Original article

Biological control of *Diaphorina citri* (Hemiptera: Psyllidae) in Guadeloupe by imported *Tamarixia radiata* (Hymenoptera: Eulophidae)

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Biological control of *Diaphorina citri* (Hemiptera: Psyllidae) in Guadeloupe by imported *Tamarixia radiata* (Hymenoptera: Eulophidae).

Abstract — **Introduction**. The Asian citrus psyllid, *Diaphorina citri* Kuwayama, is particularly harmful to citrus plantations as it is one of the two psyllid vectors of the citrus greening disease or Huanglongbing. This disease, which limits the longevity of trees, is due to an intracellular bacterium which mainly affects the phloem. **Past experience of biological control of *D. citri* in Reunion Island**. In Reunion Island, this disease, detected in 1967, was the main obstacle to the development of the cultivation of citrus fruit. Control of the vector *D. citri*, by introducing from India and multiplying on site the specific parasitoid *Tamarixia radiata* (Waterston), was carried out during 1978 when nearly 4600 adult parasitoids were released. The success of *T. radiata* combined with the distribution of healthy plant material was at the origin of the revival of the cultivation of citrus fruit in Reunion Island. **Programme for a biological control of *D. citri* in Guadeloupe**. In Guadeloupe, *D. citri* was reported in January 1998. No trace of insect parasitism was found and only one fungus, *Hirsutella citriformis* Speare, proved, under certain conditions, capable of regulating the populations of this psyllid. Taking into account the experience in Reunion Island and that the greening disease has not shown up in Guadeloupe, the introduction of *T. radiata* from Reunion Island was made in January 1999. About 1000 *T. radiata* were released all over the Guadeloupe islands. At present, just a little more than 1 year after the last releases, the parasitoid is established and present in almost all the citrus orchards where psyllid populations have decreased considerably. Quite favorable biological characteristics play a great part in explaining the excellent effectiveness of *T. radiata* in biological control programmes against *D. citri*.

France (Reunion) / France (Guadeloupe) / *Citrus* / *Diaphorina citri* / *Tamarixia radiata* / insect control / biological control organisms

Contrôle biologique de *Diaphorina citri* (Hemiptera : Psyllidae) en Guadeloupe par importation de *Tamarixia radiata* (Hymenoptera : Eulophidae).

Résumé — **Introduction**. Le psylle asiatique des agrumes *Diaphorina citri* Kuwayama est particulièrement dangereux pour les plantations d'agrumes car c'est l'un des deux psylles vecteurs de la maladie du greening des agrumes ou Huanglongbing. Cette maladie, qui limite la longévité des arbres, est due à une bactérie intracellulaire qui affecte principalement le phloème. **Expérience passée sur le contrôle biologique de *D. citri* à l'île de la Réunion**. À l'île de la Réunion, le greening détecté en 1967 était l'obstacle principal du développement de la culture des agrumes. Le contrôle de son vecteur *D. citri*, par introduction à partir d'Inde et multiplication sur place de son parasitoïde spécifique *Tamarixia radiata* (Waterston), a été effectué en 1978 ; près de 4600 adultes du parasitoïde ont alors été dispersés. Le succès de *T. radiata* combiné avec la distribution de plants sains a été à l'origine de la renaissance de la culture des agrumes à l'île de la Réunion. **Programme pour un contrôle biologique de *D. citri* à la Guadeloupe**. La présence de *D. citri* en Guadeloupe a été rapportée pour la première fois en janvier 1998. Une enquête menée alors n'a révélé aucune trace de parasitisme d'insecte et seul un champignon, *Hirsutella citriformis* Speare, a prouvé, dans certaines conditions, être capable de contrôler les populations de ce psylle. À partir de l'expérience de l'île de la Réunion et en considérant le fait que la maladie du greening n'avait pas encore été détectée en Guadeloupe, une introduction de *T. radiata* à partir de l'île de la Réunion a été faite en janvier 1999. Environ 1000 adultes de *T. radiata* ont été libérés sur l'ensemble de la Guadeloupe. Actuellement, à peine plus de 1 an après le dernier lâcher, le parasitoïde est bien établi et présent dans presque tous les vergers d'agrumes où les populations de psylles ont considérablement diminué. Les caractéristiques biologiques tout à fait favorables de l'insecte jouent un grand rôle pour expliquer l'excellente efficacité de *T. radiata* dans des programmes de lutte biologique contre *D. citri*.

France (Réunion) / France (Guadeloupe) / *Citrus* / *Diaphorina citri* / *Tamarixia radiata* / lutte anti-insecte / auxiliaire de lutte biologique

1. Introduction

Citrus greening disease, or Huanglongbing, represents one of the major obstacles in cultivating citrus fruit in the numerous Asian and African countries where it is present [1–4]. Indeed, plantations affected by this disease decline rapidly; their longevity is reduced and thus incompatible with an economic, profitable activity. Between 1960 and 1980, decisive studies were carried out aiming at a better understanding of this disease and its vectors [5–7]. The disease was shown to be an intracellular bacterium affecting principally the phloem [8–10] and significant differences were found between bacterial strains from Africa and Asia [11, 12], the latter having been characterized and named recently as *Liberobacter asiaticum* [13, 14].

The specific vectors of this disease are two psyllid species found initially in two distinct regions [15]. One species, *Diaphorina citri* Kuwayama (*figure 1*) originating from the eastern region, is present in South East and Southern Asia; the other species, *Trioza erytreae* (Del Guercio) originating from the Ethiopian region, is confined to zones of high altitude in tropical Africa [3]. The presence, at the same time, of the greening disease and its two vectors in the Indian Ocean islands of Reunion and Mauritius as well as in Saudi Arabia is most probably due to the transport of contaminated plants. The presence of the Asian psyllid, *D. citri*, in the neotropical region has likely the same origin. This psyllid is now reported in Brazil [16, 17], Honduras [18], Uruguay [19] and just recently in Guadeloupe [20], Florida [21] and Cuba in 1999 (Fernandez[1], pers. com.). Even though the greening disease has not yet been detected in the neotropical region, the presence of the vector *D. citri* is a serious threat to citrus plantations which cover wide areas in this region.

The bacterium responsible for the greening disease may also be spread easily by the grafting of budwoods collected on contaminated plants. The use of healthy plant material makes it possible to avoid this type of dissemination.

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2. Review of past experience of biological control of *D. citri* in Reunion island

In Reunion Island, citrus greening disease was first reported on the island by Moreira [22]. The following year, its presence was confirmed by experimentations of laboratory transmission and by observation in the field of the two species of psyllid vectors: *T. erytreae* and *D. citri* [23]. At that time, the populations of the African psyllid *T. erytreae* were particularly abundant on citrus fruit in zones of high altitude while *D. citri* was mainly localized in the dry and hot regions of the leeward coast, at altitudes generally below 500 m. While the control of the psyllid vectors was mainly achieved by intensive chemical spraying, Catling [24] suggested the introduction of parasitoids originating from the same country as these psyllids. This was the option chosen in Reunion Island from 1975 to 1980 [25]. *T. erytreae* was controlled in a spectacular way by the introduction of the parasitoid *Tamarixia dryi* (Waterston) (Hymenoptera: Eulophidae) from South Africa [26] and was totally eliminated in a few years from its habitat. The parasitoid's survival depended on a related species of host-psyllid [27]. For the purpose of this paper, we will review mostly some aspects of the Asian psyllid bioecology and its control by means of *Tamarixia radiata* (Waterston).

Since the early work of Husain and Nath [28], the biology of *D. citri* has been studied by Pande [7] and, more recently, by Tsai and Liu [29]. *D. citri* develops uniquely on plants of the Rutaceae family, mainly on *Citrus* spp. and *Murraya* spp. The adult is small in size (2.8–3.2 mm), with a spotted grey body and forewings forming a roof over the abdomen. These semi-transparent wings are greyish in appearance with a brownish stripe starting at the top half and ending at the apex. The adults are quite mobile, they jump when they are disturbed and fly off readily. The feeding posture is characteristic, the body forms an angle of 30° with the surface of the vegetation. Mating occurs generally 1 to 3 d after the emergence of the adults when conditions are favorable (presence of young

Figure 1.
Adult of the Asian citrus psyllid, *Diaphorina citri* (D. Vincenot, Suad / Cirad).

Figure 2.
Nymphs of *Diaphorina citri* on a citrus shoot, with characteristic honeydew excretion (D. Vincenot, Suad / Cirad).

Figure 3.
Adult of *Tamarixia radiata*, with mummies of *Diaphorina citri* nymphs, on leaves of *Murraya paniculata* (A. Franck, Cirad).

Figure 4.
Nymph of *Diaphorina citri* infected by the entomopathogenic fungus *Hirsutella citriformis* (J. Étienne, Inra).

shoots) and egg-laying begins within the 24 h following mating. On the most favorable host-plant, the mean fecundity per female reaches 857 eggs [29], always laid at the base of buds or at the tip of young shoots. The eggs, which measure about 0.3 mm long, are wide at the base and tapered at the top. Freshly laid, they are pale yellow and turn a brick orange color near hatching time. The larvae are mobile and go through five instars. The first three develop mainly on the buds or young shoots. In the last two instars, the larvae often migrate to very young stems. In the course of their development, the larvae secrete a characteristic white, waxy honeydew (*figure 2*). The total duration of development from egg to adult requires 16.9–17.6 d at 25 °C [29]. The mean female longevity is 39.6–47.5 d at 25 °C [29]. However, outside periods of host-plants vegetative growth, the adults can live for several months awaiting favorable conditions [28].

Only one endoparasitoid of *D. citri* larvae, *Diaphorencyrtus aligarhensis* (Shaffee, Alam and Agarwal) (Hymenoptera: Encyrtidae), was found in 1975 in several areas of Reunion Island though its importance was not specified at the time. It has been regularly observed since, but its impact on Asian psyllid populations remains limited compared to *T. radiata* [27, 30].

The planned introduction of *T. radiata* from India to control *D. citri* necessitated organizing the rearing of this psyllid so as to be able to multiply the parasitoid. The rearing of *D. citri* took place in a greenhouse in potted plants of *Citrus* spp. sufficiently developed to be pruned so as to produce numerous buds and young shoots indispensable for psyllid egg-laying. In the hot period (February–March), on plants at a suitable vegetative stage, mating takes place 2 to 3 d after the emergence of adults, egg-laying starts 24 h later, incubation of eggs lasts about 4 to 6 d and larval development 10 to 15 d [25].

T. radiata develops as an idiobiont ectoparasitoid on *D. citri* larvae. The females lay their eggs on the ventral side of the larvae in their third to fifth instar. When hatched, the larva of the parasitoid sucks the hemolymph from the host and, at the end of development, it fixes the remains of the dead psyllid larva ("mummy") onto the plant surface to pupate under this protection (*figure 3*). Experiments at various constant temperatures between 20 °C and 30 °C showed that development time decreases with the rise of the temperature: from 17 d at 20 °C, it is only 8 d at 30 °C. Within the same range of temperatures, the duration between egg-laying and mummification also decreases with the rise of the temperature [31].

The adult emerges by piercing a round hole in the thorax. Within the range 20–35 °C, adult longevity also decreases when the temperature rises: it goes from 37 d at 20 °C to 8 d at 35 °C [31]. Male longevity is slightly shorter than that of females. Feeding with honey improves fecundity and increases adult longevity. On the contrary, when they are fed water only, or in the absence of food, the survival of adults is very short [31]. The biotic potential of the parasitoid was studied by Quilici *et al.* [32]. The species shows an arrhenotokous parthenogenesis. At the most favorable temperatures, 25 °C and 30 °C, the female fecundity reaches around 300 eggs. In the laboratory, the average number of host mummies produced by the female is 115, with a maximum of 230. At 20 °C and 35 °C, fecundity is lower and the average number of mummies produced by the female is no more than 30 or so. Ovipositing females show a preference for the fifth (last) larval instar of the host. In the offspring of mated females, females are more numerous than males and represent 66–68% of emerged adults. Superparasitism remains fairly low and appears when the level of parasitism is high [32]. The females of this parasitoid also exhibit host feeding on psyllid larvae, which increases their impact on their host populations [33].

The planned introduction of *T. radiata* necessitated prospecting in India. A mission carried out in the Punjab [34] made it possible to collect parasitized larvae of *D. citri* and bring them to Reunion Island. A total of nearly 500 larvae alive and dead were delivered. The plant material of Indian origin which was used to transport the *D. citri*

larvae was destroyed on arrival. The live larvae of *D. citri* were transferred onto sections of citrus branches from Reunion Island. The rearing of these larvae occurred in plastic boxes. Sections of branches were renewed every 2 d. From this rearing, *D. citri* adults were obtained, as well as 32 adults belonging to three *Psyllaephagus* spp. (Hymenoptera: Encyrtidae) and eight *T. radiata* adults.

Taking into account the low number of *T. radiata* adults obtained and so as to avoid losing these parasitoids, the first generation was obtained using *D. citri* larvae (3rd and 4th stage) isolated on portions of young branches placed in plastic boxes. Four males and 10 females were obtained from this first generation. It was noticed that changing the sections of branches caused a significant mortality of the psyllid larvae before the parasitoids could complete their development. Thus, the 10 females obtained were placed directly onto the plants with psyllid larvae and covered by fine tissue cylinders. After 8 d of presence with the parasitoids, the psyllid larvae were collected and placed in boxes to obtain parasitoids. Over 8 months (May–December 1978), more than 5000 *T. radiata* adults were obtained.

The rearing of the parasitoid was carried out on a continual basis from May to December 1978, and releases took place all through this period. A total of nearly 4600 adults were released in 14 regions [25] and recaptures were carried out on several occasions in several regions of the island (St. Denis, St. André, St. Gilles and Cambaie) where psyllid larvae had become very rare. Though the acclimatization of the parasitoid was quickly achieved, it took about 2 years before the populations of *D. citri* were significantly limited [27]. Since the middle of the eighties, *D. citri* is now almost absent from citrus orchards and maintains just a few residual populations on the ornamental Rutaceous *Murraya paniculata* (L.) Jack. (orange jessamine) which is among its preferred host-plants [30, 35].

The excellent biological control achieved on the two psyllid vectors in Reunion enabled the local cultivation of citrus fruits to develop over an area of 400 ha. It also served as a basis to the development of integrated control methods aimed at all citrus pests [30, 36]. In another geographical area, *T. radiata* was also later on successfully introduced in Taiwan for the biocontrol of *D. citri* [37, 38].

3. Programme for a biological control of *D. citri* in Guadeloupe

3.1. Materials and methods

3.1.1. Preliminary survey

Soon after the discovery of *D. citri* in Guadeloupe in January 1998, a survey was conducted in all citrus producing areas of the island to determine the extent of the psyllid distribution as well as the eventual presence of indigenous natural enemies. On the whole, some 40 sites were inspected by visual control of adults and sampling of young shoots brought back to the laboratory to look for eggs or larval instars.

3.1.2. Introduction of *T. radiata*

The introduction of *T. radiata* was also the option chosen in Guadeloupe in 1999 to control the populations of the Asian psyllid. A hundred or so larvae parasitized by *T. radiata* arrived in Guadeloupe from Reunion Island on January 4th, 1999. During transport, 23 males and 51 females emerged and died. A total of 3 males and 20 females, alive when the parcel arrived, were released the same day in an orchard in Morne-à-l'Eau (Grande-Terre island) which was highly infected by the psyllid but had not had any chemical treatment. The following day, 1 male and 6 females emerged from the remaining mummies and were released in Lamentin (Basse-terre Island) on a highly infested, untreated lemon tree.

3.2. Results and discussion

3.2.1. Preliminary survey

D. citri was discovered in Guadeloupe in January 1998, in the region of Baie-Mahault and Lamentin (Basse-Terre island). From

the results of the survey, two periods can be distinguished in the year 1998:

– From January to July, the infestation appeared low with small scattered colonies of psyllids found in some districts of Grande-Terre (Pointe-à-Pitre, Gosier, Sainte-Anne) and Basse-Terre (Petit-Bourg, Goyave, Capesterre-Belle-Eau).

– From August to December, the infestation became generalized, with spectacular outbreaks occurring in various parts of the French department. In less than 1 year, encouraged most likely by the winds of the cyclone George (September 1998), this psyllid invaded all the orchards of the region as well as citrus trees isolated here and there in gardens at low altitudes. Taking into account the marked relief of Basse-Terre, the psyllid settled in a coastal strip no higher than 500 m in altitude whereas it is reckoned that it occupies all of Grande-Terre whenever its host-plants are present.

During 1998, no parasitism was found on *D. citri*. Just one ladybird *Coelophora inaequalis* (F.) (Coleoptera: Coccinellidae) was observed, in 2 out of 35 samples, feeding on young *D. citri* larvae during outbreaks of the invasion period. Its role in the control of this psyllid is probably minor. More efficient but as yet insufficient, an entomopathogenic fungus, *Hirsutella citriformis* Speare (*figure 4*), was detected in 5 out of 35 samples. It attacks *D. citri* larvae and adults mainly in humid zones (north of Basse-Terre) in periods of heavy rainfall. In these conditions, more than 80% of adult psyllids and larvae are often infected. This fungus is largely widespread in the tropical zone and is not restricted to *D. citri* as it also attacks various other Homoptera (K. Hodge, pers. com.).

The results of the preliminary survey showed that the indigenous natural enemies were obviously insufficient for limiting the populations of *D. citri*. Bearing in mind the results obtained in Reunion Island, the introduction of *T. radiata* from this island was planned.

3.2.2. Introduction of *T. radiata*

Surveys carried out in the two release sites at the end of January 1998 showed that *T. radiata* had started to settle in both sites. In April, a sample of some 500 psyllid larvae of various instars was collected in the orchard in Morne-à-l'Eau, from which 48 males and 97 females of *T. radiata* emerged, which corresponds to a parasitism rate of about 29%. These adults were used to set up a rearing of this parasitoid on *D. citri* larvae reared on *Murraya paniculata*. This plant is easier to develop in a pot than citrus plants and very well suited to the development of the Asian psyllid.

From May to November 1999, the increase in *T. radiata* made it possible to release a total of 971 parasitoids: 419 in nine districts of Grande-Terre and 552 in eleven districts of Basse-Terre. One year after the last releases, *T. radiata* was present not only in the zones where it was released but also all over the department of Guadeloupe. Its impact obviously contributed to the sharp decrease in the populations of *D. citri* that was observed after the biocontrol programme.

Hyperparasitoids are known to be able to considerably limit the action of the primary parasitoid in the zone of origin of *D. citri* as is the case in certain regions of India [6]. Similarly, the impact of various introductions of *T. radiata* in Asian countries has been extremely limited by the presence of hyperparasitoids, as in the Philippines [39] or in Indonesia [40]. In Guadeloupe, it is advisable to follow the evolution of this new biological equilibrium between *D. citri* and *T. radiata* in the years to come, and to check at the same time that no local hyperparasitoid develops to the detriment of the primary parasitoid introduced.

An integrated pest control in citrus plantations should also be carried out in order to avoid the rash use of insecticides during periods of vegetative growth. This would encourage, undoubtedly, the outbreaks not only of *D. citri* but of other pests [*Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), *Toxoptera citricida (*Kirkaldy) (Homoptera: Aphididae)] which attack the same plant stage and which are usually controlled by a series of beneficials.

4. Conclusion

In Reunion Island, as from 1980, the cultivation of citrus fruit was given a new impetus despite the presence of greening disease. This impetus was brought about by the scientific studies which made it possible to set up a rational control based on the biological control of the psyllid vectors (release of 4600 *T. radiata* adults), the elimination of trees contaminated by the greening bacterium and the distribution of healthy plant material. These measures which were adopted 20 years ago are still efficient today.

In Guadeloupe, although citrus greening disease has not been detected, the recent arrival of *D. citri* in this French department prompted us to introduce its specific parasitoid. The release of a total of about 1000 *T. radiata* adults in 20 districts of the islands allowed the parasitoid to establish and spread to most of the zones infested by *D. citri*.

The success of the biological control of *D. citri* in Reunion Island is largely due to the biological characteristics of *T. radiata* (very short cycle compared to the host cycle, quick colonization and dispersion, high fecundity) and to the fact that the introduction was carried out by carefully avoiding the importation of any hyperparasitoid. In Guadeloupe, though the populations of *D. citri* clearly decreased after the introduction of *T. radiata*, it will be necessary in the near future to quantify more precisely the impact of the parasitoid, both on *Citrus* and on alternative rutaceous host-plants. Also, a close watch should be kept on all the citrus trees of the islands so as to detect, as early as possible, eventual foci of the greening disease and take the necessary, immediate action.

This effective control method is considered to have considerable potential for the Caribbean in the near future, as was underlined in a recent "Regional Seminar on the Phytosanitary Situation of Citrus in the Caribbean", organized by Cirad (Centre de coopération Internationale en Recherche Agronomique pour le Développement) and CTA (Technical Centre for Agricultural and Rural Cooperation), held in Pointe-à-Pitre, Guadeloupe, November 28th – December 1st, 2000.

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Control biológico de *Diaphorina citri* (Hemiptera: Psyllidae) en Guadalupe por importación de *Tamarixia radiata* (Hymenoptera: Eulophidae).

Resumen — **Introducción**. La psila asiática de los cítricos *Diaphorina citri* Kuwayama es especialmente peligrosa para las plantaciones de cítricos ya que es una de las dos psilas vectores de la enfermedad del greening de los cítricos o Huanglongbing. Esta enfermedad, que limita la longevidad de los árboles, es causada por una bacteria intracelular que afecta principalmente al floema. **Experimento sobre el control biológico de *D. citri* en la isla de la Reunión**. En la isla de la Reunión, el greening detectado en 1967 era el principal obstáculo para el desarrollo del cultivo de cítricos. El control de su vector *D. citri*, mediante introducción a partir de la India y multiplicación *in situ* de su parasitoide específico *Tamarixia radiata* (Waterston), se realizó en 1978 esparciendo cerca de 4600 adultos del parasitoide. El éxito de *T. radiata* combinado con la distribución de plantas sanas fue el origen del renacimiento del cultivo de cítricos en la isla de la Reunión. **Programa para un control biológico de *D. citri* en Guadalupe**. Se informó de la presencia de *D. citri* en Guadalupe en enero de 1998. El estudio llevado a cabo no reveló ninguna huella de parasitismo de insecto y sólo un hongo, *Hirsutella citriformis* Speare, demostró, en ciertas condiciones, ser capaz de controlar las poblaciones de esta psila. Partiendo del experimento de la isla de la Reunión y teniendo en cuenta que la enfermedad del greening aún no se había detectado en la Guadalupe, se efectuó una introducción de *T. radiata* desde la Reunión en enero de 1999. Se liberaron aproximadamente 1000 adultos de *T. radiata* en el conjunto de Guadalupe. Actualmente, cuando hace un poco más de un año desde la última suelta, el parasitoide está bien establecido y se halla presente en casi todos los huertos de cítricos. Las poblaciones de psilas han disminuido considerablemente en estos huertos. Las muy favorables características biológicas del insecto son fundamentales para explicar la gran eficacia de *T. radiata* en los programas de control biológico contra *D. citri*.

Francia (Reunión) / Francia (Guadalupe) / *Citrus* / *Diaphorina citri* / *Tamarixia radiata* / control de insectos / organismos para control biológico