STANDARD OPERATING PROCEDURE

Production of Tamarixia radiata and Diaphorencyrtus aligarhensis

April 12, 2017

INTRODUCTION

The Asian citrus psyllid (ACP) (*Diaphorina citri*) is an economically important pest of citrus. It was first detected in the United States in Florida in 1998. ACP has since spread to all citrus producing areas of the United States, including California.

Damages caused by ACP include distortion and death of new shoots, and excretion of honeydew, which allows the growth of sooty mold. Most importantly, however, ACP vectors the bacterium, *Candidatus* Liberibacter asiaticus, which causes citrus greening disease or "huanglongbing" (HLB). This is the most serious disease of citrus in the world, and causes reduced production and eventual death of infected trees.

The California Department of Food and Agriculture (CDFA) conducts a classical biological control program for the suppression of ACP using two imported parasitoids: *Tamarixia radiata* and *Diaphorencyrtus aligarhensis*, reared inside specially-designed containment facilities under a departmental permit.

This document serves as the Standard Operating Procedure for all facilities conducting ACP biological control under departmental permit in California. It will be reviewed periodically and updated as new or improved techniques are developed.

Table of Contents

CHAPTER 1. FACILITIES

1.1 General Facility Requirements

Production of parasitoids of herbivorous insects requires all three trophic levels: the host plant, the host insect, and the parasitoid. Separate rooms for each parasitoid species, host insects, and host plants are needed to avoid cross contamination. When planning the layout of the facility, location, traffic patterns, and number of rooms needed for plant production should be considered to mitigate contamination and assist in keeping the plant and host supply pest free, as well as a subset of pesticide-free plants for ACP production. To further minimize the effects of pest outbreaks, large facilities should utilize multiple rooms for each production element (parasitoid, host insect, & host plant), breaking up production into smaller manageable units.

1.2 System Requirements

The systems necessary for the production of ACP parasitoids include heating, cooling, lighting, and fertigation, aeration, and screening. The table below provides information pertaining to each of the systems necessary in an ACP parasitoid production facility.

Table 1. System descriptions and purchasing information.

System	Description	Manufacturer and Model # (if applicable)
Heater	Space heater to control minimum temperature	Hot Dawg Modine, Model Size 30-125, length 30 inch
Evaporative Cooling Pad	Moistened for maximal cooling	According to equipment specifications
Louver	For low level cooling	MotorMaster, PRIVA (S016530, IP66, Motor Control)

System	Description	Manufacturer and Model # (if applicable)
High Pressure Sodium and Metal Halide Lights	For maintaining optimal photoperiod, 14:10 L:D	Sun System
Benches	4 one gallon pots fit tightly into a 1 sq. ft. area. This can be used to calculate bench dimensions. Each bug dorm is 2.5 ft. by 2.5 ft. on the outside.	Agra Tech 5.5 ft. wide, expanded galvanized metal, rolling to optimize space usage
Environmental Control System	Computer operated cooling, heating, irrigation, etc.	Growmaster Procom
Fertigation System	For automatic watering and fertilizing of plants (not in cages), 1:100 feed rate, 20:20:20 Fertilizer	Dosatron (D45RE15, 20 GPM)
Screening	To prevent ingress and egress of insects. At least 60 x 60 mesh.	Green-Tek No-Thrips insect screen
Circulation Fans	To evenly distribute temperature	J&D Manufacturing Horizontal Air Flow (HAF) Fan

1.3 Rooms Types

Use of several rooms is necessary to isolate different components of production and mitigate damage from system failures and pest outbreaks.

Two general types of rooms are used in this program: Plant Production Rooms and Insect Production Rooms. Plant production occurs in four separate rooms: Seedling Room, Plant Staging Room, Plant Recovery Room and Recovered Plant Room.

Insect production occurs in the ACP Production Rooms and the two Parasitoid Production Rooms. Table 2 provides information for the different room types.

Room	Purpose
Seedling Room	Used to cultivate host plants. Isolating seedlings reduces contamination from recycled plants.
Plant Staging Room	Temporary storage for plant preparation going into or coming out of production. Infested plants from can also be removed another system for treatment. Treating and prepping plants in an isolated room can mitigate the spread of pests.
Plant Recovery Room	Stores plants that have been clipped down after use in production until they regrow and become fit to reuse (recycled).
Recovered Plant Room	Stores plants that are suitable for production. Harsh chemicals cannot be used and infested plants must be removed.
ACP Production Rooms	Production of ACP to provide starting material for parasite production. Isolated rooms to avoid contamination from parasitoids.
Parasitoid Production Rooms	For production of parasitoids. Divides production into 6 isolated rooms to limit the spread of pests.

Table 2. Purpose of room types.

1.4 Permit Compliance

Facility operators must obtain and comply with a permit issued by the CDFA Permits and Regulations. Permit conditions will include:

- Removal of leaves or treatment of plants before moving;
- Wearing of lab coats inside the greenhouses where insects are produced;
- Treatment of waste or items contaminated with ACP by freezing at -50 for 24 hours before disposal;
- Installation and use of screen material and double doors in all insect greenhouses

Additional permit conditions may apply.

Permit information and application can be found at:
https://www.cdfa.ca.gov/plant/permitsandregs.html

1.5 The CDFA Facility

CDFA produces *T. radiata*, *D. aligarhensis,* ACP, and host plants at three locations in Riverside, Kern, and Los Angeles counties.

Figure 1. Arial view of Arvin facility in Kern County

Figure 1. 40 X 60 foot Greenhouse at Cal Poly

Table 3. CDFA facility details

Location	Room	Description	Total square feet.
Mt. Rubidoux (Riverside County)	Seedling Room		8,800
	Recovered Plant Room		1,600
	Plant Staging Room		629
	T. radiata Production Rooms		4,000
	D. aligarhensis Production Rooms		807

Location	Room	Description	Total square feet.
Arvin (Kern County)	ACP Production Rooms		8000
Cal Poly Pomona (Los Angeles County)	Plant Production Rooms		2,160
	T. radiata Production Rooms		2,160

CHAPTER 2. HOST PLANT PRODUCTION

Figure 5. Production room

2.1 ACP Host Plants Used for Production

Bergera koenigi (curry) (formerly *Murraya koenigi L.), a* relative of citrus in the Rutaceae family, is the host plant of the Asian citrus psyllid used by CDFA for the production of ACP biological control agents. Curry is the preferred host due to its fast, abundant, and regular production of flush. It is also not known to carry HLB. The curry in this production system is augmented by a lemon rootstock variety (*Citrus volkameriana)* or "volk."

Production of the biological control agents depends on maintaining an adequate supply of mature, pest- and insecticide-free host plants with the right stage and amount of flush present. Other ACP host plants may be used instead of curry following similar procedures outlined in this document. However, the health of the host plant is critical. If host plants are unhealthy, infested with pests (e.g., aphids, mites, or mealybugs), contaminated with insecticides, or do not have suitable new growth, production of biological control agents will collapse.

2.2 Plant Production Material and Equipment

Material and equipment used for plant production are provided in Table 4 below.

Materials	Equipment	Pest Control Products
Pectinase	Strainer	Tempo SC Ultra
Bleach	Mesh Flats	JMS Stylet-Oil
Seedling Soil Mix	Seed Flats	Safer Brand Insect Killing Soap
Mature Plant Soil Mix	Tall Pots	Optigard Ant Bait Gel
Goggles	1 Gallon Pots	Akari 5SC
Lab coats	Dosatron with drip system	
Nitrile Gloves Gardening Gloves		
Jack's Professional Fertilizer 20:20:20		
1:100 feed rate		

2.3 Seed Preparation

Curry seeds are collected in the field. Seeds ripen in late fall through winter and need to be planted as soon as possible to reduce mortality.

The following steps should be followed to prepare seeds for planting:

- Remove seed from fruit and discard pulp;
- Rinse with water and strain;
- Soak seeds in pectinase solution for 12 hours;
- Rinse with water then soak in 10% bleach solution for 15 minutes;
- Rinse bleach solution off with water and air dry

2.2 Seed Germination

Table 4 Material and equipment used for plant production

Prepare a 1.5 foot x 2 foot seed flat with seedling mix. Make sure there is proper drainage.

Evenly spread 200 to 300 seeds in the seed flat and cover with ¼ inch of soil (about the same width as the seed).

Water as needed

2.3 Transplanting

Transplant seedlings into 1 gallon pots with the Mature Plant Soil Mix at around 3 to 5 months old.

If space is limited, seedlings can be transplanted to 3¼ inch pots at 3 to 5 months and then transferred to gallon pots at 7 months.

Once the seedling's main stem is approximately 1 centimeter thick, the plants are ready for production. Some seedlings grow faster than others but this usually occurs at around 12 months.

2.4 Repotting

Every 6-12 months, plants should be repotted into the same pot with new potting mix. After removing the plant from the pot, keep half of the soil, trim root ball, and then add new soil. Leave dirt around main portion of the roots. If plants are root bound, twist off at the bottom (trim).

2.5 Pruning

Prune plants as needed to induce flush growth and for pest control. If plants are not producing flush after 3-5 months, it may be necessary to prune the apical meristem to stimulate new growth.

Plants should be pruned in a manner that produces abundant oviposition sites and feeding locations. See Figure 6. for ideal plant structure.

Figure 6: The plant on the left has hardened and unfolded foliage removed and abundant oviposition and feeding sites. The plant on the right has oviposition sites but little feeding area

CHAPTER 3. HOST PLANT QUANTITY

3.1 Introduction

Tamarixia radiata has a six-week development cycle. ACP host plants, *B. koenigi* (curry) and *C. volkameriana* (volk), used to produce the host insects must be mature, pest- and insecticide-free and have an adequate amount of flush.

Host plants used in insect production are depleted of water and nutrients and must be rotated out of production after the six-week development period so they can recover. Recovery time is six weeks. Not all plants will fully recover, some plants may not have adequate flush to go back into production, and some plants may die.

Maintaining an adequate supply of suitable host plants will help prevent insect production collapse. This means that a steady supply of new plants must to be fed into the system to replace plants that have lost production potential or died.

This chapter explains how to calculate the number of host plants needed for full insect production, considering each of the following factors:

- Number of cages and plants used in production
- Plant recovery time
- Percent of recovered host plants have adequate flush for continued production
  Expected mortality rate of host plants

3.2. Number of Cages and Plants in Production

In the first week of production, set up thirty cages, each with eight curry plants and one volk plant. This results in 240 curry and 30 volk plants in production in the first week.

Each subsequent week for the next six weeks, the same number of cages, each with eight curry plants and one volk plant, are set up. At the end of the first six-week period, 1,440 curry plants and 180 volk plants will be in active insect production.

3.3 Plant Recovery Time

At the end of the six-week insect development period, the host plants must be rotated out of the cages and into the plant recovery rooms. Plant recovery takes six weeks. Every week starting at the end of the first six-week insect development period, the same number of plants that entered production (240 curry and 30 volk) are rotated out.

Calculation: #Weeks to Recovery (times) #Plants Used Weekly = # Recovering Plants

3.4 Percent Usable Recovered Plants

Once recovered, only 18% of curry plants (43 out of 240 plants rotated weekly) may have adequate flush suitable to re-enter production. Therefore, each week, an additional 197 curry plants (1,182 plants over the six-week insect development period) must be available to select from. (Calculation not done for volk.)

Calculation: #Curry per Week / .18 = # Recovered Curry Needed

3.5 Replacement Plants (% Mortality)

Not all of the plants survive this process and remain suitable for production. While mortality may differ between facilities, we found 6.3% mortality rate of our entire curry stock over a period of six months. At this rate, approximately 531 mature replacement plants need to be obtained annually.

- Calculation: (Total Curry Stock X % Mortality/100) X 2 = #Replacement Curry Plants Needed Annually

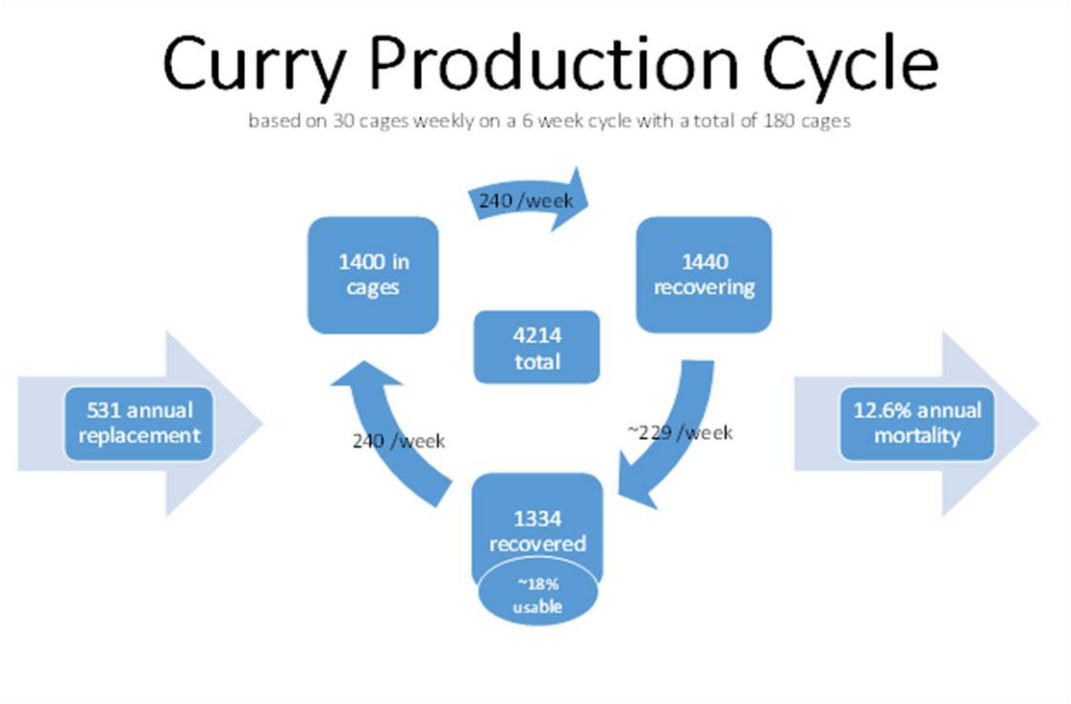


Figure 7. Curry production cycle

3.6 Total Host Plants Needed at Facility

Considering the factors outlined above, it is estimated that a starting stock of 4,214 curry and 640 volk are needed to maintain successful insect production.

Table 5 shows the number of plants that will be in active insect production in each of the first six weeks, and the total in production during the first six-week period.

Table 5. Plants in active insect production

Cycle Parameter	Curry	Volk
# Plants Per Cage	8	1
Usable Plants Recovered	18%	unknown
# Plants Rotated Per Week	240	30
Total Stock Requirements Calculation		
Total In Cages	1440	180
#Recovering	1440	180
#Recovered	1334	167
Total Stock Required	4214	527
12.6% Annual Mortality on Entire Stock		

CHAPTER 4. HOST PLANT CARE

4.1 Introduction

Healthy host plants with suitable new growth are required for insect production. Under- or over-watering and insufficient nutrition may decrease the health of the plants and negatively affect insect production. Additionally, if plants are infested with pests (e.g., aphids, mealybugs, and mites), contaminated with insecticides, or do not have suitable new growth, production will collapse.

This chapter provides information pertaining to host plant care before, during, and after the insect production cycle. Although this protocol only addresses the hosts, *B. koenigi* (curry) and *C. volkameriana* (volk), similar principles can be applied to any host plant used.

4.2 Watering

Watering needs will vary based on individual plant conditions, weather and seasonal changes, and production cycle progression. Large plants with lots of foliage will require more water than a small plant. Though seasonal change is predictable, daily weather is not and watering must be adjusted accordingly.

During the production cycle, plants are drained of water and nutrients by phloem feeding ACP. As ACP grow in size and number, the plant will need more water to support the ACP nymphs. Finally, when the plants are cut back, transpiration decreases and they require very little water. It is important to assess individual plants for under- or over-watering and accommodate watering schedules for hot and cold weather.

Under-Watering

Too little water can result in plant death or affect production if the plant survives. Early ACP nymphs are especially vulnerable because they cannot move to and feed on older leaves. When young flush wilts, nymphs are forced to intensify their feeding until both they and the flush are dead. Saucers can help prevent wilt by supplying plants with enough water to support nymphal growth and also aid in recovering wilted plants. Plants are under-watered if saucer and soil are dry and the plants are wilting.

The following steps should be used when assessing and correcting under-watered plants:

- Water the plant thoroughly. If under-watering is severe, the soil may repel water (depending on the soil type).
- Check to see if the water is draining rapidly into the saucer.
- Leave the saucer full and check in a few hours.
- If the saucer is empty after this time, fill it again and check again in another 2 hours or so.
- Plant should no longer be absorbing more water and the excess can be removed.
- Keep the soil moist but not waterlogged.
- Monitor plants individually for wilt and dry soil.
  Check for wilted plants during the hottest time of day.

Over-watering

An excess of water can cause algae growth leading to rot smell, encourage fungal gnats and cause damage to the roots due to a lack of oxygen. Ultimately over-watering will cause the plant to die and production to fail.

Small plants typically suffer more from overwatering. It is important to catch overwatering before the roots are damaged and growth is affected.

The following steps should be used when assessing and correcting over-watered plants:

- Check saucers before each scheduled watering

- If saucers are filled from previous watering, that plant may suffer from overwatering.
- Empty saucers
- Do not give additional water
- Check the plant at the next scheduled watering.
- If the soil is still damp at the second water check, continue to let it dry.
- If there are concerns about it drying out over the weekend, give it a reduced portion of water.
- Remove saucer if it smells of rot

4.3. Weather-Related Watering

Hot Conditions

Hot weather can occur any time of the year in California. If weather is sunny with daily high temperatures consistently in the high 80's or 90's watering schedules must be adjusted accordingly, even in the middle of winter.

The following watering schedule should be implemented in hot weather conditions:

- Plants should be checked every day and will likely need water every other day.
- Watering Monday, Wednesday and Friday is suggested
- If plants are not going to be checked over a long weekend or weather is especially hot, fill the saucer half way with water to keep the plants from wilting.

Cooler/Overcast Conditions

If daily high temperatures are in the low 80's or below and it is frequently overcast or raining, overwatering plants can become a problem regardless of season. The following watering schedule should be implemented in cooler/overcast conditions:

- Adjust watering schedules to two days a week.
- Avoid leaving standing water.

4.4 Fertilizing

Plants should have Osmocote® Classic 14:14:14 time-release nutrient beads already in the soil (1 teaspoon/gallon). However, the addition of liquid fertilizer with micronutrients in proper quantities can benefit new growth under the stress of ACP feeding.

The following steps should be following when adding liquid fertilizer:

- Add (3 ounce/gallon 20:20:20 Jack's Professional® Fertilizer) once a week via watering can or hose attachment.
- Do not over fertilize the plants. Watch for burn.

4.5 Pruning

Removing older leaves through pruning can mitigate wilting by decreasing transpiring surfaces on plants with abundant new growth. However, pruning is not always necessary. Mature foliage may benefit plants with small new growth by providing feeding area for larger nymphs and microclimates for adult ACP to escape the heat and stay moist. Older leaves may also provide a path for nymphs to access other plants.

Pruning, if conducted, should always occur before plants enter insect production cycle. Do not prune when new growth is too small. Pruning should be conducted:

- when humidity is high and mold is likely to be present;
- if foliage is densely packed and unusable;
- to mitigate wilt;
- to manage pests;
- to remove oiled or contaminated leaves.

4.6 Pest Control

Whenever possible avoid using plants with pest problems. Thrips and mites attack the young foliage making it unusable for ACP. They have also been found to feed on ACP eggs in some cases. Bud mites destroy young flush reducing oviposition sites.

If pests cannot be avoided, plants can be treated in one of the following methods:

- Remove unnecessary leaves
- Rinse with a pressure hose and then drench with Safer Soap.
- Allow to sit for 10 minutes; rinse again.
- The spray nozzle should be held close to the plant to physically dislodge pests.
- Volck Oil applied at least 2 weeks before being used in production.
- Spray plants with water nozzle before use
- If fungal gnats or other soil pests are an issue, netting can be tied around the pot to keep pests from emerging into the cage.

CHAPTER 5. HOST PLANT SELECTION

5.1 Introduction to Host Plant Selection

This production system uses the ACP host plants *Bergera koenigi* (curry) augmented by a lemon rootstock variety called *Citrus volkameriana* or "volk" which flushes throughout the year. Curry is a fast growing relative of citrus in the Rutaceae family that also produces abundant flush throughout the year. It has a pinnate leaf structure with an odd number of leaflets and is not known to carry HLB making it highly desirable for production systems.

When choosing which plants to use for ACP production, flush quality, plant size, and pest-cleanliness are critical factors to consider. If plants are unhealthy, infested with pests, contaminated with insecticides, or do not have suitable new growth, production will collapse.

Figure 8. Healthy B. koenigi

5.2 Flush Quality

Successful ACP production depends on the host plants having adequate oviposition and feeding sites. Plants must have new growth for oviposition, and more mature foliage for larger nymph feeding. Mature foliage also provides microclimates for adult ACP to escape the heat and stay moist, and a path for nymphs to access other plants.

Oviposition Sites

Adult ACP only lay eggs on new growth. Most eggs are laid in the young folded leaflets of the curry plant. Eggs may also be laid in the crevices and hairs further down large pinnate leaves at the base of the tender leaflets. (*See figure 9)* When selecting plants to enter into ACP production, always choose plants with:

- 10 or more oviposition sites
- tender, lighter green leaves
- a small un-expanded leaflet at the apex of the leaf.

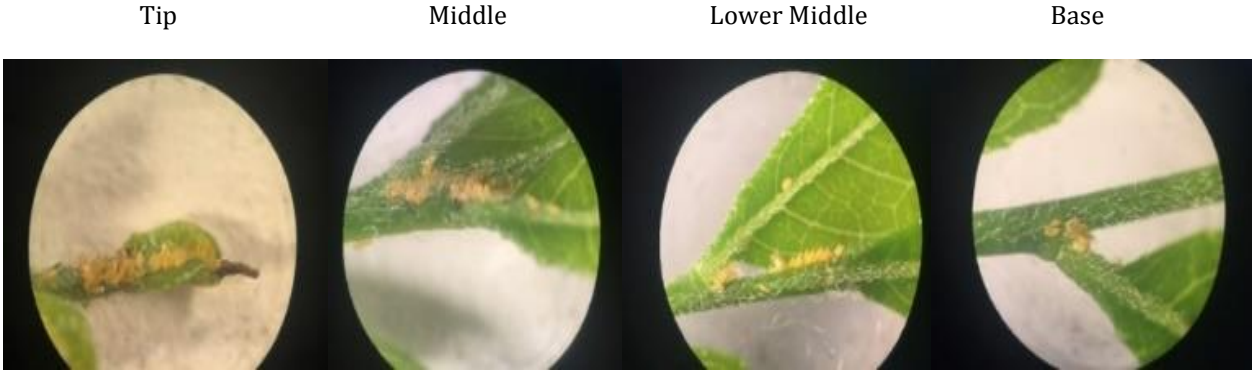


Figure 9: ACP eggs on a single curry leaf at four points from tip to mid leaf at the base of the leaflets

Feeding Sites

ACP nymphs must have room to grow and feed. If plant material is hardened or woody, young nymphs cannot insert their mouthparts into the plant, forcing them to cluster around and overwhelm small flush points. Overfeeding can kill the flush tips and excess honeydew from overcrowding can suffocate nymphs. Choose plants with long green stems and varying leaf ages.

Figure 10 shows curry plants with hardened and unfolded foliage removed. The plant on left has over 13 oviposition sites and abundant feeding area. The plant on the right has shorter flush against a woody stem where young nymphs will not be able to move and feed.

Figure 10: Plants with varying amounts of feeding and oviposition sites.

Figure 11 shows curry plants with both dark and mature leaves and new growth with oviposition sites. The plant on the left has suitable feeding and oviposition sites. The plant on the right has suitable oviposition sites but expanded foliage for older nymphs.

Figure 11: Plants with varying amounts of feeding and oviposition sites.

5.3 Plant Size

When stocking insect cages with plants, the goal is to provide plants with adequate new growth for oviposition, and overlapping branches which will aid nymph dispersal to new feeding areas. Typically, nine one-gallon plants (eight curry and one volk) with saucers can fit per cage.

Avoid stocking cages with plants that are so large that oviposition sites are blocked due to overcrowding. *(See figure 12)*

If plants are small with minute foliage, saucers can be removed and more plants added per cage to increase egg laying potential.

Figure 12. A plant with ACP overcrowding on flower buds.

5.4 Pest Cleanliness

Whenever possible, avoid using plants with pest problems. Thrips and mites attack the young foliage making it unusable for ACP, and have also been found to feed on ACP eggs in some cases. Bud mites destroy young flush, reducing oviposition sites. If pests cannot be avoided, plants can be treated as described in in Section 4.6.

5.5 Pruning

Pruning can be beneficial, but it is not always necessary. Pruning can mitigate wilt by decreasing transpiration surfaces on plants with abundant new growth. Pruning can also mitigate mold caused by high humidity, and can be used to manage other pests or to remove oiled, contaminated, or densely packed and unusable leaves. When conducted, pruning should occur before placing plants in cages.

CHAPTER 6. ACP INOCULATION

6.1 Introduction

Production of *T. radiata* and *D. aligarhensis* production requires a sustained, controlled population of the host insect (ACP). To achieve this, ACP must be reared for two purposes:

- To be used as host for the two parasitoids
- To be used to maintain a replacement population of ACP

This chapter provides guidelines for ACP inoculation, including the selection of the individuals used, inoculation method and timing, ACP dispersal, and collection and storage methods. Some aspects of ACP inoculation will differ depending on the purpose for which the ACP will be used.

ACP inoculation occurs during the first week after cage set-up, with egg laying beginning during the second week of the six-week parasitoid production cycle.

All insect production is carried out in screened cages. A number of producers make cages that are suitable for ACP and parasitoid production, and they can be made in-house. CDFA uses "Bug Dorms" as they are relatively cheap (https://shop.bugdorm.com/).

6.2 Selection of ACP Used for Inoculation

When selecting ACP for inoculation, consider the source, maturity stage, and ratio of ACP to plant number per cage.

Source

Use ACP that are reared, collected, and stored appropriately to eliminate disease potential and increase vitality.

Maturity Stage

Select the maturity stage of the ACP used for inoculation based on the purpose for which the ACP will be used.

For parasitoid production purposes, select mature ACP that are ready to lay eggs. This will result in an earlier parasitoid production start time, thereby extending the parasitoid production period, and resulting in higher numbers of parasitoids produced. However, the life span of mature ACP is about 30 days under greenhouse conditions, therefore, ACP productivity will decline over the six-week period.

For maintaining a replacement population of ACP, select newly emerged ACP. Although it can take three to five days for egg development and laying to begin, the productivity period of newly emerged ACP will last longer than that of mature ACP, resulting in higher numbers of ACP produced over the six-week period.

Vigor

The collection, storage, and dispersal processes can be stressful and/or damaging to ACP. (See Sections 6.3-6.4 for methods to reduce stress and damage during collection and storage.)

Strive to select ACP that appear healthy and vigorous in the vials for inoculation. ACP that look like they are having difficulty walking or are balling up at the bottom of the vial instead of crawling may be too damaged or stressed to lay eggs.

Ratio of ACP to Plant Number per Cage

The number of ACP needed will depend on the number and quality of plants, especially the number of flush points available. Ideally, in a cage of nine plants with adequate oviposition and feeding sites, inoculate with:

- 300-500 ACP

If not all of the flush points are being utilized, more newly emerged ACP can be used. However, if young nymphs are overcrowded and smothering each other in honey dew, fewer mature ACP, or more plants, may be needed.

6.3 ACP Dispersal

Ideally, ACP that were collected and stored properly will disperse evenly throughout all the plants in the cage. However, when first released from vials, ACP typically fly to the top of the cage and then settle onto the plants. This behavior can result in heat stress (temperatures can be over 10 degrees hotter at the top of the cage than in the room or the lower section of the cage) or disproportionate egg laying when settling on select plants.

To encourage even distribution and reduce heat stress:

- Tap the vials of ACP onto the plants spreading them evenly over the plants.
- Place the vials right side up in the shade in or beside the pots and allow any remaining ACP to crawl out.
- Place a strip of white paper at the top of the cage to shade it from the harsh afternoon sun. Do not make the squares to big because the plants at the bottom are not as warm and need the sunlight.
- Check the cage and vial for dead insects over the next few days. If insects are dead at the bottom of the cage and few ACP are seen on the flush, add fresh ACP and check collection and storage procedures.

If overcrowding is a problem on some plants and not others it is possible to clip branches with 3rd or older instars and place the leaves or branches onto unoccupied flush to distribute ACP. They will move onto other flush as long as the branches are touching.

6.4 ACP Collection and Storage

ACP are sensitive to desiccation, heat and light exposure, and time away from a food source. ACP can be exposed to drying and damaging conditions during collection and when stored in vials. These conditions can lead to ACP mortality, or may cause "sub-lethal" damage which may result in ACP that are unable to lay eggs.

To reduce stress and damage during collection and storage:

- Keep collection time to five minutes or less per vial
- Keep vials cool to reduce ACP activity. Do not use an ice pack; moisture in the vial will condense into droplets that can drown the ACP.

- Store vials out of direct sunlight and away from hot surfaces.
- Increase humidity in the vials. Pack down a damp cotton ball into the vial. Pack tightly to prevent ACP from becoming caught in the fibers. Cut a square of shop towel and pack it down on top of the cotton ball. If condensation occurs, reduce the amount of water used.
- Place collected ACP into cages the same day if possible. The longer the insects are stored away from food the less fit they will be. If this is not possible, store in a cool dark place with the moist cotton ball in the vial.

CHAPTER 7. *TAMARIXIA RADIATA* INOCULATION

7.1 Introduction

Tamarixia radiata are synovigenic-autogenous which means that while they do not require host feeding to develop their first set of eggs, host feeding is needed to continue to egg development.

For continuous production, schedule a set of cages to be set up once a week for six weeks, allowing each set of cages to run for six weeks before breaking down.

To designate a number of cages to set up per week, divide the number of available cages by the number of weeks in the cycle (6). After six weeks, the first set of cages set up will be ready to breakdown. Subsequent weeks will include a new set up, emergence, and breakdown each week

Table 8 shows an optimal six-week schedule of events.

Cycle Week	Event
1	*Bergera koenigi* (curry) and *Citrus volkameriana* (volk) are placed in sanitized cages and inoculated with ACP
2	ACP egg laying and nymph development
3	*T. radiata* are introduced in two batches to parasitize 4th and early 5th instars
4	*T. radiata* may begin emergence near end of week
5	*T. radiata* emergence
6	*T. radiata* emergence

Table 8. Schedule of events

7.2 *T. radiata* Inoculation

In order to maximize *T. radiata* production, the following factors must be considered:

- Ratio of *T. radiata* to ACP nymphs
- Inoculation Timing
- Number of Inoculations
- Condition of *T. radiata*

Ratio of *T. radiata* to ACP Nymphs

Fourth and fifth instar ACP nymphs are preferred for *T. radiata* parasitism while younger nymphs are more often used for host feeding. A female *T. radiata* is capable of killing 200 (to 300) nymphs through host feeding in her lifetime.

To reduce the effect of host feeding, use a ratio of approximately 1 parasitoid to 80 ACP nymphs when introducing *T. radiata.* Estimate the number of ACP nymphs by counting a half inch section (front and back) and comparing density **(**~1,000 nymphs per curry).

Inoculation Timing

When introduced to the cage, *T. radiata* tend to fly up to the top of the cage, where temperatures are likely to be hotter. If temperatures are too hot the *T. radiata* may die from exposure. To avoid this issue, introduce *T. radiata* into the cages in the morning. Allowing them to have cooler temperatures and time before sunset to find their way to the nymphs may help prevent mass death.

Number of Inoculations

Inoculating cages with *T. radiata* multiple times over the six-week production cycle will reduce the impact of host feeding (See Figure 15). ACP nymphs may become 4th instars while they are still very small and preferred for feeding by *T. radiata*. Putting too many *T. radiata* in with small nymphs may quickly become a massacre. Breaking up *T. radiata* inoculation into multiple batches will reduce the feeding impact on the young nymphs while promoting parasitism on older nymphs. Figure 15 shows when multiple inoculations could occur in the six-week production cycle.

F**irst inoculation**

Inoculate cages with 50 *T. radiata* when ACP 4th instars begin to develop. This will occur approximately 13 days after ACP inoculation, but frequently varies depending on temperature and when the eggs were laid. The majority of the ACP nymphs may only be 3rd instars. Monitor nymph development carefully to avoid missed opportunities for parasitism and to avoid excessive host feeding.

Second inoculation

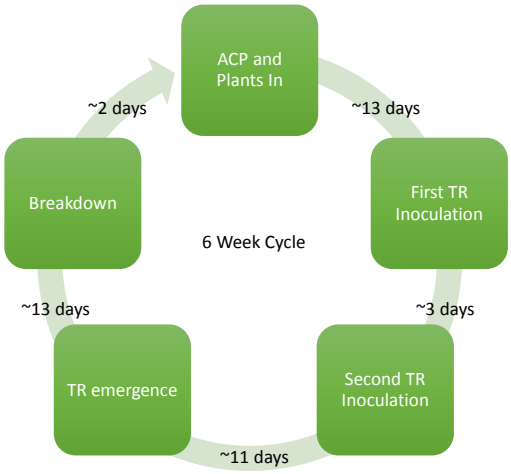
Check the cages two and three days after the first inoculation. If the majority of the nymphs are at the 4th and 5th instar stages, introduce the second batch of 50 *T. radiata*.

Third Inoculation (if needed)

If there are many nymphs with a wide range of nymphal stages including 1st and 2nd instars, plan a 3rd inoculation or increase the number released at the 2nd inoculation.

If there are very few nymphs in the cages, it may be best avoid a second inoculation altogether.

Figure 15. Multiple inoculations can occur during the six-week production cycle.



Tamarixia radiata Conditions

It is preferable to use *T. radiata* that are under four days old. If older wasps are used, ensure they have had adequate nutrition during storage. Storing *T. radiata* with only water for five days or more will cause egg reabsorption, reducing egg load to almost zero. Honey water provides limited nutrients but also lowers egg load. Inoculation using parasitoids in this condition can increase host feeding and delay egg laying, excluding older instars of ACP nymphs from potential parasitism.

T. radiata Emergence

Tamarixia radiata emergence should be evident approximately 11 days after inoculation with adult parasitoids.

Emerging *T. radiata* typically fly to the top of the cage, especially when warm. However, they often return to plants and are not readily visible. Check the stems, the top of the leaves and especially the undersides of the leaves for mummies with exit holes to help determine if emergence has begun.

After week five, production should be slowing and completely finished by week six. At this time, plants are ready for breakdown.

If ACP take longer to develop and *T. radiata* inoculations are delayed, production may still be going strong or even just starting during the sixth week. In this case, it may be better to postpone the breakdown and allow emergence to continue in the cages, especially if the inoculation took place less than less than 2 weeks prior.

If space is limited and keeping the cycle on a schedule is important, clipping the branches and storing them in a separate emergence cage may be the best option.

Production Numbers

Typical production generates on average 1000 *T. radiata* per cage.

CHAPTER 8. *DIAPHORENCYRTUS ALIGARHENSIS* PRODUCTION

Introduction

The production of the parasitoid *Diaphorencyrtus aligarhensis* resembles the production of *T. radiata* with the exceptions that it is a seven-week cycle and the 2nd and 3rd ACP nymphs are parasitized instead of the 4th and 5th instars. This rearing system, by necessity also utilizes indoor production rooms for emergence of the parasitoids.

Table 13 below provides the schedule of events during the *D. aligarhensis* production cycle.

Cycle Week	Schedule Summary
1	*Bergera koenigi* (curry) and *Citrus volkameriana* (volk) are placed in sanitized cages and inoculated with ACP at the end of the week.
2	ACP: egg laying and nymph development.
3	*D.aligarhensis* are introduced in two batches to parasitize 2nd and early 3rd instars.
4	
5	Plants with mummies are transferred to indoor emergence room and *D.aligarhensis* may begin to emerge.
6	*D.aligarhensis* emergence.
7	*D.aligarhensis* emergence.
8	Breakdown cages at the beginning of the week to prepare for the next set up

Table 13. D. aligarhensis production cycle events.

Production Room Setup

Production of *D. aligarhensis* resembles that of *T. radiata* with the following differences:

1. Place plants in cages as described for *T. radiata*, except use 15 curry and one volk.

2. Inoculate with 1,000 ACP

3. Begin inoculations (two) with *D. aligarhensis* after approximately 11 days. Sex ratios vary significantly from vial to vial so count the number of females per vial. Only females will be used for inoculation.

    - Inoculate with about 75 females when 2nd and 3rd ACP instars just start developing (about 11 days after ACP inoculation)
    - About three days later, inoculate with another 75 females.

4. Plant Transfer: This is an optional step to save greenhouse space in natural light and may be skipped if rearing space is available.

    - Transfer plants with mummies to emergence room
    - Transfer plants with associated cage tag one cage at a time to avoid miss labeling plants
    - Set grow lights to a 14:10 light dark schedule
    - Plants may need less water under artificial lighting
    - Once plants are transferred, clean out cages as described in Chapter 11 to prepare for new production

Emergence

D. aligarhensis will begin to emerge approximately five days after transfer. Expect approximately 1,000 *D. aligarhensis* per cage.

Collection

Collect insects as previously described. *D. aligarhensis* tend to hop away quickly and disperse themselves throughout the cage. Check for insects hiding on plants.

Labeling

Label vials as previously described, with the addition of the number of males and females collected.

- Use one vial per cage to record number of females and males produced per cage
- Number collected may be less than 100
- Do not collect from a different cage with the same vial
- Male and female counts can be done after collection is complete

Breakdown

Begin breakdown procedures approximately 20 days after plant transfer. Clean out cages and room as previously described at the beginning of the week to prepare for new plants to be transferred to the emergence room.

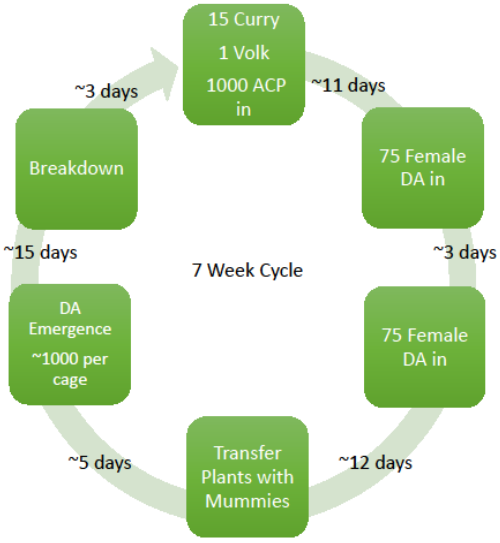


Figure 27. D. aligarhensis production cycle.

CHAPTER 9. BREAKDOWN

This chapter describes the cleaning procedures for the rearing rooms and cages and the plant material disposal method.

Cleaning Rearing Room

1. Remove all plant material and dirt from the floor and benches.

2. Check the room for any pest issue such as ants, mites, or thrips (etc.)

   If ants are present, rinse the trails with soapy water (as room allows), bait the room, and if possible leave the room empty until ants or other pests are gone.

   Occasionally spiders get into the cages and create nuisance webs causing problems for production and collection. Cages should be checked and excessive spider populations in the room should be dealt with, however these typically come in on the plants or around the edges of the pots and should be stopped there.

   If aphids, mites, mealybugs, thrips or other pests are in room or cages after using bleach and rinsing with water, it is best to leave the room empty.

   If pest treatment is necessary, use a chemical that degrades rapidly.

Cleaning Cages

1. Collect remaining parasitoids and ACP from the cages

2. Make necessary cage repairs

3. Remove cage tags and wipe them down with alcohol.

4. Check cages for mold and pests.

   If cages are mold/pest free and look relatively clean rinse the cages with soap and water. Scrub as needed and wipe down the front of the cage with vinegar where you want high visibility. Dry quickly to prevent spots

   If mold and/or pest insects are present, wipe down the entire cage with a 1:30 bleach solution. Let it sit for 10 minutes and then rinse. If mold is bad, a higher concentration may be needed. Keep area well ventilated and prevent bleach from contaminating vinegar, soap and other cleaning agents.

5. Check cages for visibility, if cages are cloudy wipe them down with vinegar water.

6. Remove and wash plastic saucers. Soak plant saucers in bleach water, scrub and rinse

Contaminate Disposal

Any plant material exposed to ACP during the process (eg. old clippings) must be quarantined and treated to ensure any ACP present are dead.

- Double bag contaminate.
- Freeze at -50° C for 24 hours.
- Dispose of in a trash receptacle.
- Containers with plant or insect material must be sanitized similarly.

Plant Recycling

Plants that have been used for insect production should be handled in the following manner before transfer to the plant recovery greenhouses:

1. Clip foliage and green stems from plants leaving as many main branches as possible. Curry has compound leaves that look like branches with small leaves. Remove these. If plants are extremely tall with no branches clip the main stem to about 5 inches to let the plant bush out.

2. Remove defoliated plant from cage. Spray with hose. Spray with foam gun. Rinse and spray again.

3. Visually inspect and transfer plants to recovery greenhouse.

4. Transfer clippings to clipping cages as quickly as possible to avoid death of insects. Do not leave clippings in plastic bags. Condensation and heat will kill mummies and emerging adult parasitoids. Store in parasitoid production conditions. Place 5-6 cages worth of clippings in a small bug dorm labeled with the date, room number

CHAPTER 10. INSECT COLLECTION AND STORAGE

10.1 Introduction

Insect collection is conducted using one of the following methods:

- Passive Collection Apparatus (PCA)
- Vacuum Pump

This chapter provides guidelines for establishing and using each method, as well as insect storage procedures after collection.

10.2 Passive Collection Apparatus (PCA)

Introduction

Passive collection is light intensity and temperature dependent. Insects are more active and likely to move through the mesh into the collection jar when temperatures are high and the sun is overhead. However, hot air being funneled into the jar can kill the insects if left for too long. Monitoring the temperature of the bags and jars is critical for effective collection and reducing mortality.

Materials and Equipment

The tables below list the material and equipment needed for the passive collection apparatus.

Tables 9-11. Equipment and material for the passive collection apparatus.

Item	Description	Purchasing Information
Table Saw	Or Rotary tool	
Drill Press		
Hole Saw		
Grinder		
Drill		
$^{1}/_{16}$ inch drill bit		
Kormacel plastic board squares	3 inch by 3 inch Kormacel plastic board squares with rounded corners	Plastic Place
White Pypropylene Cap		Model No. S-18012 at ULINE
Clear Plastic Round Wide-Mouth Jar	16 oz. with a 2 $^{3}/_{16}$ inch	Model No. S-19465 at ULINE
#4 Flat Phillips Screws	preferably $^{3}/_{8}$ inch long	Lowe's
Black 95-96 gallon trash bags	3.0 mil thick, 61 inch W by 68 inch H	Plastic Place
Black plastic sheeting	4-6 mil thick, 3 ft. wide, desired length varies based on length and number of benches being fitted	Amazon.com
Screw Eye Hooks	½ inch by ½ inch or larger eye (1.5 inch by 5/8 in)	
Bamboo Stakes	~10 inch by ½ inch	
Mason line	Size #1 braided nylon, mildew resistant	
White twist ties		
S70 Silicon Rubber O-Ring	30 X 140 or smaller 2 ¼ inch inner diameter and 2 $^{7}/_{16}$ inch outer diameter	The O-Ring Store LLC

Cloth Mesh	**Fine nylon, ~1 mm diameter holes**	
Sewing equipment	Needle and white or black thread.	
Weldbond Universal Adhesive	Glue for alternate filter creation technique.	
Seal from jar lid	White plastic seal found in the jar lids. Used to plug opening of PCA on the cage. Used to create alternate filter (circle is cut in seal and mesh is glued to mesh).	
Wax paper	Used to place alternate filter on while drying for easy removal.	

Materials	Equipment
Jars Insect Filter 3 mil black plastic bags 4 mil black plastic sheets	Passive Collection Apparatus (PCA) on Bug Dorm PCA Stopper

Assembly

Follow these steps for assemble of the passive collection apparatus:

1. Cut Kormacel plastic board into 3 inches by 3 inches squares

2. Use drill press with hole saw to cut holes into the center of the 3 inches by 3 inches squares and plastic lids.

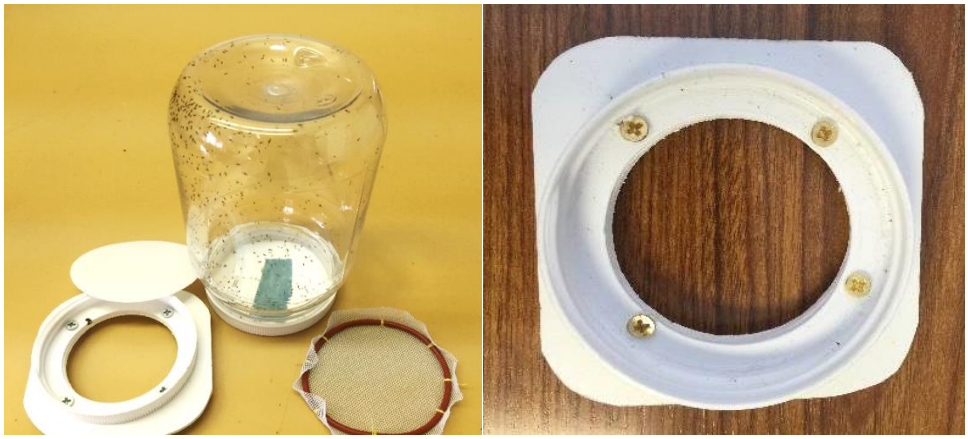
3. Grind down corners and sharp edges. Smooth with file if needed.

4. Pre-drill holes in squares and lid for screws with $\sim 1/16$ inches or slightly larger drill bit. To make the screws lay flush with the lid, create a groove for each screw by using a larger bit and setting the drill to reverse. This will help the screw lie flush so that the filter sit flat on the lid.

5. Mark matching holes for lid and square with a sharpie and twist tie the lid to the square. These parts will not be interchangeable.

6. Tip: shorter screws (#4, $3/8$ inches) are easier to screw in

Figure 16 shows components of a Passive Collection Apparatus.



Attachment to Bug Dorms

Follow these steps to attach the PCA to the Bug Dorm:

1. At the top, secure Bug Dorm plastic between the lid and square and tighten down #2 or #4 screws (shorter screws ¼ to $^{3}/_{8}$ inches long are easier to work with). Place the board as high as possible angling the board so that one of the rounded edges points to the top of the Bug Dorm. The insects will try to get to the highest point. Let the board overlap with the seam of the Bug Dorm at the top and sides to give more support. This will also help prevent trapping insects between the board and the Bug Dorm plastic as they make their way towards the light.

2. Support the Kormacel square from the inside of the Bug Dorm while tightening the screw to avoid ripping or deforming the plastic.

Figure 17. Preparing a Passive Collection Apparatus

Affixing Passive Collection Apparatus Support Structure

1. This structure prevents the plastic at the top of the Bug Dorm from deforming over time under the weight of the jar and Kormacel structure.

2. Make a small hole in the bottom corner of the Kormacel board so that the eye hook will be easier to screw inch Screw in ½ inches by ½ inches eye hooks into the bottom corner of the Kormacel board through the Bug Dorm plastic being careful to not stretch the Bug Dorm plastic or leave gaps between the Kormacel board and the Bug Dorm.

3. Place 10 inches bamboo stakes (about ¼ inch diameter- chosen for cost and light weight) on top of the fiberglass rods supporting the Bug Dorm and affix the ends to the fiberglass using twist ties or other suitable material.

4. Take a 6 inches or so piece of braided Mason line and loop it through the eye hook. Tie the string loosely to the bamboo. It just needs to be tight enough to take the pressure when pressing down with the jar.

Figure 18 (right) PCA Support Structure

Filter Insert Creation

Follow these steps to create the filter insert:

1. Tape the rubber o-ring to the top of the jar lid and place the mesh over the o-ring so that the mesh is flat but not taut. Over stretching the mesh can cause the o-ring to not sit flat in the lid and can deform the mesh allowing ACP to get through.

2. Use a second o-ring to hold the mesh firmly in place.

3. Sew through the mesh close to the o-ring keeping individual holes from stretching and loop the thread 4 or 5 times at 8 points evenly spaced around the o-ring. Placing a finger on the top of the mesh can help prevent stretching from sewing.

4. Avoid using yellow thread as it could be attractive to ACP

Figure 19. Steps for filter insert creation

Procedure for PCA Use

1. Collection conditions **r**equire direct natural light, overhead light angle (afternoon)**,** warm temperatures

2. Passive Collection Set Up

    - Attach Passive Collection Apparatus to Bug Dorm
    - Place black plastic sheeting beneath Bug Dorm, it should extend beyond the bottom edges of the cage
    - Remove stopper from PCA lid
    - Place mesh filter in the PCA lid
    - Filter can be left in the lid for future use with the stopper placed on top when not collecting

3. Attach. Make sure mesh filter is lying flat. If filter is twisted or wrinkled ACP may get through edges that have popped up

4. Cut a hole the size of the jar in the top of the bag. Do not make the hole too large

5. Cover Bug Dorm quickly and completely with 3mil black trash *bag (see Figure 20)* so that only the collection jar pokes through the top of the bag

6. Wasps will follow the light down to the bottom corners and holes in the plastic. Covering the Bug Dorm quickly prevents wasps from crawling down. Tuck the edges of the trash bag under the bottom of the Bug Dorm. Constrict the hole around the jar with a rubber band so that light only enters the cage through the jar.

Collection Duration

Duration of collection time is temperature dependent. At moderate temperatures (low 80s), collection should last for 30 minutes. Insects should be moving slowly. Jars should not be hot to touch.

At hot temperatures (high 80s to 90s), collection should last for 5-15 minutes. If the bag and jar feel hot, adjust time to avoid killing insects. Insects should move quickly through the filter with few left in Bug Dorms.

Do multiple collections if insects are left in Bug Dorms.

Check mesh filter for dead insects during and after use. Few *T. radiata* should be left in the Bug Dorm after successful collection.

Figure 20. Bug dorm with black plastic bag ready for passive collection.

Other Light Sources

Other light sources are not as effective. High pressure sodium and similar lights produce too much heat above the jars and cook the insects. Check filter for dead insects to adjust collection time and light intensity. Overnight or long collections with black lights unsuccessful so far.

Passive Collection Troubleshooting

If the passive collection does not seem to work, check the position of the insects.

1. If insects are alive in the Bug Dorm but not moving from the Bug Dorm into the jar:
    - Increase the temperature in the room or Bug Dorm
    - Increase the collection time to 30 minutes
    - Increase the light intensity or adjust the angle to overhead

2. If insects are alive in the Bug Dorm but clustered in the Bug Dorm:
    - Check for gaps or holes in the bag that expose light to that part of the Bug Dorm.
        - Bottom of the Bug Dorm 4mil black plastic bench lining
        - Bottom corners of the Bug Dorm between the bag and the bench lining
        - Sides of the bag
        - Top hole around the collection jar. If hole is too wide *T. radiata* may not move to the filter
        - Check for creases in the Bug Dorm where *T. radiata* get stuck. Is plastic around the jar sagging? *T. radiata* often crawl to the highest point.

3. If insects are dead at the bottom of the Bug Dorm it may be due to possible overheating or starvation.
    - Check temperature in the Bug Dorm at hottest time of the day

- Provide honey
- Collect more frequently

4. If insects are dead on top of the mesh filter, insects are most likely overheating in the Bug Dorm or jar.
    - Decrease temperature
    - Decrease collection time to 10-15 minutes

5. If insects are not visible, it is possibly due to late emergence, poor emergence, or they are hiding on the plants or dead.
    - Check for mummies.
        - Mummies with exit holes means that *T. radiata* have emerged. Tap plants and check bottom, sides and plants to find *T. radiata*
        - Mummies without exit holes means that *T. radiata* have not yet emerged
        - No mummies, only healthy live nymphs or adult ACP means that parasitism did not take place.
    - Check timing of *T. radiata* inoculation
    - Tap the plants and check for *T. radiata* on the bottom, sides and top of the Bug Dorm
    - Check for dead *T. radiata* on the leaves and bottom of the Bug Dorm. There should only be a small number dead from the parent generation.

10.3 Active Collection - Vacuum Pump Collection

Introduction

When using vacuum pressure to collect insects it is important to consider what kind of physical damage or stress the insect may be under due to the collection apparatus, the handling time, storage conditions and density in the vial. Some stresses lead to immediate death, however sub lethal effects can also occur that affect the fitness or decrease shelf life.

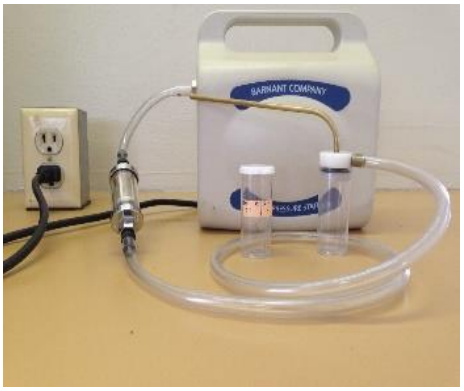


Figure 21. Vacuum pump connected to an aspirator head and collection vial.

Figure 22. Aspirator head used for vacuum pump collections.

Materials and Equipment

The table below lists the material and equipment needed for vacuum pump collection.

Item	Description	Purchasing Information
Aspirator	**Vacuum pump**: Barnant Co. model no. 400-3910, 115V **Aspirator head attachment** **Vials**: Bug-Vac #1, 1 inch diameter **9 Dram polystyrene snap cap vials** with a 1 inch (25.4 millimeter) inside diameter **Bug-Vac #2**, 1 1/8 inch diameter **12 dram polystyrene snap cap vials** with a 1 1/8 inch inside diameter **Plastic tubing**: 1 cm diameter (3/4 centimeter inside diameter) **In-line fuel filter**	http://store.clarksonlab.com/4003910.aspx http://www.roseentomology.com/Aspirator%20pages/BV1%20page.htm# http://www.thorntonplastics.com/9-dram-vial.html Briggs&Stratton part no. 691035
Shop towel	Scott Shop Towels	
Honey	Wild flower or local raw	
Water		
Cooler		
Foam Pad		
Ice Pack		
Incubator	Percival model 1-30BLL	

Table 12. Material and equipment for vacuum pump apparatus

Procedure for Use

1. Place pump on a secure dry surface

2. Keep extension cord dry and out of the walk ways

3. Turn on pump and check pressure. Pump should not be blowing air. Pressure should be no more than 35 psi. Overly high suction pressure could damage or even kill insects as they hit the sides of the collection tube or the wall of the vial.

4. Place the tip of the aspirator over or angled towards the insect. Be careful not to squish the insect against the side of the cage. After collection affix each vial with a tape collection tag (Details in Sec 5.3)

5. Look for *T. radiata* in the part of the cage nearest the sun. *T. radiata* frequently fly to the top of the cage after emergence and when it is warm.

6. If *T. radiata* are not visible, tap the plants and check the sides and bottom of the cage. *T. radiata* should fly up from the bottom in a few minutes. Repeat tap if needed

7. Avoid collecting for longer than 10 minutes per vial. Over exposure to vacuum conditions may damage and desiccate insects.

8. Do not collect too many insects into a single vial. This may also cause unnecessary stress. Optimal density is 100 *T. radiata* or ACP per 9 dram vial. Larger vials may hold more. Insects are counted individually as they are being collected with the aspirator.

9. A vial of ACP should not sound like popping corn. If insects are too active it is best to cool them down, put them in a dark place, collect fewer per vial or release them as soon as possible to keep them fit.

10.4 Handling and Temporary Storage

Once collected, using either the passive or active collection methods, insects should be kept out of direct sunlight and away from hot surfaces. Cooling down the insects so that they are less active in the vials may keep them from jumping and damaging themselves against the side of the vials, however they should never sit directly on an ice pack. Coolers should be equipped with a foam pad about an inch thick (depending on the pad) between the ice pack and the vials of insect and kept out of direct sunlight.

Vials of *T. radiata* are stored in a dark incubator at 18° C with honey water. Shop towels are cut into half inch squares and placed in a 50-75% honey solution. Drain excess liquid. Use forceps to transfer honey squares to caps. Dab off any excess liquid to prevent *T. radiata* from getting stuck in the honey. Vials of *T. radiata* stored in this way have survived for more than 2 weeks.

Cooler Preparation

- Place ice pack on the bottom of the cooler
- Place a foam pad on top of ice pack
- Do not let vials of insects touch the ice pack directly
- Place vial in cooler on top of pad after collection
- Keep cooler in the shade

Figure 23. Cooler with ice pack and foam pad.

Figure 24. Cooler with foam on top of ice pack in preparation for insect storage.

Storage Vial Diet Preparation

- Cut shop towel into half inch squares
- Soak squares of shop towel with 50-75% honey in water solution
- Drain excess liquids from lids so that no standing solution is on the lid. Insects will drown in droplets of honey water.
- Place squares on lid and dab excess liquid from lid. Use clean forceps to avoid contamination.
- Honey water should secure the square in place.
- Recap vials and store in a Ziploc in the cooler.

Figure 26. Collection vials with honey food sources (blue squares) attached to lids.

CHAPTER 11. DATA COLLECTION

11.1 Introduction

Data collected should include the number of plants per Bug Dorm, the number of ACP introduced per Bug Dorm, and the number of *T. radiata* collected per Bug Dorm.

Additional information such as pest treatments, variety of plants, and soil types can also be recorded.

To manage this information, each room is designated with a unique alphanumeric code. Bug Dorms in each room are also numbered.

A data tag, referred to as a "Cage Tag", is associated with each cage and labeled with room/cage codes, the number of insects introduced into the cage, and the date of introduction.

The number of *T. radiata* produced are recorded by tagging each collection vial with the room/cage code, date, and number of *T. radiata* collected. These tags are referred to as a "Collection Tags". At the end of the day, all data is transferred to excel spreadsheets via a "Room Datasheet" that compiles all of the Cage Tag data for a specific room.

11.2 Material and Equipment

Materials	Equipment	Programs
Sharpie® Ultra Fine Point Marker VWR® laboratory tape Velcro® tape dispenser data sheets scissors	Computer Printer Laminator with plastic	MS Excel

Table 6. Material and equipment for data collection

11.3 Data Tags and Data Sheets

Table 7 lists the purpose for each of the data collection documents and the information to be collected on each.

Data Tags and Data Sheets	Description
Cage Tags	A physical tag attached to each cage used to monitor the progression of each cage during a cycle. Includes room code, cage number and number/type of insects introduced into the Bug Dorm and the date for each introduction event. Cage Tags are printed, laminated, and attached to the cages with Velcro. The soft side of the Velcro is attached to the tags by convention and distance between Velcro sections should be standardized so that tags are interchangeable.
Room Datasheets	A physical sheet that compiles the information from all of the Cage Tags for one room (or set up) onto one sheet that is easily transferred to an excel sheet. If the Bug Dorms are otherwise numbered and plants are not transferred to a different location during the cycle, Room Datasheets can be used without Cage Tags.
Collection Tags	Physical tags for labeling individual collection vials and jars at the time of collection. Data includes production room code, cage code, date, collector's initials and number of parasitoids collected. VWR Laboratory Tape is used to generate Collection Tags. Collection Tag data is entered into an Excel spreadsheet at the end of collection. Collection Tags are useful for tracking the number of parasitoids in a vial or jar and can be easily transferred to release books or Cage Tags to monitor where specific insects go.
Inoculation and Collection Spreadsheets	An Excel spreadsheet used to organize production data digitally. Individual sheets are then printed and stored in binder as hard copies. Spreadsheets can also be copied to a cloud service such as Google Drive to back up data. Creating one sheet per event prevents errors in previous datasheets.
ACP Inoculation Sheet	Data includes room/cage code, dates of inoculation, type and number of plants per cage, and type and number of ACP per Bug Dorm for one cycle

Data Tags and Data Sheets	Description
TR Inoculation Sheet	Data includes room/cage code, dates of inoculation, and number of parasitoids per Bug Dorm for one cycle
Collection Sheet	Data includes room/cage codes, date, and number of parasitoids collected for one day of collection
Master sheets	Individual sheets from each category are compiled to create 3 master sheets to be used in Summary Data
Summary Data	Pulls data from the 3 master sheets for data analysis

Table 7. Description of data tags and sheets

11.4 Collection Tag Guidelines

The following information should be placed on Collection Tags.

- Date
- Place date on each vial in upper left corner of Collection Tag (tape). A single vial is assumed to be collected all on the same day. You do not need to write the date multiple times
- Format: mm/dd/yr (e.g. June 17th, 2016 would be 6/17/16)
- Place room code beneath the date followed by the cage# (R#-C# or 2A-10)
- If collecting from a dorm of clippings, include all cage numbers marked on the dorm (Room#-Cage#-Cage#) e.g.: 2B 1-10
- Make a new label for each new cage collected from. (i.e.; if you collect from 3 cages, make 3 labels indicating how many parasitoids you got from each cage *(See Figures 13 B-D).*
- Quantity of parasitoids collected from each cage. Place "QTY" in the top right of the label with the number collected beneath it or to the right.
- The total per vial should add up to 100
- Collectors Initials. Write your 2 letter initials on the vial to the right of the Quantity on the first tag. You do not need to write your initials multiple times on a vial

Log in Collected Tags Daily

CHAPTER 12. TROUBLESHOOTING

What to check when there is low or no production

12.1 Introduction

There are many variables that can cause production to struggle or collapse making it difficult to determine the cause or causes. This section outlines critical points that can be checked on a regular basis to reduce the risk of collapse in the order they occur in the production cycle sequence.

Potential issues

- ACP not laying eggs
- ACP eggs not hatching
- Nymphs not developing before parasitoid inoculation
- Nymphs disappearing before parasitoid inoculation
- Nymphs disappearing after *T. radiata* inoculation
- Parasitoids not emerging
- Parasitoid low emergence
- Parasitoids dying in vials

Checks

- Plant Check
- ACP Check
- Pest Check
- Wilt Check
- Parasitoid Age
- Parasitoid Number
- Parasitoid Collection

1) ACP not laying eggs
   a) **Plant Check:** Before going in the cages, check plants for:
      i) Adequate oviposition and feeding sites (see section 5.2)
      ii) Pests (see section 5.4)
      iii) Pesticide residues
   b) ACP Check (see section 6.2 & 6.3)

i) Before going in the cages, check collected vials of ACP for:
(1) General activity
(2) Balling up at the bottom
(3) Humidity
(4) Maturity
ii) After going into the cages check for:
(1) Grouping
(2) Heat stress

2) ACP Eggs not hatching
a) Predation by pest insects
b) Competition from pest insects
3) Nymphs not developing or dying before parasitoid inoculation
a) Temperature
b) Overcrowding
c) Pesticide
d) Predation or competition by pest
4) Nymphs disappearing before parasitoid inoculation
a) Check bottom of cage for dead nymphs
b) Pest check
5) Nymphs disappearing after *T. radiata* inoculation
a) Host Feeding
i) Ratio of *T. radiata* to ACP nymphs
ii) Age of nymphs
iii) Age of *T. radiata*
b) Early ACP emergence
6) Parasitoids not emerging
a) Mummy check
i) If yes then:
(1) tap plants
ii) Check bottom of cage for dead parasitoids
b) Mold check
7) Parasitoid low emergence
a) Mummy check
i) If yes
(1) Collection Methods
(2) Check bottom of cage for dead parasitoids
(a) Temperature
(b) Pest
(c) Lack of food
ii) Few mummies, lots of ACP adults

(1) Inoculation timing
(2) Parasitoid health/egg load
iii) Few mummies, few ACP adults
(1) Check inoculation

8) Parasitoids dying in vials
a) Age
b) Collection Methods
i) Desiccation
ii) High Pressure
iii) Overcrowding
c) Storage Conditions/ Handling
i) Temperature
ii) Humidity
iii) Diet
(1) Mold
(2) Dry honey pad