Manual for the mass production of the parasitoid *Tamarixia radiata* on its host, *Diaphorina citri* (Asian citrus psyllid), cultured on *Murraya paniculata* (Orange Jasmine) in Belize

TCP/BZE/3402 – Assistance to manage Huanglongbing (HLB) in Belize
Manual for the mass production of the parasitoid *Tamarixia radiata* on its host, *Diaphorina citri* (Asian citrus psyllid), cultured on *Murraya paniculata* (Orange Jasmine) in Belize

AGP: TCP/BZE/3402

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<tr>
<td>ACP</td>
<td>Asian citrus psyllid</td>
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<tr>
<td>BAHA</td>
<td>Belize Agricultural Health Authority</td>
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<td>CGA</td>
<td>Belize Citrus Growers Association</td>
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<td>CPBL</td>
<td>Citrus Products of Belize Limited</td>
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<td>CREI</td>
<td>Citrus Research and Education Institute</td>
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<td>FAO</td>
<td>Food and Agriculture Organization of the United Nations</td>
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<td>GOB</td>
<td>Government of Belize</td>
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<td>HLB</td>
<td>Huanglongbing (formerly Citrus Greening Disease)</td>
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<td>SLC</td>
<td>(FAO) Sub-regional Office for the Caribbean</td>
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<td>TCP</td>
<td>Technical Cooperation Programme (of the FAO)</td>
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Background

The citrus industry is very important to the economy of Belize in terms of employment, livelihood of rural communities, exports and local production. Since May 2009, the industry has been under threat of devastation from Huanglongbing (HLB), formerly known as Citrus Greening Disease. In response to the outbreak, industry stakeholders including growers, government and processors have undertaken a national response to reduce the incidence and spread of the disease, which is vectored by an insect called the Asian citrus psyllid (ACP), *Diaphorina citri*. All citrus varieties are susceptible to HLB and there is currently no known cure for the disease. Management of the disease (via reduced incidence of the vector and improved plant health) is the only viable option to mitigate the impact of HLB on the production and productivity of the groves.

Based on a request for assistance from the Government of Belize (GOB), the project TCP-BZE-3402 “Assistance to manage Huanglongbing in Belize” was formulated under FAO’s Technical Cooperation Programme (TCP) to fill gaps by enhancing the country’s capacity to reduce the impact of HLB.

A mid-2009 delimiting survey showed that HLB incidence was greatest along the east coast and was prevalent in the small coastal towns and villages that border the commercial citrus groves in the Stann Creek District. By 2010, nearly all back-yard citrus trees in the coastal communities in this area were infected, and some trees and groves showed obvious signs of decline. Since pesticide application is not recommended and reduction in vector populations in abandoned orchards and backyard citrus is critical, the use of effective natural enemies in a biological control programme became an important consideration. The parasitoid, *Tamarixia radiata* (which was fortuitously introduced to Belize) is one of the potential natural enemies used extensively for ACP control in many countries. Methods have already been developed for its mass production under different conditions. Under the project, suitable infrastructure for the production of *D. citri* and *T. radiata* was constructed and existing production methods were modified / adapted to suit Belize conditions.

The present Manual outlines the requirements and describes the procedures for the mass production of the parasitoid *T. radiata* on its *D. citri* host in Belize. It is collated by the Lead Technical Officer¹ for the project in close collaboration with the staff of the Citrus Research and Education Institute (CREI – the Research and Development arm of the Citrus Growers’ Association of Belize - CGA).

The key requirements for the mass production of the *T. radiata* are:

1. Three screenhouses:
   - One screenhouse for the production and maintenance of clean host plants, *M. paniculata*
   - A screenhouse or an appropriately-outfitted laboratory for the production of *D. citri*

¹ Plant Production and Protection Officer of FAO’s Sub-Regional Office for the Caribbean (SLC)
• A screenhouse or an appropriately-outfitted laboratory for the production of *T. radiata*
2. Host plants *Murraya paniculata* (orange jasmine)
3. *D. citri* (free of the bacterium that causes HLB)
4. Parasitoids *T. radiata*
5. Entomological equipment – aspirators with clear plastic vials for insect collection; binocular microscope; dissection needles; yellow traps.
6. Other materials: laboratory / field coats; black plastic bags for the host plants; clear plastic bags for collection of field samples.

In view of the existing situation in Belize, i.e. lack of appropriately-outfitted laboratories but availability of outdoor space and suitable weather conditions, it was decided that the production of *D. citri* and *T. radiata* would be carried out in screenhouses on the premises of the CGA/CREI.

**Screenhouses**

The CGA has contributed an existing screenhouse, about 7 m x 10 m, for the production of clean host plants. Based on the available space and funds, two screenhouses, each 7.5 m x 10.5 m (20 ft x 32 ft) x 9ft (height) were constructed under the project: one for the mass production of *D. citri* and the other for the production of *T. radiata* (Photo 1). The two structures are about 50 cm off the ground, with a concrete foundation. Inside, the floor is covered with gravel.

![Photo 1](screenhouses.jpg)

**Photo 1.** Screenhouses for rearing of ACP and *Tamarixia radiata*.

All three screenhouses have a double-door system to prevent the entry of pests. In addition, the two newly-constructed screenhouses have a foot-bath (Photo 2) which is
aimed at preventing the entry of specific soil-borne diseases. The sides of the
screenhouse are made of psyllid-proof mesh and the top is covered with high-grade
clear plastic that allows sufficient light into the structure. The sides and top are
reinforced with a strong wooden frame, comprising small rectangles and cross-bars.
Two air-vents on the roof facilitate air circulation and removal of warm air from the
screenhouse.

Photo 2. Close-up of (left) the screenhouse and (right) the double-door system with foot-
bath

Frames and Cages

*D. citri* and *T. radiata* are produced in cages, 80 cm × 80 cm × 80 cm (3 ft × 3 ft × 3 ft) in
size. The frame and bottom of the cages are made of wood. The three sides and part
of the cage front comprise of psyllid-proof mesh and the top of clear Plastic. A wooden
door 2 ft × 2 ft at the front of the cage is hinged at one end and has a latch at the
other end to close / lock the door.

The cages are placed on large wooden frames, 3 m (10 ft) long × 75 cm (2.5 ft) wide. To
maximize the available space, each frame has two shelves, the first shelf being 45 cm
(18 in) off the ground and the second shelf at 100 cm from the first. Each shelf holds
three cages and each frame holds six cages. Eight such frames are accommodated in
the screenhouse for ACP production (Photo 3, left). In the screenhouse for *T. radiata*
production, four frames are housed along the length of the screenhouse and one
frame along the width at the closed end (Photo 3, right). Each cage may hold up to 3-4
potted plants, but the actual number used per cage depends on several factors
including availability and size of plants and air-flow inside the cage.
Host plants

The orange jasmine, *Murraya paniculata*, has been successfully used in Florida (USA) and Jamaica to mass-produce both *D. citri* and *T. radiata*. It grows much faster than citrus and produces new flushes throughout the year; it is also readily available and relatively easy to mass-produce from seeds and from cuttings.

Orange jasmine is an evergreen shrub (or occasionally a small tree) that can live for many years. It grows to 2-3 metres in height and is easily managed by pruning\(^2\). The branches and twigs are slender and abundant at all heights. The alternate leaves are pinnately compound with three to nine leaflets alternating on the rachis. The leaflets are 1-5 cm long, dark-green, stiff and ovate. The white flowers are fragrant, five-petalled and borne in small clusters near the branch ends; they develop into shiny, red elliptic fruits about 1 cm long. One or two light green seeds are embedded in the bitter, watery pulp. The seeds are tear-drop shaped, rounded or flattened on one side depending on whether there are one or two seeds per fruit.

Orange jasmine flowers irregularly throughout the year. Fruits can be collected from the plants and the seeds removed and air-dried. Seeds require between 20 and 50 days for germination, which is hypogea. Seedlings quickly develop deep root systems and grow at a moderate rate.

However, it is important to note that the plants are attacked at times by white flies, scale insects and nematodes. Heavy whitefly and scale attacks can result in sooty mold on the upper surface of the leaves. Powdery mildew is also known to attack pruned plants. In the event that any of these infestations occur in the screenhouse, the infested leaves should be immediately removed. Depending on the pest and where it occurs, the plant should be pruned and allowed to regrow before being used in the production cycle. If the soil is treated with a systemic nematicide, then treated pots should be

\(^2\) http://www.fs.fed.us/global/lfif/pdf/shrubs/Murraya%20exotica.pdf
labelled and set aside: such plants should be used only after the nematicide effects have worn-off.

Production of plants

There are two methods of producing orange jasmine plants for use in the screenhouses:

- From seeds
- From cuttings

Production from seeds

The optimum number of host plants for the mass production facility in Belize is about 1000 plants. Extensive plant production activities should continue until this number is reached. Thereafter, the number of new plants being produced can be reduced to about 50 plants per month. This is because plants used in the production of D. citri and T. radiata will be returned to the host plant pool and re-used for another 3-4 cycles or until the plants are no longer vigorous.

The orange jasmine plants are currently grown from seeds, planted 1.5 cm deep in seed beds. The seedlings are then transplanted to small pots of size 11 cm diameter × 30 cm tall. Seven small pots with seedlings are then placed in a bigger pot measuring 32 cm diameter × 30 cm high (Photo 4), which serves as a reservoir for water. The pots are watered until the larger pot has 2-3 cm of water; this water is absorbed as necessary by the roots of the plants in the pots.

PHOTO 4 Seedlings recently transplanted from seed-beds to small pots (left); Seven small pots with seedlings placed inside larger pots (right).

The following is information on production of orange jasmine plants from seeds (including seed extraction, treatment, germination and plant care3) and is adapted to suit Belize’s conditions:

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1. Berries are collected from the orange jasmine trees once they are of an orange-red colour. The berries are soaked in a bowl of water for 48 hours to soften the skin around the seeds.

2. The berries are rubbed together to loosen the skin and crack the seed flesh while they are still in the bowl of water. The cracked berries are soaked for another 12 hours or until the berries separate and release the seeds: the seeds float to the top of the water.

3. The seeds are removed from the water and set on a paper towel. The shallow indentation that runs along the length of the seeds is located and pierced carefully with a fingernail to separate each seed into two halves, which are two individual seeds.

4. Seeds do not store well and should be used as soon as possible after extraction

5. Seeds are soaked in water for 48 hours before sowing: this gives a high germination rates (95% +).

6. The seedbed soil is be moistened with water until it feels like a damp sponge. The mixture comprises 4 parts sterile potting soil and 1 part rice hull as an alternative to commercial mixture. A clean seedbed pot is filled with the damp medium until it has a depth of at least 6 inches.

7. The seeds are sown into the seed bed at 1.5 cm (3/4 inch) depth, 0.5 cm (1/4 inch) apart, and then lightly covered with soil.

8. Seedbeds are placed in an area that receives indirect sunlight.

9. The soil moisture is monitored daily and the soil misted with water as needed to keep it evenly moist. Seed sprouts appear, usually in seven to 14 days.

10. After germination, the seed bed is moved to an area that receives bright, indirect sunlight. The seedlings are transplanted into pots once they are 15 cm (6 inches) tall.

11. The plant is pruned back to just two or three leaves after each shoot has produced six or seven leaves. This encourages bushier growth.

Production from cuttings:

Orange jasmine plants are easy to propagate from cuttings, and are fairly fast growing. Cutting-grown plants may be better-suited for D. citri production, as they do not seed prolifically and produce a bushy growth faster and better than plants produced from seed. "Almost mature" wood cuttings, taken from neither green nor brown wood, work best once they are allowed to dry for a few hours. Older wood cuttings can also be used if ‘almost mature’ cuttings are not available.

Cuttings can be taken from the ends of branches. Sections of 9-12 cm (4-5 inches) of stem are stripped of leaves on the lower third, moistened slightly, dipped in root hormone and tapped gently to remove excess powder. The end with the hormone powder is then buried at an angle of about 45 degrees in a moist medium of sharp-sand placed on soil or peat moss (Photo 5). Under warm, humid conditions, the cuttings start new roots in several weeks. A good indicator of success is new growth from the dormant buds. Once the new flush appears, the plant can be transplanted to a growing container (Photo 6).

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4 http://www.ehow.com/info_10066625_reproduction-techniques-blooming-orange-jasmine.html#ixzz2llrlLklo
Photo 5. Preparation\(^5\) and planting\(^6\) of cuttings

Photo 6. (left) Two cuttings planted per bag (14cm diameter x 30cm high). (right) Cuttings with sprouted shoots.

**Maintenance of plants**

Currently, newly-planted *Murraya* plants grown in black plastic bags are maintained under a mesh-roof screenhouse with a careful management regimen designed to maintain best health and fitness. Frequent watering of the plants is not required during the rainy season, but this carries the risk of over-watering during periods of heavy and / or continuous rainfall. During dry periods, plants are watered daily and far as possible, watering in the evening is avoided. Fertilizers (slow release 10-10-10) are applied once a month. The plants are numbered sequentially and inspected once a week for early detection of pests and diseases. Affected plants are treated in accordance with the problem encountered. The status of the plants and any treatment(s) applied are

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\(^6\) [http://www.bbc.co.uk/gardening/basics/techniques/propagation_hardwood1.shtml](http://www.bbc.co.uk/gardening/basics/techniques/propagation_hardwood1.shtml)
recorded on a datasheet (Annex 1) and entered into a database. The records facilitate traceability should any further problems be encountered.

The tip of the new plants is cut when they reach a height of 30-40 cm. This induces the production of side-shoots, which are allowed to grow until they reach a height of 40-50 cm. The tips are cut again and once there is new flush growth, the plants are ready for use in the production of D. citri.

Plants which have been used for one production cycle of D. citri and T. radiata, can be reused. These recycled plants are returned to the clean-plant pool. Before being moved to the screenhouse with the clean plants, all the leaves are removed and the plants are re-numbered. It is also ensured that the area around the base of the plant is clean and free of dead leaves, weeds and any evidence of pests etc. The plants are then placed in the screenhouse and allowed to re-grow. The plants are watered, fertilized and inspected in accordance with established protocols. The plants are ready for use once they have sufficient (5-10) shoots with new flush growth.

Plants that are no longer healthy or fit for D. citri / T. radiata production are discarded away from the production areas to prevent possible contamination.

Psyllid production

The psyllid being mass-produced is Diaphorina citri.

Psyllid description and life-cycle

Adult psyllids are 3-4 mm long with a light brown head. The forewing is broadest in the apical half, mottled, and with a brown band extending around periphery of the outer half of the wing (Photo 7 left). The antennae have black tips with two small, light brown spots on the middle. Live D. citri are covered with whitish, waxy secretion, giving a dusty appearance. Male and female D. citri can be distinguished from each other by the shape of the abdomen. The end of the abdomen in males is curved upward while in females the abdomen is straight and long with a pointed end (Photo 7 right).

Eggs of D. citri are about 0.3 mm long, elongate, almond-shaped, thicker at base, and tapering toward the other end (Photo 8 left). Each female typically lays 300-800 eggs. Eggs are laid on tips of growing shoots on and between new leaves. Newly-laid eggs are pale, turning yellow and finally orange before hatching. Females place the eggs on plant tissue with the long axis vertical to the surface of the leaf. The eggs hatch in about 4 days.

There are 5 nymphal instars. First instar nymphs are 0.25 mm long, while the fifth instars are 1.5-1.7 mm in length (Photo 8 right). The colour is yellowish-orange, with large wing pads in 4th and 5th instars and large filaments on the apical plate of the abdomen. The duration of the life cycle is affected by temperature. A life-cycle of 11-15 days has been reported at 25-26 °C. Under temperature-controlled growth-chamber conditions
(26±1°C, 75-80% relative humidity (RH), with artificial illumination) in Malaysia, the life cycle was 18.5 days on OJ. Adults may live for several months.

Photo 7. D. citri adult (left)\(^7\) and female\(^8\) (right)

Photo 8. Eggs (left) and five developmental stages (right) of D. citri\(^9\)

**Psyllid culture establishment and maintenance**

Both field-collected adults and younger stages can be used to establish D. citri cultures. The insects must be collected from areas that are currently not affected by HLB or from trees that do not show signs of HLB infection. This will ensure that the orange jasmine (OJ) plants being used for D. citri and T. radiata production do not become infected, thus lengthening their life in the production system.

Field collected, ovipositing females are usually mated. It is nevertheless a good strategy to ensure that some males are also collected. Laboratory experiments in Florida indicate that females mate every day and that multiple matings are necessary for continued oviposition by females.

In each cage, up to 20 field-collected female D. citri adults are released and allowed to oviposit for a week, thus ensuring that a mixed-age population develops on the plants. This is necessary because when the psyllids are transferred the parasitoid

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\(^7\) http://entnemdept.ufl.edu/creatures/citrus/acpsyllid.htm
\(^8\) http://www.fao.org/docrep/u5000e/U5000E01.GIF
\(^9\) http://entnemdept.ufl.edu/creatures/citrus/acpsyllid.htm
production area, adult *T. radiata* females not only oviposit on 4/5th instars but they also feed on younger instars in order to obtain proteins.

At the end of the week, the plants are gently shaken to dislodge the adult *D. citri*. The plants are then moved to another cage and the cage is labelled appropriately with the date(s) of oviposition. Clean plants are introduced in the original cages with adults for a second round of oviposition. More adult *D. citri* can be added to the oviposition cage if necessary. Photo 9 depicts a colony of *D. citri* eggs and various instars on an orange jasmine plant housed in the screenhouse in Belize.

![Photo 9. Colony of eggs and instars of *D. citri* on *M. paniculata* housed in cages in the screenhouse (indicated by arrows).](image)

In each production cycle, about 80% of plants are used for *T. radiata* production and the remaining plants are used for maintenance of the cultures.

For *D. citri* destined to be used for maintenance of cultures: the nymphs are allowed to remain on the plants until they become adults. The newly-emerged adults are aspirated from the cage and depending on the size of the plants on which the adults are to be released, 20-30 females and at least 5-10 males are collected and moved into the oviposition cage with clean Orange Jasmine plants.

For *D. citri* destined to be used for *T. radiata* production: the nymphs are allowed to develop until the oldest nymphs reach 4/5th instars and the entire cage is then moved to the *T. radiata* production screenhouse.
Parasitoid Production

The parasitoid being mass-produced is *Tamarixia radiata*, which is already present in Belize and reportedly has high parasitism efficiency. A single female *T. radiata* can kill more than 500 *D. citri* through a combination of host feeding and parasitism.

Parasitoid description and life-cycle\(^\text{10}\)

Adult *T. radiata* (Photo 10) are small black wasps (0.92-1.04 mm long) with eyes that are widely-separated and head slightly larger in width than length. The wings are hyaline with pale yellow veins. Sexual dimorphism is marked between adult male and female. Males are slightly smaller than females in total length and wing expanse, and their antennae are 1.5 times longer than those of females. The female ovipositor is barely protruding.

Female *T. radiata* feed on honeydew excreted by *D. citri* and obtain protein from *D. citri* nymphs for egg development. They feed on the haemolymph of young host nymphs accessed through ovipositor-induced punctures.

Photo 10. (left to right) *Tamarixia radiata* male, female\(^\text{11}\); female\(^\text{12}\) and mummy\(^\text{13}\) (scale not known)

**Eggs:** The adult female lays one or occasionally two eggs beneath a *D. citri* nymph. However, only one *T. radiata* larva usually reaches the adult stage and thus *T. radiata* is regarded as a solitary parasitoid. An adult female can deposit up to 300 eggs.

**Larva:** The newly hatched *T. radiata* larva attaches itself to the nymph and feeds on haemolymph of the host, eventually killing it. The length and width of the first instar is 0.28 mm × 0.11 mm and of the fourth instar 1.14 mm × 0.59 mm, respectively.

**Pupa:** Pupation takes place under the dead, mummified ACP nymphs and new adults emerge through a hole on the thorax or head of the parasitized mummy. The female to male sex ratio is 1.8 to 3.2, depending upon the origin of the colony and the rearing conditions.

Under experimental conditions (26±1°C, 70% RH) in Florida, the total developmental period (egg to adult) was 11.4 days. The egg, larval, prepupal and pupal stages were

\(^{10}\) http://entnemdept.ufl.edu/creatures/beneficial/wasps/tamarixia_radiata.htm\#top  
\(^{11}\) http://nbaii.res.in/Featured_insects/Tamarixia-radiata.php  
\(^{12}\) http://www.insectimages.org/browse/detail.cfm?imgnum=5196067  
\(^{13}\) http://ucanr.edu/sites/ACP/files/169960display.jpg
completed in 1.9, 4.0, 0.6 and 4.9 days, respectively. Average temperature in Belize\textsuperscript{14} is around 26°C (range 23-28°C), with maximum temperature averaging 28°C (range 26-30°C) and minimum 23°C (range 21-26°C). On average, females lived for 23.6 days and males 11.4 days. Males were capable of multiple matings. However, mating had no effect on longevity of adults.

Parasitoid culture establishment

There are two ways to obtain individuals of \textit{T. radiata} for establishment of a culture: (1) field-collection of adult male and female parasitoids and (2) adults emerging from field-collected mummies placed in clear plastic vials. In both cases, adult \textit{T. radiata} are collected in individual vials and separated into males and females. Depending on the numbers available, 15-20 adults are aspirated into a single vial ensuring that a few males are present in each batch of insects. The parasitoids are then released in a cage with large numbers of mixed-stage \textit{D. citri} provided as food and for parasitism. It is important to keep in mind that a single female \textit{T. radiata} may need as many as 400-500 psyllids during its lifetime or 150-200 \textit{D. citri} per week. It is anticipated that \textit{T. radiata} numbers will be limited at the start of culture establishment. Therefore, the \textit{T. radiata} must be collected from the cage one week later and transferred to another batch of plants with \textit{D. citri} for oviposition / parasitization.

Once sufficient numbers of \textit{T. radiata} are available, the adults may be allowed to remain in the same cage until the next generation emerges.

Parasitoid collection and handling

About 8-10 days after introducing the \textit{D. citri}-infested plants to \textit{T. radiata}, the plants / branches with parasitized \textit{D. citri} can be moved to the collection cage, which has a collection bottle or vial at the top. Before the vial is fixed on the cage, droplets of honey are spread on a small sheet of wax paper and left hanging in the vial as food for the wasps. It is necessary to test which concentration of honey works best under Belize conditions – pure honey or a mixture with different concentrations with up to 50% water.

Once \textit{T. radiata} start emerging, the collection vials are changed twice daily. The \textit{T. radiata} are moved to another cage and aspirated into clear plastic vials, 20 per vial (15 females and 5 males). A ‘ladder’ made of a folded 4-5 mm wide and 2-3 cm long white paper is placed in the vial as a resting place for the wasps. The \textit{T. radiata} should be stored in a cool-box immediately after collection and should be released in the field within a few hours after being collected. If the parasitoids are to be stored for a longer period e.g. a few hours or up to a day, then they should be provided with tiny droplets of honey for food, placed on the cover of the vial. In any case, they should be kept in the vials for no longer than 24 hours.

A cool-box is a styrofoam container with a snug cover or a cooler (Photo 11a, b) used for the storage of the \textit{T. radiata}. One or two freeze-packs (Photo 11c) or sealed plastic

\textsuperscript{14} http://www.weatherbase.com/weather/weather.php3?s=38587&set=metric
bags with ice are placed at the bottom of the box and several sheets of newspapers or thick absorbent paper is placed on the top of the ices packs. This helps maintain the vials with the wasps at approximately 5-8°C. The clear plastic vials containing the *T. radiata* are covered with a thick paper towel to prevent condensation in the cool-box. It is advisable to pack the parasitoid vials in small containers (Photo 11d) to prevent them from being agitated in the cool-box during transport.

**Photo 11.** Cool-box: Styrofoam (a) and plastic (b); ice-pack (c) and container for vials (d)

**Recommendation for the extraction of adult *Tamarixia radiata*.**

The establishment of a dark extraction room (7 ft × 8 ft × 10 ft) with one main natural lighted (day light) window (2 ft × 2 ft) and containing aerated shelves is recommended to place cuttings with parasitized nymphs for emergence of the adult wasps. After emerging from mummified nymph, the *T. radiata* adults will then move toward the light of the window and remain on there and could be easily collected with the use of an aspirator. A room specifically for this technique can be built or a room may be adapted in an existing structure that has opaque walls and roof with access to natural light. However, this incurs (needs) additional funding.

**Routine schedule of activities**

A routine should be established for the entire production system to ensure that all the necessary activities are done on schedule.

The Data Sheet in Annex 1 can be used to record routine observations as well as any recommendations and remarks. The information should be entered into a database (Microsoft Excel or Access). It is important to periodically review the database to identify trends which may indicate system issues with the production system. This can also provide an opportunity for continuous improvement of the production systems.

**Plant Production / Propagation:**

1. Plant propagation activities should be carried out at least once a month: these include planting of seeds as well as establishing cuttings of Orange Jasmine.
2. Weekly observations must be carried out on all plants. Each plant should be examined closely and carefully, and any signs of the presence of pests and
diseases should be recorded on the Data Sheet (Annex 1), together with actions carried out (e.g. affected plant moved out of the propagation area or insects removed manually etc.). The information should be entered as soon as possible into the database (Excel or Access).

3. Watering and fertilization of plants should be carried out as necessary.
4. M. paniculata should be free of weeds that compete with them for nutrients.

Psyllid Production:

1. Each week, one day should be set aside for D. citri production activities, comprising:
   a. removal of D. citri from plants on which they were released the previous week;
   b. release of D. citri on plants in as many cages as necessary / possible, up to a maximum of 45 cages;
   c. observations on the plants and on D. citri development recorded on the Data Sheets. Every single cage / plant in the area must be observed and the data recorded, together with any problems and action to be taken; in each production cycle, it is important to record approximate numbers of D. citri produced per plant and / or per cage, as any unexpected decline in numbers can be easily picked up and remedied;
   d. transfer of M. paniculata with appropriate stage(s) into the T. radiata production facility.
2. M. paniculata should be watered and fertilized as necessary
3. M. paniculata should be free from weeds that compete with them for nutrients.

Parasitoid Production:

1. Each week, one day should be set aside for release of T. radiata. The activity should comprise:
   a. removal of T. radiata from plants on which they were released the previous week;
   b. releases of parasitoids in as many cages as necessary / possible, up to a maximum of 35 cages;
   c. Observations on M. paniculata, as well as D. citri and T. radiata should be recorded on the Data Sheets. Every single cage / plant in the area must be observed and the data recorded, together with any problems and action to be taken;
2. M. paniculata should be watered and fertilized as necessary
3. Field release of T. radiata: Once all three production systems are working well, then there is a continuous and sustained emergence of T. radiata. The parasitoids should be collected as often as possible and released, not only in backyard gardens and abandoned fields, but also farmers’ fields, as long as the required stages of D. citri are present.

After many generations, the vigour and vitality of the parasitoid cultures declines due to erosion of genetic material from continuous culturing and inbreeding. It is therefore
It is advisable to periodically (at least once every two months) collect and introduce wild (field-collected) populations of T. radiata into the parasitoid production system. This ensures that cultures remain vigorous and productive.

**Field release and monitoring impact of the Parasitoid Tamarixia radiata**

**Selection of release sites**

The sites for the release of the wasps should be selected at a time when there is availability of *D. citri* nymphs at appropriate stages in the backyard citrus or abandoned groves. Field monitoring of environmental conditions throughout the year for both *D. citri* and *T. radiata* should be done. This is in order to determine the time of the day that is most suited for field releases and also the time of the year *T. radiata* that is most effective in controlling the population of *D. citri* in backyards and abandoned groves.

**Field Monitoring and Release of Parasitoids**

Field monitoring will also provide information for establishing a programme of release. For example, if every three months there is a flush pattern and availability of *D. citri* nymphs and records showing a decrease in the presence of *T. radiata* in the area, this will indicate how often releases should be carried out in an area. The amount of *T. radiata* released would depend on the size of the release area. For example, as a rule of thumb, about 50 *T. radiata* adults should be released in backyards with trees in less than a 25-yard area. In the case of abandoned groves, 100 *T. radiata* should be released every 100 yards within the grove.

**Evaluation of Effectiveness of Parasitoids**

The data sheet for recording field release information is at Annex 2. To evaluate the effectiveness of the parasitoid in controlling *D. citri*, it is necessary to determine the percentage of parasitism as follows:

\[
\text{Percentage parasitism} = \frac{\text{Parasitized nymphs}}{\text{Total nymphs}} \times 100
\]

The control of ACP by *T. radiata* should be evaluated in selected site before any wasps are released in order to establish a basis for comparison with data from subsequent assessments.

Sampling for the initial as well as subsequent assessments should be done on 10-20 infested shoots (5-10 cm length) collected in the field and brought to the laboratory for evaluation. Living nymphs may be host to any stage of *T. radiata* (egg, larva, prepupa). In addition, dead nymphs or mummies with *T. radiata* pupa or exit holes may
also be present. Therefore, the following steps are recommended for each field collected sample:

1. Count the number of mummies with exit holes from which parasitoids have already emerged
2. Count the number of mummies from which parasitoids have not yet emerged
3. Count, collect and rear each live nymph until it becomes an adult or a mummy
4. Record the data in the datasheet (Annex 3).
5. By adding up various columns (Annex 3), the total number of nymphs collected, number of psyllid adults that emerged and number of nymphs that were parasitized is determined. This data is inserted in a formula (as above) to obtain percentage parasitism.

Plotting initial population levels of the parasitoids against subsequent levels would show the impact of field release of *T. radiata* on the psyllid populations.

**Protocols and Sanitation**

A number of protocols and procedures must be put in place in order to minimize problems and ensure that the production systems remain efficient and secure.

A. Appropriate notices should be displayed on all three screenhouses, indicating their function i.e. Plant Propagation / Maintenance area, *D. citri* Production area and *T. radiata* Production area.

B. Movement between the areas should be uni-directional, as follows:

\[
\text{M. paniculata Production area} \quad \downarrow \\
\text{D. citri Production area} \quad \downarrow \\
\text{T. radiata Production area}
\]

*Under no conditions should this movement flow be changed.*

*If it becomes absolutely critical to enter one of the areas, then appropriate measures should be taken to ensure that security standards are maintained.*

C. It is essential that clean sanitary conditions are maintained in all three screenhouses at all times.

D. Cages should be periodically washed with soap and water and air-dried.

E. The cages should be checked regularly for holes that would permit the escape of insects (*D. citri, T. radiata*) kept inside; these should be fixed forthwith.
Annex 1. Data Recording Sheets – Clean *Murraya paniculata*, *Diaphorina citri*, and *Tamarixia radiata*

**Citrus Growers’ Association – Citrus Research and Education Institute (CREI), Belize**

*Mass production of M. paniculata, D. citri and T. radiata - Data recording sheet*

<table>
<thead>
<tr>
<th>Plant ID</th>
<th>Observations / Recommendations</th>
<th>Remarks / Action taken</th>
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Citrus Growers’ Association – Citrus Research and Education Institute (CREI), Belize
Field Release of Mass-produced *Tamarixia radiata* - Data recording sheet

<table>
<thead>
<tr>
<th>Date of release</th>
<th>Number of male/female</th>
<th>Address of area of release</th>
<th>ACP population at time of release</th>
<th><em>T. radiata</em> population at time of release</th>
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**Citrus Growers’ Association – Citrus Research and Education Institute (CREI), Belize**

**Field Collection of *Tamarixia radiata* - Data recording sheet**

<table>
<thead>
<tr>
<th>Date of collection</th>
<th>Number of mummies with exit holes (A)</th>
<th>Number of mummies with no exit holes (B)</th>
<th>Number of live psyllids collected (C)</th>
<th>Number of live psyllids that became mummies (D)</th>
<th>Total number of psyllids (A+B+C)</th>
<th>Number of psyllids parasitized (A+B+D)</th>
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Annex 4. Glossary of Terms

The following is a glossary of the most commonly-used terms in biological control programmes.\(^{15}\)

Biological control or biocontrol:

- Pest control strategy making use of living natural enemies, antagonists, competitors or other biological control agents [ISPM No. 3, 1996; revised ISPM No. 3, 2005; formerly biological control (biocontrol)]
- The use of living organisms, such as predators, parasitoids, and pathogens, to control pest insects, weeds, or diseases. Typically involves some human activity.
- Using one type of living organism to affect a change in the population density of another organism. In agriculture, refers to using insects, mites, entomopathogens (fungi, bacteria) or nematodes to control common plant pests.\(^{'}\)
- The use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be.

Biological control agent: A natural enemy, antagonist or competitor, or other organism, used for pest control [ISPM No. 3, 1996; revised ISPM No. 3, 2005]

Classical biological control: the use of exotic biological control agents imported from its native home into the target area against exotic pests that have arrived without their natural enemies

Conservation biological control: Modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effects of pests.

Cuticle: The outer skin, epidermis, shell, or exoskeleton of an arthropod.

Economic: The point at which a pest infestation can longer be controlled while retaining profitability. OR. At which point a pest infestation becomes bad enough to begin negatively affecting the crop to the degree that it will diminish its saleability.

Ecosystem: A dynamic complex of plant, animal and micro-organism communities and their abiotic environment interacting as a functional unit [ISPM No. 3, 1996; revised ICPM, 2005]

Ectoparasite: A parasite organism which works from the outside in. Living outside its host. *Tamarixia radiata* is an ectoparasitoid.

Efficacy: A non-quantitative measure of effectiveness. Example: Spraying pesticides diminishes the biocontrol agent's efficacy.

Endoparasite: A parasite which works from the inside out, dwelling within its host.

Entomogenous: A parasitic organism which lives in or on arthropods.

---

Entomology: The scientific study of insects, thus one who studies insects is an entomologist.

Entomopathogenic: An organism which can kill arthropods by way of poisoning, either through its own toxins or those it harbors - generally fungi, bacteria or nematodes.

Entomophagous: An organism which uses arthropods for sustenance (food)

Establishment: Perpetuation, for the foreseeable future, of a pest within an area after entry [FAO, 1990; revised FAO, 1995; IPPC, 1997; formerly established]

Fumigant: A substance which produces a gas, vapor, fume, or smoke intended to kill a pest.

Generation: Period from any given stage in the life cycle to the same life stage in the offspring. Typically from egg to egg

Genus, Genera (pl.): A group of evolutionarily related species, sharing one or a number of characteristics.

Honeydew: The sugary liquid excreta of certain insects (Homoptera) such as aphids, psyllids and scales

Host: The organism in or on which a parasitoid lives; a plant on which an insect feeds.

Host pest list: A list of pests that infest a plant species, globally or in an area [CEPM, 1996; revised CEPM, 1999]

Host range: A Species capable, under natural conditions, of sustaining a specific pest or other organism [FAO, 1990; revised ISPM No. 3, 2005]

Infestation: Presence in a commodity of a living pest of the plant or plant product concerned. Infestation includes infection [CEPM, 1997; revised CEPM, 1999]

Inoculation biological control: The intentional release of a living organism as a biological control agent with the expectation that it will multiply and control the pest for an extended period, but not permanently.

Insect: An arthropod having 3 body parts (head, thorax, abdomen) and six legs. Most, but not all, are winged as adults.

Insectary: An operation or system mass-producing insects, mites or nematodes for use in biocontrol activities.

Instar: The stage of an insect's life between successive molts, for example the first instar is between hatching from the egg and the first molt.

Integrated pest management (IPM): An approach to the management of pests in which all available control options, including physical, chemical, and biological controls, are evaluated and integrated into a unified program

International Plant Protection Convention (IPPC): as deposited with FAO in Rome in 1951 and as subsequently amended [FAO, 1990]

International Standard for Phytosanitary Measures (ISPM): An international standard adopted by the Conference of FAO, the Interim Commission on phytosanitary measures or the Commission on phytosanitary measures, established under the IPPC [CEPM, 1996; revised CEPM, 1999]
International Standards, Guidelines and Recommendations: For plant health, the international standards, guidelines and recommendations developed under the auspices of the Secretariat of the IPPC in cooperation with regional organizations operating within the framework of IPPC.

Introduction:
- The release of a biological control agent into an ecosystem where it did not exist previously (see establishment) [ISPM No. 3, 1996]
- The entry of a pest resulting in its establishment [FAO, 1990; revised FAO, 1995; IPPC, 1997]

Inundation biological control: The use of living organisms to control pests when control is achieved exclusively by the released organisms themselves.

Inundative biological control: The release of large numbers of mass-produced biological control agents or beneficial organisms with the expectation of achieving a rapid effect [ISPM No. 3, 1996; revised ISPM No. 3, 2005]

Invasive alien species: An alien species whose establishment and spread threaten ecosystems, habitats or species with economic or environmental harm.

Legislation: Any act, law, regulation, guideline or other administrative order promulgated by a government [ISPM No. 3, 1996].

Metamorphosis: Pertains to the development of insects. Some insects (e.g. moths and butterflies) undergo a complete metamorphosis going from egg, to numbered larval instars, to the drastic pupal stage to, adulthood. Others (whiteflies, psyllids, scales) undergo an incomplete metamorphosis, going from egg, to numbered nymphal stages, to adulthood, all seemingly more gradual and less dramatic.

Monitoring: Means the continuous investigation of a given population or subpopulation, and its environment, to detect changes in the prevalence of a disease or characteristics of a pathogenic agent.

Monitoring: Ongoing survey to verify the characteristics of a pest population [FAO, 1995]

Life Cycle: The sequence of events that occurs during the lifetime of an individual organism.

Mass-reared: Produced in large numbers, as in natural enemies produced for release program.

Microbial insecticide: A preparation of microorganisms (e.g., viruses or bacteria) or their products used to suppress insect pest populations.

Morphology: Form or structure of an organism.

Moult: An insect undergoing metamorphosis, whether complete or incomplete, will moult or shed its cuticle as it gets older and out-grows its old cuticle.

Mummies: A type of pupal casing created by the host insect’s exoskeleton, usually as a result of parasitism by (insect) parasitoids.
National Plant Protection Organization: Official service established by a government to discharge the functions specified by the IPPC [FAO, 1990; formerly Plant Protection Organization (National)]

Native organism: An organism occurring within its known or consensual range (as documented in scientific publications).

Natural control: The suppression of pest populations by naturally occurring biological and environmental agents.

Natural enemies:
  - Living organisms found in nature that kill, weaken, or reduce the reproductive potential of other organisms.
  - An organism which lives at the expense of another organism in its area of origin and which may help to limit the population of that organism. This includes parasitoids, parasites, predators, phytophagous organisms and pathogens [ISPM No. 3, 1996; revised ISPM No. 3, 2005]

Naturally occurring: A component of an ecosystem or a selection from a wild population, not altered by artificial means [ISPM No. 3, 1996]

Nectar: The sugary liquid secreted by many flowers.

New introduction: The human-mediated movement of a species outside its present distribution.

Niche: The attribute of an organism which defines the boundaries within which it can carry out its life processes.

Nonindigenous species: Any species or other viable biological material that enters an ecosystem beyond its historic range, including any such organism transferred from one country into another.

Non-native species
  - A species or race that does not occur naturally in an area; i.e. it has never occurred there or its dispersal into the area has been mediated by humans.
  - (= alien species): A species, subspecies or lower taxon, introduced (i.e. by human action) outside its natural past or present distribution; includes any part, gametes, seeds, eggs, or propagules of such species that might survive and subsequently reproduce.

Nymph:
  - The immature stage, following hatching from the egg, of an insect that does not have a pupal stage.
  - An intermediate development stage of an insect which undergoes an incomplete metamorphosis. Like the Larva, this too is a numbered instar.

Occurrence: The presence in an area of a pest officially recognized to be indigenous or introduced and/or not officially reported to have been eradicated [FAO, 1990; revised FAO, 1995; ISPM No. 17; formerly occur]

Order: A taxonomic subdivision that contains groups of related families or superfamilies; usually ending in -ptera in insects.
Organism: Any active, infective, or dormant, or stage of life form of an entity characterized as living, including plants, bacteria, algae, fungi, mycoplasma, mycoplasma-like entities, protozoa, vertebrate and invertebrate animals, as well as entities such as viruses, viroids, plasmids, phages or any living entity, related thereto, whether natural, genetically modified, living modified, or otherwise.

Oviposition: The laying or depositing of eggs.

Ovipositor: The egg-laying apparatus of a female insect, typically located at the posterior end of the abdomen. Some ovipositors can lay eggs inside the host, some only external of a host or other surface, and others serve more than one purpose. Some social wasps have a modified ovipositor. It is designed to not only deposit eggs (in the cells they build) but can penetrate skin and deliver toxins (a stinger)

Parasite:
- An organism that lives in or on another organism (the host) during some portion of its life cycle.
- An organism which lives on or in a larger organism, feeding upon it [ISPM No. 3, 1996]

Parasitoid:
- An animal that feeds in or on another living animal, consuming all or most of its tissues and eventually killing it.
- An insect parasitic only in its immature stages, killing its host in the process of its development, and free living as an adult [ISPM No. 3, 1996]

Pathogen: Micro-organism causing disease [ISPM No. 3, 1996]

Pest: Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products [FAO, 1990; revised FAO, 1995; IPPC, 1997].

Pest record: A document providing information concerning the presence or absence of a specific pest at a particular location at a certain time, within an area (usually a country) under described circumstances [CEPM, 1997]

Pest status: Presence or absence, at the present time, of a pest in an area, including where appropriate its distribution, as officially determined using expert judgement on the basis of current and historical pest records and other information [CEPM, 1997; revised ICPM, 1998]

Plant pest: Any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen.

Plant quarantine: All activities designed to prevent the introduction and/or spread of quarantine pests or to ensure their official control [FAO, 1990; revised FAO, 1995]

Precautionary approach: Measures to implement the Precautionary Principle. A set of agreed cost-effective measures and actions, including future courses of action, which ensures prudent foresight, reduces or avoids risk to the resources, the
environment, and the people, to the extent possible, taking explicitly into account existing uncertainties and the consequences of being wrong (FAO 1995, 1996).

Predator: A natural enemy that preys and feeds on other animal organisms, more than one of which are killed during its lifetime [ISPM No. 3, 1996]

Pupa, Pupae (pl.): The nonfeeding stage between the larva and adult in insects with complete metamorphosis. It’s the stage after the final larval instar (the brief transition is pre-pupal). A cocoon is a pupa.

Quarantine:
- The facility and/or process by which live organisms and any of their associated organisms can be held/or reared in complete isolation from the surrounding environment.
- Official confinement of regulated articles for observation and research or for further inspection, testing and/or treatment [FAO, 1990; revised FAO, 1995]

Release: Intentional liberation of an organism into the environment [ISPM No. 3, 1996]

Sampling: Estimating the density of organisms (pests or natural enemies) or damage by examining a defined portion of the crop.

Sanitary and phytosanitary measure: Any measure applied a) to protect human, animal or plant life or health (within a Member’s Territory) from the entry, establishment or spread of pests, diseases, disease carrying organisms; b) to prevent or limit other damage (within the Member’s Territory) from the entry, establishment or spread of pests.

Species: A group of individuals similar in structure and capable of interbreeding and producing fertile offspring. They are different in structure from other such groups and do not interbreed with them.

Specificity: A measure of the host range of a biological control agent on a scale ranging from an extreme specialist only able to complete development on a single species or strain of its host (monophagous) to a generalist with many hosts ranging over several groups of organisms (polyphagous) [ISPM No. 3, 1996]

Stage (life stage): A distinct period in the development of an organism (e.g., for some insects, egg, larval, pupal, and adult stages).

Standard: Document established by consensus and approved by a recognized body, that provides, for common and repeated use, rules, guidelines or characteristics for activities or their results, aimed at the achievement of the optimum degree of order in a given context [FAO, 1995; ISO/IEC GUIDE 2:1991 definition]

Surveillance: An official process which collects and records data on pest occurrence or absence by survey, monitoring or other procedures

Survey: An official procedure conducted over a defined period of time to determine the characteristics of a pest population or to determine which species occur in an area [FAO, 1990; revised CEPM, 1996]

Vector: Any living or non-living carrier that transports living organisms intentionally or unintentionally.