Control of the *Candidatus* Liberibacter asiaticus vector with plant extracts and with plant extracts and biorational products in Mexican lime

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Abstract. In Mexico, Huanglongbing HLB has become endemic in all Mexican lime producing areas. This disease is transmitted by the insect *Diaphorina citri* (Hemiptera: Liviidae). The objective of the study was to evaluate different plant extracts and biorational products for the control of *D. citri* in mexican lime. Two evaluations were made under field conditions; the first one included five treatments, with previous sampling and at 8, 20 and 27 days after the application of the treatments. In the second, eight treatments were evaluated, with prior sampling and at 6, 21 and 27 days. The response variable was the number of *D. citri*. A completely randomized experimental design with ten repetitions was used. A test of normality and homogeneity of variances was applied to the data, and they were processed through an analysis of variance and separation of means using Tukey ($p \leq 0.05$). The sweet clover extract (6.0 mL L^{-1}) at 20 days decreased the population density by 59.2%, and Pyrifluquinazon (0.58 mL L^{-1}) at 6 days the decrease was 31.3%. All the extracts showed to be a sustainable alternative for the management of *D. citri*.

Keywords: *Diaphorina citri,* Phytosanitary, Huanglongbing, botanical concentrates.

The disease known as citrus yellow dragon or "Huanglongbing" (HLB) has had devastating effects on the world citrus industry. China, Brazil, the United States, Spain and Mexico are the main citrus-producing countries with presence of the disease. HLB affects species of the Rutaceae family, and practically all cultivated citrus species in the world. It causes production losses, fruit deformation, and even the death of the trees (Cazares, 2014). In

Mexico, the disease was detected for the first time in July 2009 in backyard-growing Mexican lime trees in the coastal community of Tizmín, in the state of Yucatan. During 2010, new instances of the disease were detected in the states of Campeche, Colima, Sinaloa, and Michoacan. HLB is now endemic in all lime-producing areas in Mexico (Robles-González *et al.*, 2014).

The insect vector associated with HLB and citrus is *Diaphorina citri* (Hemiptera: Liviidae), which causes direct and indirect damage. It causes direct damage through the feeding of nymphs and adults, which extract large amounts of sap from the leaves and petioles and excrete honeydew. Sooty mold fungi develop on the latter, covering the leaves, reducing photosynthesis and potentially causing tree defoliation. Furthermore, when feeding, they inject a toxin that stops terminal growth and causes malformations in the leaves and shoots, which prevents normal plant growth. The indirect damages are even more serious. The pest transmits one of the most important bacteria, called *Candidatus* Liberibacter spp., which disables the phloem and causes the HLB disease, also known as "Citrus Greening" (Garza, 2014).

Given the importance of this disease, it is urgently necessary to establish efficient sampling and surveillance protocols for HLB and its vector. These protocols should be backed by studies on its development and consider the use of locally-proven management technologies to address this serious problem (Mora-Aguilera *et al.*, 2014). Recently evaluated strategies for the management of HLB in infected plants include the use of antibiotics such as oxytetracycline and penicillin. The latter, applied with a surfactant, yielded the best results in terms of bacterial control, although both had good results by increasing tree growth and improving fruit quality (Zhang *et al.*, 2021). Another strategy for the management of HLB is the use of inducers of systemic acquired resistance (SAR), such as acibenzolar-S-methyl (ASM), 2,6-dichloroisonicotinic acid (INA), and salicylic acid (SA), applied individually as foliar sprays to the soil and by injection into the trunk. Although no treatment improved fruit quality, they significantly reduced both the severity of the disease, the vector population and fruit drop, while also increasing yield (Li *et al.*, 2021).

Regarding the insect vector, in the State of Mexico, Mexico, Bautista *et al.* (2014) studied the chemical control of *D. citri* in Persian lime under greenhouse conditions. They evaluated six chemical treatments: imidacloprid + betacyflutrin, spirotetramat, Imidacloprid, thiamethoxam + lambda cyhalothrin and paraffinic petroleum oil. After 48 h, imidacloprid and paraffinic oil caused 83 and 100% mortality, respectively.

Another study carried out in commercial citrus orchards located in California, USA, evaluated a biological control program for *D. citri* that began in 2012 and lasted 10 years. The control program was based on the parasitoids *Tamarixia radiata* (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae), species originating from Punjab, Pakistan, presumably from the area of origin of *D. citri*. The pest population decreased by approximately 70% due to a combination of nymphal parasitism by *T. radiata* and predation by hoverfly generalist predators (Hoddle *et al.*, 2022). Chen *et al.* (2022) evaluated the toxicity of four insecticides against *D. citri* in citrus plants in Florida USA: bifenthrin, cyantraniliprole, dimethoate, and thiamethoxam. They reported the following resistance ratios in field populations of *D. citri*: 6.67–11.33, 3.20–36.37, 12.50–82.50, and 4.60–10.08%, respectively. The present study aimed to evaluate different plant extracts and biorational products for the control of *D. citri* in Mexican lime.

MATERIALS AND METHODS

Two field evaluations of insecticides used for the control of *D. citri* were carried out in a two-year-old Mexican lime orchard established in the Valle de Apatzingán Experimental Field [CEVA] of the National Institute of Forestry, Agriculture and Livestock Research [INIFAP], in the municipality of Parácuaro, Michoacán, Mexico. The experimental field is located at 19° 00' 44.10'' N and 102° 13' 38.57'' W, 346 masl (Google Earth, 2021).

Treatments. The first field evaluation (1st EV) began in March 2020. Five treatments were evaluated, including a control treatment (Table 1).

The second field evaluation [2nd EV] began in June 2020. Eight treatments were evaluated, including a control treatment (Table 2).

Study variable. The response variable was the number of *D. citri* adults. A sampling (*a priori*) was made before the application of the treatments to use as a reference point.

Samplings. In the 1st EV, field samplings were carried out at 8, 20 and 27 days after the application (daa) of the treatments. In the 2nd EV, field samplings were carried out at 6, 21 and 27 daa. The sampling was done by beating branches. The sample unit was taken to be a tree branch at a

Table 1. Treatments used for the control of *D. citri* in Mexican lime in the first field evaluation.

Número	Tratamiento	Dosis mL L^{-1}	Producto comercial / Compañía / País
T1	Spirotetramat 5.87%	0.5	Palgus® / Corteva / México
T2	Extracto compuesto de ajo [*Allium sativum* (Liliaceae)], manzanilla [*Matricaria chamomilla* (Asteracee) y ruda [*Ruta graveolens* (Rutaceae)] 10%	4.0	BioCrack® / Berni Labs / México
T3	Extracto de cítricos + queratina 10%	4.0	Fractal® / Berni Labs / México
T4	Extracto de meliloto *Melilotus indicus* (Fabaceae) Solución base al 50%, 500 g de la planta por L de alcohol	6.0	Producto artesanal / México
T5	Testigo (agua)	-	-

Table 2. Treatments used to control *D. citri* in Mexican lime in the second evaluation.

Número	Tratamiento	Dosis mL L^{-1}	Producto comercial / Compañía / País
T1	Testigo (agua)	-	-
T2	Tolfenpirad al 15%	0.9	Hachi Hachi / Nichino / México
T3	Tolfenpirad al 15%	1.2	Hachi Hachi / Nichino / México
T4	Tolfenpirad al 15%	1.9	Hachi Hachi / Nichino / México
T5	Pyrufluquinazon al 20,2%	0.3	Pyriflu / Nichino / México
T6	Pyrufluquinazon al 20,2%	0.4	Pyriflu / Nichino / México
T7	Pyrufluquinazon al 20,2%	0.6	Pyriflu / Nichino / México
T8	Pyrufluquinazon al 20,2%	0.3	Pyriflu / Nichino / México

height of 1.5 m. Each branch was hit three times with a wooden spade. The insects that fell on a 38 x 21 cm purple board were quantified (Miranda-Salcedo *et al.*, 2019).

Experimental design and statistical analysis. The experimental design was completely randomized, with five and eight treatments in the 1st and 2nd EV, respectively. Ten independent replicates were done for each treatment. The experimental unit was a tree.

The data on the number of insects were subjected to a Box-Cox (1964) Logistic transformation so that the assumptions of additivity, normality and constant variance were approximately satisfied. The distribution of the new transformed variables is close to normal (Peña and Peña, 1986; Castaño-Vélez, 2011). Normality and homogeneity tests were also performed [Post Hoc], as well as an analysis of variance and Tukey's mean comparison test ($p \leq 0.05$). All statistical analyses were performed with the statistical software Statistica v.13.3 (StatSoft Inc., 2017).

RESULTS

The results of the test for variance homogeneity showed a satisfactory coincidence on the straight line in both evaluations. Likewise, the samples showed a normal distribution that corroborated the reliability of the experiment.

1st EV. The analysis of variance showed no statistically significant differences ($p \leq 0.05$) between treatments.

The results of the Tukey's test ($p \leq 0.05$) showed that the treatments had different effects at 8, 20 and 27 daa (Table 3). At 8 daa, treatments T3 and T4 were associated with a decrease in the *D. citri* population were, with statistically significant differences compared to the other treatments. Treatment T5 was associated with an increase in the *D. citri* population. At 20 daa, treatment T2 was associated with a *D. citri* population decrease that was significantly different compared to the other treatments. Treatment T1 was associated with the lowest numerical value at all sampling dates

Cuadro 3. Effect of treatments on *D. citri* Tukey (=*p ≤ 0.05) for the first evaluation.

Tratamiento	Previo	Días después de la aplicación		
		8	20	27
T1	3.48 a	1.16 b	0.69 a	2.30 b
T2	3.03 a	2.28 a	1.53 ab*	3.12 ab*
T3	3.51 a	1.87 ab*	2.23 b	3.46 a
T4	2.18 a	2.00 ab*	0.89 a	4.11 a
T5	2.04 a	2.30 a	1.91 b	4.15 a

T1= Testigo (agua); T2= Tolfenpirad 0.9 mL L^{-1}; T3: Tolfenpirad 1.2 mL L^{-1}; T4=Tolfenpirad 1.9 mL L^{-1}; T5= Pyrifluquinazon 0.3 mL L^{-1}; T6= Pyrifluquinazon 0.4 mL L^{-1}; T7= Pyrifluquinazon 0.6 mL L^{-1}; T8= Pyrifluquinazon 0.3 mL L^{-1}.
* Medias con distinta letra en la columna son estadísticamente diferentes (=*p ≤ 0.05).

and with all treatments. Treatment T4 showed a numerical value below 1. In the sampling carried out at 27 daa, treatment T2 showed a statistically significant difference compared to the other treatments. Treatment T1 showed a value below the previous sampling and T3, T4 and T5 showed numerical values ranging from 3 to 4 psyllids, above the previous sampling.

2nd EV. The analysis of variance showed no statically significant ($p \leq 0.05$) differences between the treatments in the population means recorded in all the samplings. They were statistically the same.

The results of the Tukey's test ($p \leq 0.05$) showed statistically significant difference between all the samplings, including the previous one (Table 4). At 6 daa, only T7 showed a significant difference, while treatments T2 and T3 showed the lowest numerical values (i.e. the greatest decrease in the population of psyllids) compared to the other sampling dates. At 21 daa, T5, T6 and T7 were statistically equal, showing increased insect populations. Treatments T2, T4 and T8 showed showed significant differences between them with decreases in the population of *D. citri*. In the sampling carried out at 27 daa, only T4 and T5 showed significant differences. Treatment T3 showed a numerical value below zero, evidencing a greater decrease in the population of *D. citri*.

DISCUSSION

The present work corroborated a previous study on the control of Asian psyllid populations in Mexican lime plants. The results showed that there is a sustainable alternative for the management of psyllid populations using commercial and botanical insecticides.

Although the results of Tukey's test ($p \leq 0.05$) for spirotetramat were numerical, this insecticide controlled the population of *D. citri* in all the samplings and at 20 daa the effect was 80.1% greater than in the previous sampling. Therefore, it continues to represent an alternative for the control of this psyllid. Effective use of insecticides requires carrying out field samplings to determine the damage threshold of the pest before making the decision to apply a product. Miranda-Ramírez *et al.* (2021) observed that Spirotetramat caused the greatest effects at 20 daa, with a psyllid population decrease of 36.9%. Fiaz *et al.* (2018) evaluated this

Cuadro 4. Effect of the treatments on *D. citri* Tukey (=*p ≤ 0.05) for the second evaluation.

Tratamiento	Previo	Días después de la aplicación		
		6	21	27
T1	3.37 ab*	2.61 d	3.15 b	2.94 a
T2	2.24 a	0.13 a	1.61 cd*	1.21 b
T3	2.81 ab*	0.13 a	1.35 c	0.77 d
T4	3.90 b	0.52 a	1.94 acd*	1.65 bc*
T5	2.08 a	1.91 b	2.58 ab*	2.79 a
T6	2.51 a	1.94 bc	2.87 ab*	2.39 a
T7	4.11 b	2.51 cd*	2.82 ab*	3.05 a
T8	2.40 a	1.62 b	2.26 abcd*	1.99 bcd*

T1= Testigo (agua); T2= Tolfenpirad 0.9 mL L^{-1}; T3: Tolfenpirad 1.2 mL L^{-1}; T4=Tolfenpirad 1.9 mL L^{-1}; T5= Pyrifluquinazon 0.3 mL L^{-1}; T6= Pyrifluquinazon 0.4 mL L^{-1}; T7= Pyrifluquinazon 0.6 mL L^{-1}; T8= Pyrifluquinazon 0.3 mL L^{-1}.
* Medias con distinta letra en la columna son estadísticamente diferentes (=*p ≤ 0.05).

insecticide in doses of 90 g a.i. per ha^{-1} against *D. citri* affecting citrus plants. They reported that the maximum decrease of the psyllid population mean caused by spirotetramat was observed at 3 daa, ranging between 57 and 66%.

Compared to the evaluated extracts, citrus extract + keratin showed a reduction in the population of the density of the psyllids at 8 and 20 daa (1.8 psyllids, 46.5% and 2.2 psyllids, 36.4%, respectively). Miranda-Salcedo *et al.* (2020) observed that the citrus extract + keratin showed values of 1.2 *D. citri* in the previous sampling. At 3, 7 and 18 daa, the values were 0, 0.3 and 0.6 in Mexican lime, respectively. The extracts of garlic + chamomile and rue, and the sweet clover extract showed a repellent effect on insects up to 20 daa, with values of 1.5 psyllids. Agroecological management possibly played a role, especially the presence of weeds between rows, which maintain the presence of natural enemies of potential pests. Molina *et al.* (2022) noted that one of the most important plant species that have been used to control insects is garlic due to its active compounds allicin and allyl propyl disulfide. Romo-Asunción *et al.* (2016) reported that the species of the genus *Melilotus indicus* and *M. albus* contain several bioactive compounds such as terpenes, sterols, and polyphenols.

Tolfenpyrad proved to be a good alternative for the reduction of Asian psyllid populations in Mexican lime. However, the intensive use of this insecticide through calendar applications can lead to the development of resistance in insects. In this regard, Tang *et al.* (2020) found that foliar applications of Tolfenpyrad in citrus, at a dose of 50 mg kg of active ingredient, had an efficacy of 78.1% against *D. citri*, exerting its effect from 3 to 30 daa.

Miranda-Ramirez *et al.* (2021) observed that Pyrifluquinazon, applied in doses of 0.5 mL L^{-1} to the foliage of Mexican lime trees, decreased the psyllid population by 87.7% at 3 daa. However, at 6 daa the effect was null. This differs with the findings of the present work, in which Pyrifluquinazon, applied in doses of 0.5 mL L^{-1}, caused a decrease in the insect population of more than 31.31% at 21 daa. However, in doses of 0.3 and 0.4 mL L^{-1}, there was an increase in the psyllid population at 21 dda. This confirms that the dose and the time of application are important factors for achieving a reduction of psyllid populations.

CONCLUSIONS

It was possible to obtain an alternative for the sustainable management of *D. citri* using products based on citrus extract + keratin, compound extracts of garlic + chamomile and rue, and sweet clover extract.

Tolfenpyrad showed a significant decrease in the populations of the Asian psyllid in Mexican lime. However, excessive use of this molecule can lead to the development of resistance in the psyllid.

It is advisable to monitor the psyllid population weekly to make the decision to apply an insecticide. Moreover, control products with low environmental impact should be rotated using the correct doses.

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