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ORIGINAL PAPER

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

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Abstract

The development of new strategies to control pest insects is 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* (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® indicates that none of the concentrations differed significantly from the control. In addition, the 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

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 of *Nesidiocoris tenuis* with 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*.

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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 (Campos et al. 2017; Sankarganesh et al. 2017; Sylla et al. 2017; Biondi et al. 2018; Han et al. 2018; Mansour et al. 2018). This South American native moth has a life cycle of about 26–75 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 *Tuta absoluta* on tomato crops may reach 100% since the larvae feed on leaves, flowers, stems and fruits (Desneux et al. 2010, 2011). 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; Biondi et al. 2018). 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; Desneux et al. 2007; Biondi et al. 2013; Roditakis et al. 2015). Safer alternatives are required to control pests, for example through using natural insecticides, which have been considered eco-friendly 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, 2013; Desneux et al. 2005). 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, 2007).

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: Aleyrodidae), 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 behavior 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; Biondi et al. 2016).

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; Chaieb et al. 2018). 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, 2018; Pérez-Aguilar et al. 2018). 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) and disturb associated biocontrol services (Lu et al. 2012; Mohammed et al. 2018). 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 days 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 10 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

In our experiments, we used Prev-Am® (ORO AGRI International Ltd.) 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 a synthetic 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 5 s 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 11 days, the number of live offspring (eggs until 3rd instar larvae) of *T. absoluta* per life stage was measured under stereoscope microscope (10×).

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 1 L 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. Afterward, each predator was transferred in one Petri dish (10 cm diam. × 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 min 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 24 h with the 150 *T. absoluta* eggs, and the predation rate was recorded after 12 h and 24 h. In both access, twenty replicates were performed per treatment.

Survival assessment of *N. tenuis*

The tomato plants (25 days 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 1 h under laboratory conditions. *Nesidiocoris tenuis* (2 days 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):

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

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 nonparametric 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®

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]				Presence of *Nesidiocoris tenuis*[1]			
	Eggs	1st instar	2nd instar	3rd instar	Eggs	1st instar	2nd instar	3rd instar
T. absoluta +								
Control	2.85 ± 0.98[a]	6.40 ± 0.73[a]	36.50 ± 2.82[ab]	39.60 ± 4.81	0.45 ± 0.24	2.00 ± 0.43[ab]	14.00 ± 1.94[bc]	12.15 ± 2.15[bc]
Prev-am 10%	0.95 ± 0.45[ab]	1.15 ± 0.23[cd]	28.45 ± 2.22[bc]	35.05 ± 3.06	0.00 ± 0.00	1.40 ± 0.31[b]	20.45 ± 2.31[b]	13.15 ± 1.49[bc]
Prev-am 50%	0.00 ± 0.00[b]	0.90 ± 0.22[d]	22.40 ± 2.14[c]	26.05 ± 2.41	0.10 ± 0.10	0.85 ± 0.22[b]	8.45 ± 1.11[c]	5.75 ± 0.79[c]
Prev-am 100%	0.80 ± 0.48[ab]	3.20 ± 0.58[b]	33.35 ± 2.38[abc]	36.10 ± 2.76	0.35 ± 0.18	3.65 ± 0.67[a]	21.20 ± 2.43[b]	16.70 ± 2.25[b]
Lambda-cyhalothrin	0.00 ± 0.00[b]	3.02 ± 0.61[bc]	43.25 ± 4.43[a]	36.15 ± 3.38	0.00 ± 0.00	1.95 ± 0.42[ab]	31.2 ± 3.90[a]	36.65 ± 3.04[a]
$F_{4,95}$	4.81	18.06	7.29	1.86	2.09	5.64	11.56	31.41
P	< 0.001	< 0.001	< 0.001	0.123	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 ($p < 0.05$)

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).

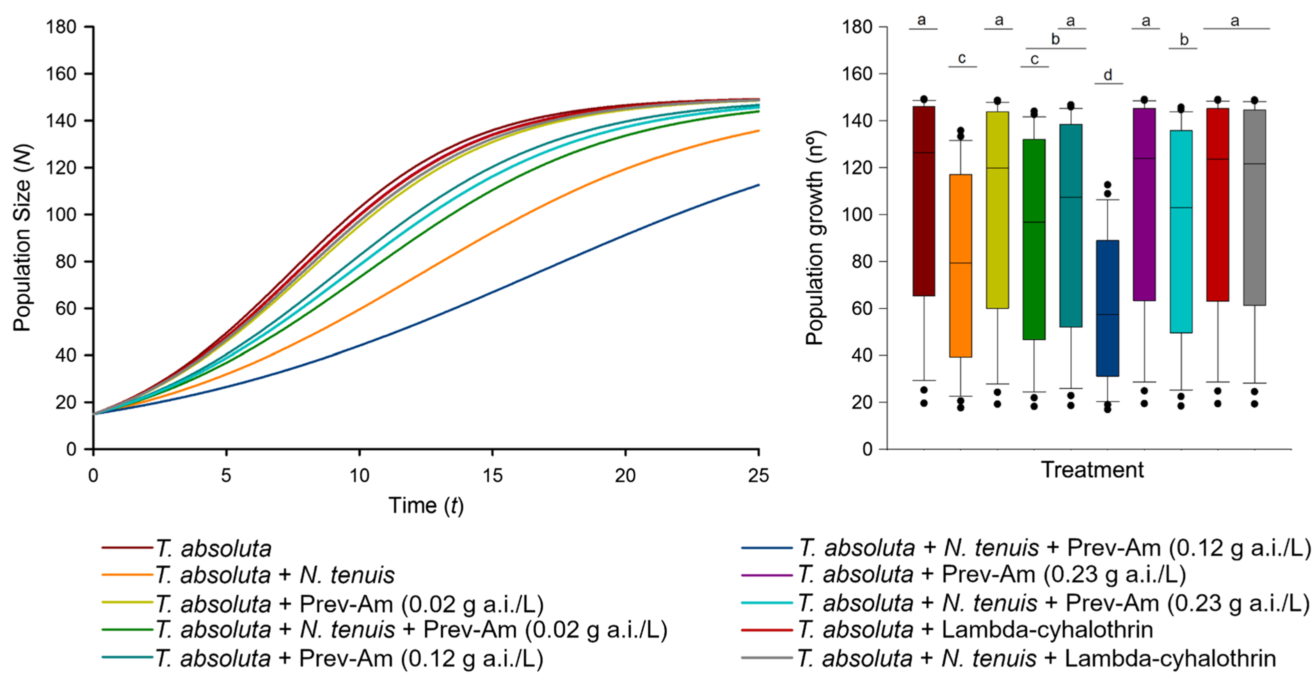

Fig. 1 Curves and box plots of the estimated population curves of *Tuta absoluta* submitted to different treatments. Box plots indicate the median and dispersion (lower and upper quartiles and outliers) population growth. The box plots with the different lower case letters are significantly different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

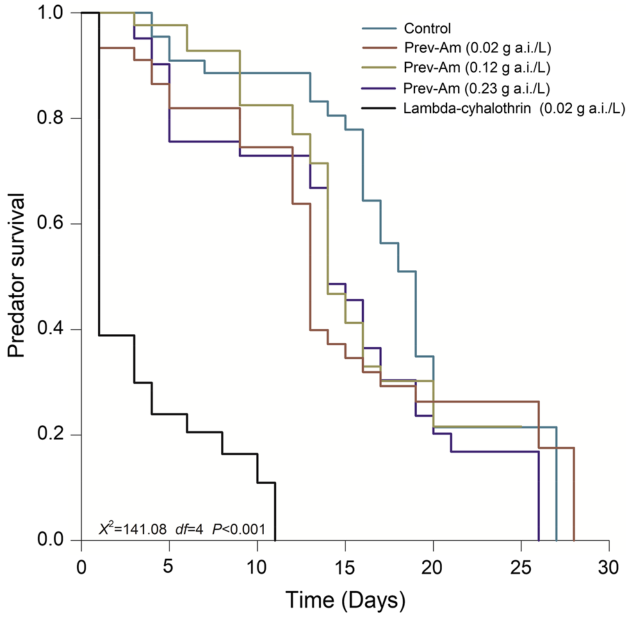

Fig. 2 Survival curves of *Nesidiocoris tenuis* adults exposed to tomato plants treated with a conventional (lambda-cyhalothrin) and a botanical (Prev-Am®) insecticide. Survival curves with different letters were significantly different (Log-rank test, $\alpha = 0.05$)

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, $\chi^2 = 141.08$, $df = 4$, $p < 0.001$) (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 ($\chi^2 = 68.3$, $df = 2$, $p < 0.001$). *Nesidiocoris 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 was significantly lower at both times of evaluation (12 and 24 h) 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 12 h ($\chi^2 = 20.0$, $df = 5$, $p < 0.001$). In addition, after 24 h, 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 and lemon oils 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 highlights the necessity to

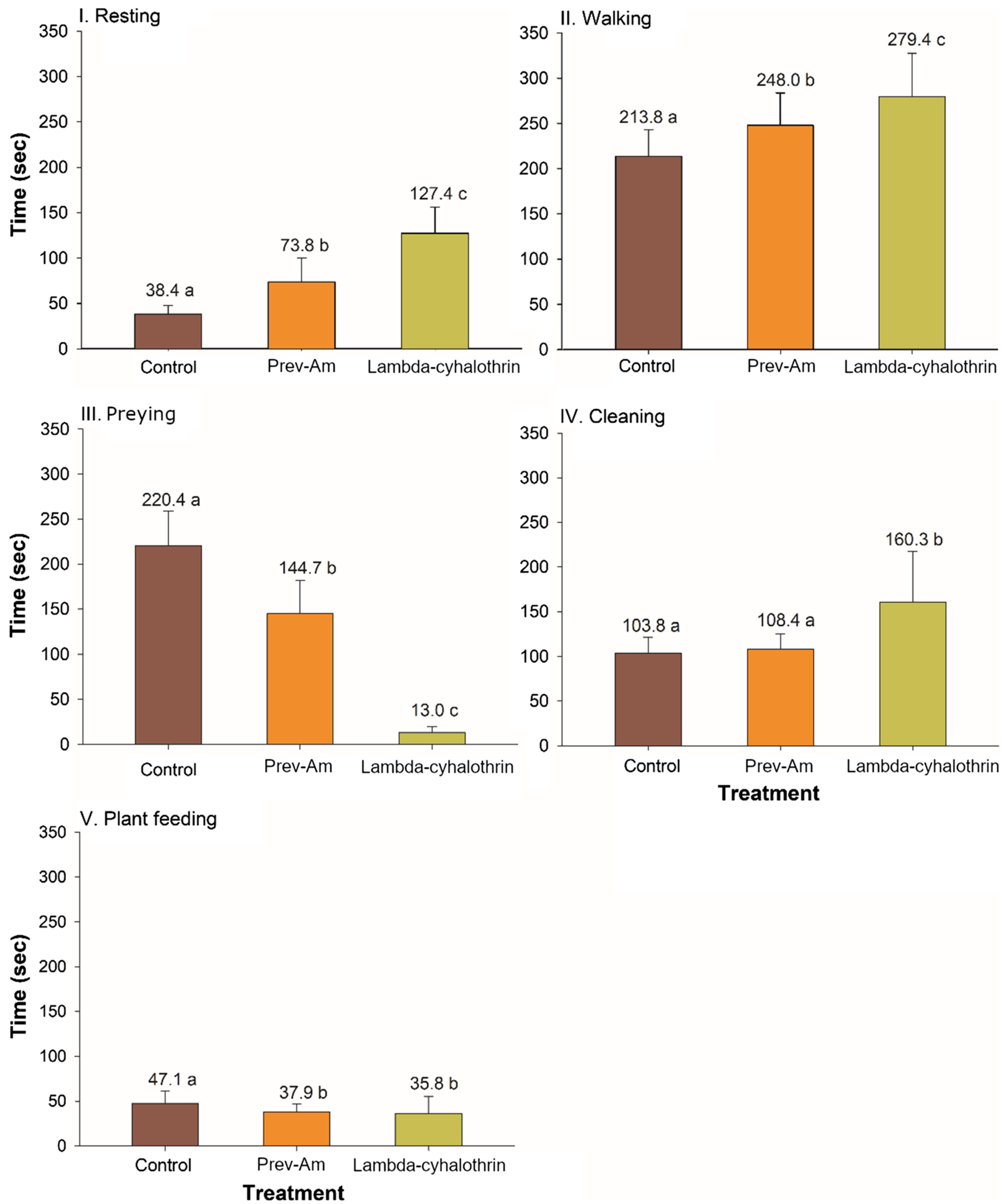

Fig. 3 Time spent (seconds) in resting, preying, plant feeding, walking and cleaning (mean ± SE) by *Nesidiocoris tenuis* females on plants treated with either distilled water (control), Prev-Am® or lambda-cyhalothrin. Each repetition was observed for a period of 10 min. Behavior traits with different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

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

Table 2 Canonical loadings (between canonical structure) of the canonical axes for the *Nesidiocoris tenuis* behavior submitted to different treatments

Behavioral response	Canonical axes	
	1st	2nd
Behavior of *Nesidiocoris 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

Bold indicates the main contributors of each axis

*Significant axes

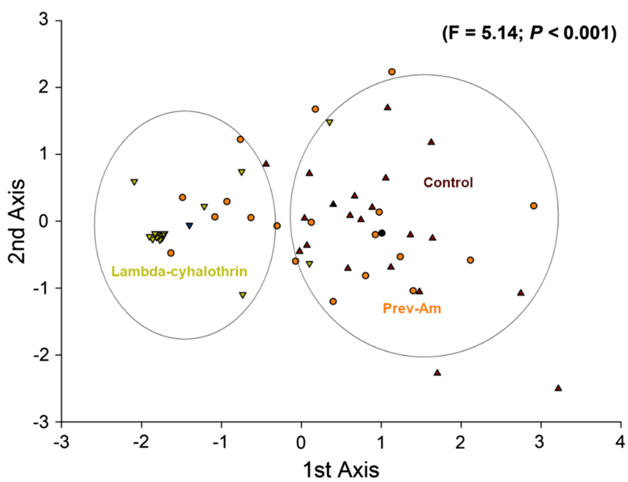

Fig. 4 Ordination (CVA) diagrams showing the divergence in *Nesidiocoris tenuis* behavior (see Table 2) when submitted to different treatments. The solid symbols represent the individual replicates. The large circles indicate treatments that are not significantly different by the approximated F test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

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

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 (12 h and 24 h)

Treatments	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

insecticide exposure (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, other studies found no or little sublethal effects of Prev-Am® on the reproduction of the parasitoids *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae) and *Bracon nigricans* Szépligeti (Hymenoptera: Braconidae), and on the predator *O. laevigatus* (Mansour et al. 2011; Biondi et al. 2012, 2013).

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 is 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 12 h of predation. However, at 24 h 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 were 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

MAS, ND, MRC, A-VL, AB and LZ conceived and designed the experiment. MAS and MRC performed the bioassays. MAS, MMH, MRC and LCP analyzed the data. MAS, LCP, GAC, MMH, MRC and A-VL 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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