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IMPACT OF RAINFALLS ON THE ACARICIDE PROPARGITE WITH AND WITHOUT ADDITION OF ADJUVANTS FOR THE CONTROL OF THE MITE *Brevipalpus yothersi*

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Abstract-Rainfalls may affect the efficiency of pesticides due to a decreased deposit of active ingredient on plants. This should be considered in spraying with acaricides, the main strategy used for the management of citrus leprosis, the main viral disease of the Brazilian citrus culture. Citrus leprosis disease is transmitted by *Brevipalpus* mites, mainly *B. yothersi*. The objective of this study was to evaluate the effects of artificial rains on the efficiency of the acaricide propargite in combination or not with adjuvants for the control of the mite *B. yothersi*. The experiment was conducted using orange plants grown in pots kept under greenhouse conditions. The acaricide propargite was evaluated isolated and with addition of the adjuvants polydimethylsiloxane (20mL cp/100 L of water) and a mixture of phosphatidylcholine (lecithin) and propionic acid (500 mL cp/100 L of water). After the application of the acaricide with and without the adjuvants, the plants were subjected to a 10-mm artificial rainfall at different time intervals: 5 minutes, and 1 and 12 hours after application. Thereafter, *B. yothersi* mites were transferred to plants for survival evaluation. The artificial rainfall affected negatively the efficiency of propargite isolated and in mixture. The addition of the adjuvant phosphatidylcholine and propionic acid impaired the efficiency of propargite due to an increased runoff of the broth and a decrease in the deposit of active ingredients on plants. We conclude that the acaricide propargite should not be used in combination with the adjuvants polydimethylsiloxane and mixture of phosphatidylcholine and propionic acid in rainy conditions.

Terms for indexation: Citrus leprosis disease, *Citrus sinensis*, CiLV, *Brevipalpus phoenicis, Spray deposition*.

IMPACTO DA CHUVA SOBRE O ACARICIDA PROPARGITE COM E SEM ADIÇÃO DE ADJUVANTES NO CONTROLE DO ÁCARO *Brevipalpus yothersi*

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Resumo-Chuvas podem afetar a eficiência dos agrotóxicos devido à redução do depósito de ingrediente ativo sobre as plantas. Esse é um fator que deve ser considerado nas pulverizações com acaricidas, principal estratégia utilizada no manejo da leprose-dos-citros, principal doença viral da citricultura brasileira. A leprose-dos-citros é transmitida por ácaros *Brevipalpus,* com destaque para *Brevipalpus yothersi*. O objetivo do trabalho foi avaliar o efeito de chuva artificial sobre a eficiência do acaricida propargite em mistura ou não com adjuvantes no controle do ácaro *B. yothersi*. O experimento foi conduzido utilizando plantas de laranja cultivadas em vasos e mantidas em casa de vegetação. O acaricida propargite foi avaliado isolado e com adição dos adjuvantes polidimetilsiloxano (20mL p.c./100L de água) e mistura de fosfatidilcoline (lecitina) e ácido propiônico (500mL p.c./100L de água). Após a aplicação do acaricida com e sem os adjuvantes, as plantas foram submetidas a chuva artificial de 10 mm, em diferentes intervalos de tempo, a 5 minutos, 1 e 2 horas após a aplicação. Em seguida, foram transferidos para as plantas ácaros *B. yothersi* para avaliação da sobrevivência. A chuva artificial afetou negativamente a eficiência do propargite aplicado isolado e em mistura. A adição do adjuvante fosfatidilcoline e ácido propiônico prejudicou a eficiência do propargite, devido ao maior escorrimento da calda e à redução do depósito de ingrediente ativo sobre as plantas. Conclui-se que o acaricida propargite não deve ser utilizado em associação com os adjuvantes polidimetilsiloxano e mistura de fosfatidilcoline e ácido propiônico em condições de chuva.

Termos para indexação: Leprose-dos-citros, *Citrus sinensis*, CiLV, *Brevipalpus phoenicis*, Depósito de calda.

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Introduction

Citrus leprosis disease is the major virus disease in citrus culture in Brazil, the largest producer and exporter of orange juice in the world (LARANJEIRA et al., 2015). The virus causing the disease (CiLV- *Citrus leprosis virus*) is transmitted by mites of the genus *Brevipalpus* (BASTIANEL et al., 2010; RODRIGUES and CHILDERS, 2013). The symptoms of citrus leprosis appear in leaves, branches and fruits (BASTIANEL et al., 2010). Symptomatic branches dry gradually, leading to plant death; in fruits, the symptom is characterized by concentric maroon spots usually surrounded by a yellowish halo, resulting in premature fruit drop (BASTIANEL et al., 2010; LARANJEIRA et al., 2015).

Until 2015, *Brevipalpus phoenicis* (Geijskes) (Acari: Tenuipalpidae) was mentioned as the only vector species of citrus leprosis in Brazil. However, by using microscopy and molecular biology techniques, it was verified that the main vector of citrus leprosis in Brazil actually is *Brevipalpus yothersi* Baker (Acari: Tenuipalpidae), a species confused with *B. phoenicis* for several decades in Brazil (BEARD et al., 2015, ROY et al., 2015). Mineiro et al. (2015) stated that *B. yothersi* is the predominant species in commercial citrus orchards in the state of São Paulo.

The control of the mite-vector by spraying of acaricides has been the main strategy to manage the disease in orchards (KITAJIMA et al., 2010; ANDRADE et al., 2013). Among the acaricides used, propargite stands out because it has been frequently used by citrus growers for many years. However, sprays are often inefficient, resulting in vector control failures. Several factors are related to spraying inefficiency, especially rainfall, which generally negatively affects the efficiency of pesticides due to a reduction in product deposition on plants (PICK et al., 1984; GAUTAM et al. 2016).

Several authors reported negative effects of rainfalls after spraying of pesticides (DINARDO-MIRANDA et al., 2004; BRÉVAULT et al., 2009, GASKIN and STEELE, 2009; PANNACCI et al., 2010; ANDERSON et al., 2014; BORGES et al., 2015; DECARO et al., 2016). A study conducted by Gautam et al. (2016) showed that artificial rainfalls reduced the efficiency of insecticides against the fly *Drosophila suzukii* Matsumura (Diptera: Drosophilidae). Pree et al. (1984) found that 15-18 mm artificial rainfalls were sufficient to reduce the deposition of the insecticide phosmet on peach plants by 20-30%.

In order to reduce the harmful effects of rainfalls on citrus fruits, it is common to use adjuvants, mainly oils (vegetable and mineral) and adhesive spreaders, aiming to increase or maintain the efficiency of sprays (OLIVEIRA et al., 1997; FOUNTAIN et al. 2010). A number of adjuvants act by reducing surface tension and increasing leaf spreading and leaf penetration (OLIVEIRA et al., 1997; RYCKAERT et al., 2007; SASAKI et al., 2015). Furthermore, adjuvants may be used to minimize the effects of rain on the deposition of the plant protection product (OLIVEIRA et al., 1997; FOUNTAIN et al., 2010).

Some adjuvants are used to reduce the impact of rainfalls on the active ingredient, e.g., by increasing the penetration of the active ingredient into the plant tissue (OLIVEIRA et al., 1997; RYCKAERT et al., 2007; SASAKI et al., 2015; FOUNTAIN et al., 2010). In citrus cultivation, several adjuvants are used. They belong to groups of activating adjuvants acting to improve the activity of the pesticide, and useful adjuvants, which act as a facilitator of the spraying process (STOCK and BRIGGS, 2000; OLIVEIRA and ANTUNIASSI, 2011). Conceptually, Hazen (2000) emphasized that adjuvants change the chemical and/or physical properties of the broth, improving the performance of the applied phytosanitary product in mixture.

In the literature, there are studies that have shown positive and negative effects of the addition of adjuvants on the spray broth (FOUNTAIN et al., 2010; HUNSCHE et al., 2011; ARRUÉ et al., 2014; OLIVEIRA et al., 1997; OLIVEIRA et al., 2003; ANDRADE et al., 2010; FERREIRA et al., 2013; MELO et al., 2015). Such differences are attributed to intrinsic properties of pesticides in relation to mixtures with adjuvants (PICK et al., 1984; ARRUÉ et al., 2014). Gautam et al. (2016) found that the addition of the adjuvant Nu Film P (Nu Film 17®) contributed to increase the residual effects of some insecticides, while for others there was a decrease or no changes in the residual effect.

Among the main adjuvants used in Brazilian citriculture, surfactants are highlighted. These products act by reducing the surface tension, facilitating or improving emulsification, dispersion, wetting and adhesion of phytosanitary product molecules on plants (KIRKWOOD et al., 1999; OLIVEIRA and ANTUNIASSI, 2011). Therefore, the addition of adjuvants of the surfactant category to acaricides, widely used in Brazilian citriculture, may increase the efficiency of applications, contributing to reduce losses caused by citrus leprosis disease. The objective of this study is to evaluate the influence of artificial rains on the efficiency of the acaricide propargite with and without the addition of adjuvants for the control of *B. yothersi*.

Materials and methods

Brevipalpus yothersi's colony

The colony of *B. yothersi* began with mites being collected in a citrus orchard located at the Faculty of Agrarian and Veterinary Sciences, UNESP, campus of Jaboticabal, state of São Paulo, Brazil (FCAV/UNESP). To confirm the species, some specimens were mounted on optical microscopy slides in Hoyer's medium, as proposed by Krantz and Walter (2009). The mites were examined using a Zeiss microscope Axion Imager A2 with phase contrast (Beard et al., 2015). The collected mites were multiplied in the laboratory using orange fruits cv. Valencia *Citrus sinensis* (L.), and kept in a laboratory at 25 ± 1°C, relative humidity of 70 ± 10%, and photoperiod of 14 h (CHIAVEGATO, 1986).

Experimental design

An experiment was conducted in a greenhouse to evaluate the addition of adjuvants and artificial rainfalls on the acaricide propargite (Acarit 720 EC®, 100 mL/100L Millenia Agro Sciences S/A). We used seedlings of cv. Valencia oranges with approximately one year grafted using Swingle citrumelo [*Poncirus trifoliata* (L.) Raf. x *Citrus paradisi* Macfad.]. The seedlings were planted in 5-L pots containing 2:1:1 of soil, sand and cattle manure substrate. Three plants were used per treatment.

The experiment was installed in randomized complete blocks using a 3 x 3 factorial design (adjuvant x artificial rainfall time after application) and an additional treatment with the application of the acaricide propargite. The first factor was composed of levels without addition of adjuvants, with addition of polydimethylsiloxane (Vertex Premium RS®, 20 mL/100 L Fertec), and the mixture phosphatidylcholine (lecithin) and propionic acid (LI-700®, 500 mL/100 L Loveland Products). The second factor (artificial rainfall) was a 10-mm water slide applied 5 minutes, 1 hour and 12 hours after the application of the acaricide.

Application of products

The application of the acaricide with and without adjuvants was carried out with a pressurized costal spray at 3 bar equipped with a bar composed of two TLX2 Teejet® tips. The broths were sprayed on orange seedlings up to beyond the point of drainage, considering the broth retention capacity of the plant.

Artificial rainfall

In order to carry out the artificial rainfall, a circular platform equipment was installed 2.9 meters high inside the greenhouse. The equipment consisted of six spray nozzles (model FL10 TeeJet®) which, at the pressure used, produced droplets with a mean diameter of > 500 μm, considered as extremely thick (ASAE, 2000). Nozzles were installed 0.25 meters apart on the circular platform. To perform the artificial rainfall of 10-mm, the equipment was adjusted to operate at a pressure of 137.8 kPa for 6.25 minutes. The droplets provided a wet area of approximately 1.75 cm^2 upon reaching the soil.

Transfer of mites

Immediately after the application of the products and the artificial rainfall in the greenhouse, the plants were taken to the laboratory. After complete drying the water over the plants, two leaves of each plant were marked, demarcating a leaf area of 2.5 cm^2 in diameter surrounded with entomological glue (Tanglefoot®) to avoid the escape of mites. In each area, ten stereoscopic adult females of *B. yothersi* from the laboratorycolony were placed under a microscopy stereomicroscope. Mite mortality assessments were performed at 1, 3 and 5 days after transfer (DAT).

Statistical analysis

The data obtained were transformed into arc sen √efficiency(%)/100 for normalization and subjected to analysis of variance (ANOVA). The means were compared by Tukey test at 5% probability using the software *Agroestat* (BARBOSA and MALDONADO, 2015). The percentages of mortality (Efficiency) were calculated using the Abbott's formula (1925), and a control (without application and without artificial rainfall) was used as reference.

Results and discussion

The adjuvant factors and the waiting time for artificial rainfall were significant for both evaluations carried out after the transfer of mites (Table 1). However, the interaction between factors was significant only at 3 days after transfer of mites (DAT), after the applications (Table 1). In the first evaluation, we observed that the treatments differed from the additional treatment only with the application of propargite, indicating that the factors directly interfered with the effects of the acaricide on mites (Table 1). The treatment with propargite presented a mean control percentage of 97.2%, while the means of the other treatments was 60.4%.

Among the adjuvants, the mixture of phosphatidylcholine and propionic acid (LI-700) reduced the most the efficacy of propargite (Table 1). There was a significant decrease in treatment efficiency with the phosphatidylcholine + propionic acid treatment in both evaluations (Table 1). On the other hand, the adjuvant polydimethylsiloxane did not affect the control efficiency of the acaricide ($p<0.05$) when compared to the treatment without addition of adjuvants (Table 1). For this adjuvant, the 1-hour waiting time between the application and the 10-mm artificial rainfall reduced the mean control percentage of mites (Figure 1). Therefore, the lowest efficiency was verified for the treatment using the adjuvant phosphatidylcholine mixed with propionic acid and the 1-hour waiting time (Figure 2).

Hypothetically, the adjutants promoted less deposit of active ingredient since they increased the dispersion of the broth and reduced the diameter of droplets (SASAKI et al., 2015). Adjuvants are used to increase the efficiency of the phytosanitary product, acting as a reducing agent of surface tension of spray droplets and/or increasing the viscosity of the liquid (CUNHA and ALVES, 2009; CUNHA et al., 2010). However, adjuvants may reduce broth deposit in leaves (OLIVEIRA et al., 1997, DECARO et al., 2016). The decrease in the surface tension causes droplets to scatter better, but they aggregate on the leaf surface, forming larger droplets that flow away and increasing the loss process (OCAMPO-RUIZ and MATUO, 1994).

The decrease in surface tension was correlated with the decrease in the deposit of broth according to a study conducted by Decaro et al. (2016) for the same treatments used in this study. In field conditions, Oliveira et al. (1997) reported that there were no differences in the retention of broth for the acaricide propargite (Omite 720 BR CE Arysta LifeScience) when associated with the adjuvants since it already has adjuvants in its composition, agreeing with the results found in this study.

The 1-hour waiting time interfered the most with the effects of propargite (Table 1), causing a drastic decrease in the efficiency of the product after a 1-hour waiting time for the artificial rainfall. For the other waiting times (5 minutes and 12 hours), in the first evaluation, the average efficiency was equal to or higher than 74.0%, whereas for the 1-hour time the result was only 33%. In the second evaluation, the control was 100.0% at the times 5 minutes and 12 hours, but in the 1-hour time, it was approximately 82.0%. It became clear that rainfalls after application reduce the efficiency of propargite. Rainfall possibly removed some of the active ingredients deposited on the leaf by washing, affecting the efficiency of the acaricide. Studies on other pests and products presented similar results (MULROONEY and ELMORE, 2000; BRÉVAULT et al., 2009).

The time between application and precipitation may also influence the efficiency of the plant protection product. Similar results were reported by other authors for the control of arthropod pests, diseases and weeds (DINARDO-MIRANDA et al., 2004; BRASVAULT et al., 2009; GASKIN and STEELE, 2009; PANNACCI et al., 2010; ANDERSEN et al., 2014). Most studies indicate that the lower the application time and the rainfall, the lower the deposition of the phytosanitary product. Decaro et al. (2016) reported that the deposition (μg per cm^2) of propargite and spirodiclofen on orange leaves was lower after rainfalls, mainly one hour after spraying, corroborating with the results of this study.

The use of adjuvants in some situations promotes reductions in broth volumes, reducing production costs and increasing the operational capacity (OLIVEIRA et al., 1997). However, an incorrect use of such products may exert adverse effects, reducing spray efficiency. In this study, it was evident that the acaricide propargite, under rainfall conditions, should not be indicated in mixture with the adjuvants polydimethylsiloxane and the mixture of phosphatidylcholine and propionic acid.

Table 1- Percentage of mortality (Efficiency) of *Brevipalpus yothersi* mites alive at 1 and 3 days after transfer (DAT) in the experiment with the acaricide Propargite.

Causes of variation	1 DAT		3 DAT	
	F^2	P	F^2	P
Factor A: adjuvant	6.73**	0.0028	4.76*	0.0133
Factor B: rainfall time	12.89**	< 0.0001	9.05**	0.0005
Interaction adjuvant x rainfall time	0.35^{NS}	0.8446	4.76**	0.0027
Treatment ad. x Factorial	9.81**	0.0031	0.90^{NS}	0.3466
Blocks	0.69^{NS}	0.6317	1.42^{NS}	0.2351
Factor A: adjuvant	**Efficiency (%)**[1]		**Efficiency (%)**[1]	
Without adjuvant	70.04 a		100.00 a	
Polydimethylsiloxane	68.18 a		96.53 ab	
Phosphatidylcholine + propionic acid	43.19 b		85.80 b	
Factor B: rainfall time	**Efficiency (%)**[1]		**Efficiency (%)**[1]	
5 minutes	74.00 a		100.00 a	
1 hour	32.98 b		82.33 b	
12 hours	74.44 a		100.00 a	

Original data transformed into arcsine of Efficiency root (%)/100. Data presented for percentages of mortality are original data. [1]Means followed by the same lowercase letter in rows do not differ by Tukey test at 5% probability. [2]F Test. [3]p-value (level of probability of significance). NS-not significant, (**) significant at 1%, (*) significant at 5% probability.

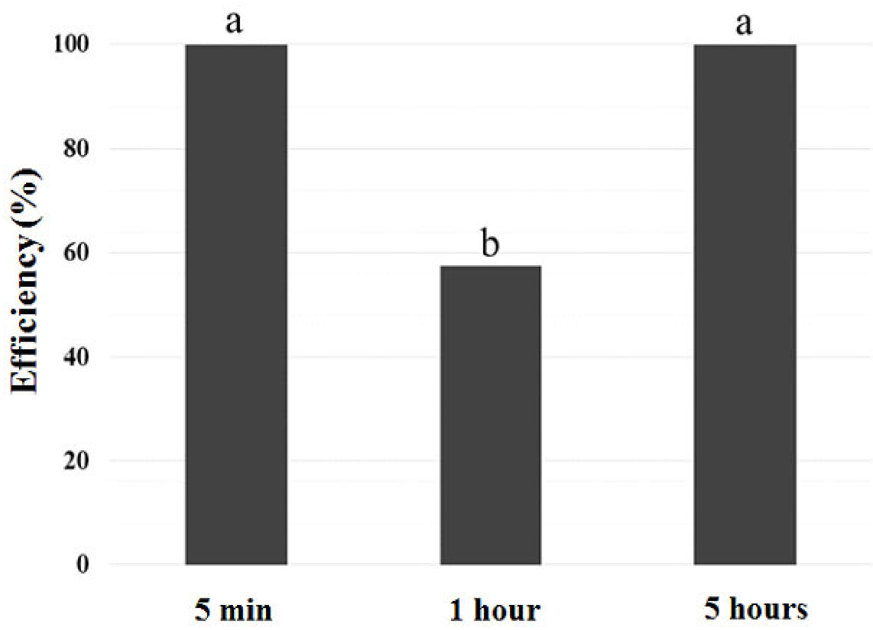

Figure 1- Percentage of mortality (Efficiency) of *Brevipalpus yothersi* mites 3 days after application (3DAT) in treatments with the adjuvant propionic acid for different waiting times of artificial rainfall. Results extracted from the unfolding between the factors adjuvant and waiting times for rainfall.

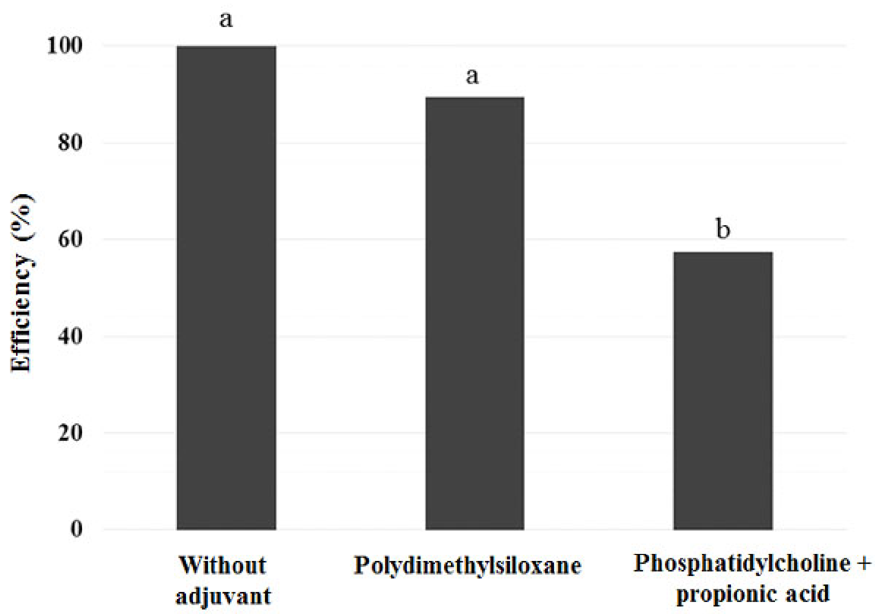

Figure 2- Percentage of mortality (Efficiency) of *Brevipalpus yothersi* mites 3 days after application (3DAT) in treatments with 1-hour rainfall waiting time with or without adjutant. Results extracted from the unfolding between the factors adjuvant and waiting times for rainfall.

Conclusions

The addition of the adjuvant phosphatidylcholine (lecithin) + propionic acid to the acaricide propargite decreased the control efficiency of *B. yothersi.*

The adjuvant polydimethylsiloxane, associated with the acaricide propargite, does not increase the control efficiency of *B. yothersi*.

Rainfalls of 10 mm after the application of propargite isolated or in mixture with the adjuvants negatively affected the control efficiency of the mite *B. yothersi*.

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