Thiamethoxam and imidacloprid drench applications on sweet orange nursery trees disrupt feeding and settling behavior of *Diaphorina citri* (Hemiptera: Liviidae)

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Abstract

BACKGROUND: Chemical control is the most used method for management of *Diaphorina citri*, the vector of the phloem-limited bacteria associated with citrus Huanglongbing (HLB) disease. The objectives of this study were: to determine the influence of soil-drench applications of neonicotinoids (thiamethoxam and imidacloprid) on the probing behavior of *D. citri* on citrus nursery trees, using the electrical penetration graph (EPG) technique; and to measure the *D. citri* settling behavior after probing on citrus nursery trees that received these neonicotinoid treatments.

RESULTS: The drench applications of neonicotinoids on citrus nursery trees disrupt the *D. citri* probing, mainly for EPG variables related to phloem sap ingestion, with a significant reduction (≈ 90%) in the duration of this activity compared to untreated plants, in all assessment periods (15, 35 and 90 days after application). Moreover, both insecticides have a repellent effect on *D. citri*, resulting in significant dispersal of psyllids from treated plants.

CONCLUSIONS: This study clearly demonstrates the interference of soil-applied neonicotinoids on feeding and settling behavior of *D. citri* on citrus nursery trees, mainly during the phloem ingestion phase. These finding reinforce the recommendation of drench application of neonicotinoids before planting nursery trees as a useful strategy for HLB management.

Keywords: Asian citrus psyllid; Huanglongbing; neonicotinoids; electrical penetration graph; pest management.

1 INTRODUCTION

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is a vector of the phloem-limited bacteria *Candidatus* Liberibacter asiaticus (Las) and *Ca*. L. americanus (Lam), which are associated with citrus Huanglongbing (HLB) disease.[1-3] HLB is considered the most serious and devastating disease of citrus in the world, due to the severe tree decline (mainly in young trees), yield reduction (fruit drop) and no resistant citrus varieties. [1] Currently, this disease is present in the major citrus-growing regions of the world, and in Brazil, the largest orange juice producer, around 38 million trees were already eradicated in the last nine years. There are no curative measures available to control the HLB. Therefore, disease management is based solely on preventive measures such as: planting healthy citrus nursery trees, inspection and eradication of HLB-symptomatic trees and *D. citri* control.[4] However, disease suppression will be achieved mainly when HLB-measures are applied on a regional scale.[5]

The chemical control is the most used and effective method for management of *D. citri*.[6-8] The current recommendation is to spray contact insecticides (foliar application) on nonbearing and bearing citrus trees, and systemic soil-drench or trunk application insecticides on nonbearing citrus trees in order to protect the new flushes. These last two application methods provide a longer psyllid control period than foliar application and with less impact on beneficial insects.[9,10]

The group of the neonicotinoids is the main chemical class of insecticides for controlling piercing-sucking insects. The success of this chemical class is due to its mode of action [nicotinic acetylcholine receptor (nAChR) agonists], broad-spectrum of efficacy, systemic action in the plant, long residual activity and application versatility (e.g. foliar, soil and trunk).[11,12] The neonicotinoids imidacloprid and thiamethoxam are the most common systemic insecticides used in citrus worldwide. In Brazil, one of the most ordinary practices used by citrus growers is to apply these neonicotinoid insecticides by drench on nursery trees few days before planting for the control of psyllid, sharpshooters, aphids and leafminer, and this kind of application can provide an effective control (mortality ≥80%) up to 90 days in the field.[13] In Vietnam, a high psyllid mortality (>80%) was observed when neonicotinoid insecticides were applied by drench on citrus seedlings as well.[14] However, this percentage of mortality was maintained for 90 days in greenhouse conditions, while in the field it was just kept for 60 days. These insecticides when applied as a soil drench, on citrus groves around 2 years old, were able to reduce significantly *D. citri* population for 6 – 11 weeks after application.[15-17] This variation in the period of control may be related to the rates of insecticides, different citrus varieties used and environmental conditions (soil and climate). Most studies involving insect vectors and insecticides are focused on the control efficacy by measuring insect mortality. Insecticides may also affect vector feeding behavior with potential impacts on pathogen transmission, but these effects have been rarely examined because the study of feeding behavior depends on techniques that are more specialized.

Detailed studies of feeding behavior of piercing-sucking insects were made possible with the development of electrical penetration graph (EPG) systems.[18,19] This technique was used to characterize the probing behavior of several hemipterans, mainly for aphids, on plants treated with insecticides.[20-26] This kind of work is important to elucidate how the insecticides interfere in the probing behavior of insect vectors and consequently how they affect the transmission process (acquisition and inoculation) of phytopathogens.

Psyllid feeding behavior studies gained greater emphasis in the last years, due to the increased importance of diseases associated with *Candidatus* Liberibacter spp. The probing behavior of *D. citri* was characterized by the description of five EPG waveforms using the DC system.[27] More recently, two new waveforms related to pathway phase were described by Cen *et al*.[28] Regarding the effect of insecticides on the probing behavior of *D. citri* using the EPG technique, studies were done with pymetrozine, imidacloprid and aldicarb.[29-31] Serikawa *et al*.[30] observed that soil-drench application of imidacloprid on sour orange (*Citrus aurantium* L.) seedlings disrupts the probing behavior of *D. citri*. However, the effect of neonicotinoids on the probing behavior of *D. citri* have not yet been studied on commercial citrus nursery trees, which are commonly treated with these insecticides to avoid early infection by the pathogen after planting in the field.

Therefore, the primary objective of this study was to determine the influence of soil-drench applications of neonicotinoids (thiamethoxam and imidacloprid) on the probing behavior of *D. citri* on citrus nursery trees, using the EPG technique; and secondly to measure the settling behavior of *D. citri* after probing on citrus nursery trees that received these neonicotinoid treatments. This study is important because the distribution of systemic insecticides in small plants like seedlings compared to larger plants such as citrus nursery trees could be completely different and consequently affect the probing behavior of *D. citri* in a different way. This is the first study that investigates the combined effects of soil-applied neonicotinoids on probing and settling behavior of *D. citri*, using a widely planted sweet orange canopy cultivar (′Valencia′) as test plant.

2 MATERIAL AND METHODS

2.1 Insects and plants

Adults of *D. citri* were reared on *Murraya paniculata* L. (Rutaceae) plants in a climate-controlled room at 25 ± 2 °C, photoperiod of 14:10 (L:D) and 60 ± 10% of RH to obtain Las-free psyllids of similar age for the assays. Adults with 10 to 15 days after their emergence were removed from the rearing colony and maintained for a 24-h acclimation period on seedlings of sweet orange [*Citrus sinensis* (L.) Osbeck].

One-year old sweet orange nursery trees (70-80 cm tall), 'Valencia' (*C. sinensis*), grafted on Swingle citrumelo [*Citrus paradise* Macf. × *Poncirus trifoliata* L. (Raf.)] rootstock, were kept in plastic bags (4 L) with *Pinus* substrate (MultplantCitrus®; Holambra, SP, Brazil) and used in all experiments.

2.2 Insecticide application

Nursery trees from the same lot were pruned and immediately treated by drench with the following insecticides: imidacloprid (Provado® 200 SC; Bayer, Belford Roxo, RJ, Brazil) at a rate of 0.35 g active ingredient/plant; and thiamethoxam (Actara® 250 WG; Paulinia, SP, Brazil) at a rate of 0.25 g a.i /plant. The insecticides were diluted in 50 ml of water/plant before drenching the substrate; by the time of application, the substrate in the bags was damp but not saturated. This is the same approach used by citrus growers before planting. After insecticide application, the plants were kept in a greenhouse under similar light and temperature conditions. For each experiment, a group of untreated plants was included as a control.

2.3 Electrical Penetration Graph (EPG) recording

The monitoring of the probing and feeding behavior was performed with a DC-EPG device, (Model GIGA-8, EPGsystems, Wageningen, The Netherlands), whose original detailed description was made by Tjallingii.[19,32] The recordings were performed with 100× gain and the analogic EPG signal was converted to digital through a Di-710 A/D card (Dataq® Instruments, Akron, OH). A Duo core® desktop computer was used for EPG data acquisition and analysis using Stylet+ software (EPG Systems, Wageningen, The Netherlands).

Adult females were attached to the EPG device as described by Bonani *et al.*[27] Each psyllid was placed on the abaxial surface of the leaf and monitored for 6 h, at 15, 35 and 90 days after application (DAA). At 15 DAA, the plants presented young shoots (15-20 cm length) with not fully expanded leaves; at 35 DAA, shoots with 20-25 cm in length with fully expanded leaves were used. After that, the plants were transplanted to plastic pots (20 L) with *Pinus* substrate and at 90 DAA a second flush occurred and shoots similar to 15 DAA were used. In the case of 90 DAA, due to the plant size, shoots were detached from the plants, and a second cut in the stem was made with the shoots immersed in water to avoid cavitation and then, they were immediately placed in plastic bottles (250 ml) full of water (one shoot per bottle). For 15 and 35 DAA the plant electrode was inserted in the substrate close to the trunk of the plant; and for 90 DAA the plant electrode was inserted in the water inside the bottle.

The process of monitoring was performed in a climate-controlled room (25 ± 2 °C) with artificial light provided by six fluorescent lights (240 W). The number of replicates per treatment per period of application was 20 recordings. Two plants from each treatment were randomly located in a Faraday cage per EPG recording. After each recording, the plants and insects were replaced and randomly arranged in the cage.

The recorded EPG data were analyzed according to the following waveforms described by Bonani *et al.*[27]: *C* (salivary sheath secretion and other stylet pathway activities); *D* (first contact with phloem); *E1* (putative salivation in phloem sieve tubes); *E2* (phloem sap ingestion); and *G* (probably xylem sap ingestion). Relevant EPG response variables were calculated from the recorded data using the EPG-Excel Data Workbook developed by Sarria *et al.*[33] These variables were separated into non-sequential: number of probes per insect (NPI), number of waveform events per insect (NWEI), waveform duration per insect (WDI), waveform duration per event

(WDE) and proportion of individuals that produced a specific waveform type (PPW); and sequential: time to event per insect.[27,34,35] The data of waveform duration and count were log-transformed and square root-transformed, respectively, to improve homogeneity and reduce variability. After transformation the data showed a normal distribution according to D'Agostino-pearson's test and were subjected to conventional analysis of variance (ANOVA). The means were compared using the Fisher Least Significant Difference (LSD) test ($P<0.05$). Statistical analysis was performed using the Statistica 7.1 software (StatSoft, Tulsa, OK, USA). For the PPW variable, the difference among treatments was compared by a Chi-square 3x2 ($P<0.05$) using the Bioestat 5.0 software.[36]

2.4 Settling behavior of *D. citri* after probing on nursery citrus trees treated with systemic insecticides

Nursery citrus trees were pruned 30 days before the experiment and part of them treated with thiamethoxam or imidacloprid as described in item 2.2. This study was performed inside a greenhouse (20-30°C, photophase of 11 h and 50 ± 10% of RH), using observation chambers (100 × 100 × 100 cm), with an aluminum structure and transparent walls (PVC film) in order to facilitate observation, except the cage top, which was covered with anti-aphid screen. Each observation cage had an untreated and a treated tree with either thiamethoxam or imidacloprid. Prior to the experiment, adult psyllids of mixed gender were marked with fluorescent powder (yellow or pink, DayGlo, Cleveland, OH, USA) using the methodology described by Nakata[37] and kept on sweet orange seedlings for a 24-h acclimation period. Marked psyllids were placed in a plastic vial (5 cm diameter by 6 cm high) for a 1-h starvation period; then groups of 50 insects marked with different colors (yellow or pink) were confined on a single branch (new shoot with 15-20 cm length) of both treated and untreated plants using a sleeve cage. After 30 min, enough time for *D. citri* individuals to settle on the shoot and start probing (Bonani *et al*.,)[27] the sleeve cage (as well as insects that remained on the sleeve cage) of each plant was removed and the number of psyllids settled was counted, between 8:30 and 9:00 am. At various periods following sleeve removal (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 24, 27, 32 and 48 h), assessments were done for each plant (treated and untreated) to determine the percentage of insects that: 1) remained on the original plant; 2) moved from the original plant to the other plant; 3) landed on the observation chamber walls or ceiling; or 4) died. Twelve replicates (observation chambers) were used per insecticide treatment. The percentage data were arcsin-square root transformed to improve homogeneity and reduce variability, and then subjected to t-test ($P<0.05$), using the Statistica 7.1 software (StatSoft, Tulsa, OK, USA).

3 RESULTS

3.1 Electrical Penetration Graph (EPG) recording

At 15 DAA there were non-significant differences in mean number of probes (NPI) and waveform events per insect (NWEI) between treated and untreated plants for all waveform types (Table 1). However, at 35 DAA, the mean number of waveform *C* (salivary sheath secretion and other stylet pathway activities), *D* (first contact with phloem) and *E1* (putative salivation in phloem sieve tubes) was significantly lower on treated than on untreated plants, but did not differ between imidacloprid and thiamethoxam treatments (Table 2). The mean number of phloem sap ingestion (*E2*) events was significantly lower on thiamethoxam and imidacloprid treated plants compared with untreated plants. At 90 DAA (Table 3), *D. citri* produced significantly fewer waveform *C* events on untreated than on treated plants. In relation to waveform *G* (xylem ingestion), there were non-significant differences among treatments for any of the evaluation periods.

Considering the mean waveform duration per insect (WDI), *D. citri* remained longer in non-probing (*Np*) activities on treated plants with thiamethoxam and imidacloprid than on untreated plants in all assessment periods (15, 35 and 90 DAA) (Tables 1-3). At 15 and 90 DAA (Table 1 and 3), there were non-significant differences among the treatments for waveforms *C*, *D* and *E1*, whereas at 35 DAA (Table 2), waveform *C* durations was significantly shorter on treated than on untreated plants. The mean duration of waveform *E1* was shorter on thiamethoxam and imidacloprid treatments than on untreated plants at 35 DAA. However, in all assessment periods, the mean duration of phloem sap ingestion (*E2*) per insect was significantly shorter on treated than on untreated plants, with a reduction of 95 and 91% at 15 DAA, 86 and 81% at 35 DAA and 90 and 87% at 90 DAA for thiamethoxam and imidacloprid treatments, respectively (Tables 1-3). For waveform *G,* the variable WDI was not statistically compared, because in all treatments and assessment periods there were just few events per insect (1-5) and low proportion of psyllids that performed this activity (5-20%). In all assessment periods for both insecticides the mean duration of *G* per insect was lower than 10 min, while on untreated it was longer than 10 min.

Regarding the mean waveform duration per event (WDE), there were no significant differences in non-probing (*Np*) activities among treatments at 15 DAA; however, at 35 and 90 DAA *D. citri* remained longer in *Np* on treated than on untreated plants (Tables 1-3). For waveform *C* there were non-significant differences among treatments at 15 and 35 DAA, but at 90 DAA the duration of *C* was significantly shorter on treated than on untreated plants. In relation to waveform *D*, significant differences were observed only at 90 DAA, in which the duration of waveform *D* was significantly shorter on treated than on untreated plants. For waveform *E1,* there were non-significant differences among treatments in all assessment periods. In contrast, the mean duration of phloem ingestion (*E2*) events was significantly longer on untreated than on treated plants with either thiamethoxam or imidacloprid (Tables 1-3). Among the psyllids that performed *E2* in all assessment periods, 66 and 50% were able to produce an *E2* > 10 minutes on thiamethoxam- and imidacloprid-treated plants, respectively, whereas on the untreated plants the percentage was 93%. For waveform *G*, WDE was not statistically compared due to the low number of events available.

Regarding the proportion of psyllids that produced waveform *C* and waveforms related to the phloem (*D* + *E1* and *E2*), the treatments did not differ (P>0.05) in all periods of assessment (Table 4). Likewise, for the sequential variables, the time to perform the first probe and waveform events related to the phloem (*D*, *E1* e *E2*) from the start of the recording, no differences were observed among treatments in all periods of assessments (Table 5).

3.2 Settling behavior of *D. citri* after probing on nursery citrus trees treated with systemic insecticides

The percentage of *D. citri* settled on thiamethoxam- and imidacloprid-treated plants decreased rapidly with time, with significant differences compared to untreated plants (P < 0.05) since 0.5 and 1 h after release for thiamethoxam and imidacloprid, respectively (Fig. 1A and 2A). At the final assessment (48 h after release) most psyllids remained on untreated plants, whereas the percentage of psyllids that stayed on treated plants was nearly zero. This low percentage of psyllids on treated plants was partially due to movement of these insects to the other plant (Fig. 1B and 2B) or to the observation chamber walls or ceiling (Fig. 1C and 2C), but mainly because they died throughout the experiment (Fig. 1D and 2D).

During the first assessments, the percentage of psyllids that moved from treated to untreated plants and vice versa was similar. However, significant differences ($P < 0.05$) were observed after 6 (imidacloprid) and 8 h (thiamethoxam) of release, because the percentage of psyllids that moved from treated to untreated plants increased, while the percentage of psyllids that moved from untreated to treated plants decreased, mainly in the case of thiamethoxam (Figure 1B and 2B). The percentage of psyllids that dispersed from thiamethoxam-treated plants to the observation chamber walls and ceiling was significantly higher ($P < 0.05$) than that from untreated plants in only four of the eight first assessments (Fig. 1C). In the case of imidacloprid-treated plants, however, the percentage of psyllids dispersed on the chamber walls and ceiling was higher than that observed for individuals from untreated plants in most assessments (Fig. 2C).

Regarding psyllid mortality, significant differences ($P < 0.05$) were observed between treated and untreated plants since the first and second assessment for thiamethoxam and imidacloprid, respectively. The percentage of mortality increased over time reaching 83 and 56% for insects from thiamethoxam- and imidacloprid-treated plants, respectively, at the end of the experiment, whereas for untreated plants it was around 20% (Fig. 1D and 2D).

4 DISCUSSION AND CONCLUSIONS

The probing behavior of phloem-feeding insects can be divided mainly in a pathway phase that occurs in non-vascular tissues (activities prior to first phloem contact) and a phloem phase that occurs after phloem contact (activities related with salivation and ingestion).[38,39] The phloem phase represents the predominant activity of *D. citri* probing behavior.[27] The present study showed that drench applications of thiamethoxam and imidacloprid on sweet orange nursery trees disrupt the probing and settling behavior of *D. citri*. This was especially true for EPG variables related to phloem sap ingestion (waveform *E2*) and, in all assessment periods, a significant reduction in the duration of this activity was observed on plants treated with these neonicotinoid insecticides. The discrimination of treated plants occurs mainly when *D. citri* starts feeding in the phloem and after a short period of ingestion most psyllids withdraw their stylets from the plant. This fact can explain, in part, the longer time spent by this insect in non-probing (*Np*) activities on treated compared to untreated plants. Thus, these compounds (thiamethoxam and imidacloprid) act as feeding deterrents for *D. citri* when soil-applied on citrus nursery trees. Likewise, Serikawa *et al*.[30] observed a reduction in the phloem ingestion durations when *D. citri* feeds on sour orange seedlings treated with imidacloprid by drench, but in that case, the reduction was not statistically significant. The reduction of phloem sap ingestion duration and longer periods of *Np* activities were reported for the potato psyllid *Bactericera cockerelli* (Sulc) on potato plants treated with imidacloprid as well.[40]

In general, drench applications on citrus nursery trees seemed not to interfere with probe initiation and some variables related to stylet pathway phase, regardless of the insecticide used. This is evidenced by the similarity in the time necessary for *D. citri* to perform the first probe and reach phloem vessels (waveforms *D*, *E1* and *E2*) on treated and untreated plants. Moreover, the proportion of individuals that produced waveforms related to phloem was similar among the treatments. Unlike our study, Serikawa *et al*.[30] observed more differences in the EPG variables related with pre-phloem level, and most of the psyllids were not able to reach the phloem on treated plants. In that study, sour orange seedlings with height of 20-30 cm were used and imidacloprid was applied at a rate of 0.32 g per plant, whereas in our study, sweet orange nursery trees about 70-80 cm tall were used and the rates of thiamethoxam and imidacloprid applications were 0.25 and 0.35 g per plant, respectively. Therefore, the differences observed between the studies could be related to the size of the plants and rates used; on the smaller plants (seedlings) used by Serikawa *et al.*[30] the insecticide concentration was probably higher, which may have affected the probing behavior of *D. citri* in a different way.

One of the limitations of EPG technique is the fact that the insect is connected to a wire, which can limit its movement. Thus, additional studies using free insects are important to further understand the effects of insecticides on the feeding behavior of piecing-sucking insects. The data from the settling behavior experiment show that drench applications of thiamethoxam or imidacloprid have a repellent effect on *D. citri,* resulting in significant dispersal of psyllids from treated plants to the observation chamber walls and ceiling or to the untreated plant. However, the dispersal did not occur immediately; significant differences in the percentage of psyllids that left the treated plant were observed one hour (imidacloprid) and two hours (thiamethoxam) after the confinement (sleeve) cage was removed. Moreover, the percentage of psyllids that moved from treated to untreated plants and vice versa was similar during the first assessments, with a significant increase in the percentage of psyllids that moved from treated to untreated plants throughout the experiment. Therefore, this settling behavior experiment provided further evidence that the interference factors on the probing behavior of *D. citri* on citrus nursery trees treated with neonicotinoids were more related to the phloem ingestion phase. Altogether, our EPG and settling behavior data suggest that most *D. citri* individuals were able to detect the presence of the insecticides when they started ingesting in the sieve elements, and after that, part of them abandoned the treated plants and the others died. Likewise, soil applications of imidacloprid in potato may cause both a feeding deterrent and a repellent effect on the psyllid *B. cockerelli*.[41]

The results of this paper are important to understand how neonicotinoids insecticides can interfere on Las and Lam acquisition and inoculation by *D. citri* in citrus. Bonani *et al.*[27]

observed that Las acquisition occurred exclusively when *D. citri* was able to ingest from the phloem (waveform *E2*) for a period of 1 h on infected plants, with a 6% efficiency. The waveform *E2* was associated with *Candidatus* Liberibacter solanacearum (Lso) acquisition by *B. cockerelli* on tomato as well; however, in this case the threshold to acquire Lso was 6.9 min of phloem sap ingestion.[42] In our study, most psyllids did not perform a waveform *E2* >10 min on treated plants, regardless of the insecticide used. Therefore, our results suggest that treatment of citrus nursery trees with neonicotinoids is efficient to prevent the acquisition of Las or Lam. In case some psyllids are able to acquire bacteria, there is still a latency period of Las in *D. citri* of at least a week before the psyllids become infective.[43] Considering that most insects in the present study died within 48 hours following exposure to young shoot of plants that received drench applications of thiamethoxam or imidacloprid, it is reasonable to speculate that the probability of acquisition and subsequent transmission of these bacteria by psyllids that land on treated citrus nursery trees is close to zero.

For *D. citri* the waveform *E1* may be associated with salivation in the sieve elements.[27] This waveform was correlated with the inoculation of circulative plant viruses in the phloem by whitefly and aphids during salivation in the sieve elements.[44,45] Waveform *E1* was recently associated with the inoculation of Lso by *B. cockerelli*.[42] In our study, we neither observed a significant reduction in the number of psyllid adults that produced waveforms *E1* nor in the duration of this waveform per event, except at 35 DAA, when the number and duration of *E1* per insect were reduced. Likewise, Serikawa *et al*.[30] found a significant reduction in these two EPG variables (number and duration of *E1* per insect) for *D. citri* when imidacloprid was applied via drench on smaller citrus plants (seedlings). Therefore, the interference in these EPG variables by insecticides could reduce the probability of Liberibacter inoculation in citrus plants by *D. citri*. In potato, the use of imidacloprid applied to the soil significantly reduced transmission of *Candidatus* Liberibacter psyllaurous by *B. cockerelli* when compared to untreated plants.[41] The efficacy of soil-applied neonicotinoids in preventing or reducing inoculation of vascular-restricted pathogens by other hemipteran vectors has also been reported.[46-48] Thus, possible effects of neonicotinoids on vector inoculation should be investigated for the *D. citri*-Liberibacter-citrus pathosystem.

This study clearly demonstrates the interference of soil-applied neonicotinoids on feeding and settling behavior of *D. citri* on citrus nursery trees, mainly during the phloem ingestion phase and until 90 days after application. In São Paulo State, Brazil, this kind of application on nursery citrus trees has good efficacy (mortality>80%) on psyllid control for a period of around 100 days.[9,13] Therefore, our results reinforce the recommendation of drench application of neonicotinoids before planting nursery trees as a useful strategy for HLB management. Moreover, this is the first report showing the effects of thiamethoxam on probing behavior of *D. citri*, which in general were similar to those observed for imidacloprid. Thus, both neonicotinoid insecticides can be applied via drench in citrus nursery trees aiming at a reduction in the probability of HLB spread in young citrus orchards.

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Table 1. Means (± SE) of EPG variables for 6-h monitoring of *Diaphorina citri* on *Citrus sinensis* nursery trees treated by drench with neonicotinoid insecticides (15 days after application).

EPG variables[b]	Thiamethoxam	Imidacloprid	Control	F	df	P
NPI	4.10 ± 0.51a[a]	3.95 ± 0.44a	4.40 ± 0.49a	0.2550	57	0.7757
NWEI						
Np	5.00 ± 0.51a	4.80 ± 0.42a	4.85 ± 0.51a	0.314	57	0.969
C	4.80 ± 0.56a	5.00 ± 0.40a	4.95 ± 0.55a	0.104	57	0.901
D	0.65 ± 0.13a	0.80 ± 0.17a	0.90 ± 0.12a	0.810	57	0.449
E1	0.65 ± 0.13a	0.80 ± 0.17a	0.85 ± 0.11a	0.059	57	0.552
E2	0.60 ± 0.11a	0.65 ± 0.13a	0.80 ± 0.09a	0.919	57	0.404
G	0.05 ± 0.05a	0.25 ± 0.12a	0.10 ± 0.07a	1.402	57	0.254
WDI[c]						
Np	284.94 ± 14.02a	261.52 ± 16.61a	158.57 ± 25.78b	9.190	57	0.000
C	68.44 ±14.28a	79.41 ± 15.67a	65.35 ± 17.22a	1.151	57	0.323
D	0.45 ± 0.05a	0.77 ± 0.21a	0.54 ± 0.11a	1.317	37	0.280
E1	0.85 ± 0.17a	1.19 ± 0.19a	1.40 ± 0.35a	1.222	37	0.306
E2	7.64 ± 2.19b	13.87 ± 2.72b	161.27 ± 29.32a	34.558	37	0.000
G	3.15 ± NA	4.63 ± 3.59	47.70 ± 13.61	---	---	---
WDE[c]						
Np	56.99 ± 9.21a	54.48 ± 8.23a	32.69 ± 5.83a	2.213	290	0.111
C	14.26 ± 3.10a	15.88 ±3.64a	13.20 ± 2.65a	0.680	292	0.507
D	0.42 ± 0.06a	0.58 ± 0.11a	0.48 ± 0.05a	0.769	43	0.469
E1	0.79 ± 0.16a	0.90 ± 0.16a	1.31 ± 0.22a	2.208	43	0.122
E2	7.64 ± 2.19b	12.80 ± 2.55b	161.27 ± 29.32a	35.925	38	0.000
G	3.15 ± NA	3.70 ± 2.89	47.70 ± 13.61	---	---	---

[a] Averages followed by the same letter, in the same row, do not differ significantly (P > 0.05) using Fisher LSD test

[b] NPI (number of probes per insect), NWEI (number of waveform events per insect), WDI (waveform duration per insect) and WDE (waveform duration per event).

[c] Values in minutes.

Table 2. Means (± SE) of EPG variables for 6-h monitoring of *Diaphorina citri* on *Citrus sinensis* nursery trees treated by drench with neonicotinoid insecticides (35 days after application).

EPG variables[b]	Thiamethoxam	Imidacloprid	Control	F	df	P
NPI	3.70 ± 0.45a[a]	3.95 ± 0.46a	4.90 ± 0.62a	1.3426	57	0.2693
NWEI						
Np	4.50 ± 0.47a	4.65 ± 0.41a	5.30 ± 0.59a	0.608	57	0.547
C	4.55 ± 0.49b	4.80 ± 0.43b	7.00 ± 0.83a	4.268	57	0.018
D	0.65 ± 0.13b	0.80 ± 0.22b	1.65 ± 0.33a	4.643	57	0.013
E1	0.65 ± 0.13b	0.80 ± 0.22b	1.75 ± 0.40a	4.715	57	0.012
E2	0.65 ± 0.13b	0.50 ± 0.11b	1.05 ± 0.17a	3.572	57	0.034
G	0.20 ± 0.09a	0.05 ± 0.05a	0.75 ± 0.43a	2.149	57	0.125
WDI[c]						
Np	253.21 ± 21.68a	252.86 ± 18.77a	122.17 ± 13.28b	7.816	57	0.010
C	92.80 ± 22.66b	93.23 ± 17.80b	140.61 ± 18.05a	3.655	57	0.032
D	0.88 ± 0.18a	0.94 ± 0.22a	1.50 ± 0.30a	1.382	36	0.264
E1	1.74 ± 0.37b	2.59 ± 0.70b	4.22 ± 0.72a	3.359	36	0.045
E2	16.69 ± 3.11b	22.09 ± 11.32b	117.50 ± 20.38a	19.723	34	0.000
G	6.10 ± 1.58	2.66 ± NA	13.63 ± 4.93	---	---	---
WDE[c]						
Np	56.27 ± 7.76a	54.38 ± 7.93a	23.05 ±3.60b	7.026	286	0.010
C	20.39 ± 5.33a	19.42 ± 3.29a	20.09 ±2.66a	0.016	324	0.983
D	0.81 ± 0.17a	0.65 ± 0.06a	0.73 ±0.07a	0.209	59	0.811
E1	1.61 ± 0.35a	1.78 ± 0.34a	1.93 ±0.34a	0.028	61	0.972
E2	15.41 ± 1.92b	22.09 ± 11.32b	83.93 ±16.72a	6.071	41	0.004
G	6.10 ± 1.58	2.66 ± NA	4.54 ±1.75	---	---	---

[a] Averages followed by the same letter, in the same row, do not differ significantly (P > 0.05) using Fisher LSD test

[b] NPI (number of probes per insect), NWEI (number of waveform events per insect), WDI (waveform duration per insect) and WDE (waveform duration per event).

[c] Values in minutes.

Table 3. Means (± SE) of EPG variables for 6-h monitoring of *Diaphorina citri* on *Citrus sinensis* nursery trees treated by drench with neonicotinoid insecticides (90 days after application).

EPG variables[b]	**Thiamethoxam**	**Imidacloprid**	**Control**	**F**	**df**	**P**
NPI	4.40 ± 0.56a[a]	3.95 ± 0.44a	3.05 ± 0.56a	2.3042	57	0.1090
NWEI						
Np	5.35 ± 0.56a	4.85 ± 0.43a	3.35 ± 0.60b	5.313	57	0.007
C	5.40 ± 0.60a	5.80 ± 0.62a	3.70 ± 0.67b	4.437	57	0.016
D	0.95 ± 0.15a	1.75 ± 0.40a	1.15 ± 0.20a	1.937	57	0.153
E1	0.95 ± 0.15a	1.75 ± 0.40a	1.15 ± 0.20a	1.937	57	0.153
E2	0.85 ± 0.13a	1.00 ± 0.15a	0.75 ± 0.12a	0.778	57	0.463
G	0.05 ± 0.05a	0.15 ± 0.08a	0.20 ± 0.12a	0.735	57	0.483
WDI[c]						
Np	278.34 ± 11.83a	277.00 ± 14.88a	118.96 ± 28.37b	16.595	57	0.000
C	61.23 ± 12.10a	55.63 ± 7.44a	73.18 ± 14.44a	0.236	57	0.789
D	0.62 ± 0.10a	1.02 ± 0.27a	1.13 ± 0.19a	1.980	43	0.155
E1	1.96 ± 0.60a	2.86 ± 0.67a	2.88 ± 0.70a	0.866	43	0.427
E2	22.30 ± 2.64b	28.29 ± 10.99b	228.88 ± 23.33a	66.780	42	0.000
G	1.87 ± NA	3.24 ± 0.61	25.94 ± 10.40	---	---	---
WDE[c]						
Np	52.03 ± 7.46a	57.11 ± 8.10a	35.51 ± 8.88b	4.512	268	0.011
C	11.34 ± 2.31b	9.59 ± 1.10b	19.78 ± 4.28a	5.238	295	0.005
D	0.49 ± 0.08b	0.47 ± 0.03b	0.74 ± 0.05a	10.947	74	0.000
E1	1.55 ± 0.47a	1.31 ± 0.28a	1.88 ± 0.40a	1.053	74	0.353
E2	19.68 ± 2.22b	22.63 ± 8.97b	213.62 ± 25.80a	62.211	49	0.000
G	1.87 ± NA	3.24 ± 0.61	19.45 ± 6.68	---	---	---

[a] Averages followed by the same letter, in the same row, do not differ significantly (P > 0.05) using Fisher LSD test

[b] NPI (number of probes per insect), NWEI (number of waveform events per insect), WDI (waveform duration per insect) and WDE (waveform duration per event).

[c] Values in minutes.

Table 4. Proportion of individuals (*Diaphorina citri*) that produced a specific waveform type (PPW) (n=20) on *Citrus sinensis* nursery trees treated by drench with neonicotinoid insecticides, during a 6-h time period

Waveform	Thiamethoxam	Imidacloprid	Control	χ^2	df	P
15 DAA [a]						
C	20	20	20	---	---	---
D + E1	12	12	16	2.400	2	0.301
E2	12	12	15	1.318	2	0.517
35 DAA						
C	20	20	20	---	---	---
D + E1	12	11	16	3.077	2	0.214
E2	12	10	15	2.679	2	0.262
90 DAA						
C	20	20	20	---	---	---
D + E1	15	16	15	0.186	2	0.911
E2	15	16	14	0.533	2	0.765

[a] Days after application

Table 5. Mean (± SE) time to event per insect (minutes) for 6-h monitoring of *Diaphorina citri* on *Citrus sinensis* nursery trees treated by drench with neonicotinoid insecticides, at different time intervals after application.

EPG variables	Thiamethoxam	Imidacloprid	Control	F	df	P
15 DAA[b]						
First probe from start of recording	23.29 ± 9.58a[a]	19.52 ± 7.29a	17.45 ± 6.82a	0.188	57	0.835
First D from start of recording	98.67 ± 16.21a	132.84 ± 28.59a	108.14 ± 19.22a	0.106	37	0.899
First E1 from start of recording	99.10 ± 16.23a	133.44 ± 28.62a	108.58 ± 19.20a	0.107	37	0.899
First E2 from start of recording	100.41 ± 16.25a	136.10 ± 28.69a	110.52 ± 20.46a	0.132	36	0.876
35 DAA						
First probe from start of recording	27.22 ± 8.13a	28.69 ± 6.18a	26.98 ± 8.03a	0.170	57	0.843
First D from start of recording	130.04 ± 23.74a	110.39 ± 20.31a	111.84 ± 15.53a	0.139	36	0.870
First E1 from start of recording	130.89 ± 23.70a	107.15 ± 19.74a	112.56 ± 15.55a	0.214	36	0.807
First E2 from start of recording	132.58 ± 23.72a	115.79 ± 20.62a	124.95 ± 15.77a	0.943	34	0.910
90 DAA						
First probe from start of recording	15.30 ± 3.16a	12.85 ± 3.49a	41.70 ± 17.42a	0.394	57	0.675
First D from start of recording	95.19 ± 11.68a	71.18 ± 9.16a	120.00 ± 26.04a	1.101	43	0.341
First E1 from start of recording	97.72 ± 12.33a	71.81 ± 9.11a	120.70 ± 26.08a	1.112	43	0.338
First E2 from start of recording	99.78 ± 12.40a	82.21 ± 9.47a	121.65 ± 21.69a	0.711	42	0.496

[a] Averages followed by the same letter, in the same row, do not differ significantly ($P > 0.05$) using Fisher LSD test

[b] Days after application

Figure Legend

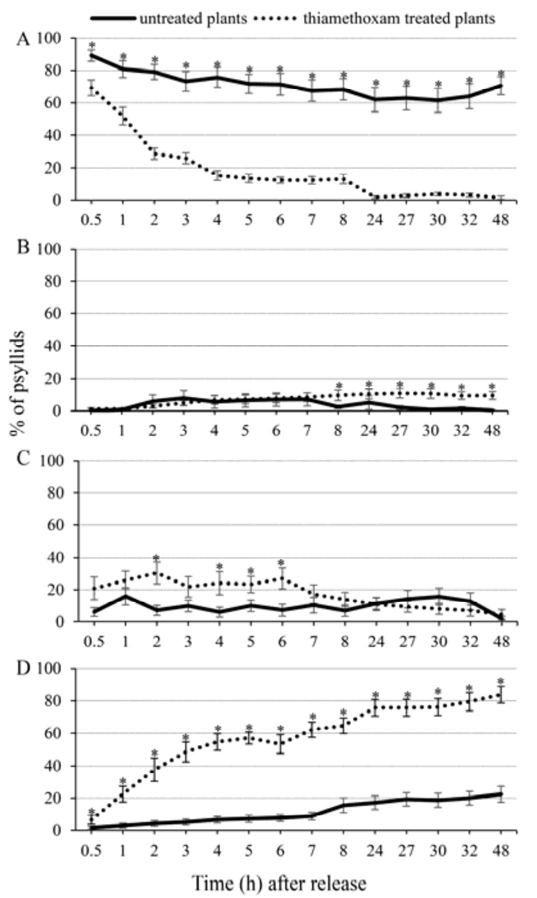

Fig. 1 Mean (± SE) percentage of marked Diaphorina citri that: (A) remained on the original plant (Citrus sinensis nursery trees treated with thiamethoxam or untreated); (B) moved from the original plant to the other plant; (C) moved from either thiamethoxam-treated or untreated plants to the observation chamber walls and ceiling; or (D) died. Asterisk indicates significant difference by t test (P < 0.05) between treated and untreated plants in a given time interval after release. Thiamethoxam application was done 30 days before to start the experiment.

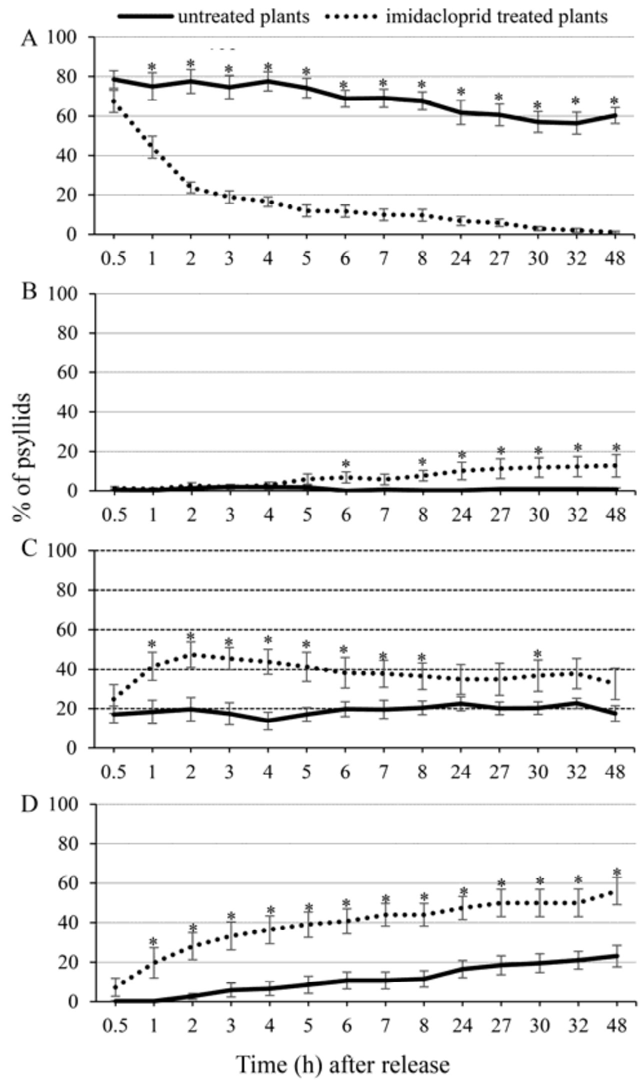

Fig. 2 Mean (± SE) percentage of marked Diaphorina citri that: (A) remained on the original plant (Citrus sinensis nursery trees treated with imidacloprid or untreated); (B) moved from the original plant to the other plant; (C) moved from either imidacloprid-treated or untreated plants to the observation chamber walls and ceiling; or (D) died. Asterisk indicates significant difference by t test ($P < 0.05$) between treated and untreated plants in a given time interval after release. Imidacloprid application was done 30 days before to start the experiment.