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Differential Efficacy of Insecticides According to Crop Growth: The Citrus Psyllid on Citrus Plants

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1. Introduction

Citrus greening is a destructive disease of citrus trees in subtropical and tropical regions. The pathogen of this disease in Asian countries, *Candidatus* Liberibacter asiaticus, is transmitted by the citrus psyllid, *Diaphorina citri* Kuwayama (Huang et all, 2004). Since no direct control of the pathogen has been established yet, the current management of this disease relies on the control of the vector, especially by insecticides (Halbert & Manjunath, 2004; Yang et al., 2006). Many studies have been performed to examine the efficacy of various insecticides on the control of psyllids, revealing that neonicotinoids such as imidacloprid or thiamethoxam are very effective on the psyllid (Hayashikawa et al., 2006; Yasuda et al., 2006; Srinivasan et al., 2008; Gatineau et al., 2010; Ichinose et al., 2010a). These insecticides are characterised by their long-continued residue effect on the pest, up to several months (Yasuda et al, 2007), and their reducible effects on the psyllid population can continue even after the concentration of the insecticides is decreased below the lethal level (Boina et al., 2009).

Important issues in the use of insecticides are 1) how frequently an insecticide should be applied in year, 2) how much the insecticide should be used in each application, and 3) when the insecticide application should be performed in the year. For the third case, insecticides will be unnecessary when target pests are few due to their seasonal activities or other factors that affect the pest population. For example, the application of insecticides in winter in Florida allows the omission of insecticide uses in subsequent several months when psyllids are likely increased (Quresh & Stansly, 2010). On the other hand, more frequent insecticide application may be required for the control of the psyllid in the tropics where the insect can maintain its population at higher densities throughout the year. Gatineau et al. (2010) reported that sufficient reduction of psyllids was attained either by monthly application of imidacloprid or fortnightly application of fenobucarb in citrus orchards of southern Vietnam. The weather in this region is characterised by the tropical climate with

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two distinct seasons in the viewpoint of precipitation, dry season with low precipitation from November to April and wet season with frequent heavy rains from May to October. Serious problem in the management of citrus greening by insecticides in southern Vietnam is raised in the cultivation of king mandarin, which is the most predominant cultivars in southern Vietnam. When trees grew in the stage to yield fruits, usually one and half to two years after planting, the efficacy of neonicotinoids is significantly reduced or even lost despite either the dose or the mode of application (Ichinose et al., 2010b). The control efficiency on the psyllid on grown trees was not implemented by either the increase of insecticide dose up to 100 times or the change of the application mode, not only soil drench but also trunk injection, trunk painting, or leaf spray. Thus, studies were urgently needed to establish control methods for the psyllid on grown trees.

The following five experiments were thus performed for the control of the psyllid on the grown king mandarin tree: 1) the residue effect of five insecticides, imidacloprid, thiamethoxam, clothianidin, methidathion and fenobucarb, were tested by the spray on the whole body of citrus trees cultured in a net house; 2) two application modes, soil drench and spray, of imidacloprid and methidathion were compared in a net house; 3) imidacloprid, methidathion or the mixture of these two insecticides were sprayed on three year old citrus trees, and their efficacy on the psyllid and aphids were counted for four weeks after the application. In the course of these experiments, the selling of methidathion was banned in Vietnam. Hence, we replaced this insecticide for dimethoate, which were tested in the following experiments: 4) imidacloprid, dimethoate, the mixture of these two insecticides, pymetrozine, or dinotefuran was applied on three years old trees and the mortality of psyllids were compared between these treatments; 5) imidacloprid, dimethoate or the mixture of these two insecticides were applied on three years old citrus trees and psyllids and other citrus pests were counted for four weeks after the application. More efficient uses of insecticides were attempted to establish not only for the control of the psyllid but also other citrus pests.

2. Experiments on the control of psyllids by insecticides

All experiments in this report were carried out in the province of Tien Giang, southern Vietnam, about 70 km south-west to Ho Chi Minh. The altitude is generally less than 10 m. This region lies in the tropical zone with two seasons: wet season with relatively higher temperatures from May to October and dry season with relatively lower temperatures from November of the year to April of the succeeding year. Means of annual, minimum and maximum temperatures are 28.0ºC, 23.7ºC, and 32.3ºC, respectively. Annual precipitation is about 2500 mm. Total area for fruit crops in the Mekong Delta Region of Vietnam, more southern areas than Ho Chi Minh, is 282,200 ha, and 77,300 ha (27.4%) area is used for citrus. The most common variety of citrus crops cultivated in this region is king mandarin, which is sensitive to citrus greening (Koizumi et al, 1997). Mean area of farm per family is about 0.5 ha. Various managements for citrus greening are performed in this region, and the most efficient management seems to be the combination of the use of disease-free seedlings and bimonthly application of neonicotinoids (Ichinose & Kano, 2006). Only king mandarin, *Citrus nobilis* Loureiro, was used in our studies, which were carried out under the research project for CG between Japan International Research Centre for Agricultural Sciences (JIRCAS) and Southern Horticultural Research Institute of Vietnam (SOFRI) owing to the predominance of king mandarin in this region and high economic return compared to the investment for the cultivation. All trees were originated from seedlings produced at SOFRI under the condition of no invasion of CG. These seedlings are referred to as disease-free seedlings hereafter.

2.1 Quickness in and longevity of insecticide efficacy

This experiment was attempted to evaluate the efficiency of one organophosphate, methidathion, and one carbamate, fenobucarb. These two insecticides were compared with three neonicotinoids, imidacloprid, thiamethoxam and clothianidin, since the efficacy of the latter insecticides had been examined and their efficacy was already known (Ichinose et al., 2010a). Hence, using seedlings of king mandarin, residue effect of five insecticides was tested: imidacloprid (0.20 g/tree), thiamethoxam (0.17 g), clothianidin (0.19 g), methidathion (0.40 g) and fenobucarb (0.50 g). In the end of February 2009, 30 disease-free seedlings of king mandarin were purchased at the division of seedling production of SOFRI, and 18 seedlings were randomly chosen among them. These selected seedlings were randomly separated into six groups with the same number, three, and each group was treated on 5 March 2009 by no insecticide or any of the above insecticides. In the application, three solutions were prepared for each neonicotinoid treatment: neonicotinoid was dissolved in water to obtain a solution of 20 ml in the total volume so that the dose in the solution would be as in the above quantity. Each 20 ml solution was applied to each seedling on the soil surface on the pot. Methidathion and fenobucarb were diluted by water 1000 times in volume, thus 400 ml and 500 ml respectively, and each solution was sprayed on the whole body of seedlings in each treatment. The above treatments provided a completely randomized design with three replicates in each treatment. These seedlings were maintained in a net house at SOFRI.

One leaf was collected from each tree on the day of the application before spraying, one, two, five, 10, 15, 30, 45, 60 and 90 days after the application. Each leaf collected on each day was placed in a transparent plastic cup of 500 ml just after the collection. Psyllids were collected one day before each leaf-collection day and left in a laboratory for one day. These psyllids transferred in a plastic box with plaster of paris of 1 cm thickness in the bottom for humidity and kept there for about one day for the starvation condition. Just after the preparation of the leaf, two psyllids were randomly selected from the box and released in each cup. The survival of each psyllid in each cup was recorded at every hour until six hours after the release, at 8, 12, 24, 36, 48 and 72 hr. The residue effect of the insecticides was determined by the mortality of psyllids in each cup. The mean of the mortality of the psyllid in the control cup at each observation hour on each observation day was calculated and used to correct the psyllid mortality in cups with insecticide-treated leaves at the same observation time on the same day by using the Abbott's correction formula. The detail of the mortality calculation is described in Ichinose et al. (2010a).

2.1.1 Efficacy of methidathion and fenobucarb

It is difficult to determine the effective mortality attained by insecticide for the management of CG. At this moment, a mortality of 80% was taken as an efficient one in this study, and the effective period was conveniently defined as the days during which mean mortalities were over this level.

Methidathion showed quick, high lethal effect from one to 10 day within 12 hr after the application (Fig. 1A), but such high mortalities were not attained in 15 d after the application (Fig. 1B). High mortalities were not seen in fenobucarb treatment throughout this experiment. Although mortalities were increased in 12 h after the release on 1 to 10 d after the application, the mean mortality was lower than 80 % (Fig. 1C). Although the mortality at 72 hr after the release on the 15th d was a little higher than 80 %, no high mortalities were seen at any time on any day after this day (Fig. 1D). Both methidathion and fenobucarb completely lost its efficacy on the 45th d.

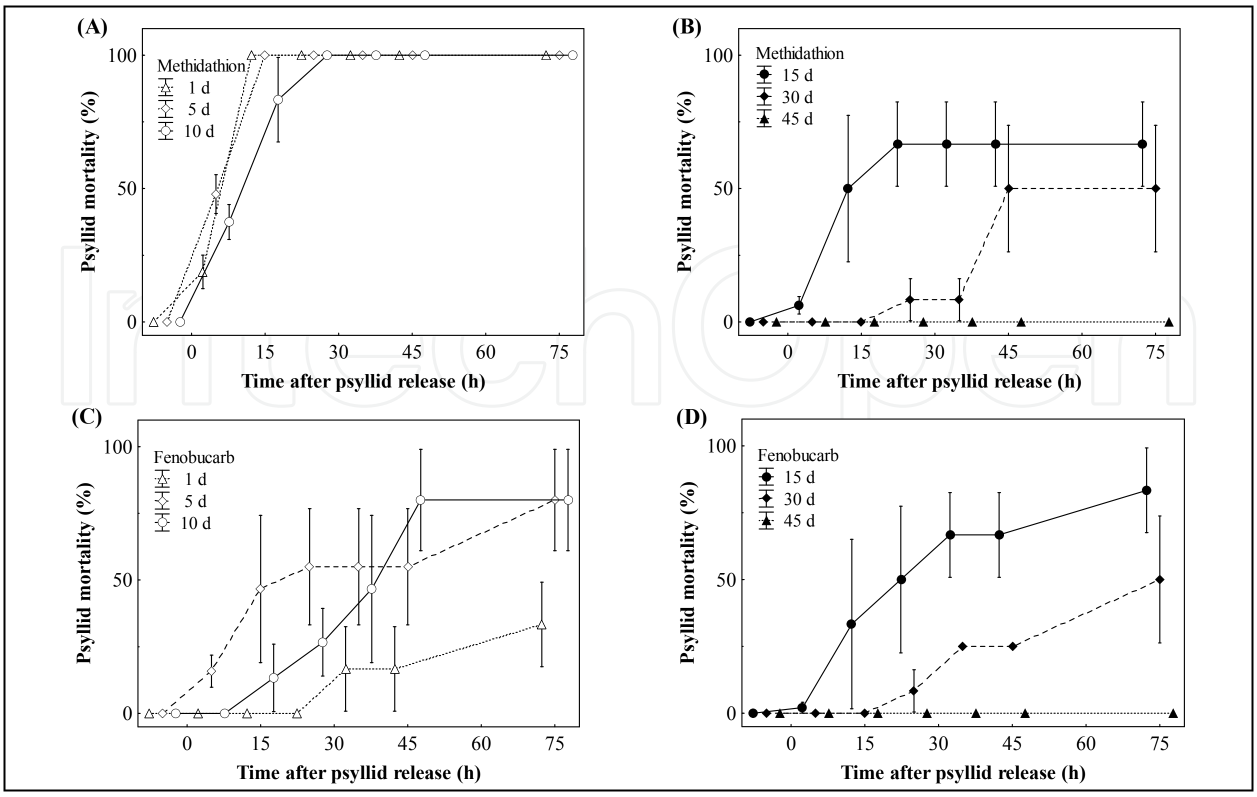

Fig. 1. Mortality of psyllids released on leaves treated with thiamethoxam one to 10 d (A) and 30 to 90 d (B) after the application. Similarly the mortality of psyllids on leaves treated with fenobucarb is shown in C and D, respectively. All mortalities were corrected by the Abbott's formula with using the data of the control treatment. Bars indicate the standard errors of the means.

2.1.2 Efficacy of neonicotinoids

The mortality of psyllids released on leaves treated with imidacloprid was not high until 24 h after the release of psyllids on the leaf until the 10th day after the application (Fig. 2A). Although the mortality reached 100 % after 72 hr even on the 1st to 5th day, it took also 72 hr to reach this mortality on the 60 d and no such high mortalities were attained thereafter (Fig. 2B). Both thiamethoxam and clothianidin needed 10 days after the application to reach high mortalities,> 80 %, at 72 h after the release (Fig. 2C, E) and no high mortalities were attained before this time throughout the experiment (Fig. 2D, F). Mortalities on the 90th day were lower in both thiamethoxam and clothianidin. Hence, three neonicotinoids showed similar quickness of both the lethal effect and the residue effect, although imidacloprid seemed to affect quickly psyllids in the early periods after the application.

These results indicate that the application of methidathion with a frequency of every 10 days would control psyllids best among the insecticides examined. If any of three neonicotinoids are used, similar results in the control of psyllids could be expected among them. Application time of every two month can be recommended. Although the probability of the transmission of the CG pathogen by the psyllid is not known well, methidathion would reduce the infection more efficiently than the neonicotinoids owing to its quickness to reach the 100% lethal effect. If adults eclosed from nymphs grown on infected trees would transmit the pathogen more efficiently than those infested by the pathogen after eclosing (Inoue et al. 2009), the efficacy of the CG reduction would be similar between methidathion and imidacloprid. Since three neonicotinoids showed similar residue effect but imidacloprid seemed to be better in the quickness, imidacloprid was examined in the succeeding experiments.

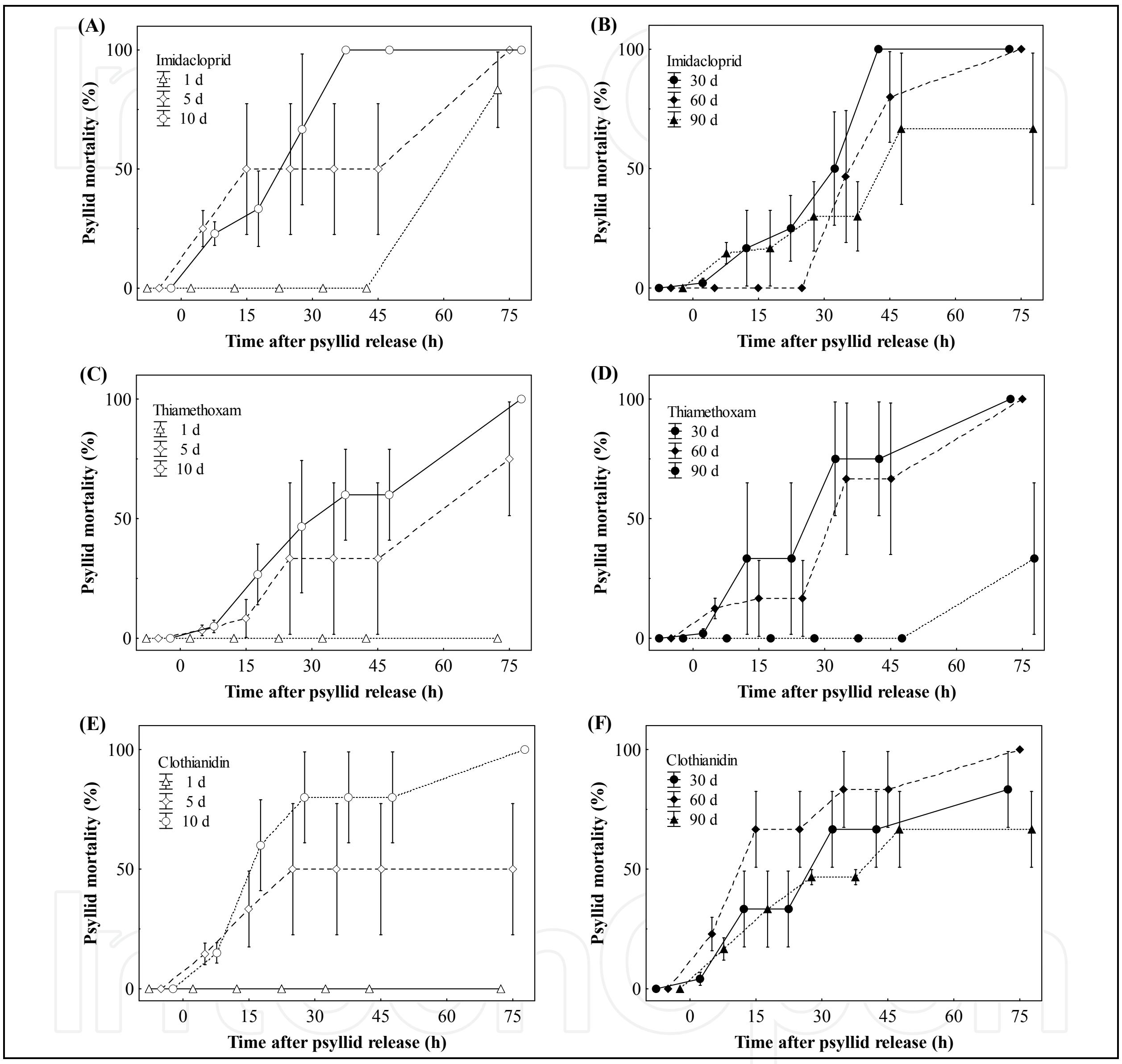

Fig. 2. Mortality of psyllids released on leaves treated with imidacloprid one 10 d (A) and 30 to 90 d (B) after the application. Similarly mortalities of psyllids on leaves treated with thiamethoxam and clothianidin are shown in C-D and E-F, respectively. All mortalities were corrected by the Abbott's formula with using the data of the control treatment. Bars indicate the standard errors of the means.

2.2 Effect of the application mode of insecticide: spray and soil drench

In this experiment, it was attempted to test the application mode of imidacloprid and methidathion. In particular, focused issue was the extension of the residue effect of methidathion by soil drench. Insecticides sprayed on leaves would be flown away off by raining, while those applied on soil surface by drenching would be less affected by rain. Thus, the efficacy of these insecticides applied by leaf spray or soil drench was compared. On 18 March 2008, 25 seedlings of king mandarin were purchased at SOFRI, and were separated into five groups in the same number. Any of the following treatment was randomly distributed to the groups: no insecticide, spray of 0.2 g imidacloprid in 300 ml water, soil drench of 0.2 g imidacloprid diluted to 20 ml by water, spray of 0.40 g methidathion in 400 ml water, and soil drench of 0.40 g methidathion diluted to 20 ml by water. On the day of the seedling purchase, seedlings were treated as in the above experiment, and the evaluation of the efficacy of each insecticide by each application mode was evaluated similarly.

2.2.1 Comparison of application mode

The psyllid mortality at 12 h after the release was generally low, except for imidacloprid by spray or drench on the 10th day after the application and methidathion by spray from first to fifth day (Table 1). If mortality over 80% was taken as "effective" residue effect, the best efficacy at 12 h was attained by methidathion by spray (Table 1). The mortality at 24 h was still under the effective level in the treatment of imidacloprid by spray or drench on any day except for the 10th day, although the days over this level in the methidathion treatment by spray was extended until 10th day. Methidathion by soil drench was still under the level on any day. The psyllid mortality at 72 h after the release was over the effective level on fifth to 30th days in the treatment of imidacloprid by spray or drench. Methidathion by spray was effective at this time on the first to 15th days after the application, but this insecticide by soil drench was effective only on 15th day.

Insecticide applied	Application mode	Time (h)	Max mortality (%)	Start (d)	End (d)
Imidacloprid	Spray	12	100.0	10	10
Imidacloprid	Drench	12	100.0	10	10
Methidathion	Spray	12	100.0	1	5
Methidathion	Drench	12	50.0	n/a	n/a
Imidacloprid	Spray	24	100.0	10	30
Imidacloprid	Drench	24	100.0	10	30
Methidathion	Spray	24	100.0	1	10
Methidathion	Drench	24	66.7	n/a	n/a
Imidacloprid	Spray	72	100.0	5	30
Imidacloprid	Drench	72	100.0	5	30
Methidathion	Spray	72	100.0	1	15
Methidathion	Drench	72	83.3	15	15

Table 1. The maximum mean of the psyllid mortality observed at 12 or 72 hours after the release on leaves treated with imidacloprid or methidathion applied by leaf spray or soil drench. The first day when the mean mortality was over 80 % and the last day when the mean was reduced below the level again are shown as start and end, respectively. The treatment in which a mortality over 80% was not attained is shown by "n/a".

These results indicate that imidacloprid could effectively control psyllids in 72 h from fifth day on after the application, but the effective level would be only on 10th day unless it would take 24 hours to transmit pathogen efficiently. Imidacloprid by spray did not show quick lethal effect even at 72 h after the release of psyllid until fifth day after the application. It provided similar results with this insecticide by soil drench in both quickness and residue effect. This suggests that either permeability or systemics of imidacloprid would be delayed even on leaves. Methidathion by soil drench should be avoided, since this application would not bring good controls in either quickness of efficacy or longevity. In this point, methidathion by spray could be expected for better management than imidacloprid owing to its quickness in the termination of psyllids after infestation on plants. However, the relatively shorter residue effect would need more frequent application of the insecticide which would not be preferred by farmers. Although either imidacloprid or methidathion was not effective if they were applied on grown trees in field (Ichinose et al., 2010b), it has been reported that imidacloprid effectively control psyllid populations in field (cf. Srninivasan et al., 2008; Boina et al., 2009; Gatineau et al., 2010). Thus, the application of imidacloprid and methidathion in field was examined also in this study.

2.3 Application of methidathion and imidacloprid in field

The results of the above two experiments indicate that methidathion can quickly eliminate psyllids probably within 24 hr after application but the efficiency could continue shorter than a half month. On the other hand, neonicotinoids, especially imidacloprid, could maintain high efficacy for longer than one month, although it would take five to 10 days to reach such high efficacy and three days would be needed to attain effective control level. These results also provide an expectation that the mixture of these two insecticides would have quick and long-lasting efficacy on the psyllid. Thus, the efficacy of individual insecticides and the mixture of them were examined in field. Two orchards, located in Cai Be, about 100 km south-west of Ho Chi Minh, were selected for this study. The area size of each orchard was extended about 0.35 ha, in which three year old king mandarin were planted in 2.5 m distance both between trees and lines. The total numbers of king mandarin trees in these two orchards were 386 and 378. The experiment in this section was carried out from July 2009 to June 2010. Before the study, both orchards were divided into four parts, in each of which three lines lied and 16 to 18 trees were planted on each line. Hence, these divided parts had 54 to 56 trees. Any of the following four treatments was randomly distributed to a part of each orchard: no insecticide application as control; spray of 0.40 g methidathion dissolved in 400 ml water per tree; spray of 0.20 g imidacloprid dissolved in 400 ml water per tree; spray of the mixture of 0.40 g methidathion and 0.20 g imidacloprid in 400 ml water per tree. Based on the results in the second experiment, high residue effect in the plots with imidacloprid was expected to be maintained for one month, although residue effect in the plots with only methidathion would be kept high less than two weeks. Thus, the insecticide spray was carried out once in the early of every month during the study period. In this experiment, 15 trees were selected systematically: every seven trees from the first tree were examined. For the evaluation of insecticide efficacy, psyllid adults were counted individually, and nymphs were counted in a unit of colony. Colony was defined as a group of nymphs on one new shoot. Besides the count of the psyllid, aphids and scale insects were also counted in a colony unit as in the psyllid nymph. The efficacy of each insecticide treatment was evaluated in the numbers of these pests, compared with the control treatment. These counts were performed within three days after the insecticide application and once every week thereafter until the end of the month. The mean numbers of psyllid adults and nymph colonies, aphid and scale colonies were calculated for individual selected trees over all samplings. These numbers were used for statistical analyses to evaluate the effects of treatments on these insects.

2.3.1 Insecticide efficacy on the psyllid

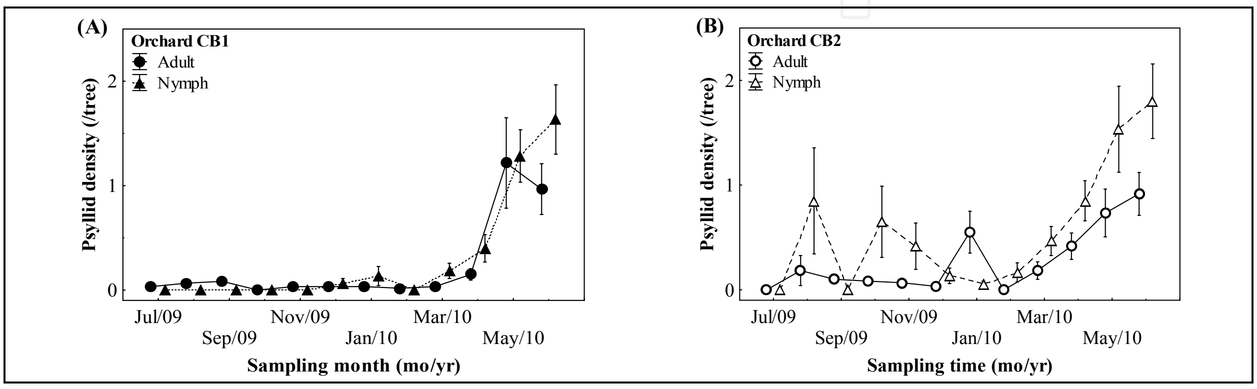

Fig. 3. Densities of psyllid adults and nymph colonies in the plot without any insecticide for the control of psyllids in two old orchards, CB1 (A) or CB2 (B). Bars indicate the standard errors of the means.

The means of adult and nymph colony counts on each tree in every month were calculated. Psyllids in the control plot without insecticide in two orchards were expected to be free from the influence of insecitide. Then, the densities of both adults and nymph colonies in these plots were compared between sampling times. Both of adults and nymphs increased in April to June 2010, but were generally much fewer in the other months (Fig. 3 A, B).

The population of psyllid adults and nymphs were compared between treatments with using the data obtained after April 2010. In these analyses, the means of these counts were calculated for every sampling time to trace the time-dependent change of the residue effect of insecticides. The effects of insecticide treatment on the psyllid was evaluated by MANOVA, in which the means of the psyllid counts were compared among orchards and treatments. The numbers were significantly different between orchards ($F_{2,\ 111}$ = 4.229, P = 0.017) but not between treatments ($F_{6,\ 222}$ = 1.680, P = 0.170). The interaction of orchard and treatment was not significant ($F_{6,\ 222}$ = 1.349, P = 0.237). Although there were significant differences in the numbers of psyllids between treatments, apparent differences in the numbers were hardly found in the change of the population density of the psyllid between the treatments through the above sampling periods (Fig. 4A - F). Furthermore, no distinct decreases in the psyllid numbers were seen at the first sampling time just after each insecticide spray. These results means that these insecticides by spray did not effectively reduce psyllids in the field, although the application mode and dose both followed those recommended as effective in the former experiments.

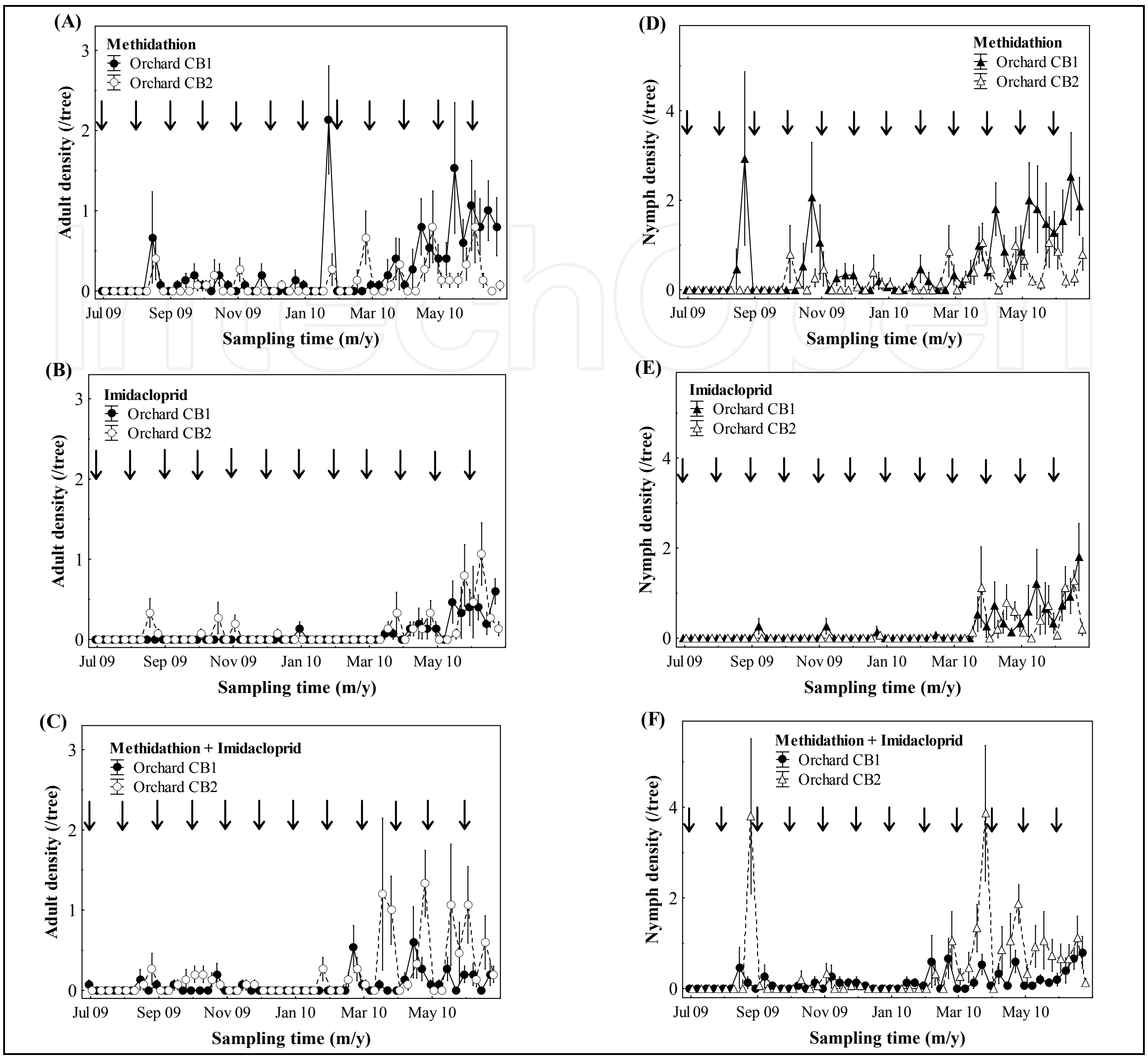

Fig. 4. Densities of adults (A-C) and nymph colonies (D-F) in two orchards in Cai Be, southern Vietnam. Each orchard was divided into four plots, and one was used as control without any insecticide and the other three plots treated by either methidathion 0.40 g/tree, imidacloprid 0.20 g/tree or the mixture of these two insecticides in two orchards. Arrows show the time when insecticides were sprayed. Bars indicate the standard errors of the means.

2.3.2 Insecticide efficacy on the aphid and scale insect

Aphids increased in November 2009 to March 2010 in both orchards, although a smaller increase was seen in July 2009 in CB2 (Fig. 5A, B). In both orchards, monthly application of insecticides reduced the aphid population, but the efficacy continued for one to two weeks only in both orchards. In particular, the aphid population seems to have been less affected by imidacloprid. On the other hand, scales insects increased until november 2009, and showed little increase thereafter (Fig. 5C, D). In CB2, scales were fewer throughout this study than in CB1. Thus, the scale population was effectively suppressed just after the application of methidathion, but the residue effect was maintained for less than two weeks. The application of imidacloprid led the scale rather to increase more than the no-insecticide treatment.

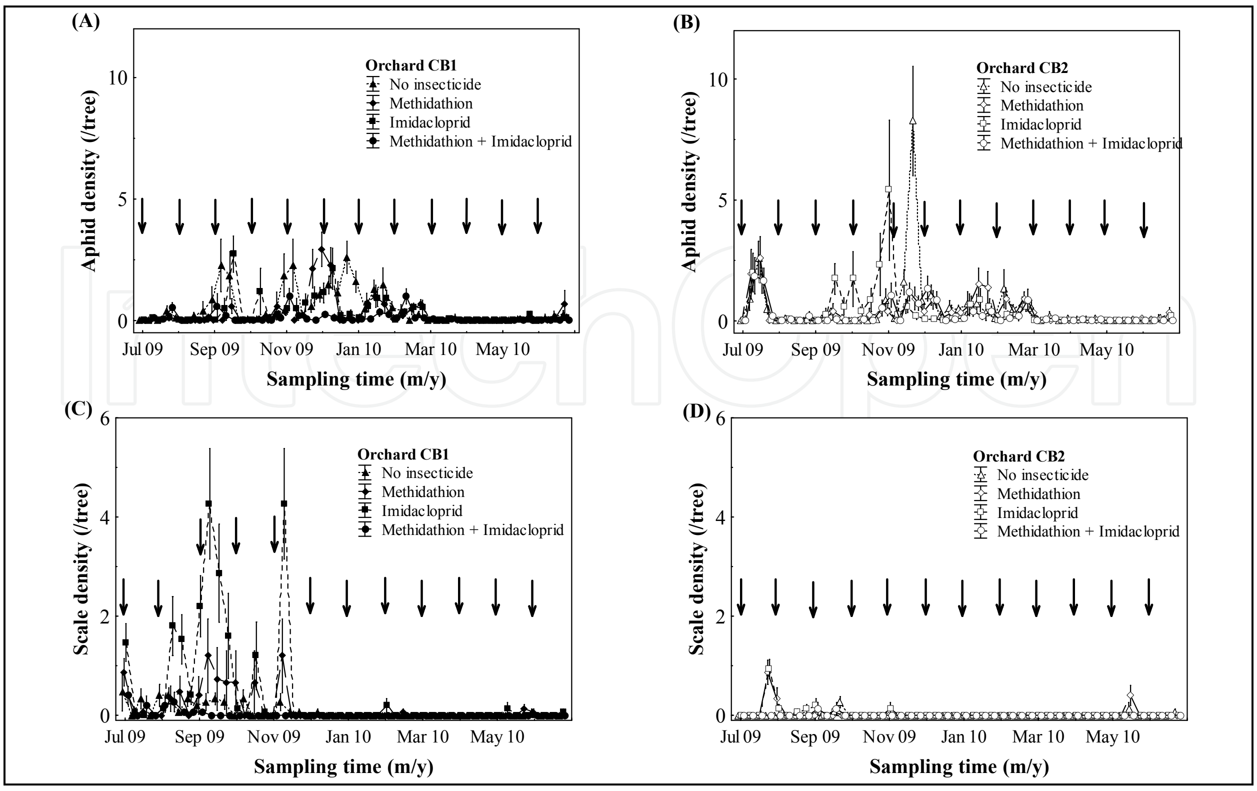

Fig. 5. Densities of aphid (A, B) and scale insect (C, D) colonies in two two-years old orchards, where seedlings had been planted either in May 2007 (CB1) or in November 2007 (CB2). Each orchard was divided into four plots, to each of which any of the four treatments were randomly distributed: no insecticide use for the control of psyllids, 0.40 g/tree methidathion by spray, 0.20 g/tree imidacloprid by spray, or 0.40 g methidathion and 0.20 g imidacloprid/tree by spray. Arrows indicate the time when insecticides were sprayed. Bars show the standard errors of the means.

Univariate ANOVA was used to evaluate the effect of treatment on the aphid and scale. The mean numbers of the aphid colony was significantly different both between orchards ($F_{1, 112}$ = 4.958, P = 0.028) and between treatments ($F_{3, 112}$ = 3.803, P = 0.012). The interaction of these two variables was significant ($F_{3, 112}$ = 2.754, P = 0.046). Similarly, the number of scales were significantly different both between orchards ($F_{1, 112}$ = 12.983, P < 0.001) and between treatments ($F_{3, 112}$ = 8.936, P < 0.001). The interaction of these two variables was significant ($F_{3, 112}$ = 3.718, P = 0.014).

These results indicate that aphids could be effectively controlled by either methidathion or imicloprid, but the efficacy would be continued for two weeks at longest. Scale insects would be controlled effectively by methidathion, but imidacloprid should be avoided for the control of the scale.

2.4 Efficacy of insecticides replaced for methidathion

Unfortunately, the sale of methidathion was discontinued in 2010 in Vietnam. According to local vendors, this was not due to any problem of methidathion per se, but farmers were likely to avoid using this insecticide due to its relatively higher prices in the market of Vietnam. Irrespective of the reason of the ban, it was urgently needed to find other insecticides that could be replaced for methidathion. Candidate insecticides should have been those which could be easily purchased in Vietnam and would be expected to show similar efficacy as methidathion. For these conditions, dimethoate was selected, since it belongs to the same insecticide group with methidathion, "organophosphate". Two other insecticides, dinotefuran and pymetrozine, were also examined as well. For this experiment, 12 two-year old king mandarin trees were randomly selected in an orchard, located in Cai Be, about 120 km south-east to Ho Chi Minh. The mean (± SEM) of circumference of these trees just above the grafted part was 156.2 ± 22.8 mm. This experiment was consisted of four treatments, no insecticide, dimethoate, dinotefuran and pymetrozine, each of which was randomly distributed to three trees, producing three replicates for each. The doses of dimethoate, dinotefuran and pymetrozine were 0.40 g/tree, 0.20 g/tree and 0.50 g/tree, respectively. These insecticides were dissolved in water to become 400 ml in total volume, and sprayed on the whole body of the tree. The spray was executed on 11 May 2010, and there was no precipitation for longer than one month after this day. Two leaves of each tree were collected on the day just before the insecticide application, one, two, five, 10, 15, 20, 30, 45, 60, and 90 days after it. These leaves were treated as in the previous experiments for the test of insecticide efficacy. The mean of psyllid mortality on two leaves of each tree on each collection day was calculated, and the mean was used as the mortality which was achieved at the observation time on the day. The mean of the mortality on the control tree at a given time on a given day was calculated, and incorporated into the Abbott's formula to correct the mortality on insecticide-treated leaves at the same time on the same day.

2.4.1 Insecticide efficacy on the psyllid

High lethal effect of dimethoate was attained only at 72 hr after the release of psyllids only one day after spray, and decreased thereafter (Fig. 6). The psyllid mortality at 12 hr on this day was only about 60%. The results mean that dimethoate was inferior in both quickness and residue of the efficacy on the psyllid to methidathion. Hence, it could be expected that field application of dimethoate would show mortalities as high as methidathion on one day after the spray, and its effectiveness would be much lower thereafter with allowing the psyllid population to be recovered earlier. Dinotefuran showed similar effect as dimethoate until this day, but its residue effects was likely to be higher than dimethoate thereafter. The mortality of psyllids at 72 hr by dinotefuran was smaller than 80% after five days. These results in this experiment indicate that both quickness and residue effects of dinotefuran

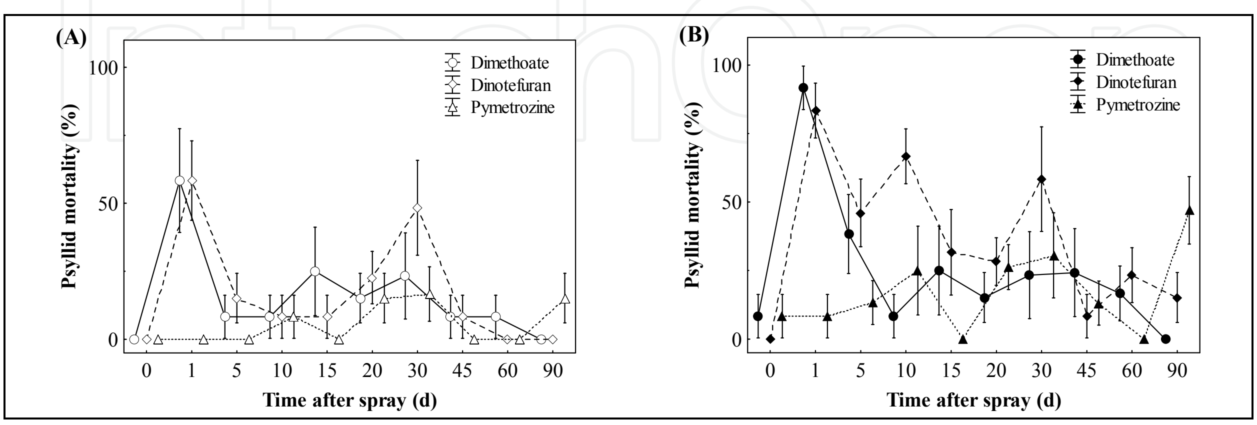

Fig. 6. Mortality of psyllids at 24 (A) and 72 (B) hours after released on leaves collected on the days after application of dimethoate, dinotefuran, or pymetrozine. The psyllid mortality was corrected by the Abbott's formula. Bars indicate the standard error of the mean.

were lower than those of imidacloprid, and thus dinotefuran should be much less effective on the control of psyllids in field than that of imidacloprid. The lethal effect of pymetrozine was always lower through this experiment, never attaining mortality over 50%. Based on the results, dimethoate and imidacloprid were examined in the following experiment.

2.5 Efficacy of dimethoate in field

The efficacy of dimethoate on the psyllid in field was examined as in the experiments of methidathion. The two orchards which had been used for the experiment of methidathion were also used for this experiment. Each of the two orchards was divided into four plots, to each of which any of the four treatments was randomly distributed: no insecticide, spray of 0.80 g dimethoate per tree, spray of 0.20 g imidacloprid, and spray of 0.80 g dimethoate and 0.20 g of imidacloprid per tree. These treatments were carried out in the early of every month from July 2010 to November 2010. The dilution and application mode of insecticides were same as in the experiment of methidathion. Psyllids, aphids, and scale insects were counted in a couple of day after the insecticide application, and once a week after then. This experiment was carried out from July 2010 to November 2010.

2.5.1 Insecticide efficacy on the psyllid

Adults and nymphs increased in July to September 2010, but few were present in the other months in both orchards (Fig. 7 A, B). Although adults were likely to be more in CB1 than in CB2 and nymphs were more in CB2 than in CB1 in the early period of this experiment, no apparent differences in these numbers were seen between the two orchards thereafter.

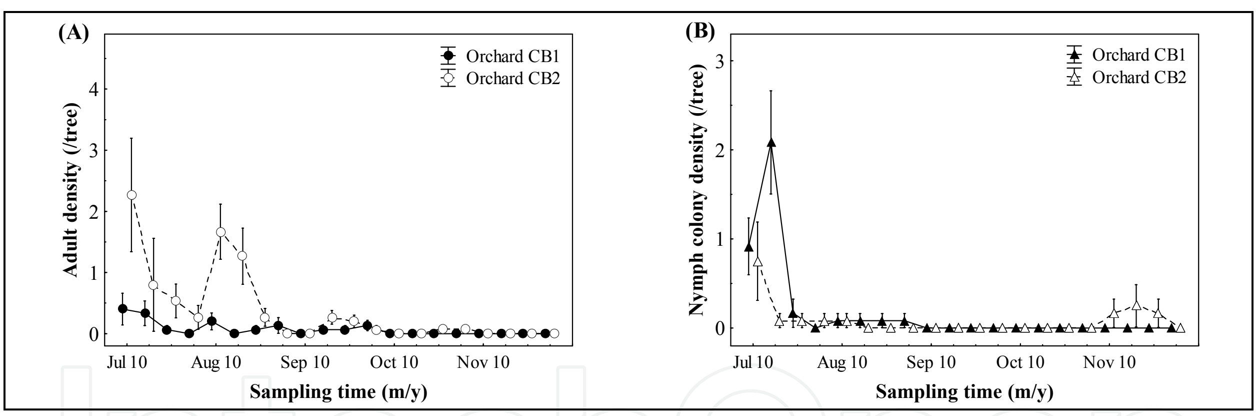

Fig. 7. Densities of adult (A) and nymph colonies (B) of the psyllid in plots without any insecticide for the control of psyllids in two old orchards, where seedlings had been planted either in May 2007 (CB1) or in November 2007 (CB2). Each orchard was divided into four plots, to each of which any of the four treatments were randomly distributed: no insecticide use for the control of psyllids, 0.40 g/tree dimethoate by spray, 0.20 g/tree imidacloprid by spray, or 0.40 g dimethoate and 0.20 g imidacloprid/tree by spray. Bars indicate the standard errors of the means.

Despite few occurrences of psyllids in the control plot after September, both adults and nymphs were counted at higher densities in plots with insecticide treatments in these months (Fig. 8). The first and second applications of dimethoate was followed by the increase of nymph colonies in both orchards, although following applications of this insecticide resulted in the decrease of both adults and nymph colonies for less than two weeks after the application (Fig. 8A, D). Adults in the plot with imidacloprid treatment

maintained low densities throughout this experiment in CB1. Distinct reduction of nymph colonies by imidacloprid was seen only in August, however, and nymph colonies rather increased in the other months (Fig. 8B, E). The application of the mixture of these two insecticides resulted in decreases of both adult and nymph psyllids throughout this experiment, except for the nymph in July in CB2.
The effects of insecticide treatment on the psyllid was evaluated by MANOVA, in which the means of the counts of adult and nymph psyllids per sampling time were culculated for individual trees and compared among orchards and treatments. The numbers were significantly different between orchards ($F_{2,\ 98}$ = 23.016, P < 0.001) but not between treatments ($F_{6,\,196}$ = 2.058, P = 0.060).The interaction of orchard and treatment was significant ($F_{6,\,196}$ = 3.272, P = 0.004).

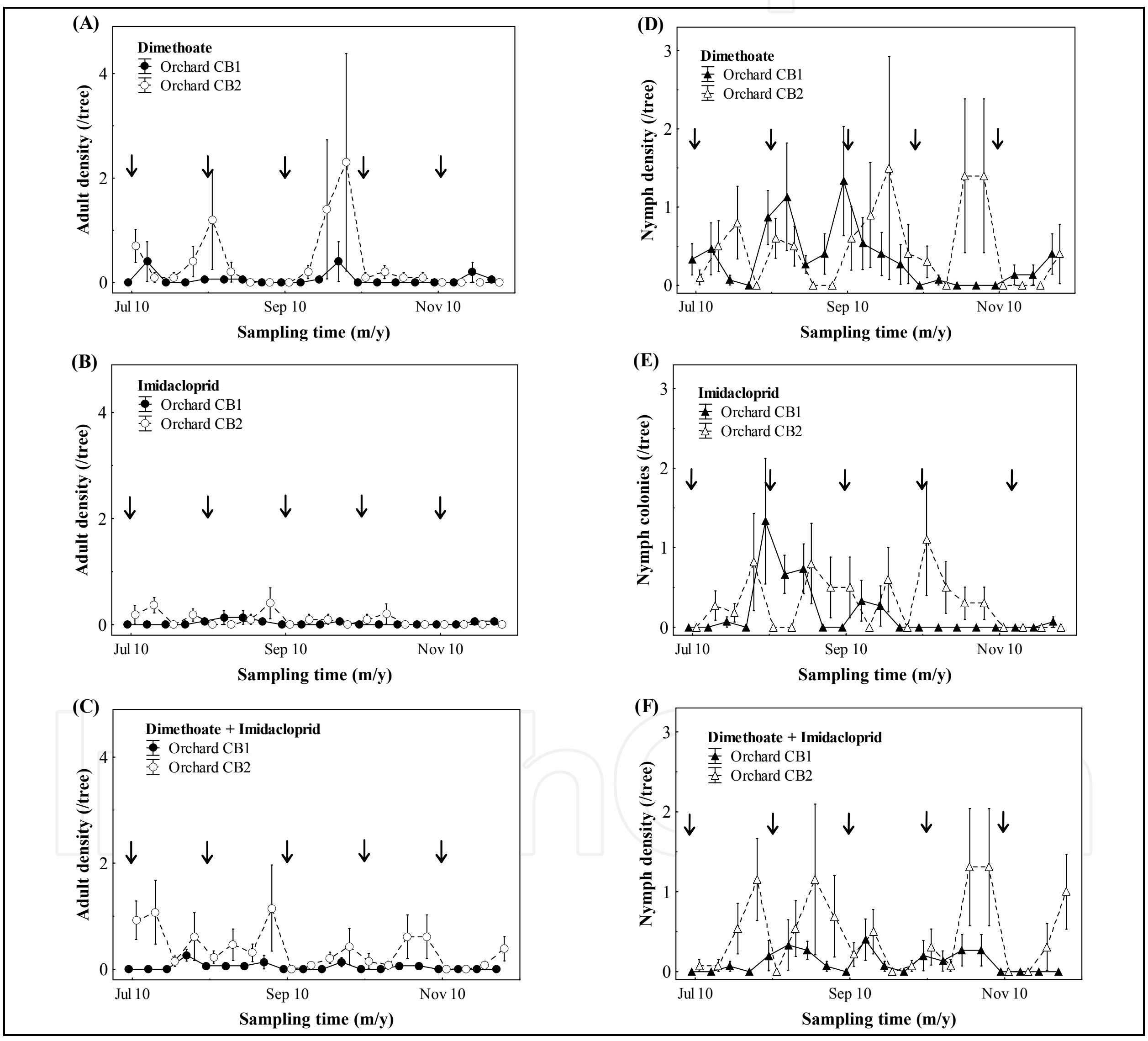

Fig. 8. Densities of adults (A-C) and nymph colonies (D-F) in plots treated by either dimethoate 0.40 g/tree, imidacloprid 0.20 g/tree or the mixture of these two insecticides in two orchards, where seedlings had been planted either in May 2007 (CB1) or in November 2007 (CB2). Arrows show the time when insecticides were sprayed. Bars indicate the standard errors of the means.

Any insecticide did not show long residue effects in this experiment. Furthermore, psyllids were increased in plots with insecticide treatments when few or no psyllids were found in the control plot. In particular, psyllids were likely to be more in insecticide-treated plots after September 2010 than in the control plot, even though psyllids were reduced just after the insecticide application. The interative factors in the increase in these plots are still unknown. One possible explanation seems to be predators of the psyllid that had been eliminated by insecticide application and their populations could not be recovered soon. Other interesting results were the difference in the the efficacy of imidacloprid on adults and on nymph colonies. Adults were well controlled by imidaclprid, but nymphs appreared to be free of the insecticide effects. However, adults in the plot with the treatment of the mixture of two insecticides were as many as those in the plot with only dimethoate. In the experiment of methidation application in field, imidaclprid did not show such a control effect on either the adult or the nymph. The few occurrences of adults in this experiment seem to have been involved in other confounding factors. Furthermore, adult psyllids were likely to be more in CB2 than in CB1, although no apparent differences in the nymph numbers were seen. Thus, any insecticide effectively reduced psyllid populations just after the application, but the residue effect could not continue for longer than two weeks.

2.5.2 Insecticide efficacy on the aphid and scale insect

In the control plot, the increase of aphids was not necessarily correspondent between two orchards (Fig. 9A). In each orchard, only one increase was observed: in September in CB1 and in July in CB2. On the other hand, scale insects increased similarly in these two orchards: one increase in August and the other in September to October (Fig. 9B). These densities were compared with those in plots with insecticide treatments. Generally, aphid populations after insecticide application were reduced in both orchards, but increased in one to two weeks later (Fig. 10A, B). In October to November, their populations were recovered in the plots with dimethoate or imidacloprid treatment in two weeks after the application. Although scale insects were found more in CB2 than in CB1, poulation densities of the scale insect were likely to be higher in the plots with the treatment by only imidacloprid in both orchards (Fig. 10C, D). Application of dimethoate effectively suppressed the scale population, but imidacloprid led the scale rather to increase more than the no-insecticide treatment. The population of scale insects were particularly higher in CB2

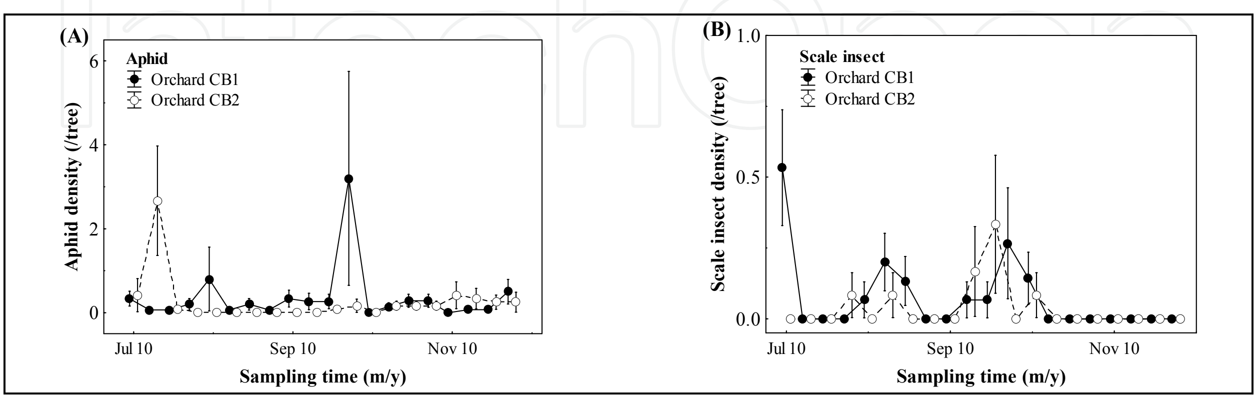

Fig. 9. Densities of aphid (A) and scale insect colonies (B) in plots without insecticide treatment for the control of psyllids in two orchards, where seedlings had been planted either in May 2007 (CB1) or in November 2007 (CB2). Bars indicate the standard errors of the means.

during the late August to early October than in the control plots. In the plots with the mixture of both insecticide, populations were maintained lower than the others.

Univariate ANOVA was used to compare the effects of insecticide treatments on the aphid or scale insect population. The aphid population density was significantly different between treatments ($F_{3,\,99}$ = 4.078, P = 0.009) but not between orchards ($F_{1,\,99}$ = 0.253, P > 0.05). The interaction of these two variables was not significant ($F_{3,\,99}$ = 0.319, P > 0.05). The population density of scale insects was significantly different both between orchards ($F_{1,\,99}$ = 6.206, P = 0.014) and between treatments ($F_{3,\,99}$ = 3.443, P = 0.020). The interaction of orchard and treatment was not significant ($F_{3,\,99}$ = 0.506, P > 0.05). These results indicate that aphids could be effectively controlled by either dimethoate or imidacloprid but the efficacy would be continued for two weeks at longest. Imidacloprid would more effectively control aphids than dimethoate. Scale insects would be controlled effectively by dimethoate, but imidacloprid should be avoided for the control of the scale. Hence, when aphids are dominant and scales insects were few, either dimethoate or imidacloprid could be used. However, when scales were abundant, imidaclprid should be avoided even for the control of aphids, and dimethoate should be selected. If both pests are abundant, the mixture of these insecticides would be considered. It should be noted that the residue effects in any insecticide application would continue for two weeks at longest.

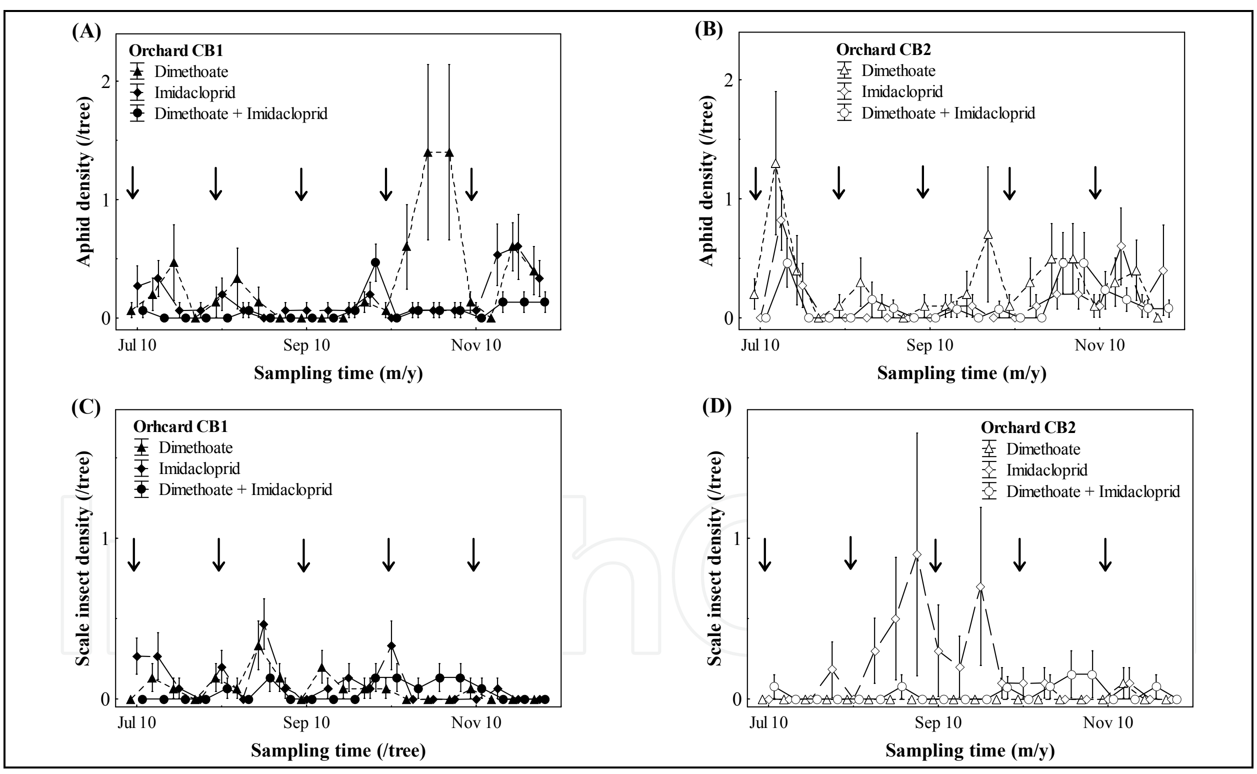

Fig. 10. Densities of aphid (A, B) and scale insect (C, D) colonies in two orchards, where seedlings had been planted either in May 2007 (CB1) or in November 2007 (CB2). Each orchard was divided into four plots, to each of which any of the four treatments were randomly distributed: no insecticide use for the control of psyllids, 0.40 g/tree dimethoate by spray, 0.20 g/tree imidacloprid by spray, or 0.40 g dimethoate and 0.20 g imidacloprid/tree by spray. Arrows indicate the time when insecticides were sprayed. Bars show the standard errors of the means.

3. Conclusion

When trees are young, usually until one and a half years after planting in southern Vietnam, neonicotinoids by soil drench are expected to control psyllids effectively for two months after the application. Methidathion by spray can keep high residue effects for a half month for the control of psyllids on seedlings. This insecticide can quickly kill psyllids in 12 hr after their infestation on plants, and neonicotinoids did not attain such quick effect on the psyllid. This is the advantage in the use of methidathion. Dimethoate showed similar lethal effects on the psyllids as methidathion, although both its quickness and residue effects were inferior to those of methidathion. Field application of imidacloprid, methidathion, dimethoate, and the mixture of imidacloprid with any of the two organophosphates showed that these insecticides showed similar effects on the control of psyllids: their residue effect was maintained for less than two weeks and no insecticides succeeded in attained high lethal effect to eradicate psyllids on trees even in a couple of days after the spray. It should be noted that dimethoate could not control aphids as much as imidacloprid or its mixture with dimethoate but imidacloprid would lead the increase of scale insects after the application. The application of any insecticide examined in this study did not lead these pests to be eradicated for even one week after the application. These results indicate that the application of insecticides cannot be expected to attain perfect protection of citrus trees from CG infection once the tree grew. Furthermore, since nymphs could increase in two weeks after the application without elimination, even secondary infection would not be avoided when citrus trees have grown to a stage of fruit yielding. The application of insecticide would only reduce more or less the probability of the second infection of trees by CG in the orchard.

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5. References

Boina, D.R.; Onagbola, E.O., Salyani, M. & Stelinski, L.L. (2009). Antifeedant and sublethal effects of imidacloprid on Asian citrus psyllid, *Diaphorina citri*. *Pest Management Science*, 65, 870-877.

Gatineau, F.; Bonnot, F., Yen, T. T. Hong, Tuan, D. H., Tuyen, N. D., & Truc, N. T. N. (2010). Effects of imidacloprid and fenobucarb on the dynamics of the psyllid *Diaphorina* *citri* Kuwayama and on the incidence of *Candidatus* Liberibacter asiaticus. *Fruits*, Vol.65, pp. 209-220

Halbert, S.E. & Manjunath K.L. (2004). Asian citrus psyllids (Sternorhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. *Florida Entomologist*, Vol.87, pp. 330-353.

Hayashikawa, S., Suenaga H. & Torigoe H. (2006). Insecticidal activity of some insecticides on Asian citrus psyllid, *Diaphorina citri* Kuwayama. *Kyushu Plant Protection Research*, 52, 71-74. (In Japanese with an English summary)

Hung, T.-H.; Hung S.-C., Chen C.-N., Hsu M.-H. & Su H.-J. (2004). Detection by PCR of *Candidatus* Liberibacter asiaticus, the bacterium causing citrus huanglongbing in vector psyllids: application to the study of vector–pathogen relationships. *Plant Pathology*, Vol.53, pp. 96-102.

Ichinose, K.; Bang, D.V., Tuan, D.H. & Dien, L.Q. (2010a). Effective use of neonicotinoids for protection of citrus seedlings from invasion by *Diaphorina citri* (Hemiptera: Psyllidae). *Journal of Economic Entomology*, Vol.103, pp.127-35

Ichinose, K. & Kano, T. (2006). Citrus greening disease and its management in the Mekong Delta Region of Vietnam. *Shokubutsuboeki*, Vol.60, 302-307. (In Japanese)

Ichinose, K.; Miyaji, K., Matsuhira, K., Yasuda, K., Sadoyama, Y., Tuan, D.H. & Bang, D.V. (2010b). Unreliable pesticide control of the vector psyllid *Diaphorina citri* (Hemiptera: Psyllidae) for the reduction of microorganism disease transmission. *Journal of Environmental Science and Health Part B*, Vol.45, pp.466-472.

Inoue, H.; Ohnishi, J., Ito, T., Tomimura, K., Miyata, S., Iwanami, T. & Ashihara, W. (2009). Enhanced proliferation and efficient transmission of *Candidatus* Liberibacter asiaticus by adult *Diaphorina citri* after acquisition feeding in the nymphal stage. *Annals of Applied Biology*, Vol.155, pp.29-36.

Koizumi, M.; Prommintara, M., Linwattana, G. & Kaisuwan, T. (1997). Epidemiological aspects of citrus huanglongbing (greening) disease in Thailand. *JARC*, Vol.31, pp. 205-211.

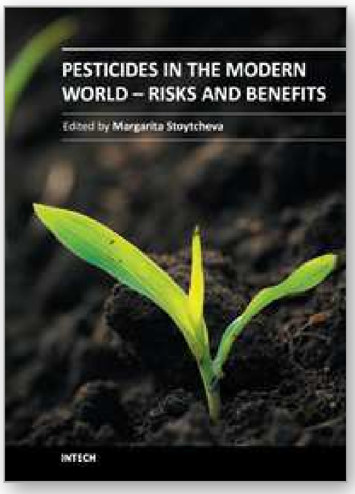
Quresh, J.A.; Stansly, P.A. (2010). Dormant season foliar sprays of broad-spectrum insecticides: An effective component of integrated management for *Diaphorina citri* (Hemiptera: Psyllidae) in citrus orchards. *Crop Protection*, Vol.29, pp. 860-866.

Srinivasan, R., Hoy, M.A., Singh, R. & Rogers, M.E. (2008). Laboratory and field evaluation of Silwet L-77 and kinetic alone and in combination with imidacloprid and abamectin for the management of the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae). *Florida Entomologist*, Vol. 91, pp.87-100.

Yang, Y.; Huang, M., Beattie, G. A. C., Xia, Y., Ouyang, G. & Xiong, J. (2006). Distribution, biology, ecology and control of the psyllid *Diaphorina citri* Kuwayama, a major pest of citrus: a status report for China. *International Journal of Pest Management*, Vol.52, pp. 343-352.

Yasuda, K.; Ooishi, T. & Kawamura, F. (2006). Effect of insecticides on adults and larvae of Asian citrus psyllid, *Diaphorina citri* (Homoptera: Psyllidae). *Kyushu Plant Protection Research*, Vol.52, pp. 75-78. (In Japanese with an English summary)

Yasuda K.; Yoshitake H., Ooishi T., Toudou A. & Uechi N. (2007). Effect of high-density scatter of the infiltration shift insecticide on invasion by adult and egg-laying individuals of the Asian citrus psyllid, *Diaphorina citri* (Homoptera: Psyllidae). *Kyushu Plant Protection Research*, Vol.53, pp.95-98. (In Japanese with an English summary).



Pesticides in the Modern World - Risks and Benefits
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This book is a compilation of 29 chapters focused on: pesticides and food production, environmental effects of pesticides, and pesticides mobility, transport and fate. The first book section addresses the benefits of the pest control for crop protection and food supply increasing, and the associated risks of food contamination. The second book section is dedicated to the effects of pesticides on the non-target organisms and the environment such as: effects involving pollinators, effects on nutrient cycling in ecosystems, effects on soil erosion, structure and fertility, effects on water quality, and pesticides resistance development. The third book section furnishes numerous data contributing to the better understanding of the pesticides mobility, transport and fate. The addressed in this book issues should attract the public concern to support rational decisions to pesticides use.