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Toxicity of an acetogenin-based bioinsecticide against *Diaphorina citri* (Hemiptera: Liviidae) and its parasitoid *Tamarixia radiata* (Hymenoptera: Eulophidae)

Leandro Do Prado Ribeiro[1], Mônica Silva Santos[2], Gabriel Luiz Padoan Gonçalves[2], and José Djair Vendramim[2]*

Abstract

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is currently the most important insect pest affecting citrus worldwide due to its relation (as insect vector) with huanglongbing (greening) disease. To determine an alternative tool for *D. citri* control, this study evaluated the insecticidal activity of ethanolic extract from *Annona mucosa* Jacq. (Magnoliales: Annonaceae) seeds (ESAM), which has the acetogenin rolliniastatin-1 as its major compound, against *D. citri*. ESAM caused high mortality in both 3rd instar nymphs (LC_{50} = 429.43, 247.95, 148.16, 96.89, and 57.76 mg/L after 24, 48, 72, 96, and 120 h of exposure, respectively) and adults (LC_{50} = 5,359.00, 2,464.00, 1,507.00, and 795.51 mg/L after 48, 72, 96, and 120 h of exposure, respectively), showing higher effectiveness than Azamax® 1.2 EC (azadirachtin + 3-tigloylazadirachtol, positive control) at the recommended concentration, which showed insecticidal effects only on nymphs. At a sublethal concentration (LC_{25}), ESAM caused significant reductions in feeding and oviposition of *D. citri* adults. However, the adult emergence of the ectoparasitoid *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) was reduced when exposed (by contact) to ESAM in its larval stage. In a greenhouse trial (seedlings cultivated in vases), the insecticidal activity of formulated ESAM was superior to that of Azamax® 1.2 EC, showing a residual effect of approximately 6 d (effectiveness > 80%). The effectiveness of ESAM (> 99%) for *D. citri* control also was confirmed in a commercial sweet orange farm (field trial). In light of these results, ESAM can constitute a useful component in the framework of *D. citri* integrated pest management, mainly in domestic orchards and organic systems.

Key Words: botanical insecticide; *Annona mucosa*; deterrent; selectivity; IPM

Resumo

O psilídeo-dos-citros, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), é o mais importante inseto-praga da citricultura mundial na atualidade devido sua relação (inseto vetor) com o huanglongbing (greening). De modo a detectar ferramentas alternativas de manejo, foi avaliada, primeiramente, a atividade inseticida do extrato etanólico de sementes de *Annona mucosa* Jacq. (Magnoliales: Annonaceae) (ESAM), o qual possui a acetogenina roliniastatina-1 como composto ativo majoritário, sobre *D. citri*. ESAM causou alta mortalidade de ninfas de 3° instar (CL_{50} = 429,43; 247,95; 148,16; 96,89 e 57,76 mg/L após 24, 48, 72, 96 e 120 horas de exposição, respectivamente) e de adultos (CL_{50} = 5.359,00; 2.464,00; 1.507,00 e 795,51 mg/L após 48, 72, 96 e 120 horas de exposição, respectivamente), mostrando uma eficácia superior ao bioinseticida Azamax® 1,2 EC (azadiractina + 3-tigloilazadiractol, controle positivo) testado na concentração registrada, o qual mostrou ação somente sobre ninfas. Em concentração subletal (CL_{25}), ESAM causou significativa redução na alimentação e oviposição de *D. citri*. Entretanto, a emergência de adultos do seu ectoparasitoide, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), foi reduzida quando em contato com o ESAM no estágio larval. Em um teste em casa de vegetação (mudas cultivadas em vasos), a atividade inseticida do ESAM formulado foi superior ao Azamax® 1,2 EC, com efeito residual de aproximadamente seis dias (eficácia > 80%). A eficácia do ESAM (> 99%) no controle de *D. citri* foi também confirmada em um cultivo comercial de laranjeira-doce (teste de campo). Diante desses resultados, ESAM pode ser um componente útil para o manejo integrado de *D. citri*, especialmente em pomares domésticos e sistemas orgânicos.

Palavras Chave: inseticida botânico; *Annona mucosa*; deterrentes; seletividade; MIP

Huanglongbing (HLB), also known as citrus greening disease, is currently the main phytosanitary problem of the citrus industry worldwide (Alemán et al. 2007; Grafton-Cardwell et al. 2013). In Brazil (the largest citrus producer worldwide), the disease is caused by *Candidatus* Liberibacter asiaticus and *Candidatus* Liberibacter americanus (Coletta-Filho et al. 2004; Teixeira et al. 2005). These phloem-limited bacteria are transmitted in a persistent manner by the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Hall et al. 2012). Although this insect has occurred naturally within Brazil's citrus groves for more than 60 yr without causing economic damage (Costa Lima 1942), in 2004, its relationship with HLB transmission was discovered in orchards of São Paulo State (Leal et al. 2010). The disease has since spread rapidly to other citrus producing regions (Belasque Jr et al. 2010), requiring significant multi-institutional research efforts to contain its advance and economic impact.

As all citrus species farmed are HLB susceptible and varieties tolerant to the disease's expression are still unavailable (Grafton-Cardwell et al. 2013), control of the insect vector is considered essential to reduce

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disease spread in orchards (Hall et al. 2012; Boina & Bloomquist 2015). In this context, the use of botanical derivatives is an important tool in integrated *D. citri* management in organic orchards, a market niche with huge potential for expansion in Brazil (Turra et al. 2014). Although a strategy still poorly exploited and used, studies have demonstrated the potential of botanical derivatives for *D. citri* control, including extracts from *Azadirachta indica* A. Juss (Sapindales: Meliaceae) (Shivankar et al. 2000; Borad et al. 2001; Shivankar et al. 2003; Weathersbee III & McKenzie 2005; Khan et al. 2012), *Vitex negundo* L. (Lamiales: Lamiaceae), and *Acorus calamus* L. (Acorales: Acoraceae) (Shivankar et al. 2000), essential oils of aromatic plants (Mann et al. 2011, 2012), and, more recently, a matrine-based biopesticide (Zanardi et al. 2015). However, it is still necessary to perform studies aiming to detect new sources of botanical insecticides that can be used in managing this pest species, especially plant species with great abundance under neotropical conditions.

In our current research program aiming to detect new sources of insecticides/acaricides, compounds derived from *Annona mucosa* Jacq. (Magnoliales: Annonaceae) were chosen as this plant is observed to cause pronounced lethal and sublethal effects in some pest species of agricultural importance, such as *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) (Ribeiro et al. 2013), *Panonychus citri* (McGregor) (Prostigmata: Tetranychidae) (Ribeiro et al. 2014c), *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae), and *Myzus persicae* (Sulzer) (Aphidomorpha: Aphididae) (Ribeiro et al. 2014a). Based on the promising initial results, biomonitored fractionation was conducted, indicating that the toxicity of this *A. mucosa* derivate is due to synergy of structurally diverse acetogenins, the majority being the bis-tetrahydrofuranic acetogenin rolliniastatin-1 (Ribeiro 2014). Despite the potential of derivatives from Annonaceae in arthropod pest management, little is known regarding their action against sucking insects that transmit phytopathogens.

Due to its importance in establishing a natural biological balance, preservation of the control exercised by natural enemies (entomophagous and entomopathogenic) should be considered in a screening program of new insecticide compounds, thus allowing for reduced economic costs and environmental impacts from applying these xenobiotics. Under the citrus growing conditions in Brazil, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) is the main parasitoid of *D. citri* (Gómez-Torres et al. 2012) because of its high parasitism efficiency, large dispersal capacity, and good field adaptation (Étienne et al. 2001). In addition, this ectoparasitoid has been used in classical biological control programs for *D. citri* in Brazil (Diniz et al. 2012; Gómez-Torres et al. 2012) and in other citrus producing countries (Étienne et al. 2001; Williams et al. 2013). A previous study (Ribeiro et al. 2014b) found that derivatives from *A. mucosa* are compatible with 3 species of entomopathogenic fungi, but to our knowledge, no previously published study has evaluated the impact of derivatives from these promising Annonaceae on entomophagous insects.

Given these considerations, the present study aimed to compare the toxicity of *A. mucosa* ethanolic seed extract (ESAM) and a limonoid-based commercial bioinsecticide on *D. citri* in laboratory, semi-field (greenhouse), and field experiments. In addition, we evaluated the effects of ESAM on oviposition and feeding behavior of *D. citri* adults and its impact on *T. radiata* ectoparasitoid emergence in the laboratory.

Materials and Methods

TEST INSECTS

The *D. citri* (nymphs and adults) and *T. radiata* specimens used in the bioassays were obtained from a population reared in the laboratory under controlled conditions (26 ± 2 ℃, 70 ± 10% RH, and 14:10 h L:D photoperiod). Seedlings (approx. 15 cm) of orange jessamine (*Murraya paniculata* [L.] Jacq.; Sapindales: Rutaceae), considered as one of the most suitable host species for *D. citri* (Michaud & Olsen 2004), were used for rearing purpose. For multiplication of *T. radiata*, orange jessamine seedlings were used and were infested with 4th and 5th instar nymphs of *D. citri* (host for the immature stage) and honey to feed the adults as described by Gómez-Torres et al. (2012).

CRUDE EXTRACT: SOURCE AND PREPARATION

The *A. mucosa* seeds used to prepare the crude extract were obtained from mature fruit collected on 17 Mar 2011 from specimens grown on the "Luiz de Queiroz" College of Agriculture campus, Piracicaba, São Paulo, Brazil (22°42'28.5"S, 47°37'59.6"W; altitude: 534 m). A voucher specimen, previously identified by Dr. Heimo Rainer (Department of Systematics and Evolution of Higher Plants, University of Vienna, Vienna, Austria), was deposited in the ESA herbarium of the Department of Biological Sciences at ESALQ/USP in Piracicaba, São Paulo, Brazil, under registration number 120985.

To prepare the extracts, the seeds were dried in an oven at 40 °C for 48 to 72 h and subsequently ground in a knife mill. The powder obtained was stored in sealed glass and kept refrigerated (approx. −10 °C) until use. Organic extract was obtained using the ethanol (99.5%) soaking technique (in a 1:5 ratio, w/v) as previously described (Ribeiro 2014).

BIOASSAYS

Laboratory Tests

All laboratory trials were conducted in a climate-controlled room (26 ± 2 °C, 70 ± 10% RH, and 14:10 h L:D photoperiod) under a completely randomized design.

Toxicity of the Extract Obtained Compared with a Limonoid-based Bioinsecticide

To conduct the bioassays, lime (*Citrus limona* L. Osbeck var. 'Cravo'; Sapindales: Rutaceae) seedlings grown in plastic tubes were previously pruned, and after shoots were emitted (2–3 cm long), they were used as experimental units.

Effect on Nymphs. The insecticidal action (via residual contact) of the *A. mucosa* ethanolic seed extract (ESAM) on *D. citri* 3rd instars was compared with that of a limonoid-based bioinsecticide (azadirachtin [6,220.15 g/L] + 3-tigloylazadirachtol [2,596.60 mg/L]; Azamax® 1.2 EC, UPL Brasil Ltda., Campinas, São Paulo, Brazil), commercialized in Brazil to manage *D. citri* on citrus (Agrofit 2014). For this goal, 'Cravo' lime seedlings were sprayed with the treatments via a micro-atomizer (Arprex® model 5A, Mogi das Cruzes, São Paulo, Brazil) coupled to a pneumatic pump adjusted to provide a pressure of 0.5 kgf/cm^2. For all treatments, the spray volume used was 2 mL of solution per seedling, stipulated based on preliminary tests to obtain complete and uniform coverage (point of runoff).

The concentration used in both treatments (extract and positive control) was 2,500 mg/L, which is recommended for Azamax® 1.2 EC to control *D. citri* in Brazil (Agrofit 2014). Due to its rapid degradation, the limonoid levels (azadirachtin + 3-tigloylazadirachtol) in the formulation was quantified using the analytical method described by Forim et al. (2010) at the moment of product usage. The negative controls consisted of the solvents used to solubilize the extract and the commercial bioinsecticide (acetone:deionized water [1:3, v/v] and deionized water, respectively).

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After applying the treatments, the seedlings were kept in a climate-controlled room for 2 h to dry the residue. Next, the seedlings were arranged in cages (2 L) constructed as described by Zanardi et al. (2015). Subsequently, ten 3rd instars were transferred from the rearing stock to each seedling using a fine brush and a stereoscopic microscope. Six replicates were used for each treatment level (n = 60). Nymph mortality was evaluated every 24 h for 5 d using a stereoscopic microscope. Dead nymphs were considered to be those that were dried and did not react to the touch of a fine brush.

Effect on Adults. The same procedures and experimental units used in the test with nymphs were employed to evaluate the insecticidal action on *D. citri* adults. However, the concentration of ESAM and commercial bioinsecticide used was 10,000 mg/L (4 times the concentration recommended for Azamax® 1.2 EC in controlling nymphs), defined based on previous tests. After applying the treatments and drying the residue, each seedling was infested with 10 adults (non-sexed) from the rearing stock aged between 5 and 8 d old. Similar to the previous test, 6 seedlings (replicates) per treatment (n = 60) were used, and the mortality of the exposed insects was evaluated daily for 5 d.

Concentration–Response Curve

To estimate LC_{50} and LC_{90}, corresponding to the levels necessary to cause 50 and 90% mortality, respectively, in the exposed insect population (separately by stage), preliminary tests were performed to determine the concentrations that caused a 95% insect mortality and a mortality level similar to that obtained in the control. Based on these results, 6 concentrations were established for testing (intervals: 31.25 – 1,000 mg/L for nymphs and 250 – 10,000 mg/L for adults) based on the procedures described by Finney (1971). The same experimental procedures described for the previous test were adopted for these estimates, and the mortality evaluations were performed daily for 5 d.

Estimated Mean Lethal Time (LT_{50})

LT_{50} (time necessary to kill 50% of the population) values of ESAM for *D. citri* nymphs and adults were estimated at different concentrations (125; 250; 500; and 1,000 mg/L [nymphs] and 1,000; 2,000; 3,981; 6,309; and 10,000 mg/L [adults]). For this purpose, the same aforementioned experimental procedures were adopted, and mortality was evaluated daily for 5 d.

Evaluating Deterrent Effects of ESAM on Oviposition and Feeding

Deterred Oviposition. The deterrent effect of ESAM on *D. citri* adult oviposition was evaluated at the previously estimated LC_{25} (exposure time = 120 h) using a test without opportunity to choose (confinement). The same experimental units and procedures afore mentioned were adopted for this bioassay. The acetone:deionized water solution (1:3, v/v) used to solubilize the extract served as the negative control.

After drying the residues, the seedlings were isolated into cages (2 L) and infested with 5 *D. citri* adult couples per seedling, and already fertilized females were selected (expanded abdomen with yellow-orange color; Skelley & Hoy 2004; Wenninger & Hall 2007). Ten plants were used for each treatment, totaling 50 couples per treatment. After 48 h of infestation, the number of eggs oviposited on each plant was counted using a stereoscopic microscope.

Deterred Feeding. Discs of leaves from sweet orange (*Citrus sinensis* [L.] Osbeck var. 'Pêra'; Sapindales: Rutaceae) (3.5 cm wide) were submerged in an extract solution (in the LC_{25} previously estimated for *D. citri* adults [exposure time = 120 h]) for 5 s and resuspended in acetone:deionized water (1:3, v/v). After applying the treatment, the discs were kept in a climate-controlled room on a paper towel for 2 h to dry the residue. Next, the discs were placed on Petri dishes (3.5 cm wide) containing solidified agar:deionized water solution (2.5% [w/v]).

In each Petri dish, 10 non-sexed adults aged between 5 and 8 d old were released with 10 replicates per treatment level (n = 100). A filter paper disc was added to the top of each Petri dish. The paper discs were thus kept face down in the Petri dishes to collect the honeydew excreted by the confined insects according to the procedure described by Boina et al. (2009). After 48 h, the filter paper discs were removed and submerged into ninhydrin:acetone solution (1% [v/v]) for 3 min. After 24 h, the discs were then scanned, and the honeydew drop area was estimated using Quant software version 1.0.1 (Vale et al. 2001).

Effect of ESAM on the Ectoparasitoid *Tamarixia radiata*

Orange jessamine branches infested with 4th and 5th instars of *D. citri* from the rearing stock were placed on buds of orange jessamine seedlings reared in plastic tubes (50 mL) for spontaneous migration of the nymphs. After 24 h (the period necessary for attachment and natural distribution of the nymphs on seedlings), nymphs were counted using a stereoscopic microscope. Next, the seedlings infested with the nymphs were placed into cages (2 L) that were infested with 10 *T. radiata* females for each *D. citri* nymph. The parasitoid females remained in contact with the *D. citri* nymphs for 48 h for the occurrence of parasitism. After this period, the parasitoid females were removed, and the plants containing the nymphs were kept in the respective cages in a climate-controlled room.

Four days after removing the parasitoid, the parasitized *D. citri* nymphs (mummified) were counted and sprayed with the extract at the LC_{90} previously estimated for *D. citri* adults (exposure time = 120 h), adopting the same aforementioned equipment and procedures. Acetone:deionized water (1:3, v/v) was used as a control, and 10 replicates (seedlings) were used for each treatment. Evaluation was performed after 9 d of applying the treatments, counting the number of parasitoids that emerged in each experimental unit.

GREENHOUSE TEST WITH FORMULATED ESAM (POTTED SEEDLINGS)

Sweet orange seedlings (approx. 80 cm) kept in pots (10 L) were sprayed with an aqueous emulsion of ESAM containing 5 g/L of Tween 80® (Ribeiro et al. 2014a) until the point of runoff using a Guarany® sprayer backpack equipped with a constant-flow full cone nozzle (FullJet®). The concentration of the extract used corresponded to the LC_{90} previously estimated for *D. citri* adults (exposure time = 120 h). Azamax® 1.2 EC bioinsecticide was used as a positive control, and the solvents employed to solubilize the formulated extract (methanol:water [1:10, v/v] + Tween 80® [0.5%, v/v]) and commercial bioinsecticide (deionized water) were used as negative controls.

At 3 h (time 0 = period necessary for drying the residues) and 1, 3, 6, 12, and 24 d after spraying, leaves from the apical portion of the treated seedlings were covered with acrylic cages (5 × 4 × 2 cm) and infested with 10 non-sexed adults aged between 5 and 8 d old, with 5 replicates per treatment level (n = 50). After this, the infested seedlings were kept in a greenhouse, and after 5 d, the mortality of the adults exposed in each treatment was evaluated.

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EFFICACY OF FORMULATED ESAM UNDER FIELD CONDITIONS (COMMERCIAL CITRUS FARM)

The efficacy of the formulated ESAM compared with a limonoid-based bioinsecticide (Azamax® 1.2 EC) was evaluated in a commercial sweet orange orchard (*C. sinensis* var. 'Valência'; approximately 4 yr old) with plants grown 4 × 6 m apart, as implemented in Piracicaba, São Paulo, Brazil (22°42'30"S, 47°38'0"W). In this orchard, no pesticides were applied for 12 mo before the onset of the experiment.

Branches from the apical portion of the plants of the plot were selected randomly and marked. Next, the plants were sprayed, using the same equipment described previously until the point of runoff. The concentration of the formulated ESAM used corresponded to the LC_{90} previously estimated for *D. citri* adults (exposure time = 120 h). The bioinsecticide Azamax® 1.2 EC (10,000 mg/L) was used as a positive control, and the solvents employed to solubilize the formulated extract (methanol:water [1:10, v/v] + Tween 80® [0.5%, v/v]) and commercial bioinsecticide (deionized water) were used as negative controls. Five replicates (plants) were used for each treatment.

After applying and drying the residue, selected branches were covered with a cage of voile fine tissue (20 × 15 cm) and infested with 20 non-sexed adults aged between 5 and 8 d old (n = 100). The mortality of the exposed insects was evaluated 5 d after infestation.

DATA ANALYSES

Generalized linear models (GLM) (Nelder & Wedderburn 1972) with quasi-binomial, quasi-Poisson, and Gaussian distributions were used for data analysis of mortality ratios, *D. citri* egg counts, and honeydew drop area, respectively. In all cases, the goodness of fit was tested using half-normal plots of probabilities with simulated envelope (Hinde & Demétrio 1998). When the treatments differed significantly, multiple comparisons (Tukey test, $\alpha = 0.05$) were performed using the glht function of the multicomp package, with the P values adjusted for the treatments with qualitative levels, whereas non-linear regressions were used to compare the treatments with quantitative levels. All analyses were performed using R statistical software version 2.15.1 (R Core Team 2012). The mortality data obtained in the semi-field and field tests were corrected using the formula proposed by Schneider-Orelli (1947).

To estimate the lethal concentrations (LC_{50} and LC_{90}), a binomial model with complementary log–log link function (gompit model) was used, using the Probit Procedure (SAS version 9.2; SAS Institute 2011). In turn, to estimate mean lethal time (LT_{50}), the method proposed by Throne et al. (1995) was used for probit correlated data analysis.

Results

The yield of the obtained extract from the maceration process of *A. mucosa* seeds in ethanol at a 1:5 (w/v) ratio was 18.79% (g of extract/g of seed powder). Regardless of the *D. citri* stage (nymphs or adults), the insecticidal action of ESAM was higher than that of the limonoid-based bioinsecticide (Azamax® 1.2 EC) used as a positive control, which was only effective in controlling nymphs (Table 1). ESAM caused complete mortality of exposed insects when tested at concentrations of 2,500 mg/L and 10,000 mg/L, respectively, for nymphs and adults.

Depending on the exposure time, ESAM caused high mortality in *D. citri* nymphs (LC_{50} = 429.43, 247.95, 148.16, 96.89, and 57.76 mg/L after 24, 48, 72, 96, and 120 h of exposure, respectively; Table 2) and *D. citri* adults (LC_{50} = 5,359.00, 2,464.00, 1,507.00, and 795.51 mg/L, after 48, 72, 96, and 120 h of exposure, respectively; Table 2). Similarly, the mean lethal time (LT_{50}) estimated was concentration dependent with significantly increased mortality throughout the exposure time (LT_{50} [nymphs] = 76.41, 41.99, 17.50, and 10.31 h at concentrations of 125, 250, 500, and 1,000 mg/L, respectively; LT_{50} [adults] = 91.90, 84.35, 47.93, 41.43, and 31.23 h at concentrations of 1,000, 2,000, 3,981, 6,309, and 10,000 mg/L, respectively; Table 3).

Table 1. Mortality (mean ± SE) of 3rd instar nymphs and adults of *Diaphorina citri* exposed to residual ethanolic extract from *Annona mucosa* seeds (ESAM) or commercial limonoid-based bioinsecticide (Azamax® 1.2 EC, positive control). Note: The treatments were tested at concentrations of 2,500 mg/L and 10,000 mg/L for nymphs and adults, respectively.

	Mortality (%)[a]	
Treatment	Nymphs	Adults
ESAM	100.00 ± 0.00	100.00 ± 0.00
Azamax® 1.2 EC	91.47 ± 3.06 a	30.00 ± 6.32
Control (acetone:water [1:3, v/v])	6.67 ± 3.33 b	7.50 ± 4.78
Control (water)	5.00 ± 3.41 b	14.00 ± 5.09
F	75.904	3.8607 ^{ns}
df	2, 15	2, 15
P value	< 0.0001	0.05368

[a]Means followed by different letters in a column indicate a significant difference between the treatments (GLM with quasi-binomial distribution followed by a Tukey post hoc test, $P < 0.05$); ^{ns}: not significant.

Regarding sublethal effects, ESAM (at the LC_{25} estimated for adults [exposure time = 120 h]) significantly reduced the number of deposited eggs and the feeding of *D. citri* adults, which indicates that this product has deterrent action on oviposition and feeding (Figs. 1 and 2).

Despite the potential bioactive effects on *D. citri*, ESAM reduced the emergence of ectoparasitoid *T. radiata* adults (Fig. 3) when its larval stage (2nd instar) was exposed (by contact) to the extract applied at the LC_{90} estimated for controlling *D. citri* adults (exposure time = 120 h).

The results obtained in the greenhouse test (semi-field) also confirmed the higher efficacy of ESAM compared with the limonoid-based bioinsecticide (Azamax® 1.2 EC) used as a positive control (Fig. 4). Under the same conditions, ESAM exhibited persistence of approx. 6 d (efficacy > 80%), showing a pronounced decrease in its insecticidal action after this period (Fig. 4). In contrast, Azamax® 1.2 EC caused higher adult mortality between 3 and 6 d after application, corroborating its systemic action and demonstrating reduced translocation speed in the citrus plants within the first days after leaf application.

In the test conducted in the Valencia sweet orange orchard, the formulated ESAM, when tested at the LC_{90} estimated under laboratory conditions (4,463.00 mg/L), exhibited high efficacy (> 90%) at controlling *D. citri* adults (Table 4). Despite the lower concentration used, the level of activity caused by this extract was significantly higher than that of the Azamax® 1.2 EC insecticide used as a positive control, which was tested at a concentration of 10,000 mg/L.

Discussion

Our results, obtained under laboratory, semi-field, and field conditions, indicate promising insecticidal action of ESAM for *D. citri*, and its efficacy levels were higher than those of a commercial limonoid-based bioinsecticide (Azamax® 1.2 EC, positive control) used to manage this pest species in Brazilian citrus orchards. Corroborating the toxicity of ESAM for sucking insects, our previous study (Ribeiro et al. 2014a) demonstrated that this extract has superior aphidicidal action (against *M.s persicae*) compared with commercial acetogenin-based (Anosom® 1 EC) and pyrethrin-based (Insect Spray®) bioinsecticides in laboratory and semi-field tests, without having any phytotoxic effects on the plant species (cabbage and citrus) used in the study.

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Table 2. Estimated LC_{50} and LC_{90} (in mg/L) and confidence interval of ethanolic extract from *Annona mucosa* seeds (ESAM) for 3rd instar nymphs and adults of *Diaphorina citri* at different exposure times.

Exposure time (h)	Stage	*n*[a]	Slope ± SE (*P* value)	LC_{50} (CI)[b]	LC_{90} (CI)[b]	χ^{2} [(c)]	df[d]	h.[e]
24	Nymphs	300	2.85 ± 0.36 (< 0.0001)	429.43 (348.34–515.91)	1,133.00 (903.06–1,570.00)	0.98	3	0.32
	Adults	—	—	> 10,000.00	—	—	—	--—
48	Nymphs	300	2.08 ± 0.31 (< 0.0001)	247.95 (173.57–319.70)	937.60 (706.96–1,444.00)	3.29	3	1.09
	Adults	420	1.98 ± 0.54 (0.0003)	5,359.00 (2,526.00–7,304.00)	21,663.00 (14,710.00–65,942.00)	2.26	4	0.56
72	Nymphs	300	1.66 ± 0.29 (< 0.0001)	148.16 (82.80–210.49)	785.40 (561.78–1,343.00)	3.90	3	1.30
	Adults	420	1.93 ± 0.35 (< 0.0001)	2,464.00 (1,281.00–3,503.00)	10.299.00 (7,994.00–14,790.00)	2.62	4	0.65
96	Nymphs	300	1.71±0.30 (< 0.0001)	96.89 (48.15–143.66)	490.06 (358.52–770.67)	3.00	3	1.00
	Adults	420	1.84 ± 0.20 (< 0.0001)	1,507.00 (1,121.00–1,886.00)	6,732.00 (5,390.00–9,021.00)	4.97	4	1.24
120	Nymphs	300	1.55 ± 0.29 (< 0.0001)	57.76 (23.53–91.98)	344.53 (249.02–543.05)	1.02	3	0.34
	Adults	420	1.60 ± 0.19 (< 0.0001)	795.51 (507.11–1,083.00)	4,463.00 (3,513.00–6,050.00)	5.96	4	1.49

[a]*n*: Number of insects tested.
[b]CI: Confidence interval at 95% error probability.
[c]χ^{2}: Pearson chi-square value.
[d]df: Degrees of freedom.
[e]h.: Heterogeneity factor.

In addition to the lethal toxicity of ESAM, our laboratory results also showed that at sublethal levels, it has pronounced deterrent effects on feeding and oviposition, effects that can affect the demography and population dynamics of *D. citri*, a hypothesis to be tested in the field in further studies. Moreover, compounds that inhibit feeding or alter feeding behavior can affect a phytopathogen's ability to transmit via insect vectors (Halbert & Manjunath 2004) because acquisition of the bacteria associated with HLB by *D. citri* makes salivation and ingestion of the phloem sap necessary (Bonani et al. 2010). The phloem is where the bacteria are found inside citrus plants (Batool et al. 2007). Given the perspective of applying

Table 3. Estimated mean lethal time (LT_{50}, in h) and confidence interval of ethanolic extract from *Annona mucosa* seeds (ESAM) for 3rd instar nymphs and adults of *Diaphorina citri* at different levels.

Concentration (mg/L)	*n*[a]	Slope ± SE (*P* value)	LT_{50} (CI)[b]	χ^{2} [(c)]	df[d]	h.[e]
			Nymphs			
125	50	2.33 ± 0.37 (< 0.0001)	76.41 (64.87–92.48)	0.69	3	0.29
250	50	2.35 ± 0.36 (< 0.0001)	41.99 (33.08–49.93)	1.43	3	0.47
500	50	1.52 ± 0.36 (< 0.0001)	17.50 (5.87–27.01)	2.94	3	0.98
1,000	50	2.57 ± 0.69 (< 0.0001)	10.31 (2.34–16.92)	0.97	3	0.32
			Adults			
1,000	70	3.29 ± 0.43 (< 0.0001)	91.90 (81.92–105.96)	2.71	3	0.90
2,000	70	3.05 ± 0.39 (< 0.0001)	84.35 (74.85–97.07)	1.99	3	0.66
3,981	70	2.67 ± 0.34 (< 0.0001)	47.93 (40.59–54.92)	1.64	3	0.55
6,309	70	2.90 ± 0.34	41.43 (34.89–47.43)	2.22	3	0.74
10,000	70	3.89 ± 0.44 (< 0.0001)	31.23 (26.54–35.44)	2.90	3	0.97

[a]*n*: Number of insects tested.
[b]CI: Confidence interval at 95% error probability.
[c]χ^{2}: Pearson chi-square value.
[d]df: Degrees of freedom.
[e]h.: Heterogeneity factor.

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Table 4. Percentage mortality (mean ± SE) of *Diaphorina citri* adults 120 h after applying an aqueous emulsion of ethanolic extract from *Annona mucosa* seeds (ESAM) or a commercial limonoid-based bioinsecticide (Azamax® 1.2 EC, positive control) in a test conducted in a commercial 'Valência' sweet orange orchard.

Treatment	Concentration (mg/L)	Mortality (%)[a]	C.E.[b]
ESAM	4,463.00 (= LC_{90})	99.04 ± 0.95 a	98.93
Azamax® 1.2 EC	10,000.00	60.48 ± 8.82 b	56.57
Control (methanol:water [1:10, v/v] + Tween 80® [0.5%, v/v])	—	10.00 ± 6.12 c	—
Control (deionized water)	—	9.00 ± 5.33 c	—
F		36.028	
df		3, 16	
P value		< 0.0001	

[a]Means followed by different letters in a column indicate a significant difference among the treatments (GLM with quasi-binomial distribution followed by a post hoc Tukey test, $P < 0.05$).
[b]C.E.: Control efficacy calculated by the Schneider-Orelli (1947) formula.

phagodeterrent compounds in managing phytopathogen-transmitting insect pests, the interference of ESAM in *D. citri* feeding behavior will be the subject of a future study using the electrical penetration graph technique.

Typically, the bioactivity of ESAM is due to the synergy of compounds from different chemical classes (especially acetogenins, alkaloids, and triglycerides) and/or of different polarities (Ribeiro et al. 2013), where the bis-tetrahydrofuranic acetogenin rolliniastatin-1 is the major active component (Ribeiro 2014). Acetogenins are considered potent complex I inhibitors (NADH: ubiquinone oxidoreductase) of the mitochondrial electron transport system and NADH: oxidase of the plasma membrane, which induces cellular apoptosis (programmed cell death), perhaps as a result of ATP deprivation (Tormo et al. 1999). Lately, acetogenins have attracted much interest due to their promising insecticidal action (Alali et al. 1999; Ribeiro et al. 2013) and repellence/deterrence of feeding and oviposition (Blessing et al. 2010). Studies on the structure–activity relationship have proven that acetogenins with adjacent bis-tetrahydrofuranic rings and 3 hydroxyl groups (e.g., rolliniastatin-1) have more pronounced entomotoxicity compared with acetogenins containing other distributions of functional groups in their structure (He et al. 1997).

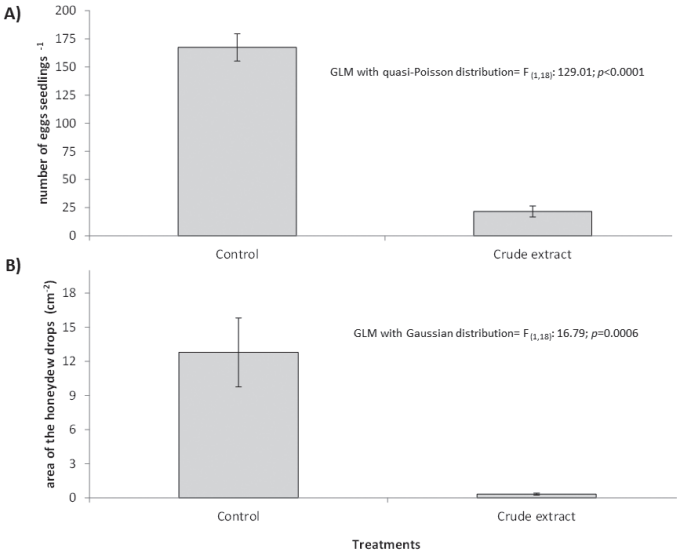


Fig. 1. Effect of ethanolic extract from *Annona mucosa* seeds (ESAM) on oviposition preference of copulated *Diaphorina citri* females (A) and on feeding in non-sexed *D. citri* adults (B). Both tests were conducted without opportunity to choose, and the concentration of the extract used was equivalent to the LC_{25} (224.92 mg/L) previously estimated for *D. citri* adults (120 h of exposure).

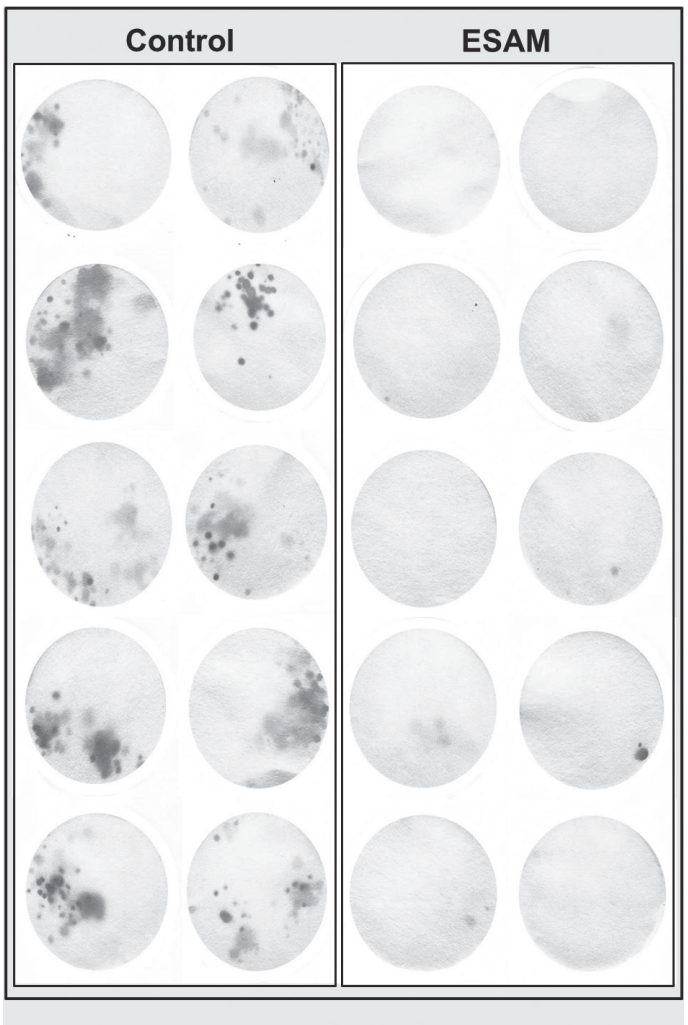


Fig. 2. Filter paper discs (experimental units) of the control and treatment with ethanolic extract from *Annona mucosa* seeds (ESAM). Dark points inside the discs comprise honeydew drop areas excreted by *Diaphorina citri* adults exposed to the referred treatments, which were stained with a ninhydrin:acetone solution (1%, v/v).

Although a deeper evaluation of the possible chronic effects of ESAM on the ectoparasitoid *T. radiata* is necessary, both under laboratory and field conditions, our results showed a negative impact of the extract on the emergence of this natural enemy. However, Leatemia &

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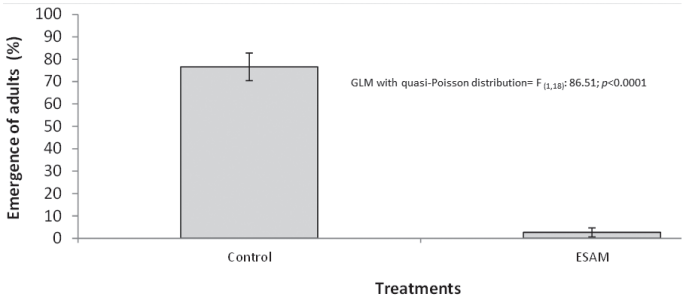


Fig. 3. Percentage of emergence of *Tamarixia radiata* adults exposed to ethanolic extract from *Annona mucosa* seeds (ESAM) during their larval stage. The concentration of extract used was equivalent to the LC_{90} (4,463.00 mg/L) estimated for *D. citri* adults (120 h of exposure).

Isman (2004), using direct spraying and residual contact tests, found variation in the susceptibility of generalist predators to an extract from *Annona squamosa* L. (Magnoliales: Annonaceae) seeds, wherein larvae of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) were less susceptible than adults of *Orius insidiosus (*Say) (Hemiptera: Anthocoridae). However, the compatibility of derivatives from *A. mucosa* with entomopathogenic fungi (*Beauveria bassiana* Bals.-Criv. Vuill. [Hypocreales: Cordycipitaceae] isolate ESALQ-PL63; *Isaria fumosorosea* Wize [Hypocreales: Cordycipitaceae] isolate ESALQ-1296; and *Metarhizium anisopliae* [Metschn.] Sorokin [Hypocreales: Clavicipitaceae] isolate ESALQ-E9) (Ribeiro et al. 2014b) is a positive aspect for including it in integrated *D. citri* management programs. Several studies have demonstrated the potential of entomopathogens in managing this insect vector (Hoy et al. 2010; Avery et al. 2011; Guizar-Guzman & Sanchez-Peña 2013), and commercial formulations of mycoinsecticides aiming to control *D. citri* are under development for the Brazilian market (Ribeiro et al. 2014b).

In addition to aspects related to the agronomic efficacy of these natural derivatives, it is necessary to carefully evaluate their possible effects on non-target organisms (especially mammals) and their behavior in the environment. In a preliminary approach, González-Coloma et al. (2002) found that cells from the ovary of a mammal (Chinese hamster) were less sensitive (approx. 400 times) to the acetogenin rolliniastatin-1 than cells from *Spodoptera frugiperda* Smith & Abbot (Lepidoptera: Noctuidae) (*Sf*9), although enzymatic and immunochemical studies have revealed high similarity between the enzymes involved in cellular respiration in insects, mammals, and fungi (Lümmen 1998). However, membrane factors dependent on the structure and metabolic capacity of inactivating acetogenins in different groups can provide different levels of sensitivity (Ribeiro 2014), an aspect that should be investigated further.

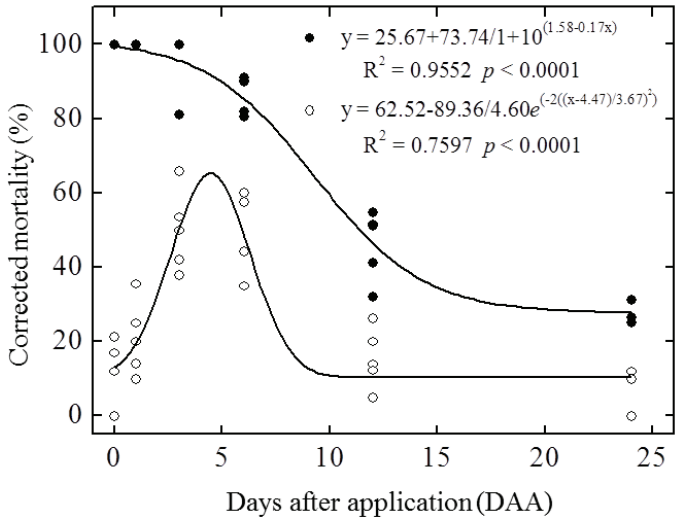


Fig. 4. Corrected mortality (Schneider-Orelli 1947) of *Diaphorina citri* adults exposed to residue on 'Valência' sweet orange seedlings treated with an aqueous emulsion of ethanolic extract from *Annona mucosa* seeds — ESAM (●) — or the commercial limonoid-based bioinsecticide Azamax® 1.2 EC (○) used as a positive control, at different periods after application in a semi-field trial (greenhouse). The mortality levels were lower than 7.5% in the controls.

Based on the results obtained, we conclude that ESAM has promising bioactivity for *D. citri* and may constitute a useful component for managing this pest species in Brazil and in other citrus producing countries, especially in domestic orchards and organic citrus production systems. Given this perspective, studies on optimizing extraction processes and formulations should be conducted, especially to enable controlled-release nanoformulations that provide increased residual effects of bioinsecticides developed based on *A. mucosa* seed extract.

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References Cited

Agrofit. 2014. Phytosanitary Agrochemicals System [online]. Ministry of Agriculture, Livestock and Food Supply, Brazil. http://agrofit.agricultura.gov.br/agrofit_cons/principal_ agrofit_cons(last accessed 14 Dec 2014).

Alali FQ, Liu XX, McLaughlin EJL. 1999. Annonaceous acetogenins: recent progress. Journal of Natural Products 62: 504-540.

Alemán JH, Baños H, Ravelo J. 2007. *Diaphorina citri* y la enfermedad huanglongbing: una combinación destructiva para la producción citrícola. Revista de Protección Vegetal 22: 154-165. (In Spanish)

Avery PB, Wekesa VW, Hunter WB, Hall DG, McKenzie CL, Osborne LS, Powell CA, Rogers ME. 2011. Effects of the fungus *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) on reduced feeding and mortality of the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae). Biocontrol Science and Technology 21: 1065-1078.

Batool A, Iftikhar Y, Mughal SM, Khan MM, Jaskani MJ, Abbas M, Khan IA. 2007. Citrus greening disease — a major cause of citrus decline in the world — a review. Horticultural Science 34: 159-166.

Belasque Jr J, Barbosa JC, Massari CA, Ayres AJ. 2010. Incidência e distribuição do huanglongbing no estado de São Paulo, Brasil. Citrus Research and Technology 31: 1-9. (In Portuguese)

Blessing LT, Colom OA, Popich S, Neske A, Bardón A. 2010. Antifeedant and toxic effects of acetogenins from *Annona montana* on *Spodoptera frugiperda*. Journal of Pest Science 83: 307-310.

Boina DR, Bloomquist JR. 2015. Chemical control of the Asian citrus psyllid and of huanglongbing disease in citrus. Pest Management Science (in press).

Boina DR, Onagbola EO, Salyani M, Stelinski LL. 2009. Antifeedant and sublethal effects of imidacloprid on Asian citrus psyllid, *Diaphorina citri*. Pest Management Science 65: 870-877.

Bonani JP, Fereres A, Garzo E, Miranda MP, Appezzato-Da-Gloria B, Lopes JRS. 2010. Characterization of electrical penetration graphs of the Asian citrus psyllid, *Diaphorina citri*, in sweet orange seedlings. Entomologia Experimentalis et Applicata 134: 35-49.

Borad PK, Patel MJ, Vaghela NM, Patel BH, Patel MG, Babubhai HP, Patel JR. 2001. Evaluation of some botanicals against citrus leafminer (*Phyllocnistis citrella*) and psylla (*Diaphorina citri*) on kagzilime (*Citrus aurantifolia*). Indian Journal of Agricultural Sciences 71: 177-179.

Coletta-Filho HD, Targon MLPN, Takita MA, De Negri JD, Pompeu-Junior J, Machado MA. 2004. First report of the causal agent of huanglongbing ('*Candidatus* Liberibacter asiaticus') in Brazil. Plant Disease 88: 1382.

Costa Lima AM. 1942. Insetos do Brasil, Homoptera. Escola Nacional de Agronomia, Rio de Janeiro, Brazil. (In Portuguese)

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Diniz AJF, Alves GR, Parra JRP, Yamamoto PT. 2012. *Tamarixia radiata*: uma alternativa para controle do psilídeo dos citros. Citricultura Atual 1: 12-14. (In Portuguese)

Étienne J, Quilici S, Marinal D, Franck A. 2001. Biological control of *Diaphorina citri* (Hemiptera: Psyllidae) in Guadeloupe by imported *Tamarixia radiata* (Hymenoptera: Eulophidae). Fruits 56: 307-315.

Finney DJ. 1971. Probit Analysis. Cambridge University Press, Cambridge, United Kingdom.

Forim MR, Fernandes MFG, Cass QB, Fernandes JB, Vieira PC. 2010. Simultaneous quantification of azadirachtin and 3-tigloylazadirachtol in Brazilian seeds and oil of *Azadirachta indica*: application to quality control and marketing. Analytical Methods 2: 860-869.

Gómez-Torrez ML, Nava DE, Parra JRP. 2012. Life table of *Tamarixia radiata* (Hymenoptera: Eulophidae) on *Diaphorina citri* (Hemiptera: Psyllidae) at different temperatures. Journal of Economic Entomology 105: 338-343.

González-Coloma A, Guadaño A, Inés C, Martínez-Díaz R, Cortes D. 2002. Selective action of acetogenin mitochondrial complex I inhibitors. Zeitschrift für Naturforschung C 57: 1028-1034.

Grafton-Cardwell EE, Stelinski LL, Stansly PA. 2013. Biology and management of Asian citrus psyllid, vector of the huanglongbing pathogens. Annual Review of Entomology 58: 413-432.

Guizar-Guzman L, Sanchez-Peña R. 2013. Infection by Entomophthora sensu stricto (Entomophthoromycota: Entomophthorales) in *Diaphorina citri* (Hemiptera: Liviidae) in Veracruz, Mexico. Florida Entomologist 96: 624-627.

Halbert SE, Manjunath KL. 2004. Asian citrus psyllid (Sternorryncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. Florida Entomologist 87: 3330-3353.

Hall DG, Richardson ML, Ammar ED, Halbert SE. 2012. Asian citrus psyllid, *Diaphorina citri*, vector of citrus huanglongbing disease. Entomologia Experimentalis et Applicata 146: 207-223.

He H, Zeng L, Ye Q, Shi G, Oberlines N, Zhao G, Njoku C, McLaughlin J. 1997. Comparative SAR evaluations of annonaceous acetogenins for pesticidal acitivity. Journal of Pesticide Science 49: 372-378.

Hinde J, Demétrio CGB. 1998. Overdispersion: models and estimation. Computational Statistics and Data Analysis 27: 151-170.

Hoy MA, Singh S, Roger ME. 2010. Evaluations of a novel isolate of *Isaria fumosorosea* for control of the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae). Florida Entomologist 93: 24-32.

Khan I, Zahid M, Khan GZ. 2012. Toxicity of botanic and synthetic pesticide residues to citrus psyllid *Diaphorina citri* Kuwayama and *Chrysoperla carnea* (Stephens). Pakistan Journal of Zoology 44: 197-201.

Leal RM, Barbosa JC, Costa MG, Junior JBJ, Yamamoto PT, Dragone J. 2010. Distribuição espacial de huanglongbing (greening) em citros utilizando a geoestatística. Revista Brasileira de Fruticultura 32: 808-818. (In Portuguese)

Leatemia JA, Isman MB. 2004. Toxicity and antifeedant activity of crude seed extracts of *Annona squamosa* (Annonaceae) against lepidopteran pests and natural enemies. International Journal of Tropical Insect Science 24: 150-158.

Lümmen P. 1998. Complex I inhibitors as insecticides and acaricides. Biochimica et Biophysica Acta 1364: 287-296.

Mann RS, Rouseff RL, Smoot JM, Castle WS, Stelinski LL. 2011. Sulfur volatiles from *Allium* spp. affect Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), response to citrus volatiles. Bulletin of Entomological Research 1: 89-97.

Mann RS, Tiwari S, Smoot JM, Rouseff RL, Stelinski LL. 2012. Repellency and toxicity of plant-based essential oils and their constituents against *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). Journal of Applied Entomology 136: 87-96.

Michaud JP, Olsen LE. 2004. Suitability of Asian citrus psyllid, *Diaphorina citri*, as prey for ladybeetles. BioControl 49: 417-431.

Nelder JA, Wedderburn RWM. 1972. Generalized linear models. Journal of the Royal Statistical Society A 135: 370-384.

R Core Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ribeiro LP. 2014. Exploring genetic biodiversity: secondary metabolites from Neotropical Annonaceae as a potential source of new pesticides. Ph.D. thesis, College of Agriculture, University of São Paulo, Piracicaba, Brazil.

Ribeiro LP, Vendramim JD, Bicalho KU, Andrade MS, Fernandes JB, Moral RA, Demétrio CGB. 2013. *Annona mucosa* Jacq. (Annonaceae): a promising source of bioactive compounds against *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae). Journal of Stored Products Research 55: 6-14.

Ribeiro LP, Akhtar Y, Vendramim JD, Isman MB. 2014a. Comparative bioactivity of selected seed extracts from Brazilian *Annona* species and an acetogenin-based commercial bioinsecticide against *Trichoplusia ni* and *Myzus persicae*. Crop Protection 62: 100-106.

Ribeiro LP, Mota LHC, D'Alessandro CP, Vendramim JD, Delalibera Jr I. 2014b. In vitro compatibility of an acetogenin-based bioinsecticide with three species of entomopathogenic fungi. Florida Entomologist 94: 1395-1403.

Ribeiro LP, Zanardi OZ, Vendramim JD, Yamamoto PT. 2014c. Comparative toxicity of an acetogenin-based extract and commercial pesticides against citrus red mite. Experimental and Applied Acarology 64: 87-98.

SAS Institute. 2011. Statistical analysis system: getting started with the SAS learning. Version 9.2. SAS Institute Inc., Cary, North Carolina, USA.

Schneider-Orelli O. 1947. Entomologisches Praktikum. Sauerlander, Aarau, Switzerland.

Shivankar VJ, Rao CN, Singh S. 2000. Studies on citrus psylla, *Diaphorina citri* Kuwayama: a review. Agricultural Reviews 21: 199-204.

Shivankar VJ, Rao CN, Singh S. 2003. Evaluation of plant based biopesticides against psylla, *Diaphorina citri* Kuwayama and *Mallada boninensis* Okamoto against insect pests of citrus *In* Koul O, Dhaliwal GS, Marwaha SS, Arora JK [eds.], Biopesticides and Pest Management. Campus Books International, New Delhi, India.

Skelley LH, Hoy MA. 2004. A synchronous rearing method for the Asian citrus psyllid and its parasitoids in quarantine. Biological Control 29: 14-23.

Teixeira DC, Saillard C, Eveillard S, Danett JL, Costa PI, Ayres AJ, Bové J. 2005. '*Candidatus* Liberibacter americanus', associated with citrus huanglongbing (greening disease) in São Paulo State, Brazil. International Journal of Systematic and Evolutionary Microbiology 55: 1857-1862.

Throne JE, Weaver DK, Chew VB, James E. 1995. Probit analysis of correlated data: multiple observations over time at one concentration. Journal of Economic Entomology 88: 1510-1512.

Tormo JR, Gallardo T, Aragón R, Cortes D, Estornell E. 1999. Annonaceous acetogenins as inhibitors of mitochondrial complex I. Current Topics in Phytochemistry 2: 69-90.

Turra C, Vian CEF, Nielsen FAG, Santos PS, Penteado LFF. 2014. Overview of the Brazilian citriculture certification. Journal of Agricultural and Environmental Ethics 27: 663-679.

Vale FXR, Fernandes-Filho E, Liberato JR. 2001. Quant — Quantificação de doenças: versão 1.0.1. Universidade Federal de Viçosa, Viçosa, Brazil. (In Portuguese)

Weathersbee III AA, McKenzie CL. 2005. Effect of neem biopesticide on repellency, mortality, oviposition and development of *Diaphorina citri* (Homoptera: Psyllidae). Florida Entomologist 88: 401-407.

Wenninger EJ, Hall DG. 2007. Daily timing of mating and age at reproductive maturity in *Diaphorina citri* (Hemiptera: Psyllidae). Florida Entomologist 90: 715-722.

Williams T, Arredondo-Bernal HC, Rodríguez-del-Bosque LA. 2013. Biological pest control in Mexico. Annual Review of Entomology 58: 119-140.

Zanardi OZ, Ribeiro LP, Ansante TF, Santos MS, Bordini GP, Yamamoto PT, Vendramim JD. 2015. Bioactivity of a matrine-based biopesticide against four pest species of agricultural importance. Crop Protection 67: 160-167.

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