Detrimental sublethal effects hamper the effective use of natural and chemical pesticides in combination with a key natural enemy of Bemisia tabaci on tomato
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Detrimental sublethal effects hamper the effective use of natural and chemical pesticides in combination with a key natural enemy of *Bemisia tabaci* on tomato

Marianne A Soares, Geraldo A Carvalho, Mateus R Campos, Luis C Passos, Marcelo M Haro, Anne-Violette Lavoir, Antonio Biondi, Lucia Zappalà and Nicolas Desneux

Abstract

Background: *Bemisia tabaci* (Hemiptera: Aleyrodidae) represents one of the greatest threats to agricultural crops. Chemical control is the primary tool used in integrated pest management (IPM) programs. However, release of the predator *Nesidiocoris tenuis* (Hemiptera: Miridae) on tomato plants is a highly recommended control tactic. The objective of this study was to evaluate the efficacy of a commercial borax plus citrus oil (BCO) product against *B. tabaci* in the presence and absence of *N. tenuis*. The synthetic insecticide lambda-cyhalothrin was used as a positive control. We also evaluated the sublethal effects of BCO on the behavior and predation rate of *N. tenuis*.

Results: Our results demonstrated that BCO, alone and at its maximum recommended field rate for *B. tabaci*, was not effective in controlling the pest under laboratory conditions. Application of BCO simultaneous with *N. tenuis* release did not reduce the increase in the *B. tabaci* population. Effective control of *B. tabaci* was achieved using only *N. tenuis*. However, synthetic lambda-cyhalothrin pyrethroid, used here as a control, caused high pest mortality and led to on-site extinction of *N. tenuis*, which did not occur for insects exposed to BCO. Lambda-cyhalothrin and BCO significantly affected the foraging behavior of *N. tenuis*, reducing the predation rate, especially following exposure to lambda-cyhalothrin.

Conclusion: The insecticide lambda-cyhalothrin achieved satisfactory results in suppressing *B. tabaci*, but was harmful to *N. tenuis*. Additionally, lambda-cyhalothrin and BCO affected predator behavior.

Keywords: biological control; ecotoxicology; integrated pest management; predatory mirids; whitefly

1 INTRODUCTION

The silverleaf whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (MED - biotype Q), is one of the most devastating pests of several crops, and is of global economic importance. The whitefly is highly polyphagous, feeding on and damaging ~500 host plants, including ornamentals, vegetables, legumes, cotton, and corn. This herbivore can cause damage to plants through direct phloem feeding; however, the greatest threat is due to the transmission of plant pathogens during feeding. In addition, *B. tabaci* excretes ‘honeydew’ while feeding on the plant, favoring the growth of opportunistic fungi, reducing plant photosynthesis, and consequently causing yield losses.

Management of *B. tabaci* populations and the plant diseases transmitted by this polyphagous insect represents a challenge for farmers and researchers. Difficulty in controlling whiteflies results from their rapid population growth, ability to develop resistance to conventional insecticides, and their biological development characteristics in that all life stages (eggs, nymphs and adults) remain protected in the abaxial leaf surface of the host plant. Tomato production systems usually require the use of insecticides to control *B. tabaci*, and in most cases, this tool is used in an inadequate manner. This practice has triggered resistance to several conventional insecticides with different modes of action in various *B. tabaci* populations. Cases of resistance are
related to modulators of nicotinic acetylcholine receptors (e.g., neonicotinoids), molecules that act on the nerves and muscles of insects (e.g., pyrethroids, organophosphates compounds, and diamides) and a wide variety of growth-regulator insecticides.\textsuperscript{10,12-14}

The appropriate use of zoophagous predators is a safe alternative to pesticides for controlling pests in tomato crops. Additionally, these predators can be combined with other natural enemy insects (e.g., parasitoids), natural pathogens (e.g., fungi, nematodes, bacteria, and viruses) or selective insecticides (e.g., the diamide chemical group).\textsuperscript{15-21} Among the natural enemies studied for the control of Solanaceae pests, are the predators \textit{Macrolophus pygmaeus} (Rambur) and \textit{Nesidiocoris tenuis} (Reuter) (Hemiptera: Miridae), which are considered to be efficient naturally occurring and/or commercialized agents for biological control against several herbivorous arthropods in Europe, Asia, and Africa.\textsuperscript{22-25} These predators are able to feed on various small pests (e.g., whiteflies, aphids, mites, and lepidopterans);\textsuperscript{26-28} move over the trichomes of plants to a satisfactory degree, and females lay their eggs endophytically in tomato leaves.\textsuperscript{29}

Because of possible mortality (acute toxicity) and several sublethal physiological and behavioral effects of non-selective insecticides in beneficial arthropods,\textsuperscript{30,31} active compounds derived from plants may be an alternative to other types of pesticides for use in integrated pest management (IPM).\textsuperscript{32-37} Botanical pesticides may be used as isolated substances or complex mixtures, and their range of action includes their use as insecticides, fungicides, nematicides, and bactericides.\textsuperscript{38} However, before including new products in IPM programs, it is necessary to know the effects of these botanical insecticides on target and non-target species. Approaches that use population models can provide useful population dynamics predictions based on individual-level parameters.\textsuperscript{39,40} Thus, the harmful effects of insecticides on population levels of pests and natural enemies can be estimated based on the life history parameters of these organisms.\textsuperscript{41-46}

This study explored the potential of a commercial product based on borax plus citrus oil (BCO) combined with the predator \textit{N. tenuis} as a tool for whitefly control. The objectives of this study were to: (i) provide information on the population growth of \textit{B. tabaci} following exposure to BCO and a standard synthetic insecticide (lambda-cyhalothrin) in the presence and absence of \textit{N. tenuis}; (ii) determine the influence of the botanical insecticide and conventional insecticide on population growth in \textit{N. tenuis}; and (iii) evaluate possible sublethal effects on the foraging behavior and predation rate of \textit{N. tenuis} following exposure to BCO and lambda-cyhalothrin.

\section*{2 MATERIALS AND METHODS}

\subsection*{2.1 Biological materials}

Bioassays were performed at the National Agronomic Research Institute (INRA; Sophia-Antipolis) in France under controlled laboratory conditions (25 ± 2 °C, 75 ± 10% relative humidity (RH) and 16:8 h light/dark photoperiod). Tomato and tobacco plants (\textit{Solanum lycopersicum} cv. Marmande and \textit{Nicotiana tabacum} var. Wild) were cultivated in a climatic chamber (25 ± 2 °C, 75 ± 5% RH and 16:8 h light/dark photoperiod) on a commercial substrate (Tournesol®, Nice, France) in plastic pots (2 L), without receiving pesticide applications.

\textit{Bemisia tabaci} (biotype Q) was maintained on tobacco plants in a climatized room (25 ± 2 °C, 75 ± 5% RH and 116:8 h light/dark photoperiod). Whiteflies were kept on two tobacco plants (20 and 40 days old) in an anti-aphid cage made by covering a wooden cage (100 × 100 × 75 cm\textsuperscript{3}) with an insect-proof net (50 mesh). Plants were irrigated three times a week. Once a month, a new tobacco plant was offered to the colony and the oldest plant was discarded.

The predator \textit{N. tenuis} and an alternative prey \textit{Ephestia kuehniella} Zeller (Lepidoptera: Pyralidae) were supplied by Koppert Biological Systems (Almeria, Spain). Predators were kept on tobacco plants (~ 40 days old) in a plastic cage (60 × 60 × 40 cm\textsuperscript{3}) covered by an insect-proof net (50 mesh). \textit{Nesidiocoris tenuis} was fed on \textit{E. kuehniella} eggs. Adult predators were transferred to a new plant once a week, on which females could lay eggs over a period of 1 week. Thus, predators of a similar instar were kept together inside the anti-aphid cage to minimize cannibalism by larger insects.\textsuperscript{47}

\subsection*{2.2 Insecticides}

Prev-Am\textsuperscript{48} (borates tetrasodium salts and 60 g of orange oil per liter; ORO AGRI Internacional Ltda., Gennevilliers, France), referred to as BCO in this study, is produced from sweet orange peel oil and is used as an alternative pest control agent in agroecosystems. To evaluate the demographic parameters of \textit{B. tabaci} and \textit{N. tenuis}, bioassays were performed using three BCO concentrations, namely, the maximum concentration recommended to control whiteflies in the field (0.2352 g a.i. L\textsuperscript{-1}), half of the maximum concentration (0.1176 g a.i. L\textsuperscript{-1}), and 10 % of the maximum concentration (0.0235 g a.i. L\textsuperscript{-1}). The predation bioassay was performed using only the maximum concentration of this product.

Karate Zeon\textsuperscript{49} 100 EC (Syngenta International Ltd., Milano, Italy) is a commercial formulation of the pyrethroid insecticide lambda-cyhalothrin, which is widely used to control pests in agricultural systems. It was used as a positive control at the maximum recommended concentration to control whiteflies (0.0237 g a.i. L\textsuperscript{-1}) in the bioassays of demographic parameters and predation behavior.

The given concentration were obtained by diluting Karate Zeon\textsuperscript{4*} and Prev-Am\textsuperscript{4*} in distilled water, which alone served as negative control.

\subsection*{2.3 Estimating the demographic parameters of \textit{Bemisia tabaci} and \textit{Nesidiocoris tenuis}}

The bioassays aimed to determine the mean offspring and the population growth of \textit{B. tabaci} (ten treatments, of which five had added predators and five had no predators) and \textit{N. tenuis} (five treatments). In both bioassays, 20 replicates were performed per treatment.

Adults of \textit{B. tabaci} and \textit{N. tenuis} were exposed to dry residues of lambda-cyhalothrin and BCO on tomato leaves. Tomato leaves with fully expanded leaflets (~ 15 cm\textsuperscript{2}, composed of five leaflets) were immersed in BCO (in one of the three concentrations: 0.2352, 0.1176 and 0.0235 g a.i. L\textsuperscript{-1}), lambda-cyhalothrin (0.0237 g a. i. L\textsuperscript{-1}) or distilled water for 5 s. The leaves were left to dry for 1 h and placed inside a system composed of overlapping plastic cups, as an experimental unit proposed by Biondi et al.\textsuperscript{50} The plastic cup (700 mL, length: 15 cm) had a hole in the bottom center to allow the leaf stem to reach a second plastic cup (350 mL, length: 11 cm) below that contained water. Subsequently, a fine mesh net was fixed in the upper opening of the first upper cup to permit ventilation and prevent insects from escaping.

To evaluate the demographic parameters of \textit{B. tabaci}, four pairs of whiteflies (~ 2 days old) were maintained in each experimental unit. In treatments comprising \textit{B. tabaci} plus predator, one \textit{N.}
tenuis female49 (~2 days old) was also added. Additionally, to evaluate the demographic parameters of N. tenuis, two pairs49 of predators (~8 days old) were maintained on tomato leaves in the experimental unit described above.

Eleven days after the beginning of each bioassay, living offspring of the whiteflies and predators at each stage of life cycle were counted under a stereomicroscope (×40 magnification). The evaluation time was that proposed by Walthall and Stark.50

2.4 Effects of insecticides on the foraging behavior and predation rate of Nesidiocoris tenuis

Bioassays to determine behavioral response and predation rate of N. tenuis were performed using B. tabaci (second instar) nymphs as prey, and were performed with three treatments (BCO, lambda-cyhalothrin and untreated control). Twenty replicates were performed for each treatment.

Untreated tomato leaves were previously offered to B. tabaci adults for 24 h so the insects could lay eggs. After 13 days, excess nymphs were removed, leaving 150 whitefly nymphs (second instar) in each replicate.

Nesidiocoris tenuis females49 (~2 days old) were kept on treated tomato leaves, and starved for 24 h. Leaves were treated with a BCO or lambda-cyhalothrin solutions, both at the maximum concentrations recommended for the control of whiteflies, as previously described. Distilled water was used as a negative control.

Predators were subsequently isolated in Petri dishes (10 cm diameter × 2 cm height) containing an untreated tomato leaflet infested with B. tabaci nymphs in an agar–water solution (1%). Petri dishes were closed with Teflon® film to prevent insects escaping.

Predator activity was recorded for 10 min per replicate. Thus, five actions were simultaneously recorded in real time: walking, cleaning, feeding on plants, preying on B. tabaci and resting. Time spent on each action was recorded using ETHOWATCHER® software.51

The second step was to evaluate the predation rate of N. tenuis. Thus, after the behavioral assay, predators were kept in the Petri dish described above, and their predation rate (i.e., the number of preyed on whitefly nymphs) was recorded 12 and 24 h after the start of the experiment.

2.5 Statistical analyses

Data were subjected to tests of normality (Shapiro–Wilk)52 and homoscedasticity (Bartlett).53 Subsequently, the number of B. tabaci and N. tenuis nymphs at each instar was subjected to an analysis of variance (PROC ANOVA) and Tukey’s test to detect differences between the treatments (P < 0.05) (PROC GLM).

The demographic parameters of B. tabaci and N. tenuis were evaluated using the instantaneous rate of increase (r), based on the equation proposed by Walthall and Stark50 as follows:

\[
\frac{dN}{dt} = rN - \frac{N}{K}
\]

Where N is the initial population size, which represents the initial number of B. tabaci or N. tenuis used in each experimental unit. r represents the per capita growth rate (capacity of each B. tabaci or N. tenuis to produce descendants). In earlier trials, the carrying capacity of the arenas was determined, based on the approximate number of insects in natural colonized plants, which was represented by the K value; (K-N) is the unused capacity. Growth curves were adjusted using Kaplan–Meier estimators from the non-parametric procedure (PROC LIFETEST). Similarities between the time–response curves were tested in paired comparisons (χ² log-rank test) between the curves.

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

Finally, differences in the predation rates between times and among treatments were analyzed by Generalized Linear Models (GLM’s) following a Poisson distribution. All analyses were performed in SAS v. 9.2 (SAS Institute, Cary, NC, USA), except for the predation rate analyses, which were performed using ‘R’ 3.4.4 (R Foundation for Statistical Computing, Vienna, Austria).

3 RESULTS

3.1 Estimating the demographic parameters of Bemisia tabaci

In the absence of N. tenuis, whitefly females laid more eggs on treatments exposed to distilled water and the minimum concentration of BCO (Table 1). Bemisia tabaci females reduced their oviposition when exposed to half-maximum and maximum concentrations of BCO. The lowest number of eggs was reported for insects in distilled water treatment (Table 1).

The number of nymphs found at the maximum concentrations of BCO and lambda-cyhalothrin treatments was lower than with other treatments. For second instar nymphs, the highest number of offspring was found in treatments containing the minimum BCO concentration; the lambda-cyhalothrin treatment had the lowest number of nymphs. At the third instar, there were no significant differences in the numbers of offspring for insects exposed to minimum and half the recommended BCO concentrations. Finally, the lowest number of nymphs was found in the lambda-cyhalothrin treatment (Table 1).

In the presence of N. tenuis, the lowest number of B. tabaci eggs was obtained in the control and lambda-cyhalothrin treatments. Regarding the first instar of B. tabaci, the lowest number of nymphs was observed in the distilled water and lambda-cyhalothrin treatments. For the second instar of B. tabaci, the largest number of descendants was found in treatments containing the half and maximum concentrations of BCO. In addition, a lower number of B. tabaci nymphs was observed in the distilled water and lambda-cyhalothrin treatments. With respect to the third instar, the largest number of B. tabaci nymphs was observed at the half-maximum concentration of BCO. Additionally, no significant differences were observed between treatments composed of distilled water, maximum BCO concentration and lambda-cyhalothrin (Table 1).
Table 1  Mean (± SE) number of eggs laid and emerged individuals reaching the first, second or third instar in the progeny of Bemisia tabaci exposed to dry residues of borax plus citrus oil (at 10, 50 and 100% label concentration), lambda-cyhalothrin (treated control) and distilled water (untreated control)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Eggs</th>
<th>First instar</th>
<th>Second instar</th>
<th>Third instar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>325.9 ± 12.1 a</td>
<td>38.3 ± 2.8 a</td>
<td>308.9 ± 9.1 a</td>
<td>55.6 ± 4.9 a</td>
</tr>
<tr>
<td>BCO 10%</td>
<td>300.1 ± 10.2 a</td>
<td>37.4 ± 2.9 a</td>
<td>274.1 ± 6.9 ab</td>
<td>40.4 ± 4.0 b</td>
</tr>
<tr>
<td>BCO 50%</td>
<td>221.4 ± 13.9 b</td>
<td>26.8 ± 2.5 b</td>
<td>246.6 ± 14.9 b</td>
<td>37.2 ± 3.4 b</td>
</tr>
<tr>
<td>BCO 100%</td>
<td>117.4 ± 14.3 c</td>
<td>5.9 ± 0.9 c</td>
<td>167.7 ± 12.1 c</td>
<td>14.2 ± 1.8 c</td>
</tr>
<tr>
<td>Lambda-cyhalothrin</td>
<td>1.3 ± 0.5 d</td>
<td>0.1 ± 0.1 c</td>
<td>1.1 ± 0.4 d</td>
<td>1.1 ± 0.4 d</td>
</tr>
<tr>
<td>F&lt;sub&gt;4,95&lt;/sub&gt;</td>
<td>139.58</td>
<td>67.69</td>
<td>150.05</td>
<td>43.81</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Absence of Nesidiocoris tenuis*  

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Eggs</th>
<th>First instar</th>
<th>Second instar</th>
<th>Third instar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>17.1 ± 3.0 c</td>
<td>1.6 ± 0.3 c</td>
<td>27.1 ± 4.2 c</td>
<td>1.5 ± 0.4 c</td>
</tr>
<tr>
<td>BCO 10%</td>
<td>68.4 ± 7.0 b</td>
<td>5.7 ± 0.8 b</td>
<td>104.1 ± 10.3 a</td>
<td>11.4 ± 2.1 b</td>
</tr>
<tr>
<td>BCO 50%</td>
<td>109.9 ± 11.0 a</td>
<td>20.6 ± 1.6 a</td>
<td>103.5 ± 7.5 a</td>
<td>26.2 ± 1.5 a</td>
</tr>
<tr>
<td>BCO 100%</td>
<td>88.1 ± 12.2 ab</td>
<td>8.0 ± 1.0 b</td>
<td>66.5 ± 9.5 b</td>
<td>5.2 ± 1.4 c</td>
</tr>
<tr>
<td>Lambda-cyhalothrin</td>
<td>1.4 ± 0.4 c</td>
<td>0.5 ± 0.2 c</td>
<td>1.3 ± 0.4 c</td>
<td>1.2 ± 0.4 c</td>
</tr>
<tr>
<td>F&lt;sub&gt;4,95&lt;/sub&gt;</td>
<td>32.55</td>
<td>71.26</td>
<td>38.49</td>
<td>61.84</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Presence of Nesidiocoris tenuis*  

*Values followed by the same letter within a column are not significantly different by Tukey’s HSD test (P ≤ 0.05).

The treatment consisting solely of B. tabaci, B. tabaci + BCO (minimum concentration), and B. tabaci + BCO (half concentration) resulted in the greatest population growth capacity. Bemisia tabaci + N. tenuis resulted in fewer B. tabaci nymphs. However, the lowest number of B. tabaci offspring was found on tomato leaves treated with lambda-cyhalothrin (Fig. 1).

3.2 Estimation of demographic parameters for Nesidiocoris tenuis

For the three BCO concentrations and lambda-cyhalothrin, the number of N. tenuis nymphs that emerged from tomato leaves was significantly lower than for insects exposed in the untreated control. Emergence of first and fourth instar nymphs was significantly lower in BCO treatments (three concentrations) than in the untreated control. With respect to second instar nymphs, the three concentrations of BCO also reduced emergence. The half and maximum recommended concentrations of BCO were more toxic for the third instar nymphs. Nesidiocoris tenuis adults did not reproduce when exposed to lambda-cyhalothrin (Table 2).

Although the three BCO concentrations reduced the mean number of offspring of N. tenuis, analysis of the demographic parameters showed positive population growth for predators exposed to BCO treated leaves (Fig. 2). In addition, the increase in the population of predators exposed to the minimum BCO concentration was similar to that for the untreated control. However, the same was not observed for insects exposed to lambda-cyhalothrin, which did not show any capacity for population growth following exposure to the synthetic insecticide (Fig. 2).

3.3 Effects of insecticides on the foraging behavior and predation rate of Nesidiocoris tenuis

CVA indicated significant global differences in the behavior of the predator insects when subjected to different treatments (Wilks λ = 0.61; F = 2.93; df (num/den) = 10/106; P = 0.002) (Table 3).

The CVA diagram suggests that the lambda-cyhalothrin and BCO treatments affected predator behavior compared with the untreated control (Fig. 3). The first axis (P = 0.002) explained 98% of the observed differences (Table 3). Higher canonical loads were observed in cleaning and predation behavior on the first axis, which were responsible for most of the divergence observed between the treatments.

N. tenuis individuals exposed to BCO and lambda-cyhalothrin predated fewer B. tabaci nymphs than those in the untreated control. Moreover, the interaction between time factors and treatments showed significant differences in the reduction in the predation rate (χ² = 8.42, df = 2, P = 0.015). The lowest number of predated B. tabaci nymphs was observed for predators exposed to lambda-cyhalothrin, and the highest number was observed for predators treated with distilled water (χ² = 67.40, df = 2, P < 0.001). In all treatments, predators preyed more during the first evaluation period (12–24 h) (χ² = 1443.62, df = 1, P < 0.001) (Fig. 4).

4 DISCUSSION

Insecticides have been widely used in tomato and other crops due to their effective pest control and rapid action against susceptible populations of B. tabaci. However, environmentally safe approaches (e.g., combining natural enemies and selective products) should be prioritized to avoid a build-up of insecticide resistance. In our study, the B. tabaci population was able to increase when exposed to dry residues of BCO on tomato leaves. Furthermore, a seemingly additive relationship between this botanical insecticide and N. tenuis was observed, although a greater decrease in whiteflies nymphs was observed in the treatment where N. tenuis acted alone. In addition, the insecticide lambda-cyhalothrin provided the greatest control of B. tabaci. By contrast, predators exposed to lambda-cyhalothrin exhibited the
greatest reduction in population growth and predation rate. The behavioral characteristics of \textit{N. tenuis} were affected when predators were exposed to BCO and lambda-cyhalothrin.

Most toxicological studies involving botanical insecticides are based on the acute mortality of target species and natural enemies. However, after exposure to harmful compounds, insects can compensate their individual mortality in the population dynamics. Thus, insects are able to show logistic growth in the population, even after the mortality of some individuals. In our study, \textit{B. tabaci} adults were able to reproduce and, consequently, increase their population even after exposure to BCO. The alternative hypothesis is as follows: (i) whiteflies are more susceptible to BCO during the nymphal stadium than during the egg and adult stages; (ii) serosal cells present in eggs could protect the embryo from BCO toxic compounds; and (iii) BCO does not have a large residual effect by contact against \textit{B. tabaci}. By contrast, whiteflies exhibited a decline in demographic parameters after exposure to lambda-cyhalothrin, which was also observed for \textit{N. tenuis}. \textit{Nesidiocoris tenuis} is an effective predator of \textit{B. tabaci} on tomato plants in greenhouses and open fields. Release of this predator in tomato crops may result in >80\% \textit{B. tabaci} control. Despite the benefits provided by \textit{N. tenuis} in terms of pest control, in some cases, use of insecticides and botanical insecticides is necessary in agricultural systems, especially when pests reach the economic damage threshold in crops. Our results showed that BCO and lambda-cyhalothrin caused a significant reduction in the emergence of \textit{N. tenuis} nymphs. However, the sublethal effect on predator reproduction when exposed to both products cannot be considered similar. \textit{Nesidiocoris tenuis} exposed to BCO showed an increase in its population growth curve. The same was not observed for lambda-cyhalothrin, which compromised the reproduction of the predators.
BCO did not reduce the longevity of *N. tenuis*, as reported for lambda-cyhalothrin, after exposure to dry residues of these products.64 Another important generalist predator, *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae), did not have its longevity altered when exposed to BCO (at field concentration), suggesting that BCO has no effect on this biological trait in these predators. In turn, the number of *O. laevigatus* offspring was reduced after exposure to BCO.48 However, no effects were observed on the survival and reproduction of adult females of the scale insect parasitoid *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae) and the tomato leafminer parasitoid *Bracon nigricans* Szépligeti (Hymenoptera: Braconidae) when exposed to dry BCO residues.43,65

*D*-Limonene (the primary component of BCO) is a monocyclic terpene produced by plants as a secondary metabolite and is often abundant in citrus peel.66 Interestingly, the mode of action of *d*-limonene-based biopesticides is similar to that described for lambda-cyhalothrin, which acts on the nervous systems of insects.67–70 This property could explain the results of our bioassays, in which both commercial products had detrimental effects on the behavioral response of *N. tenuis*. The undesirable sublethal effects on the predator behavioral response may lead to reductions in the reproductive rate, host search capacity and prey capture.64,67,71,72 In a behavioral follow-up study, we offered *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) as prey for *N. tenuis*, and the predators spent more time walking and resting after exposure to BCO during the assessments.64 Moreover, the predation rate was lower compared to that of the untreated insects. Despite the results described above, in the same report, lambda-cyhalothrin was more harmful to *N. tenuis* than BCO.64

The services provided by natural enemies during crop protection may be direct, by predation and/or parasitism, and indirect,
by inhibiting the presence of herbivores, because these insects can recognize the chemical signals released by predators and/or parasitoids. Consequently, the importance of using harm-less insecticides can be emphasized because, even with lower predation rates, the presence of predators is indirectly beneficial for tomato crops. In our results, N. tenuis had the highest predation rate in B. tabaci nymphs during the first evaluation, due to the fasting time (24 h) before exposure to prey in the experimental areas. Furthermore, both compounds promoted a reduction in the number of B. tabaci nymphs preyed on by N. tenuis. The same products decreased the total predation rate of N. tenuis over T. absoluta in a laboratory bioassay. The undesirable interference in predatory behavior after insecticide exposure can be characterized by the: (i) repellent effect, (ii) anti-alimentary properties, and (iii) interruption in the ability to locate prey. We suggest that hypothesis (iii) is applicable here because both commercial products could trigger random movements and increase insect restlessness.

In summary, despite the smaller effect of BCO on the demographic parameters of B. tabaci, use of this type of botanical insecticide should be prioritized instead of lambda-cyhalothrin in IPM programs. BCO could act simultaneously with N. tenuis against B. tabaci, which was not noted for predators exposed to lambda-cyhalothrin. Bemisia tabaci was exposed via residual contact with dry residues; however, direct topical spraying on nymphs and adults may provide more effective pest control. Consequently, direct topical spraying could have additional negative effects on the predator. Thus, to achieve the maximum potential of both control methods, BCO should be employed at first aiming to reduce the B. tabaci population. Since this commercial product is considered to have low persistence in agronomic environments (~7 days, according to the manufacturer), N. tenuis could be released as a second line of defense on tomato crops. It may also be worth evaluating the sublethal effects of BCO on N. tenuis under commercial conditions, as this predator could avoid treated tomato plants, thus reducing the damage caused to its biological characteristics by pesticides.


54 Calvo FJ, Bolckmans K and Belda JE, Release rate for a pre-plant application of *Nesidiocoris tenuis* for *Bemisia tabaci* control in tomato. *BioControl* 57:809–817 (2012).


56 Zappala L, Siscaro G, Biondi A, Mollá O, González-Cabrera J and Urbanbeja A, Efficacy of sulphur on *Tuta absoluta* and its side effects


