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Control of *Diaphorina citri* and selectivity to *Pentilia egena* by insecticides applied at ultra-low volume (ULV) on citrus orchards

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Summary

The chemical control of greening vector is still the most widely used method for reducing the incidence of that disease in citrus orchards in Brazil. However, it is an expensive strategy due the number of applications and collateral effects of reducing natural enemies. Use of smaller spraying volumes with newer machines may be benificial in reduce costs and impacts to natural enemies. The aim of this study was to evaluate the efficiency of the ultra-low volume application (ULV) to control adults of citrus psyllid, *Diaphorina citri*, and the selectivity to the ladybug, *Pentilia egena*, in citrus orchards. The insects were confined in cages from 25 March to 15 April 2010, at SGS GRAVENA Research Station, in Jaboticabal, SP, Brazil. The statistical design adopted was the randomized blocks, with six treatments and four replications. The application was made on 25 March 2010, with the insecticides: lambda-cialothrin (0.4 $L.ha^{-1}$), dimethoate (2 $L.ha^{-1}$), etofenprox (0.5 $L.ha^{-1}$) and imidacloprid (0.4 $L.ha^{-1}$) using 10 $L.ha^{-1}$ spraying volume in comparison to imidacloprid (0.4 $L.ha^{-1}$), sprayed by 1000 $L.ha^{-1}$, by gun sprayer. Cages made of voile were fixed on branches of plants and 5 adult psyllids were confined per replication, totaling 20 insects per treatment, at 0, 1, 3, 7, 10 and 14 days after spraying. The mortality assessments were performed 0, 1, 2, 3, 4 and 7 days after the insect's confinement. The evaluation of the natural enemies' presence, the ladybug *Pentilia egena* in the stages of larva and adult was performed at 0, 1, 3, 7, 10 and 14 days after treatments application, by surrounding each useful plant for 2 mins. All insecticides were effective in controlling adult psyllids up to 7 days after application and moderately to highly toxic to larvae and adults of *P. egena* when used in ULV or traditional spraying volume.

Key words: Sprayer, spraying, IPM, HLB, natural enemy

Introduction

The greening or HLB, caused by *Candidatus* Liberibacter asiaticus and *Candidatus* Liberibacter americanus was detected in the Sao Paolo State, the largest orange producer in Brazil, in 2004 (Colleta-Filho *et al*., 2004, Teixeira *et al*., 2005). There is no known sources to HLB resistance to citrus species grown commercially in Brazil and there is no curative method for the control of HLB that may be used in commercial orchards. Thus, preventing the infection of plants is crucial reduce disease sources. Currently in Brazil, the recommended control comprises planting healthy seedlings, elimination of diseased plants, elimination of *Murraya paniculata* (host of *Candidatus* Liberibacter) and control of the HLB vector, the psyllid *Diaphorina citri* (Belasque Jr. *et al*., 2010). Chemical sprays still being the main method to control of this disease's vector. However, this solution for eliminating disease presents problems to the applicator, consumers and the environment (Bergamin *et al*., 2008). The use of insecticides for vector control also has the side effect of reducing the population of natural enemies in orchards (Stansly *et al*., 2008), besides the high cost. The ladybug *Pentilia egena* is the main predator of mealybugs: *Selenaspidus articulatus*, *Parlatoria Ziziphi*, *Unaspis citri*, *Parlatoria cinerea*, and other Hemiptera from Diaspididae family. The population of ladybug is falling in citrus due to the toxic effect of frequently applied insecticides, often more than two applications per month for the control of *D. citri* vector of HLB (Gravena, 2005). Due to lack of application technologies and new products with low impact to man, natural enemies and environment are very important to develop methods that present efficiency and selectivity in citrus orchards.

In this context, we aimed in this work to compare the efficiency of high volume spraying to ultra-low volume (ULV) for control of *D. citri* and the selectivity of *P. egena* in citrus orchards (*Citrus sinensis*, Osbeck).

Material and Methods

Mortality of adults of Diaphorina citri *(Kuwayama, Hemitptera: Psyllidae)*

The experiment was conducted from 25 March to 15 April 2010, at the SGS GRAVENA Research Station in the municipality of Jaboticabal – Sao Paolo State, Brazil (coordinates: 21.3263S, 48.3277W and altitude of 579 m), in citrus orchard. The trials were placed in a two years old orchard, of plants spaced at 6 m × 4 m, 2 m high with young branches and fruits at stage BBCH = 31-39 and 79.

The statistical design used was a randomized block design (RBD) with six treatments and four replications. Each plot consisted of 288 m^2 with 12 plants (three planting rows with four plants each), being considered useful for evaluating the two central plants in the central row.

Table 1. *Insecticides evaluated, active ingredients and dosages per hectare for the control of citrus psyllid,* D. citri. *Jaboticabal, 2010*

Treatments	Active ingredient	Chemical group	Dosages		
			Spray volume $L.ha^{-1}$	g or mL c.p. ha^{-1}	g or mL a.i. ha^{-1}
1-Karatê Zeon 50 CS	lambda-cyhalothrin	pyrethroid	10	400	20.0
2-Perfekthion 400 EC	dimethoate	phosphorus	10	2000	800.0
3-Provado 200 CS	imidacloprid	neonicotinoid	10	400	80.0
4-Trebon 100 CS	etofenprox	diphenyl ether	10	500	50.0
5-Provado 200 CS	imidacloprid	neonicotinoid	1000	400	80.0
6-Control	-	-	-	-	-

The insecticides chosen for this research aimed to alternate groups and chemical modes of action for resistance management of the *D. citri* (Table 1). The spraying was done on 25 March 2010, from 10 a.m. to 2 p.m., at temperatures between 25.6°C and 30.5°C, relative humidity at 57 to 67%, winds about 2.3 $km.h^{-1}$. In treatments 01 to 04 a sprayer equipped with a pneumatic nozzle brand Pulsfog Pulverizadores - Model AT - 100 was used at ultra-low volume (ULV) of 24 mL per plant equivalent to 10 $L.ha^{-1}$. In the treatment 05, a conventional standard, a hand sprayer with two sprayers guns (KO Máquinas Agrícolas) with hydraulic hollow cone nozzles was used, providing a spraying volume of 2.40 L per plant equivalent to 1000 $L.ha^{-1}$ operating at the pressure of 1034.2 kPa. In the branches of the plants voile cages were installed where five psyllids were confined per plot totaling 20 insects per treatment at 0, 1, 3, 7,10 and 14 days after spraying. The evaluations of mortality were performed at 0, 1, 2, 3, 4 and 7 days after the confinement of insects. The psyllids used in the research came from a mass production of this insect in citrus seedlings and *Murraya paniculata*, surrounded by the voile cages.

The original data were transformed $(x + 0.5)^{0.5}$ and then subjected to analysis of variance by F test and the averages were compared by Tukey test ($P \leq 0.05$). The efficiency of the insecticides to control of psyllids in citrus plant was calculated using Abbott's formula (1925).

Insecticide selectivity to Ladybug Pentilia egena *(Mulsant, Coleoptera: Coccinellidae)*

The evaluation of insecticide selectivity for the ladybug was evaluated at: 0, 1, 3, 7, 10 and 14 days after the spraying. To evaluate the ladybugs population present naturally in the experimental field (larvae and adults) it was observed for 2 mins around one plant per plot, the entire treetops as far as the evaluator could see, quantifying the number of individuals present (Gravena, 2005). The statistical design was a randomized block with six treatments and four replications. Original data were transformed by $(x + 0.5)^{0.5}$ and submitted to analysis of variance (F test) and the averages were compared by Tukey test ($P \leq 0.05$). The degree of toxicity of the insecticides was determined according to the classification proposed by Hassan *et al*. (1991).

The formula of Henderson & Tilton (1955) was used for calculating the percentages of efficiency, considering the average number of insects evaluated, aiming to constitute in a reduction index of natural enemies.

Results and Discussion

Mortality of adults of Diaphorina citri *(Kuwayama, Hemitptera: Psyllidae)*

It was observed that when adults of psyllid were confined in the same day of the insecticides application, all treatments resulted in 100% of mortality of insects. With psyllids confined one day after insecticides application, it was found that the treatments were effective, with efficiencies greater than 80% except for ethofenprox that was efficient only after 4 days (Table 2).

Table 2**.** *Average number of survived* D. citri *confined 1 day after the insecticide application and evaluated at 1, 2, 3, 4 and 7 days after the confinement. Jaboticabal, 2010*

Treatments	Dosages (mL or g c.p. ha^{-1})	Spray volume L.ha^{-1}	Average number of *D. citri* survived at 1 day after the application, in hours after the confinement				
			1 day 27 Mar	2 days 28 Mar	3 days 29 Mar	4 days 30 Mar	7 days 2 Apr
1- Lambda-cyhalothrin	400	10	0.2[1] b[2] **94**[3]	0.2 b **94**	0.2 b **94**	0.2 b **94**	0.2 b **94**
2- Dimethoate	2000	10	0.0 b **100**	0.0 b **100**	0.0 b **100**	0.0 b **100**	0.0 b **100**
3-Imidacloprid	400	10	0.0 b **100**	0.0 b **100**	0.0 b **100**	0.0 b **100**	0.0 b **100**
4-Etofenprox	500	10	1.0 b **71**	1.0 b **71**	0.8 b **76**	0.6 b **81**	0.4 b **88**
5-Imidacloprid	400	1000	0.0 b **100**	0.0 b **100**	0.0 b **100**	0.0 b**100**	0.0 b **100**
6-Control	-	-	3.4 a	3.4 a	3.4 a	3.2 a	3.2 a
Coeficient of Variation			26.76	26.76	23.29	24.23	19.72

[1]Transformed data by $(x+0.5)^{0.5}$, [2]Values followed by the same letter in the column do not differ significantly according to Tukey test ($P<0.05$), [3] Percentage of efficiency calculated by Abbott's formulae.

In the confinement 3 days after insecticide application, both volumes were efficient and significantly different from the control up to 7 days (Table 3). When psyllids were confined seven days after application, there was significant reduction in number of insects alive two days after the confinement, remaining until the last evaluation (Table 4). For confinement at 10 and 14 days after the application, the percentages of *D. citri* reduction were smaller than 80%, indicating that the insecticides at the dosages and spray volumes evaluated were efficient up to 7 days after application (Tables 5 and 6). This was in line with the expected reduction of pesticide deposit residues on citrus plants due to biotic factors such as the absorption of active ingredients by the plant itself, the biological degradation of molecules by plants and microorganisms and abiotic factors such as insolation, alternating periods of higher and smaller relative humidity and drag of molecules by the wind.

The results were similar in efficiency for application made at 10 $L.ha^{-1}$ (ULV) and 1000 $L.ha^{-1}$ (conventional standard) using imidacloprid (400 $mL.ha^{-1}$).

Table 3. *Average number of survived* D. citri *at 3 days after the insecticide application and evaluated at 1, 2, 3, 4 and 7 days after the confinement. Jaboticabal, 2010*

Treatments	Dosages (mL or g c.p. ha^{-1})	Spray volume $L.ha^{-1}$	Average number of *D. citri* survived at 3 days after the application, in hours after the confinement				
			1 day 27 Mar	2 days 28 Mar	3 days 29 Mar	4 days 30 Mar	7 days 4 Apr
1- Lambda-cyhalothrin	400	10	1.0[1] b[2] **76**[3]	0.6 b **83**	0.4 b **89**	0.4 b **89**	0.2 b **94**
2- Dimethoate	2000	10	0.8 b **81**	0.6 b **83**	0.4 b **89**	0.0 b **100**	0.0 b **100**
3-Imidacloprid	400	10	0.6 b **86**	0.4 b **89**	0.2 b **94**	0.2 b **94**	0.0 b **100**
4-Etofenprox	500	10	0.4 b **90**	0.2 b **94**	0.2 b **94**	0.2 b **94**	0.2 b **94**
5-Imidacloprid	400	1000	0.6 b **86**	0.4 b **89**	0.2 b **94**	0.0 b **100**	0.0 b **100**
6-Control	-	-	4.2 a	3.6 a	3.6 a	3,6 a	3.4 a
Coeficient of Variation			20.38	22.91	24.11	17.87	17.56

[1]Transformed data by $(x+0.5)^{0.5}$, [2]Values followed by the same letter in the column do not differ significantly according to Tukey test ($P<0.05$), [3] Percentage of efficiency calculated by Abbott's formulae.

Table 4. *Average number of survived* D. citri *at 7 days after the insecticide application and evaluated at 1, 2, 3, 4 and 7 days after the confinement. Jaboticabal, 2010*

Treatments	Dosages (mL or g c.p. ha^{-1})	Spray volume $L.ha^{-1}$	Average number of *D. citri* survived at 7 days after the application, in hours after the confinement				
			1 day 2 Apr	2 days 3 Apr	3 days 4 Apr	4 days 5 Apr	7 days 8 Apr
1- Lambda-cyhalothrin	400	10	1.0[1] b[2] **74**[3]	0.6 b **81**	0.6 b **81**	0.2 b **94**	0.2 b **93**
2- Dimethoate	2000	10	1.0 b **74**	0.2 b **94**	0.0 b **100**	0.0 b **100**	0.0 b **100**
3-Imidacloprid	400	10	0.8 b **79**	0.4 b **88**	0.4 b **88**	0.4 b **88**	0.4 b **87**
4-Etofenprox	500	10	1.0 b **74**	0.6 b **81**	0.6 b **81**	0.6 b **81**	0.6 b **80**
5-Imidacloprid	400	1000	0.8 b **79**	0.6 b **81**	0.2 b **94**	0.0 b **100**	0.0 b **100**
6-Control	-	-	3.8 a	3.2 a	3.2 a	3.2 a	3.0 a
Coeficient of Variation			19.26	19.37	19.82	20.07	20.2

[1]Transformed data by $(x+0.5)^{0.5}$, [2]Values followed by the same letter in the column do not differ significantly according to Tukey test ($P<0.05$), [3] Percentage of efficiency calculated by Abbott's formulae.

Table 5. *Average number of survived* D. citri *at 10 days after the insecticide application and evaluated at 1, 2, 3, 4 and 7 days after the confinement. Jaboticabal, 2010*

Treatments	Dosages (mL or g c.p. ha^{-1})	Spray volume $L.ha^{-1}$	Average number of *D. citri* survived at 10 days after the application, in hours after the confinement				
			1 day 5 Apr	2 days 6 Apr	3 days 7 Apr	4 days 8 Apr	7 days 11 Apr
1- Lambda-cyhalothrin	400	10	2.0 ab **38**	2.0 ab **38**	1.8 ab **40**	1.6 ab **47**	1.4 a **50**
2- Dimethoate	2000	10	1.2 b **63**	1.2 bc **63**	1.2 b **60**	1.2 b **60**	1.0 a **64**
3-Imidacloprid	400	10	1.6 b **50**	1.6 bc **50**	1.6 b **47**	1.4 b **53**	1.0 a **64**
4-Etofenprox	500	10	1.2 b **63**	1.0 c **69**	1.0 b **67**	1.0 b **67**	1.0 a **64**
5-Imidacloprid	400	1000	1.6 b **50**	1.4 bc **56**	1.2 b **60**	1.0 b **67**	1.0 a **64**
6-Control	-	-	3.2 a	3.2 a	3.0 a	3.0 a	2.8 a

Coeficient of Variation	14.85	12.61	13.84	16.39	26.98

[1]Transformed data by $(x+0.5)^{0.5}$, [2]Values followed by the same letter in the column do not differ significantly according to Tukey test ($P<0.05$), [3] Percentage of efficiency calculated by Abbott's formulae.

Table 6. *Average number of survived* D. citri *at 14 days after the insecticide application and evaluated at 1, 2, 3, 4 and 7 days after the confinement. Jaboticabal, 2010*

Treatments	Dosages (mL or g c.p. ha^{-1})	Spray volume L.ha^{-1}	Average number of *D. citri* survived at 14 days after the application, in hours after the confinement				
			1 day 9 Apr	2 days 10 Apr	3 days 11 Apr	4 days 12 Apr	7 days 15 Apr
1- Lambda-cyhalothrin	400	10	2.8[1]a[2]**22**[3]	2.4 a **20**	2.2 a **27**	2.0 a **29**	1.4 a **46**
2- Dimethoate	2000	10	2.6 a **28**	2.6 a **13**	2.2 a **27**	1.8 a **36**	1.4 a **46**
3-Imidacloprid	400	10	3.0 a **17**	2.8 a **7**	2.2 a **27**	2.2 a **21**	2.0 a **23**
4-Etofenprox	500	10	2.2 a **39**	2.0 a **33**	1.6 a **47**	1.4 a **50**	1.4 a **43**
5-Imidacloprid	400	1000	2.8 a **22**	2.4 a **20**	2.2 a **27**	2.2 a **21**	1.8 a **31**
6-Control	-	-	3.6 a	3.0 a	3.0 a	2.8 a	2.6 a
Coeficient of Variation			11.6	12.57	19,64	20.15	22.06

[1]Transformed data by $(x+0.5)^{0.5}$, [2]Values followed by the same letter in the column do not differ significantly according to Tukey test ($P<0.05$), [3] Percentage of efficiency calculated by Abbott's formulae.

Insecticide selectivity to Ladybug Pentilia egena *(Mulsant, Coleoptera: Coccinellidae)*

The insecticides lambda-cyhalothrin and dimethoate showed low toxicity to *P. egena* larvae, according to the classification of Hassan *et al*. (2001) one day after the application and moderate toxicity from three to 10 days after the application. Treatments with imidacloprid at the volumes of 10 and 1000 L.ha^{-1} of spray liquid and ethofenprox were highly toxic from one to ten days after application. After 14 days after application, the larvae population in the control reduced dramatically indicating that this period is inappropriate to consider toxic effect of the products (Table 7).

Table 7. *Average number of* P. egena *ladybug larvae and percentage of density reduction in days after the application in citrus. Jaboticabal, 2010*

Treatments	Dosages (mL or g c.p. ha^{-1})	Spray volume L.ha^{-1}	Average number of *P. egena* ladybug larvae and percentage of density reduction in days after the application					
			0 25 Mar	1 26 Mar	3 28 Mar	7 1 Apr	10 4 Apr	14 8 Apr
1- Lambda-cyhalothrin	400	10	97.5[1]a[2]	62.5b **40**[3]	24.5b **71**	24.0b **70**	18.8b **71**	11.0b **84**
2- Dimethoate	2000	10	110.0a	70.0b **41**	33.3b **65**	24.5b **69**	18.9b **71**	10.8b **86**
3-Imidacloprid	400	10	90.0a	22.5c **77**	4.8c **94**	4.3c **93**	0.0c **100**	0.0c **100**
4-Etofenprox	500	10	100.0a	18.8c **82**	7.0c **92**	4.5c **94**	0.0c **100**	0.0c **100**
5-Imidacloprid	400	1000	102.5a	19.5c **82**	6.5c **89**	3.3c **96**	0.0c **100**	0.0c **100**
6-Control	-	-	107.5a	115.0a	93.8a	77.0a	71.3a	76.3a
Coeficient of Variation			6.2	8.4	15.3	19.7	8.8	9.0

[1]Transformed data by $(x+0.5)^{0.5}$, [2]Values followed by the same letter in the column do not differ significantly according to Tukey test ($P<0.05$), [3] Percentage of efficiency calculated by Henderson & Tilton formulae.

When evaluating toxicity to adults of *P. egena* at one day after application, dimethoate presented no harmful effect, lambda-cyhalothrin showed moderate toxicity, whereas the treatments with imidacloprid at the volumes of 10 and 1000 L of spray liquid per ha and ethofenprox were highly toxic (Table 7). At 3 days after the application, the insecticide dimethoate showed low toxicity, while lambda-cyhalothrin, imidacloprid at 10 and 1000 L of spray liquid per ha and ethofenprox were highly toxic (Table 7). From 7 to 14 days after spraying, the pesticide dimethoate presented moderate toxicity to adults of *P. egena*, while lambda-cyhalothrin, imidacloprid at 10 and 1000 $L.ha^{-1}$ of spray liquid and ethofenprox were highly toxic (Table 8).

In summary, the treatments performed were from moderately to highly toxic for larvae and adults of the *P. egena* when used in ULV, according the same results for the selectivity achieved in the research conducted by Stansly *et al*. (2008). In treatments where the insecticide imidacloprid had been used (400 $mL.ha^{-1}$), only varying the spray liquid volumes of 10 $L.ha^{-1}$ (ULV) and 1000 $L.ha^{-1}$ (conventional standard), the results were similar for both volumes and highly toxic to larvae and adults of the natural enemy *P. egena*.

Table 8. *Average number of* P. egena *ladybug adults and percentage of density reduction in days after the application in citrus. Jaboticabal, 2010*

Treatments	Dosages (mL or g c.p. ha^{-1})	Spray volume $L.ha^{-1}$	Average number of *P. egena* ladybug adults and percentage of density reduction in days after the application					
			0 25 Mar	1 26 Mar	3 28 Mar	7 1 Apr	10 4 Apr	14 8 Apr
1- Lambda-cyhalothrin	400	10	5.3[1]a[2]	2.0ab **62[3]**	0.5b **81**	1.8b **85**	0.5b **94**	0.8b **90**
2- Dimethoate	2000	10	3.0a	2.3b **23**	1.0b **34**	1.0b **57**	0.8b **65**	1.2b **60**
3-Imidacloprid	400	10	4.5a	0.0c**100**	0.3b **87**	0.5b **86**	0.0c **100**	0.0c **100**
4-Etofenprox	500	10	4.8a	0.3c **94**	0.3b **88**	0.5b **85**	0.0c **100**	0.0c **100**
5-Imidacloprid	400	1000	3.5a	0.8c **77**	0.3b **83**	0.5b **81**	0.0c **100**	0.0c **100**
6-Control	-	-	6.5a	6.5a	3.3a	5.0a	5.0a	7.0a
Coeficient of Variation			18.0	22.1	35.5	23.4	25.5	24.4

[1]Transformed data by $(x+0.5)^{0.5}$, [2]Values followed by the same letter in the column do not differ significantly according to Tukey test ($P<0.05$), [3] Percentage of efficiency calculated by Henderson & Tilton formulae.

Conclusion

The ULV spraying for the control of citrus psyllid was as efficient as the traditional volume of 1000 $L.ha^{-1}$ and resulted in no different mortality of *P. egena*, enabling further research and development with the technology to be used in the field.

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