination between rooms. Before leaving a room the lab-coat should be removed, and clothing should be brushed off vigorously to dislodge stray insects. When possible, having dedicated staff for each room will provide the greatest protection.
- (3) **Equipment movement:** All equipment should be thoroughly wiped down with a wet cloth before entering a greenhouse, ACP rearing room, or *Tamarixia* rearing room. Provided the general procedures as outlined throughout the SOP are followed, there is minimal likelihood for contamination when progressing in the direction of the general work-flow. Equipment should never be moved counter to the general work-flow unless it is thoroughly cleaned and inspected for potential cross contamination, this sort of movement poses significant risks to the rearing system and should be avoided when possible.
- (4) **Facilities:** The facilities segregate plant production, ACP rearing, and *T. radiata* propagation in three separate areas. Doors to rooms are checked regularly to ensure that they seal tightly. This prevents escape of ACP and *T. radiata* and keeps pests from entering.
- (5) **Benches:** Bench legs are placed in small containers holding soap and water or mineral oil to prevent ants from accessing the cages.
- (6) **Cages:** Cages are used to contain and isolate the plants, ACP and *T. radiata* during the rearing processes. The cages are thoroughly cleaned at the end of each rearing cycle and stored in a clean room. Ensure electrical cords are not touching the cage which will permit ants to access the cage.

- e) **RECYCLING PLANTS:** After plants have passed through a rearing cycle, they must be thoroughly pruned, cleaned, and treated with the soap and oil mixture [see 15] to prevent pest outbreaks. Plants are then given a three-month recovery period before being used in subsequent rearing cycles, label plants with the current date. At three months make plants available for use in the rearing system. When sufficient greenhouse space is not available, plants are stored outside temporarily. Subsequently, they are pruned, cleaned, and treated with the soap and oil mixture before being placed back inside the greenhouse.

PLANT CARE SCHEDULE

2D	D	W	Q	Y	N
Twice Daily AM/PM	Daily	Weekly	Quarterly	Yearly	As Needed

Monitoring Procedure	
2D	Scout greenhouses for pest problems (see d)Error! Bookmark not defined.].
2D	Verify environmental controls and monitoring systems are functioning properly.
2D	Document temperature, humidity and any pests noted for each greenhouse in the respective Room Logbook.
Plant Maintenance	
D	Inspect soil moisture of plants and water as needed.
W	Prune double the number of plants needed for future rearing as indicated by the production schedule.
Q	Fertilize all plants with the slow-release fertilizer at a rate of ½ tsp per gallon of the plant container.
Y	Fertilize all plants with the micronutrient blend at a rate of 1 tsp per gallon of the plant container.
Y	Lime all plants with dolomitic lime at the rate of ¾ tsp per gallon of the plant container.
Pest Management	
D	Sweep floors and ensure proper operation of drainage systems.
N	Decide on required treatments based on pest species and severity noted during scouting as outlined in SOP [see d)].
N	Discuss planned pesticide applications with the production manager prior to application. Soap and oil applications are excluded from this requirement
N	Document any treatments to be made in respective Room Logbook(s).
N	Place appropriate signage and markers as necessary [see (b)].
N	Don necessary PPE as required. This is found on the pesticide label.
N	Prepare treatment as necessary; only the required quantity should be prepared.
N	Apply prepared treatment only to the areas that need treatment and have been properly marked as such.
N	Clean PPE, application equipment, and self as necessary to prevent contamination.
Recycle Plants	
D	Prune, clean and treat with soap and oil mixture all plants within the designated recycle staging area.
D	Label newly recycled plants with the current date.
D	Move plants that have been rested for three months back into the production cycle.

SECTION III. ASIAN CITRUS PSYLLID (*Diaphorina citri*) REARING



Figure 7a: ACP eggs.

Figure 7b: Fifth instar ACP nymphs.

Figure 7c: ACP adult.

a) EQUIPMENT AND SUPPLIES

EQUIPMENT	SUPPLIES
1) Dehumidifier 2) Aspirator 3) Automatic timer for lights 4) T5VHO lighting fixtures (48 in.) 5) Insect rearing cages	1) Log books 2) 50ml vials with caps 3) Latex gloves 4) Disposable respirators 5) Sponge or cardboard cutout (to fit around base of plant)

b) **ENVIRONMENTAL CONDITIONS:** Maintain these environmental conditions for the entire psyllid rearing period until transfer to the *T. radiata* rearing cages.

- i) **Temperature:** $27 \pm 2^{\circ} \text{C}$
- ii) **Relative humidity:** $50 \pm 10\%$
- iii) **Lighting:** 14L:10D (6:00AM to 8:00PM)

(1) Automatic timers used to control the cage lighting system are checked daily for proper operation.

- (2) At the end of the workday, all other overhead lights are turned off. This is easily overlooked when the cage lighting system is turned on.

c) OVIPOSITOIN CAGE:

i) Plant Selection and Preparation for Rearing Process:

(1) Selecting new growth:

Flush must be neither too young nor too old; use new growth that is branched with at least two shoots, one 1 ½ to 2 ½ inch shoot and one ½ to 1-inch shoot. All stages of ACP rely on the initial quality and quantity of flush supplied.

- (2) Plant inspection:** Plants selected for rearing ACP must be inspected [see d)] to eliminate pests.



Figure 8: High quality orange jasmine flush.

- (3) Plant cleaning:** Plants must be cleaned thoroughly [see 14] before being placed into the oviposition cages to ensure insecticidal soaps, oils, and other contaminants are removed.

- ii) Cage setup:** A specified number of approximately 24 in. tall plants for ACP oviposition are placed into each oviposition cage (12 plants for 24 in. W x 25 in. H x 24 in. D cages or 30 plants for 31 in. W x 36 in. H x 36 in. D cages). The tallest plants are located on one side and the shortest on the other side of the cage. The cage label indicates the date oviposition began; and the plants remain in the cage for three days of ACP oviposition.

- (1) Psyllids for oviposition:** The number of adult female psyllids released in the oviposition cage depends on the number of shoots with flush, and the estimated number of adult psyllids remaining from the previous oviposition cycle. About 20 adult psyllids are maintained in an oviposition cage for each shoot with high quality flush. Many adult psyllids remain in a cage after the plants containing eggs are removed and new plants added. The remaining psyllids are supplemented to maintain the number and age of psyllids required to produce the prescribed quantity of eggs.

(a) Number of adult psyllids required in an oviposition cage:

- (i) Total adult psyllids = 20 x avg. number of shoots with flush per plant x number of plants
- (ii) Adults needed to replenish cage = total adult psyllids – (remaining adult psyllids x 0.8)

(iii) Number of psyllid vials required = adults needed to replenish cage / 150

(b) **Releasing adults:** Obtain required quantity of vials containing previously collected psyllid adults from an environmental chamber. Open vials inside the oviposition cage and place them on the potting mix surface with the opening resting on the plant stem or the rim of the plant pot.

(c) **Reevaluation:** A few hours after the initial psyllid release, inspect the flush for psyllid abundance. If a significant amount of flush appears to be un-infested, then there are two potential remedies.

(i) Psyllids may aggregate on the flush of taller plants or those near the edge of the cage. If there are shoots with flush supporting an overabundance of psyllids, shake the plants to dislodge the psyllids onto nearby plants. Reposition plants as necessary.

(ii) If it appears that there are insufficient adult female psyllids for the required level of oviposition then release additional psyllids.

iii) **Ending Cage Cycle:** After three days in the oviposition cage, separate the plants from the adult psyllids to prevent further oviposition. Nymphal stages must have no more than 1-2 instars of separation. This will allow for the most efficient processing. At the end of the oviposition period, complete the following:

(1) **Transfer plants from an oviposition cage to a development cage:** Record on the cage label the date the plants are introduced and when they are to be removed from the development cage and perform the required procedures.

(a) Reach through the sleeve in the door and cover the potting soil surface with a cardboard cutout surrounding the trunk at the base of the plant to prevent adult psyllids from falling onto the potting soil surface.

(b) Shake the plant vigorously without breaking off new growth.

(c) Remove the plant from the oviposition cage through the sleeve in the door.

(d) Aspirate the remaining adult psyllids from the plants [see (1)].

(e) Inspect plants for pests [see d)].



Figure 9: Removing a plant from an ACP oviposition cage.

(f) Record the current date on the container label.

(g) Place the clean plant in the development cage.

(h) Release any aspirated adult psyllids back into the oviposition cage.

(2) **Cleaning:** After each cycle, the plastic trays holding the plants should be removed and thoroughly cleaned, any debris in the cage should be swept up and the bottom of the cage should be wiped down with a damp cloth. Quarterly, all psyllids are removed; fully wipe down each cage [see (3)] and perform necessary maintenance [see i)(4)].

d) **DEVELOPMENT CAGE:** Allow the majority of developing ACP nymphs to reach the 4th instar. This takes 8-9 days within the development cage. This is the ideal time for transferring nymphs to *T. radiata* rearing cages for oviposition. After this point, allow no more than 1-2 additional days before *T. radiata* oviposition. If the nymphs cannot be used within this time period, they should be utilized for ACP colony maintenance.



Figure 10: Waxy threads of honeydew produced by developing ACP nymphs.

e) **COLONY MAINTENANCE:** A continuous supply of ACP adults is required for maintaining ACP production. For psyllid colony production, some plants in a development cage are held for psyllid production rather than being transferred to *T. radiata* rearing cages. In this case, collection of adult psyllids begins 15 days after plants are transferred into the development cage. To supply a sufficient number of ACP adults, 3-4 plants should be withheld for colony maintenance for each scheduled ACP oviposition cage. ACP may be collected from colony maintenance cages for up to two weeks after eclosion.

i) **Collection of ACP adults:** ACP adults are collected in a vial (50ml) attached to an aspirator [see (1)], and then sorted [see (1)].

ACP GENERAL MAINTENANCE SCHEDULE

2D	D	W	Q	Y	N
Twice Daily AM/PM	Daily	Weekly	Quarterly	Yearly	As Needed

Monitoring Procedure	
2D	Scout cages for pest problems [see d].
2D	Verify environmental controls and monitoring systems are functioning properly.
2D	Verify proper operation of lighting system and timers.
2D	Document temperature, humidity and any pests noted for each rearing room in the respective Room and/or ACP production logbook.
Plant Maintenance	
D	Inspect soil moisture of plants and water as needed.
Pest Management	
D	Remove any infested materials that were identified from cages.
D	Wipe down benches, work surfaces, and walls of any contamination.
D	Sweep floors and ensure proper operation of drainage systems.
D	Check water levels for table leg basins.
D	Verify no cables, hoses, or other objects are creating a pathway for ants.
Cage Maintenance	
D	Inspect all cages for tears, rips, or cracks in the meshing, acrylic, edges and door seals.
Q	Remove all ACP and perform full cleaning on oviposition cages [see i](3)].
Colony Maintenance	
W	Select 3-4 plants, from plants which have been given three days of ACP oviposition, for each oviposition cage scheduled two weeks in advance. Place these in a clean development cage to be used for colony maintenance.

ACP REARING SCHEDULE

Oviposition Cage Setup	
N	Select the appropriate number of plants for the oviposition cage to be used. Plants should be selected based on flush quality and quantity from the designated set of plants [see c)i)(1)].
N	Wash all selected plants thoroughly [see ii)].
N	Label container of each clean plant with current date and place into the oviposition cage.
N	Gather appropriate number of sorted adult ACP for oviposition. This is based on flush quality and is approximately 20 ACP per flush [see c)ii)(1)].
N	Release ACP into cage.
N	Record all of the required information into the designated ACP production logbook.
N	Reassess ACP release; if needed gather and release more ACP and document in the designated ACP production logbook.
Ending Oviposition Cage Cycle (Three days after setup)	
N	Shake off ACP adults on a plant to keep them inside oviposition cage, remove the plant, and aspirate any remaining ACP. Do this until all plants are removed from the oviposition cage.
N	Record the date transferred on each container's label and place plants into a development cage.
N	Record the date of transfer on the development cage.
N	Record the date of transfer in the designated ACP production logbook.
N	Remove and clean fiberglass plant trays with soap and water.
N	Sweep bottom of cage, wipe with a damp cloth and return cleaned plant trays to the proper position.
N	Release aspirated ACP.
Development Cage	
N	Move any plants remaining in the development cage for over 11 days into a colony maintenance cage.
N	Clean development cage thoroughly at the end of each cycle [see i)(3)].
Sorting Collections [see SORTING PROCEDURE]	

SECTION IV. *Tamarixia radiata* REARING

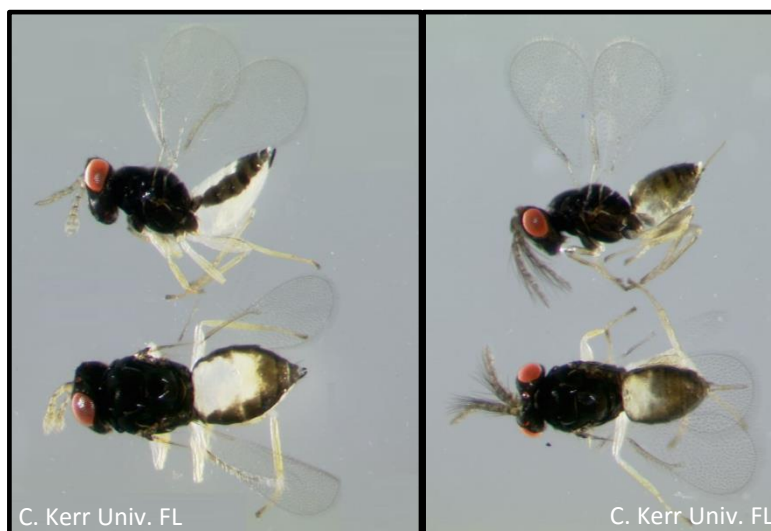


Figure 11a: Female *Tamarixia radiata*. **Figure 11b:** Male *Tamarixia radiata*.

a) EQUIPMENT AND SUPPLIES

EQUIPMENT		SUPPLIES	
1)	Dehumidifier	1)	Log books
2)	Aspirator	2)	50ml plastic vials with caps
3)	Insect rearing cages	3)	Honey
4)	Black fabric cover for cage	4)	Paper towels
5)	Automatic timer for lights		
6)	T5VHO lighting fixtures (48 in.)		
7)	Sorting Hood		
8)	Environmental chamber for storage		
9)	Plastic collection jars (2 1/2 in. dia. x 5 in.		
H)			
10)	Rolling utility carts		

b) ENVIRONMENTAL CONDITIONS: Maintain the environmental conditions for the entire *T. radiata* rearing period until the adult *T. radiata* progeny are transferred to the environmental chamber for short-term storage.

- i) **Temperature:** 27±2°C
- ii) **Relative humidity:** 50±10%
- iii) **Lighting:** 14L:10D (6:00AM to 8:00PM)

(1) Automatic timers used to control the cage lighting system are checked daily for proper operation.

- (2) At the end of the workday, all other overhead lights are turned off. This is easily overlooked when the cage lighting system is turned on.
- c) **Food source:** During the entire cycle and throughout storage, *T. radiata* are provided with honey-soaked paper towels for additional nourishment.

i) **Preparation:**

- (1) Working over a clean paper towel to absorb excess honey, spread out a standard commercial grade paper towel. Using a large brush, lightly coat one side of paper towel with honey and remove the excess.



Figure 12: Honey soaked paper towel cut into strips for collection vials.

- (2) Place a second layer of paper towel on top of the first, sandwiching the layer of honey between the two and press lightly.
- (3) Roll up the paper towel and store at room temperature in a sealed jar until used.

ii) **Use:**

- (1) For the rearing cages, the honey-soaked paper towel is cut into roughly 2 in. x 2 in. squares and hung from the top of the *T. radiata* rearing cage with a twist-tie.
- (2) For the collecting jars, the honey-soaked paper towel is cut into roughly 2 in. x 2 in. squares; one piece is pressed firmly against the inside of the jar and another piece is pressed firmly against the inside of the lid.
- (3) For the collecting vials, the honey-soaked paper towel is cut into ¼ in. x 3 in. strips. One strip is pressed firmly against the inside of each vial.

d) ***Tamarixia radiata* REARING CAGE:**

- i) **Cage Setup:** When the majority of the ACP nymphs reach the 4th instar in the development cage, plants with the nymphs are transferred into the *T. radiata* rearing cage. A specified number of plants for *T. radiata* rearing are placed into each oviposition cage (10 for 24 in. W x 25 in. H x 24 in. D cages or 24 for 31 in. W x 36 in. H x 36 in. D cages). Based on the cage size and infestation rate, the required number of *T. radiata* adults is transferred from storage into the rearing cage for oviposition on the nymphs.

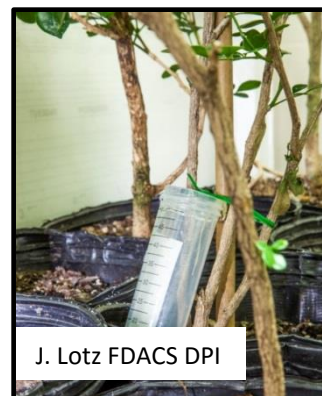


Figure 14: Releasing *T. radiata* adults for oviposition on ACP.

- (1) The number of adults released depends on the estimated number of psyllid nymphs on each plant, but is generally within the range of 450 - 550 per cage.
 - (2) *T. radiata* adults are released by placing the vial inside the rearing cage, uncapping and positioning it upright on the potting mix with the opening facing up and resting the top against the trunk of the plant.
- ii) ***Tamarixia radiata* Collection:** Collection of newly emerged *T. radiata* adults begin 10 days after the cage is setup and continues for up to 8 days. Normal collection times are from 10:00 am to 4:00 pm.

- (1) The general procedure for daily *T. radiata* collection is as follows (Except for the final day of collection).

(a) **Place collection jar:** Remove the restricting mesh from the opening for the collection jar at the top of the *T. radiata* rearing cage and place a clean collection jar upside down in the opening. Indicate the cage number, date, and strain on the jar label.

(b) **Attracting *T. radiata* to the collection jar:** Cover the cage with the black cloth hood with the black plastic sheet on top to create a point source of light through the collection jar to attract *T. radiata* adults into the jar.

(c) **Replacing the collection jar:** During normal collection times, the collection jars should be replaced hourly. Label another collection jar then remove and cap the jar full of wasps and place the newly labeled collection jar upside down in the opening quickly to prevent the *T. radiata* from escaping.



Figure 15: Removing restricting mesh for *T. radiata* adult collection.



Figure 16: Collection jar full of *T. radiata* adults.

Sort the full collection jar or store in the environmental chamber until you are ready to sort.

- (d) Ending the daily collection:** When sufficient *T. radiata* have been collected, as determined by daily release and rearing quotas, or before leaving for the day remove the black cloth from the cage to avoid overheating. Any unsorted jars should be stored in the environmental chamber overnight and sorted the following day.

NOTE: If a cage does not have a collecting jar at the top, adults must be aspirated directly from the cage [see (1)]. Shaking the plants will cause many adults to land on the side and top inner surfaces of the cage making aspiration easier.

- (2)** The procedures for the final day of collection are as follows.

(a) Plant removal

- (i)** Select one plant at a time. Vigorously shake the plant to dislodge any attached *T. radiata*. Then remove the plant from the cage and place it on a cart. Aspirate adults that remained on the plant, or that can be reached inside the cage [see (1)]. When 150 adults have been collected, tap the bottom of the vial on a hard surface to force adults away from the opening. Remove the vial from the aspirator and quickly cap it. Attach a new vial to the aspirator and continue collecting *T. radiata*.
- (ii)** Trim off all growth that supported psyllid nymphs or eggs [see i)], set the plant aside for final cleaning, and proceed to trim the next plant.
- (iii)** When all of the plants have been removed, aspirate all of the remaining *T. radiata* from inside the cage.
- (iv)** Fully wipe down each cage and perform necessary maintenance [see i)]. Clean all of the plants [see ii)], and take them to the designated area to be recycled [see e)].



Figure 17: Removing plant from *T. radiata* rearing cage.



Figure 18: Aspirating *T. radiata* from inside rearing cage.

- (3) Collections for rearing stock:** *Tamarixia* collected for subsequent rearing operations and for the colony maintenance should be collected from the 5th or 6th day of the collection cycle, preferably later in the afternoon, to ensure sufficient females are collected. Between 70%-80% females is desirable. Collection procedures are the same as those outlined above however, collection vials should be labeled as rearing stock and the labels must include the date of collection, number of males and females, and their strain. Rearing stock must be used within two days from the date of collection.
- iii) **Sorting:** After collection, the *T. radiata* are sorted in a hood to ensure that no contamination occurs and that vials contain the proper number of wasps [see **(1)**].

***Tamarixia* GENERAL MAINTENANCE SCHEDULE**

2D	D	W	Q	Y	N
Twice Daily AM/PM	Daily	Weekly	Quarterly	Yearly	As Needed

Monitoring Procedure	
2D	Scout cages for pest problems [see d].
2D	Verify environmental controls and monitoring systems are functioning properly.
2D	Verify proper operation of lighting system and timers.
2D	Document temperature, humidity and any pests noted for each rearing room in the respective Room Logbook.
Pest Management	
D	Remove any infested materials that were identified from cages.
D	Wipe down benches, work surfaces, and walls of any soil or other contamination.
D	Sweep floors and ensure proper operation of drainage systems.
D	Check water levels for table leg basins.
D	Verify no cables, hoses, or other objects are creating a pathway for ants.
Plant Maintenance	
D	Inspect soil moisture of plants and water as needed.
Cage Maintenance	
D	Inspect all cages for tears, rips, or cracks in the meshing, acrylic, edges and door seals.
N	Clean rearing cages thoroughly at the end of each cycle [see i)(3)].
Food Preparation	
N	Spread a thin layer of honey on one side of a paper towel and then press a second paper towel over the honey layer. Blot off any excess honey and store in a sealed jar [see c)i)].
N	Cut honey-soaked paper towels into 2 in. x 2 in. squares for use in rearing cages and ¼ in. x 3 in. strips for use in collection vials.

***Tamarixia* REARING SCHEDULE**

Rearing Cage Setup	
N	Identify the designated ACP development cage that has been given a minimum of 8 days of development after the date of transfer as labeled on the cage and plant container.
N	Verify the majority of ACP nymphs are 4 th instar.
N	Select the appropriate number of plants based on cage size, 12 for small or 24 for large cages, and place them on a rolling cart.
N	Transport plants quickly to the designated <i>T. radiata</i> rearing room.
N	Place plants into a clean <i>T. radiata</i> rearing cage.
N	Affix two 2 in. x 2 in. squares of honey-soaked paper towels to top of cage with twist ties.
N	Gather and release the appropriate number of <i>T. radiata</i> into the rearing cage [see i)]
N	Record all required information into the <i>Tamarixia</i> production logbook.
Rearing Cage Collection Period (10 days after setup for a duration of 8 days.)	
D	Remove the restricting mesh and simultaneously place a clean collection jar, supplied with a fresh piece of 2 in. x 2 in. honey-soaked paper towel, upside down into the collection opening and twist on loosely. Do this for all collection jars on cage.
D	Cover the cage with the black cloth hood then place the black plastic cutout over this. Ensure light can enter the cage through the collection jars only.
N	Label collection jar lids with the strain, cage number, and collection date.
H	Change out the collection jar(s) with newly prepared collection jars. Quickly place the new jar while simultaneously capping the full jar with a labeled lid.
H	Sort collection jars or store them in an environmental chamber.
D	When finished collecting for the day, fold the front half of the cloth hood along with black plastic cut out to the back of the cage so that at least half of the cage is uncovered.
D	Record daily data in the designated <i>Tamarixia</i> production logbook.
Final Collection for a Rearing Cage (18 days after setup)	
N	Select one plant at a time and vigorously shake off any <i>T. radiata</i> adults on the plant then remove the plant from the rearing cage.
N	Aspirate any <i>T. radiata</i> that remain on the plant into a collection vial.
N	Trim off any growth that supported ACP nymphs or eggs then set on a cart.
N	Continue removing the plants as described above until all plants have been removed.
N	Aspirate all <i>T. radiata</i> adults remaining inside the rearing cage.
N	Change collection vials whenever 150 <i>T. radiata</i> have been collected.
N	Record the total quantity of <i>T. radiata</i> collected from the cage in the designated <i>Tamarixia</i> production logbook.
N	Take plants to the designated sinks and clean them thoroughly [see ii)].
N	Place cleaned plants in the designated area to be recycled.

N	Clean the rolling cart.
N	Clean the rearing cage thoroughly [see i)(3)].
Sorting Collections [see SORTING PROCEDURE]	

SECTION V. STORAGE AND SHIPMENT

a) **STORAGE CONDITIONS:** The *T. radiata* are stored for short periods before being used for rearing or shipped to release sites.

i) **Environmental Conditions:** Vials containing *T. radiata* are stored in an environmental chamber.

(1) **Temperature-** $20 \pm 2^{\circ}\text{C}$

(2) **Relative humidity-** $55 \pm 5\%$

(3) **Photoperiod-** 14L:10D (6:00AM to 8:00PM)

ii) **Storage Duration:** Storage periods for the *T. radiata* are to be kept as short as possible because prolonged storage reduces egg numbers and parasitization rates. The *T. radiata* are stored for three days or less before shipment and release in the field.

b) **SHIPPING *T. radiata*:** Prepare *T. radiata* for shipment as close to the pickup time as is reasonable. Ensure the oldest *T. radiata* are shipped first.

(1) Place a frozen icepack in the bottom of the shipping container.

(2) A foam pad is placed immediately above the icepack to separate it from the storage vials.

(3) A prepaid and preaddressed U.S. Postal Service shipping label is included to facilitate return of the packaging materials.

(4) Shipments are sent via overnight courier, Monday-Thursday, and are picked up at approximately 3:30PM.



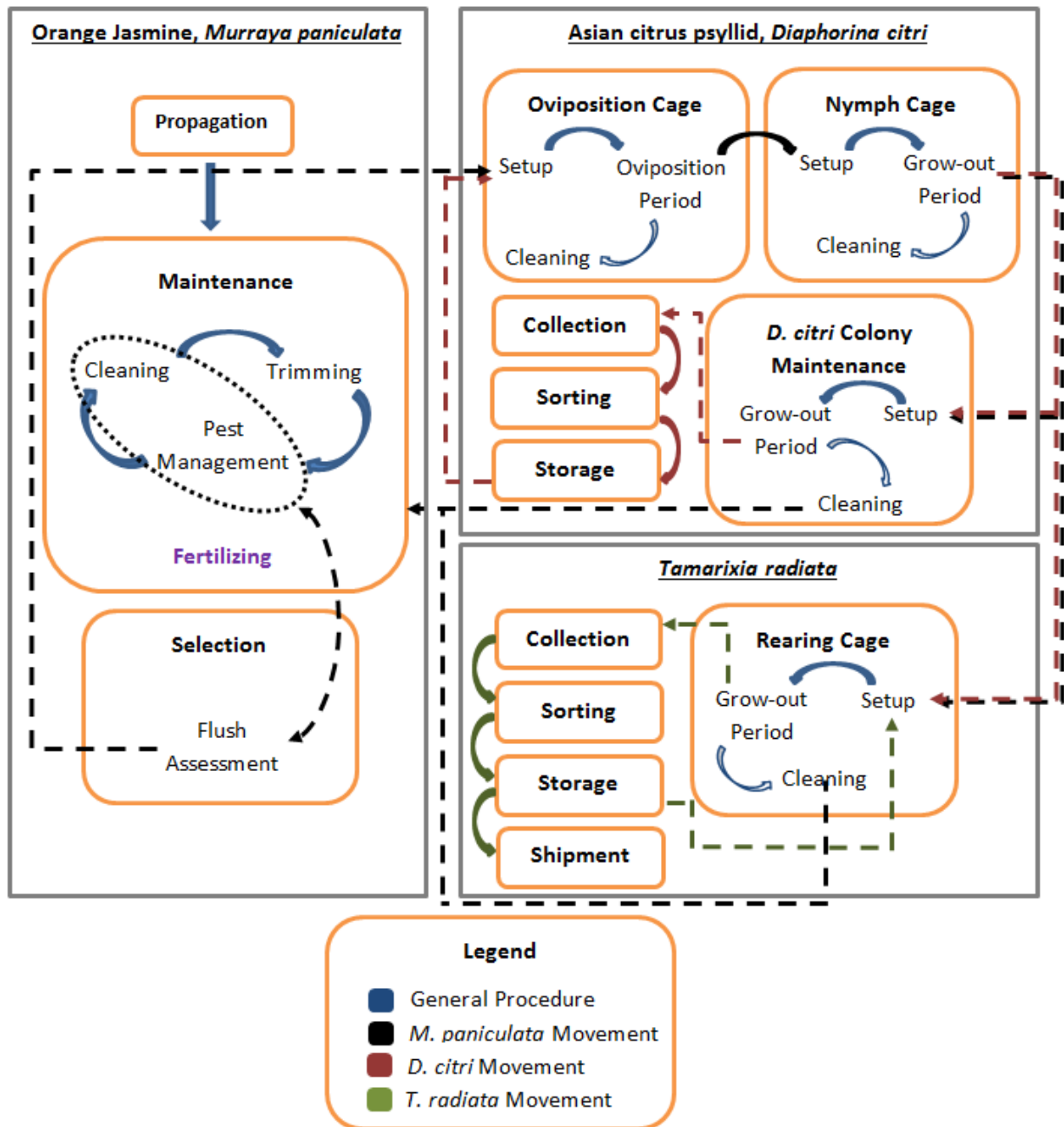
Figure 19: Packaging *T. radiata* for shipment.

SHIPPING SCHEDULE

2D	D	W	Q	Y	N
Twice Daily AM/PM	Daily	Weekly	Quarterly	Yearly	As Needed

Monitoring Procedure	
2D	Verify environmental controls and monitoring systems are functioning properly.
2D	Document temperature and humidity of the environmental chamber in the respective environmental chamber logbook.
D	Ensure only <i>T. radiata</i> or ACP, as designated, are stored within the environmental chamber.
Shipping	
N	Gather required number of <i>T. radiata</i> vials to be shipped. Select oldest vials first.
N	Package vials in accordance with this SOP [see b)]
N	Bring sealed packages to designated area for pickup.
Receiving Returned Materials	
N	Remove shipping labels
N	Open container and store individual components in the proper location.

APPENDIX A: REARING SYSTEM FLOWCHART



APPENDIX B: PEST IDENTIFICATION

Asian citrus psyllid, *Diaphorina citri*



Cowpea aphid, *Aphis craccivora*



Brown soft scale, *Coccus hesperidum*

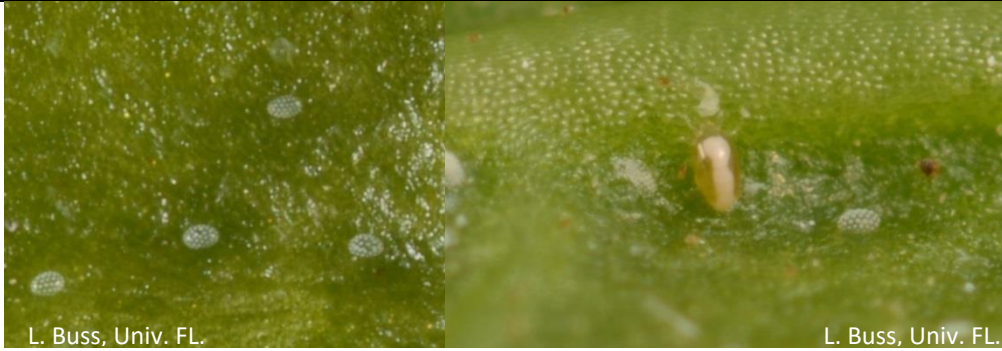


Citrus snow scale, *Unaspis citri*



APPENDIX B: PEST IDENTIFICATION

Broad mite, *Polyphagotarsonemus latus*



Citrus red mite, *Panonychus citri*



Twospotted spider mite, *Tetranychus urticae*

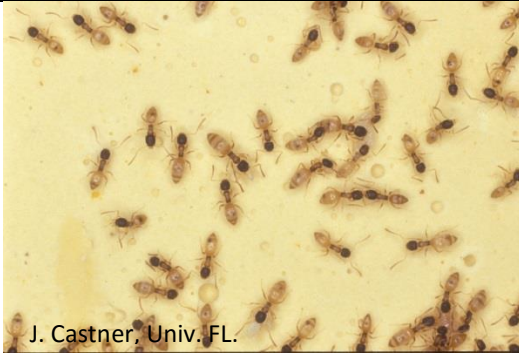


Sixspotted mite, *Eotetranychus sexmaculatus*



APPENDIX B: PEST IDENTIFICATION

Ghost ant, *Tapinoma melanocephalum*



J. Castner, Univ. FL.



L. Buss, Univ. FL.

Tawny crazy ant, *Nylanderia fulva*



L. Buss, Univ. FL.



L. Buss, Univ. FL.

Red imported fire ant; *Solenopsis invicta*



L. Buss, Univ. FL.



L. Buss, Univ. FL.

APPENDIX C: SAMPLE LOG WORKSHEETS

ROOM LOG

[illegible]

APPENDIX C: SAMPLE LOG WORKSHEETS

ACP PRODUCTION LOG

Oviposition

Room Log:		<u>COMMENTS</u>
Cage:		
Release Date:		
# of Plants:		
# of Psyllids Released:		
		Technician: _____

Development

Cage:		<u>COMMENTS</u>
Transfer Date:		
		Technician: _____

DAY	PEST/PATHOGEN PROBLEMS	COMMENTS
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		

APPENDIX C: SAMPLE LOG WORKSHEETS

Tamarixia PRODUCTION LOG

Room Log:		<u>COMMENTS</u>
Cage:		
Release Date:		
# of <i>Tamarixia</i> Released:		
# of Plants:		
Nymph Source Log:		
Psyllid Oviposition Period:		
		Technician: _____

Development (10 Days)

<u>COMMENTS:</u>

Collection (8 Days)

DAY	TAMARIXIA COLLECTED	PSYLLIDS COLLECTED	PESTS FOUND	COMMENTS
1				
2				
3				
4				
5				
6				
7				
8				
TOTAL				