Do combinations of insecticides and acaricides influence spray droplet formation and the interaction with citrus leaves?

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Abstract
In agriculture, the mixing of pesticides in tanks is a common practice. However, it is necessary to previse possible physical-chemical implications of this practice, which may affect the efficiency of the treatments performed. Therefore, the objective of this study was to evaluate the effects of the addition of acaricide to insecticidal spray mixtures on the formation of spray droplets and the interaction with citrus leaves. The experimental design was totally randomized, in a (2 × 3 + 1) factorial scheme for seven treatments. Factor A corresponded to the spray mixture used (isolate or in the mixture). Factor B corresponded to the insecticides tested (lambda-cyhalothrin + thiamethoxam, phosmet, and imidacloprid) and the control consisted of a spray mixture with spirodiclofen only. Nine replications were performed for characterization of the spray droplet size spectrum and four replications for the analysis of the surface tension and the contact angle. The mixture of pesticides showed positive results in terms of application safety. The addition of acaricide to insecticide spray mixtures reduced the surface tension and contact angle of droplets on the adaxial surface of orange leaves. There was an increment in volume median diameter (VMD), a significant reduction in the volume of droplets with drift-sensitive size and improvement in the uniformity of droplet size. Therefore, the addition of acaricide to an insecticide spray mixture positively influenced spray droplet formation and the interaction with citrus leaves providing better coverage and droplet size fractions with an appropriate size for safe and efficient application.

Keywords: contact angle, coverage efficiency, droplet size, surface tension, tank mixture

Introduction
Citrus crops face phytosanitary problems related to the occurrence of disease vectors (insects and mites), which lead to reduced productivity and longevity of orchards, and depreciation of fruit quality. Among vector-borne diseases, citrus leprosis and HLB-Huanglongbing, transmitted by the mites Brevipalpus spp. and by the psyllid Diaphorina citri Kuwayama, 1908 (Bastianel et al. 2010; Bassanezi et al. 2013) are responsible for the greater demand for phytosanitary treatments and the consequently higher citrus production costs (Neves et al. 2002; Belasque Jr. et al. 2010; Van Leeuwen et al. 2015).

The application of pesticides by spraying is the main strategy adopted by farmers to keep the population of these vectors at low densities and the mixture of products is a common practice since these pests can occur at the same time in the field (Parra et al. 2010; Laranjeira et al. 2015). However, the combination of pesticides in a tank-mix can modify the physicochemical characteristics of the spray mixture and affect the values of the surface tension, pH, electrical conductivity and spray mixture stability. These parameters can, in turn, influence the efficacy of phytosanitary treatments (Maciel et al. 2010; Petter et al. 2013).
The physicochemical properties of the spray mixture can significantly change the process of droplet formation by changing their diameter and uniformity in size (Marmottant and Villermaux 2004), which influences the destination of droplet deposit. The droplet size is significant regarding susceptibility to drift and evaporation, penetration capacity, in addition to the deposit, spreading of the droplets, and distribution on the targets (Prokop and Kejklicek 2002; Andrade et al. 2010).

Studies on the interference of the physicochemical properties of the tank-mix on the formation, transport, and deposit of droplets on targets are essential because these investigations can allow for the evaluation of the efficiency and safety of the application (Hewitt 2008). Concerns about the interactions between products in sprayer tanks have been increasing among farmers, but there is a lack of information about associated consequences. Therefore, there is a gap between the stages of development and appropriate usage.

The objective of this work was to evaluate the effects of the addition of acaricide to insecticidal spray mixtures on the surface tension, droplet spectrum characteristics, as well as the contact angle of the droplets on the surface of citrus leaves.

Materials and Methods

The work consisted of three complementary experiments based on the analysis of surface tension, the contact angle of droplets on the surface of orange leaves [Citrus sinensis (L.) Osbeck] and the characterization of the spray droplet spectrum.

The spray liquids consisted of lambda-cyhalothrin + thiamethoxam [15 ml · 100 l−1 (Engeo® Pleno SC – Syngenta, India and Huddersfield, England)], phosmet [150 g · 100 l−1 (Imidan® 500 WP – Cross Link, Brazil)], imidacloprid [20 ml · 100 l−1 (Provado® 200 SC – Bayer CropScience AG, Germany)] and the acaricide spiromitrofen [25 ml · 100 l−1 (Envidor® 240 SC; Bayer CropScience AG, Germany)], as a control. The spray mixtures were prepared with deionized water at an ambient temperature of 25 ± 2°C and a relative humidity of 65 ± 5%.

The experiments were performed in a completely randomized design in a 2 × 3 + 1 factorial scheme, with nine repetitions for the characterization of the spray droplet spectrum and four repetitions for the analyses of surface tension and contact angle. Factor A corresponded to the spray mixture used (insecticides isolated or mixed with acaricide). Factor B corresponded to the insecticides tested (lambda-cyhalothrin + thiamethoxam, phosmet, and imidacloprid) and the control consisted of a spray mixture with only spiromitrofen.

The mix of products was performed in the following order of preparation: water addition + solid formulation addition + liquid formulation addition, following NBR 13875 published in ABNT (2015).

Surface tension and droplet contact angle

Measurements of both parameters were performed in the Nucleus of Study and Development at the Technology of Application laboratory with an automatic tensiometer (OCA-15 Plus, Dataphysics Germany), following the methodologies of Decaro JR et al. (2015) and Ferreira et al. (2013). For surface tension measurements, the droplets were formed at the end of a needle coupled to a precision syringe of 500 µl, which was evaluated by the pendant drop method. In this approach, a CCD (charge-coupled device) high speed, high definition camera was used to capture images, and software was used to analyze the drop image by axis asymmetry (ADSA – Axisymmetric Drop Shape Analysis). The surface tension was determined using the Young-Laplace equation. The 20 sec data generated after drop formation was considered to characterize the tested spray mixture with respect to the inflection point of the dynamic surface tension curve. The droplet volume used during the surface tension measurement was 4 µl.

To determine the contact angle, droplets were deposited on the surface of orange leaves. In the process of acquiring droplet contact angles, longitudinal 5 × 1 (cm) rectangles of orange leaves were cut and fixed in a press so that any undulations on the analyzed surface would not interfere with the readings. The droplet volume used during the measurements of the contact angle was 2 µl. The evaluation of surface tension and droplet contact angle was carried out in a room with a controlled ambient temperature of 25 ± 2°C and relative humidity of 65 ± 5%.

Characterization of the spray droplet spectrum

To perform spraying, a hollow cone spray nozzle was used (TXA 8002, Teejet®). Droplet size spectrum analysis was performed directly based on a laser beam diffraction method using a particle sizer (Mastersizer S®, Malvern Instruments Co.), well described by Griesang et al. (2017). It is based on the diffraction of light by the droplets, wherein the droplet diameter is inversely proportional to the angle of deviation of the light (Fernandes et al. 2007).

The spray nozzle was installed 40 cm away from the laser beam and the spray was driven by compressed air at a pressure of 0.5 MPa, which was held constant with a precision air pressure regulator. The spray liquids were prepared with deionized water in an
ambient temperature of 25 ± 2°C and relative humidity of 65 ± 5%. The ambient conditions during the experiments were: an air temperature of 26°C, a relative humidity of 55% and the absence of wind.

To characterize the droplet size spectrum, the values of the volume median diameter (VMD), the percentage of the volume with droplet diameters less than 100 μm (V < 100) and the uniformity of the droplets (SPAN) were used. The SPAN was obtained from the droplet sizes that related to the spray volume at 10, 50 and 90% (Dv0.1, Dv0.5, and Dv0.9), using the following equation (Malvern 1997):

$$ \text{SPAN} = \frac{Dv0.9 - Dv0.1}{Dv0.5} $$

The SPAN value indicates the variation of the droplet size i.e., the smaller the value, the more uniform the spray droplet spectrum.

### Statistical analysis

The data for the surface tension, contact angle, VMD, V < 100, and the SPAN were subjected to analysis of variance by the F test and the means were compared using the Tukey test (p > 0.05). with the statistical program AgroEstat.

Pearson’s correlation test was performed between the variables of surface tension and contact angle, VMD and between V < 100 and SPAN, at 5% probability.

### Results

#### Surface tension

The addition of acaricide to an insecticide spray mixture reduced the surface tension (F = 88.79; p < 0.0001). There was a statistically significant difference between the insecticides when they were evaluated separately (F = 4.75; p = 0.0198). However, when combined with acaricide, the lambda-cyhalothrin + thiamethoxam insecticide showed a lower surface tension value compared to the others. There was significant interaction between the spray mixture and insecticide factors (F = 7.13; p = 0.0043). Therefore, the factors can be considered interdependent (Table 1). The additional treatment (spirodifenol) differed from the other treatments evaluated (F = 366.14; p < 0.0001), presenting lower values.

#### Droplet contact angle

Differences were observed among the analyzed factors regarding the contact angle of droplets on the adaxial surface of the orange leaves (Table 2). The addition of acaricide to the insecticidal spray mixtures reduced the contact angle of the droplets. Among the insecticides, imidacloprid showed a greater contact angle compared to the others. The contact angle of the control did not differ from other treatments (F = 2.84; p = 0.1068), with a value of 84.09.

The correlation coefficient was significant (p = 0.0279) and positive for the surface tension and contact angle for isolated insecticides (R = 0.6307). However, it was not significant (p = 0.6565) for the mixture of insecticide and acaricide (R = 0.1434). The addition of acaricide to insecticides reduced the dependence between factors.

#### Characterization of the spray droplet spectrum

Both the VMD, the V < 100, and the SPAN were influenced by the spray mixture constitution. The VMD values of the droplets presented significant differences for isolated insecticide spray mixtures or in a mixture with spirodifenol (F = 53.24; p < 0.0001), and for the insecticides used (F = 16.02; p < 0.0001). The combination of insecticide and acaricide resulted in increases in the VMD value, except for insecticide imidacloprid (Fig. 1). For the insect-only spray mixture, imidacloprid presented the highest value of VMD. Among the insecticides combined with acaricide, phosmet presented a higher VMD value (134.57 μm). It was observed that the interaction between the evaluated factors was

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>Spraying liquid</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>alone</td>
<td>mixture</td>
</tr>
<tr>
<td>Lambda-cyhalothrin + thiamethoxam</td>
<td>72.26 aA</td>
<td>67.42 bB</td>
</tr>
<tr>
<td>Phosmet</td>
<td>71.90 aA</td>
<td>70.33 bA</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>72.91 aA</td>
<td>68.96 bAB</td>
</tr>
</tbody>
</table>

LSD line (5%) 1.32; LSD column (5%) 1.60; CV (%) 1.29

Means followed by different lowercase letters in the lines and upper case in the columns differ significantly from each other by the Tukey’s test (p > 0.05).

LSD – least significant difference, CV – coefficient of variation
significant \( (F = 14.83; p < 0.0001) \). The additional treatment did not differ from the other treatments, with the value 126.83 μm \( (F = 1.31; p = 0.2567) \).

The \( V < 100 \) differed between the isolated and mixed products \( (F = 61.90; p < 0.0001) \) and between the different insecticides \( (F = 22.55; p < 0.0001) \). There were significant differences in the interaction between the evaluated factors \( (F = 7.27; p = 0.0016) \) (Fig. 1).

The spray mixture formed by the insecticide only presented a higher percentage of drops smaller than 100 μm in relation to the spray mixture combined with acaricide. Regardless of the type of spray mixture for the different insecticides, lambda-cyhalothrin + thiamethoxam presented a higher \( V < 100 \). The additional treatment (acaricide control) did not differ from the other treatments \( (F = 3.66; p = 0.0609) \), with 26.42% of \( V < 100 \).

The uniformity of the droplet size spectrum was altered by the presence of acaricide \( (F = 53.51; p < 0.0001) \) and by the different insecticides evaluated \( (F = 41.13; p < 0.0001) \) and the results resembled those obtained for the variable \( V < 100 \). There was significant interaction between factors A and B \( (F = 5.68; p = 0.0057) \). Among the insecticides evaluated, the droplet size spectrum generated by lambda-cyhalothrin + thiamethoxam presented the least uniform droplet spectrum regardless of the type of spray mixture. The uniformity of the additional control (acaricide) differed from the other treatments \( (F = 6.11; p = 0.0165) \), presenting a value of 0.95.

### Table 2. Analysis of variance and tests of significance for the contact angle (degree) formed by drops applied on the adaxial surface of orange leaves in a function of different factors (spray mixture and insecticides)

<table>
<thead>
<tr>
<th>Causes of variation</th>
<th>( F )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spraying liquid (A)</td>
<td>5.71*</td>
<td>0.0263</td>
</tr>
<tr>
<td>Insecticides (B)</td>
<td>24.44**</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>A vs. B</td>
<td>0.38 ns</td>
<td>0.6856</td>
</tr>
<tr>
<td>Control vs. Factorial</td>
<td>0.34 ns</td>
<td>0.5664</td>
</tr>
<tr>
<td>CV</td>
<td>4.73</td>
<td></td>
</tr>
<tr>
<td>Spray mixture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alone</td>
<td>84.77 a</td>
<td></td>
</tr>
<tr>
<td>Mixture</td>
<td>80.94 b</td>
<td></td>
</tr>
<tr>
<td>LSD (S%)</td>
<td>3.33</td>
<td></td>
</tr>
<tr>
<td>Insecticides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lambda-cyhalothrin + thiamethoxam</td>
<td>78.74 b</td>
<td></td>
</tr>
<tr>
<td>Phosmet</td>
<td>79.05 b</td>
<td></td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>90.78 a</td>
<td></td>
</tr>
<tr>
<td>LSD (S%)</td>
<td>4.95</td>
<td></td>
</tr>
</tbody>
</table>

Means followed by * or ** differ significantly from each other by the Tukey test, \( p > 0.01 \) or \( p > 0.05 \), respectively. Means followed by different lowercase letters in the columns differ significantly from each other by the Tukey test \( (p > 0.05) \). LSD – least significant difference; CV – coefficient of variation; ns – not significant.
The correlation coefficient was significant and negative between VMD and V < 100 for both isolated insecticides (p = 0.0143; R = −0.6831) and the insecticide and acaricide mixture (p = 0.011; R = −0.7012).

The Pearson correlation coefficient was significant (p = 0.0206) and positive for the isolated insecticides (R = 0.6559) between the parameters V < 100 and SPAN, and there was no significant correlation between the insecticide and acaricide (p = 0.0947; R = 0.5041).

Discussion

Surface tension and droplet contact angle

The mixing of pesticides in tanks intended for different uses is a current practice which is mainly used to save operational resources. However, it is necessary to know the possible physical, chemical and biological implications of this practice that could compromise the efficacy of the treatments performed (Andrade et al. 2013).

In general, we observed that the spray mixture surface tension was reduced with the addition of the acaricide, from 72.91 to 67.42. This is an indication of the spreading of droplets on the tested leaves and the greater coverage of the leaf’s surface. A positive correlation was recorded as has been observed in other research (Yu et al. 2009; Decaro JR et al. 2014). This effect may be related to the modification of the molecular orientation in the active ingredients, or the interaction of the liquid and the leaf’s surface (lost and Raetano 2010).

For insecticide spraying of only the tested mixtures, the values were similar to that obtained for water (74 mN·m⁻¹). This implies that this kind of spray mixture has high surface tension values and the droplets tend to maintain high contact angles on the surface where they settle. When the contact angle exceeds 90°, the surface is considered as hydrophobic, whereas lower values characterize the surface as hydrophilic (lost and Raetano 2010). The constituents of the leaf’s epidermis have a significant influence on this spreading, and when the interfacial tension (spray mixture-leaf) is greater than the surface tension of the liquid, it results in greater spreading (lost and Raetano 2010).

In a field application, high surface tensions and contact angles would imply a lower potential for coverage of the target. This would require an increase in the application volumes or the use of smaller droplets. Considering that orange crop plants usually require coverage of a large leaf surface, the volume requirement is already high. For targets of low mobility and difficult access to spraying, such as Brevipalpus spp., mites, the use of spray mixtures with high surface tensions and contact angles can, therefore, result in more expensive applications (Decaro JR et al. 2015).

Thereby, the adoption of alternatives that improve the coverage of the treated surface, such as the use of adjuvants in the spray mixtures as the products evaluated in this study, can contribute to the satisfactory coverage of the targets without the need to increase the volume of application. This characteristic can have direct implications on the costs of phytosanitary treatments due to the operational performance of the sprayer by using less water per area unit. However, the costs of the adjuvants should not be greater than the operational costs provided by the alternative of application with higher volumes. In addition, alternatives that modify the characteristics of the spray mixture may result in a reduction of the expected biological effect for the control of the targets.

Characterization of spray droplet size

The composition of phytosanitary spray mixtures based on multiple components can influence the viscosity and surface tension of the spray mixture by acting on the internal structure of the jet, with an overall effect on the size of the droplets formed (Butler-Ellis et al. 2001). The addition of acaricide to the insecticidal spray mixtures resulted in increases of VMD values, SPAN reduction and %vol < 100 μm with combinations, compared to isolated products. Generally, the increase in droplet size leads to less coverage of leaves in the field. However, with the increased spread of the droplets and the reduction in the volume of droplets susceptible to drift, the combination of products can produce positive results.

The uniformity of the droplet size spectrum determined with SPAN can be altered based on the composition of the spray mixture (Costa et al. 2017). In this way, the addition of acaricide to the insecticides can facilitate a more homogenous droplet size spectrum. According to the values obtained for the Pearson correlation coefficient, there was a negative correlation between the VMD and V < 100 factors for both the isolated insecticides and the mixture with the acaricide. Therefore, with an increase of the VMD values, there was a decrease in the V < 100 (Baesso et al. 2014; Costa et al. 2017). An increase of the VMD or a reduction of the amount in droplets smaller than 100 μm can result...
in less drift risk (Dorr et al. 2013; Oliveira et al. 2015). This can, in turn, reduce the risk of contamination in adjacent areas.

A positive correlation was found for the $V < 100$ and SPAN factors, meaning that a more uniform droplet size spectrum leads to a reduction in $V < 100$. Considering that droplets are classified according to sizes into several classes (ASABE 2009), the very fine class can suffer significant spray losses due to drift and evaporation. Droplets with a size greater than those recommended for the application can be lost by a ricochet and superficial runoff. Obtaining a jet of spray mixture with droplets of uniform size spectrum means lower losses (Ferreira et al. 2013). It is worth mentioning that the use of the class of fine droplets is recommended when greater coverage and penetration is required in the canopy of plants, as well as those produced in this research, and that classes of medium or coarse droplet sizes are better for application under conditions of greater risk of drift (Cunha et al. 2007; De Oliveira and Antuniasi 2011; Almeida et al. 2014, 2016; Triloff et al. 2014). However, little is known about the drop diameter ratio that provides coverage of a leaf’s surface that results in effective pest control. The ideal coverage for the response to biological phenomenon will also depend on the interactions of the molecules and the formulation of the product with the target (Almeida et al. 2016).

**Conclusions**

The combinations of tested products showed notably positive results on the safety of application. A significant reduction of the drift susceptible fraction of droplets and an increase in the VMD value were observed. In addition, the improvement in the uniformity of the droplet size spectrum is worth mentioning, because it resulted in a larger fraction of droplet uniformity with the appropriate size for the application. In addition, a decrease in surface tension improved spreading of the drops on the target surface, resulting in increased coverage values and consequently greater control of biological targets.

**References**


