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ASSESSMENT OF SUBLETHAL EFFECTS ON BEHAVIOR AND DEMOGRAPHIC TRAITS OF INSECTICIDES EMPLOYED IN TOMATO CROPS ON MIRID PREDATORS

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Prof. Dr. Geraldo Andrade Carvalho
(Orientador)

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MARIANNE ARAÚJO SOARES

AVALIAÇÃO DOS EFEITOS SUBLETAIS NO COMPORTAMENTO E PARÂMETROS DEMOGRÁFICOS DE MIRÍDEOS PREDADORES EXPOSTOS A INSETICIDAS EMPREGADOS EM CULTIVOS DE TOMATE

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RESUMO GERAL

O controle da traça-do-tomateiro, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) e da mosca-branca, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), na cultura do tomateiro, é feito principalmente com uso de inseticidas químicos. O controle biológico de pragas é seguro ambientalmente; entretanto, é ainda pouco utilizado no Brasil devido à falta de estudos a respeito da bioecologia dos inimigos naturais, bem como dos possíveis efeitos de inseticidas sintéticos e de bioinseticidas sobre estes organismos. Neste contexto, o presente trabalho teve como principais objetivos: (*i*) avaliar os efeitos do bioinseticida Prev-am® e do inseticida lambda-cyhalothrin no crescimento populacional das pragas *T. absoluta* e *B. tabaci*, e do predador *Nesidiocoris tenuis* (Hemiptera: Miridae); (*ii*) determinar o crescimento populacional de *T. absoluta* e *B. tabaci* após contato com Prev-am® e lambda-cyhalothrin, na presença e ausência do predador *N. tenuis*; (*iii*) avaliar os efeitos subletais destes produtos na longevidade, comportamento e predação de *N. tenuis*, e (*iv*) determinar os efeitos letal e subletais dos inseticidas (spinetoram, chlorantraniliprole + abamectin, triflumuron e tebufenozide), recomendados para a cultura do tomateiro, nas características biológicas e comportamentais de *Macrolophus basicornis* (Hemiptera: Miridae). Os experimentos foram conduzidos em condições de laboratório, utilizando o delineamento inteiramente casualizado. Visando determinar o crescimento demográfico de *B. tabaci* e *T. absoluta*, insetos adultos foram expostos ao Prev-am®, lambda-cyhalothrin e água destilada, na ausência e presença de *N. tenuis*. Após 11 dias da implementação dos experimentos, o número de descendentes vivos por ínstar destes herbívoros foi registrado. Os experimentos para avaliar o comportamento de *N. tenuis* foram realizados em placas de Petri, após a exposição do predador às máximas concentrações de Prev-am® e lambda-cyhalothrin. *Bemisia tabaci* e *T. absoluta* foram utilizadas como presas e avaliou-se predação, caminhamento, repouso, limpeza e fitofagia. Para quantificar os efeitos letal e subletais de inseticidas sobre *M. basicornis*, realizaram-se estudos de exposição das ninfas a plantas tratadas e de aplicação direta em predadores adultos. Adicionalmente, verificaram-se os efeitos dos inseticidas no comportamento de voo de *M. basicornis*. Os resultados obtidos referentes ao bioensaio com *T. absoluta* evidenciou que o predador *N. tenuis* apresentou potencial em reduzir o crescimento da população desta praga. Lambda-cyhalothrin e Prev-am® reduziram a capacidade predatória de *N. tenuis*. Entretanto, ao contrário do verificado para lambda-cyhalothrin, o biopesticida não alterou o comportamento do predador. Referente aos bioensaios realizados com *M. basicornis*, abamectin causou efeito letal, enquanto os demais inseticidas (spinetoram, chlorantraniliprole + abamectin, triflumuron e tebufenozide) provocaram efeitos subletais. Quanto ao estudo realizado com *B. tabaci* foi constatado que o bioinseticida Prev-am® não foi capaz de reduzir a população da praga e comprometeu o crescimento populacional de *N. tenuis*. Entretanto, Prev-am® apresentou menor toxicidade ao predador comparado ao lambda-cyhalothrin. O uso dos inseticidas químicos e do bioinseticida, avaliados no presente trabalho, deve ser realizado de forma criteriosa, sendo que outros estudos em semicampo e campo devem ser realizados para comprovação ou não da sua toxicidade.

Palavras-chave: *Solanum lycopersicum*. Mosca-branca. Traça-do-tomateiro. Controle biológico. Biopesticida. Seletividade.

GENERAL ABSTRACT

The control of the South American tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and the whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) on tomato crops is based mainly using chemical compounds. The biological control of pests represents an alternative environmental safe. However, this method is used few due to the lack of studies concerning the bioecology of natural enemies, as well as the possible effects of insecticides and bioinsecticides upon these organisms. The objective of this study was (i) to evaluate the effects of the bioinsecticide Prev-am® and insecticide lambda-cyhalothrin in the population growth of *T. absoluta* and *B. tabaci* pests, as well as the *Nesidiocoris tenuis* (Hemiptera: Miridae) predator; (ii) to determine the population growth of *T. absoluta* and *B. tabaci* after Prev-am® and lambda-cyhalothrin contact, in the presence and absence of *N. tenuis*; (iii) to evaluate the sublethal effects of these products in the longevity, behavior and predation of *N. tenuis*; and (iv) to determine the lethal and sublethal effects of insecticides (spinetoram, chlorantraniliprole + abamectin, triflumuron and tebufenozide) in the biological and behavioral characteristics of *Macrolophus basicornis* (Hemiptera: Miridae). The experiments were carried out laboratory conditions, using the completely randomized design. Aiming to determine the demographic growth of *B. tabaci* and *T. absoluta*, the adults were exposed to Prev-am®, lambda-cyhalothrin and distilled water, in the presence and absence of *N. tenuis*. The number of insects emerged was recorded after 11 days the experiment was set up. The bioassays aiming to evaluate the *N. tenuis* behaviour were carried out into Petri dishes, after the predators exposition to the maximum concentration of Prev-am® and lambda-cyhalothrin. *Bemisia tabaci* and *T. absoluta* were used as prey; regarding the predator behavioral response were assessed predation, resting, cleaning and plant feeding. Aiming to quantify the lethal and sublethal effects of insecticides upon *M. basicornis*, nymphs were exposed to plants treated, and adults directly treated. In addition, the insecticides effects in the *M. basicornis* flight response was assessed. The results concerning the *T. absoluta* bioassays showed a high capacity of *N. tenuis* to reducing the pest population growth. Lambda-cyhalothrin and Prev-am® reduced the predatory capacity of *N. tenuis*. One the other hand that was verified to lambda-cyhalothrin, the biopesticide did not alter the predator behavior. In the bioassays performed to *M. basicornis*, abamectin caused lethal effect on the predator, while the other insecticides (spinetoram, chlorantraniliprole + abamectin, triflumuron, tebufenozide) caused sublethal effects. Regarding the study using *B. tabaci*, the biopesticide Prev-am® was unable to reducing the population pest, and compromised the population growth of the predator. However, Prev-am® showed smaller toxicity on predator compared to lambda-cyhalothrin. The use of insecticides and bioinsecticide need be employed carefully, and experiments should carried in standard conditions to prove the toxicity of compounds evaluated in this work.

Keywords: *Solanum lycopersicum*. Whitefly. South American tomato pinworm. Biological control. Biopesticide. Selectivity.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

O tomateiro (*Solanum lycopersicum* L.) é uma hortaliça da família Solanaceae, sendo cultivado em área de aproximadamente cinco milhões de hectares em várias partes do mundo, com produção mundial de aproximadamente 17 milhões de toneladas anualmente (FAO, 2017). No Brasil, de acordo com o Levantamento Sistemático da Produção Agrícola (LSPA), a produção de tomate atingiu em torno de 4,5 milhões de toneladas. Os principais estados produtores são Goiás com 32,4% da produção nacional, São Paulo com 21,1%, Minas Gerais com 16,7%, Bahia com 4,5% e Santa Catarina com 4,4% (IBGE, 2018). Dentre os fatores que ocasionam perda de produtividade, destacam-se os artrópodes-praga, que ocorrem em todos os estádios fenológicos do tomateiro. Os principais insetos pragas que acometem o tomateiro são a traça-do-tomateiro, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) e a mosca-branca, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae).

A traça-do-tomateiro foi reportada pela primeira vez em 1950 devido aos danos causados em cultivos de tomateiro; entretanto, esse lepidóptero recebeu maior atenção mundial em 2006, quando foi reportada no continente Europeu e recebeu status de praga invasora (BIONDI et al., 2018; MANSOUR et al., 2018). Após ser detectada pela primeira vez na Espanha, a traça-do-tomateiro rapidamente se disseminou para outros países da Europa, alcançando também os continentes Africano e Asiático. Os danos indiretos são causados pela alimentação das lagartas, as quais se alimentam do mesofilo foliar provocando a formação de galerias ou minas nas folhas do tomateiro. Entretanto, em altas infestações, podem atacar os frutos, causando danos diretos (DESNEUX et al., 2011; TROPEA GARZIA et al., 2012). Devido à voracidade da *T. absoluta* e da ausência de medidas eficientes de controle, essa praga pode acarretar prejuízos de até 100% em cultivos de tomate (DESNEUX et al., 2010; CAMPOS et al., 2017; BIONDI et al., 2018).

A mosca-branca representa uma das maiores ameaças ao tomateiro, sendo considerada uma das 100 pragas mais invasivas do mundo (RAMOS et al., 2018). A alta adaptabilidade da *B. tabaci* é devido ao seu hábito polífago, sendo descrita em 54 países, causando danos em diversos cultivos agrícolas (CABI, 2018). Os danos causados pelas moscas brancas podem ser diretos, pela sucção da seiva da planta, e consequente aparecimento do fungo da fumagina associado ao *honeydew* liberado por meio de sua alimentação; ou indiretos, pela transmissão de 150 tipos de vírus, o que representa a maior ameaça para a produção dessa solanácea

(NAVAS-CASTILLO et al., 2011; THOMPSON, 2011). A classificação taxonômica da *B. tabaci* é controversa, com alterações constantes, sendo uma espécie representada por um complexo de biótipos (espécies irmãs). De acordo com a ocorrência em tomateiro, os biótipos são descritos em New World (antes biótipo A), MEAM1 - Middle East Asia Minor 1 (biótipo B) e MED-Mediterranean (biótipo Q) (DE BARRO et al., 2011; LIU et al., 2012; BOYKIN et al., 2013; MASOOD et al., 2017). Devido ao grande número de biótipos da *B. tabaci*, o seu controle é difícil, principalmente em função da existência de populações resistentes aos inseticidas (PERRING, 2001; JONES et al., 2003; HOROWITZ et al., 2005).

O controle da traça-do-tomateiro e da mosca-branca é realizado basicamente pelo uso de produtos sintéticos, os quais geralmente são aplicados de forma indiscriminada (GUEDES; PICANÇO, 2012). Isto acarreta em uma série de fatores indesejados, como efeitos letal e subletais sobre insetos benéficos (parasitoides, predadores e polinizadores), ressurgência de pragas secundárias e seleção de populações de pragas resistentes (DESNEUX et al., 2007; SOHRABI et al., 2016; HADDI et al., 2017; RODITAKIS et al., 2017). Apesar da efetividade do controle químico para populações de pragas susceptíveis, o emprego dos demais métodos de controle pode minimizar os efeitos negativos dos produtos fitossanitários ao ambiente e ao próprio homem.

Como alternativa viável ao controle de pragas, destacam-se os programas de controle biológico de pragas da Europa, que obtiveram resultados aplicáveis com o uso de predadores da família Miridae (CALVO et al., 2009; CALVO et al., 2012). Destacam-se as espécies *Nesidiocoris tenuis* Reuter e *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) (Sánchez & Lacasa, 2008), que são produzidas comercialmente para controle de moscas-branca, afídeos e da traça-do-tomateiro (GABARRA et al., 1990; URBANEJA et al., 2012). Estes predadores apresentam alta taxa reprodutiva em plantas de tomateiro, possuem alta capacidade predatória e são capazes de se deslocarem facilmente sob os tricomas do tomateiro. No Brasil, apesar dos recentes estudos reportando o potencial do mirídeo *Macrolophus basicornis* (Stal) (Hemiptera: Miridae) como predador da *T. absoluta*, existem entraves para sua comercialização em virtude principal da falta de estudos que demonstrem sua compatibilidade com os demais métodos de controle (VAN LENTEREN et al., 2017).

Além do uso do controle biológico, os inseticidas botânicos, que são produzidos a partir de metabólitos secundários de plantas desempenham importante papel na proteção de cultivos de tomateiro contra patógenos e herbívoros (CIRIMINNA et al., 2014; CAMPOLO et al., 2017). Os biopesticidas atuam diretamente causando a morte dos insetos-praga e indiretamente por meio da redução alimentar e de sua oviposição (PAVELA; BENELLI, 2016). Produtos comerciais à base de óleo extraído de plantas são classificados como ambientalmente seguros, devido à baixa toxicidade e persistência em campo. Entretanto, estudos visando obter informações a respeito de sua toxicidade para inimigos naturais, vêm demonstrando que esses compostos podem ser nocivos, e neste caso indesejáveis em programas de Manejo Integrado de Pragas (MIP). Desta forma, é de extrema importância a realização de pesquisas que busquem informações que permitam o uso integrado de inseticidas botânicos e ou sintéticos químicos com agentes de controle biológico de pragas para o sucesso de programas de MIP (GUEDES et al., 2016; SOARES et al., 2019).

Neste contexto, o presente trabalho teve como principais objetivos: (*i*) avaliar os efeitos dos inseticidas Prev-am® e lambda-cyhalothrin no crescimento populacional das pragas *T. absoluta* e *B. tabaci*, e do predador *N. tenuis*; (*ii*) determinar o crescimento populacional de *T. absoluta* e *B. tabaci* após o contato Prev-am® e lambda-cyhalothrin, na presença e ausência do predador *N. tenuis*; (*iii*) avaliar os efeitos subletais deste biopesticida na longevidade, comportamento e predação de *N. tenuis*, e (*iv*) determinar os efeitos letal e subletais de inseticidas recomendados para a cultura do tomateiro nas características biológicas e comportamentais de *M. basicornis*.

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SEGUNDA PARTE – ARTIGOS

ARTIGO 1

BOTANICAL INSECTICIDE AND NATURAL ENEMIES: A POTENTIAL COMBINATION FOR PEST MANAGEMENT AGAINST *Tuta absoluta*

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Botanical insecticide and natural enemies: a potential combination for pest management against *Tuta absoluta*

Key message

- New strategies based on the use of natural enemies and biopesticides to control *Tuta absoluta* are desirable for Integrated Pest Management (IPM).
- The combination between *Nesidiocoris tenuis* and Prev-am® (at half of the recommended concentration) showed enhanced efficacy against *T. absoluta*.
- Presence of *N. tenuis* significantly reduced *T. absoluta* population growth.
- Lambda-cyhalothrin and Prev-am® could disturb behavioral response of *N. tenuis*.

ABSTRACT

The development of new strategies to control pest insects are required, in combination with conventional pesticides or replacing them. Essential oils produced from botanical extracts used in management programs should be effective against pests and selective to natural enemies. *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the most destructive pests of solanaceous crops in the world, and a possible management strategy consists of releases of the predator *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae), along with botanical applications. The objective of this study was to evaluate the effects of Prev-am® oil on *T. absoluta* offspring, either with or without the predator *N. tenuis*, as well as the oil's effects on *N. tenuis* predatory behavior and longevity. The oil's effects were compared with distilled water (control) and a synthetic pesticide (lambda-cyhalothrin). The response of populations to lambda-cyhalothrin was similar to that with Prev-am®, compared to the control, showing that *N. tenuis* had higher capacity to reduce *T. absoluta* populations. The survival analysis of predators exposed to Prev-am® indicate that none of the concentrations differed significantly from the control. In addition, the CVA (Canonical Variate Analysis) indicated significant overall differences in the predator behavior submitted to different treatments, suggesting that synthetic pesticide treatment affected predator behavior when compared to control and Prev-am®. Reduction in predatory voracity of *N. tenuis* adults exposed to leaves treated with pesticide and biopesticide was significant compared to the control treatment. The results obtained could improve IPM programs against *T. absoluta* through the Prev-am® applications and *N. tenuis* releases.

Keywords: Natural product, Predatory Mirid, South American Tomato Pinworm, Ecotoxicology, Biological control**.**

INTRODUCTION

Biological invasions caused by insect-pests represent major threats to agroecosystems and agricultural crops (Asplen et al. 2015; Xian et al. 2017; Biondi et al. 2018). Alien species are responsible for reducing yields, increasing the use of pesticides and subsequently increasing production costs (Ragsdale et al. 2011; Wan and Yang 2016). The South American tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) has been considered a worldwide invasive pest, which invaded Europe in 2006 and spread throughout the Afro-Eurasian supercontinent (Desneux et al. 2010; Desneux et al. 2011; Campos et al. 2017; Sankarganesh et al. 2017; Sylla et al. 2017; Biondi et al. 2018). This South American native moth has a life cycle of about 26 to 75 days, according to the upper and lower developmental thresholds (34.6 and 14°C), and generational overlap has been observed in the field (Guedes and Picanço 2012; Martins et al. 2016). The yield loss caused by *T. absoluta* on tomato crops may reach 100% since the larvae feed on leaves, flowers, stems, and fruits (Desneux 2010). Conventional control based on insecticide use is extremely difficult, since the larvae exhibit an endophytic habit, staying inside the leaf mesophyll or fruits, protected from most of the chemical compounds (Biondi et al. 2018) and because acquired resistance to numerous insecticides of different modes of action (Roditakis et al. 2018).

Many insecticides are used to control *T. absoluta*, and most of them are incompatible with integrated pest management (IPM) programs applied in tomato crops (Campos et al. 2014; Roditakis et al. 2015). Pesticides can cause several disturbances on environment, such as soil and ground water contamination, effects on wildlife and human health, selection of resistant populations of target organisms, resurgence of secondary pests and negative effects on beneficial arthropods (Siqueira et al. 2000; Biondi et al. 2013; Roditakis et al. 2015; Desneux et al. 2007). Safer alternatives are required to control pests, for example through using natural insecticides, which have been considered ecofriendly and easily degradable products (Mossa 2016, Nollet and Rathore 2017). Botanical insecticides are used to control herbivores and pathogens, based on their natural chemical defenses (Regnault-Roger et al. 2012; Campolo et al. 2017). Such practices present low risk to animals and humans and minimal on crops, consequently reducing the environmental disturbance (Pavela and Benelli 2016).

The combination between biopesticides and natural enemies is advisable for IPM or organic farming to control *T. absoluta* (Campolo et al. 2017; El Hajj et al. 2017). To optimize such strategies, the potential lethal and sublethal effects of biopesticides on natural enemies must be tested (Biondi et al. 2012; Biondi et al. 2013). The predator–prey interactions in the environment are extremely complex, and some disruption due to contaminants, such as pesticides, can change interaction variables between species (Köhler and Triebskorn 2013). Each organism has a specific sensitivity, which mediates its density, through the direction and magnitude of each imbalance top-down or bottom-up trophic cascades on crops (Abrams 1995; Relyea and Auld 2005). For example, *T. absoluta* demographic parameters are dependent on parasitoids and predators; however, natural enemies could be highly sensitive, according to dose, time of exposure and kind of pesticides (Desneux et al. 2004a).

Nesidiocoris tenuis Reuter (Hemiptera: Miridae) is a predator of several important pests on tomato crops, such as *T. absoluta*, the whiteflies, *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodiade), thrips, leafminers and aphids (Perdikis and Lykouressis 2002, 2004; Blaeser et al. 2004; Calvo et al. 2012; Devi et al. 2002; Perdikis and Arvaniti 2016). However, *N. tenuis* exhibits a zoophytophagous behaviour and occasionally can be considered a pest, due to the habit of feeding on plants when the insects' prey are scarce on crops (Coll and Guershon 2002; De Clercq 2002).

A novel biopesticide, Prev-Am®, composed of orange oil, salt borax and biodegradable surfactants, being used against insects and mites, is able to cause repellent action and to penetrate into the cuticle of pests, causing cellular disruption (Hafsi et al. 2012). Prev-Am® is essentially composed of cold-pressed *Citrus* peel oil, whose main compound is d-limonene (> 90%), a monoterpene with high insecticidal activities (Ciriminna et al. 2014). Nevertheless, few studies explored the capacity of such biopesticide to protect crops and their potential impact on the demographic parameters of the pests.

The most common laboratory procedure method estimates the effects of chemicals on beneficial arthropods through a median lethal dose (LD_{50}) or lethal concentration (LC_{50}) (Stenersen 2004). Different from these classical toxicological laboratorial studies, demographic analyses provide data related to variations in an insects population stability owing to exposure to chemicals, depending on the mode of action of each product (Herbert et al. 2004; Drobnjaković et al. 2016; Passos et al. 2017). Sublethal effects of pesticides or biopesticides may impair the behavior and physiology of the natural enemies of pests (Desneux et al. 2007; Biondi et al. 2013). Therefore, the aim of this study was to evaluate the influence of Prev-Am® oil on demographic parameters of *T. absoluta* population growth, in the presence or absence of the predator *N. tenuis*. In addition, Prev-Am® oil effects on *N. tenuis* predatory behavior and longevity were also evaluated.

MATERIALS AND METHODS

Biological materials

Tomato and tobacco plants (*Solanum lycopersicum cv* Marmande and *Nicotiana tabacum*, respectively) were grown in climatic chambers (25± 2 °C, 75± 5% RH, and 16L: 8D photoperiod), in a commercial substrate (Tournesol®) in 2L pots, until the plants reached 60 cm high, 40-d old.

The *T. absoluta* colony was set up from individuals (±190) collected in July of 2009 at the French National Institute for Agricultural Research (INRA), Alénya, France. The colony was established in laboratory under controlled conditions (25 ± 2 °C, 75 ± 5% RH and photoperiod of 16L: 8D) using a system of frame box (60 × 40 × 40 cm), covered with nylon mesh. Tomato plants (60 cm high) were placed into to the frame box with *T. absoluta* adults offering a substrate to females oviposition. Posteriorly, each ten days, the plants with *T. absoluta* eggs were moved to another cage, and new plants were provided before the larvae started to hang from the mines. The insects were kept in three cages, following this concept – 1) oviposition, 1st and 2nd instar larvae, 2) 3rd and 4th instar larvae, pupae, and 3) adults.

Koppert Biological Systems (Almeria, Spain) provided the alternative prey and *N. tenuis* adults to be used in the bioassays; the adults were kept in commercial bottles with 500 individuals dispersed in inert material. During the experiments, predators were supplied with UV sterilized *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs for feeding.

Biopesticide and synthetic pesticide

Prev-Am® (ORO AGRI International Ltd.) is a commercial biopesticide representing an alternative to control pests in organic farming. In our experiments, we used it to assess demographic effects on *T. absoluta* and the survival of predators under three doses at the maximum recommended (0.2352 g a.i./L), half concentration (0.1176 g a.i./L) and ten per cent (0.0235 g a.i./L) to control pests on tomato crop. These concentrations were obtained by diluting Prev-am® in distilled water. Based on demographic results, bioassays were performed using the maximum rate only.

Karate Zeon® (Syngenta International Ltd.) is an insecticide belonging to the pyrethroid group (lambda-cyhalothrin), widely used in agricultural systems. As a positive control, this compound was used only at the maximum recommended concentration (0.0237 g a.i./L), in the demographic bioassays with *T. absoluta*; longevity, behavior and predation by the predator were determined. This concentration was obtained by the dilution of lambda-cyhalothrin in distilled water. Distilled water was used as negative control in each bioassay.

Demographic bioassays for *T. absoluta*

The bioassays were carried out at the French National Institute for Agricultural Research (INRA, Sophia-Antipolis) in France, in laboratory under controlled conditions (25 ± 2 °C, 75 ± 10% RH, 16:8 L.D.). They were performed by exposing *T. absoluta* adults to dry residues of Prev-am® and lambda-cyhalothrin on tomato leaves. Twenty tomato leaves were dipped in a 1L-beaker during 5s containing either Prev-am® (one of the three concentrations: maximum recommended, half concentration or 10%), or lambda-cyhalothrin (positive control), or distilled water (negative control). Then leaves were dried for 1 h on filter paper. One treated tomato leaf (around 15 cm, composed by 5 leaflets) was cut and placed into a system comprising two superimposed plastic cups, as described by Biondi et al. (2012). The first plastic cup (700 mL, length: 15 cm) had a central hole on its bottom to allow the stem of the tomato leaf to reach the water present in a second (bottom) plastic cup (350 mL, length: 11 cm). An organdy mesh screen was fixed on the upper opening of the larger cup to allow ventilation.

Three *T. absoluta* female adults were introduced in the bioassay with one *N. tenuis* female (2 days-old) in treatments with predator. Twenty replicates were performed per concentration/treatments. After eleven days, the number of live offspring (eggs until 3rd instar larvae) of *T. absoluta* per life stage was measured under stereoscope microscope (10x).

Behavior and the predation rate of *N. tenuis*

Bioassays of behavioral response and predation rate of *N. tenuis* were assessed on *T. absoluta* eggs. Female predators (2 days-old) were kept for 24 h without food on treated tomato leaves. The leaves were dipped in a 1L beaker, as previously described in a solution of Prev-Am® or the synthetic insecticide lambda-cyhalothrin, both in the maximum recommended dosage to control pests on tomato crops. Distilled water was used as negative control. Afterwards, each predator was transferred in one Petri dish (10 cm diam. x 2 cm ht.) containing an untreated tomato leaf on water-agar solution (1%). The predator was kept with 150 *T. absoluta* eggs in the Petri dish covered with Teflon® to prevent insect escape. The time spent (in seconds) by the predators in each action (walking, cleaning, plant feeding, preying and resting) was recorded during 10 minutes per repetition. The data were accounted in real time using the software ETHOWATCHER®, simultaneously for each behavior that *N. tenuis* exhibited (Crispim Jr et al. 2012). Then, the predator was kept 24h with the 150 *T. absoluta* eggs, and the predation rate was recorded after 12h and 24h. In both access, twenty replicates were performed per treatment.

Survival assessment of *N. tenuis*

The tomato plants (25-day-old) were sprayed with one of three doses of Prev-Am® (maximum recommended, half concentration, ten percent) or with lambda-cyhalothrin synthetic insecticide (in maximum recommended concentration). The products were sprayed on plants, until run-off, using a 1.5 L power-pack aerosol hand sprayer. Subsequently, the plants were left to dry for 1h under laboratory conditions. *Nesidiocoris tenuis* (2 day-old) adults were placed on plants with fresh dry residues, and a fine mesh bag was attached over one tomato leaf to avoid the escape of predators. *Ephestia kuehniella* eggs were provided daily as a food source for the predators. The experiment was carried out with 5 tomato plants per treatment, and 6 insects per plant, in laboratory controlled conditions. The predator survival was assessed daily until death.

Data analysis

The differences in the same life stage (emerged insects) among the treatments were subjected to analysis of variance and Tukey's HSD test when appropriate (PROC GLM). Normality and homoscedasticity assumptions were checked (PROC UNIVARIATE).

The demographic parameter assessed was the instantaneous rate of increase (r_i) at the population level, based on the equation proposed by Walthall and Stark (1997):

$$ri = \frac{\mathrm{Ln}\left(\frac{\mathrm{N_f}}{\mathrm{N_0}}\right)}{\Delta \mathrm{t}}$$

N_f is the final number of alive insects, N_0 is the initial number of insects and Δt is the interval time (days) from the start to the end of the laboratory trials (Stark et al. 1997;

Walthall and Stark 1997). Then, influence of the treatments in population growth was estimated using the following model:

$$\frac{dN}{dt} = rN\frac{K - N}{K}$$

N represents the starting population size, r is a *per capita* growth rate, K is an environmental carrying capacity, and (K-N) is an unused capacity.

Estimated population growth curves were analyzed using Kaplan-Meyer estimators from the non-parametric procedure LIFETEST. Overall, similarities among time-response curves were tested by χ^2 log-Rank test and the pairwise comparisons were performed among curves.

Differences in the bioassays of the predation behavior effects of pesticides and predation were analyzed by Generalized Linear Models (Nelder and Wedderburn 1972). In addition, multiple comparisons (Tukey's test, $p < 0.05$) were performed using the "glht" function of the multicomp package. Datasets did not show a normal distribution when we tested the normality and homogeneity of variance using Shapiro-Wilk and Bartlett's tests ($p < 0.05$), respectively.

A canonical variate analysis (CVA) of the predator behaviors (resting, preying, plant feeding, walking and cleaning) subjected to different treatments was performed to recognize their eventual differences and the main contributing behavior for observed differences (PROC CANDISC with Distance statement).

Survival curves were generated from the proportion of surviving insects from the beginning to the end of the experiment. The predator survival curves were performed using the Kaplan–Meier estimators (Log-rank method).

All statistical analyses were run using SAS software version 9.2 (SAS Institute 2008), except behavioral and predation rate analyses were prepared using "R" software 3.4.4 (R Development Core Team 2016).

RESULTS

Demographic bioassays for *T. absoluta*

The number of emerged individuals in *T. absoluta* offspring changed with the concentrations of the products and presence or absence of *N. tenuis* (Table 1). In treatments without natural enemies, the post-set-up time of the experiment showed that the Prev-Am® oil concentrations and lambda-cyhalothrin caused significant differences in 1st instar offspring, and the control treatment resulted in a higher number of insects compared to other treatments ($F_{4, 95}$= 18.06, $p < 0.001$). However, no significant differences were observed to 2nd instar immatures among the control and Prev-Am® in the maximum concentration and lambda-cyhalothrin, except for the half concentration Prev-Am® with the lowest offspring values ($F_{4, 95}$= 7.29, $p < 0.001$). Lastly, neither lambda-cyhalothrin nor any concentration of Prev-Am® differed significantly from control on 3rd instar offspring.

When the predator was present, the overall means in 1st instar offspring treated with Prev-Am® and lambda-cyhalothrin did not show significant differences compared with the control, being the offspring in treatments with Prev-Am® half and minimum concentration slightly (nevertheless insignificant) fewer offspring in treatments ($F_{4, 95}$= 5.64, $p < 0.001$). The comparisons of 2nd and 3rd instar offspring show that lambda-cyhalothrin treatments resulted in the highest number of *T. absoluta* larvae compared with other treatments ($F_{4, 95}$= 11.56, $p < 0.001$; $F_{4, 95}$= 31.41, $p < 0.001$) (Table 1).

According to demographic parameters of *T. absoluta*, the response to lambda-cyhalothrin in presence or absence of *N. tenuis* did not show significant differences when compared with the control. In addition, treatments comprised of Prev-Am® did not show significant differences in reduction of tomato borer reproductive parameters when compared with control, except the treatment comprised of *T. absoluta* and Prev-Am® (half concentration). The reduction of *T. absoluta* population was higher in treatment with Prev-Am® (half concentration) and *N. tenuis*, compared to treatments without *N. tenuis*, besides *T. absoluta* with lambda-cyhalothrin, and *T. absoluta* with Prev-Am® (maximum concentration) (Fig. 1).

Survival assessment of *N. tenuis*

The survival analysis of mirid predators exposed to insecticide residues indicated significant differences among lambda-cyhalothrin and treatments of Prev-Am®. However, none of the Prev-Am® concentrations differed significantly from control (Log-rank test, χ^2 = 141.08, $df = 4$, $p < 0.001$) (Fig. 2).

Bioassay on the behavior and predation rate of *N. tenuis*

The behavior time was allocated to five main activities: Resting, preying, plant feeding, walking and cleaning (Fig. 3). There were significant differences in resting time among the control and the exposed insects ($\chi^2 = 105.3$, $df = 2$, $p < 0.001$). *Nesidiocoris tenuis* exhibited a lower amount of time walking in control treatment compared to insects in Prev-Am® and lambda-cyhalothrin treatments, with a higher walking activity in the treatment with the conventional insecticide ($\chi^2 = 86.9$, $df = 2$, $p < 0.001$). A significant increase on number of eggs preyed by *N. tenuis* in control was observed compared with Prev-Am® treatments. The predation had a drastic reduction when insects were exposed to lambda-cyhalothrin ($\chi^2 = 144.9$, $df = 2$, $p < 0.001$). Cleaning time was not different between control and Prev-Am®, however it was significantly lower when insects are exposed to lambda-cyhalothrin ($\chi^2 = 68.3$, $df = 2$, $p < 0.001$). *N. tenuis* showed significant differences in time spent on plant feeding, with a decrease after exposure to Prev-Am® and lambda-cyhalothrin when compared with the control ($\chi^2 = 61.1$, $df = 2$, $p < 0.001$) (Fig. 3).

A CVA analysis indicated significant overall differences in the predator behavior submitted to different treatments (Wilks' $\lambda = 0.45$; F = 5.14; *df* (num/den) = 10/106; $p < 0.0001$) (Table 2). The CVA diagram suggests that pesticide treatment affected predator behavior when compared with the other treatments (Fig. 4). The first axis ($p < 0.001$) explained 96% of the observed differences (Table 2). Higher canonical loads were observed in preying and cleaning for the 1st axis, which were responsible for most of the observed divergence among treatments.

Reductions in predation rates by *N. tenuis* exposed to treated leaves were significant when compared with the control treatment (Table 3). However, the number of *T. absoluta* eggs consumed by predators exposed to lambda-cyhalothrin were significantly lower at both times of evaluation (12 and 24h) and also for total consumption at the end of the bioassay, compared with the Prev-Am® and control. The number of eggs preyed was significantly lower when *N. tenuis* were exposed to Prev-Am®, except in the first twelve hours ($\chi^2 = 20.0$, $df = 5$, $p < 0.001$). In addition, after 24h, the number of eggs preyed by *N. tenuis* was significantly different among treatments, being highest in the control ($\chi^2 = 31.8$, $df = 2$, $p < 0.001$) (Table 3).

DISCUSSION

Despite the large number of essential oils tested against pest insects, only few have been tested against the tomato borer. In addition, an even smaller number of studies have assessed the efficacy of new biopesticides to *T. absoluta* and the impact on its natural enemies. Other authors reported a lower toxicity caused by Prev-Am® for *T. absoluta* larvae, where the mortality values found did not exceed 20% during 7 days after the biopesticide exposure (Hafsi et al. 2012). However, population dynamics prediction depends on the fecundity *per capita* of females, besides the abiotic and biotic influences. Campolo et al. (2017) reported that sweet orange oil and lemon oil could effectively control *T. absoluta*, especially eggs and larvae inside mines. The present study suggests that *T. absoluta* is more susceptible to Prev-am® than to lambda-cyhalothrin, since most of the offspring analysis showed significant differences compared to control. Besides, the present study indicated a probable resistance of this *T. absoluta* population to lambda-cyhalothrin, as was documented by other authors (Silva et al. 2011; Haddi et al. 2012; Roditakis et al. 2013). Therefore, the pest resistance toward conventional insecticides highlight the necessity to develop alternative products, such as biopesticides derived from plants.

Recent studies based on deleterious effects of essential oils demonstrated the wide range of insects affected (Sosa and Tonn 2008; Miresmailli and Isman 2014; Campolo et al. 2017). However, Prev-Am® did not reduce longevity of adult *N. tenuis*, as opposed to lambda-cyhalothrin, which did. According to Biondi et al (2012), Prev-Am® did not affect the survival of the predator *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae); likewise, we found that none of the tested concentrations of Prev-Am® reduced longevity of *N. tenuis*. The modest effect of Prev-am® on survival may be due to the mode of exposure, where the insects were in contact with dry residues on leaves (Regnault-Roger et al. 2012).

Sublethal behavioral effects are determinant measures of exposure to pesticides and biopesticides, potentially affecting the mobility, search and preying capacity of predators. Our results showed that there were significant differences in resting and walking time of *N. tenuis* among the treatments (Fig. 2). Likewise, other studies verified disturbance in mobility of insects exposed to lambda-cyhalothrin, correlated with increased or decreased walking time after insecticide exposure (Haynes 1988; Desneux et al. 2007; Cordeiro et al. 2010). According to the scientific literature, essential oils (Mossa 2016) and lambda-cyhalothrin (Desneux et al. 2004b) act on the insect nervous system. Therefore, we observed significant differences in preying and plant feeding time of *N. tenuis* among the treatments (Fig. 2).

These changes in the predator behavior may lead to reduction in the capacity of prey location and capture. However, another study (Mansour et al. 2011) did not find any effect of Prev-am® on offspring production in the parasitoid *Anagyrus pseudococci* (Girault) (Hymentoptera: Encyritidae), an important measure of sublethal effects. As a result, Prev-am® has been recommended for IPM in vineyards.

Insecticides may interfere in the feeding of exposed predators in three ways: repellent effects, antifeedant properties and disruption in the ability to locate food in the environment (Desneux et al. 2007). Therefore, the use of less harmful products to control pests are required to minimize the sublethal effects on predators. As observed in our experiments, the biopesticide Prev-Am® was less harmful to *N. tenuis* compared to lambda-cyhalothrin, since it was similar to the control in the first 12h of predation. However, at 24h and in the total predation by *N. tenuis*, adverse effects were observed. The food intake may have been affected by the haphazard movement and increased restlessness after exposure of predators to Prev-Am® residues (He et al. 2012).

Predators could affect prey populations through direct consumption or by triggering anti-predator reactions in prey (Lima and Dill 1990). Behavioral responses exhibited by prey include decreased feeding, moving to lower quality foliage or physiological stress when the predators are present (Slos et al. 2009; Barnier et al. 2014). As a matter of fact, treatments with the presence of *N. tenuis* caused a significant reduction on *T. absoluta* demographic parameters. Meanwhile, except when the predators were exposed to lambda-cyhalothrin the tomato borer population increased, probably due to the lethal effects of the insecticide on the predator. These results clearly demonstrated the impact of *N. tenuis* on *T. absoluta* population growth, shown by the offspring mortality (Fig. 1).

Population levels cannot be based on individual cases of insect mortality, since populations are able to compensate mortality points through the ecological dynamic systems (McNair et al. 1995; Stark et al. 2004). However, comparing the curves of population dynamic, the interaction with Prev-Am® at the half concentration and *N. tenuis* provide the better results to reduce the population of *T. absoluta*, besides that a lower number of descendants was observed, perhaps because the repellent effects of Prev-Am®. Repellent effects caused by essential oils, such as reduced time spent by adults on plants are directly correlated with reductions in offspring number (Isman 2000; Cordeiro et al. 2010). Consequently, it caused a decrease of demographic parameters of this pest; probably, the effects of limonene and *N. tenuis* act additively or synergistically to reduce demographic parameters of the tomato borer.

In summary, the results obtained could be useful for IPM programs against *T. absoluta*, through the combination of Prev-Am® application and biological control. Besides, we have to consider the side effects of Prev-Am® in behavioral parameters of *N. tenuis* due to the fact that the bioassays were conducted in laboratory conditions. It is important to emphasize that Prev-Am® is an environmentally-safe product, and the sublethal effects caused in *N. tenuis* could be diluted in tomato fields; consequently, the present botanical insecticide could be more harmless to predators. However, the timing of application of Prev-Am® must depend on *N. tenuis* release time, therefore the persistence of the product on foliage needs to be properly assessed. Like other natural products, the activity period of Prev-Am® is short, promoting a better compatibility with natural enemies.

Author contributions statement

MS, ND, MC, AL, AB, LZ conceived and designed the experiment. MS, MC performed the bioassays. MS, MH, MC, LP analyzes the data. MS, LP, GC, MH, MC, AL led the writing of the manuscript. All authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights This article does not contain any studies with human participants or animals (other than insects) performed by any of the authors.

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Table 1 Number of laid-eggs and emerged individuals reaching the 1st, 2nd or 3rd instar (mean ± SE) in the progeny of *Tuta absoluta* submitted to different treatments

Treatment		**Absence of *Nesidiocoris tenuis*[1]**			
		Eggs	**1st instar**	**2nd instar**	**3rd instar**
T. absoluta +	Control	2.85 ± 0.9 a	6.40 ± 0.7 a	36.50 ± 2.8 ab	39.60 ± 4.8
	Prev-am 10%	0.95 ± 0.4 ab	1.15 ± 0.2 cd	28.45 ± 2.2 bc	35.05 ± 3.1
	Prev-am 50%	0.00 ± 0.0 b	0.90 ± 0.2 d	22.40 ± 2.1 c	26.05 ± 2.4
	Prev-am 100%	0.80 ± 0.5 ab	3.20 ± 0.6 b	33.35 ± 2.3 abc	36.10 ± 2.7
	Lambda-cyhalothrin	0.00 ± 0.0 b	3.02 ± 0.6 bc	43.25 ± 4.4 a	36.15 ± 3.4
$F_{4, 95}$		4.81	18.06	7.29	1.86
P		<0.001	< 0.001	< 0.001	0.123
		Presence of *Nesidiocoris tenuis*[1]			
		Eggs	**1st instar**	**2nd instar**	**3rd instar**
T. absoluta +	Control	0.45 ± 0.2	2.00 ± 0.4 ab	14.00 ± 1.9 bc	12.15 ± 2.1 bc
	Prev-am 10%	0.00 ± 0.0	1.40 ± 0.3 b	20.45 ± 2.3 b	13.15 ± 1.4 bc
	Prev-am 50%	0.10 ± 0.1	0.85 ± 0.2 b	8.45 ± 1.1 c	5.75 ± 0.8 c
	Prev-am 100%	0.35 ± 0.2	3.65 ± 0.7 a	21.20 ± 2.4 b	16.70 ± 2.25 b
	Lambda-cyhalothrin	0.00 ± 0.0	1.95 ± 0.4 ab	31.2 ± 3.9 a	36.65 ± 3.0 a
$F_{4, 95}$		2.09	5.64	11.56	31.41
P		0.088	< 0.001	< 0.001	< 0.001

[1]Values followed by the same letter in a column are not significantly different by Tukey's HSD test ($P < 0.05$).

Table 2. Canonical loadings (between canonical structure) of the canonical axes for the *Nesidiocoris tenuis* behavior submitted to different treatments. Bold type indicates the main contributors of each axis and asterisks indicate the significant axes.

Behavioral response	**Canonical axes**	
	1st	**2nd**
Behavior of *N. tenuis*		
Restining	0.50	0.87
Preying	**1.00**	-0.09
Plant feeding	0.98	-0.19
Walking	0.97	0.25
Cleaning	**0.99**	0.15
$F_{appr.}$	5.14	0.49
Proportion	0.96	0.04
P	< 0.001*	0.74
Eigenvalue	1.12	0.03

Table 3. Mean consumption (± SE) of *Tuta absoluta* eggs by *Nesidiocoris tenuis* females exposed to treated and untreated tomato leaves, during 24 h in two times after treatment-exposure (12h and 24h).

Treatment	**Consumption – 24 h recovery period**		
	0 - 12 h	**12 - 24 h**	**Total (24 h)**
Control	125.8 ± 4.7 Aa	18.8 ± 3.7 Ab	144.6 ± 2.7 A
Prev-am	122.2 ± 4.8 Aa	13.1 ± 2.0 Bb	135.3 ± 4.8 B
Lambda-cyhalothrin	39.0 ± 12.3 Ba	7.1 ± 2.3 Cb	46.1 ± 14.5 C

Data followed by different capital letters are significantly different within the same column among the treatments and different lower case letters are significantly different within the same row among the time.

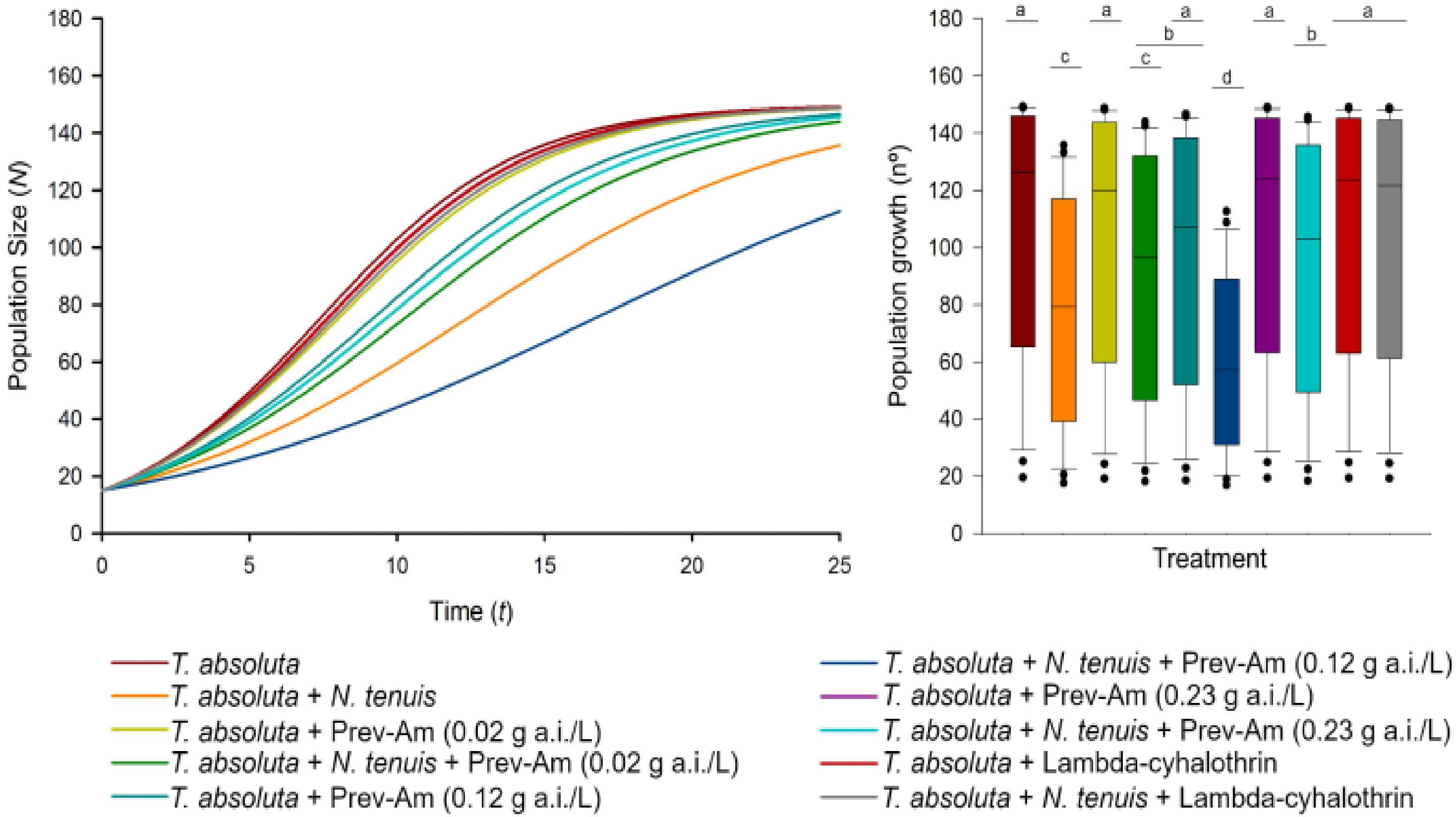

Figure 1. Curves and box plots of the estimated population curves of *Tuta absoluta* submitted to different treatments. Box plots indicate the median and dispersion (lower and upper quartiles, and outliers) population growth. Box plots with the different lower case letters are significantly different by pairwise comparison in χ^2 log-Rank test ($p <$ 0.05)

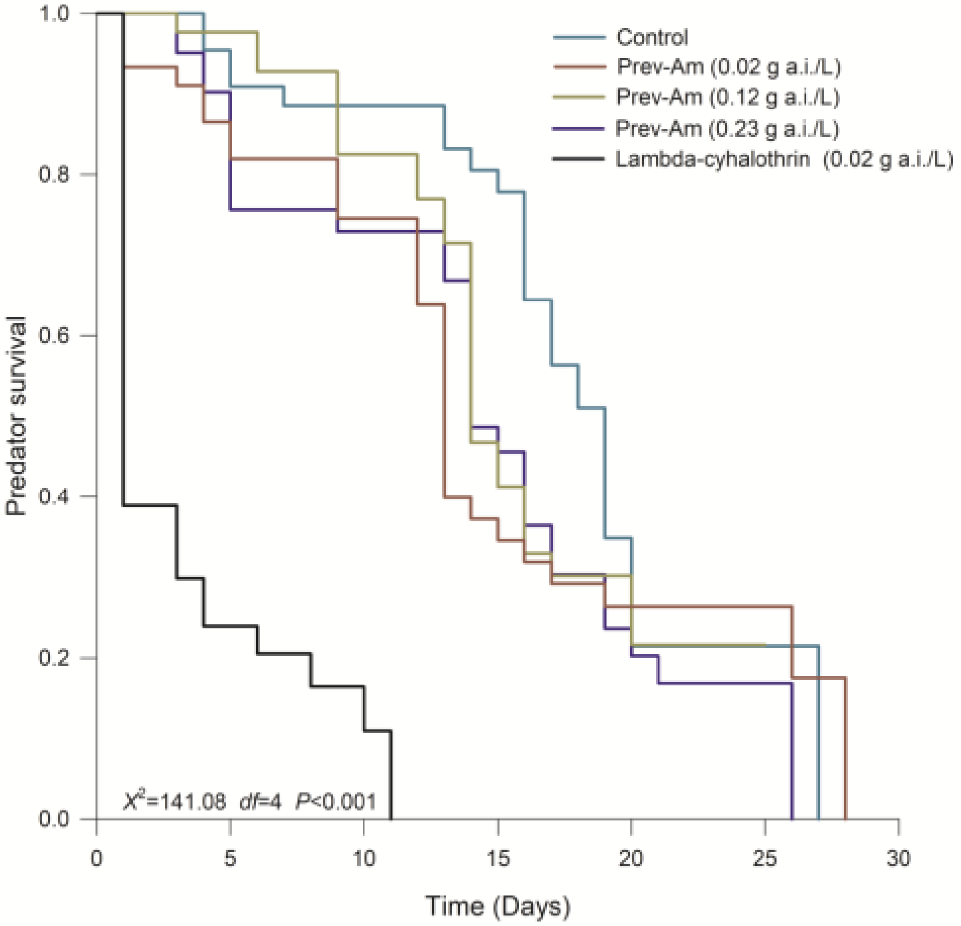

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Figure 2. Survival curves of *Nesidiocoris tenuis* adults exposed to tomato plants treated with traditional (lambda-cyhalothrin) and botanical insecticides. Survival curves with different letters were significantly different (Log-rank test, α = 0.05).

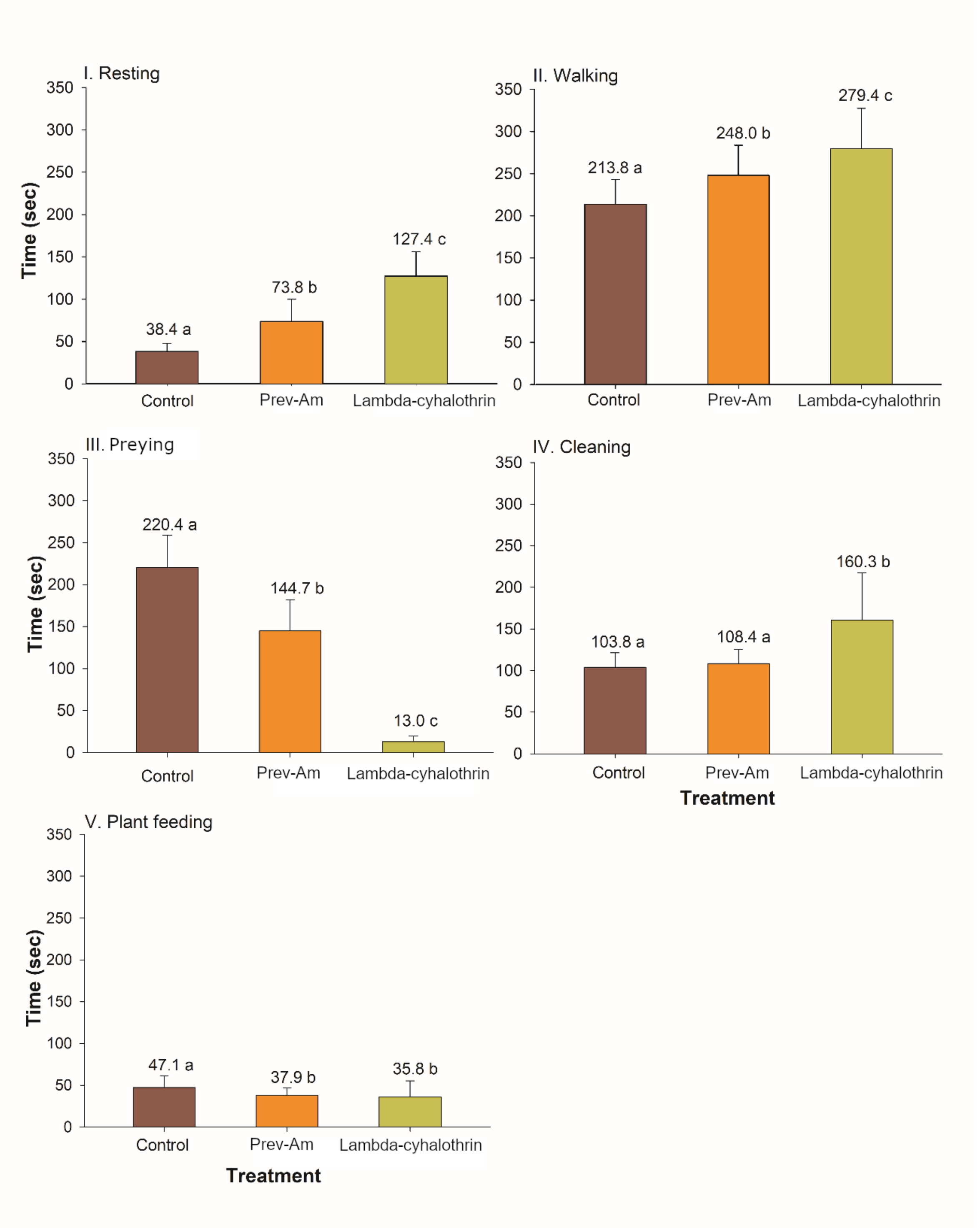

Figure 3. Time spent (seconds) in resting, preying, plant feeding, walking and cleaning (mean ± SE) by *Nesidiocoris tenuis* females on plants treated with either distilled water (control), orange oil or lambda-cyhalothrin. Each repetition was observed for a period of 10 minutes. Behavior traits with different letters among treatments indicate statistically significant differences (GLM-with Poisson distribution, followed by Tukey test, $p<0.05$)

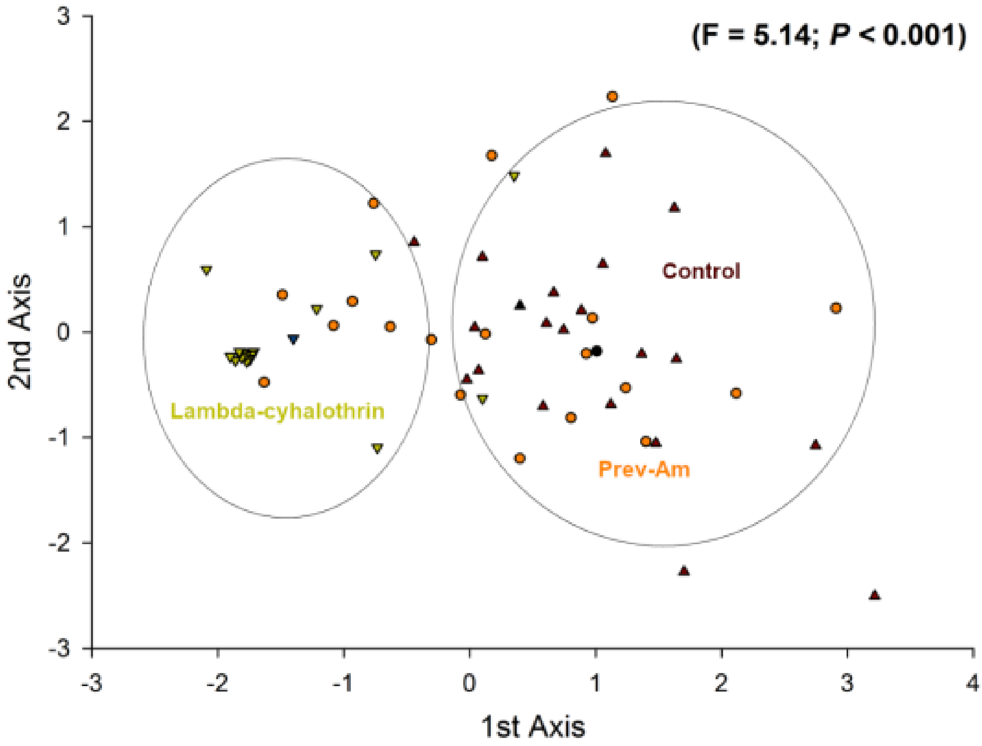

Figure 4. Ordination (CVA) diagrams showing the divergence in *Nesidiocoris tenuis* behavior (see Table 2) when submitted to different treatments. The solid symbols represent the individual replicates. The large circles indicate treatments that are not significantly different by the approximated F-test (p < 0.05), based on the Mahalanobis (D2) distance between class means.

ARTIGO 2

SIDE EFFECTS OF INSECTICIDES COMMONLY USED AGAINST *Tuta absoluta* ON THE PREDATOR *Macrolophus basicornis*

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Side effects of insecticides commonly used against *Tuta absoluta* on the predator *Macrolophus basicornis*

Key message

- The risk assessment of insecticides could provide new information for enhancing the management of *Tuta absoluta*.
- The use of mirid predators to control *Tuta absoluta* is recommended for sustainable IPM.
- This study provides information on the side effects of five insecticides commonly used to control *Tuta absoluta* on the predator *Macrolophus basicornis*.
- The insecticide abamectin caused high mortality in nymphs and adults of *Macrolophus basicornis*.
- All insecticides exhibited negative side effects on *Macrolophus basicornis*.

ABSTRACT

Macrolophus basicornis (Stal) (Hemiptera: Miridae) is a promising biological control agent against tomato pests, mainly the South American tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Nevertheless, the amount of pesticides used in tomato crops could compromise the effectiveness of *M. basicornis* in pest control. Thus, the present research aims to evaluate the lethal and sublethal effects of five insecticides (spinetoram, chlorantraniliprole + abamectin, triflumuron, tebufenozide, and abamectin) commonly used in tomato crops on *M. basicornis*. Third instar nymphs were exposed to dry residues of insecticides on tomato seedlings, and adults were directly sprayed using a Potter precision tower. Abamectin caused the highest mortality rate (79.98%) of *M. basicornis* nymphs. Females exposed to spinetoram during the nymphal stage showed a reduction in tibia length. Except for spinetoram, all other insecticides significantly influenced adult longevity. All insecticide treatments caused a reduction in female offspring. However, the growth of males and females (F_1 generation) issued from adults treated did not differ significantly from the control. In the predator flight bioassay, males in the first evaluation showed a reduction in flight activity following exposure to chlorantraniliprole + abamectin and to tebufenozide. Overall, all insecticides tested caused negative effects on *M. basicornis*.

Keywords: Predatory mirid, Ecotoxicology, Risk assessment, Beneficial arthropods, Chemical substances

INTRODUCTION

The tomato plant, *Solanum lycopersicum* L., is an important horticultural species cultivated worldwide (Olaniyi et al. 2010; Bhowmik et al. 2012). Many pests can cause qualitative and quantitative losses to this crop. The South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is considered the most destructive of those pests, and without control measures, its damage can cause up to 100% production loss (Desneux et al. 2010; Campos et al. 2017; Biondi et al. 2018; Mansour et al. 2018). Through larvae feeding, immature stages penetrate and create mines in the leaf mesophyll, reducing the plant's photosynthetic capacity (Desneux et al. 2011; Tropea Garzia et al. 2012). In addition, damage to stems causes necrosis that reduces plant development. *Tuta absoluta* is able to feed on fruits, generating direct visual harm to commercial products (Biondi et al. 2018). Since the 1950s, this pest, native to South America, has been reported to cause damage to tomato crops, while since 2006, *T. absoluta* status has been changed to "invasive pest" after spreading across Europe, Africa, and Asia. It is considered to be a major threat to the international tomato trade (Desneux et al. 2010; Desneux et al. 2011; Campos et al. 2017; Sankarganesh et al. 2017; Sylla et al. 2017; Biondi et al. 2018; Han et al. 2018; Mansour et al. 2018).

Zoophytophagous predators belonging to the Miridae and Anthocoridae families are important natural enemies in agricultural systems, acting against whiteflies, aphids, thrips, mites, and lepidopterans (Urbaneja et al. 2012; Zappalà et al. 2013; Mollá et al. 2014; Perdikis and Arvaniti 2016; Salehi et al. 2016). In South America, mirid predators have been receiving attention as promising biological agents against *T. absoluta*, especially *Macrolophus basicornis* (Stal) (Hemiptera: Miridae). This predator is able to walk and reproduce on tomato plants, and its preying capacity on *T. absoluta* can reach an average of 331.0 eggs in the nymphal stage and 100.9 eggs per day in the adult stage (Bueno et al. 2013; Van Lenteren et al. 2017).

Despite the high number of natural enemies acting against *T. absoluta*, the primary method for its control is the application of insecticides (Guedes and Picanço 2012; Campos et al. 2017; Biondi et al. 2018; Mansour et al. 2018). Chemical control is required to suppress pests when an economic threshold is reached, and thus the identification of products that are not harmful to beneficial organisms and that are respectful of the environment is extremely important in integrated pest management (IPM) programmes (Guedes and Picanço, 2012). However, the incorrect employment of pesticides in pest management causes biological disturbances such as the selection of resistant populations and pest outbreaks (Siqueira et al.

2000a, b; Haddi et al. 2012; Campos et al. 2014; Sohrabi et al. 2016; Haddi et al. 2017; Roditakis et al. 2017). Furthermore, non-target insects may experience lethal and sublethal effects after exposure to pesticides (Passos et al. 2017; Pérez-Aguilar et al. 2018; Desneux et al. 2007; Müller et al. 2017).

The first ecotoxicological studies concerning *M. basicornis* have considered teflubenzuron, methoxyfenozide and chlorantraniliprole to be safe to this mirid, and therefore, combined use might be advised (Passos et al. 2017, 2018). Nevertheless, it is necessary to obtain more information on other pesticides commonly applied in tomato pest management. In this context, the aim of the present research was to evaluate the lethal and sublethal effects of insecticides usually recommended for tomato crops on the predator *M. basicornis*. The results obtained could improve the management of *T. absoluta*, a pest that is controlled in Brazil mainly with the use of insecticides. In the laboratory, we assessed the effect of the direct contact of nymphs (i.e., mortality and growth measurement) with treated plants and direct application of insecticides on adults (i.e., longevity, offspring rate, growth measurement and flight capacity).

MATERIALS AND METHODS

Insects

Macrolophus basicornis colony was initiated with adults (~200) collected from untreated open-field tobacco plants in Lavras, Minas Gerais, Brazil. The insects were transported to the Laboratory of Ecotoxicology and Integrated Pest Management (Federal University of Lavras) and identified based on visual morphological characteristics, following the key of mirid species proposed by Ferreira and Henry (2011). Under laboratory conditions, the insects were kept in acrylic boxes (60 x 30 x 30 cm) containing a tobacco plant (*cv*. TNN) (50 cm in height with four to six fully expanded leaves) as oviposition substrate and a water source. We provided *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs (Insecta Agentes Biológicos, Lavras, MG, Brazil), which were sprinkled on the tobacco leaves for nymphs and adults of *M. basicornis,* as a food source. Once a week, a new tobacco plant was supplied to sustain the predator colony, and the plant with *M. basicornis* eggs was transferred to an insect-free cage. Hence, the predators with the same ages were kept in the same cage. The rearing and bioassays were maintained in an air-conditioned room at 25 ± 2 °C, 70 ± 10% relative humidity, and a photoperiod of 12:12 h (L:D).

Insecticides

The insecticides evaluated were spinetoram, the mixture chlorantraniliprole + abamectin, triflumuron, tebufenozide, and abamectin. They were applied at their maximum recommended dosages for *T. absoluta* control in tomato crops in Brazil (Table 1). Distilled water was used as the control treatment.

Exposure of nymphs to fresh residues

Tomato seedlings *cv.* Santa Clara (approximately 15 cm high) were sprayed with the insecticide solutions using a 1 L power-pack aerosol hand sprayer. Insecticides were sprayed directly towards seedlings from a distance of 30 cm until runoff, approximately 0.6 mL of insecticide solution per plant. Treated seedlings were left in a ventilated laboratory for two hours to obtain fresh dried residues. Then, treated seedlings and ten third instar nymphs of *M. basicornis* were placed inside polyvinyl chloride (PVC) tubes (30 cm ht. x 15 cm diam.) containing *E. kuehniella* eggs *ad libitum* as a food source. The top of the PVC tube was covered with a fine mesh, and the bottom was supported on a Petri dish (8 cm ht. x 20 cm diam.) to prevent insects from escaping. Preliminary tests did not indicate cannibalism among the nymphs in the same experimental arena. Nymphal mortality was checked at 24 h intervals for three days. Insects were considered dead if no movement was observed after touching them with a thin paintbrush. Six replicates with ten *M. basicornis* individuals were used per treatment.

When nymphs became adults, the left posterior leg in each specimen was removed from the body and measured with a digital pachymeter (Mtx®, 0-150 mm) under a stereoscopic microscope (40x). Often, the hind tibia length is utilized to evaluate adult size, as each part of an insect's body grows proportionally (Shingleton et al. 2007; Soler et al. 2007). Twenty replicates were assessed for each sex per treatment.

Direct spraying on adults

Macrolophus basicornis adults (~2 days old) collected from laboratory colonies were kept in tubes (8 cm ht. x 3 cm diam.) and subjected to carbon dioxide (CO_2) anaesthesia for 3 s. The predators were then placed in a Petri dish (2 cm ht. x 15 cm diam.) and treated with direct spraying under a Potter precision tower (Burkard Scientific Co., Uxbridge, UK; pressure = 100 kPa, deposited volume = 1.5 ± 0.5 mL/cm²). Treated insects were maintained on seedlings of tomato *cv.* Santa Clara (approximately 15 cm high) inside the PVC tubes containing *E. kuehniella* eggs, as described previously. Thereafter, the mortality and survival of adults were recorded daily. The bioassay was performed with six repetitions per treatment, each containing *M. basicornis* adults (5 males and 5 females).

Subsequently, to evaluate insecticide effects on predator reproduction, twelve couples were formed and maintained on tomato seedlings inside the PVC tubes. The number of nymphs hatched was counted daily until the adults died. The nymphs (F_1 generation) were maintained on tomato seedlings (as described above) until they reached adulthood. When the insects became adults, the left posterior leg in each specimen was removed from the body and measured with a digital pachymeter (Mtx®, 0-150 mm) under a stereoscopic microscope (40x). Twenty replicates were evaluated for each sex per treatment.

Behavioural assessment - flight response

To assess the predator's flight response, surviving insects directly sprayed with insecticides (except for abamectin, which caused higher mortality rates) were tested. As previously described in the adult bioassay, *M. basicornis* adults were subjected to carbon dioxide (CO_2) anaesthesia for 3 s. Then, the predators were placed in a Petri dish (2 cm ht. x 15 cm diam.) and treated under a Potter precision tower. Twenty-four hours after treatment, the predators from the Petri dishes were collected using an aspirator and placed into the PVC tubes (30 cm ht. x 15 cm diam.). To develop a short-range flight test, a barrier of talcum powder (Êxodo Científica®) was applied on the PVC tube wall to prevent the predators from climbing. A transparent Petri dish (2 cm ht. x 20 cm diam.) was covered with entomological glue and placed on top of the tube to serve as a trap for flying predators. The number of insects attached to the Petri dish was recorded at two times (2 h and 4 h) after the experiment was set up. The bioassay was performed with five repetitions per treatment, each containing *M. basicornis* adults (10 males and 10 females).

Data analysis

The bioassay data were submitted to homoscedasticity (Bartlett 1937) and normality (Shapiro and Wilk 1965) tests. However, the bioassay data did not follow a normal distribution and were submitted to generalized linear models (GLMs). The mortality of nymphs was fitted in a GLM analysis (binomial family). The predator tibia length data in the bioassays linked to nymphs and adults were tested using a GLM analysis (quasi-Poisson family) with two factors (treatment and sex). To assess adult survival, curves were generated using Kaplan-Meier estimators (log-rank method) from the proportion of surviving insects from the beginning to the end of the experiment. The offspring data were fitted in a GLM analysis (quasi-Poisson family). Adult flight capacity data were assessed using a GLM analysis (Poisson family) with two factors (treatment and sex). All statistical analyses were carried out using R software 3.4.4 (R Development Core Team, 2017).

RESULTS

Exposure of nymphs to fresh residues

After 24 h, spinetoram, abamectin + chlorantraniliprole and tebufenozide did not cause significant mortality rates to nymphs when compared to the control, whereas triflumuron and abamectin affected nymphal survival ($X^2 = 178.4$, $df = 5$, $p < 0.001$, Table 2). At 48 h ($X^2 = 201.5$, $df = 5$, $p < 0.001$) and 72 h ($X^2 = 103.1$, $df = 5$, $p = 0.009$) and considering the overall mortality incidence ($X^2 = 278.88$, $df = 5$, $p < 0.001$), only abamectin was significantly different from the control.

Based on tibia length, insecticides affected the predator's size, with differences between males and females. The results showed a significant effect of spinetoram on *M. basicornis* growth, with the female tibia being smaller than the male tibia ($F = 6.80$, $df = 1$, $p = 0.009$). When exposed to spinetoram, the females' tibia length in the nymphal stage was reduced. Significant differences were observed among the treatments for males. A reduction in tibia length among other treatments was observed, in which males exposed to spinetoram and abamectin + chlorantraniliprole were smaller than those treated with triflumuron ($F = 11.05$, $df = 4$, $p < 0.001$) (Table 2).

Direct spraying on adults

The insecticides affected the survivorship of *M. basicornis* males and females. Regarding male survival, spinetoram was the least toxic product and did not differ significantly from the control. Triflumuron and tebufenozide were harmful to males, reducing their longevity (Fig. 1a) ($X^2 = 250.48$, $df = 5$, $p < 0.001$). Concerning female survival, neither spinetoram nor tebufenozide differed significantly from the control. However, triflumuron reduced the longevity of females (Fig. 1b) ($X^2 = 257.87$, $df = 5$, $p < 0.001$). In both sexes, compared with the other treatments, abamectin and abamectin + chlorantraniliprole were more toxic to *M. basicornis*, causing the first and second highest sublethal effects on predator longevity, respectively (Fig. 1a and 1b).

All treatments differed significantly from the control regarding female offspring rate (F = 328.61, *df* = 5, $p < 0.001$). Spinetoram, triflumuron and tebufenozide reduced the number of descendants, whereas no nymphs were observed in the abamectin + chlorantraniliprole and abamectin treatments (Fig. 2).

Tibia growth of the predators varied significantly among treatments, as well as between males and females. Significant differences between male and female tibia length were verified only for tebufenozide, and the tibias of females were smaller than those of males (F = 9.64, *df* = 1, $p = 0.002$). Concerning males, a reduction in the tibia length was observed for triflumuron compared to spinetoram (F = 3.38, *df* = 3, $p = 0.019$). Significant differences were not observed for females (Fig. 3).

Behavioural assessment - flight response

Macrolophus basicornis flight capacity was affected by the insecticides. Nonetheless, no significant differences between males and females were observed. In the first evaluation (2 h), a reduction in male flight activity induced by chlorantraniliprole + abamectin and tebufenozide was verified ($X^2 = 24.88$, *df* = 4, $p < 0.001$); however, the same reduction did not occur in treated females ($X^2 = 32.60$, *df* = 4, $p = 0.062$). Nevertheless, in the second evaluation (4 h), no significant differences were observed in the flight activity of predators between the sexes ($X^2 = 29.35$, *df* = 1, $p = 0.30$) and treatments ($X^2 = 30.39$, *df* = 4, $p = 0.17$) (Table 3).

DISCUSSION

In general, the five insecticides showed different levels of negative side effects on *M. basicornis*. Therefore, abamectin-active substances may induce stronger effects on nymphs and adults of this predator. A reduction in the *M. basicornis* descendant rate was observed for all insecticides tested. Predator longevity was reduced by abamectin, abamectin + chlorantraniliprole, triflumuron and tebufenozide, and premature death in treatments with abamectin greatly compromised the offspring rate. The hind tibia measurement is a useful tool to determine an insect’s growth pattern; therefore, it may be directly correlated with the natural enemy’s fitness (Charleston et al. 2005; Castañé et al. 2006; Passos et al. 2017). Interestingly, only females exposed to spinetoram during the nymphal stage were smaller than control. *Macrolophus basicornis* males showed a decrease in flight activity when exposed to abamectin + chlorantraniliprole and to tebufenozide. The disturbances in the predator’s orientation could undermine the search for prey and affect mate-finding ability (Desneux 2007; Nachtigall et al. 2018).

Spinetoram is a semi-synthetic spinosyn that acts as an allosteric modulator of the nicotinic acetylcholine receptor (Crouse et al. 2001). According to the results obtained in the present study, spinetoram did not cause a lethal effect on *M. basicornis* nymphs and adults. In contrast, the natural enemies *Aphelinus mali* (Hald) (Hymenoptera: Aphelinidae) and *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) employed for orchard pest management showed acute mortality that was greater for adults than immature life stages following exposure to spinetoram (Mills et al. 2015). Despite the low lethal effect in nymphs, contact with spinetoram in the nymphal stage reduced the size of females. The sublethal effects of insecticides on predator size may affect predator fitness and mating, reducing their efficiency in controlling pests (Desneux et al. 2007). Nevertheless, a reduction in tibia length in adults (F_1 generation) descended from insects exposed to treated plants was not observed. The reduced fertility shown by *M. basicornis* might compromise predator population growth. For example, spinetoram negatively affected demographic parameters of the predator *Deraeocoris brevis* (Uhler) (Hemiptera: Miridae) after adult exposure (negative intrinsic rate of population increase - r_m). In addition, the mortality of adults caused a decrease in reproductive coefficient values, affecting the number of predator offspring (Amarasekare and Shearer 2013). Biondi et al. (2012a) report spinosyn-based products as controversial insecticides because extension specialists and agribusiness companies often recommend them for IPM programmes. However, several sublethal effects (e.g., physiological and behavioural effects) generated by these insecticides have been reported on natural enemies and pollinators.

Abamectin acts on the chloride channels, triggering negative effects in locomotion, feeding and behavioural sensory inputs (Wolstenholme 2012). In our research, abamectin was more toxic to *M. basicornis* nymphs and adults than other insecticides. Similarly, previous studies have reported abamectin to be detrimental to *M. basicornis* adults exposed to tomato plants treated in laboratory trials; the same effects happened to nymphs under greenhouse conditions (Passos et al. 2017, 2018). Regarding other hemipteran predators, *Orius insidiosus* (Say) and *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) were also susceptible to abamectin, suggesting incompatibility between these predators and the insecticide abamectin (Biondi et al. 2012b; Moscardini et al. 2013). Abamectin could be slightly harmful when used in greenhouse environments due to rapid photolysis under sunlight, and the combination of this active substance with *M. basicornis* should be avoided (Bostanian and Akalach 2004; Passos et al. 2017).

Commercial formulations combining different active substances are considered a promising tool for insecticide resistance management of herbivorous insects, since different chemical groups have different modes of action (Ghanim and Ishaaya 2011). The chlorantraniliprole + abamectin mixture did not cause mortality or differences in growth rate in *M. basicornis* nymphs. Nevertheless, this insecticide was harmful to *M. basicornis* adults, reducing predator longevity and affecting reproduction and male flight response. In contrast, chlorantraniliprole did not cause lethal, sublethal or transgenerational effects on nymphs and adults of *M. basicornis* (Passos et al. 2017, 2018). Martinou et al. (2014) also considered chlorantraniliprole to be safe to the predator *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae), despite its side effects on feeding behaviour. Therefore, abamectin may account for the majority of the harmful effects observed, a consequence of the combination of these active substances in the blend. An alternative hypothesis would concern the quantity of active substance in the chemical solution recommended; according to the label dosage, the quantity of abamectin (60 mL/100 L) solution to treat the plants in the mixture is close to half that of the abamectin (100 mL/100 L) single treatment employed in the experiments (Table 1). This phenomenon may explain the sublethal effects in the longevity and offspring of *M. basicornis* adults, even when exposed to a lower concentration of abamectin. There is a lack of studies involving the side effects of insecticide mixtures on natural enemies; thus, it remains unclear whether these molecules are safe when combined and could be used as a complement in biological control programmes.

The insect growth regulator (IGR) group could interfere with chitin synthesis, affecting the development and growth of insects (Merzendorfer 2013). Triflumuron and tebufenozide belong to the IGR group, which is considered safe for a wide array of non-target organisms (Sun et al. 2015). Nevertheless, beneficial arthropods exposed to the IGR group may respond differently, according to the active substance used. For example, after comparing the mortality rates of triflumuron and tebufenozide, only triflumuron increased the lethal effects of *M. basicornis* nymphs in the first exposure period. On the other hand, our findings show sublethal effects of both triflumuron and tebufenozide on *M. basicornis* adults, diminishing their longevity and offspring rate and making clear the importance of assessing insect life span. Regarding other hemipteran predators, tebufenozide caused low mortality to *Orius insidiosus* (Say) and *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae), which suggests that taxonomically close species might be affected differently by the same kind of insecticide (Pietrantonio and Benedict 1999; Elzen 2001; Angeli et al. 2005). Teflubenzuron, another IGR, is more harmful to *O. laevigatus* through oral exposure than through direct contact, inducing a reduction in egg hatching and high mortality in nymphs (Van de Veire et al. 2002; Angeli et al. 2005). As stated by Passos et al. (2018), teflubenzuron may reduce the tibia length of *M. basicornis* females (F_1 generation) through the transovarial activity of insecticides. The effects of chemical compounds in subsequent generations occur due to epigenetic mechanisms based on maternal transference, where heritable changes in gene expression could be triggered by environmental factors (e.g., pesticides) (Collotta et al. 2013).

Studies reporting the risk assessment of insecticides for non-target insects supply data about the selectivity and efficacy of synthetic chemicals, therefore increasing the opportunity to integrate chemical and biological control (Gentz et al. 2010). *Macrolophus basicornis* was negatively influenced by all insecticides evaluated. However, abamectin was the most toxic product, causing a high lethal effect in both life stages of the predator. In addition, the employment of abamectin + chlorantraniliprole, triflumuron and tebufenozide together with *M. basicornis* would not be sustainable in IPM. The least toxic insecticide was spinetoram, as the adult's longevity was not affected, which suggests a possibility of synchronizing insecticide spraying and *M. basicornis* release on tomato crops. However, we ought to consider that the bioassays were carried out in standard conditions and future studies under realistic environments (e.g., tomato field) are required. Hence, the noxious effects observed in *M. basicornis* may decrease owing to the degradation of chemical compounds caused by weather conditions (e.g., temperature and rainfall) on agricultural systems. This study provides useful insights towards improving the integrated management of *T. absoluta*, taking into consideration safe control alternatives for native predators in South America.

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Table 1. Details of insecticides tested at the maximum recommended field rate for their non-target effects on *Macrolophus basicornis*.

Active ingredient	Trade name (supplier)	Chemical sub-group	Main group and primary site of action[a]	Dosages[b]
Spinetoram	Delegate 250 WG® (Dow AgroSciences)	Spinosyns	Nicotinic acetylcholine receptor (nAChR) allosteric modulators	16 g
Chlorantraniliprole + Abamectin	Voliam Targo SC® (Syngenta)	Diamides and Avermectins	Ryanodine receptor modulators and glutamate-gated chloride channel (GluCl) allosteric modulators	60 mL
Triflumuron	Certero 480 SC® (Bayer)	Benzoylureas	Inhibitors of chitin biosynthesis, type 0	30 mL
Tebufenozide	Mimic 240 SC® (Iharabras S.A.)	Diacylhydrazines	Ecdysone receptor agonists	50 mL
Abamectin	Vertimec 18 EC® (Nortox)	Avermectins	Glutamate-gated chloride channel (GluCl) allosteric modulators	100 mL

[a] According to the standards of the Insecticide Resistance Action Committee (IRAC 2018)

[b] Rate ha^{-1} = 100 L

Table 2. Effects on mortality and adult tibia length of *Macrolophus basicornis* nymphs exposed to treated tomato seedlings.

Treatment	Nymph mortality (%) [a]				Predator tibia length [b]	
	24 h [c]	48 h [c]	72 h [c]	Total mortality [c]	Females [d]	Males [d]
Control	0 ± 0.00a	6.66 ± 0.03a	1.66 ± 0.01a	8.32 ± 0.03a	1.75 ± 0.06 Abc	1.77 ± 0.07 Aab
Spinetoram	3.33 ± 0.02a	8.33 ± 0.03a	1.66 ± 0.01a	13.32 ± 0.04a	1.68 ± 0.08 Aa	1.74 ± 0.11 Ba
Chlorantraniliprole + Abamectin	3.33 ± 0.02a	3.33 ± 0.02a	0.00 ± 0.00a	6.66 ± 0.03a	1.72 ± 0.07 Aab	1.75 ± 0.07 Aa
Triflumuron	10 ± 0.03b	3.33 ± 0.02a	1.66 ± 0.01a	14.96 ± 0.04a	1.79 ± 0.05 Ac	1.83 ± 0.05 Ab
Tebufenozide	6.66 ± 0.03a	5.00 ± 0.02a	6.66 ± 0.03a	18.32 ± 0.05a	1.80 ± 0.08 Ac	1.80 ± 0.07 Aab
Abamectin	31.66 ± 0.06c	36.66 ± 0.06b	11.66 ± 0.04b	79.98 ± 0.05b	-	-

[a] Mean data ± SE (GLM - binomial family)
[b] Mean data ± SD (GLM - quasi-Poisson)
[c] Means followed by different letters in the columns are significantly different (Tukey test, $p < 0.05$)
[d] Means followed by different capital letters are significantly different within the same row between the sexes, and different lower case letters indicate significant differences within the same column among the treatments (Tukey test, $p < 0.05$)

Table 3. Flight capacity of *Macrolophus basicornis* adults after insecticide exposure.

Treatment	Flight response (0 - 2 h)[a]		Flight response (2 - 4 h)[a]	
	Females[b]	Males[b]	Females[b]	Males[b]
Control	1.80± 0.15 a	1.80± 0.08 b	6.60 ± 0.18 a	6.80 ± 0.18 a
Spinetoram	1.20± 0.22 a	2.00± 0.27 b	7.00 ± 0.19 a	6.40 ± 0.27 a
Chlorantraniliprole + Abamectin	0.40 ± 0.05 a	0.00± 0.00 a	4.40± 0.18 a	4.80 ± 0.19 a
Triflumuron	2.00 ± 0.14 a	0.80± 0.04 b	5.20 ± 0.26 a	7.20 ± 0.13 a
Tebufenozide	0.60 ± 0.05 a	0.20 ± 0.04 a	6.20± 0.18 a	7.80 ± 0.11 a
Abamectin	-	-	-	-

[a] Mean data ± SD (GLM - Poisson)

[b] Means followed by different letters in the columns are significantly different (Tukey's test, $p < 0.05$)

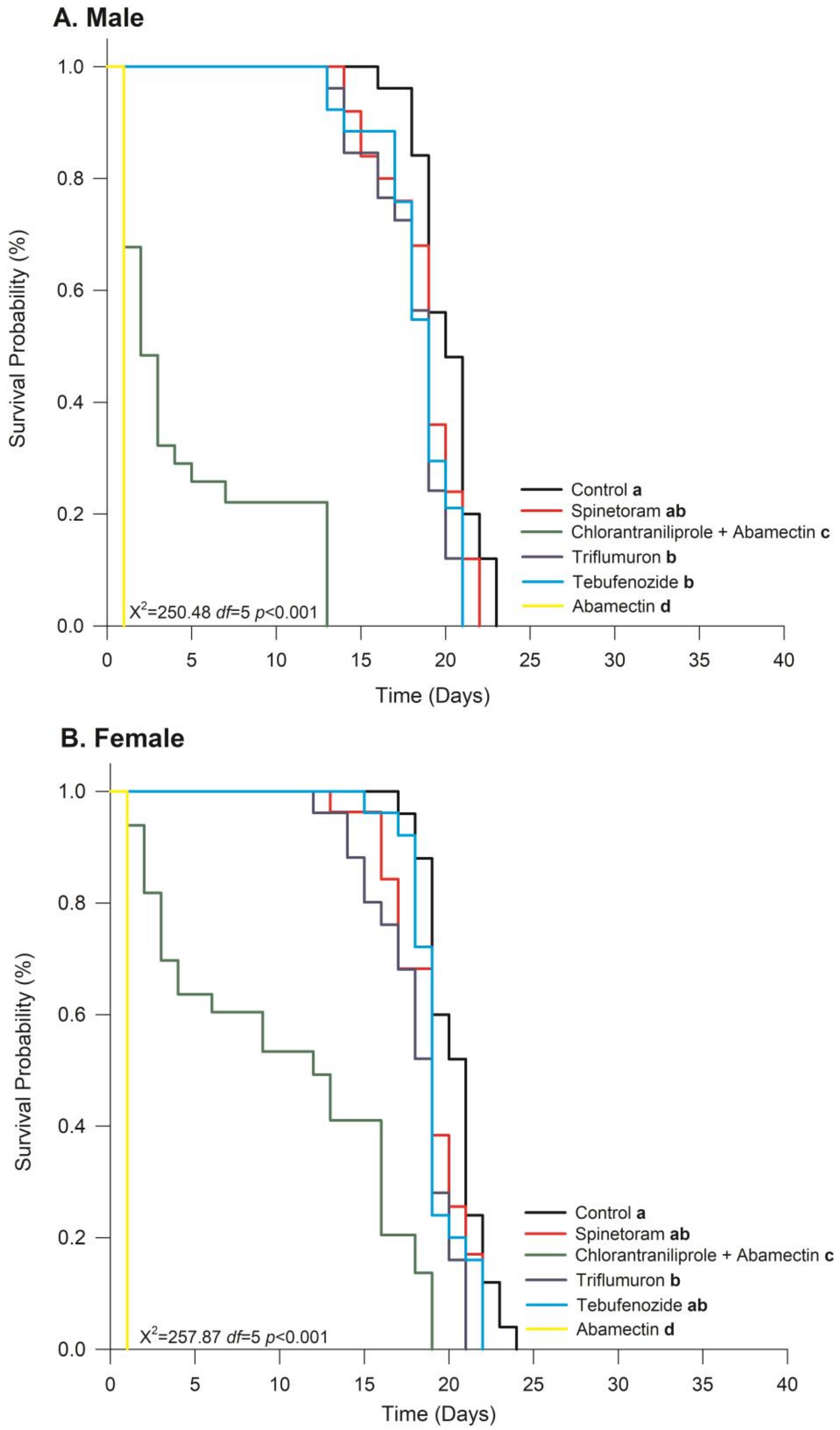

Figure 1. Survival curves of *Macrolophus basicornis* adults (A. Male and B. Female) directly treated by insecticides. Survival curves with different letters were significantly different (log-rank test, $\alpha = 0.05$).

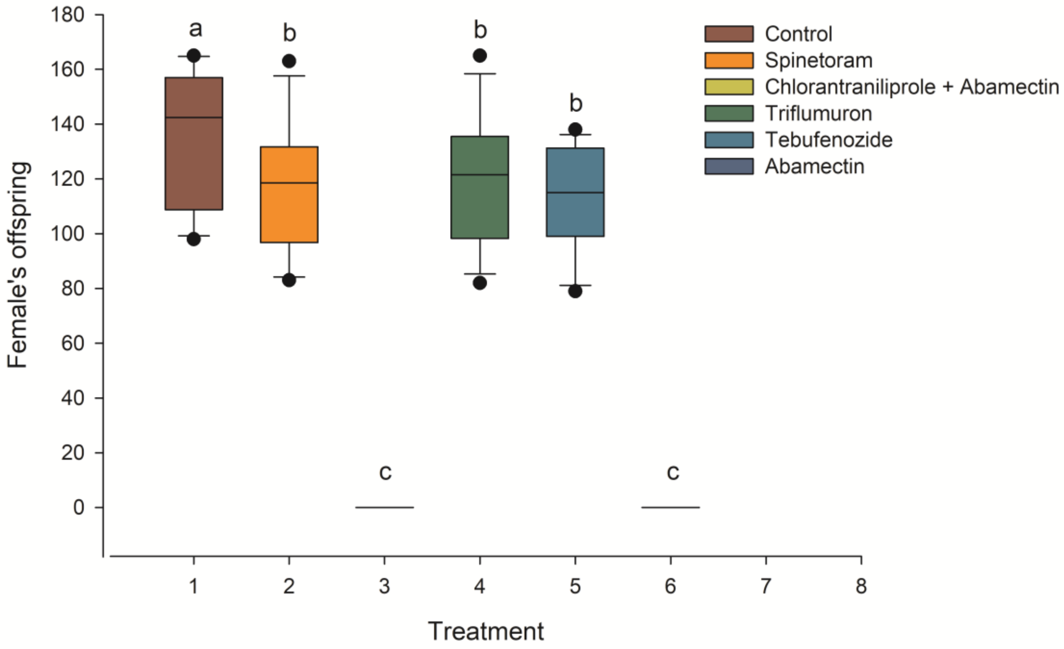

Figure 2. Bars represent *Macrolophus basicornis* offspring. Boxplots show the median, interquartile range, minimum/maximum range. Points are values that fall outside the interquartile range. Bars with different letters were significantly different (GLM, quasi-Poisson family; Tukey test, $p < 0.05$).

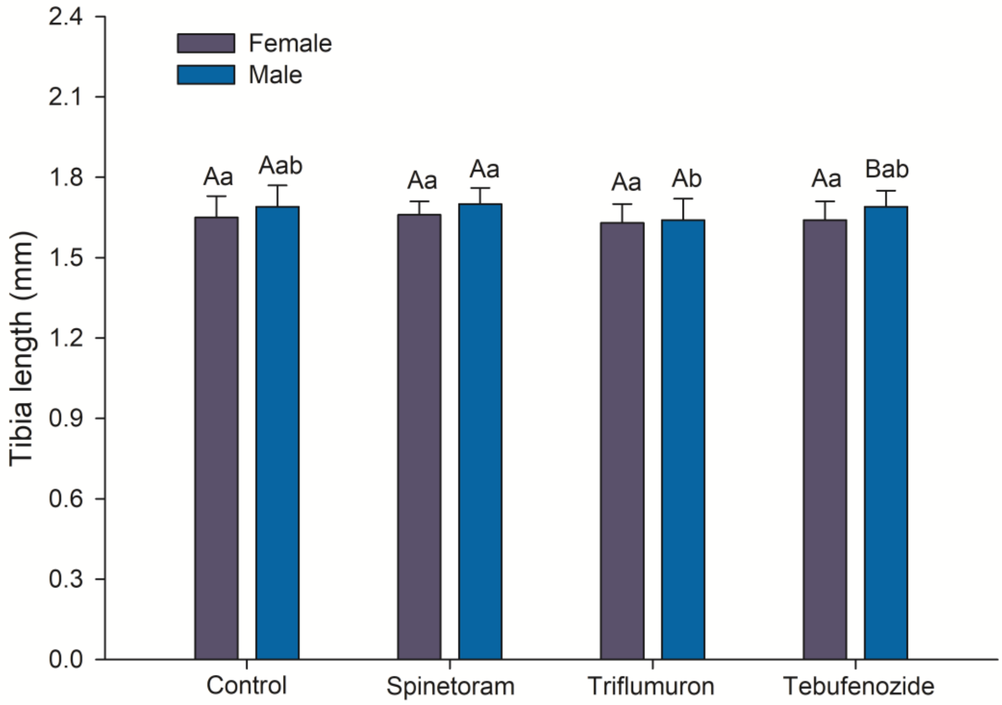

Figure 3. Sublethal effect on tibia length (mean ± SD) of *Macrolophus basicornis* adults (F_1 generation) following direct insecticide spraying on adults. Different capital letters indicate significant differences between the sexes, and different lower case letters indicate significant differences among the treatments (GLM, quasi-Poisson family; Tukey test, $p < 0.05$).

ARTIGO 3

SUBLETHAL EFFECTS PREVENT EFFECTIVE COMBINATION OF BIOPESTICIDE AND CHEMICAL PESTICIDE TOGETHER WITH A KEY NATURAL ENEMY OF *Bemisia tabaci* ON TOMATO

The present manuscript was prepared according to the Chemosphere journal rules

Sublethal effects prevent effective combination of biopesticide and chemical pesticide together with a key natural enemy of *Bemisia tabaci* on tomato

Highlights

1. The insecticide lambda-cyhalothrin achieved satisfactory results on suppressing *Bemisia tabaci* population, yet it was harmful to the predator *Nesidiocoris tenuis*.

2. *Nesidiocoris tenuis* played a key role in reducing *Bemisia tabaci* population.

3. The predator's behavior was affected by lambda-cyhalothrin and Prev-am.

4. The compatibility between plant-derived active compounds and natural enemies could be an efficient strategy for pest control.

ABSTRACT

The silverleaf whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (MED - biotype Q), represents one of the greatest threats to many cultivated vegetable crops, owing to its direct feeding damage and plant virus transmission. Chemical control is the main tool employed in Integrated Pest Management (IPM) programs for controlling this pest. However, it can result in *B. tabaci* populations' outbreaks due to development of insecticide resistance and side effects on natural enemies. Thus, as an alternative to control whiteflies, releases of the zoophytophagous predators, such as *Nesidiocoris tenuis* (Hemiptera: Miridae) in tomato crops, is highly recommended in Europe. However, a complementary tactic, such as the use of selective insecticides, is usually recommended to enhance pest control. Hence, the aim of this study was to assess the effectiveness of the citrus oil-based commercial product Prev-am® against *B. tabaci* in the presence and absence of *N. tenuis*. In addition, we evaluated sublethal effects of this botanical insecticide on the *N. tenuis* behavior and predation rate. Our findings demonstrate that Prev-am®, alone and at its maximum label dose, was not effective to control *B. tabaci*, and the interaction between this botanical insecticide and *N. tenuis* is not able to reduce the *B. tabaci* population rise. An effective *B. tabaci* control was thus achieved by using *N. tenuis* alone. Nevertheless, the synthetic pyrethroid, lambda-cyhalothrin, here tested as standard reference, caused a high mortality of the pest, and led to the extinction of *N. tenuis*, which not occurred with Prev-am®. Yet, lambda-cyhalothrin and Prev-am® significantly affected the *N. tenuis* foraging behavior, by reducing the predation rates, especially in after the exposure to lambda-cyhalothrin. These results stress the importance of including only selective insecticides use a harmless commercial product to the predator *N. tenuis* in tomato crops.

Keywords: Natural compounds, Predatory Mirid, Biological control, whitefly, Ecotoxicology

INTRODUCTION

The silverleaf whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (MED - referred to as biotype Q), is one of the most devastating pests of several crops with worldwide economic importance (Oliveira et al., 2001; De Barro et al., 2011). Among the approximately 500 host plants of *B. tabaci* ornamental plants, vegetables, grain legumes, cotton and maize are included (De Barro et al., 2011; Horowitz et al., 2011; Quintela et al., 2016). This pest can damage plants through phloem-feeding, but the greatest threat is the transmission of plant pathogens (Navas-Castillo et al., 2011; Gilbertson et al., 2015). Moreover, while feeding on the plant *B. tabaci* excretes honeydew, favoring the growth of opportunistic fungi and reducing the plant photosynthesis. *Bemisia tabaci* has been classified as a complex of at least 37 cryptic (sibling) species, which means that are morphologically similar, however exhibit biochemical or molecular polymorphism among them (De Barro et al., 2011; Liu et al., 2012; Boykin et al., 2013; Masood et al., 2017). This variations results on differences in the characteristics, such as plant preference, specificity in natural enemies' attraction, expression of resistance to insecticides, and in plant virus-transmission (Perring, 2001; Jones et al., 2003; Horowitz et al., 2005).

The management of *B. tabaci* populations, as well as the plant diseases transmitted by this polyphagous insect, represents a challenge for farmers and researches. It occurs due to fast populations' growth, capacity to develop resistance towards conventional insecticides, and their biological development characteristics, since all life stages (eggs-nymphs-adults) remain protected in the abaxial part of the leaves (Oliveira et al., 2001; Horowitz et al., 2011). Tomato production systems often requires the use of insecticides to control *B. tabaci*, and most of the time, this tool is used in a neglected manner (Palumbo et al., 2001). In fact, many *B. tabaci* populations presented resistance to several conventional insecticides from different modes of action (MoA) (Liang et al., 2012). Resistance cases were related to modulators of insect nicotinic acetylcholine receptors (e.g. neonicotinoids), molecules that act on insects' nerves and muscles (e.g. pyrethroids, organophosphates and diamides), and a great range of growth regulator insecticides (Palumbo et al., 2001; Roditakis et al., 2009; Bass et al., 2015; Dângelo et al., 2018).

The proper employment of zoophytophagous predators is a safer alternative to control pests on tomato crops, which can be combined with other natural enemies, as well as selective pesticides (Lykouressis et al., 2009; Bonato et al., 2011; Perdikis and Arvaniti, 2016; Passos et al., 2017, 2018). Among the natural enemies studied to control solanaceous pests, the predators *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) are considered efficient fortuitous and/or commercialized agents of biological control against various herbivorous arthropods in Europe, Asia and Africa (Zappalà et al., 2013; Biondi et al., 2018; Mansour et al., 2018; Han et al., 2019). These predators are able to feed on several small pests (e.g. whiteflies, thrips, aphids, mites, and lepidopterans), and they also satisfactory reproduce and walk over tomato plant trichomes (Wheeler and Krimmel, 2015).

Due to the possible mortality (acute toxicity) and several sublethal effects (e.g. physiological and behavioral) caused on beneficial arthropods by non-selective insecticides (Desneus et al., 2007), plant-derived active compounds can be an alternative in Integrated Pest Management (IPM) programs (Campolo et al., 2017; Chaieb et al., 2018). Botanical pesticides can be employed through isolated substances or complex mixtures, and the range of action encompasses the effects of insecticides, fungicides, nematicides, and bactericides (Isman, 2006). Nevertheless, prior including novel insecticides in IPM schemes, it is necessary to know the effects of biopesticides on target and non-target species. The approaches using ecological models may provide useful prediction of population dynamics based on individual level endpoints (Drobnjaković et al., 2016). Thus, noxious effects caused by insecticides in populations levels of pests and natural enemies can be estimated based on life-history parameters (Stark et al., 1997; Stark et al., 2004; Biondi et al., 2013; Amarasekare et al., 2016).

The present research explored the potential of the citrus oil-based commercial product, Prev-am®, as a tool for whitefly control, combining this biopesticide with the predator *N. tenuis*. The goals of this research were (i) provide information about the population growth of *B. tabaci* after Prev-am® and a synthetic standard insecticide, lambda-cyhalothrin exposure, as well as in the presence and absence of *N. tenuis*; (ii) determine the influence of biopescticide and conventional insecticide in the population growth of *N. tenuis*, and (iii) assess possible sublethal effects on foraging behavior and predation of *N. tenuis* after Prev-am® and lambda-cyhalothrin exposure.

MATERIALS AND METHODS

Biological materials

The bioassays were carried out at the Institut National de la Recherche Agronomique (INRA, Sophia-Antipolis) in France, in laboratory under controlled conditions (25 ± 2 °C, 75 ± 10% RH, and 16L:8D photoperiod). Tomato and tobacco plants (*Solanum lycopersicum* cv Marmande and *Nicotiana tabacum* var Wild) were grown from seeds in a climatic chamber (25± 2 °C, 75± 5% RH, and 16L:8D photoperiod), in a commercial substrate (Tournesol®) in 2L plastic pots, without any pesticide application.

Bemisia tabaci (biotype Q) was reared on tobacco plants in a climatic room (25± 2 °C, 75± 5% RH, and 16L:8D photoperiod). The whiteflies were maintained on tobacco plants (40 day-old), inside an anti-aphid cage (100 ×100 × 75 cm), and the plant was watered three times per week. One time per month, a new tobacco plant was offered to the colony and the oldest was discarded.

The predator *N. tenuis* and its alternative prey, *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) were provided by Koppert Biological Systems (Almería, Spain). The predators were reared on tobacco plants (40 day-old) inside an anti-aphid cage (60 × 60 × 40 cm), and fed with *E. kuehniella* eggs. Adult predators were transferred to a new plant weekly, in order to females lay eggs. In this way, insects belonging to coetaneous cohorts were kept together minimizing cannibalism.

Insecticides

Prev-Am® 60 a.i./L (ORO AGRI International Ltd.) is based on cold-pressed citrus peel oil, used as an alternative to control sucking pests in agroecological farming systems. In order to evaluate *B. tabaci* and *N. tenuis* demographic parameters, the bioassays were performed using three concentrations of Prev-Am®, being them the maximum field-registered concentration for the tomato crop (0.2352 g a.i./L), half (0.1176 g a.i./L) and ten per cent (0.0235 g a.i./L). Subsequently, the predation bioassay was performed using only the maximum dose.

Karate Zeon® 100 EC (Syngenta International Ltd.) is a commercial formulation of the pyrethroid insecticide lambda-cyhalothrin, widely used to control pests in agricultural systems. This compound was used as a positive control, in the maximum recommended dosage (0.0237 g a.i./L), in the bioassays of demographic parameters and predation behavior of *N. tenuis*. The concentrations were obtained by the dilution of Karate Zeon® and Prev-Am® in distilled water. Distilled water was used as negative control the bioassays.

Demographic effects on *Bemisia tabaci* and *Nesidiocoris tenuis*

The bioassays were performed by exposing the pest (*B. tabaci*) and predator (*N. tenuis*) adults to dry residues of lambda-cyhalothrin and Prev-am® on tomato leaves. Tomato fully expanded leaves (~15 cm, composed by 5 leaflets) were dipped in the insecticide solutions for 5s in the Prev-am® (one of the three concentrations: 0.2352 g a.i./L, 0.1176 g a.i./L and 0.0235 g a.i./L, respectively), or lambda-cyhalothrin (0.0237 g a.i./L), or distilled water. Leaves were left drying for one h and placed inside a system composed by superposed plastic cups, as proposed by Biondi et al. (2012). Thereby, the top plastic cup (700 mL, length: 15 cm) had a hole in the bottom center allowing to the stem of the leave to reach a second plastic cup below (350 mL, length: 11 cm) with water. Subsequently, a fine mesh net was fixed on the upper opening of the first top cup to allow ventilation and prevent the escape of insects. The bioassays with the *B. tabaci* and *N. tenuis* were established with twenty replicates per treatment.

To assess the demographic parameters of *B. tabaci*, four couples of whiteflies (~2-day old) were kept in each experimental unit. In the treatments with *N. tenuis*, one female (2-days old) of the predator was also added. Subsequently, the *B. tabaci* offspring was evaluated per life stage eleven days after the experiment set-up, under a stereoscope microscope (40x).

For assessing the demographic parameters of exposed *N. tenuis,* the bioassay was set up with two couples of predators (~8-day old) placed inside in the same experimental unit described above. Afterwards, the number of live progeny per each life stage was evaluated eleven days after the experiment set-up.

Effects on *Nesidiocoris tenuis* foraging behavior and predation

The bioassays to determine the behavioral response and predation rate of *N. tenuis* were performed using *B. tabaci* nymphs (2nd instar) as prey. *Nesidiocoris tenuis* females (2-days old) were starved for 24h in treated tomato leaves. As described previously, the leaves were treated with a Prev-am® or lambda-cyhalothrin solution, both in the maximum recommended concentration for controlling tomato pests. Distilled water was used as negative control. The tomato leaflet was offered previously to *B. tabaci* adults during 24h to oviposition. After 13 days, the excess of nymphs was removed, leaving 150 2nd instar whiteflies in each repetition. Subsequently, the predators were isolated in Petri dishes (10 cm diam. x 2 cm ht.) containing an untreated tomato leaflet infested with *B. tabaci* nymphs over an water-agar solution (1%). The dishes were closed with a Teflon® film to prevent insect escape. During ten minutes per repetition, the activity of the predators was recorded. Thus, five actions were simultaneously registers in real time: walking, cleaning, plant feeding, preying and resting. The time spent in each action was recorded in the software ETHOWATCHER® (Crispim Junior et al., 2012). The second step was to evaluate the predation rate of *N. tenuis*. Thus, after the behavioral assay, the predators were kept in the experimental unit described above, and predation rate (i.e., the number of whitefly nymphs preyed) was recorded 12 and 24 hrs after from experiment set up.

Statistical analysis

Firstly, raw data were submitted to normality (Shapiro-Wilk) and homoscedasticity (Bartlett) tests (Bartlett, 1937; Shapiro and Wilk, 1965). Thereafter, the number of *B. tabaci* and *N. tenuis* nymphs of each instar was fitted to analysis of variance (PROC ANOVA) and Tukey's HSD test (PROC GLM).

The demographic parameters of *B. tabaci* and *N. tenuis* were assessed using the instantaneous rate of increase (r_i), based on the equation proposed by Walthall and Stark (1997):

$$\mathrm{R}i = \frac{\mathrm{Ln}\left(\frac{\mathrm{N_f}}{\mathrm{N_0}}\right)}{\Delta \mathrm{t}}$$

Where N_f refers to the final number of alive insects, N_0 is the initial insects number, and Δt is the interval time (days) from the beginning until the end of the laboratory experiments (Stark et al., 1997; Walthall and Stark, 1997). Afterwards, the population growth of *B. tabaci* and *N. tenuis* was estimated following the model below:

$$\frac{dN}{dt} = rN\frac{K - N}{K}$$

Where N means the initial population size, r is a *per capita* of growth rate, K is an environmental carrying capacity, and (K-N) is an unused capacity.

The growth curves were fitted using Kaplan-Meyer estimators from the non-parametric procedure (PROC LIFETEST). The similarities among time-response curves were tested the pairwise comparisons (χ^2 log-Rank test) among curves.

A canonical variate analysis (CVA) of the predator behavior (resting, preening, plant feeding, walking and cleaning) subjected to different treatments were performed to recognize their eventual differences and the main contributing behavior for observed differences (PROC CANDISC with Distance statement). Additionally, behavioral trais were subjected to analyses of variance and Tukey´s HDS test, if appropriate (PROC GLM). The normality and homoscedasticity assumptions were checked (PROC UNIVARIATE).

Finally, the differences in predation rate between the time and among treatments were performed by Generalized Linear Models (GLMs) SAS software version 9.2 (SAS Institute, 2008), except the predation rate analyses which was fitted in the “R” software 3.4.4 (R Development Core Team, 2016).

RESULTS

Demographic effects on *Bemisia tabaci*

Regarding the treatments in the absence of *N. tenuis*, the whitefly females laid the highest amount of eggs in the treatments exposed to distilled water and the minimum concentration of Prev-am®. *Bemisia tabaci* females reduced the oviposition when exposed to the half concentration of Prev-am® and a highest reduction was observed for the highest concentration of this product. Moreover, in the lambda-cyhalothrin treatment the lowest number of eggs was found. Concerning the 1st instar, the number of nymphs found in distilled water treatment did not differ significantly from insects exposed to the minimal concentration of Prev-am®. A reduction in the number of insects was observed for the half concentration of Prev-am®. The number of nymphs found in the maximum concentration of Prev-am® and lambda-cyhalothrin treatments was lower than the other treatments. For the 2nd instar, the highest number of progenies were verified to insects maintained in the distilled water and in the minimum concentration of Prev-am®. The reduction in the number of nymphs was significant for the maximum concentration of Prev-am®. Lambda-cyhalothrin was the most toxic compound to the 2nd instar of *B. tabaci*. For the 3rd instar of *B. tabaci*, the highest number of nymphs was observed in the distilled water treatment. There was no differences in the progeny of insects exposed to the minimum and half concentrations of Prev-am®. Still, fewer nymphs were found for females maintained in the maximum concentration of Prev-am®. Lastly, the smallest number of 3rd nymphs was found in the lambda-cyhalothrin treatment (Table 1).

Concerning the treatments in the presence of *N. tenuis*, the highest number of *B. tabaci* eggs was found in the half and maximum concentrations of Prev-am®. Yet, no significant differences were observed in the minimal and maximum concentrations of Prev-am®. The distilled water and lambda-cyhalothrin treatments resulted in a reduced number of *B. tabaci* eggs. Regarding the 1st instar of *B. tabaci*, the number of nymphs was higher in the half concentration of Prev-am®. In the treatments with the minimal and maximum concentrations of Prev-am®, the number of *B. tabaci* nymphs reduced significantly. The smallest number of nymphs was observed in the distilled water and lambda-cyhalothrin treatments. For the *B. tabaci* 2nd instar nymphs, the highest number of descendants was found in the treatments with half and maximum concentrations of Prev-am®. In the maximum concentration Prev-am® treatment, the second highest number of nymphs was verified. In addition, the smallest number of *B. tabaci* nymphs was observed in distilled water and lambda-cyhalothrin treatments. Regarding the 3rd instar, the highest number of *B. tabaci* nymphs was verified in the half concentration of Prev-am®. The minimum concentration of Prev-am® was the treatment with the second highest number of *B. tabaci* nymphs. Finally, no significant differences were observed among the treatments comprised by distilled water, maximum concentration of Prev-am® and lambda-cyhalothrin (Table 1).

For the establishment of *B. tabaci* on tomato leaves, treatments comprised only by *B. tabaci*, along with *B. tabaci* + Prev-am® (minimum concentration) and *B. tabaci* + Prev-am® (half concentration) showed the highest population increases. The second highest increase of *B. tabaci* population was verified in the *B. tabaci* + *N. tenuis* + Prev-am® (half concentration) and *B. tabaci* + Prev-am® (maximum concentration) treatments. The third higher progeny values were observed for *B. tabaci* + *N. tenuis* + Prev-am® (minimum concentration) and *B. tabaci* + *N. tenuis* + Prev-am® (maximum concentration) treatments. Finally, *B. tabaci* + *N. tenuis* was the fourth treatment with the less of *B. tabaci*, and the lowest *B. tabaci* progeny was found in the leaves treated with lambda-cyhalothrin (Figure 1).

Demographic effects on *Nesidiocoris tenuis*

The number of *N. tenuis* nymphs emerged from tomato leaves treated with Prev-Am® (all concentrations) and lambda-cyhalothrin was significantly lower than the untreated control. Regarding the emergence of 1st and 4rd instar nymphs , for the three concentrations of Prev-Am® the number of nymphs was significantly smaller than in the control. Concerning 2nd instar nymphs, the three oil doses also reduced the nymphs emergence; however, among the tested doses, a lower number of nymphs was verified to the maximum concentration of Prev-am®. On the other hand, the half and maximum doses of Prev-Am® were more toxic to 3rd instar nymphs. In addition, *N. tenuis* adults did not reproduced when exposed to lambda-cyhalothrin (Table 2).

Although the three tested Prev-am® concentrations reduced the progeny of *N. tenuis* exposed to treated leaves, the analysis of demographic parameters showed that the number of insects could rise (Figure 2). In addition, the increase of population curve of predators exposed to Prev-am® minimum concentration was similar to the control. However, the same was not observed for insects submitted to lambda-cyhalothrin, with no capacity of population increase after exposure to the synthetic insecticide (Figure 2).

Effects on the *Nesidiocoris tenuis* foraging behavior and predation rate

The CVA analysis indicated significant overall differences in the predator behavior submitted to different treatments (Wilks' $\lambda = 0.61$; $F = 2.93$; $df_{(num/den)} = 10/106$; $P = 0.003$) (Table 3). The CVA diagram suggests that treatment with lambda-cyhalothrin and Prev-Am® affected predator behavior when compared with control (Figure 3). The first axis ($P = 0.003$) explained 98% of the observed differences (Table 3). Higher canonical loads were observed in preening and cleaning for the 1st axis, which were responsible for most of the observed divergence among treatments.

Regarding the predation rates of *B. tabaci* exhibited by *N. tenuis*, the predators exposed to Prev-am® and lambda-cyhalothrin preyed fewer *B. tabaci* nymphs than the predators in the untreated control treatment. In addition, the interaction between time and treatments factors showed significantly differences on reduction of predation rate ($\chi^2 = 8.42$, df = 2, $P = 0.015$). The lowest number of *B. tabaci* preyed was observed for predators exposed to lambda-cyhalothrin, and the highest by predators from the distilled water treatment

(χ^2 = 67.40, df = 2, P < 0.001). In all treatments, the insects preyed more in the first evaluation time (1-12h) than in the second time (12-24h) (χ^2 = 1443.62, df = 1, P < 0.001) (Figure 4).

DISCUSSION

Insecticides have been widely used due to their effectiveness on pest control rapid action against susceptible *B. tabaci* populations (Naranjo et al., 1996). However, environmentally safe approaches have to be prioritized to avoid resistant pest population peaks (e.g. combining natural enemies and selective products) (Guedes et al., 2016). In our study, the population of *B. tabaci* is able to grow when exposed to fresh residues of Prev-am®. Moreover, an apparently antagonistic relation between this biopesticide and *N. tenuis* was observed, since a greater decrease of *B. tabaci* progeny was verified only in the presence of this predator. On the other hand, lambda-cyhalothrin promoted the highest control of *B. tabaci*. Meanwhile, predators exposed to lambda-cyhalothrin showed the greatest reduction in population growth and predation rate. The behavioral traits of *N. tenuis* were affected in the same way by Prev-am® and lambda-cyhalothrin.

Most toxicological studies involving botanical insecticides are based on acute mortality of target species and natural enemies (Regnault-Roger et al., 2012). After the exposure to noxious compounds, the insects may offset the mortality points in the population dynamics (McNair, 1995; Stark et al., 2004). In our study, *B. tabaci* adults were able to reproduce, and consequently to increase their population even after Prev-am® exposure. The alternative hypotheses would be concerning (i) the whiteflies are more susceptible in nymphal stages than eggs and adults to Prev-am®, (ii) the penetration of Prev-am® residues into *B. tabaci* eggs is low, and (iii) Prev-am® do not provide a large residual effect against *B. tabaci*. In contrast, the whitefly exhibited a decline on demographic parameters after lambda-cyhalothrin exposure, and this was also observed for *N. tenuis*.

Nesidiocoris tenuis is an effective predator of *B. tabaci* on tomato under greenhouse and open field conditions (Calvo et al., 2012). Releases of this predator in tomato crops may result in more than 80% of *B. tabaci* control (Calvo et al., 2009). Despite the benefits provided by *N. tenuis* to pest control, in some cases the use of insecticides and biopesticides are required in agricultural systems, mainly when pests reach the economic damage threshold level on crops (Zappalà et al., 2012; Madbouni et al., 2017). Our results confirmed that Prev-

Am® and lambda-cyhalothrin caused a significant reduction in *N. tenuis* nymph's emergence in endpoint analysis. However, the sublethal effect on predator's reproduction exposed to both products cannot be considered similar. After the predator exposure on Prev-Am®, *N. tenuis* was still able to increase its population. The same was not verified to lambda-cyhalothrin, which compromised the reproduction of the predators.

Prev-am® did not reduced the longevity of *N. tenuis*, as was reported for lambda-cyhalothrin, after exposed to dried residues (Soares et al., 2019). Another important generalist predator, *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae), did not have its longevity altered, suggesting that Prev-Am® have no effect on this biological trait to these predators. On the other hand, *O. laevigatus* offspring was reduced after Prev-am® exposure (Biondi et al., 2012). However, no effects were noticed on the survival and reproduction of adult females of the mealybug parasitoid *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae) and of the moth parasitoid *Bracon nigricans* Szépligeti (Hymenoptera: Braconidae) exposed to dry residues of Prev-am® (Mansour et al., 2011; Biondi et al., 2013).

The D-limonene (major component of Prev-am®) is a monocyclic terpene produced by plants, as secondary metabolites, often abundant in citrus fruit peel (Ciriminna et al., 2014). Interestingly, the mode of action of biopesticides based on D-Limonene is similar with described for lambda-cyhalothrin, acting upon the nervous systems of insects (Desneux et al., 2004; Malacrinò et al., 2016; Mossa, 2016). This could explain the results obtained in our bioassays, where both commercial products caused detrimental effects in *N. tenuis* behavioral response. Undesirable sublethal effects on predator's behavioral response may lead to reductions of reproductive rates, host-finding and prey-feeding (Desneux et al., 2004; Müller, 2018). In a companion behavioral study, we offered *T. absoluta* as prey for *N. tenuis*, and the predators spent more time walking and resting after Prev-am® exposure. In addition, its predation rate was lower compared to non-treated insects. Despite the results described above, in the same report, lambda-cyhalothrin was more harmful to *N. tenuis* than Prev-Am® (Soares et al., 2019).

The services provided by natural enemies in crop protection can be direct, by the predation and/or parasitism, and indirect, inhibiting the presence of herbivorous, sincethese insects can recognize chemical cues released by predators and/or parasitoids (Nomikou et al., 2003; Messelink et al., 2012). Consequently, it may highlight the importance to use harmless insecticides, because even with a lower predation rate, the presence of predators are indirectly beneficial for tomato crops. In our findings, *N. tenuis* showed the highest predation rate on *B. tabaci* nymphs in the first evaluation, due to the starvation time (24 h) prior to the exposition to the prey in the experimental arenas. In addition, both compounds promoted a reduction in the number of *B. tabaci* nymphs preyed by *N. tenuis*. The same products decreased the total predation rate of *N. tenuis* upon *T. absoluta* in laboratory bioassay (Soares et al., 2019). The undesirable interferences in the predatory behavior after insecticide exposure may be characterized by (i) repellent effects, (ii) antifeedant properties, and (iii) disruption in the ability to locate food (Desneux et al., 2007; Müller, 2018). We suggest that the hypothesis (iii) is applicable in our results, because both commercial products could trigger haphazard movement and increased the restlessness in insects (He et al., 2012).

In summary, despite the smaller reduction of Prev-am® on the demographic parameters of *B. tabaci*, the use of this kind of insecticide should be prioritized for inclusion into IPM programmes that expect the concurrent activity of natural enemies. However, it is important to highlight that *B. tabaci* was exposed by residual contact to dry residues, and that the direct topical spraying upon adults can allow a stronger pest control. Another important point that may be worthy of further investigation is the assessment of Prev-am® sublethal effects on *N. tenuis* under standard conditions, since of this predator may avoiding treated tomato plants, thus reducing the damage caused by insecticides on its life traits.

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Table 1. Mean number (±SE) of eggs laid and emerged individuals reaching the 1st, 2nd or 3rd instar in the progeny of *Bemisia tabaci* exposed to dry residues of Prev-am® (at 10, 50 and 100% of its label concentration), of lambda-cyhalothrin (treated control) and to distilled water (untreated control). On the bottom of the table, the statistical results of nymphs number produced by adults submitted to different treatments.

Treatment		**Absence of *Nesidiocoris tenuis*[1]**				**Presence of *Nesidiocoris tenuis*[1]**			
		Eggs	**1st instar**	**2nd instar**	**3rd instar**	**Eggs**	**1st instar**	**2nd instar**	**3rd instar**
B. tabaci +	Distilled water	325.9 ± 12.1 a	38.3 ± 2.8 a	308.9 ± 9.1 a	55.6 ± 4.9 a	17.1 ± 3.0 c	1.6 ± 0.3 c	27.1 ± 4.2 c	1.5 ± 0.4 c
	Prev-am 10%	300.1 ± 10.2 a	37.4 ± 2.9 a	274.1 ± 6.9 ab	40.4 ± 4.0 b	68.4 ± 7.0 b	5.7 ± 0.8 b	104.1 ± 10.3 a	11.4 ± 2.1 b
	Prev-am 50%	221.4 ± 13.9 b	26.8 ± 2.5 b	246.6 ± 14.9 b	37.2 ± 3.4 b	109.9 ± 11.0 a	20.6 ± 1.6 a	103.5 ± 7.5 a	26.2 ± 1.5 a
	Prev-am 100%	117.4 ± 14.3 c	5.9 ± 0.9 c	167.7 ± 12.1 c	14.2 ± 1.8 c	88.1 ± 12.2 ab	8.0 ± 1.0 b	66.5 ± 9.5 b	5.2 ± 1.4 c
	Lambda-cyhalothrin	1.3 ± 0.5 d	0.1 ± 0.1 c	1.1 ± 0.4 d	1.1 ± 0.4 d	1.4 ± 0.4 c	0.5 ± 0.2 c	1.3 ± 0.4 c	1.2 ± 0.4 c
$F_{4, 95}$		139.58	67.69	150.05	43.81	32.55	71.26	38.49	61.84
P		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[1]Values followed by the same letter in a column are not significantly different by Tukey's HSD test ($p \leq 0.05$)

Table 2. Mean number (±SE) of *Nesidiocoris tenuis* 1st, 2nd, 3rd and 4th instars produced by females exposed to dry residues of Prev-am® (at 10, 50 and 100% of its label concentration), of lambda-cyhalothrin (treated control) and to distilled water (untreated control). On the bottom of the table, the statistical results of nymphs number produced by adults submitted to different treatments.

Treatment		Developmental stages of natural enemies[1]			
		1st instar	**2nd instar**	**3rd instar**	**4th instar**
N. tenuis +	Distilled water	8.9 ± 0.7 a	20.6 ± 1.1 a	19.5 ± 1.4 a	7.4 ± 0.5 a
	Prev-am 10%	5.5 ± 0.6 b	14.3 ± 1.2 b	14.6 ± 1.1 b	5.0 ± 0.4 b
	Prev-am 50%	4.5 ± 0.6 b	11.5 ± 1.4 b	9.7 ± 1.4 c	4.5 ± 0.6 b
	Prev-am 100%	3.5 ± 0.5 b	6.7 ± 0.5 c	8.0 ± 0.6 c	4.2 ± 0.5 b
	Lambda-cyhalothrin	0.0 ± 0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 c
$F_{4, 95}$		30.81	57.43	48.09	32.32
P		< 0.001	< 0.001	< 0.001	< 0.001

[1]Values followed by the same letter in a column are not significantly different by Tukey's HSD test ($P \leq 0.05$)

Table 3. Canonical loadings (between canonical structures) of the canonical axes for the *Nesidiocoris tenuis* foraging behavior previously exposed to dry residues of Prev-am® (at 10, 50 and 100% of its label concentration), of lambda-cyhalothrin (treated control) and to distilled water (untreated control). Bold type indicates the main contributors of each axis and asterisks indicate the significant axes.

Behavior Predator	**Canonical axes**	
	1st	**2nd**
Restining	-0.55	0.47
Preening	0.68	0.08
Plant feeding	0.73	-0.34
Walking	-0.37	0.15
Cleaning	**-0.99**	-0.92
$F_{appr.}$	2.93	0.11
Proportion	0.98	0.01
P	0.002*	0.974
Eigenvalue	0.61	0.01

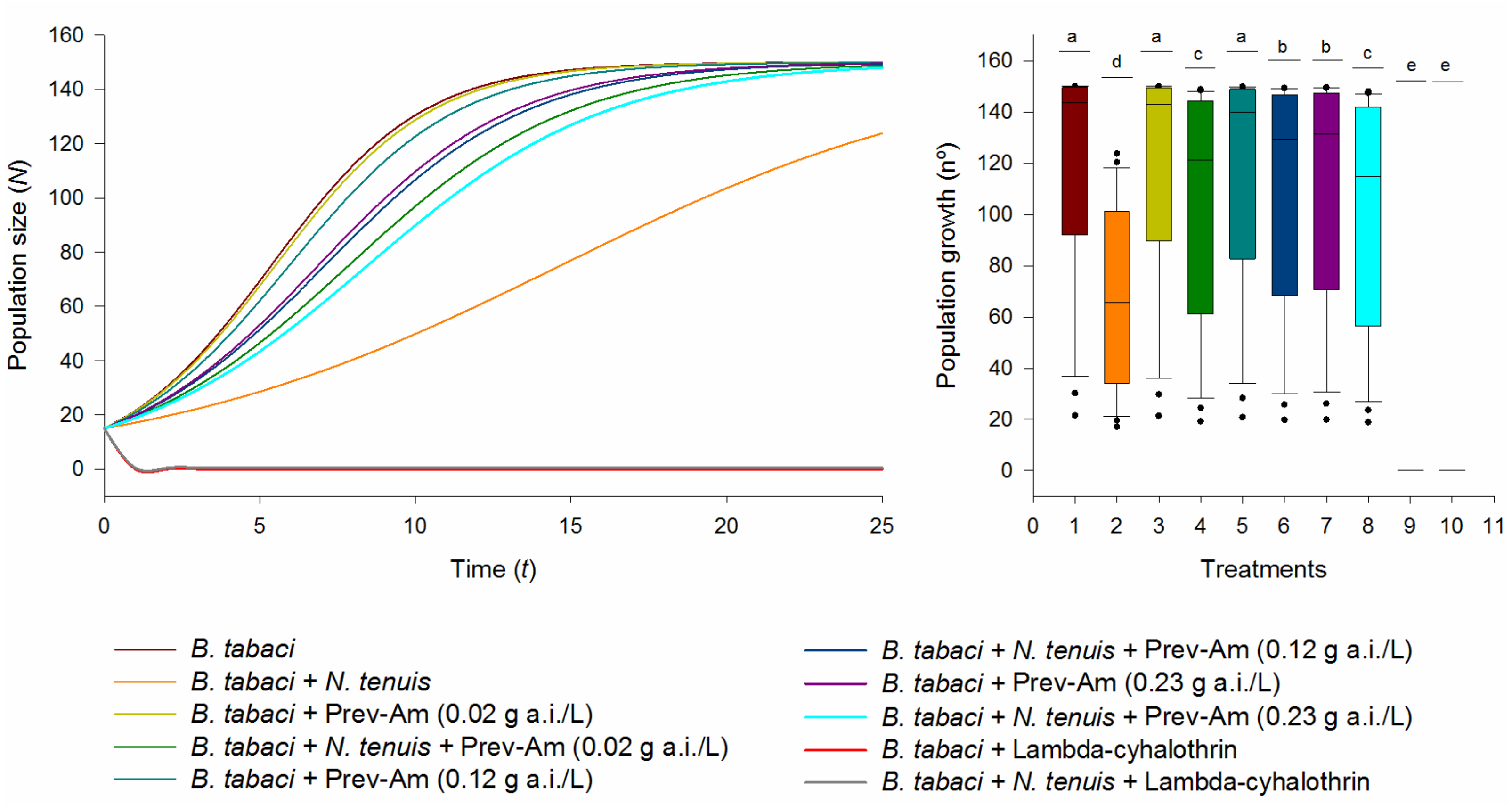

Figure 1. Curves and box plots of the estimated population curves of *Bemisia tabaci* exposed to dry residues of Prev-am® (at 10, 50 and 100% of its label concentration), of lambda-cyhalothrin (treated control) and to distilled water (untreated control). Box plots indicate the median and dispersion (lower and upper quartiles and outliers) population growth. The box plots with the different lower-case letters are significantly different by pairwise comparison in χ^2 log-Rank test ($P \leq 0.05$).

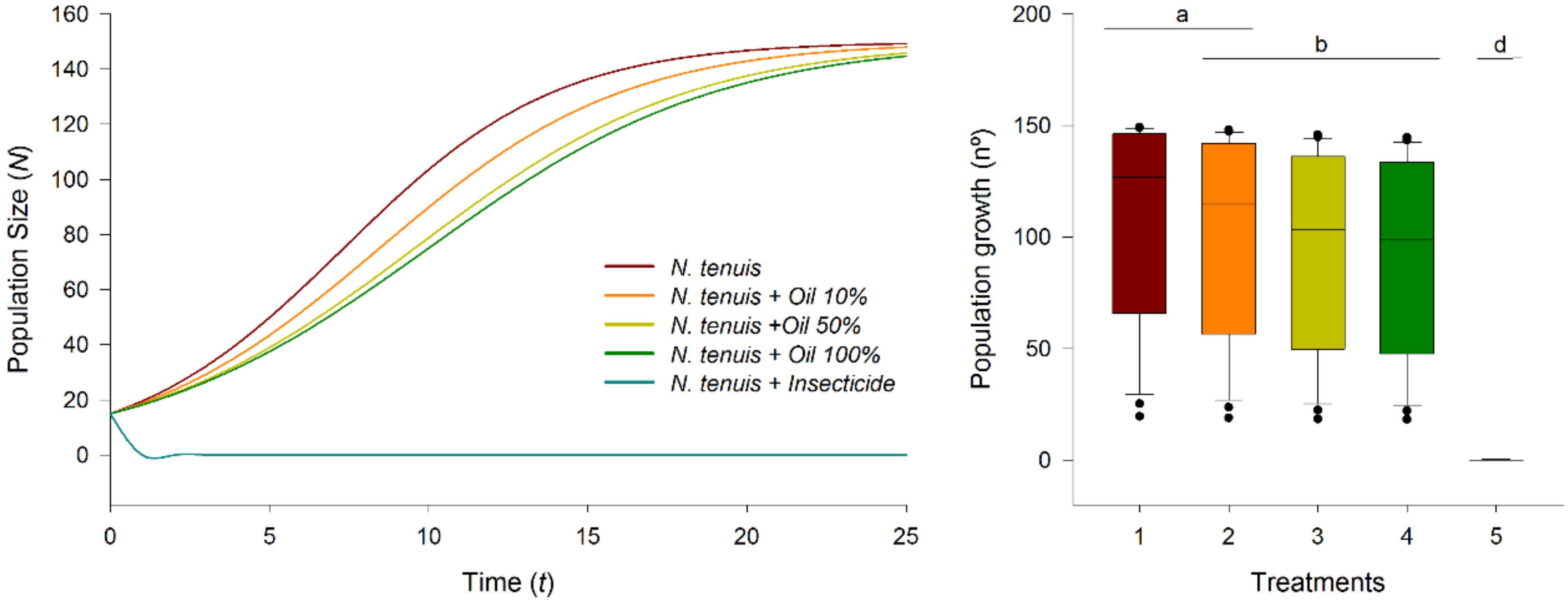

Figure 2. Curves and box plots of the estimated population curves of *Nesidiocoris tenuis* exposed to dry residues of Prev-am® (at 10, 50 and 100% of its label concentration), of lambda-cyhalothrin (treated control) and to distilled water (untreated control). Box plots indicate the median and dispersion (lower and upper quartiles and outliers) population growth. The box plots with the different lower case letters are significantly different by pairwise comparison in χ^2 log-Rank test ($P \leq 0.05$).

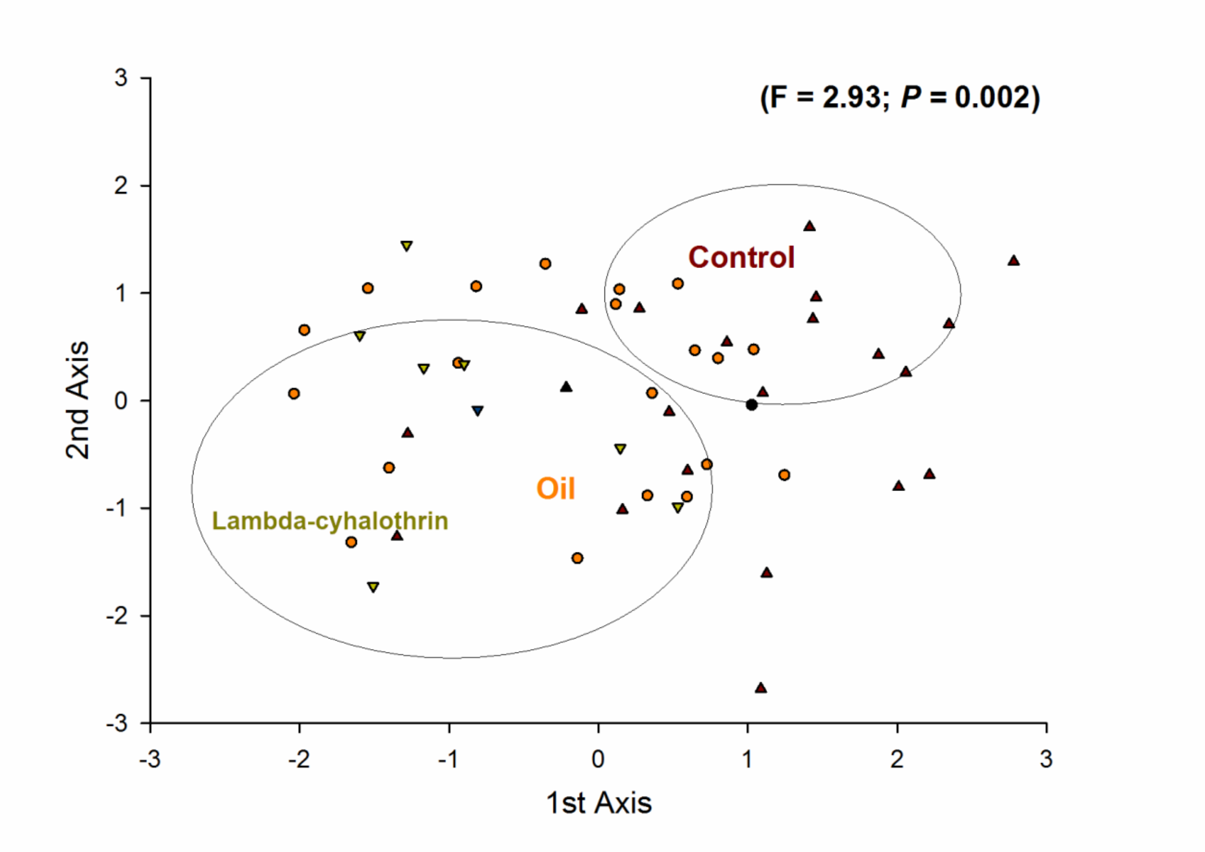

Figure 3. Ordination (CVA) diagrams showing the divergence in predator behavior when exposed to dry residues of Prev-am® (at 10, 50 and 100% of its label concentration), of lambda-cyhalothrin (treated control) and to distilled water (untreated control). The solid symbols represent the individual replicates. The large circles indicate treatments that are not significantly different by the approximated F-test ($P \leq 0.05$), based on the Mahalanobis (D^2) distance between class means.

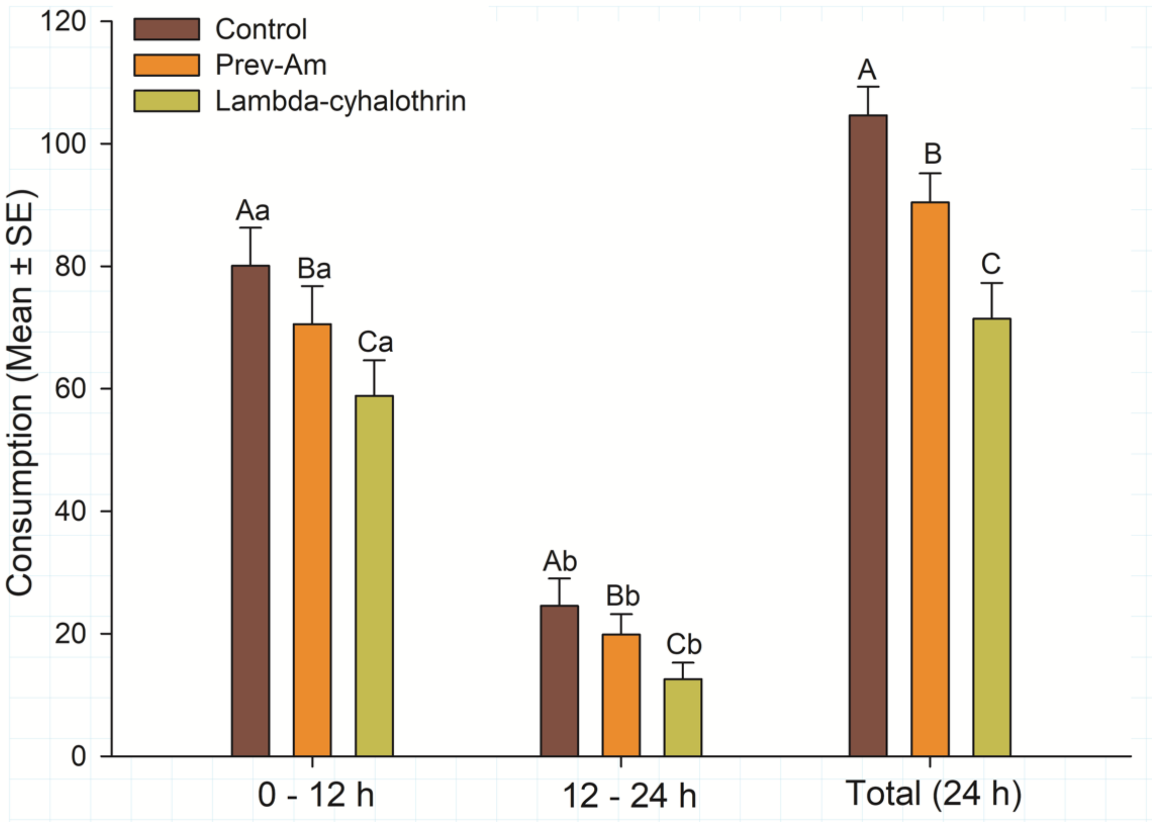

Figure 4. Mean (±SE) consumption of *Nesidiocoris tenuis* females preying upon *Bemisia tabaci* after exposure to dry residues of Prev-am® (at 100% of its label concentration), lambda-cyhalothrin (treated control) and distilled water (untreated control), in two times after exposure to the treatments (0 – 12 h and 12 - 24 h), and the total consumption after 24 h. Bars followed by different capital letters are significantly different among the treatments, and different lower case letters are significantly different among the two-time consumption (GLM-with Poisson distribution, followed by Tukey's test, $P \leq 0.05$).

CONSIDERAÇÕES FINAIS

Os resultados do presente trabalho demonstram que o predador *N. tenuis* apresenta potencial para estabelecer-se em folhas de tomateiro, bem como reduzir as populações de *T. absoluta* e *B. tabaci* em condições de laboratório. Entretanto, o uso inadequado de lambda-cyhalothrin pode comprometer o seu desempenho como predador, diretamente por meio do efeito letal, ou indiretamente ao afetar negativamente suas características biológicas e comportamentais. O bioinseticida Prev-am®, apesar de apresentar baixa persistência no ambiente, causa efeitos subletais em *N. tenuis*. Desta forma, recomenda-se que Prev-am® deva ser utilizado de forma criteriosa em campos de tomateiro onde se realizam liberações inoculativas de *N. tenuis*. O uso dessincronizado dos dois métodos de controle pode ser considerado uma boa estratégia, podendo-se aplicar primeiramente o bioinseticida Prev-am® para abaixar a população da praga e posteriormente realizar as liberações de *N. tenuis* como segunda linha de defesa do cultivo.

O predador *M. basicornis* apresenta grande potencial como agente de controle biológico de *T. absoluta* e *B. tabaci*; entretanto, o principal entrave para que isto ocorra, está relacionado ao baixo número de estudos realizados com esse mirídeo, principalmente em condições reais de cultivos de tomate. No presente estudo, constatou-se que todos os inseticidas (spinetoram, chlorantraniliprole + abamectin, triflumuron e tebufenozide) avaliados apresentam efeitos negativos sobre este predador. Desta forma, é recomendada a realização de novas pesquisas visando avaliar os efeitos destes compostos em condições de semicampo e campo para confirmação da sua toxicidade e possível uso em programas de MIP visando a integração dos dois métodos de controle, biológico e químico.