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Toxicity of an azadirachtin-based biopesticide on *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) and its ectoparasitoid *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae)

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ABSTRACT

The bioactivity of an azadirachtin-based biopesticide (Azamax™ EC) was assessed on nymphs and adults of the Asian citrus psyllid *Diaphorina citri* Kuwayama using different product application methods. Its compatibility with the ectoparasitoid *Tamarixia radiata* (Waterston) was also assessed. Exposure of *D. citri* nymphs to azadirachtin by direct contact + residues, to dry residues or systemically led to mortality in laboratory bioassays. For adult psyllids, the mortality was at most 67% after 120 h of exposure to dry residues, even if using 16-fold more active ingredient than for nymphs. In the field, the biopesticide exhibited high efficacy (~90%) in controlling nymphs, similar to that achieved with the insecticide imidacloprid (Provado® 20 SC, at 40 mg a.i. L^{-1}) used as positive control. Azadirachtin was harmless to *T. radiata* larvae and pupae, but caused 77.8% adult mortality after 24 h of exposure. Despite the high toxicity to adults, the biopesticide had a low duration of harmful activity (~3 days). Given the high efficacy against *D. citri* nymphs, reduced effects on the immature stages of the ectoparasitoid *T. radiata* and low duration of harmful activity, the biopesticide is an important alternative for *D. citri* population management and can be used as a substitute or in rotation with current synthetic insecticides in integrated pest management programs.

1. Introduction

Huanglongbing (HLB), previously known as citrus greening, is one of the most important and destructive diseases of citriculture worldwide. In Brazil, the disease symptoms are associated with the bacteria "*Candidatus* Liberibacter americanus" and "*Candidatus* Liberibacter asiaticus", whose vector is the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), considered one of the most important crop pests in the country (Bové, 2006; Belasque-Jr. et al., 2010a,b). Due to the high susceptibility of cultivated citrus species to bacteria and ineffectiveness of curative methods biocontrol of the disease, HLB management has been performed proactively by planting healthy seedlings and, later, with the elimination of infected plants (reduction of inoculum) and insect vector control (Belasque-Jr. et al., 2010b).

Among the strategies for HLB management, application of synthetic insecticides of broad spectrum activity (particularly organophosphates, pyrethroids and neonicotinoids) has been the main tactic used by citrus growers to reduce population levels of the pest and prevent spread of the disease. However, the heavy use of pesticides can cause serious problems to human health and environmental contamination (Ribas and Matsumura, 2009). Moreover, overuse pesticide has caused the selection of resistant pests to the main active ingredients (Tiwari et al., 2011), increasing outbreaks of spider mites, scales and caterpillars (Cutler et al., 2009) due to biological changes, also known as the hormesis effect (characterized by inversion of the biological response as the reduction in development time, increase in fertility, longevity, etc.) caused by continuous exposure to sublethal doses of pesticides (Guedes and Cutler, 2014). Furthermore, the use of pesticides increases the mortality of natural enemies responsible for the natural control of

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these pests (Yamamoto and Bassanezi, 2003), increasing production costs and reducing the technique effectiveness and the environmental sustainability of production systems (Ribeiro et al., 2014).

Given these problems and the growing consumer demand for healthier food produced in low-input systems, the use of biological control agents, particularly the ectoparasitoid *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), has been an important alternative for *D. citri* population suppression, present in different production systems (Paiva and Parra, 2012), especially in those where the use of synthetic insecticide is not permitted (ecologically-based production system), and tactics for insect vector control are even scarce. *T. radiata* preferentially develops on *D. citri* nymphs from the third to fifth instar (Étienne et al., 2001). In addition to parasitism, adults can act as predators of eggs and nymphs of the first and second instars (Hoy and Nguyen, 2001), increasing the potential of this natural enemy as a biological control agent. Previous studies have revealed that the percentage of parasitism by *T. radiata*, ranging from 27.5 to 80% (Gómez-Torres et al., 2006), has reduced to a maximum of 25.7% with the increased use of insecticides to control the insect vector because of the progress of HLB spreading to new producing regions (Paiva and Parra, 2012). Therefore, the adoption of strategies that contribute to the preservation of *T. radiata* and other natural enemies in production areas is very important to allow biological control and reduce the problems associated with HLB.

In this context, crude extracts, fractions or compounds isolated from the secondary metabolism of plants have been explored as an alternative to replace or reduce the dependence on conventional synthetic insecticides for pest control in citrus groves (Weathersbee and McKenzie, 2005). Despite the high efficacy, a very limited number of biopesticides has been used in field conditions (Isman and Grieneisen, 2014), due to the lack of quality, stability and standardization of these compounds in the environment (Corrêa and Salgado, 2011). The recent promulgation and implementation of a specific legislation for the registration of products for use in organic agriculture (Brasil, 2009) is enabling the registration of new biopesticides based on the Brazilian flora (Zanardi et al., 2015). However, before any recommendation, it is necessary to thoroughly evaluate these products on the target species under different conditions (laboratory, semi-field and field) and their interactions in the different trophic levels of the distinct agroecosystems, guaranteeing the sustainability and credibility of the technology.

Among the bioactive plant compounds, azadirachtin, abundantly found in *Azadirachta indica* A. Juss (Meliaceae) (a plant commonly known as neem), has demonstrated high potential for use against pests of agricultural importance in different production systems due to its high insecticide and acaricide activities and rapid degradation in the environment (Charbonneau et al., 2007; Grimalt et al., 2011; Biondi et al., 2012). Azadirachtin, a limonoid with different modes of action, acts mainly as a repellent, antifeedant and insect growth inhibitor, and it interferes with the mating behaviour, fecundity and fertility of female arthropod pests with different eating habits (Weathersbee and McKenzie, 2005; Abedi et al., 2014; Sánchez-Ramos et al., 2014). Besides activity against arthropod pests, azadirachtin-based products may also cause deleterious effects on natural enemies, especially parasitoids. Lyons et al. (2003) found that the use of azadirachtin on eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) parasitized by *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae), significantly reduced the females survival. Ruiu et al. (2008) demonstrated that azadirachtin-based formulation (Oikos 25 Plus, at 20 mg a.i. L^{-1}) caused a slight reduction in their lifespan (25.1% and 15.7%, males and females, respectively) and reproduction rate (27.5%) of *Muscidifurax raptor* Girault (Hymenoptera: Pteromalidae), whereas Abedi et al. (2014) found that the emergence rate of *Habrobracon hebetor* Say (Hymenoptera: Braconidae) was reduced to 39.0% when larvae were dipped in solutions of azadirachtin-based biopesticides (NeemGuard and BioNeem, at 43.5 and 10.2 mg a.i. L^{-1}). However, the lethal and sublethal effects of azadirachtin-based biopesticides on the *T. radiata* ectoparasitoid have not been investigated.

Currently, several azadirachtin-based commercial formulations are commercially available for use in agriculture. In Brazil, the biopesticide is available in an emulsifiable concentrate formulation [Azamax™ EC, UPL – United Phosphorus do Brazil Ltda., Indianápolis, São Paulo, Brazil (http://www.uplbrasil.com.br/)], and it is authorized for use in the control of arthropod pests in different crops and production systems (Agrofit, 2014). Moreover, the biopesticide is certified by the Biodynamic Institute (IBD) for use in organic production systems without a withdrawal period, allowing its application during fruit harvest, without the risk of leaving toxic residues in the final product. Despite the large number of published studies demonstrating the efficacy of azadirachtin-based biopesticides on arthropod pests, few studies have demonstrated the biopesticide action on *D. citri* nymphs and adults and its toxicity on the ectoparasitoid *T. radiata*. Considering the potential association of the biopesticide with *T. radiata* release for the management of vector *D. citri* in citrus groves, the objective of this study was to evaluate the toxicity of the azadirachtin-based formulation on nymphs and adults of the Asian citrus psyllid and assess its compatibility with the ectoparasitoid *T. radiata*.

2. Materials and methods

2.1. Insects

The insects used in the bioassays were from populations under laboratory conditions [temperature of 26 ± 2 °C, relative humidity (RH) of 70 ± 10% and 14 L: 10 D photoperiod]. For the rearing of *D. citri*, seedlings of *Murraya paniculata* (L.) Jack of the Rutaceae family and commonly known as orange Jasmine were used as the substrate for feeding nymphs and adults and oviposition of the females, as described by Nava et al. (2007). For multiplication of *T. radiata*, orange Jasmine seedlings were used and were infested with 4^{th} and 5^{th} instar nymphs of *D. citri* (host for the immature stage development) and honey to feed the adults as described by Gómez-Torres et al. (2012).

2.2. Biopesticide characterization

The biopesticide assessed was available as an emulsifiable concentrate formulation (Azamax™ EC, 11.4 g a.i. L^{-1}) containing limonoids, azadirachtin (main component) and 3-tigloylazadirachtol, at concentrations of 8.9 and 2.5 g L^{-1}, respectively. The content of these compounds was previously measured by liquid chromatography and mass spectroscopy analyses using the method described by Forim et al. (2010). The concentrations tested in different bioassays were defined based on the total composition of biopesticide (Azamax™ EC, at 11.4 g a.i. L^{-1}).

2.3. Bioassays

All of the laboratory bioassays were conducted under controlled conditions (temperature of 26 ± 2 °C, RH 70 ± 10% and 14 L: 10 D h photoperiod). The field bioassays (efficacy on *D. citri* nymphs and persistence on *T. radiata* adults) were conducted in a commercial orchard.

2.3.1. Systemic action of the biopesticide on D. citri nymphs and adults

The systemic toxicity of the biopesticide was evaluated on 3rd instar nymphs and adults of *D. citri* at 5–10 days of age. To this, seedlings of Rangpur lime [*Citrus reticulata* Blanco × *Citrus sinensis* (L.) Osbeck (Rutaceae)] grown in 150 mL plastic containers, containing Multiplant Citrus® [Terra do Paraíso: Substrato para Plantas, Holambra, SP (http://agroserra-rs.com.br/substratos-terra-do-paraiso.html)] substrate, were previously pruned at 5 cm to stimulate growth. After the emergence of sprouts (2–3 cm length), the seedlings were kept for 24 h without irrigation. Thereafter, a systemic application (drench) was performed with 15 mL of the solution at different treatment levels. The biopesticide concentrations tested were: 2.9, 5.7, 11.4, 22.9 and 45.8 mg a.i. L^{-1} for nymphs and 45.8, 91.5, 183, 366 and 732 mg a.i. L^{-1} for adults, both defined based on previous tests. Deionized water used to solubilize the biopesticide was considered the control.

After application of the treatments, the seedlings were taken to the greenhouse and irrigated daily with 5 mL of deionized water for 120 h. After this period, each seedling was infested with 10 *D. citri* nymphs or adults. Twelve replicates were used for nymphs ($n = 120$) and six replicates for adults ($n = 60$) at each treatment level. Thereafter, the seedlings were placed in cages (experimental units) made with 2 L transparent polyethylene terephthalate (PET) bottles with a front opening of approximately 100 cm^2, coated with voile tissue to allow gas exchange and prevent excess moisture. These bottles were mounted on plastic containers containing a polystyrene disc at the top with a central orifice (where the plastic container containing the seedling was attached to) and deionized water (200 mL) to maintain the plant turgor. The mortality of the nymphs and adults in each experimental unit was assessed after 72 and 120 h of infestation. The nymphs and adults that showed no reaction to the touch of a fine brush were considered dead. Based on the mortality data of nymphs and adults of *D. citri* obtained in the different treatment levels, the systemic acute toxicity of the biopesticide was estimated. The estimated of the LC_{50} and LC_{90} (concentrations required to kill 50% and 90% of the population) were used as a criterion to determine the systemic acute toxicity on *D. citri* nymphs. However, due to the low mortality of *D. citri* adults, it was not possible to estimate LC_{50} and LC_{90} values for this development stage of the insect. In this case, for acute toxicity of the azadirachtin-based biopesticide the corrected mortality was calculated based on the number of dead insects at different concentrations of product and in the control (deionized water) using the Abbott (1925) formula.

2.3.2. Residual contact action of the biopesticide on D. citri nymphs and adults

To evaluate the residual toxicity of the biopesticide on *D. citri* 3rd instar nymphs and adults at 5–10 days of age, Rangpur lime seedlings were grown in tubes (50 mL), containing Multiplant Citrus® [Terra do Paraíso: Substrato para Plantas, Holambra, SP (http://agroserra-rs.com.br/substratos-terra-do-paraiso.html)] substrate and used as experimental unit. Initially, the seedlings were pruned at 5 cm to stimulate growth. Upon the emergence of sprouts (2–3 cm in length), the seedlings were subjected to 2 mL of spray solution using a microatomizer coupled to a pneumatic pump adjusted to provide a pressure of 0.7 kg cm^{-2}. The biopesticide was solubilized in deionized water at concentrations of 2.9, 5.7, 11.4, 22.9 and 45.8 mg a.i. L^{-1} for nymphs and 45.8, 91.5, 183, 366 and 732 mg a.i. L^{-1} for adults; a control treatment (deionized water) was also used.

After application of the treatments, the seedlings were kept under controlled conditions (temperature of 26 ± 2 °C, RH 70 ± 10%) for 2 h to dry the residues. After this period, 10 psyllid nymphs or adults were transferred from the breeding cage to each seedling. Twelve replicates were used for nymphs ($n = 120$) and six replicates for adults ($n = 60$) for each treatment level. After infestation, the seedlings were kept under controlled conditions and mortality of nymphs and adults were evaluated 72 and 120 h after the infestation using the same procedure and criteria described in item 2.3.1. Based on the mortality data obtained in the different treatment levels, the lethal concentrations (CL_{50} and CL_{90}) for nymphs and the corrected mortality to *D. citri* adults exposed to residual of biopesticide were estimated as described in item 2.3.1.

2.3.3. Direct + residual contact action of the biopesticide on D. citri nymphs

The direct + residual toxicity of the biopesticide was assessed on *D. citri* nymphs. To achieve this, Rangpur lime seedlings containing 2- to 3-cm-length sprouts were previously infested with 10 3rd instar nymphs of the psyllid and were used as the experimental unit. Twenty-four hours after infestation, the experimental units (seedlings containing the nymphs) were sprayed with 2 mL of solution at the same concentrations of the biopesticide and control described in item 2.3.2.

The other procedures and number of replicates were the same as described in item 2.3.1. Based on the mortality data obtained in the different treatment levels, the lethal concentrations (CL_{50} and CL_{90}) for nymphs and the corrected mortality to *D. citri* adults exposed to residual of biopesticide were estimated as described in item 2.3.1.

2.3.4. Biopesticide efficacy on D. citri nymphs in the field

Biopesticide efficacy was assessed in a commercial groves of Pera-Rio sweet orange [*C. sinensis* (L.) Osbeck (Rutaceae)], grafted on Rangpur lime, at four years of age in the municipality of Mogi Mirim (latitude 22°32′41″ S; longitude 43°07′44″ W), São Paulo, Brazil, with natural infestation of *D. citri*.

Prior to the application of treatments, branches infested with *D. citri* nymphs were marked and the insects of each branch were counted to assess the initial infestation level of the pest. Next, the plants were sprayed with a biopesticide concentration of 2.9 mg a.i. L^{-1} with a Jacto® backpack sprayer equipped with a full cone nozzle (FL-5VS) to the run off point. The activity of the biopesticide was compared with a negative (deionized water) and a positive control [imidacloprid insecticide (Provado® 20 SC, at 40 mg a.i. L^{-1}) – Bayer CropScience Ltda., São Paulo, SP]. This insecticide is registered with the Ministry of Agriculture, Livestock and Supply (MAPA) and has been widely used for *D. citri* management in Brazilian citrus groves (Agrofit, 2014). Four replicates (50 plants $replicate^{-1}$) were used for each treatment.

The evaluation was performed 72 h after application of the treatments, counting the live nymphs on each branch. The efficacy of the biopesticide was calculated based on the average number of nymphs recorded in the pre-sampling (time 0) and after 72 h of the application of the treatments using the formula of Henderson and Tilton (1955). The instantaneous rate of population growth (*ri*) was calculated using the following formula: $ri = \ln(Nf/No)/\Delta t$ where: Nf = final number of live nymphs in the final assessment (72 h), No = initial number of live nymphs in the pre-sampling (time 0), and Δt = time (h) between the pre-sampling and final assessment (Stark and Banks, 2003). Positive and negative *ri* values indicate an increase and a decrease in the population, respectively, whereas $ri = 0$ values indicate that the population has remained stable during the assessment period.

2.3.5. Biopesticide toxicity against T. radiata larvae and pupae

For this assessment, orange Jasmine branches infested with 4th and 5th instar nymphs of *D. citri* from the rearing kept in the

laboratory were placed on shoots of seedlings of this plant and were grown in plastic containers (50 mL) for spontaneous migration of nymphs. After 24 h, the branches were removed, and the nymphs were counted on each seedling. Subsequently, each seedling with nymphs was put in a cage (experimental unit), and a female *T. radiata* were released for each 10 *D. citri* nymph. The parasitoid females remained in contact with the *D. citri* nymphs for 48 h for parasitism. After this period, the parasitoid females were removed, and the nymphs were kept in their cages. After four (effect on larvae) and nine (effect on pupae) days from the removal of the parasitoid females, the parasitized (mummified) *D. citri* nymphs were counted and submitted to the biopesticide spray at a concentration of 2.9 mg a.i. L^{-1} (corresponding to the LC_{90} estimated to *D. citri* nymphs in item 2.3.3). The biopesticide toxicity was compared with the same controls mentioned in item 2.3.4. Treatments were applied as per item 2.3.2. Each treatment was repeated 10 times. The assessment was performed after 10 (effect on larvae) and 5 (effect on pupae) days following the treatments, counting the emerged parasitoids in each experimental unit. The toxicity of the products on larvae and pupae was calculated based on the number of parasitoids emerged in the treatments and negative control (deionized water) using the formula of Henderson and Tilton (1955).

2.3.6. Acute toxicity of the biopesticide on T. radiata adults

The acute toxicity of the biopesticide on *T. radiata* adults was assessed using the residual contact bioassay. To that end, 3.5-cm-diameter discs of Pera-Rio sweet orange leaves were sprayed with 2 mL of each treatment solution in a Potter tower (Burkard Scientific Co., Uxbridge, UK), adjusted at the pressure of 0.7 kg cm^{-2}, to obtain a spray deposition of 1.8 ± 0.1 mg cm^{-2} according to the criteria established by Pesticides and Beneficial Organisms working group of the International Organization for Biological Control of Noxious Animals and Plants, West Palaearctic Regional Section (IOBC/WPRS) for toxicity studies of pesticides on natural enemies (Hassan et al., 1994). The biopesticide was applied at a concentration of 2.9 mg a.i. L^{-1} and compared with the same controls mentioned in item 2.3.4. After application of the treatments, the discs were maintained under controlled temperature conditions for 2 h to dry the residues and then were placed in Petri dishes (3.5-cm diameter) containing a non-gelified layer of agar (2.5% w/v in water). After gelification, 10 parasitoid adults up to 48 h of age were transferred from rearing to each dish (experimental unit). The dishes were sealed with voile fabric to allow gas exchange and avoid excess moisture. A droplet of honey (~1 mm^3) was placed on the voile that served as food to the parasitoids during the assay period. Each treatment was repeated 10 times ($n = 100$).

Mortality of *T. radiata* adults in each experimental unit was assessed 24 h after infestation, and insects that had no reaction to the touch of a fine brush were considered dead. Based on the mortality data, the acute toxicity was estimated for each treatment using the Abbott (1925) formula.

2.3.7. Duration of the harmful activity of biopesticide on T. radiata adults

Due to the high acute toxicity of the biopesticide on *T. radiata* adults in the laboratory, the duration of the harmful activity of the product was assessed in a greenhouse, as recommended by the IOBC/WPRS (Van de Veire et al., 2002). For this, 10 seedlings of Pera-Rio sweet orange trees, grown in pots (10 L), were sprayed to the run off point with the biopesticide at a concentration of 2.9 mg a.i. L^{-1}. The duration of the harmful activity was compared with the same controls mentioned in item 2.3.4. After 1, 3, 7, 10, 17, 24 and 31 days of treatment application (residual time), a treated leaf of each seedling was randomly removed, brought to the laboratory and sectioned in 3.5-cm diameter discs (experimental units), as described in item 2.3.6. Each experimental unit was infested with 10 parasitoid adults up to 48 h of age. Ten replicates ($n = 100$) were used for each treatment and residual time. Mortality of adults in each experimental unit was assessed after 24 h. The mortality data obtained in each treatment (azadirachtin-based and imidacloprid) and residual times were corrected for the negative control using the Abbott (1925) formula.

2.3.8. Data analysis

The experimental design used in all of the bioassays was completely randomized. Generalized linear models (Nelder and Wedderburn, 1972) using the quasi-binomial, quasi-Poisson and Gaussian distributions were used for data analysis of the mortality rate of the insect vector *D. citri* and parasitoid *T. radiata*, counting of *D. citri* nymphs (field test) and the instantaneous rate of population growth, respectively. The verification of the adjustment quality was performed using the normal-probability chart with a simulation envelope (Hinde and Demétrio, 1998). When significant differences were found between treatments, multiple comparisons (Tukey test, $p < 0.05$) were performed using the "*glht*" function of the "*multcomp*" package with adjustment of p values for treatments with qualitative levels, while linear and nonlinear regressions were used to compare treatments with quantitative levels, subsequently, by assessing the adjustment quality. All of these analyses were performed using "*R*" statistical software, version 2.15.1 (R Development Core Team, 2012).

The acute toxicity of the biopesticide on *D. citri* nymphs in the different bioassays and exposure times were determined based on the estimate of LC_{50} and LC_{90} of product. For this, a binomial model was used to complement the log−log link function (gompit model) using the *Probit Procedure* of the SAS software, version 9.2 (SAS Institute, 2011).

3. Results

Different levels of biopesticide toxicity occurred for *D. citri* nymphs depending on the form of application, concentration and exposure time of the insects. The highest mortality rates were found in nymphs that were sprayed and maintained on residues (direct + residual contact) of the biopesticide, followed by those exposed only to residual contact and systemic application (drench) (Table 1). Considering the relative toxicity (RT) of the biopesticide azadirachtin-based, our results showed that the *D. citri* nymphs treated directly with the product and kept on the dry residues of product (direct contact + residual) were more susceptible than those who were exposed only to contact with the residues (contact residual) or kept on plants treated systemically with the biopesticide (Table 1). Moreover, the product showed greater toxicity to *D. citri* nymphs than to adults. The 16-fold increase in the biopesticide concentrations caused a maximum 10% mortality of adults and was similar to that for control treatment (data not shown).

In a test conducted in commercial sweet orange groves with natural pest infestation, the biopesticide caused a significant reduction in the population of nymphs, 72 h after application of the product, demonstrating similar efficacy to that of the positive control [imidacloprid (Provado® 20 SC, at 40 mg a.i. L^{-1})] (Table 2).

Despite the high toxicity observed on *D. citri* nymphs in laboratory studies and proven efficacy in field conditions, the biopesticide was considered harmless for larvae and pupae of the ectoparasitoid *T. radiata* compared with the positive control (Table 3). Furthermore, our results revealed that the biopesticide did not significantly affect the rate of emergence of the parasitoid even with the death of the host (*D. citri* nymphs). Conversely, exposure of *T. radiata* adults to the product residues caused high

Table 1
Estimate of LC_{50} and LC_{90} (in mg a.i. L^{-1}) along with confidence interval of an azadirachtin-based biopesticide for *Diaphorina citri* nymphs under different forms of application and exposure times.

Application	Exposure time (h)	N[a]	Slope ± SE (p value)	LC_{50} (CI)[b]	LC_{90} (CI)[b]	χ^{2}[c] (d.f. = 3)	h[d]	RT[e]
Systemic (drench)	72	720	6.12 ± 1.25 ($p < 0.0001$)	5.4 (4.8–6.8)	8.5 (6.8–14.0)	2.274	0.758	0.18
	120	720	5.39 ± 1.49 ($p = 0.0003$)	4.9 (3.6–16.1)	7.4 (3.5–24.6)	7.495	2.498	0.16
Residual contact	72	720	1.66 ± 0.22 ($p < 0.0001$)	2.0 (1.6–2.6)	10.7 (7.3–19.3)	2.595	0.865	0.50
	120	720	1.21 ± 0.15 ($p < 0.0001$)	0.9 (0.6–1.3)	9.3 (6.1–18.3)	6.196	2.066	0.89
Direct contact + residual	72	720	2.62 ± 0.34 ($p < 0.0001$)	1.0 (0.6–1.4)	2.9 (2.0–5.5)	3.014	1.004	1.00
	120	720	2.79 ± 0.42 ($p < 0.0001$)	0.8 (0.4–1.2)	2.3 (1.6–4.7)	7.044	2.348	1.00

[a] N: number of insects tested.
[b] CI: confidence interval at 95% of probability of error.
[c] χ^{2}: Pearson' chi-squared value.
[d] h: heterogeneity factor.
[e] RT: relative toxicity [LC_{50} (systemic or residual contact)/LC_{50} (direct contact + residual)].

Table 2
Efficacy of azadirachtin-based biopesticide and imidacloprid control of *D. citri* nymphs in commercial sweet orange orchards.

Treatment	Concentration (mg a.i. L^{-1})	EC (%)[a]	Instantaneous population growth rate (ri)[b]
Azadirachtin-based biopesticide	2.9	89.7 ± 6.63 a	−2.50 ± 0.73 b
Imidacloprid (Provado® 20 SC)	40.0	92.6 ± 5.42 a	−1.96 ± 0.65 b
Control (deionized water)	–	4.3 ± 1.79 b	−0.47 ± 0.22 a
F		28.579	66.080
d.f.		2, 9	2, 9
p value		<0.0001	<0.0001

[a] Efficacy of control calculated using the Henderson and Tilton formula (1955).
[b] Means followed by the same letter in the column do not differ significantly (GLM with Gaussian distribution, followed by *post hoc* Tukey test, $p < 0.05$).

Table 3
Mortality of larvae, pupae and adults of *Tamarixia radiata* treated with insecticides.

Treatment	Concentration (mg a.i. L^{-1})	Mortality (%)[a]		
		Larvae	Pupae	Adults
Azadirachtin-based biopesticide	2.9	0.9 ± 0.37 b	4.6 ± 2.10 b	77.8 ± 4.51 a
Imidacloprid (Provado® 20 SC)	40	93.3 ± 3.79 a	62.9 ± 4.12 a	88.9 ± 5.67 a
Control (deionized water)	–	1.0 ± 0.22 b	3.3 ± 0.68 b	4.2 ± 0.98 b
F		9.087	6.336	67.245
d.f.		2, 27	2, 27	2, 27
p value		0.0011	0.0043	<0.0001

[a] Means followed by the same letter in the columns do not differ significantly (GLM with quasi-binomial distribution, followed by *post hoc* Tukey test, $p < 0.05$).

mortality (77.8%) similar to that observed with imidacloprid (Table 3). Despite its high toxicity, the biopesticide showed a rapid decrease in insecticidal activity over time (Fig. 1).

4. Discussion

In the laboratory, our results showed that the insecticidal activity of the biopesticide was more pronounced for *D. citri* nymphs than for adults, which can be assigned to the action mode/mechanism of the biopesticide. Azadirachtin-based products act by inhibiting the production of juvenile hormones and ecdysone, which is responsible for the growth of insects, causing high mortality rates when applied to immature ones (Coelho et al., 2006; Ghazawy et al., 2010). Despite the lower activity observed in *D. citri* adults in this study, azadirachtin-based products act as sterilizing, repellents, antifeedants and oviposition deterrents (Weathersbee and McKenzie, 2005; Isman, 2006), which can assist in reducing population levels of the pest, as well as reduce the dissemination of the disease in the next generations. However, sublethal effects of azadirachtin-based biopesticides on *D. citri* adults should be investigated with higher accuracy before being is recommended in the field.

Among the biopesticide application strategies, the highest values of mortality were obtained in insects exposed to direct + residual contact and residual contact of the product. Similarly, Tomé et al. (2013) while assessing the bioactivity of an azadirachtin-based formulation (Azamax™ 1.2 EC, at 6.0 mg a.i. L^{-1}) on 2nd instar larvae of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), found that the mortality was higher than 75% when insects were exposed to residual contact of the biopesticide. A high mortality of 2nd instar nymphs of *Toxoptera citricida* (Kirkaldy) (Hemiptera: Aphididae) was also reported by Tang et al. (2002) in insects exposed to residual contact of the other azadirachtin-based formulation (Neemix® 4.5 EC, at 250 mg a.i. L^{-1}). Mortality rates from 76.6 to 100% were observed in *Liriomyza sativae* (Blanchard) (Diptera: Agromyzidae) larvae exposed to direct + residual contact of azadirachtin (NeemAzal®-T/S 1 EC, at 50 and 100 mg a.i. L^{-1}, respectively) (Hossain and Poehling, 2009). Therefore, our results indicate that the efficiency of biopesticide is associated with the insect exposure mode to the active ingredients of the product. In this context, the higher insecticidal activity of biopesticide is obtained when the product is sprayed on *D. citri* nymphs.

The systemic action of the biopesticide against *D. citri* nymphs was also observed, demonstrating that bioactive compounds of the product were absorbed and transferred to the tissues of the plant canopy. The systemic action of azadirachtin-based biopesticides has already been demonstrated for several insect pests such as *Bemisia tabaci* (Gennadius) biotype B (Hemiptera: Aleyrodidae),

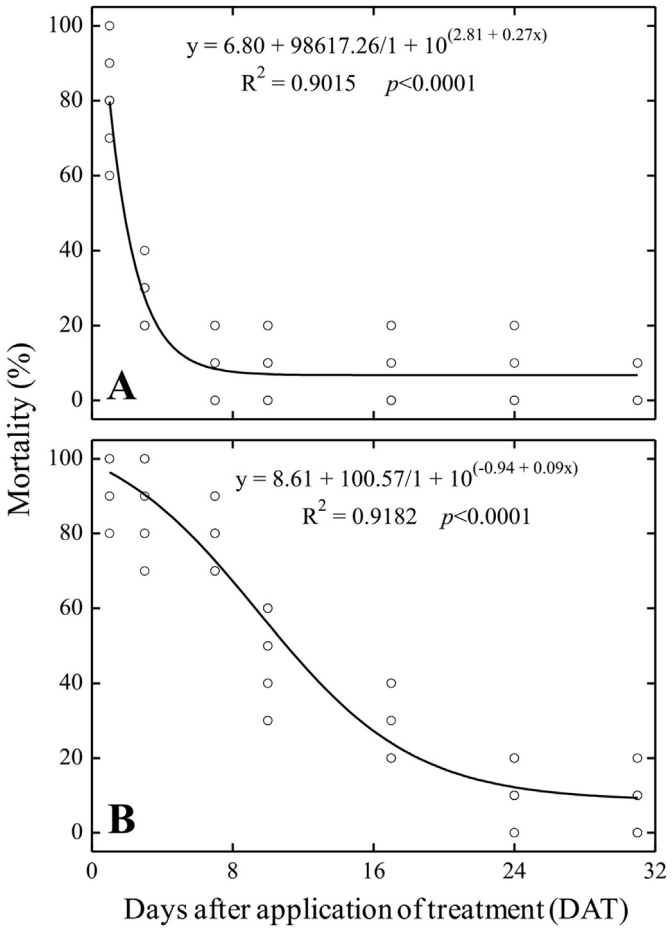

Fig. 1. Mortality of *Tamarixia radiata* adults after 24 h of exposure to azadirachtin-based biopesticide (2.9 mg a.i. L^{-1}) (A) and imidacloprid (Provado® 20 SC, at 40 mg a.i. L^{-1}) (B) with different residual times. For each treatment ten replicates were used with 10 adults of *T. radiata* of up to 48 h of age ($n = 100$). In the control treatment, mortality was less than 6%.

Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae) and *Aphis craccivora* Koch (Hemiptera: Aphididae) (Souza and Vendramim, 2005; Thoeming et al., 2006; Costa et al., 2010). Biopesticides that have systemic action are important for pest management programs as they provide greater protection time of the plants, due to the lower action of environmental factors (mainly ultraviolet radiation and temperature) that act in the degradation of the active components of biopesticides (Turek and Stintzing, 2013), and reduce the effects on non-target organisms (Yamamoto and Bassanezi, 2003). Therefore, this strategy may be an important alternative for *D. citri* management in the seedling production system and installation of new groves.

In the field, the biopesticide was highly effective against *D. citri* nymphs (efficacy ~ 90%), being equivalent to the positive control [imidacloprid (Provado® 20 SC, at 40 mg a.i. L^{-1})]. The efficacy of an azadirachtin-based formulation (NeemAzal®-T/S 1 EC, at 100 mg a.i. L^{-1}) comparable to triazophos phosphorus insecticide was observed by Ulrichs et al. (2001) in *A. craccivora* exposed to direct + residual contact of the product. Likewise, Dhingra et al. (2008), while assessing the efficacy of the other azadirachtin-based formulation (NeemAzal® T/S 1 EC, at 20 mg a.i. L^{-1}) on several okra key pests [*Abelmoschus esculentus* (L.) Moench (Malvaceae)], found that the biopesticide was similar to organochlorine endosulfan (Thiodan® 35 EC, at 500 mg a.i. L^{-1}). These results demonstrate that the biopesticide is effective and can be used as an alternative to synthetic insecticides for the management of *D. citri* nymphs in different production systems.

In addition to the promising insecticidal action against *D. citri* nymphs, the biopesticide was considered harmless (<25% reduction in survival rate) to *T. radiata* larvae and pupae, according to the criteria established by the IOBC/WPRS (Van de Veire et al., 2002). Similarly, Luna-Cruz et al. (2011) found that, when the pupae of the ectoparasitoid *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) was exposed to azadirachtin (PHC Neem® 3.2 EC) at different concentrations (156, 234 and 312 mg a.i. L^{-1}), there was no effect on the emergence of insects. Likewise, Aggarwal and Brar (2006) found that direct application of azadirachtin solutions (NeemAzal®-T/S 1 EC), at concentrations of 200 and 400 mg a.i. L^{-1}, on larvae of *Encarsia sophia* Girault & Dodd (Hymenoptera: Aphelinidae), did not affect the rate of emergence of the parasitoid. Although *T. radiata* is considered an ectoparasitoid, which could increase the susceptibility of immature stages to the pesticides used in pest management, the females lay their eggs at the bottom of the host body, whose integument serves as a physical barrier of protection for larvae and pupae of the parasitoid.

On the other hand, the exposure of *T. radiata* adults to dry residues of azadirachtin-based biopesticide indicated that the product was highly harmful (>75% mortality, IOBC/WPRS) to ectoparasitoid, being similar to the insecticide imidacloprid used as a positive control. Likewise, Stara et al. (2011) reported 100% mortality after 48 h in adults of *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae) exposed to azadirachtin residues (NeemAzal®-T/S, at 10 mg a.i. L^{-1}). However, Luna-Cruz et al. (2011) found that azadirachtin did not change the survival rate of *T. triozae* adults after 24 h of exposure to *Bactericera cockerelli* nymphs (Sulc.) (Hemiptera: Triozidae) and plants treated with different PHC Neem 3.2 EC concentrations (156, 234 and 312 mg a.i. L^{-1}). The different toxicity levels of azadirachtin-based biopesticides are associated with genetic differences, dosage, time and form of exposure of the insects to residues and mainly to the formulation used in bioassays (Cosme et al., 2009). Despite the high toxicity observed in *T. radiata* adults in the present study, the duration of the harmful activity of the biopesticide was rapidly reduced (~3 days) being considered short-lived (<5 days), according to the IOBC/WPRS. The rapid degradation of azadirachtin-based biopesticides was demonstrated by Sundaram and Curry (1994), when they found that the half-life values of azadirachtin in seedlings of *Quercus rubra* L. (Fagaceae) and *Abies balsamea* (L.) Mill. (Pinaceae) were 12 and 22 h, respectively. The low persistence of azadirachtin-based products is assigned to the sensitivity of the active principles to ultraviolet and visible radiation and temperature, which are considered the main factors of degradation of natural products (Turek and Stintzing, 2013).

Although the low residual effect is considered a disadvantage by the need for more frequent re-application of the biopesticide for pest control, this characteristic allows its use in pre-harvest, a situation where there is a need to use products with a reduced withdrawal period. Furthermore, this characteristic reduces the potential selection of insect populations resistant to the biopesticide (Georghiou and Taylor, 1977). Moreover, the low duration of harmful activity of the formulation reduces the impact on biological control agents present in the production areas due to the shorter action period of the active ingredients of the product, allowing the recolonization and establishment of ecological balance of agroecosystems. The low duration of harmful activity of the product allows the mass release of biological control agents, particularly *T. radiata*, in a short period of time after its use. Given the high efficacy of the biopesticide on *D. citri* nymphs (~90%), the reduced effects on the immature stages (≤4.6% of reduction in the survival of larvae and pupae) of ectoparasitoid *T. radiata* and low duration of

harmful activity of the product (causing ~ 30% of adult mortality, 3 days after application), the biopesticide Azamax™ EC (azadirachtin- and 3-tigloylazadirachtol-based), assessed in this study, is an important alternative for *D. citri* population management and can be used as a substitute or in rotation with current synthetic insecticides in integrated pest management programs.

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References

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265–267.

Abedi, Z., Saber, M., Gharekhani, G., Mehrvar, A., Kamita, S.G., 2014. Lethal and sublethal effects of azadirachtin and cypermethrin on *Habrobracon hebetor* (Hymenoptera: Braconidae). J. Econ. Entomol. 107, 638–645.

Aggarwal, N., Brar, D.S., 2006. Effects of different neem preparations in comparison to synthetic insecticides on the whitefly parasitoid *Encarsia sophia* (Hymenoptera: Aphelinidae) and the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae) on cotton under laboratory conditions. J. Pest Sci. 79, 201–207.

Agrofit, 2014. Sistema de Agrotóxicos Fitossanitários – Ministério da Agricultura, Pecuária e Abastecimento, Brasil. http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons (accessed 14.01.14.).

Belasque Jr., J., Bassanezi, R.B., Yamamoto, P.T., Ayres, A.J., Tachibana, A., Violante, A.R., Tank Jr., A., Di Giorgi, F., Tersi, F.E.A., Menezes, G.M., Dragone, J., Jank Jr., R.H., Bové, J.M., 2010a. Lesson from huanglongbing management in São Paulo State, Brazil. J. Plant Pathol. 92, 285–302.

Belasque Jr., J., Yamamoto, P.T., Miranda, M.P., Bassanezi, R.B., Ayres, A.J., Bové, J.M., 2010b. Controle do huanglongbing no estado de São Paulo. Bras. Citrus Res. Technol. 31, 53–64.

Biondi, A., Desneux, N., Siscaro, G., Zappalà, L., 2012. Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. Chemosphere 87, 803–812.

Bové, J.M., 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. J. Plant Pathol. 88, 7–37.

Brasil, 2009. Decreto No. 6.913, de 23 de julho de 2009. http://www.planalto.gov.br/ccivil_03/_Ato2007-2010/2009/Decreto/D6913.htm (accessed 15.03.15.).

Charbonneau, C., Côté, R., Charpentier, G., 2007. Effects of azadirachtin and of simpler epoxy-alcohols on survival and behaviour of *Galleria mellonella* (Lepidoptera). J. Appl. Entomol. 131, 447–452.

Coelho, C.A.A., Souza, N.A., Feder, M.D., Silva, C.E., Garcia, E.S., Azambuja, P., Gonzalez, M.S., Rangel, E.F., 2006. Effects of azadirachtin on the development and mortality of *Lutzomyia longipalpis* larvae (Diptera: Psychodidae: Phlebotominae). J. Med. Entomol. 43, 262–266.

Corrêa, J.C.R., Salgado, H.R.N., 2011. Atividade inseticida das plantas e aplicações: revisão. Rev. Bras. Plantas Med. 13, 500–506.

Cosme, L.V., Carvalho, G.A., Moura, A.P., Parreira, D.S., 2009. Toxicidade de óleo de nim para pupas e adultos de *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae). Arq. Inst. Biol. 76, 233–238.

Costa, J.V.T.A., Bleicher, E., Cysne, A.Q., Gomes, F.H.T., 2010. Óleo e extrato aquoso de sementes de nim, azadiractina e acefato no controle do pulgão-preto do feijão-de-corda. Pesq. Agropec. Trop. 40, 238–241.

Cutler, G.C., Ramanaidu, K., Astatkie, T., Isman, M.B., 2009. Green peach aphid, *Myzus persicae* (Hemiptera: Aphididae), reproduction during exposure to sublethal concentrations of imidacloprid and azadirachtin. Pest Manag. Sci. 65, 205–209.

Dhingra, S., Walia, S., Kumar, J., Singh, S., Singh, G., Parmar, B.S., 2008. Field efficacy of azadirachtin-A, tetrahydroazadirachtin-A, NeemAzal® and endosulfan against key pests of okra (*Abelmoschus esculentus*). Pest Manag. Sci. 64, 1187–1194.

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

Forim, M.R., Fernandes, M.F.G., Cass, Q.B., Fernandes, J.B., Vieira, P.C., 2010. Simultaneous quantification of azadirachtin and 3-trigloylazadirachtol in Brazilian seeds and oil of *Azadirachta indica*: application to quality control and marketing. Anal. Methods 2, 860–869.

Georghiou, G.P., Taylor, C.E., 1977. Operational influences in the evolution of insecticide resistance. J. Econ. Entomol. 70, 653–658.

Ghazawy, N.A., Awad, H.H., Rahman, K.M.A., 2010. Effects of azadirachtin on embryological development of the desert locust *Schistocerca gregaria* Forskål (Orthoptera: Acrididae). J. Orthopt. Res. 19, 327–332.

Gómez-Torres, M.L., Nava, D.E., Gravena, S., Costa, V.A., Parra, J.R.P., 2006. Registro de *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) em *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) em São Paulo. Bras. Rev. Agric. 81, 112–117.

Gómez-Torres, M.L., Nava, D.E., Parra, J.R.P., 2012. Life table of *Tamarixia radiata* (Hymenoptera: Eulophidae) on *Diaphorina citri* (Hemiptera: Psyllidae) at different temperatures. J. Econ. Entomol. 105, 338–343.

Grimalt, S., Thompson, D., Chartrand, D., Mcfarlane, J., Helson, B., Lyons, B., Meating, J., Scarr, T., 2011. Foliar residue dynamics of azadirachtins following direct stem injection into white and green ash trees for control of emerald ash borer. Pest Manag. Sci. 67, 1277–1284.

Guedes, R.N.C., Cutler, G.C., 2014. Insecticide-induced hormesis and arthropod pest management. Pest Manag. Sci. 70, 690–697.

Hassan, S.A., Bigler, F., Bogenschutz, H., Boller, E., Brun, J., Calis, J.N.M., Coremans-Pelseneer, J., Duso, C., Grove, A., Heimbach, U., Helyer, N., Hokkanen, H., Lewis, G.B., Mansour, F., Moreth, L., Polgar, L., Samsøe-Petersen, L., Sauphanor, B., Staubli, A., Sterk, G., Vainio, A., Van de Veire, M., Viggiani, G., Vogt, H., 1994. Results of the sixth joint pesticide testing programme of the IOBC/WPRS – Working Group "Pesticides and Beneficial Organisms". Entomophaga 39, 107–119.

Henderson, C.F., Tilton, E.W., 1955. Tests with acaricides against the brown wheat mite. J. Econ. Entomol. 48, 157–161.

Hinde, J., Demétrio, C.G.B., 1998. Overdispersion: models and estimation. Comput. Stat. Data Anal. 27, 151–170.

Hossain, M.B., Poehling, H.M., 2009. A comparative study of residual effects of azadirachtin, apinosad and avermectin on *Liriomyza sativae* (Dip.: Agromyzidae) on tomatoes. Int. J. Pest Manag. 55, 187–195.

Hoy, M.A., Nguyen, R., 2001. Classical biological control of Asian citrus psylla. Citrus Ind. 81, 48–50.

IBD, 2014. Sistema de Agrotóxicos Fitossanitários – Ministério da Agricultura, Pecuária e Abastecimento, Brasil. http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons (accessed 14.01.14.).

Isman, M.B., 2006. Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. Ann. Rev. Entomol. 51, 45–66.

Isman, M.B., Grieneisen, M.L., 2014. Botanical insecticide research: many publications, limited useful data. Trends Plant Sci. 19, 1–6.

Luna-Cruz, A., Lomeli-Flores, J.R., Rodríguez-Leyva, E., Ortega-Arenas, L.D., Huerta-De La Peña, A., 2011. Toxicidad de cuatro insecticidas sobre *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) y su hospedero *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae). Acta Zool. Mex. 27, 509–526.

Lyons, D.B., Helson, B.V., Bourchier, R.S., Jones, G.C., McFarlane, J.W., 2003. Effects of azadirachtin-based insecticides on the egg parasitoid *Trichogramma minutum* (Hymenoptera: Trichogrammatidae). Can. Entomol. 135, 685–695.

Nava, D.E., Goméz-Torres, M.L., Rodrigues, M.D.L., Bento, J.M.S., Parra, J.R.P., 2007. Biology of *Diaphorina citri* (Hem., Psyllidae) on different hosts and at different temperatures. J. Appl. Entomol. 131, 709–715.

Nelder, J.A., Wedderburn, R.W.M., 1972. Generalized linear models. J. R. Stat. Soc. 135, 370–384.

Paiva, P.E.B., Parra, J.R.P., 2012. Natural parasitism of *Diaphorina citri* Kuwayama (Hemiptera, Psyllidae) nymphs by *Tamarixia radiata* Waterston (Hymenoptera, Eulophidae) in São Paulo orange groves. Rev. Bras. Entomol. 56, 499–503.

R Development Core Team, 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, p. 1706.

Ribas, P.P., Matsumura, A.T.S., 2009. A química dos agrotóxicos: impacto sobre a saúde e meio ambiente. Rev. Lib. 10, 149–158.

Ribeiro, L.P., Zanardi, O.Z., Vendramim, J.D., Yamamoto, P.T., 2014. Comparative toxicity of an acetogenin-based extract and commercial pesticides against citrus red mite. Exp. Appl. Acarol. 64, 87–98.

Ruiu, L., Satta, A., Floris, I., 2008. Effects of an azadirachtin-based formulation on the non-target muscoid fly parasitoid *Muscidifurax raptor* (Hymenoptera: Pteromalidae). Biol. Control 47, 66–70.

Sánchez-Ramos, I., Pascual, S., Marcotegui, A., Fernández, C.E., González-Núñez, M., 2014. Laboratory evaluation of alternative control methods against the false tiger, *Monosteira unicostata* (Hemiptera: Tingidae). Pest Manag. Sci. 70, 454–461.

Sas Institute, 2011. Statistical Analysis System: Getting Started with the SAS Learning. Version 9.2. SAS Institute, Cary, NC, p. 200.

Souza, A.P., Vendramim, J.D., 2005. Efeito translaminar, sistêmico e de contato de extrato aquoso de sementes de nim sobre *Bemisia tabaci* (Genn.) biótipo B em tomateiro. Neotrop. Entomol. 34, 83–87.

Stara, J., Ourednickova, J., Kocourek, F., 2011. Laboratory evaluation of the side effects of insecticides on *Aphidius colemani* (Hymenoptera: Aphidiidae), *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae), and *Neoseiulus cucumeris* (Acari: Phytoseiidae). Pest Manag. Sci. 84, 25–31.

Stark, J.D., Banks, J.E., 2003. Population-level effects of pesticides and other toxicants on arthropods. Ann. Rev. Entomol. 48, 505–519.

Sundaram, K.M.S., Curry, J., 1994. Initial deposits and persistence of azadirachtinin fir and oak foliage after spray application of 'Margosan-O'® formulation. Pest. Sci. 41, 129–138.

Tang, Y.Q., Weathersbee, A.A., Mayer, R.T., 2002. Effect of neem seed extract on the

brown citrus aphid (Homoptera: Aphididae) and its parasitoid *Lysiphlebus testaceipes* (Hymenoptera: Aphidiidae). Environ. Entomol. 31, 172–176.
Thoeming, G., Draeger, G., Poehling, H.M., 2006. Soil application of azadirachtin and 3-tigloyl-azadirachtol to control western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae): translocation and persistence in bean plants. Pest Manag. Sci. 62, 759–767.
Tiwari, S., Mann, R.S., Rogers, M.E., Stelinski, L.L., 2011. Insecticide resistance in field populations of Asian citrus psyllid in Florida. Pest Manag. Sci. 67, 1258–1268.
Tomé, H.V.V., Martins, J.C., Corrêa, A.S., Galdino, T.V.S., Picanço, M.C., Guedes, R.N.C., 2013. Azadirachtin avoidance by larvae and adult females of the tomato leafminer *Tuta absoluta*. Crop Prot. 46, 63–69.
Turek, C., Stintzing, F.C., 2013. Stability of essential oils: a review. Compr. Rev. Food Sci. F. 12, 40–53.
Ulrichs, C.H., Mewis, I., Schnitzler, W.H., 2001. Efficacy of neem and diatomaceous earth against cowpea aphids and their deleterious effect on predating Coccinelidae. J. Appl. Entomol. 125, 571–575.
Van de Veire, M., Sterk, G., Van der Staaij, M., Ramakers, P.M.J., Tirry, L., 2002. Sequential testing scheme for the assessment of the side-effects of plant protection products on the predatory bug *Orius laevigatus*. BioControl 47, 101–113.
Weathersbee, A.A., McKenzie, C.L., 2005. Effect of neem biopesticide on repellency, mortality, oviposition and development of *Diaphorina citri* (Homoptera: Psyllidae). Fla. Entomol. 88, 401–407.
Yamamoto, P.T., Bassanezi, R.B., 2003. Seletividade de produtos fitossanitários aos inimigos naturais de pragas dos citros. Laranja 24, 353–382.
Zanardi, O.Z., Ribeiro, L.P., Ansante, T.F., Santos, M.S., Bordini, G.P., Yamamoto, P.T., Vendramim, J.D., 2015. Bioactivity of a matrine-based biopest icide against four pest species of agricultural importance. Crop Prot. 67, 160–167.