

Effects of insecticide and acaricide mixtures on *Diaphorina citri* control

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Abstract - Pesticide mixtures are commonly used to reduce production costs in agriculture. However, the consequences of such practice towards target organisms are still unknown in most cases. Therefore, this study aimed to evaluate the effects of combinations of insecticides and one worldwide used acaricide on the control of *Diaphorina citri* (Asian citrus psyllid). All insecticides alone (lambda-cyhalothrin+thiamethoxam, phosmet, and imidacloprid) and in combination with spiroticlofen were efficient in controlling *D. citri* (> 80%). No significant effects were found for combinations of lambda-cyhalothrin+thiamethoxam and phosmet insecticides with spiroticlofen. Conversely, imidacloprid caused an antagonistic effect on the control of *D. citri* when mixed with the acaricide. After all, spiroticlofen had no effect on the pH and electrical conductivity of insecticidal spray solutions. Spiroticlofen mixtures with lambda-cyhalothrin+thiamethoxam and phosmet had no effect on *D. citri* control, but combinations with imidacloprid interfered with it. **Index terms:** Huanglongbing – HLB; antagonist effect; tank mixture; Asian citrus psyllid; citrus leprosis.

Efeito da mistura de inseticidas e acaricida no controle de *Diaphorina citri*

Resumo - Misturas de agrotóxicos são comumente utilizadas para reduzir os custos de produção na agricultura. No entanto, as consequências de tal prática para organismos-alvo ainda são desconhecidas na maioria dos casos. Portanto, este trabalho teve como objetivo avaliar os efeitos de combinações de inseticidas e um acaricida utilizado mundialmente no controle de *Diaphorina citri* (psilídeo asiático dos citros). Todos os inseticidas isolados (lambda-cialotrina + tiametoxam, fosmete e imidacloprídeo) e em combinação com o espiroticlofeno foram eficientes no controle de *D. citri* (> 80%). Não foram encontrados efeitos significativos para combinações de lambda-cialotrina + tiametoxam e fosmete com espiroticlofeno. Por outro lado, o imidacloprido causou um efeito antagônico no controle de *D. citri*, quando misturado ao acaricida. Afinal, o espiroticlofeno não teve efeito sobre o pH e a condutividade elétrica das soluções de pulverização com inseticidas. Misturas de espiroticlofeno com lambda-cialotrina + tiametoxam e fosmete não tiveram efeito sobre o controle de *D. citri*, mas combinações com imidacloprídeo interferiram nele.

Termos para indexação: Huanglongbing – HLB; efeito antagônico; mistura em tanque; psilídeo asiático dos citros; leprose dos citros.

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Introduction

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Sternorrhyncha: Liviidae), has become one of the main citrus pests worldwide, especially for being a vector of the bacterial disease Huanglongbing (HLB) (HALBERT and MANJUNATH, 2004). *Diaphorina citri* adults and nymphs can transmit the causal agent of HLB, which is a group of bacteria belonging to the genus *Candidatus Liberibacter* (XU et al., 1988; PELZ-STELINSKI et al., 2010). As there are still no curative measures for the disease, the management of HLB has been mainly performed by spraying synthetic insecticides against the psyllids (BOINA and BLOOMQUIST, 2015).

After detection in 2004, the control costs of the Brazilian citrus industry have increased significantly, mainly regarding HLB monitoring, eradication of symptomatic plants, and insecticide sprays (TANSEY et al., 2015). Such costs have raised from 12 to 40% of the total costs for citrus production in Brazil (BELASQUE Jr. et al., 2010).

In this context, new alternatives have been demanded to maintain the economic viability of citrus crops. One of these alternatives consists of using spray tank mixtures, as long as several other pests may occur concomitantly. A very common example is the occurrence of *Brevipalpus* spp. (Acari: Tenuipalpidae) mites, which are citrus leprosis vectors, along with the Asian citrus psyllid. Notwithstanding the benefits, tank mixtures may also imply numerous adverse effects, such as interferences with physicochemical stability and efficiency of chemicals (REFSTRUP et al., 2010).

Some pesticide mixtures may change pH, electrical conductivity, spray-solution stability, besides interfering with the biological effect on target organism when compared to the pesticide alone (PETTER et al., 2013).

When combining spinosad and bifenthrin, Willmott et al. (2013) found an antagonistic effect for the control of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Yet Khajehali et al. (2009) verified that, when applied before, chlorpyrifos affected the efficiency of bifenthrin, which is an acaricide, used to control *Tetranychus urticae* Koch (Acari: Tetranychidae).

Moreover, Della Vechia et al. (2018) confirmed a negative effect of acaricide and insecticide mixtures for the control of *Brevipalpus yothersi* Baker (Acari: Tenuipalpidae). Given the above, it becomes also necessary to verify whether these mixtures can interfere with the control of Asian citrus psyllids and compare combinations with insecticides alone. Thus, this study aimed to investigate the effects of mixtures of different insecticides with an acaricide on *D. citri* control.

Materials and methods

Diaphorina citri rearing

To supply insects for bioassays, *D. citri* population was initiated from insects provided by the Fund for Defense of the Citriculture (FUNDECITRUS). Then, these insects were transferred to a greenhouse, at FCAV/UNESP in Jaboticabal, SP (Brazil), being kept on orange jasmine [*Murraya paniculata* (L.) Jack] placed inside cages made of voile fabric, a method adapted from Skelley and Hoy (2004).

Residual effect

We selected three insecticides and one acaricide widely used in citrus production to control *D. citri* and *Brevipalpus* spp., respectively (TIWARI et al., 2011; VAN LEEUWEN et al., 2015). The insecticides were lambda-cyhalothrin+thiamethoxam (3.7 g a.i. per 100 L water - Engeo Pleno[®] SC; Syngenta, England), phosmet (175.0 g a.i per 100 L water - Imidan[®] 50 WP; Cross Link, Brazil), imidacloprid (4.0 g a.i. per 100 L water - Provado[®] 20 SC; Bayer CropScience AG, Germany). The acaricide was spiroticlofen (6.0 g a.i. per 100 L water - Envidor[®] 24 SC; Bayer CropScience AG, Germany). The concentrations used in this experiment correspond to the highest recommended by manufacturers. As a matter of comparison, the insecticides were tested alone and in combination with the acaricide, and water as a control, so totalizing eight treatments.

Seedlings of *Citrus sinensis* L. (var. Pera) were grown in 25-L pots filled with soil, sand, and bovine manure (2:1:1; v: v: v), with no pesticide spray. Leaf discs (3.3 cm) were cut from these plants, being placed on acrylic plates (35 × 10 mm; diameter × height). These plates contained a 1.5% agar layer in water (5 mm) for leaf turgescence (as adapted from BARBER et al., 1999).

The pesticides were sprayed on the adaxial surface of leaf discs, applying 2 mL on the entire surface with the aid of a Potter Tower at 34.5 kPa (Burkard Manufacturing, Rickmansworth, Herts., United Kingdom). The active ingredient concentration in dried residues was about 1.56 mg cm⁻². After leaf discs were dried, 15 *D. citri* adult females were transferred to each of them. Prior to transfer, the insects were anesthetized with a minimal amount of CO₂ (carbon dioxide) for a short period (WENNINGER et al., 2009). With the aid of a sucker device, the insects were transferred to plates, which were wrapped with plastic film (PVC) and kept in a room (25±2°C; 65%±10%; and 14:10 h L: D photoperiod). After 24 and 48 hours of application, mortality rates were evaluated by counting the number of dead and living psyllids.

The experimental design was completely randomized, with eight treatments and five replications, which consisted of one leaf disc each. The experiment was performed twice to confirm the results, being called experiment A and B.

Physico-chemical compatibility

Physical compatibility between pesticides was assessed by a method based on the NBR 13875 (ABNT, 2014). As aforementioned, this experimental design was also completely randomized but with four replications (a 250 mL beaker each).

The used reagent was standard water with a total hardness of 20 mg.kg⁻¹ in CaCO₃ equivalent, prepared according to the NBR 13074 (ABNT, 1997). Solutions were prepared in 250 mL graduated beakers topped with metallic fabric sieve (149 µm mesh), according to the NBR NM-ISO 3310-1 (ABNT, 2010). Besides that, we used an analytical balance (0.1-mg resolution) and a graduated pipette for measurements.

Before mixing, all pesticides (insecticides and acaricide) were evaluated individually for dispersion (homogenization). All products used were evaluated at the maximum concentration recommended by the manufacturer (as cited in the previous item). Spray formulations were pipetted with a graduated pipette in beakers. Standard-water samples (150 mL), previously prepared (20 mg.kg⁻¹ of CaCO₃), were added to each beaker prior to pesticides. This beaker was inverted ten times every two seconds for good homogenization. Afterwards, it was completed with standard water up to 250 mL and inverted ten times again.

Soon after preparation, spray solutions were assessed by checking homogeneity and heterogeneity visually. These patterns consisted of flocculation, sedimentation, phase separation, lumps, oil separation, crystal formation, cream and foam (viscous supernatant layer).

pH and electrical conductivity

Spray solutions were measured for pH just before application using a benchtop pH meter (Q400AS model, Quimis[®] Company, Diadema, SP - Brazil). The pH values ranged from 0.00 to 14.00, and automatic temperature compensation was between 0 and 100 °C, the respective accuracies were ±0.02 and ±0.2 °C.

Electrical conductivity was measured with a benchtop conductivity meter (MP11P model, Marth[®] Company). Before reading, calibration was achieved with standard solutions provided by the manufacturer.

Again, the experiment was carried out in a fully randomized design, with the same treatments mentioned in item 2.2. These treatments were repeated four times, each of which consisted of one reading.

Data analysis

Data obtained on the first and second day after *D. citri* transfer were used to calculate control efficiency for both treatments using the formula proposed by Abbott (1925), in which mortality rate is corrected for control (only water). Control mortality was similar in both

experiments (A and B), being of 0 and 6% after 24 and 48 hours, respectively.

Interactions between insecticides and the acaricide were verified by comparing the observed and expected control efficiency, both alone and in combination. The expected efficiency of combined agents (M_E) was calculated using the formula: $M_E = M_A + M_I[(1-M_A).100^{-1}]$; wherein: M_A and M_I are respectively the control efficiency of acaricide and insecticide (%) separately (MORALEZ-RODRIGUEZ and PECK, 2009).

The interactions were considered additive when mixtures reached a control efficiency equals the sum of each product efficiency; synergistic, when higher than the expected; antagonistic when lower than the expected; and a non-interference when equals that of insecticide alone (REFSTRUP et al., 2010).

The expected and observed control efficiencies were compared by the Chi-square test (X^2) at 5% significance. Yet pH and electrical conductivity values underwent a descriptive analysis with the aid of AgroEstat software (BARBOSA and MALDONADO, 2013).

Results

The results obtained in both experiments (A and B) were similar ($F = 4.49$; $p = 0.08$), which enabled evaluating the effects of combinations between the acaricide and each insecticide on *D. citri* control efficiency (Figures 1 and 2).

Twenty-four hours after application, most of the spray formulations reached a control efficiency above 93.0% (Figures 1 and 2) except for that with the acaricide alone, which had a 1.70% efficiency in experiment A and 2.85% in B. Conversely, spiroadiclofen and imidacloprid mixture affected the insecticide performance in both experiments, therefore, showing an antagonistic interaction (Figures 1 and 2, and Tables 1 and 2). Despite imidacloprid, none of the other insecticides was affected by acaricide addition (Figures 1 and 2 and Tables 1 and 2).

Insecticidal spray formulations with the acaricide were physically compatible since there was no sedimentation, phase separation, flocculation, and formation of crystals or lumps, therefore, being homogeneous mixtures. Separately, the pH values of spray solutions were 6.02 ± 0.02 (lambda-cyhalothrin+thiamethoxam), 6.09 ± 0.03 (phosmet), 6.08 ± 0.01 (imidacloprid), and 6.20 ± 0.05 (spiroadiclofen). For mixtures, these values were 5.95 ± 0.02 (lambda-cyhalothrin+thiamethoxam and spiroadiclofen), 6.31 ± 0.03 (phosmet and spiroadiclofen), and 6.05 ± 0.02 (imidacloprid and spiroadiclofen). Electrical conductivity values were 19.19 ± 0.02 (lambda-cyhalothrin+thiamethoxam), 62.52 ± 0.09 (phosmet), 18.58 ± 0.05 (imidacloprid), and 18.71 ± 0.11 (spiroadiclofen). For mixtures with spiroadiclofen, these values were 19.59 ± 0.06 (lambda-cyhalothrin+thiamethoxam), 65.32 ± 0.05 (phosmet), and 18.87 ± 0.11 (imidacloprid).

Discussion

The main goal of insecticide and acaricide mixtures is to save operational and financial resources since insects and mites may occur at the same time in citrus orchards. Della Vechia et al. (2018) demonstrated a reduction in control efficiency of *B. yothersi* mites when combining spiroticlofen with phosmet (about 43%) and with imidacloprid (about 28%). Such an outcome was the basis for the development of the present study.

All the treatments containing insecticides were efficient (>80%) to control *D. citri*, either alone or in combination with the acaricide. Several studies have already proved the effectiveness of the tested insecticides against Asian citrus psyllid (SANCHES et al., 2009; YAMAMOTO et al., 2009; QURESHI et al., 2014). As already expected, spiroticlofen have no significant insecticidal effect. Richardson and Hall (2013) tested spiroticlofen to control *D. citri* and noted that, right after application, no mortality was observed for nymphs nor did it affect adult longevity; however, as time passed, the authors verified mortality of nymphs. This finding could be explained by the mechanism of action of this acaricide (lipid-synthesis inhibition), in which the time required to cause death is generally higher if compared to other acaricides (MARCIC, 2012).

By contrast, combinations of imidacloprid and spiroticlofen had an antagonistic effect, reducing insecticidal efficiency. Indeed, reductions in the efficiency of *D. citri* control are quite worrisome since they might occur together with an increase in the number of insects contaminated with *Ca. Liberibacter* (about 70%) (Manjunath et al. 2008). Distinctly, Poltronieri (2013) observed no effect on imidacloprid efficiency against *D. citri* when mixed with the fungicides thiophanate-methyl, pyraclostrobin, and difenoconazole. In wheat crops, Joshi and Sharma (2009) reported no effect of imidacloprid mixtures with propiconazole for the control of *Sitobion avenae* (Fabricius) and *Rhopalosiphum maidis* Fitch, 1856 (Hemiptera: Aphididae).

Notably, pesticide mixtures may affect the physicochemical or even biological characteristics of spray solutions (VAN LEEUWEN et al., 2007; MINTON et al., 2008; REFFSTRUP et al., 2010; PETTER et al., 2013). As a rule, the pH of spray solutions is associated with an incompatibility between the used chemicals (RAJASEKAR and MALLAPUS, 2017). In this study, however, the pH of insecticides was not influenced by adding the acaricide. The pH ranged little in mixtures if compared to the insecticides alone, but still within the expected for a good insecticide performance (from 3.5 to 6.0) (Petroff). This might have been the reason for the lack of physical incompatibility between the tested chemicals.

Electrical conductivity can be altered in insecticides according to spray formulation, which may reduce the biological efficacy of the chemical (RHEINHEIMER and SOUZA, 2000). Nevertheless, both the pH and electrical conductivity of mixtures remained similar to that of insecticides alone. Therefore, the addition of spiroticlofen to lambda-cyhalothrin, phosmet, and imidacloprid has no influence on the pH nor electrical conductivity of the studied spray formulations.

Above all, the determinations of pH and electrical conductivity are important as they influence application efficiency and accelerate pesticide degradation (RAJASEKAR and MALLAPUS, 2017). In addition, the dissociation constant of many chemicals depends on pH, what further influenced the uptake by plant tissues (CUNHA et al., 2017).

We found that imidacloprid efficiency was affected by adding spiroticlofen to spray formulation. By mixing the products, chemical changes and rearrangement of molecules may have occurred, which might have altered insecticide toxicity. Additionally, pesticide mixtures can also modify the activity of enzymes in the target organism, which are responsible for metabolic detoxification and, consequently, reducing the expected insecticide toxicity (REFFSTRUP et al., 2010).

Table 1. Percentage of observed and expected (mean \pm standard error) efficiency in control of *Diaphorina citri* and chi-square value (χ^2) of combination of spiroticlofen with lambda-cyhalothrin + thiametoxam, phosmet and imidacloprid 24 and 48 hours after application (Experiment A).

Treatments	Measurement ¹	24 hours		48 hours	
		Mortality	χ^2	Mortality	χ^2
Spiroticlofen + Lambda cyhalothrin + thiametoxam	Observed	97.5 \pm 2.50	0.06	98.57 \pm 1.42	0.00
	Expected	99.97 \pm 0.00		99.92 \pm 0.00	
Spiroticlofen + Phosmet	Observed	93.42 \pm 2.11	0.16	98.47 \pm 1.53	0.05
	Expected	97.35 \pm 2.62		99.92 \pm 0.00	
Spiroticlofen + Imidacloprid	Observed	93.32 \pm 2.11	0.44	93.32 \pm 2.11	0.44
	Expected	99.97 \pm 0.00		99.92 \pm 0.00	

¹Observed = visualized efficiency of insecticide and acaricide applied in combination; expected = sum of the efficiency of each product applied separately.

Table 2. Percentage of observed and expected (mean \pm standard error) efficiency in control of *Diaphorina citri* and chi-square value (χ^2) of combination of spiroticlofen with lambda-cyhalothrin + thiametoxam, phosmet and imidacloprid 24 and 48 hours after application (Experiment B).

Treatments	Measurement ¹	24 hours		48 hours	
		Mortality	χ^2	Mortality	χ^2
Spiroticlofen + Lambda cyhalothrin + thiametoxam	Observed	96.0 \pm 4.00	0.02	100.00 \pm 0.00	0.00
	Expected	97.33 \pm 2.67		99.98 \pm 0.00	
Spiroticlofen + Phosmet	Observed	93.33 \pm 5.16	0.07	97.15 \pm 2.85	0.07
	Expected	96.00 \pm 2.67		99.98 \pm 0.00	
Spiroticlofen + Imidacloprid	Observed	92.00 \pm 4.90	0.64	95.73 \pm 2.85	0.18
	Expected	100.00 \pm 0.00		99.98 \pm 0.00	

¹Observed = visualized efficiency of insecticide and acaricide applied in combination; expected = sum of the efficiency of each product applied separately.

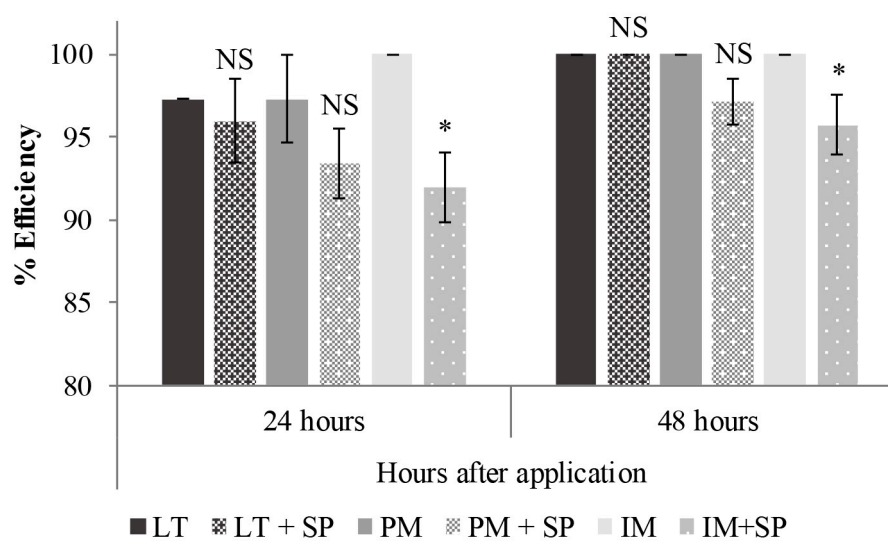


Figure 1. Efficiency (mean \pm standard error) of lambda-cyhalothrin + thiametoxam (LT), phosmet (PM) and imidacloprid (IM), alone and in combination with spiroticlofen (SP) against *Diaphorina citri* (experiment A).

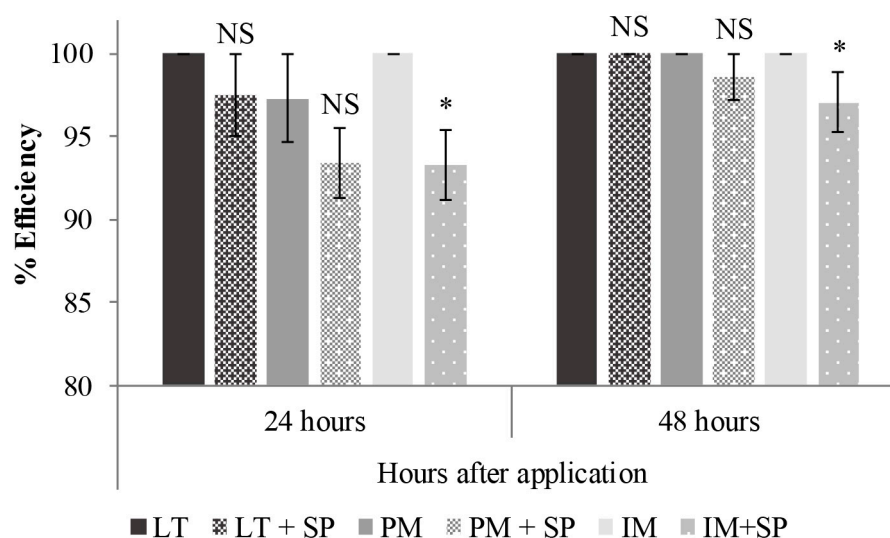


Figure 2. Efficiency (mean \pm standard error) of lambda-cyhalothrin + thiametoxam (LT), phosmet (PM) and imidacloprid (IM), alone and in combination with spiroticlofen (SP) against *Diaphorina citri* (experiment B).

Conclusions

Lastly, the addition of spiroticlofen to lambda-cyhalothrin+thiamethoxam and phosmet insecticides caused no interference for control *D. citri*; however, it compromises the efficiency of imidacloprid. According to our findings, further studies are required to understand how such antagonistic interaction between imidacloprid and spiroticlofen occurs. Another important point is verifying whether acaricide sprays before insecticides also results in such interaction.

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