*Journal of Integrated Pest Management* (*2016*) 7(1): 5; 1–11
doi: 10.1093/jipm/pmw003
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# *Tamarixia radiata* (Hymenoptera: Eulophidae) × *Diaphorina citri* (Hemiptera: Liviidae): Mass Rearing and Potential Use of the Parasitoid in Brazil

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Received 3 July 2015; Accepted 15 January 2016

## Abstract

Huanglongbing (HLB) is the most serious disease affecting citriculture worldwide. Its vector in the main producing regions is the Asian citrus psyllid, *Diaphorina citri* Kuwayama, 1908 (Hemiptera: Liviidae). Brazil has the largest orange-growing area and is also the largest exporter of processed juice in the world. Since the first detection of the disease in this country, >38 million plants have been destroyed and pesticide consumption has increased considerably. During early research on control methods, the parasitoid *Tamarixia radiata* (Waterston, 1922) (Hymenoptera: Eulophidae) was found in Brazil. Subsequent studies focused on its bio-ecological aspects and distribution in citrus-producing regions. Based on successful preliminary results for biological control with *T. radiata* in small areas, mass rearing was initiated for mass releases in Brazilian conditions. Here, we review the Brazilian experience using *T. radiata* in *D. citri* control, with releases at sites of HLB outbreaks, adjacent to commercial areas, in abandoned groves, areas with orange jessamine (a psyllid host), and backyards.

**Key words**: biological control, parasitoid, Asian citrus psyllid

Huanglongbing (HLB) or greening is currently the most important disease affecting citriculture worldwide and is widespread in almost all citrus-producing regions except the Mediterranean. Infection of groves results in complete loss of productive capacity within four years, and young citrus trees never produce (Belasque et al. 2010).

The disease is associated with three bacteria, "*Candidatus* Liberibacter asiaticus," "*Ca.* L. africanus," and "*Ca.* L. americanus," and a phytoplasma. A fifth causal agent, "*Ca.* L. caribbeanus," was recently identified in the Caribbean region (Instituto Colombiano Agropecuário [ICA] 2014). These organisms are transmitted primarily by insect vectors, the psyllids *Diaphorina citri* Kuwayama, 1908 (Hemiptera: Liviidae) and *Trioza erytreae* (Del Guercio, 1918) (Hemiptera: Triozidae). The former occurs worldwide, while the latter occurs mainly in Africa (Hall et al. 2012) and was recently found in Europe (European and Mediterranean Plant Protection Organization [EPPO] 2014).

In Brazil, *D. citri* was first recorded in 1942 (Costa Lima 1942). This pest received little attention because it directly causes only minor damage to citrus, until 2004 when HLB was first detected. Since then, >38 million citrus plants have been eliminated (Coordenadoria de Defesa Agropecuária [CDA] 2014).

No curative measures have been found for the disease, and it is managed by planting certified seedlings, eliminating symptomatic plants, and chemical control of the vector (Kimati et al. 2005). From 2004 to 2009, the citrus sector increased insecticide use by >600% in attempts to control the psyllid (Neves 2011). Because of this intensive use of pesticides, new control tactics for the vector are being sought, such as management of host plants, insecticidal plants, and biological control.

Biological control of *D. citri* can be accomplished using the parasitoid *Tamarixia radiata* Waterston, 1922 (Hymenoptera: Eulophidae) (Fig. 2), which is the most effective natural enemy. This parasitoid has been successfully used in various parts of the world (Etienne et al. 2001). In Brazil, Gómez-Torres (2009) and Diniz (2013) obtained promising results, with parasitism rates of 72.5 and 88.8%, respectively.

## Natural Enemies of *D. citri*

Natural enemies (parasitoids, predators, and pathogens) are the main factors producing natural mortality in the agro-ecosystem, and are some of the basic components of integrated pest management (IPM). Natural enemies may act separately, keeping the pest


**Fig. 1.** Life cycle of *D. citri*: (**A**) eggs; (**B**) nymphs; (**C**) adult. Biological aspects of *T. radiata*: (**D**) adult parasitizing a nymph; (**E**) egg (in circle); (**F**) prepupa; (**G**) pupa; (**H**) adult near emergence; (**I**) emergence hole of parasitoid. All these pictures was taken by fourth author Jaci Mendes Vieira.

population at acceptable levels, or they may be integrated with other methods for insect control (Kogan 1998).

Currently, the control of *D. citri* for the management of HLB is based mainly on the use of insecticides. DeBach and Rosen (1991) noted several problems that can arise from intensive use of these products, such as the elimination of natural enemies, pest resurgence, secondary pest outbreaks, and the development of pest resistance to insecticides.

According to Qureshi and Stansly (2009), natural enemies play an essential role in regulating the population of *D. citri* in the field, and the elimination of these control agents by intensive use of insecticides can increase the spread of the disease. The major predatory

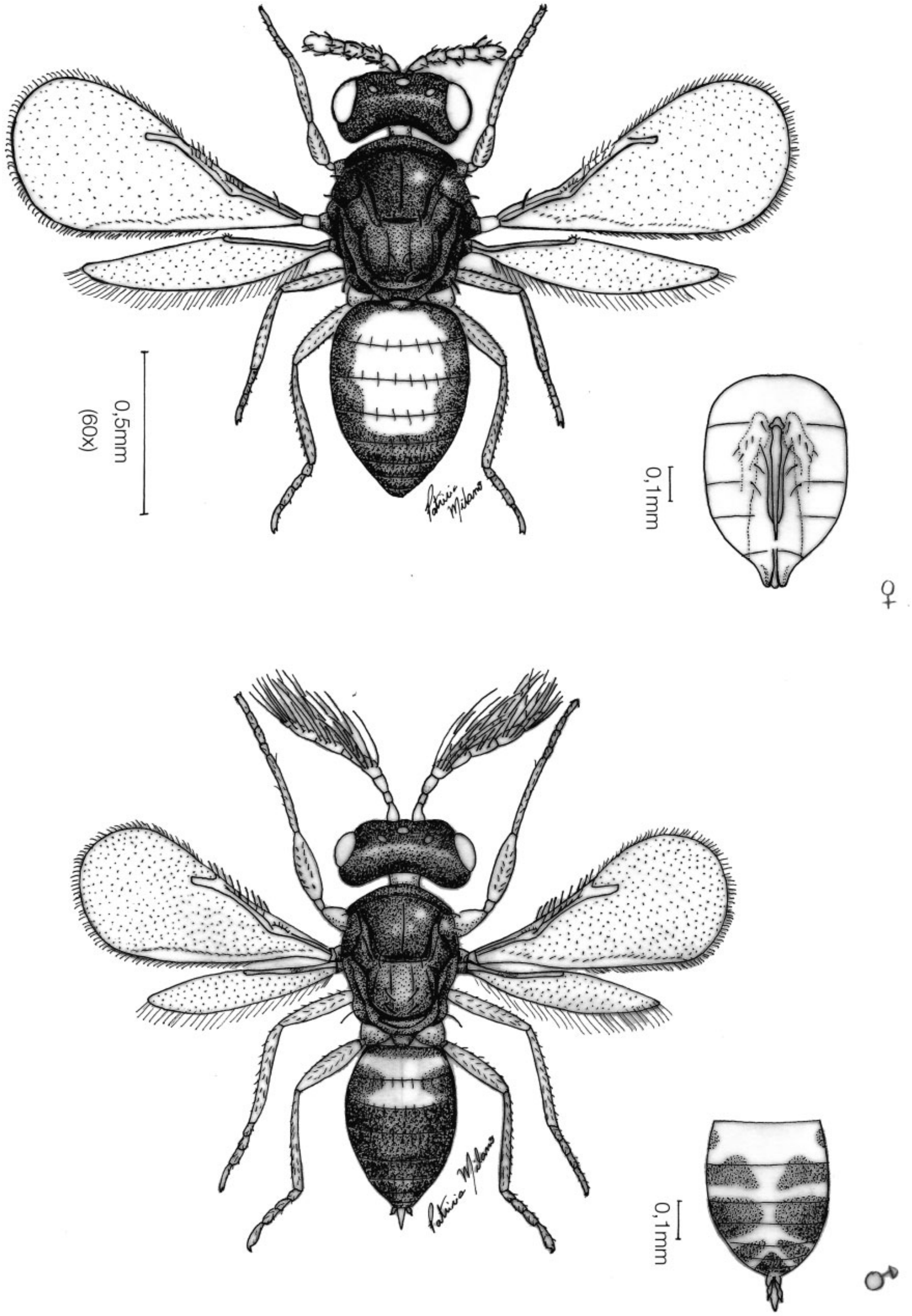


**Fig 2.** Adults of *T. radiata*: (**A**) Female; (**B**) Male. Drawing by Patrícia Milano.

species of *D. citri* in Florida, USA, are the ladybugs *Harmonia axyridis* (Pallas, 1773), *Olla v-nigrum* (Mulsant, 1866), *Cycloneda sanguinea* (L., 1763), and *Exochomus childreni* Mulsant, 1850 (Coleoptera: Coccinellidae); two lacewings, *Ceraeochrysa* sp. Adams (1982) and *Chrysoperla rufilabris* Burmeister, 1839 (Neuroptera: Chrysopidae); and an arachnid, *Hibana velox* (Becker, 1879) (Araneae: Anyphaenidae) (Michaud 2004). Coccinellids have been reported as the most important control agents in Florida. Other predators reported feeding on *D. citri* are members of the families Syrphidae and Anthocoridae (Michaud 2002, 2003).

In Puerto Rico, ladybugs are important agents to control *D. citri*; the most abundant species are *Coelophora inaequalis* (F., 1775) and *Cycloneda sanguinea limbifer* L. (Coleoptera: Coccinellidae) (Pluke et al. 2005). In Mexico, the wasp *Brachygastra mellifera* (Say, 1837) (Vespidae, Polistinae) has also been reported preying on a large number of nymphs of *D. citri* (Reyes-Rosas et al. 2011). Another species with potential for psyllid control is the mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae). This natural enemy caused a sixfold greater reduction of eggs and nymphs in the first instar of *D. citri*, compared to the control. The number of adult psyllids emerged during eight weeks was 80% lower when mites were used as control agents (Juan-Blasco et al. 2012).

In a survey of natural enemies associated with *D. citri* in Brazil, three coccinellids were found on branches containing the psyllid: *C. sanguinea*, *H. axyridis*, and *Scymnus* sp. (Pullus), as well as larvae of syrphids and lacewings. However, only one neuropteran, *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae), has been observed preying on a nymph and an adult of *D. citri* (Paiva and Parra 2012a). According to Parra et al. (2010), predators are not important in the control of *D. citri* in Brazil, probably as a result of the massive use of agrochemicals.

Two parasitoid species associated with the citrus psyllid are *Tamarixia radiata* (Waterston, 1922) (Hymenoptera: Eulophidae), an idiobiont ectoparasitoid, and *Diaphorencyrtus aligarhensis* (Shaffe, Alam and Agarwald 1975) (Hymenoptera: Encyrtidae), an endoparasitoid (Aubert 1987, Halbert and Manjunath 2004). *T. radiata* is believed to be more efficient than *D. aligarhensis* in controlling *D. citri*. A survey conducted in Réunion Island showed that *T. radiata* parasitized up to about 70% of psyllid nymphs, while *D. aligarhensis* parasitized <20% (Aubert 1987).

## Origin and Occurrence of the Parasitoid

*T. radiata* was first described by Waterston in 1922, in the genus *Tetrastichus*, from specimens collected in northwestern India in the province of Punjab, currently part of Pakistan. Graham (1987) transferred it to the current genus. Gómez-Torres (2009) found chromatic variations in different Brazilian populations of *T. radiata*, but concluded that these were adaptations to the environment and therefore the specimens belonged to the same species.

*T. radiata* occurs naturally in various citrus-growing regions in Asia and the Arabian Peninsula, namely, Nepal (Lama et al. 1988), China (Tang 1988), Indonesia (Nurhadi 1989), Thailand and Saudi Arabia (Qing 1990). Following promising results in these regions, *T. radiata* was introduced into other parts of the world, including Réunion Island (Etienne and Aubert 1980), Mauritius (Qing 1990), Taiwan (Chiu et al. 1988), the Philippines (Gavarra et al. 1990), Guadeloupe (Etienne et al. 2001), and the United States of America (USA), in the states of Florida (Hoy et al. 1999) and California (Hoddle 2012).

*T. radiata* has been reported in Brazil (Gómez-Torres et al. 2006), Puerto Rico (Pluke et al. 2008), Venezuela (Cermeli et al. 2007), Argentina (Cáceres and Aguirre 2005, Lizondo et al. 2007), Colombia (Ebratt Ravelo et al. 2011), Cuba (Peralta 2002), Mexico (González-Hernández et al. 2009), and in the state of Texas, USA (Léon and Sétamou 2010), without the need for a previous introduction.

## Bioecological Aspects of *T. radiata*

### Development and Mode of Parasitism

*T. radiata* is an idiobiont ectoparasitoid, specifically of *D. citri*. It is also arrhenotokous, i.e., unfertilized eggs originate males (Chen and Stansly 2014). The females usually lay one, or at most two eggs, on the ventral side of psyllid nymphs between the third pair of legs; if two eggs are laid, only one larva develops. Once hatched, the larva will feed on the hemolymph of the host and, at the end of this phase, it affixes the remains of the nymph to the plant to create a shield for pupation (Hoy et al. 1999) (Fig. 1). As the parasitoid develops, the psyllid nymph darkens and eventually dries; the fully developed adult of *T. radiata* then emerges through a hole in the front region of the nymph. These features allow easy identification of parasitized nymphs (Etienne et al. 2001), as well as of nymphs attacked by *D. aligarhensis*, since adults of this parasitoid emerge from the posterior region of the nymphs.

*T. radiata* adults can feed on first- to third-instar nymphs and on the eggs, and their females parasitize preferably fourth- and fifth-instar nymphs (Chu and Chien 1991, Gómez-Torres 2009, Sule et al. 2014). By feeding and parasitism, a single female parasitoid may eliminate up to 500 nymphs of the psyllid during its life (Chien and Chu 1996). Hoy et al. (1999) noted that feeding on the initial stages of *D. citri* provides essential protein for egg maturation of the parasitoid, characterizing it as a synovigenic insect (Chien 1995).

Gómez-Torres et al. (2012) found that the mean development time of *T. radiata* ranged from 17.33 to 7.60 d within a temperature range from 15 to 35°C for females. For males, the time was significantly shorter, 15.33 to 6.63 d in the same temperature range.

### Supplementary Feeding Effects

Fauvergue and Quilici (1991) found that the mean longevity of parasitoids ranged from 37 to 8 d within a temperature range from 20 to 37°C, respectively, and males have a slightly shorter longevity than females. Longevity may also be affected by supplementing the diet of adults. When fed with pure honey, or a 50% aqueous solution of honey, adult *T. radiata* lived for 24 and 21 d, respectively. On the other hand, when only water was provided, longevity fell to only 2.1 d on average. Chu and Chien (1991) found fecundity levels ranging from 98 to 156 eggs per female, when fed with honey and pollen; however, according to Pluke et al. (2008), a single female of *T. radiata* can lay up to 300 eggs throughout its life.

Diniz (2013) stated that supplementing the diet of *T. radiata* with different mixtures of honey, pollen, and yeast does not influence longevity and parasitism in laboratory tests. However, the parasitism rate of *T. radiata* is highest in the first three days of life and then declines rapidly, as can be clearly observed for all treatments in this study.

### Environmental Factors Influencing Survival and Parasitism Rate

Gómez-Torres et al. (2014) found the highest parasitism rates at 25 and 30°C (84.17 and 72.50%, respectively). The highest emergence rates (86.49 and 88.41%) were also observed at these temperatures. For these same temperatures, the authors found that the total parasitism capacity of *T. radiata* ranged from 20 to 167.42 nymphs parasitized by a female. The lowest parasitism rate was observed at 15°C. According to Quilici et al. (1992), the most favorable temperature range for parasitism is from 25 to 30°C. At these temperatures, they obtained 115 and 230 nymphs parasitized per female, respectively.

McFarland and Hoy (2001) evaluated the influence of five levels of relative humidity (RH) (7, 33, 53, 75, and 97%) on the survival of *T. radiata*, and observed greater survival of parasitoids in the three highest humidity levels. Diniz (2013), evaluating the emergence of *T. radiata* in RHs of 40, 60, 80, and 100%, found significant reductions at 40 and 100%. Similarly, Gómez-Torres (2009) obtained emergence rates of 35.6 and 35.7% in 35 and 90% RH, respectively.

In addition to climate factors, the cultivar of the host plant of *D. citri* can influence parasitism by *T. radiata*. In a field survey, Paiva and Parra (2012b) found differing parasitism rates for the most common citrus varieties grown in the state of São Paulo, from 8.6 to 35.6% in Valencia and Hamlin oranges, respectively. However, this difference was not confirmed for parasitism and development of natural enemies in laboratory tests using the same orange varieties (Alves 2012).

The reproductive success of a parasitoid is defined by its ability to locate partners for reproduction and hosts (Dicke and Grostal 2001). Thus, the host-searching behavior of females of *T. radiata* is associated with the release of volatiles by *D. citri* nymphs (Mann et al. 2010). Mann et al. (2010) found that females are more strongly attracted to citrus plants infested with nymphs, than to plants or nymphs alone. On the other hand, males responded only to females.

Different types of antennal sensilla occur in *T. radiata*; however, the number of each type differs between males and females. The most abundant type of sensillum in females is linked to host searching, while in males, it is related to perception of sexual compounds (Onagbola et al. 2009). Therefore, knowledge of the factors that influence the host-searching behavior is important.

The association of insects with facultative symbionts such as *Wolbachia* can affect the behavior as well as the biology of its host (Werren et al. 2008). Gómez-Torres (2009) found that populations of *T. radiata* free of *Wolbachia* showed lower rates of parasitism and emergence compared with a population of parasitoids with symbionts.

## Efficacy of Parasitism by *T. radiata* Around the World

*T. radiata* is considered a promising organism for the control of *D. citri*, as its use in classical biological control programs has yielded good results. In Réunion Island, HLB was first detected in 1967. *T. radiata* was imported from India, reared in the laboratory, and in 1978–1979 around 4,600 parasitoids were released in 14 different areas of the island. Recaptures of *T. radiata* showed that the parasitoid had become established. Surveys conducted during the 1980s revealed that the population of *D. citri* had suffered a massive reduction and was restricted to only some areas of orange jessamine [*Murraya paniculata* (L.) Jack] (Etienne et al. 2001).

In 1998, *D. citri* was recorded on Guadeloupe, and based on the success at Réunion Island, *T. radiata* was introduced to this island. Only 1,000 parasitoids were released, and they remained and dispersed to various citrus areas (Etienne et al. 2001). According to Halbert and Manjunath (2004), the successes achieved on Réunion and Guadeloupe are attributed to the geographical characteristics of the islands and the absence of hyperparasitoids.

The parasitoid was also introduced to the Philippines, at two separate locations. Where they were reared and released, parasitism rates ranged from 15 to 67% in 1989 and 1990 (Gavarra et al. 1990). In Taiwan, successive releases were performed on citrus and orange jessamine plants between 1983 and 1986. Parasitism rates ranged from 30 to 87%, and the parasitoid also dispersed to areas where no releases were made, proving its dispersal capacity (Chiu et al. 1988).

In Florida, *T. radiata* and *D. aligarhensis* were imported and released in the classical biological-control routine, although only *T. radiata* became established (Michaud 2002). The parasitoid has also been recorded in urban areas, as orange jessamine plants, the preferred host of *D. citri*, are widely used as hedges. These sites can serve as a source of psyllids or their parasitoids. A mean parasitism rate of 18.5% was observed throughout the year on orange jessamine hedges in four locations in Florida (Chong et al. 2010).

In 2012, 5,000 individuals of *T. radiata* were imported from Pakistan and released in southern California, and field surveys showed that the parasitoid successfully became established in the region (Hoddle 2012).

In a study conducted in Puerto Rico in 2004 and 2005, Pluke et al. (2008) recorded a mean parasitism rate of *T. radiata* of 70%, reaching up to 100% on some occasions, on citrus and orange jessamine plants. The authors observed a reduction in the population of *D. citri* under ideal conditions for its development (high availability of new shoots and warm temperatures), which they ascribed to the efficacy of the parasitoid.

In Brazil, Gómez-Torres (2009) released the parasitoid in three commercial citrus-production areas, and reported that the number of fourth- and fifth-instar nymphs of *D. citri* subsequently decreased. Parasitism rates of up to 72.75% were obtained 8 d postrelease, although these rates decreased to between 4.17 and 10% 15 d postrelease. Through surveys, Paiva and Parra (2012b) concluded that the parasitoid is widely distributed throughout the state of São Paulo. They found the highest parasitism rates during the summer, with a mean of 25.7%.

Although under laboratory conditions *T. radiata* shows high reproductive rates and capacity to control the psyllid, under field conditions in Florida, the results were variable. In the spring and summer, parasitism rates did not reach 20%, but reached 56% during the autumn (Pluke et al. 2008). These investigators did not find hyperparasitoids in the locations searched, and attributed this to extreme climate conditions in the state, as well as to the intensive use of insecticides to control *D. citri*.

The varied efficiency of the parasitoid in the different regions may be related to the degree of similarity of these locales to the center of origin of the species, as well as to the possible existence of different strains (De Leon and Setamou, 2010), which show distinct adaptations in the different regions.

Diniz (2013) conducted a study to evaluate the performance of *T. radiata* in different regions of the state of São Paulo. The author selected six areas (municipalities), characterized as backyards (without application of agrochemicals) and residential landscapes (Table 1). In each area, Diniz conducted a survey to assess natural parasitism, and later, performed eight releases of 400 parasitoids per hectare. The initial assessment indicated very low rates of natural parasitism, as three of the six municipalities showed no natural parasitism. The rate was highest (22.0%) in the municipality of Rincão, and in other areas, ranged from 0.96 to 3.5% (Pirajuí and Itapetininga, respectively). However, after the releases, the initial parasitism and establishment of *T. radiata* was high, with a mean increase of 4.3 times and a mean reduction in the nymph population of 68.6% for the six municipalities (Fig. 3). Compared with the initial rates, the parasitism rates increased from 2.1-fold in Itapetininga to 8.7-fold in Pirajuí, and the reduction of nymphs ranged from 43.3% in Anhembi to 88.8% in Cajobi. Excellent control of nymphs of *D. citri* was obtained in all areas, under different levels of HLB infestation, confirming that *T. radiata* can be an important component of integrated management of this psyllid.

**Table 1.** Characteristics of the sites used in release of *T. radiata* to control *D. citri* (Diniz 2013)

| Municipality | Plant sampled | Area sampled (ha) | Nature of the area |
|---|---|---|---|
| Anhembi | Citrus | 2.5 | Backyard |
| Cajobi | Orange jasmine | 2.5 | Residential landscape |
| Getulina | Citrus | 2.5 | Backyard |
| Itapetininga | Citrus | 2.5 | Backyard |
| Pirajuí | Orange jasmine | 1 | Residential landscape |
| Rincão | Orange jasmine | 1.5 | Residential landscape |

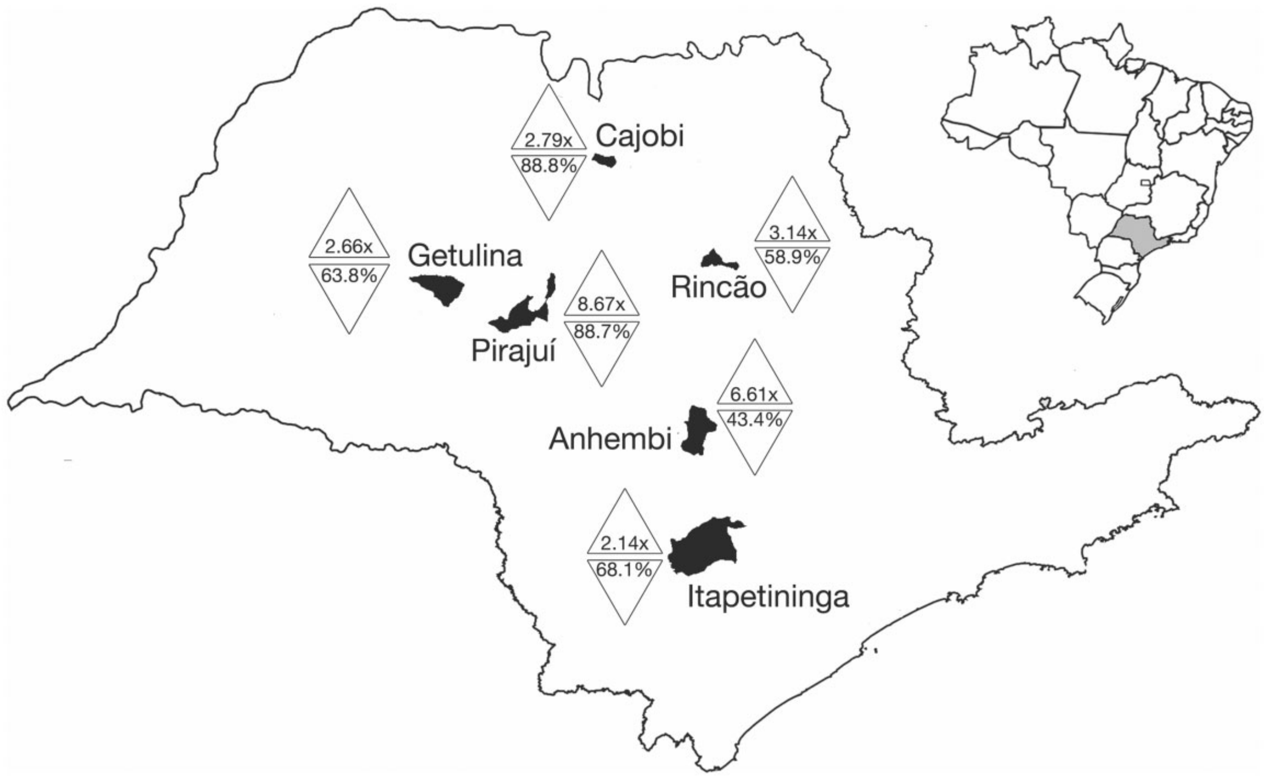


**Fig. 3.** Municipalities where *T. radiata* was released to control *D. citri* in the state of São Paulo (inset map shows location of the state in Brazil), with the mean increase of initial parasitism (up arrow) and population reduction of nymphs (down arrow) of the pest after eight releases (Diniz 2013).

Assessing the toxicity of 16 agrochemicals, insecticides, and fungicides, Hall and Nguyen (2010) found 12 agrochemicals with high or moderate toxicity to *T. radiata*. Parra et al. (2010) concluded that *T. radiata* can be used as a biological control agent of *D. citri*, as an additional component in the IPM in citrus. However, the most serious problem associated with the use of the parasitoid is the systematic use of nonselective chemicals for the control of the psyllid (Beloti et al. 2015). Parra et al. (2010) proposed a new approach to the problem, which is the release of *T. radiata* in abandoned groves, nonsprayed groves, orange jessamine areas, organic groves, and backyard groves, in order to increase the population of the parasitoids for subsequent dispersal, as practically no chemicals are used at these sites. This approach seems promising, because these sites are important sources of contamination and dispersal, and the psyllid can actively migrate over distances from 1.6 to 2.0 km to commercial areas (Ferreira 2014, Lewis-Rosenblum et al. 2015).

## Mass Rearing of *D. citri* to Produce *T. radiata*

Biological control programs involve mass rearing of natural enemies and their subsequent release to foster a rapid reduction of the pest population (DeBach and Rosen 1991). According to van Lenteren (2012), 230 species of natural enemies are commercially available. Each species is produced with particular rearing methods, which are usually proprietary. *Tamarixia radiata* is reared exclusively on nymphs of *D. citri*, and therefore depends on efficient rearing of the host for economic viability.

The first rearing system for *D. citri* and *T. radiata* was established by Skelley and Hoy (2004), using orange jessamine (*M. paniculata*), which is the preferred host of *D. citri* (Aubert 1987). Nava et al. (2007) and Alves et al. (2014) compared different hosts for *D. citri* and found that orange jessamine better supported the development of the psyllid.

The method established by Skelley and Hoy (2004) proposed a rearing system carried out exclusively in air-conditioned rooms, which, although it provides better control of species development and is ideal for small experiments, is costly for mass rearing. In Brazilian conditions, Gómez-Torres (2009) established a rearing system for *D. citri* adapted from the production system used for *Phyllocnistis citrella* Stainton, 1856 (Lepidoptera: Gracillariidae) for the purpose of rearing *Ageniaspis citricola* Logvinovskaya, 1983 (Hymenoptera: Encyrtidae). This system uses, first, citrus seedlings in tubes, and later orange jessamine seedlings in acrylic cages as hosts for the psyllid. This rearing system was also developed for use in air-conditioned rooms.

According to Bolckmans (2007), achieving economic efficiency is one of the greatest challenges of biological control, ensuring the quality of the control agents produced. In order to mass-rear parasitoids for field release, a rearing system was developed to reduce costs by rearing the psyllid in greenhouses with minimal control of environmental conditions. The parasitoid continued to be produced in air-conditioned rooms, as temperature control aids substantially in the rearing. In the proposed system, at least three structures are required: two greenhouses, one for the maintenance of the orange jessamine plants, another for rearing the psyllid, and a third, temperature-controlled greenhouse for rearing the parasitoid. This new system is described detailed below.

### Maintenance of Orange Jasmine Plants

Several sizes of orange jasmine seedlings are available in nurseries; however, seedlings planted in 1.5-liter plastic bags, with four to six flushes and about 45 cm tall, were considered most suitable for rearing in the proposed model. The seedlings were fertilized through biweekly foliar applications of 1% (NPK 20-20-20). The sporadic occurrence of aphids, mealybugs, and psyllids was recorded. Psyllids were controlled using yellow traps and spraying with a solution of neutral detergent (10%), and the plants were rinsed immediately afterward. This concentration was sufficient to eliminate the insects without causing phytotoxicity to the plants and leaving residues. Seedlings were kept in a greenhouse with automatic irrigation twice a day, and 50% shade, as according to Arrigoni-Blank et al. (2003), this light level promotes greater sprouting and vigor.

For the rearing, the apical buds were removed, to stimulate sprouting of axillary buds. At a mean temperature of 25°C, the flushes reached optimal size about a week after this pruning. The mean length of 1 cm, with the leaflets still closed is considered to be preferred by the psyllids for oviposition (Diniz 2013). When most of the flushes reached the 1-cm length, the plants were transported to the *D. citri* rearing cages and offered to the females for oviposition. A plant in good condition after pruning produces three to four sprouts per branch, and in a plant with five flushes, we obtained 15–20 sprouts suitable for oviposition.

After use for psyllid and parasitoid rearing, the plants are returned to the greenhouse and receive an application of detergent solution (10%) to eliminate any remaining insects, and are kept there for vegetative development and reuse. A plant remains suitable for the development of the psyllids for two cycles, after which the number of insects produced decreases, due to plant depletion (Diniz 2013).

## Methodology for Rearing *D. citri*

Greenhouse rearing of psyllids has the primary aim of reducing costs, as the construction and maintenance of enough air-conditioned rearing rooms to produce the needed large numbers of insects makes the whole system excessively expensive.

The greenhouse uses a pad-fan evaporative cooling system, programmed to start whenever the internal temperature reaches 28°C. On the hottest summer days, however, even with the system turned on, the internal temperature of the greenhouse can reach 35°C for a few hours. This temperature increase does not seem to affect the rearing, as the largest populations were always obtained during this period.

The rearing cages are constructed using a model with antiaphid nets with 0.87 mm mesh, preventing the psyllids from escaping and any parasitoids from entering, but allowing air circulation. The cages are cub, 47 cm on a side, supported by a frame of polypropylene tubing. Each cage contains a polypropylene tray (45 by 36 by 7 cm), large enough to accommodate six orange jasmine plants, 40 cm tall on average, in plastic bags of 15 cm diameter.

With an average of 15–20 shoots per plant, each cage has 90–120 shoots suitable for oviposition. Three hundred adult psyllids about 12 d after eclosion, i.e., at the beginning of the reproductive phase, are placed in each cage. These insects are kept in the cage for 7 d to allow oviposition, after which they are collected with the use of an electric aspirator. Given the size of the rearing project (200 cages for rearing *D. citri*), this aspirator is essential to improve the efficiency of the process.

Not all insects are recovered; on average, 60% of the insects initially placed are collected. This reduction is attributed to natural mortality. After the adults are collected, the cages with plants and psyllid eggs are transferred to another part of the greenhouse to allow the nymphs to develop to the ideal stage (fourth and fifth instars) for offering to the parasitoids, or are kept until the emergence of adults that will then be used to continue the rearing. At the mean temperature of 25°C, nymphs reach the fourth and fifth instars (ideal for parasitism) in about 12 d, and the *D. citri* adults emerge in ~18 d.

In this method, of all insects produced in all cages, 30% are used to produce adults to continue the rearing colony, and 70% to produce nymphs to be used as hosts for rearing *T. radiata*.

## Method for Rearing *T. radiata*

The parasitoid is reared in air-conditioned rooms regulated at a temperature of 25°C and a photoperiod of 14:10 (L:D) h.

The rearing cages for the parasitoids have aluminum frames, with the top and front closed with polystyrene (4 mm thick) to allow good light passage and better observation of the parasitoids, and antiaphid nets on the remaining sides (0.87 mm mesh). The cage dimensions are 97 by 45 by 45 cm (length by width by height), planned to fit on the metal shelves for optimum arrangement in the rearing room. Each shelf has three divisions, each with its own lighting system for each cage, consisting of four fluorescent lamps (20 W each).

In each rearing cage, two trays of the same type as those used to rear the psyllids are placed, and accommodate 12 plants with nymphs of *D. citri* (fourth and fifth instars). A mixture of honey and pollen (2:1) is offered to supplement the diet of the parasitoids.

The number of nymphs on the orange jasmine plants is estimated in order to calculate the number of parasitoids to be placed in each cage. The parasitoids, about 24 h old, are released at a ratio of 1:10 nymphs of *D. citri*. The parasitoids are not removed afterward.

At 25°C, the adults of *T. radiata* begin to emerge after 12 d. In order to facilitate their collection, emergence boxes are used. These boxes are made of black MDF (medium density fiberboard), fully sealed to exclude light and with dimensions of 40 by 30 by 30 cm, with an opening at the top, where a transparent glass bottle (500 ml) is attached.

Ten days after parasitism, orange jasmine flushes containing mummies (parasitized nymphs) are cut and placed in emergence boxes. Once emerged, the wasps are attracted to the light from the bottles, facilitating their collection. The flushes in these boxes are sprayed with water daily to ensure a sufficient humidity level to prevent mortality of the parasitoids at emergence, as they are particularly sensitive to desiccation at this stage (Diniz 2013). About 70% of the *T. radiata* in the collection boxes emerge in the first three days, and the rest in the following two days.

The parasitoids are collected from the bottles with aspirators of the same type used for the psyllid rearing. The wasps are placed in glass tubes one inch in diameter (200 per tube), a drop of the honey and pollen mixture is added, and the tubes are placed in polystyrene

**Table 2.** Estimated quantities of each item required for deployment of a rearing system for *D. citri* and *T. radiata,* for a production rate of 100,000 parasitoids per month

| Items | Unit | Quantity |
|---|---|---|
| Greenhouse space for plant maintenance | $m^2$ | 153 |
| Greenhouse space for rearing *D. citri* | $m^2$ | 145 |
| Laboratory space for rearing *T. radiata* | $m^2$ | 67.2 |
| Cages for rearing the psyllid | unit | 264 |
| Cages for rearing the parasitoid | unit | 60 |
| Emergence boxes | unit | 45 |
| Shelves for rearing the parasitoid | unit | 25 |
| Plants for rearing both species | unit | 6,912 |

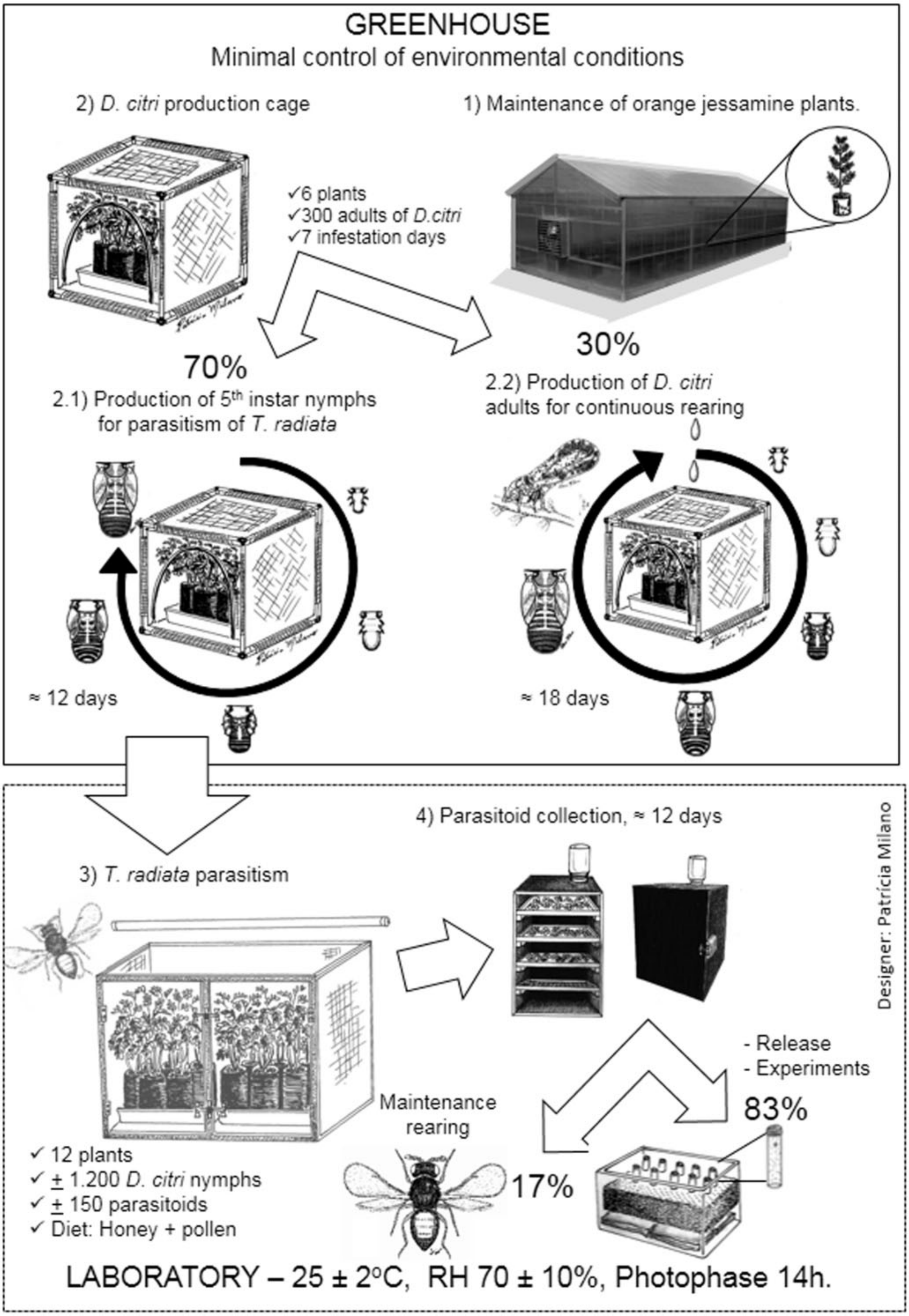


**Fig. 4.** General scheme for rearing *D. citri* and for mass rearing of *T. radiata*. The psyllids are reared in a greenhouse and the parasitoids in a laboratory, at 25°C ± 2°C, 70 ± 10% RH, and a photoperiod of 14:10 (L:D) h (Alves 2012).

boxes kept at 18°C until they are taken to the field. The releases are carried out in late afternoon or early morning. In the proposed system, 17% of the parasitoids are used for continue rearing, and 83% for field releases.

## Dimensions of the Rearing System

A proposal to produce about 100,000 parasitoids per month requires obtaining 5,000 individuals per day, for 20 d each month.

The estimated quantities of each item required for a rearing system for *D. citri* and *T. radiata* as proposed, are given in Table 2.

For the operation of the entire rearing system, daily maintenance is required for the 17 cages, i.e., 12 of *D. citri* and 5 of *T. radiata*. For the plants, at the scale proposed, 132 plants need to be pruned per day. This work requires three full-time employees. A general summary of the proposed rearing system is presented in Fig. 4.

According to the model, the estimated cost per parasitoid is US$ 0.05, including all costs involved in the rearing process and based on the type of proposed physical installation, which could be reduced. The estimate of production costs, at the scale proposed, showed that the most expensive factor in the entire system is labor, which accounts for 76.6% of the total cost. This concords with the information of Parra (2002), who reported that labor represents 70–80% of the cost of producing natural enemies.

The estimated unit cost of the parasitoids is compatible with other forms of control, and in some cases is much lower than the cost of insecticides, depending on the type of management adopted. Using the classical biological-control approach, the cost per hectare would be US$ 24.00, compatible with the cost of using natural enemies for other crops such as sugar cane, soybeans, or corn (maize).

Currently, six rearing facilities are operating in the state of São Paulo, using this model. Four of them are managed by a private orange-juice company, and another two by research institutions, each producing an average of 100,000 parasitoids per month.

## Acknowledgments

We extend our thanks to Janet W. Reid for the English revision, to the Fundo de Defesa da Citricultura (Fundecitrus) (Projeto Tamarixia 837), and Citrosuco (Projeto Tamaradiwa 1022) for financial support.

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