Original article

Effects of imidacloprid and fenobucarb on the dynamics of the psyllid *Diaphorina citri* Kuwayama and on the incidence of *Candidatus* Liberibacter asiaticus

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Effects of imidacloprid and fenobucarb on the dynamics of the psyllid *Diaphorina citri* Kuwayama and on the incidence of *Candidatus* Liberibacter asiaticus.

Abstract — **Introduction**. The effects of imidacloprid and fenobucarb insecticides on the dynamics of the psyllid *Diaphorina citri* and on the incidence of *Candidatus* Liberibacter asiaticus (*Ca*. L. a.), the putative causal agent of Huanglongbing disease (HLB), were studied in a field experiment. **Materials and methods**. The experimental design consisted of three independent 0.5-ha *Citrus* orchards planted with disease-free HLB-susceptible orange trees, located in a *Citrus* producing area seriously affected by HLB. Imidacloprid was applied monthly to the trunks in one orchard at a rate of 0.15 g a.i.·tree^{-1}; fenobucarb was sprayed fortnightly in a second orchard at a rate of 250 g a.i.·ha^{-1}. The 3rd orchard was managed as a control without insecticide applications. The total number of adult *D. citri* specimens and the percentages of trees harbouring psyllid eggs and 5th instar nymphs were monitored at fortnightly intervals in each orchard. *Ca*. L. a. incidence was assessed in each orchard by PCR at 5 months, 12 months and 24 months after planting. **Results**. Compared with the control, both the fenobucarb and imidacloprid treatments reduced adult psyllid populations by over 90% and reduced the frequency of trees harbouring eggs and 5th instar nymphs. Only imidacloprid treatments totally prevented development of a new generation of adults from eggs. Two years after planting, the prevalence of *Ca*. L. a. was 0.939, 0.745 and 0.239 in the control and in the orchards treated with fenobucarb and imidacloprid, respectively. **Discussion and conclusion**. The results indicated that, although both the insecticides used effectively reduced *D. citri* populations by killing adults and nymphs and by affecting or preventing psyllid reproduction in orchards, neither of the two insecticide treatments totally prevented transmission of *Ca*. L. asiaticus. However, due to its long-lasting effect and systemic activity, the imidacloprid treatment provided the best protection against infections, and delayed and slowed down the spread of the pathogen. Furthermore, it reduced the number of pesticide applications needed and left the way open for biological integrated pest management programmes.

Viet Nam / *Citrus* / disease control / psyllidae / *Diaphorina citri* / pathogens / insecticides

Effets de l'imidaclopride et du fénobucarbe sur la dynamique du psylle *Diaphorina citri* Kuwayama et sur l'incidence de *Candidatus* Liberibacter asiaticus.

Résumé — **Introduction**. Les effets des insecticides imidaclopride et fénobucarbe sur la dynamique du psylle *Diaphorina citri* et sur l'incidence de *Candidatus* Liberibacter asiaticus (*Ca*. L. a), l'agent causal putatif de la maladie du Huanglongbing (HLB), ont été étudiés expérimentalement au champ. **Matériel et méthodes**. Le dispositif expérimental a consisté en trois vergers indépendants de 0,5 ha d'orangers sains mais sensibles au HLB, situés dans une zone de production de *Citrus* sérieusement affectée par le HLB. L'imidaclopride a été appliqué mensuellement sur les troncs d'un vergers à la dose de 0,15 g de matière active par arbre ; le fénobucarbe a été pulvérisé tous les quinze jours dans un second verger à la dose de 250 g de matière active par hectare. Le troisième verger a été conduit en parcelle témoin sans traitement insecticide. Le nombre total de spécimens adultes de *D. citri* et les pourcentages d'arbres portant des œufs de psylle et des nymphes de 5^{e} stade ont été relevés tous les quinze jours dans chaque verger. L'incidence de *Ca*. L. a. a été déterminée dans chaque verger par PCR à la périodicité de 5 mois, 12 mois et 24 mois après plantation. **Résultats**. Comparativement au verger témoin, les traitements au fénobucarbe et à l'imidaclopride ont réduit les populations de psylles adultes de plus de 90 % et ils ont diminué la fréquence des arbres portant des œufs et des nymphes de 5^{e} stade. Seuls les traitements à l'imidaclopride ont empêché le développement d'une nouvelle génération d'adultes à partir des œufs. Deux ans après plantation, la prévalence de *Ca*. L. a. était de 0,939, 0,745 et 0,239 dans le verger témoin et dans les vergers traités avec le fénobucarbe et l'imidaclopride, respectivement. **Discussion et conclusion**. Les résultats ont indiqué que, bien que les deux insecticides utilisés réduisaient efficacement les populations de *D. citri* en tuant les adultes et les nymphes et en affectant ou empêchant la reproduction du psylle dans les vergers, aucun des deux traitements insecticides n'empêchaient totalement la transmission de *Ca*. L. a. Cependant, en raison de sa rémanence et de son activité systémique, le traitement à l'imidaclopride a fourni la meilleure protection contre les infections ; il a retardé et ralenti la propagation du pathogène. De plus, ce traitement a réduit le nombre d'applications nécessaires et permettrait la mise en place de programmes de contrôle par lutte biologique intégrée.

Viet Nam / *Citrus* / contrôle de maladies / psyllidae / *Diaphorina citri* / agent pathogène / insecticide

1. Introduction

Huanglongbing disease is one of the most serious diseases of *Citrus*. It affects yields and fruit quality, and shortens the lifespan of infected trees. The causal agent, identified as a bacterium [1, 2], is the non-cultured phloem-restricted alpha-proteobacterium '*Candidatus* Liberibacter' [3] (*Ca*. L.). Several species associated with Huanglongbing have been identified [4]. '*Ca*. L. asiaticus', the putative causal agent of the Asian form of the disease, is transmitted by the psyllid *Diaphorina citri* Kuwayama [5–7] and occurs in Asia and America [8, 9]. '*Ca*. L. africanus' occurs in Africa and is transmitted by the psyllid *Trioza erytreae* Del Guercio [10]. Recently, a new species, '*Ca*. L. americanus', probably transmitted by *D. citri*, was identified in South America [11].

At the present time, direct control of *Ca*. Liberibacters is impossible and injecting antibiotics into infected trees [12] is not economically sustainable. Given the lack of resistant or even tolerant *Citrus* varieties, the existing Huanglongbing control strategies are indirect and based on reducing or controlling psyllid vector populations using insecticides and/or biological control.

On the island of Reunion, where both the Asian and African forms of the disease, as well as their respective psyllid vectors, *D. citri* and *T. erytreae,* exist, biological control of the two psyllid species has been successfully achieved by introducing and releasing two imported hymenopteran psyllid parasitoids: *Tamarixia radiata* Waterston, which has been used against *D. citri*, and *Tamarixia dryi* Waterston against *T. erytreae* [13]. Unfortunately, such biological control cannot be implemented in most countries affected by Huanglongbing, due to the existence of indigenous hyperparasitoids that attack psyllid parasites. Hyperparasitoids are known to considerably limit the action of the primary parasitoid in the zone of origin of *D. citri,* and in certain regions of India [14]. Similarly, the impact of various *T. radiata* introductions in Asian countries has been extremely limited by the presence of hyperparasitoids, as in the Philippines [15] or in Indonesia [16].

Several insecticides, including organophosphates and pyrethroids, applied by spraying, trunk injection or soil applications have been used in psyllid and HLB disease management programmes [17–20]. All the chemicals tested have been proved effective against *D. citri* and reduced populations developing in orchards by up to 90% [21]. However, most of those insecticide management programmes had a limited effect on disease development, as the vector had a very low noxiousness threshold and because orchards were rapidly re-colonised by infectious populations developing on wild and untreated reservoir plants. Furthermore, most insecticide management programmes are only implemented during peak psyllid population periods, while insecticide coverage needs to be provided throughout the year, even when population levels are low.

In tropical and subtropical Asia, where only *D. citri* and the Asian form of the disease occur, the disease can affect all *Citrus* cultivars and species (*C. sinensis*, *C. limon*, *C. reticulata* and *C. paradisi*) and can destroy orchards within 5 years of planting. The severity of the disease, a lack of tolerant/resistant *Citrus* varieties and the impossibility of biologically controlling *D. citri* have prevented the establishment of viable *Citrus* industries. This has led to massive, excessive and, most of the time, inappropriate use of insecticides, which is incompatible with integrated pest management programmes. In addition, massive spraying of non-selective insecticides often disrupts the biological control of a range of other *Citrus* pests. Significant improvements could be achieved in minimising vector populations, disease transmission and ecological damage by implementing strategic insecticide practices and by improving pesticide applications.

Imidacloprid, a contact and systemic neonicotinoid insecticide with good persistence, has been shown to be effective not only against a wide range of insects, including *D. citri* and other vectors of plant diseases [21, 22], but also in reducing insect feeding and/or disease transmission [22–28]. In addition, imidacloprid can be used in trunk applications which protect auxiliary fauna and reduce chemical release into the environment.

Our objective was to examine how psyllid dynamics and *Ca*. L. asiaticus spread were affected by an insecticide treatment based on monthly trunk applications of imidacloprid, compared with the effectiveness of a practice conventionally implemented in Vietnam, consisting of spraying fenobucarb, a contact insecticide, at fortnightly intervals.

2. Materials and methods

2.1. Site

Experiments were conducted in the Mekong delta area of South Vietnam (Caí Bè district, Tiên Giang province) in a traditional *Citrus* producing area where *Ca*. L. asiaticus and *D. citri* are endemic. There were typical and strong Huanglongbing (HLB) disease symptoms, and large *D. citri* populations.

2.2. Field plots

The experimental design consisted of three 0.5-ha orchards, each planted with 200 HLB-susceptible *Citrus* trees and surrounded by *Citrus* orchards severely affected by HLB. The plots were planted in July 2003 with 6-month-old disease-free planting material (King mandarin, grafted on *Citrus volkameriana*), originating from the Southern Fruit Research Institute, Viet Nam, and produced from mother plants grown under insect-proof conditions. Trees were tested by PCR prior to the launch of the experiment to check for the absence of *Ca*. L. asiaticus. The trees were planted in a 3 m × 3 m planting design.

2.3. Insecticide treatments

In one orchard, a soluble concentrate of imidacloprid (200 g a.i·L^{-1}) (Confidor, Bayer, Monheim am Rhein, Germany) was applied monthly to the trunks at a rate of 0.15 g a.i. per tree: 0.75 mL of undiluted insecticide was applied on the lower part of the trunk 30 cm from the ground. The application level was based on the usage recommended by the manufacturer. In a second orchard, fenobucarb was sprayed fortnightly at the rate conventionally used by farmers, namely 250 g a.i.·ha^{-1}. The third orchard was managed as a control without insecticide applications.

2.4. Population dynamics

Each fortnight, one day before each insecticide application, we recorded in each experimental orchard the total number of adult *D. citri* specimens and the total numbers of trees harbouring psyllid eggs and 5th instar nymphs. The presence/absence of psyllid eggs and 5th instar nymphs was checked for each tree on the terminal 10 cm of each branch. The total number of adult specimens was counted on each tree by a careful inspection of the whole canopy.

Monthly means were calculated for the total number of adult *D. citri* specimens. Monthly frequencies of trees harbouring psyllid eggs and 5th instar nymphs were calculated as the monthly mean number of trees harbouring eggs or nymphs, divided by the total number of trees still alive in the plot. Data were collected from July 2003 to January 2005.

2.5. *Ca.* Liberibacter assessment

2.5.1. Sample collection

Five months, 1 year and 2 years after planting, each tree in the three experimental orchards was individually sampled for disease assessment. Each sample consisted of 20–30 leaves collected from the entire canopy of the tree. Each sample was processed, separating midribs from laminae. Midribs were cut into 1-cm-long pieces and the sample was homogenised. Each sample was then divided into three separate 1-g batches stored at –20 °C pending analysis.

2.5.2. DNA extraction

Total DNA was extracted from each batch of excised midribs using a ball-bearing

Table I.
Reduction (%) in the prevalence of *Diaphorina citri* populations in citrus orchards managed with applications of fenobucarb and imidacloprid insecticides (Mekong delta, South Vietnam).

	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Mean
	2003						2004												2005	
Reduction in the prevalence of adult *D. citri* specimens																				
A Fenobucarb	57	93.6	74.1	89.7	0	55.9	86.6	99.2	94.1	90.5	98.3	91.4	88.8	97.9	95.6	94.9	93.2	99.8	99.2	93.5
B Imidacloprid	100	100	100	96.6	90	98.5	99.7	97.9	97.7	98.9	99.2	97.3	99.2	100	99.7	100	100	100	100	99.1
Reduction in the frequency of trees harbouring *D. citri* eggs																				
C Fenobucarb	100	88.9	100	100	–	46.2	88.6	83.1	91.1	79.6	94.7	85.1	83.5	91.7	97.2	97.8	96.4	100	97.5	90.1
D Imidacloprid	100	100	100	100	–	100	100	100	96.0	100	100	99.4	100	100	100	100	100	100	100	99.7
Reduction in the frequency of trees colonised by *D. citri* 5th instar nymphs																				
E Fenobucarb	100	15.2	0	100.0	0	62.3	50.5	96.6	88.9	87.2	92.5	92.3	92.8	95.7	96.6	95.8	–	93.1	100	75.2
F Imidacloprid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	–	100	100	100

apparatus and cethyltrimethyl ammonium bromide (CTAB) buffer [29].

2.5.3. *Ca*. Liberibacter detection

All DNA extracts were individually checked for *Ca*. Liberibacter infection by using PCR amplification of *Ca*. Liberibacter 16S rDNA with the specific primer pair 16SA2-GoRev5 [30].

2.5.4. *Ca.* Liberibacter prevalence

Pathogen prevalence π was calculated on each date of the survey as the number of infected trees divided by the total number of trees still alive in the plot.

2.6. Assessment of insecticide efficiency against psyllid populations and against *Ca.* Liberibacter epidemics

The relative efficiency of fenobucarb and imidacloprid treatments against *D. citri* populations was assessed by calculating the monthly and overall reduction achieved by each insecticide treatment in (i) the total number of adult specimens, (ii) the frequency of trees harbouring eggs, and iii) the frequency of trees colonised by 5th instar nymphs. Data collected from the untreated orchard were used as references.

The effects of insecticide treatments on *Ca.* Liberibacter epidemics were assessed by comparing pathogen incidence over time. Previous temporal analysis of HLB spread showed that epidemics tended to follow a sigmoid curve [31, 32] and could be described by using the logistic model [31]. *Ca.* Liberibacter prevalence π, assessed at time t = 5 months, 1 year and 2 years after planting, were fitted to a logistic regression function $\{\pi(t) = \exp(a+bt) / [1 + \exp(a+bt)]\}$ [33]. Coefficients a, b (monthly rate of logistic increase), and the coefficient of determination R^2 were estimated by a generalised linear model procedure. Approximate confidence intervals for the time that gave rise to a *Ca.* Liberibacter prevalence π_0 were estimated using Fieller's method [33].

3. Results

3.1. Adult psyllid population dynamics

In the orchard managed without insecticide (*figure 1A*), adult specimens were found on all dates of the survey and a total of 7417 specimens was counted from July 2003 to January 2005. The adult population peaked three times in 2004: in February-March, in June-September and in December.

In orchards managed with fenobucarb and imidacloprid insecticides, no such seasonal population peak was observed (*figures 1B, 1C*, respectively). Compared with the total number of adults counted in the untreated orchard, fewer adults were found in the treated orchards: in total, 480 specimens were counted in the orchard managed with fenobucarb and 65 specimens in the orchard with imidacloprid. The overall reduction in adult populations provided by the fenobucarb treatment was 93.5% but monthly values varied from 0% (November 2003) to 99.8% (December 2004) (*table I*, row A). In the orchard managed with imidacloprid, the overall reduction in adult populations was 99.1% (*table I*, row B). Monthly reduction rates varied from 90% (November 2003) to 100%. No adults were found in the orchard treated with imidacloprid from July to September 2003, in August 2004, and from October 2004 to January 2005.

3.2. Egg-laying activity

In the untreated orchard, psyllid eggs were found on all the survey dates, except in November 2003 (*figure 1A*). The frequency of trees harbouring eggs varied from 0 (November 2003) to 0.333 (July 2004) and peaked in January, March, June-July, September and December 2004. In the orchard treated with fenobucarb (*figure 1B*), the frequency of trees harbouring eggs never exceeded 0.055 and reached a maximum in July 2004. No eggs were found in July, September and October 2003 or in December 2004. In the orchard treated with imidacloprid (*figure 1C*), eggs were only found in March and June 2004. The maximum frequency of trees harbouring eggs was 0.010.

The overall reduction in the frequency of trees harbouring eggs achieved by fenobucarb spraying was 90.1% but monthly values varied from 46.2% (December 2003) to 100% (July, September, October 2003 and December 2004) (*table IC*). In the orchard treated with imidacloprid, the overall reduction was 99.7%. Monthly reduction rates varied from 96% (March 2004) to 100% (*table ID*).

3.3. Nymph population

In the untreated orchard (*figure 1A*), 5th instar nymphs were found on all survey

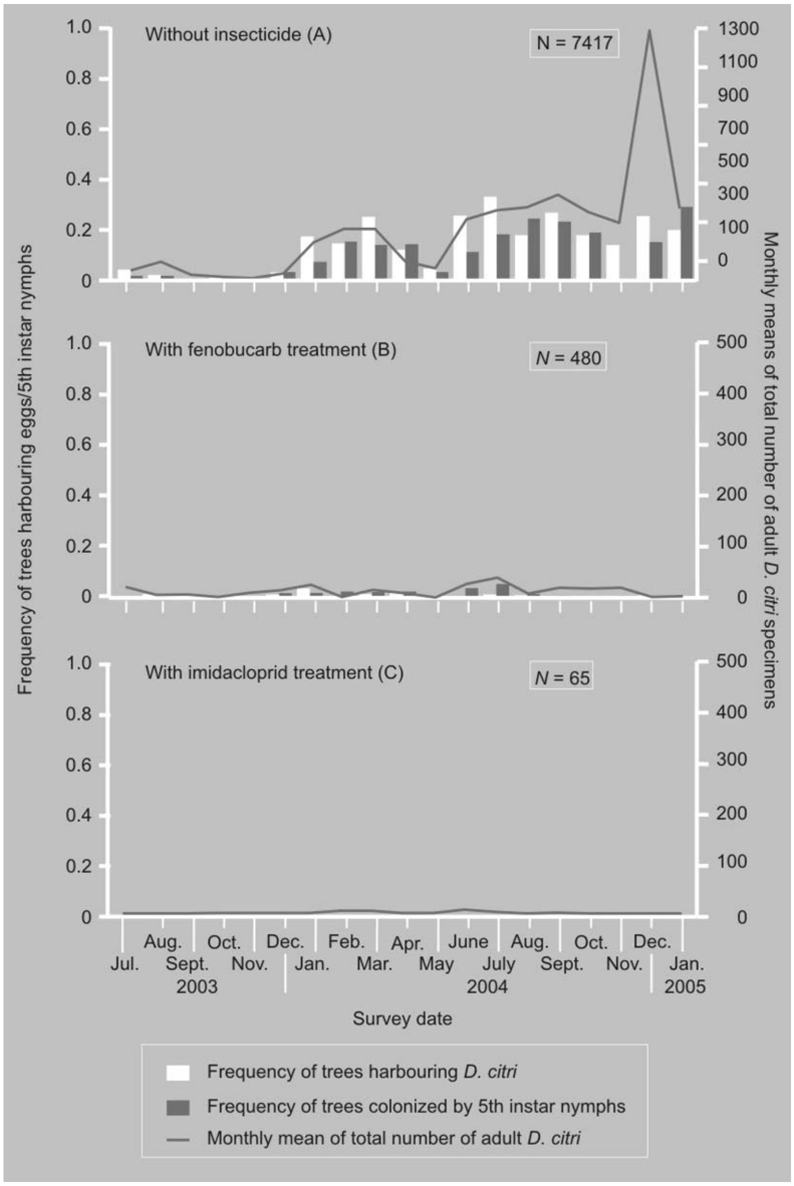

Figure 1.
Variation in the monthly average number of adult *Diaphorina citri* specimens, the frequency of trees harbouring eggs and of trees colonised by 5th instar nymphs from July 2003 to January 2005 in untreated, fenobucarb-treated and imidacloprid-treated *Citrus* orchards (*N* = total number of adult of *D. citri* specimens counted over the duration of the experiment).

Table II.
Number and prevalence of *Candidatus* Liberibacter asiaticus-infected trees in citrus orchards at 5 months (December 2003), 1 year (July 2004) and 2 years (July 2005) after planting, depending on the insecticide treatment with fenobucarb or imidacloprid (Mekong delta, South Vietnam).

Treatments	December 2003		July 2004		July 2005	
	Number of infected trees	Disease prevalence	Number of infected trees	Disease prevalence	Number of infected trees	Disease prevalence
A untreated	4	0.022	124	0.619	188	0.939
B fenobucarb	5	0.028	34	0.171	149	0.745
C imidacloprid	0	0	8	0.043	48	0.239

Table III.
Estimated values and confidence intervals of coefficients *a* and *b* of the logistic regression, and R^2 values, for Huanglongbing disease decrease in citrus orchards (Mekong delta, South Vietnam).

Treatments	a			b			R^2
	Estimate	Lower	Upper	Estimate	Lower	Upper	
Untreated	– 3.95	– 4.60	– 3.30	0.331	0.275	0.386	0.903
Fenobucarb	– 4.50	– 5.13	– 3.86	0.233	0.200	0.267	0.988
Imidacloprid	– 5.92	– 7.24	– 4.61	0.200	0.141	0.258	0.990

dates, except in November 2004. The frequency of colonised trees varied from 0.002 (September and November 2003) to 0.292 (January 2005) and peaked three times: in the February-April 2004 period, in the August-September 2004 period and in January 2005. In the orchard treated with fenobucarb (*figure 1B*), 5th instar nymphs were found on all survey dates except in July and October 2003, in November 2004 and in January 2005. The frequency of colonised trees was maximum in January 2004 (0.037). The overall reduction in colonised tree frequency achieved by fenobucarb treatments was 75.2%; monthly values varied from 0 to 100% (*table IE*). In the orchard treated with imidacloprid (*table IF*), no 5th instar nymphs were found on any survey date. Imidacloprid treatments achieved a 100% reduction in colonised tree frequency for the duration of the experiment.

3.4. *Ca.* Liberibacter prevalence

Five months after planting, *Ca.* Liberibacter prevalence was 0.022 and 0.028 in the untreated orchard and the orchard treated with fenobucarb, respectively. No infected trees were found in the plot treated with imidacloprid (*table II, figure 2*).

One year after planting, all the experimental orchards were affected by the pathogen: *Ca.* Liberibacter prevalence was 0.043 and 0.171 in the plots treated with imidacloprid and fenobucarb, respectively, and reached 0.619 in the control orchard. Two years after planting, *Ca.* Liberibacter prevalence was 0.239, 0.745 and 0.939, respectively (*table II, figure 2*).

3.5. Analysis of disease increase

For each experimental plot, the coefficients of determination (R^2) and coefficients *a* and *b* of the logistic regressions were estimated (*table III*). As parameters *a* and *b* of a given logistic regression are highly correlated, confidence regions for those parameters are more relevant than confidence intervals taken separately. Confidence regions for those parameters at levels 0.95 and 0.99 were drawn (*figure 3*). As the confidence regions do not overlap, the curves are considered as significantly different.

The logistic rates of monthly disease increase, *b*, were 0.33, 0.23 and 0.20 in the untreated orchard and the orchards treated with fenobucarb and imidacloprid, respectively (*table III*). The logistic model predicted a pathogen prevalence of 0.995, 2.3, 3.5 and 4.7 years after planting in the untreated orchard and in the orchards treated with fenobucarb and imidacloprid, respectively (*table IV*).

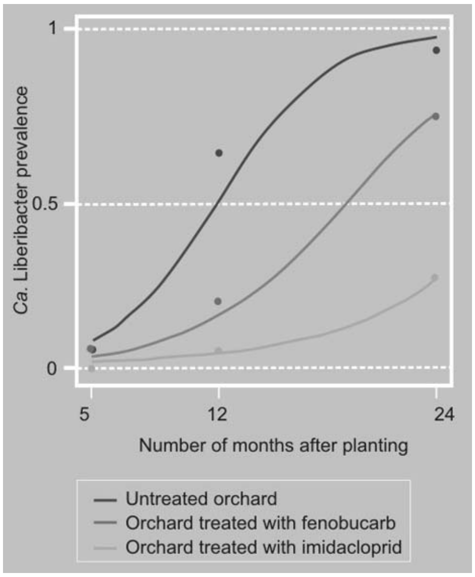

Figure 2.
Prevalence of *Candidatus* Liberibacter asiaticus and adjusted logistic regression curves in untreated, fenobucarb- and imidacloprid-treated citrus orchards.

4. Discussion

4.1. Effects of insecticide treatments on *D. citri* populations

In tropical and subtropical Asia, *D. citri* populations display seasonal fluctuations correlated with host plant development. Spring, summer and autumn flushes are followed by an increase in *D. citri* populations [34]. Data presented in this paper and collected from an untreated orchard confirmed such seasonal fluctuations and showed that *D. citri* was abundant at our experimental site for the duration of the experiment. Adult populations peaked three times in 2004: in February–March, June–September and December, which coincided with periods of new flush production and an increase in psyllid reproduction. Consequently, the psyllid population present in the untreated orchard resulted from both the arrival of specimens from surrounding foci, and multiplication of the psyllids already in the orchard.

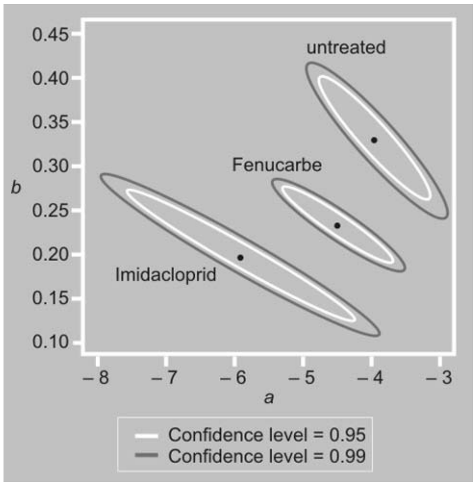

Figure 3.
Confidence regions of the parameters *a* and *b* of the logistic regression for Huanglongbing disease decrease in citrus orchards treated with fenobucarb or imidacloprid insecticides.

Data from the treated orchards indicated that both fenobucarb and imidacloprid insecticides were effective against *D. citri*: adult populations were reduced by over 90% compared with the population found in the control orchard, and no major increase was found during periods of new flush production. However, in the orchard treated with fenobucarb, adult specimens were found on all survey dates. In addition, eggs and 5th instar nymphs were frequently found in the orchard. These observations indicated that D. citri was able not only to reinvade the orchard between two insecticide sprayings, but also to stay alive long enough to reproduce inside the orchard. This suggests that, in view of the short residual activity and/or over-long intervals between two sprayings, the fenobucarb treatment assessed was not adapted to the biology of D. citri, which can produce a new adult generation within 12 to 15 days [34]. Furthermore, data showed that the reduction in adult populations achieved by fenobucarb spraying could fluctuate over time. The effectiveness of insecticide application to foliage is known to depend on the

Table IV.
Estimates and confidence intervals of the time (in months and years) giving rise to a *Candidatus* Liberibacter asiaticus prevalence of 0.995 in citrus orchards (Mekong delta, South Vietnam).

Treatments	Estimate		Lower		Upper	
	Months	Years	Months	Years	Months	Years
Untreated	27.9	2.3	25.4	2.1	31.4	2.6
Fenobucarb	42.0	3.5	38.8	3.2	46.2	3.8
Imidacloprid	56.2	4.7	48.3	4.0	70.5	5.9

climate and application conditions. Not only high rainfall that occurred during the monsoon season, but also hot temperatures and wind conditions during spraying may have led to a loss or degradation of the chemical and might explain the observed fluctuations. Hence, the psyllid population present in that orchard mainly resulted from the arrival of individuals from surrounding foci and might also have partly resulted from occasional and possible psyllid multiplication inside the orchard, depending on the residual activity of the sprayed insecticide.

In the orchard treated with imidacloprid, the adult population remained extremely low throughout the experiment, while there was a large population in the adjacent control orchard. Some eggs were observed on two survey dates only, but 5th instar nymphs were never found. These results indicate that, through their systemic activity and long-lasting chemical effect, the imidacloprid treatments remained effective over the interval between two insecticide applications and prevented establishment, build-up and multiplication of the psyllid population in the orchard. Hence, the adult specimens found in the orchard resulted solely from migrations from surrounding multiplication sites.

To sum up, the data collected in our experimental plots indicated that: (i) psyllid populations displayed seasonal fluctuations but remained present and could multiply in *Citrus* orchards throughout the year, and (ii) only the imidacloprid treatment totally prevented *D. citri* from multiplying in the orchard.

4.2. Effects of insecticide treatments on *Ca.* Liberibacter incidence

Ca. L. asiaticus was detected in the untreated orchard from the first inspection, 5 months after planting, and the incidence of infected trees reached 0.94 only 2 years after planting. An incidence of 0.995 was predicted after 2.3 years. These results indicate that *Ca.* L. asiaticus prevalence was substantial at our experimental site and confirm that young *Citrus* orchards planted in that area are highly vulnerable to the disease. The epidemic spread of *Ca.* L. asiaticus was a result of both primary transmission attributable to infectious psyllids migrating from external foci and secondary transmissions caused by infectious psyllids that multiplied on infected trees in the orchard.

Ca. Liberibacter infections occurred in the orchards treated with fenobucarb and imidacloprid. This indicates that although the two insecticide treatments could drastically reduce *D. citri* populations, neither of them could totally prevent transmission of *Ca.* L. asiaticus. This can be explained by the fact that insecticide applications did not have any effect on the arrival of viruliferous psyllids, responsible for primary transmission, developing in the vicinity on untreated wild reservoir plants or in neglected orchards. The results show that those migrating specimens, when feeding on treated plants, had enough time to inoculate *Ca.* L. asiaticus before they died. This confirms reported data demonstrating that *D. citri* can transmit *Ca.* L. asiaticus with a short exposure time of 15 min to 1 h [35]. Similar findings have been reported on the transmission of other insect-vectored viruses to plants treated with imidacloprid [23–27].

In the plot treated with fenobucarb, *Ca.* L. asiaticus was detected from the first inspection, five months after planting, and disease incidence was similar to that observed in the untreated orchard. This indicates that fenobucarb treatment, under our experimental conditions, had no major effect on the occurrence of primary infections and on disease outbreak. However, the pathogen spread more slowly than in the untreated orchard and a pathogen prevalence of 0.995 was predicted only after 3.5 years. This slower spread of the pathogen could be explained by the overall reduction in the *D. citri* population achieved by fenobucarb spraying: by limiting multiplication of the psyllid population in the orchard, fenobucarb treatments may have limited secondary spread of *Ca.* L. asiaticus and may thereby have reduced its incidence.

Ca. L. asiaticus was only detected in the orchard treated with imidacloprid 1 year after planting and the rate of pathogen spread was slower than in the plot treated with fenobucarb. Such a delay in disease outbreak and the slower spread of the pathogen can be attributed to the effectiveness of imidacloprid treatments against psyllid populations: due to long-lasting chemical activity, migrating infectious psyllids were killed as soon as they fed on treated plants, which may have reduced the occurrence of primary transmission and may have totally prevented psyllid multiplication and consequent secondary transmission.

5. Conclusion

Forty years after the discovery of its aetiological cause and description of its transmission mechanisms, Huanglongbing (HLB) disease remains one of the most serious threats and impediments hanging over worldwide *Citrus* production. The recent outbreak of the disease in North and South America, where major *Citrus* producing and exporting countries are located, demonstrates how difficult it is to control the spread of the disease, in spite of strict quarantine policies.

Our experiments conducted in Viet Nam, a country seriously affected by HLB, demonstrated, however, that a traditional insecticide management strategy could be significantly improved in order to minimise vector populations and disease transmission.

Using the systemic insecticide imidacloprid against *D. citri*, instead of conventional spraying of environment-damaging and hazardous contact chemicals, led to a drastic reduction in psyllid populations, delayed disease outbreak and slowed down its epidemic development, in spite of the small scale of our experimental orchard and high prevalence of both *Ca.* L. asiaticus and its vector. It can be assumed that coordinated implementation of such insecticide treatments by farmers in the same area would substantially reduce the overall prevalence of *D. citri* and the consequent spread of *Ca.* L. asiaticus. As an added advantage, such insecticide treatment could also control other economically important *Citrus* pests, such as aphids, vectors of viruses, *Citrus* mealybugs, scales and the *Citrus* leaf miner *Phyllocnistis citrella*, while preserving auxiliary fauna since only sap-sucking insects are targeted. Given its systemic properties, soil-applied imidacloprid has less impact on natural enemies of Asian *Citrus* psylla than many foliar contact insecticides [22, 36, 37]. Hence, this system could fit in very well with possible biological and integrated control systems. Coordinated implementation of such insecticide treatment as part of an integrated pest management programme would lead to better phytosanitary conditions in *Citrus* orchards with fewer insecticide applications.

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Efectos de imidacloprid y de fenobucarb en la dinámica de la psila *Diaphorina citri* kuwayama y en la incidencia de *Candidatus* Liberibacter asiaticus.

Resumen — **Introducción**. Se estudiaron mediante experimento sobre el terreno los efectos de los insecticidas imidacloprid y fenobucarb en la dinámica de la psila *Diaphorina citri* y en la incidencia de *Candidatus* Liberibacter asiaticus (*Ca*. L. a), el agente causal putativo de la enfermedad del Huanglongbing (HLB). **Material y métodos**. El dispositivo experimental consistió de tres vergeles independientes de 0,5 ha de naranjos sanos pero sensibles al HLB, situados en una zona de producción de *Citrus* gravemente afectada por el HLB. El imidacloprid se aplicó mensualmente sobre los troncos de uno de los vergeles en dosis de 0,15 g de materia activa por árbol; el fenobucarb se pulverizó cada 15 días en un segundo vergel en dosis de 250 g de materia activa por hectárea. Se condujo el tercer vergel en parcela testigo sin tratamiento insecticida. El número total de especímenes adultos de *D. citri* y los porcentajes de árboles portadores de huevos de psila y de ninfas del 5º estadio se recolectaron cada 15 días en cada vergel. La incidencia de *Ca*. L. a. se determinó en cada vergel por PCR en periodos de 5 meses, 12 meses y 24 meses tras plantación. **Resultados**. En comparación con el vergel testigo, los tratamientos a base de fenobucarb y de imidacloprid redujeron las poblaciones de psilas adultas en más del 90 % y disminuyeron la frecuencia de los árboles portadores de huevos y de ninfas de 5º estadio. Únicamente los tratamientos a base de imidacloprid impidieron el desarrollo de una nueva generación de adultos a partir de los huevos. Dos años después de la plantación, la prevalencia de *Ca*. L. a. fue de 0,939, 0,745 y de 0,239 en el vergel testigo y en los vergeles tratados con el fenobucarb y el imidacloprid, respectivamente. **Discusión y conclusión**. Los resultados indicaron que, a pesar de que ambos insecticidas empleados redujeran eficazmente las poblaciones de *D. citri* matando los adultos y las ninfas y afectando o impidiendo la reproducción de la psila en los vergeles, ninguno de los dos tratamientos insecticidas impidió totalmente la transmisión de *Ca*. L. a. Sin embargo por causa de su remanencia y de su actividad sistémica, el tratamiento a base de imidacloprid ofreció la mejor protección contra las infecciones; retrasó y ralentizó la propagación del patógeno. Además, dicho tratamiento redujo el número de aplicaciones necesarias y permitiría la ejecución de programas de control mediante lucha biológica integrada.

Vietnam / *Citrus* / control de enfermedades / psyllidae / *Diaphorina citri* / organismos patógenos / insecticidas